AN EXPERIMENTAL STUDY OF THE METASTASIS OF TUMOURS

by

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सुश्रुतसंहितायां, निदानस्थाने, ग्रन्थ्यपच्यर्बुद गलगण्डानां निदानं नाम्नि एकादशाध्याये अर्बुद विषयका इमे श्लोकाः ॥

"मुष्टिप्रहारादिभिरर्दितेऽङ्गे ।
मांसं प्रदुष्टं प्रकरोति शोफम् ॥१७॥
अवेदनं स्निग्धमनन्यवर्ण —
मपाकमश्मोपममप्रचाल्यम् ॥
प्रदुष्टमांसस्य नरस्य बाढ —
मेतद् भवेन्मांसपरायणस्य ॥१८॥
मांसार्बुदं त्वेतदसाध्यमुक्तम्
साध्येष्वपीमानि विवर्जयेत्तु ॥
संप्रस्रुतं मर्मणि यच्च जातम्
स्रोतःसु वा यच्च भवेदचाल्यम् ॥१९॥
यज्जायतेऽन्यत्खलु पूर्वजाते
ज्ञेयं तदध्यर्बुदमर्बुदज्ञैः ॥
यद् द्वन्द्वजातं युगपत्क्रमाद्वा
द्विरर्बुदं तच्च भवेदसाध्यम् ॥२०॥"

('SUSHRUTASAMHITA, NIDANASTHANA',Chapter XI, verses 17-20)

In a part of the body belaboured by blows from fists etc., the afflicted flesh produces a swelling 17

In a person fond of eating meat or whose tissues are vitiated, the swelling progressively increases in size. It is painless, greasy, of uniform colour, non-suppurating, hard like a stone and not movable 18
This is MANSARBUDA and it is said to be incurable.

In the(tumours, which appear) curable, the following should not be selected (for treatment): Those which have spread to vital regions, and those which if they are fixed and have arisen in Srotas (channels, e.g. mouth, eyes, ears, etc.) 19

A tumour originating in an early existing growth is designated as ADHARBUDA by Arbudadnas (Tumour experts).

Tumours which arise (in different parts of body) at the same time or those which arise one after another are DWIARBUDAS (secondary tumours) and are incurable 20

(By courtesy of Dr. V.R. Khanolkar).

TABLE OF CONTENTS

Table of Contents - Cont'd

P R E F A C E

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INTRODUCTION

Living in an era of summits and far-reaching experimentation, are we to believe in the aphorism of Oberling that CANCER is the failure of medicine? The death statistics of the more advanced countries like the U.K., the U.S.A. and Japan tell us that cancer to-day is the second greatest cause of death. The death of about 10% of all human beings is due to this dreadful and very ancient disease. This is in vivid contrast to the success of preventive and social medicine in these countries. Dreadful diseases, such as typhoid, tuberculosis and others, have been almost completely controlled, raising the life span of average person. But death due to cancer seems, on the contrary, to have increased.

In Canada 21,739 people died of cancer in 1957, i.e. 131 per 100,000 population. Cancer is a disease which generally strikes people during the period of greatest social activity. The incidence increases with the increase of age. In Vienna between 1923 and 1933, the number of inhabitants over 60 years of age was augmented by 40% and the cancer death rate increased by 30% (Khanolkar, 1945).

The cancer problem is being studied and experimentally attacked from every conceivable angle, by scientists in Botany, Genetics, Anatomy, Physiology, Chemistry, Physics and Medicine. The methods have achieved great complexity, even to the extent of fractionating the cell, the ultimate constituent of tissues, into minute pieces in trying to localize the abnormal foci of this ill understood disease. Millions of mice, rats, guinea pigs, rabbits and other animals have been sacrificed.

These sacrifices have not all been in vain, but at times led to discoveries which brought nearer the final understanding of the problem. Nobel prizes have been awarded, on occasion, to the experimenters. But the hydra-like disease always baffled human pursuit by extending out hidden tentacles, often to the surprise of the unsuspecting but ever-expectant observers. Such extensions tend to confuse the understanding of the problem, and make evident how ignorant and how limited is our vision and thought.

Though contemporary cancer research workers have opened many secret recesses, and brought to light many hidden truths about the disease, they have yet to put a stop to this deadly process which ushers in "a stealthy attack, slow yet irrevocable; the depression, so different from the buoyancy of tuberculosis; the ulceration, with its fetid secretion; the loss of flesh, giving the advanced cancer patient, with his emaciated, cadaverous face, the aspect of one dead and forgotten among the living. All these stamp on the memory of the persons either relations or attendants watching such a person die, an ineffaceable impression of suffering horror" (Oberling - translated by Woglom, 1946).

That cancer kills was known to such ancients as Hippocrates and Shusruta in the 4th century B.C. They also knew that death was effected by the involvement of vital organs. How the stealthy tentacles of the slowly progressing cancerous crab establish themselves in some of the distant organs is not well understood. This experimental study of the establishment of secondary tumours in the liver and in the mesentery was undertaken in an attempt to elucidate in some part the mechanism of metastasis formation in them.

CHAPTER I

TUMOURS IN ANCIENT INDIA

"The large vegetation of flesh which may appear at any part of the body, which may become slightly painful, which is rounded, immovable and deep seated, which has its root sunk to a considerable depth in the affected part, and which is due to the vitiation of the flesh and blood by the deranged and aggravated doshas (Vayu, Pittam and Kapham) is called an ARVUDA or a Tumour". Thus in about the 4th century B.C. was a tumour protrayed by Shusruta, a Hindoo surgeon in India.

The flesh of any part of the body hurt by an external blow, such as hurting it with a log of wood, and vitiated in consequence was thought to give rise to another sort of swelling (tumour) which was called 'MANSARVUDA' and which was also believed to originate through the action of the deranged Vayu. A mansarvuda was described as glossy, painless, non-suppurating, hard as a stone, immobile and of the same colour as the surrounding skin. Such a tumour appearing in a person addicted to the meat diet was noted to become deep seated owing to the consequent vitiation of the bodily flesh, and was found soon to lapse into an incurable type. Even curable types of Arvudam mentioned in other places were to be regarded as incurable if they appeared as in the cavity of a SROTA channel or artery, or any vulnerable joint of the body, or if they were characterized by any sort of secretion, or were immovable. An Arvudam cropping up on a previously existing tumour was called ADHYARVUDAM, and was deemed incurable. A pair of contiguous Arvuda occurring simultaneously or one after another were called DVIARVUDAM, and were again held as incurable.

An Arvuda of whatsoever type was held never to suppurate, owing to the exuberance of the damaged kapham and fat, as well as in consequence of the immobility, condensation and compactness of the damaged doshas (Vayu, Pittam and Kapham) involved in the case, or out of a specific trait of its own nature.

CHAPTER II

THE DEVELOPMENT OF KNOWLEDGE OF CANCER

The history of Cancer is as old as the subject itself. Wolff (1907) devoted two large volumes to the history of the disease and shorter accounts are those of Oberling (1952), Cowdry (1955) and Wilder (1956). These earlier reports have been used freely in the following general introduction.

Cancer was well known to the ancients. The first known description of malignant disease is found in the Egyptian Papyri in about 1500 B.C. Certain skin lesions which did not heal in spite of vigorous treatment are described. Benign tumours were also noted. In Ebers Papyrus there is very clear description of fat tumours (lipomata) and their operability. However, during this period very little was known except of the superficial tumours. The name 'Karkinos' CANCER was given by Hippocrates in the 4th century B.C., as the extensions into the surrounding tissue from a primary cancer were thought to bear some resemblance to the legs extending from the body of a crab. In Greek 'Karkinos' and in Latin 'Cancer' mean a crab. It is interesting to note that in Sanskrit the term is 'Karkara', again, a crab. Hippocrates also classified the tumours.into two classes (i) 'Carcinos', a miscellaneous group of conditions including many chronic inflammations, haemorrhoids and benign tumours, and (ii) 'Carcinoma' which spread progressively and were invariably fatal. Celsus (30 B.C. to 38 A.D.) distinguished several forms of carcinoma. The first description of local metastatic spread of cancer seems to be that given by Herodotus

in 520 B.C. Herodotus described a tumour of the breast, which burst during his period of observation and spread considerably.

Galen (150 A.D.) in his writings, so long considered to be almost infallible, classified tumours (i) according to nature - the swelling of the pregnant uterus and the breasts in puberty, (ii) exceeding nature - the callus uniting bones when they have been fractured, and (iii) contrary to nature - benign and malignant tumours. He also held that it was the concentration of 'black bile' which was responsible for the cancer. He also compared cancerous disease to a crab as follows "In the breast we often find tumour which in size and shape closely resembles the animal known as the crab. As in the latter the limbs protrude from either side, so in the tumour swollen veins radiate from its edges and give a perfect picture of a crab". It is important to note that the word "metastasis" had not yet been used, and the concept was not understood.

During the next 1500 years little progress was made in the understanding of cancer. The Arabs added to the knowledge of the symptomatology, diagnosis and treatment of malignant tumours, but no new ideas on the nature or behaviour of cancer were brought out. It is interesting that even though the phenomenon of metastasis was not established or understood, the surgeons already advocated radical dissection of the diseased part. Guy de Chauliac (1300-1368) advocated in the removal of the rests of the disease, Fabricius (1537-1619) the removal of the axillary glands in breast cancer, Marcus Aurelius Severinus (1580-1656) the extirpation of the axillary nodes for breast cancer, and Ambroise Paré (1510-1590) the total excision of the tumour.

The German surgeon Guilielmus Hildanus carried out extensive dissections, including axillary nodes. The theory that 'black bile' was the cause of the cancer, as suggested by Galen, was challenged by Paracelsus (1413-1541), who claimed that cancer was due to 'mineral salts' in the blood, and that it developed where these salts became concentrated and sought an outlet. This challenge to the reigning Galenic theory heralded the end of the period of ignorance, and was followed by great many important new discoveries, the circulation of the blood by Harvey (1628), the discovery of the lymph vessels by Olens (1652), and the development of the microscope by van Leeuwenhoek (1673). During this period post mortem examinations became more common. Even so the formation of metastases was not understood. Morgagni (1761) recognized cancer among his 700 autopsy cases but he did not recognize metastases. He found in a case of the cancer of the pylorus of the stomach extensive involvement of the liver, but did not correlate the two findings.

By the 18th century the Galenical influence had receded and a great many new theories had come into vogue concerning the aetiology of the cancer. Bichat (1821), perhaps, brought in a new era. Cancer seemed to him as an accidental formation built up in the same manner as any other portion of adventitious tissue. It is interesting that Bichat founded his observations without the aid of the microscope, and even thought that the microscope could never furnish satisfying results in studies of cancer. Descartes introduced his 'SOUR LYMPH' theory and Sylvius his 'ACIDOSIS versus ALKALOSIS' hypothesis. A most important advance was evident early in the 18th century when LeDran wrote that cancer is a local disease in its earlier stages, but that it spreads by lymphatics to regional nodes and then to the general circulation.

LeDran was supported by Bernard Peyrilhe (1735-1804). Both John Hunter (1835-37) in England and the French School felt at this time that alterations in the lymph might be responsible for cancer formation and formulated the 'COAGULATED LYMPH' theory. They thought that cancer was a systemic disease arising wherever lymph coagulated.

At the beginning of the 19th century, Raspail (1826) showed that the growth of tissues resulted from the multiplication of cells. Van Leeuwenhoek had long before discovered microscopic elements in tissues of plants which he called membranulae or vesiculae and in 1665 Hooke had given these structures the name "cellule". However, only after many years their significance was appreciated. The cell theory was enunciated by Schwann in 1839. Others who laid foundation work were Raspail, Collard and Schlieden. The microscopic examination of cancers by Gluge (1837) and above all by Johannes Müller (1838) showed that they too were composed of cells. Some thought the tumour cells came from germ cells scattered between the tissue elements, others that they differentiated from blastema, a rudimentary substance from which all cells and tissues were formed. It is during this period that the term 'METASTASIS' was first used. Joseph Claude Recamier in 1829 recorded secondary growths in the brain occurring in cases of mammary carcinoma and applied the term 'Metastasis'. Recamier also described the local infiltration of cancer and the invasion of veins by cancer. The blastema theory of the origin of the tumours was finally abandoned in favour of the doctrine of 'omnis cellula e cellula',every cell from a cell, which was announced by Leydig and was adopted by Virchow in 1885, This new belief clarified the views on the nature of cancer, for it could now be seen that a cancer is made up of cells, and that it can originate only

as a result of abnormal proliferation of cells. The concept gave rise to two hypotheses as to the origin of cancer, (i) that any cell in the body that is capable of multiplying could give rise to cancer, or in other words, that normal cells could be transferred into cancer cells, and (ii) that cells destined to become cancerous are abnormal from the very first, carrying over some structural blemish from embryonal life. Johannes Müller favoured the second hypothesis, teaching that cancer developed from morbid seeds distributed throughout the tissues. The other hypothesis also had its advocates.

In 1863 Virchow suggested that 'Chronic Irritation' was the cause of cancer. Chronic inflammatory conditions were noted to be associated with cancer formation, as, for example, in the cancers associated with lupus vulgaris, or those arising in old fistulous tracts leading down to diseased bone or associated with varicose ulcers of long duration. Unna (1896) noted the undue frequency of carcinoma in the irritated skin of sailors, and many other examples can be suggested, Kangri cancers in Kashmir, Kairo cancer in Japan, cancer of the lower lip in pipe smokers, cancer of the penis in the uncircumcized, industrial cancers such as those of chimney sweeps, cotton spinners, aniline dye workers, those who handle tar or paraffin, and those who expose themselves to X-rays or radium without adequte protection. The concept was that every injury to the tissues is followed by a state of irritation in which the cells at the site are stimulated to multiply in order that damage may be repaired. If for one reason or another, the noxious influence should persist, the irritation persists with it, and the cellular proliferation grows more and more excessive and more and more irregular. It seemed only reasonable

to infer that if such a condition of affairs were to last a long enough time, it must almost necessarily end in cancer. Indeed Virchow, the father of irritation theory, insisted that this is what actually happens. Whatever the type of chronic inflammation, whether due to burns, soot or sunlight, the result would be always the same, chronic inflammation. And chronic inflammation leads to cancer.

As early as 1829, two French investigators, Lobstein and Recamier, attributed the origin of tumours to the proliferation of embryonal cells that had persisted into adulthood. Müller, Paget, Remak, Durante, Cohnheim and many others supported and extended this view. Of these, Cohnheim was the principal exponent. He thought cancers originated from small clump of cells misplaced in the course of development. He also thought that chronic irritation helps to bring about the malignant transformation of these cells. On the other hand, Ribbert thought that the abnormally located cells were more or less isolated and not subjected to the same nervous and blood vascular regulation as are normally situated cells. He assumed that they became more and more independent, to the point of the almost complete autonomy typical of the malignant cells. The misplacement was supposed to have been brought about by chronic inflammation. Thus Ribbert's theory and Cohnheim's are both a combination of the irritation and the embryonal concepts.

Borrel in 1903 proposed that viruses might be the aetiological agents of cancer. In 1911, Rous reported the transmission of a malignant tumour of fowls by a cell-free extract. Following this Rous and Murphy (1914) showed that other avian mesoblastic tumours, myxosarcoma, osteosarcoma, angiosarcoma, and endothelioma could often be transferred from bird to bird in indefinite series by their filtered extract. The

experimental production of tar tumours by Yamagiwa and Ichikawa in 1915 temporarily damped the spirit of research in this field, but in 1932 the interest was renewed when Shope grew in rabbits a fibromatous tumour which could be propagated by cell-free extracts. Sanarelli discovered a similar tumour of rabbits which he called 'infectious myxoma'. Shope showed that the causative organisms of these two tumours were immunologically related. In 1933, Shope discovered a virus-induced papilloma of the skin in wild cotton tail rabbits. This tumour remains benign for a long time but may change to squamous cell carcinoma, metastasize and ultimately kill the animal (Shope 1933, Rous and Beard 1935, and Syverton 1952). In 1934, Luckė demonstrated a renal adenocarcinoma of frogs to be of virus origin. In this cancer the affected cells show intranuclear inclusions like those in other virus diseases. Burmester in 1947, 1952 and 1954 showed that under certain conditions, avian lymphomatosis may spread from host to host among very young chicks. Gross (1951) was the first to demonstrate the cell-free transmission of a mouse leukaemia. Subsequent confirmation of the fact was reported by further experiments of Gross (1953, 1954, 1955 and 1958), Furth (1956), Woolley and Small (1956), Dulaney (1957), Hays (1957) and Kassel (1958). In 1953, Gross found that the virus which transmitted the leukaemia in mice was also able to induce salivary tumours and fibrosarcomata in the host. Stewart (1958) announced that a 'virus' derived from mice with leukaemia was able to induce many kinds of tumours in its hosts. To-day the milk factor, which was discovered in 1933 by Bittner and his associates and by Korteweg (1934) is also thought to be a virus agent (Dmochowski, 1953).

In 1913, Fibiger described the relationship between the presence of parasitic worms and cancer by demonstrating cancer-like masses in the cardiac region of the stomach of rats. The worms which were supposed to cause the tumours were called Spiroptera neoplastica and later on Gongylonema neoplasticum. Later investigators suggested the cause of these gastric tumours was 'vitamin deficiency'. However, the matter is still not clear, and other worms are undoubtedly associated with tumour formation. Tumours of the liver, for example, are associated with Cysticercus fasciolaris (Bullock and Curtis, 1924), bilharzia with bladder tumours (Affifi 1948), and Spirocerca lupi with oesophageal sarcoma in dogs (Ribelin and Bailey, 1958).

With the unfolding of the knowledge about cancer, attempts were directed to produce cancer experimentally. One of the first attempts at the experimental production of cancer was made by Bernard Peyrilhe. He attempted to transfer human cancer to the dog. At first, it was thought that he was successful, as the tumour grew at the site of transfer, and he was awarded a prize by the Academy des Sciences et Belles-Letters de Lyon. However, further investigation proved it all to be a mistake, and the lesion induced to be an abscess. Hanau (1889) carried out the first successful transplantation of cancer from one individual to another. He took metastases from a primary carcinoma of the skin of a rat and inoculated it into the tunica vaginalis of the testicles of two other rats of the same species and found, after several weeks, scattered tumours in the peritoneal cavities of both hosts. These tumours were microscopically similar to the primary tumours. In 1881, Morau transplanted a mammary carcinoma of a mouse to other mice. He also succeeded in transferring the tumours in series of mice for several

generations. But these findings were not immediately accepted and, indeed, Hanau committed suicide with his contribution scorned. Ten years later Leo Loeb (1901) and Jensen (1903), among others, proved the correctness of these observations and transplantation of tumours became commonplace. The 'experimental' production of cancer in human beings by X-rays was noted in 1901 by Frieben (1902). Cancer of the skin developed six years after the discovery of X-rays by Roentgen on the hand of a man who used his hands freely in the manyfacture of X-ray tubes. Shortly after other cases also occurred in those exposed to X-rays. Clunet (1910) was perhaps the first to produce experimental cancer in animals. This investigator caused ulcers to develop in the skin of rats by exposure to X-rays. The ulcers were prevented from healing by repeated exposures. A sarcoma developed nine months after the start of the experiment in one animal, and two years after in a second animal. Clunet was able to transplant the latter tumour into other rats for several generations. Soot and tar had been suspected as carcinogens since the observation in 1775 of Percival Pott, the English surgeon, that carcinoma of the scrotum was unduly common in the chimney sweeps. Pott suggested that these cancers were caused by soot lodged in the rugae of the scrotum. Many attempted to induce carcinoma of the skin experimentally by applying tar or other substances to skin of various animals, but it was Yamagiwa and Ichikawa who in 1915 reported the experimental production of cancers in the ears of rabbits painted repeatedly with the tar. Thus they showed clearly that a chemical substance is capable of giving rise to cancer as well as the physical agent, X-ray, discovered earlier. In 1918, Tsutsui showed that tar could induce cancer in mouse skin, and the great volume of

work on chemical carcinogenesis summarized by Woglom in 1926 began.

Meanwhile, other advances had been made. In 1910, Carrel and Burrows; and Volpino grew cancer cells in tissue cultures. Lewis (1931) first observed the remarkable phenomenon of pinocytosis and compared the phases of mitosis in malignant and normal cells.

Maud Slye dedicated her life to the study of hereditary cancer in mice. She laid the basis for the development of the pure, closely in-- bred strains of to-day. This was a great step forward as the members of each strain inbred through a sufficient number of generations have become almost as like as identical twins. By the selective crossing of members of different strains many modern developments in cancer genetics have been made possible, and because of the near identity of members of the same pure strain the influence of environment and many other factors on cancer initiation and development and cure can be tested with assurance that the otherwise complicating factor of differences in hereditary endowments has been eliminated.

A tumour 'Crown Gall' in plants was described by Smith in 1916. He pointed out its relation to human cancers. The cause of the tumour formation was found to be a bacillus, B. tumefaciens. So far no human cancer has been shown to be caused by bacteria, yet the value of this fundamental study should not be underrated. Because by their studies on mutation and viruses, botanists and bacteriologists are in recent years contributing greatly to our knowledge of the cancer problem.

Loeb in 1919 suggested 'oestrogen' (folliculin) as a possible cause of cancer. He showed that in mice displaying a high incidence of breast cancer, cancer could be avoided if the animals were oophorectomized before they were four months old, and its frequency could be reduced if

the animals were spayed between 4 and 7 months of age, but that later oophorectomy had no effect. Lacassagne (1932) showed that weekly injections of oestrogen into female mice of a high breast cancer strain increased the incidence of breast cancer distinctly, and caused the tumours to appear earlier; but in mice of a low breast cancer strain found the injections of oestrogen were without effect. He also reported that injections of testosterone into females of high breast cancer strain prevented the development of breast cancer, perhaps by causing atrophy of the oestrogen producing ovarian tissue through its action on the pituitary. This work has been confirmed by Burrows (1935), Allen and Gardner (1941) and others.

Warburg in 1923 made a discovery which gave him the Nobel prize. He announced that cancer cells differ in their metabolism from normal tissue, in that they have a higher rate of anaerobic glycolysis. They consume more sugar and give off more lactic acid. But later investigations proved that at least 20% of normal tissues have similar rate of glycolysis to the cancer (Greenstein, 1947).

Findlay (1928) produced cancer in animals with ultraviolet rays. It is important because sunlight containing ultraviolet rays is the one carcinogen to which all of us are more or less exposed.

After 1930, a very considerable increase in our knowledge of chemical compounds producing cancer occurred. Kennaway in 1930, with his associates, succeeded in finding a tar fraction, 3,4-benzpyrene, which produced skin cancers in mice. Since then an enormous number of investigations have been carried out, and reviews by Hartwell (1951) and Shubik and Hartwell (1957) give a list of 405 compounds which were reported to cause malignant tumours in animals, and 35 others which induce

only benign tumours. The carcinogens are divided into inorganic and organic compounds. The organic compounds include aliphatic, monocyclic, bicyclic, tricyclic, tetracyclic, pentacyclic, hexacyclic and higher hydrocarbons, azo derivatives, steroids, heterocyclic compounds and unclassified substances. Among the important and common ones are 20-methylcholanthrene, a component similar to bile acids (Cook and Haslewood, 1934), urethane (Nettleship, Henshaw and Mayer, 1943), 2-acetylaminofluorene (Wilson, DeEds and Cox, 1941) and plastics (Oppenheimer and associates, 1948, 1952 and 1953).

At first, carcinogenic action was looked upon as a single, if long drawn out process. The work of Berenblum (1941), Mackenzie and Rous (1941), Rous and Kidd (1941), Friedewald and Rous (1944), Mottram (1944, a & b), Kline and Rusch (1944), Rusch and Kline (1956), Berenblum and Shubik (1947, a & b) and others showed that cancer formation may occur in two stages. After a suboptimal treatment, or a single application of a subliminal dose of a carcinogenic compound to the skin, tumours could be made to develop in considerable numbers by subjecting the pre-treated skin to certain forms of stimulation which were themselves not carcinogenic. The change from the normal to the dormant tumour cell has been named 'initiating' action and the awakening of the dormant tumour cells into actively growing tumour has been named 'promoting' action (Friedewald and Rous, 1944).

Cancer research to-day is spreading its claws like the crab itself into every conceivable field. We cannot even summarize the growing volume of work. The transformation of a solid transplantable mouse carcinoma into ascites tumour (Klein and Klein, 1951), and the

cell fractionation of Bensley and Hoerr (1934) are just two of the many important advances.

Lastly, after everything is said and done, it stands out clearly that cancer is a cellular problem (Müller, 1838) as has been conclusively shown by its successful transmission by the inoculation of a single malignant cell. This was first accomplished by Furth and Kahn (1937) employing mouse leukaemia, and later with Yoshida's ascitic sarcoma by Ishibaschi (1950) and Hosokawa (1950) by intraperitoneal inoculation. Earle (1952) cultured both single normal and malignant cells in tissue culture.

This is only a brief account of some of the landmarks in cancer research. It is not possible to approach even to the edge of the enormous volume of work and literature in this vast field. We have tried to outline the more important aspects of the subject. In later chapters the factors of importance to our immediate purpose will be taken up in more detail.

CHAPTER III

THE DEVELOPMENT OF THE CONCEPT OF METASTASIS

Metastasis is one of the significant features of malignancy. Originally the term was used to signify certain diseases or afflictions which were caused under the special influence of malign or evil spirits. Cancer was thought such a disease. It was noted that malignant tumours persisted and were associated with a tendency for softening and ulceration; that these tumours showed an infiltrating mode of growth called 'invasiveness', so that the margin of the tumour interpenetrated and destroyed the surrounding structures; that pieces of these tumours might escape to form secondary tumours in the adjacent lymph glands or be carried to distant parts of the body and there form secondary growths; that these tumours often grew again even after drastic attempts to ensure their complete destruction by radical surgical procedures; and that these tumours were associated with cachexia, a marked impairment of the general health with wasting.

It is well known that cancer kills, and this fatal issue is due mostly to the involvement of the vital organs. This involvement takes place by the invasion of the surrounding tissues and by metastasis to distant sites. This is, therefore, the aspect of malignancy that concerns us here.

It is interesting to recall that though in the 4th century B.C. in India Shusruta had already distinguished between the primary tumours ARVUDA and the secondary tumours DWIARVUDA, yet it was only in 1829 that Recamier first used the term 'METASTASIS' for these distant or separate

growths. It is also important to note that infiltration occurs not only in malignant tumours, but also in chronic inflammatory processes, in the invasion of the uterine wall by Trophoblast in pregnancy, and in other conditions. In erosions of the os uteri, for example, Friedlander and Councilman (1908) stressed the importance of invasion by inflammatory cells. Nor is metastasis a character of malignant tumours only. The term 'metastasis' originally was used to indicate transportation of any substance metal or coal dust, normal cells, placenta, bone marrow, bacteria or cancer cells from one part of the body to another. It was von Recklinghausen who first restricted the meaning of the term and used it only in connection with the pathological substances. Now it is even further restricted and used rarely except to denote secondary deposits of tumours. Willis (1953) defines metastases as those growths which are separate from the primary growth and have arisen from detached transported fragments from the primary focus. Ewing (1940) defined it as 'the formation of secondary tumours at some distance from the primary growth' and Berenblum (1958) calls a metastasis 'a secondary centre of tumour growth at a distance from the primary focus'. Our study is mainly devoted as to how these metastases of tumours are formed. Nevertheless it should be remembered that non-neoplastic tissues can also form 'metastases'. Young and Griffith (1950) give examples.

Though Recamier named metastases, he could not explain the mechanism of their formation. Virchow (1863) thought malignant tumours contained a fluid which was carried in the lymphatics, or that cancers might encroach upon the walls of veins and release a fluid which travelled in the blood stream. He thought these fluids possessed the power of producing an infection which disposed different parts of body to a

production of a mass of the same nature as the one which originally existed. Virchow also pointed out that the infectious juices might form cancerous tumours at a more distant point if they passed through the lungs without producing any change in them. He noted that certain cancers appeared to advance in a direction contrary to the current of the lymph. Although Hannover described the circulation of cancer cells in the blood before Virchow, it was Thiersch (1865) who disproved the fluid theory. He suggested that emboli of tumour cells were the cause of secondary deposits, considering cancer as a local process which spread and became systemic by the embolic phenomenon. Waldeyer (1872) extended Thiersch's theory, showing that tumour spread by the blood and lymph vessels by continuous growth as well as by cell emboli. These two workers by their direct evidence, adduced after the painful study of huge numbers of sections, finally led to the rejection of Virchow's theory of metastasis formation. Following Thiersch and Waldeyer's work, a great many investigators came to support the "mechanical" theory, agreeing that tumour cells travelled in the blood and the lymph vessels and caused secondary growths wherever they lodged. Hoggan (1878) and Stevens (1907) supported the mechanical theory. Goldmann (1906) stated that purely mechanical conditions govern the occurrence of secondary growths of lymphatic glands though he had forewarned in 1897 that 'tumour embolism is not metastasis'. Stiles (1899) upheld the embolic origin of lymph gland metastases of carcinoma of the breast as he did not find microscopically any growth in the main lymphatics between the breast and axilla, and also because secondary growths did not appear at intermediate points. Von Recklinghausen (1885) stressed that the retrograde flow in lymphatic channels caused by obstruction explained

some of the peculiarities in the location of metastasis, thus again supporting the mechanical theory.

However, Billroth (1879) opposed the mechanical theory and put a question 'what is that decides what organ suffers in cases of disseminated cancer'? The question was reviewed by Stephen Paget (1889). He pointed out that in cancer the distribution of metastasis in various organs is by no means impartial. He deduced from his observations that some organs form a more favourable site for the growth of the metastatic cells than others. He introduced the famous 'seed - soil' theory, proposing that for the establishment of metastases one must have a good 'seed', viable tumour cells, and a good 'soil', a suitable site of lodgement.

Schmidt (1903) demonstrated small emboli in small arteries of the lungs in cases of abdominal tumours, but noted that only a small proportion of these emboli gave rise to metastatic tumours, or broke through the arterial wall into the perivascular tissue. Most of them were either destroyed by organization of their ensheathing thrombus, or, while retaining the power of growth, were encapsulated and rendered harmless. Some, however, pushed forward through the organizing thrombus which surrounded them and so gave rise to new emboli and to new growths in the course of the systemic circulation.

Armstrong and Oestil (1919) also agreed that the site of a secondary tumour depends on a great many other factors besides mechanical ones. They attributed much importance to the effect of the 'metabolic processes of the tumour cells, the physiological resistance to the biogenetic relationship of the tumour cells, to the tissue soil and the quantity of tumour elements'.

In the beginning of the present century Handley (1922) added another concept, continuous permeation in the lymphatics. He considered that the lymph glands became involved by carcinoma not by embolism, but by the growth of a continuous column of cells through the lymph vessels. To explain how lymph glands may be involved without evidence of permeation in the afferent lymph vessels, he postulated that perilymphatic fibrosis might destroy the continuous line of cancer cells. In 1938, Gray maintained that the mode of spread to the lymph glands is by lymphatic emboli, that cancer affects lymphatics only in a mechanical way and that cancer cells do not remain for any length of time within the lumen of the lymphatic vessels. He was unable to confirm the permeation theory of Handley and this theory has never been widely accepted as the sole mode of lymphatic spread.

Thus the view put forward at the end of the 19th century, that metastatic disease was to be explained basically on the mechanical theory with some modifcation due to the theory of soil selection remains unchanged to-day.

CHAPTER IV

EXPERIMENTAL WORK ON METASTASIS

It is hard to review the experimental work on metastasis formation in a logical manner. The experiments have been so varied, and so often cannot be accurately compared one with another, that it has been thought best to present the work more or less in chronological order, with only minor attempts to group like studies together. The work on certain factors of particular relevance to this thesis will be considered more fully in subsequent chapters. In most of the experiments to be described transplantable tumours were used. It has been necessary to consider the tumours arising at the site of inoculation sometimes as a primary, and sometimes as a secondary. As will be seen this confusion is also unavoidable.

Hanau (1889) recorded the presence of a metastasis in the inguinal lymph glands of a female rat with primary epithelioma of the vulva, which he transplanted successfully and got dissemination over the peritoneum in the host animals. Jensen (1903) saw no metastases in the lungs or elsewhere in the mouse in which his now well-known tumour occurred, but Borrel (1903) recorded pulmonary metastases, usually in the form of emboli to small branches of the pulmonary artery, in a large proportion of mice with spontaneous breast tumours. Borrel and Haaland (1908) recorded lymphatic metastases in a case of a squamous cell carcinoma of the lower jaw in a mouse. Haaland (1905) gave a full description of the pathological anatomy of the condition with figures of the primary growth and the metastases in lymph glands.

These works showed that the metastases of the transplanted carcinoma originated in minute emboli, as noted by Borrel. Bashford, Murray and Cramer (1905) working with the Jensen tumour found metastasis in the lung as soon as 44 days after subcutaneous injection. They confirmed Borrel's finding that the earliest metastatic deposits were microscopic in size, merely distending the walls of small pulmonary arteries but not breaking through them. In later cases, all stages of invasion were followed in different areas. Numerous isolated cancer cells were found in rapid process of division. In larger arteries, emboli completely occluded the lumen of the vessels, and at places continued proliferation of the cells led to the formation of a large growth which extended beyond the limit of the blood vessels and invaded the alveoli of the lungs. These metastases in the lung illustrated the results of extension by blood stream. In metastatic growths which grew to a certain size, stroma and blood vessels were provided by the host as in a primary tumour at the site of inoculation. Bashford, Murray and Cramer (1905) also studied a different mode of extension of the Jensen tumour. When it was injected intraperitoneally, the growth involved the liver and the small intestine, forming small lobulated masses. In the liver the tumour was first seen beneath the peritoneal covering but soon penetrated deeply into the organ, destroying liver cells as it advanced. There was evidence of expansive growth, pressing upon and distorting the normal arrangement of the liver. In the intestine, the tumour grew in between the longitudinal and the circular muscle layers. There was again evidence of expansive growth pressing upon and distorting the normal arrangement of the surrounding tissues. When dense tissue

opposed its progress, the tumour assumed an infiltrative character, and extension took place in narrow columns which passed through the less resistant parts, and pressed on the other structures. In the same year, Bashford, Murray and Haaland (1908) showed that immunity or resistance could be induced by interposing inoculation of the normal tissue or of spontaneous tumour between a primary successful inoculation and a subsequent one. Murray (1908) investigated the formation of metastasis in lungs and in lymph glands in the case of mammary tumours of the mouse. Murray pointed out that in the mice extension took place mainly by the blood stream. The metastases in the lung began as miliary emboli in the terminal branches of the pulmonary arteries. The endothelium and internal elastic lamina seem to present a serious obstacle to the free growth of the cancer cells, and the emboli therefore frequently grew to a considerable size within the vessel. In this way long sausage-shaped masses were formed filling up a considerable part of the pulmonary arterial system as already described by Borrel (1903), Haaland (1905), and Bashford, Cramer and Murray (1908). Healing occurred when the nodules failed to become vascularized, becoming encapsulated by the proliferation of the endothelium, and ultimately degenerating and being replaced by sclerotic connective tissue. If, however, the nodule became vascularized, capillaries budded in from the intima of the vessel and the growth, now being better nourished, increased in size, burst through the wall of the artery and invaded the lung tissue. Murray also showed that plasma and blood have no power of destroying cancer cells directly as had been assumed by many. He emphasized that the determining factor is the cancer cell itself. He thought possible that the cells detached from the primary growth at

different times might vary in their power to establish themselves in new sites. When intraperitoneal transplantation was attempted the phenomena observed were strictly comparable with the behaviour of intravascular emboli. The cancer cells were shut off by the mesothelium from the connective tissue, the reaction of which as has been shown by Russell (1908), is essential for successful transplantation. In this way was explained the long time for which metastases remain intravascular and, incidentally, the similarity of the intraperitoneal 'rice bodies' of Ehrlich's chondroma with the unvascularized nodules of the same tumour produced in partially immunized mice.

Lewin (1908) showed that by repeated transplantation of a mammary carcinoma of the rat, it is possible to get different varieties of tumour. The animals died of cachexia and macroscopically metastases were seen in almost all organs. Levin and Sittenfield (1911) failed to obtain pulmonary growths in any of 36 rats killed 8 - 28 days after injection of sarcoma intravenously. However, a subcutaneous inoculation of the same emulsion produced 90% growth of tumours in animals. Subcutaneous implants of the Flexner Jobling rat carcinoma frequently yield spontaneous metastases and when they injected the minced tumour intravenously, pulmonary growths were obtained in 3 out of 16 rats. Weil (1913) pointed out the reason for Levin and Sittenfields failure to obtain metastases by injecting sarcoma intravenously was their defective method of preparing the tumours for injection. He used a carefully prepared emulsion of the sarcoma and obtained a high percentage of pulmonary tumours. Iwasaki (1915) following up Weil's work obtained growths in 19 out of 23 intravenous inoculations. He studied the

histological changes in the tumour emboli arrested in the lung vessels and found intravascular destruction of the majority of the tumour cells. He also showed that all the tumour cells introduced do not die inside the blood vessels, finding mitoses inside some of the vessels.

Others studied the effect of various local factors on the frequency of metastases. Some studied the effect of such factors on the "primary", others these effects in determining the site of the metastases, or the ease with which they took and grew. Worzosek (1911-12) found in mice that a transplantable tumour implanted in the tail grew more slowly than did the same tumour implanted elsewhere, but that it gave rise to metastases more frequently than did the tumour implanted elsewhere. He came to the conclusion for the frequent appearance of microscopic metastases in mice inoculated with a transplantable tumour, that the tumour must have exceptional growing energy; the mice be inoculated in a place which is not favourable for quick development of the tumour, and only mice whose organs are adapted for forming microscopic metastases should be inoculated. Tyzzer (1913), experimenting with inoculated mouse tumours, showed that massage of established subcutaneous growths markedly augmented the incidence of metastases in the lungs. In non-massaged mice visible pulmonary metastases were never found earlier than the 39th day after the subcutaneous inoculation, but 80 per cent of the massaged mice had visible pulmonary metastases on the 36th day. He also found that although incomplete amputation or incision of implanted tumours did not increase the incidence of metastases, these did grow more rapidly than usual. He suggested that this might depend on improved general condition of the mice following the removal of the bulk of the

primary growth, or perhaps because of the availability of extra growth substances for the metastases. Lubarsch (1912 & 1922) used many series of animals with implanted tumours in the hope of ascertaining what effects mechanical forces could exert on the rate of growth. He inoculated sarcomata into mice and rats in various ways; traumatized spontaneous tumours in rats and dogs with forceps, or injected homologous blood into tumours over periods of months but he was not able to observe any increase in the rate of growth of the tumours, in their morphologic structure or their mitotic rate. He pointed out that untraumatized tumours do not grow with any regularity and that considerable periods of rest may alternate with active periods of growth without apparent cause, a phenomenon that abundantly confirmed by others. Marie and Clunet (1910) found that of 145 mice dying of a particular implanted tumour none had visceral metastases, whereas of 11 animals in which the tumour had been excised, 5 presented metastases. They thought, as had Tyzzer (1913) of the implanted growths made available larger supplies of some nutritive substance for the growth of metastases. Similar findings were reported by Tadenuma and Okonogi (1923-24). The explanation is a modification of the Athrepsia hypothesis of Ehrlich (1908) and that of Apolant (1908 & 1911). This hypothesis postulated the existence of specific growth substance essential to tumour proliferation, which were abundant in susceptible animals and scanty in resistant animals. By excising part of the primary, more of this substance is made available to the metastases. Tadenuma (1923), in his experiments with fowl sarcoma, also found that excision of the implanted tumours decidedly increased the incidence of metastasis and thought the reason was perhaps, in part, the prolongation of the

life, but that the principal factor was probably the greater supply available to the metastases of some 'Threptic substance'.

Lubarsch (1912) found that mouse tumours may be made to localize in the liver around splinters implanted in this organ. Marchand (1914) implanted lycopodium spores in the peritoneum to serve as a foreign body. They were rapidly surrounded by mesothelial cells and later encapsulated by connective tissue. The granulation tissue so formed offered a most favourable locus for tumour implantation. Jobling (1910) showed that intraperitoneal injection of a suspension of a rat carcinoma killed by heat might increase the susceptibility of animals to later subcutaneous implantation of the same tumour. Loeb (1912) found that the tumour grew better in regenerating kidney than in the healthy organ. Loeb and Sweek (1913) ascribed the sluggish course of epitheliomata to the resistance offered by an inert connective tissue. Levin (1912) showed that Flexner Jobling rat tumour inoculated into the testes of rats would grow in testes previously injected with Scharlach R and ether water but not in normal testes, and suggested the chemical influence induced a 'precancerous state' in the testicle. Jones and Rous (1914) dealing with the cause of localization of secondary tumours at points of injury, felt that it was the derangement of the connective tissue rather than the mesothelium which rendered an injured peritoneal lining favourable to the lodgement and growth of the tumour fragments. Levin (1912) also showed that when an inflammatory lesion is produced in the organ previous to the inoculation of the tumour, the inoculation was more likely to be successful.

Others studied the effect of systemic factors on metastases. Levin (1913) found that in rats in which subcutaneous inocula took poorly, direct inoculation of tumour material into the liver and spleen also failed to produce tumours. But he found that in those animals in which the subcutaneous tumours developed well, the inoculation of the liver and spleen were also successful. Further experiments indicated also that the visceral inoculations were successful in those animals in which the subcutaneous tumours recurred after the excision, but failed when there was no recurrence of the subcutaneous tumours after excision. Experimenting with the Jensen sarcoma and Flexner-Jobling adenocarcinoma, he also showed that when an emulsion of one of the tumours was injected intravenously, no tumour growth formed anywhere, but when such an emulsion was inoculated into a parenchymatous organ like the liver or spleen, tumours formed in a number of cases. This formation of tumours in the liver and spleen was explained due to the change in character which the secondary cells underwent during their sojourn in the parenchymatous organs. He cited Hansemann as having made similar observations in human pathology. Levin pointed out that his experiments showed the formation of metastases is a complex phenomenon. The frequency and localization of metastatic growth differ in various tumours. Also the same tumour may form metastases in different organs in different individuals. Takahashi (1915) inoculated various tumours intravenously and found pulmonary growths in 0 - 90 per cent of animals according to the tumour used. Well differentiated squamous cell carcinoma and glandular carcinoma failed to grow in the lungs, but less differentiated tumours grew with varying degrees of success. Uhlenhuth, Händel and Steffenhagen (1911) showed that when a transplanted tumour was

removed surgically and did not recur, then was inoculated subcutaneously in a different place, the second inoculation would not take. If, however, after the removal of the first tumour there was a recurrence, then the second inoculation succeeded. These striking results were confirmed by Meidner (1912), Händel and Schönburg (1912) and by Uhlenhuth, Dold and Bindseil (1912). Apolant (1911), however, did not agree, and denied any relation between the recurrence of the first tumour after the removal and the success of the second inoculation.

The studies on the local and systemic factors which may influence metastases were continued. In 1916, Gaylord and Simpson found that anesthaesia and haemorrhage increased the susceptibility of mice to cancer transplants and augmented the growth of recurrent tumours and of metastases. Wood (1919) showed that duration of the growth also plays a part in increasing the number of metastases. Sampson (1924) claimed that manipulation of the uterus and diagnostic curretage in human patients may dislodge carcinomatous fragments into the blood vessels or into the peritoneum via the fallopian tubes. Sappington (1922) and Dial (1930) also stated that there had been cases in which unusually extensive metastases were found in patients whose primary tumours were subjected to massage. Nather (1922) implanted a carcinoma intramuscularly into 30 mice, and, when the tumours had grown, made biopsies from one half of them. Four days later the biopsied mice were found to weigh about 5 per cent more than those not biopsied, and Nather concluded that this considerable increase in body weight was due solely to an enormous propagation of tumour growth following diagnostic incision. Knox (1921), while experimenting with inoculated tumours in mice, showed that as in

experiments of Tyzzer, massage of the primary tumour increased the number of tumour emboli arrested in the lungs and increased the incidence of pulmonary metastases. Wood (1925, 1927, 1930) inoculated 400 mice with Flexner rat carcinoma and divided them into two groups when the tumour had grown. In 200, a slice of tumour was excised. Ten days later the whole tumour was excised from all 400. The animals were allowed to live for several months and were then killed. The percentage of metastases in the biopsied and unbiopsied groups were practically the same. In a subsequent experiment Wood and Tyzzer (1932) massaged transplanted tumours for a few minutes on two or three successive days and then removed the tumours surgically to prevent further metastases. The number of metastases in the lungs was greatly increased by the massage. In some cases the number was nearly doubled. Marsh (1927),using inoculated mammary tumours in mice, obtained metastases in 62 per cent of animals in which the tumours were subjected to massage, but in only 40 per cent of the unmassaged controls. Knox (1929), in her thorough review, did not doubt that sarcomas of the extremities were frequently aggravated by superficial injuries. Hellwig (1932) stated that it is true that biopsy, being a surgical procedure, could not be regarded as absolutely harmless. Besides the complications of any operation - haemorrhage, infection, unexpected injury to organs - one must consider the especial dangers pertaining to the incision in tumours, i.e. stimulation of growth and dissemination of tumour cells through blood and lymph vessels.

The possibility that radiation might augment the likelihood of metastases has also been studied. Hunter (1927) described a case of

mammary cancer with 'carcinomatosis' following treatment with radium. Bell and Datnow (1932) state that in all cases in which they saw metastases from uterine cervical cancer, radium treatment had been used and many records with implications of a similar nature have appeared in the French literature. However, Jeanneney and co-workers (1930) found the incidence of metastasis was not significantly greater in radiated than in non-radiated cases. In 1932, the subject was discussed in an editorial in American Journal of Cancer, and it was concluded that there is no evidence that radiation provokes dissemination, but that it is possible that it might increase the incidence of metastases by prolonging the life. In animals, Cirio and Balestra (1930) showed that the general X-radiation of mice bearing tar cancers markedly increased the incidence of metastases and postulated therefore that radiation weakened the resistance of tissues to disseminated tumour cells. This result is not applicable to the purely local irradiation of tumours. Roentgen ray radiation has been claimed to increase the number of metastases by others as well - Kaplan (1929), Krebs (1929) and Yamamoto (1931).

To return to the work on local and systemic factors which might influence metastasis, in 1923 Pearce and Brown studied the peculiarities of metastatic distribution of an inoculable rabbit tumour, and found that the mechanism of the circulation failed to account for these peculiarities. They postulated that if the factor of animal resistance could be eliminated, metastatic tumours would grow best in those organs where nutritive conditions were optimal. Begg (1923) showed the invasion of nerves by cancer is a comparatively common condition in skin epitheliomas of the mouse, although metastases are uncommon.

Tadenuma (1923) confirmed Gaylord and Simpson's (1916) findings, showing that fowls which under normal condition developed no metastases did so when they were bled repeatedly, and attributed this result to the loss of some defensive agent in the blood. The same author working with Okonogi (Tadenuma and Okonogi, 1924) found that loss of blood augmented the frequency of metastases in mice inoculated with carcinoma. In 1925, Bonne working with tar induced sarcomata confirmed Wrzosek's (1911-12) observation that when tumours inoculated in the tail grew slowly, but that tail grafts always produced pulmonary metastases though subcutaneous implants did not. If, however, the tail was amputated 17 days after grafting, no metastases occurred. Van Allen (1925) working with a transplantalbe epithelioma of the rabbit concluded that the susceptibility of an individual to the inroads of the primary growth is no certain index of the susceptibility to the metastasis.

In 1928, Fibiger made a study of the influence of extracts of embryo tissues on metastasis. White mice bearing tar cancers were given subcutaneous injections of emulsions of mouse embryo skin. No effect on the development of primary tar cancers was observed, but only 30 per cent of the injected animals developed metastases while metastases appeared in 58 per cent of the untreated control animals. In this connection it may be noted that resistance of animals to tumour implantation can be augmented by injection of emulsions of adult or embryo tissues. Fibiger and Möller (1928) suggested that resistance to metastasis formation might be augmented in the same way, but Casey (1932, 1941) and Chambers and Scott (1926) increased in the frequency of metastases by injecting tumour autolysate fraction into the host.

Mercier and Gosselin (1931) found node metastases in 10/106 mice with grafted tumours. They thought metastases not related to heredity, age, survival time after grafting, or rapidity of growth of tumour, but concluded that metastasis is a local phenomenon of the adaptation of the cancer cell to life in the nodes. They showed further that when 8 mice were grafted with metastases from the nodes, 6 showed metastases in their nodes. Also, the growth rate of these metastases was much higher than that of grafts from the main tumour. The experiment was thought to prove that there had been adaptation of the cancer cells to life in the node, and that after a node metastasis is formed, the cells which it liberates are able to colonize in other nodes more easily. Mercier and Gosselin (1932) also again confirmed that the grafts in the tail led to the formation of metastases, and suggested that grafts in the testis or peritoneum behaved similarly. Falks (1932) came to a different conclusion. He studied the influence of age of the inoculated rats on the development of lymph node metastasis and found that the inoculation of Jensen's sarcoma into the thigh muscles of young rats resulted almost invariably in the development of metastases in the lumbar glands of the corresponding side, while in old rats treated similarly such metastases appeared only occasionally. Foulds (1932) injected the Brown-Pearce rabbit tumour intravenously and found trypan blue injections increased the number of tumour metastases.

Besredka and Gross (1933) found a sarcoma which when inoculated subcutaneously into mice always grew, but only in 4/300 animals showed metastasis. Nevertheless, they found that injections of blood, liver, spleen, lung, and kidney taken from tumour-bearing mice 24 hours

after inoculation of the tumour produced tumours in new mice, and that liver taken 5 hours after inoculation produced new tumours when injected into new mice. Rubbing shaved skin with the tumour gave no takes, but rubbing the inner surface of a strip of skin always gave takes when the strip is replaced. Tumours so induced appeared very soon, but grew very slowly. Metastases were common after inoculation by this intradermal route. Gross (1932), Besredka and Cross (1933) and Warren and Gates (1936) showed that injections of uninjured tumour cells in ascitic fluid of cases with carcinomatous ascites gave earlier and more numerous pulmonary tumours than injections of artificially made suspensions in which many tumour cells were injured.

These conflicting reports were revised in part by Rousy, Oberling and Guérin (1936) who considered the formation of metastases after tumour grafting, and concluded that the development of metastases after excision of the grafted tumours depends on many factors. The removal of the tumour favours metastasis, the more so the bigger the tumour is, though the mechanism of this effect is unknown and probably complex. It is not solely a nutritive matter as supposed by the athreptic theory of Ehrlich (1908). The opposite effect may also occur, and partial removal of a tumour results in the destruction or inhibition of the remaining part. The effect of excision in increasing the survival time and so the length of time the tumour has to metastasize must be considered, but is not the sole explanation of the results. It is important to realize that different tumours behave in different fashion and that one cannot extend these results directly to human tumours.

Druckery, Hamperl, Herken and Rarei (1938-39) by repeated operative removal of an implanted tumour and by repeated replantations in rats showed the development of resistance against the new implantations, but also showed the occurrence in the operated rats of metastases, which were never observed in unoperated rats. Operations on the tumours were also followed by wide-spread metastases when BP-induced tumours were studied in mice. Druckery and his colleagues expressed the view that cancer operations should not be considered as a local interference only, thinking there was also a general effect. As regards the effect of operation, different tumours reacted differently, but age of the host was not important. In man, operation has been found to increase the number of tumour cells circulating in the blood, though not the number of metastases (Cole, Roberts, Watne, McDonald and McGrew, 1958; McDonald, 1957; Cruz, McDonald and Cole, 1956; Engell, 1959; Salgado, Hopkirk, Long, Ritchie, Ritchie and Webster, 1959). Schatten and Kramer (1958) found that anaesthesia, operation and cortisone administration were without effect on the number of pulmonary metastases of the S-91 melanoma or the DBA sarcoma which occurred after subcutaneous inoculation of these tumours or the number of pulmonary tumours found after intravenous injection of these tumours. Schatten (1958) also found that a primary tumour of sufficient size inhibited the development and growth of its distant metastases. Pulmonary metastases of S-91 melanoma and DBA 49 tumour that occurred in mice, became established and grew prior to the time the primary tumour became large. Removal of primary tumour 3 weeks after implantation, resulted in the establishment and rapid growth of large numbers of latent metastases. This was thought

not to be due to the operative procedure because amputation of a normal leg of a mouse with a tumour in the contralateral leg did not affect the frequency or size of pulmonary metastases.

Kahn and Furth (1938) showed that benzapyrene induced sarcoma could be transmitted with 50 cells and Furth and Kahn (1937) that mouse leukaemia could be transmitted with a single leukaemic leucocyte injected intravenously. Wagner (1938) transplanted lung, brain, kidney, spleen, lymph nodes, heart's blood from 25 animals, 17 of which carried mice ascites tumour and 8 carried subcutaneous tumours, each into two mice. With lung transplants 6 of the animals showed positive results, with brain 2 of the animals, and with heart's blood in 2 of the animals. Tumour cells were found in the lung and blood injected.

Lo (1956-57) found that after total extirpation of a subcutaneous implant of Yoshida sarcoma 9 out of 15 animals (60%) died of relapse or metastases. If an incomplete operation was performed, leaving 0.5 to 2.0 gm. of the tumour behind, the rate of relapse and of metastasis increased. Of the 35 rats operated in this way 26 (74%) died of relapse or recurrence.

CHAPTER V

MECHANISM OF METASTASIS FORMATION

This subject has been completely reviewed by Coman in 1953 and by Zeidman in 1957. The steps in the formation of metastatic tumours are (i) detachment of the cancer cells at the site of the primary tumour, (ii) invasion of the surrounding tissues, (iii) invasion of channels, (iv) carriage to distant organs, and (v) growth of these cells or emboli in the distant tissues or organs.

Detachment of the Malignant Cells:

Malignant cells cohere to one another less firmly than do normal cells of the same type (Bebe, 1904-05; Clowes, 1905; Schrek, 1943, 1949; Brunshwig, Dunham and Nichols, 1946; Carruthers and Suntzeff, 1946; McCutcheon, Coman and Moore, 1948; Zeidman, 1947; and DeLong, Coman and Zeidman, 1950). This lessening in coherence may be due to the low calcium content of many malignant cells. Another concept of adhesiveness of the cells is suggested by Tyler (1947) and Weiss (1947; 1950) who propose that links similar to those between antigen and antibodies may hold adjacent cells together. Spiegel (1955) suggested auto-antibodies which combine with antigens of the tumour cells might be responsible for the release of the neoplastic cells. It is clear that a reduction in 'cohesiveness' might facilitate the detachment of malignant cells from the primary focus.

Invasion of the Surrounding Tissues:

It is not understood why, or how malignant tumours invade the surrounding tissues. Several factors have been suggested. Tumour cells might be forced into the surrounding tissues by the pressure built up within the tumour by the proliferating cells. Some tumour cells show amoeboid movement and might invade the tissues by their own amoeboid movement (Ewing, 1940; Willis, 1952; Coman, 1942, 1946, 1947; McCutcheon, 1947, 1948; Eterline and Coman, 1950). It has also been suggested that the invasion into the surrounding tissues might be due to the peripheral growth of the cells of the malignant tumour (Ewing, 1940). Certain chemical substances, such as lactic acid, spreading factor, or hyaluronidase might be produced by the cancer cells, and by modifying the surrounding tissue might enhance the invasive power of the tumours. The formation of lactic acid by many tumours was shown by Warburg in 1923. The production of the spreading factor or hyaluronidase has been supported or disputed by many workers (Boyland, 1935; Claude and Duran-Reynals, 1934; Coman, 1946; Duran-Reynals and Stewart, 1931; Gibertini, 1942; Madinaveitia, 1940; McLean, 1941; Pirie, 1942). Proteolytic enzymes might be produced by some cancer cells (Vasiliev, 1958), and similarly enhance their invasiveness.

Invasiveness is not a property restricted to the malignant tumours. Invasive growth may be shown in inflammatory proliferation of the epithelium in non-malignant tissues (Vasiliev, 1958). The pseudoepitheliomatous hyperplasia of chancres and other inflammations is an example. In such cases, the invasive growth of the epithelium is closely connected both in time and place with inflammatory changes

in subepithelial connective tissue. In all cases, the epithelium is found to invade young connective tissue induced in various ways. Zwarsin (1947) emphasized that formation of immature connective tissue under the basal membrane always precedes the invasive growth of the epithelium. Invasive growth is also observed in normal morphogenesis in embryos and in adult animals. For instance, in pregnancy in the mouse, the mammary gland epithelium forms alveolar buds which grow into the surrounding connective tissue. Some think that before the growth of epithelium begins, the compact basal membrane disappears and a net of thin fibres forms around the ducts, and concluded that invasive growth of the mouse mammary gland epithelium is somewhat similar to inflammatory proliferation of epithelium. In both cases the formation of the 'bed' consisting of the young connective tissue precedes the epithelial invasion (Vasiliev, 1958). The relationship of the connective tissue changes and the epithelial growth may be different in different processes. It is possible that in some cases the epithelium, stimulated by some endogenous agent (such as a hormone), begins to secrete substances which induce the proliferation of connective tissues. In other cases, the proliferation of the epithelium and that of the connective tissue may be under the control of different if mutually interdependant mechanisms. However, in all cases formation of young connective tissue 'matrix' seems to be essential for the invasive growth of the non-malignant epithelium (Vasiliev, 1958). The invasion of the uterine wall by Trophoblast is another example of non-neoplastic invasion.

The phenomena of neoplastic invasion have been studied in tissue cultures. Santesson (1935) showed that the tumours most malignant

clinically were the most invasive in tissue culture, But when he tried to assess the proteolytic activity of the tumour cells, he found that the most malignant tumours were least proteolytic, and the least malignant, on the contrary, the most proteolytic. He explained this reverse relationship by suggesting that non-malignant tumours through their proteolysis destroyed the bridge (fibrin clot) that they would ordinarily use to invade connective tissue, and that malignant tumours by not digesting the plasma clot preserved such routes for migration. Like other workers before him Santesson observed a mutual stimulation between the outgrowth of tumour and the connective tissue as they approached one another. Nerve cells may be similarly stimulated in tissue culture (Levi-Montalcini and Hamburger, 1951; Levi-Montalcini, Meyer and Hamburger, 1954). Wolff (1956) showed the ability of sarcoma 180 of the mouse grown in organ culture to invade a variety of tissues of the chick embryo, and observed that the mesonephros of the chick is particularly susceptible to invasion. The process of tumour spread in all organs studied took the same pattern. When the normal tissue colonies touch one another, further migration of the normal cells is stopped, presumably by a 'contact inhibition'. Neoplastic cells, however, continue to proliferate. (Abercombie and Heaysman, 1954). In the sponge matrix tissue culture the interaction of normal tissues and of human cells derived from the carcinoma, D-189, was studied in vitro. Tumour cells readily invaded the normal tissues of the chick embryo or human foetus if they gave rise to a luxuriant outgrowth of connective tissue. The malignant cells and the explants of normal connective tissue were attracted to one another. When contact was established, malignant cells began to invade the connective tissue.

Schliech (1956) showed the presence of normal connective tissue was necessary for survival of malignant cells of the Yoshida sarcoma in vitro. Powell (1957 and 1958) suggested that monocytic cells contained in the explanted pieces of normal embryonic organs formed in vitro some substances which are essential for growth of cells of the Ehrlich mouse ascites carcinoma.

As to the formation of tumour cell aggregates, Wilson's (1907-08) work on the reaggregation of sponge cells is usually cited as the earliest experimental study on the subject. Grobstein (1956) reviewed the subject from the point of view of experimental embryology. Malignant tumours are commonly arranged in organized aggregates of cells. As tumours grow locally and spread to distant sites the organoid arrangement is usually retained. Growth consists of the multiplication of cells and either the increase in size of each aggregate or an increase in the number of aggregates, or both. Under conditions of continuing division of cells in a neoplasm without concomitant multiplication of the aggregates, the expanding mass of tumour cells would meet increasing nutritional deprivation and would finally consist of large necrotic focus with a relatively thin cortex of viable cells in contact with the tissues of the host. The successful growth of a tumour requires the presence of mechanisms for aggregate replication either within tumour cells themselves or as a result of the interactions of host and cancer. Aggregate multiplication has been demonstrated in vivo. (Leighton, 1957). In the spread of cancer, cells migrate from areas of dense population of tumour cells towards uninvolved normal tissues, presumably in response to gradients of metabolites. In highly malignant

tumours small aggregates or single infiltrating cells are able to detect minute variations in their environment in order to determine the direction of their migration. Less malignant cells are obliged to form large aggregates in order to recognize metabolic variations in the immediate environment and so to select the direction of migration suitable for continued life. Some tumour cells require environments conditioned by other cancer cells, and achieve this by forming large aggregates while remaining non-aggressive (in situ), or penetrating the surrounding matrix of connective tissue only slowly. Grobstein (1956) has suggested that increased attention be directed to permeability of the ground substance in studies on organization, differentiation and cancer biology. Tumours requiring very large aggregates for their survival might be expected to be less able to move freely through the connective tissue, since large aggregates would find the presence of reticular and collagen fibres a physical obstacle. A reduction in the aggregate size might be expected to be parallelled by increasing invasiveness. Conversely, the introduction of variations that would produce an enlargement of the aggregates might reduce invasiveness. In studies with agar-overlay preparations and in sponge matrix tissue cultures, Moscona (1957) showed that a fission occurs when small groups of cells protrude from large aggregates, and that they separate from the parent aggregate by a kind of cleavage. These techniques also offer an opportunity to examine more carefully the role if any of stromal cells in aggregate multiplication. Also, with the membrane-filter methods of Grobstein, one could explore the possible production of diffusible metabolites by either the neoplastic or the normal tissue that might alter the spatial arrangement of the other.

As pointed out by Willis (1952), the responses of the stroma of the host to an invading neoplasm are interpreted with difficulty. Often the connective tissue in a tumour results from an inductive action of the tumour cells on the host. In some it may be a response to host tissue attempting to limit further growth of the tumour.

The important role of connective tissue reactions in modifying the invasive spread of cancer cells is still under investigation. Devic with others and Vasiliev (1958) maintain that malignant cells migrate from subcutaneous grafts of mouse or rat tumours into the undifferentiated connective tissue which develops around such grafts after an initial inflammatory reaction. They suggested that this connective tissue proliferation is favourable for initial spread of malignant cells from grafted tumour fragments. Carrel (1925), Jones (1926) and Selye (1957) also found that a moderate inflammatory reaction helped the growth of a transplanted tumour. On the other hand, Kubo (1930), Chambers and Grand (1937), Pigarevsky (1952) when trying to assess the stimulant effect of an inflammatory reaction and of connective tissue proliferation on the rate of growth of transplanted tumours, found that both inhibited growth of the tumour, and Malomut, Spain, Kreisler and Warshaw (1955) and Hewitt (1956) did not find either affected tumour growth at all. Inflammation associated with extensive suppuration and tissue necrosis obviously may have a harmful effect on the tumour cells, but the majority of authors are of the opinion that the mild inflammation associated with proliferation of the connective tissue cells favours the establishment and growth of tumour transplants and the spread of metastasis of malignant cells (Vasiliev, 1958).

In this connection the influence of embryonic tissue on the growth of tumour transplants is of interest. It is reported that the percentage of positive 'takes' when tumours are implanted subcutaneously was increased if pieces of embryonic tissues were added to the grafts. (Greene, 1949; Schneyer, 1955; Vasiliev, 1958). Greene's suggestion was that the embryonic tissue either evoked stromal reactions of the host, or served as primary stroma for the transplant tumour. Accumulation of a large number of immature fibroblasts at the site of injection of suspensions of homologous embryonic tissues had been observed in rats by Vasiliev (1958). The role of embryonic tissue in these experiments might be the same as that of the undifferentiated connective tissue which develops around a growing tumour.

Invasion of Channels:

It is a common knowledge that the walls of blood capillaries, veins and lymphatics are often invaded by malignant tumour cells (Virchow, 1863; Goldmann, 1897, 1911; Ribbert, 1911; Willis, 1930) and it is generally assumed that the entrance of the tumour cells into the vascular lumina is a natural consequence of their property of invasive growth. It is therefore important to remember that non-neoplastic cells can also enter vessels. That parenchymatous cells may enter the venous channel was shown by Zenker (1862) in the case of adipose tissue, Bausch (1866), and Schmoral (1887-88) in the case of liver, and Krakower (1936) in the case of brain. Schenken and Coleman (1943) suggested in connection with haematopoietic tissue that transit of emboli would be easier to understand if a shift of the differential pressure occurred so that the extravascular pressure exceeded the intravascular one. The extravasation of

blood from lacerated vessels produces an increase in the extravascular pressure which upsets both the balance and range of the differential pressure normally prevailing in the tissues concerned (Claude Bernard, 1878). Bausch (1866), Bergman (1910), Warthin (1913), Whittaker (1939) and others suggested that fat embolism in fracture of bones might be produced by some such mechanism. Young and Griffith (1950) showed by their ingenous experiment that bodies suspended in a fluid medium surrounding a collapsible perforated tube cannot enter the lumen of the tube so long as the internal pressure is greater than the external, but that they can and do enter the lumen when the external pressure is greater than the internal pressure. The rupture of blood vascular walls and the consequent disruption of adjacent aggregates of a neoplasm would allow small aggregates of the tumour cells to enter the vascular channels if the extravascular pressure rose above the intravascular. Possibly the entrance of tumour cells into the lumen of a lymph channel is achieved by the same means.

The mechanical movement of the organs in which malignant cells are present may act in a massaging manner, periodically stretching and compressing the surrounding tissues in a way which lays open their natural, preformed crevices assisting the entry of tumour cells into the vessels by altering the extravascular and endovascular pressures as described above and by augmenting the lymphatic and venous flow from the part assists their escape. The importance of movement is supported by the animal experiments referred to before in which massage increased the frequency with which metastases occurred.

Sometimes the tumour cells may be temporarily restricted at the site of lodgement by becoming covered with a thrombus. The thrombus containing the entrapped tumour cells may enlarge and even block the vessel. But this is only a temporary halt as the neoplastic cells may infiltrate their way along the interstices in the thrombus until they reach the next larger venous trunk, as was mentioned earlier.

The number of emboli discharged by the tumour is also of importance in determining the frequency of metastases. Weil (1913) produced multiple discrete pulmonary tumours by injecting suspension of rat sarcoma into the lungs of rats, but failed to get metastases to the lungs by implanting fragments of the same sarcoma subcutaneously. The failure of the implanted rat sarcomas to produce metastases was thought due in great part to the failure to discharge viable cells in sufficient number into the circulation. This was perhaps in part due to a generalized or constitutional resistance to implantation of fresh tumours present in animals already growing large tumours. Zeidman, McCutcheon and Coman (1950) stated that the wide variation in numbers of 'spontaneous' metastases must depend on the number of emboli given off. Coman, Eisenberg and McCutcheon (1949) thought the scarcity of metastases in an organ is explained by a scarcity of emboli. They showed that in a metastasizing tumour implanted in the lungs, metastasis did not develop in any organ because tumour cells do not pass in adequate numbers from the lungs into the systemic arteries.

Cancer cells can spread to other parts of the body other ways than the blood channels, by lymphatic channels, coelomic cavities, the cerebrospinal spaces, and perhaps by epithelial lined cavities.

The subject has been thoroughly dealt by Walther (1948) and Willis (1952). Cancer cells may also enter the blood vessels by the thoracic duct from the lymphatic system. All these routes of extension of tumour cells are well known. But as our main concern is in connection with the vascular extension of the cancer cells , we will restrict our discussion to this subject.

It was originally thought that only sarcomas spread by blood stream, carcinomas spreading only by the lymphatics. Von Recklinghausen (1885) first indicated that carcinoma, too, may spread through the blood vessels. Goldmann (1897) and Schmidt (1903) both saw cancer cells in the vessels. Cancer cells were found in the blood of a patient with cancer by Ashworth (1869), and his observation has been repeatedly confirmed as by, among others, Pool and Dunlop (1934), Fawcett, Vallee and Soule (1950), Tobin and Zarquiey (1950), Engell (1955), Fisher and Turnbull (1955), Seal (1956), Sandberg and Moore (1957), Turnbull (1957), Zeidman (1957), Malmgren, Pruitt, Vecchio and Potter (1958), Pruitt, Hilberg and Kaiser (1958). In a recent investigation, Salgado, Hopkirk, Long, Ritchie, Ritchie and Webster (1959) in this Department have shown tumour cells to be present in blood drawn from the antecubital vein in 57% of 60 cases of various kinds of malignancy.

Carriage to the Distant Organs:

The earlier investigators, Goldmann for instance, concluded that the tumour cells in the blood reached the pulmonary vessels and were there stopped. This was also accepted by Borrel, Murray, and others. The ultimate destination of tumour emboli like thrombotic ones is determined largely by mechanical factors. Those that arise in the portal

area pass to the liver, those carried in the systemic veins to the lungs and those which reach the pulmonary veins to the greater arterial circulation. However, there are many exceptions. Some of these can also be explained on mechanical grounds. For instance, Batson (1940) showed in the cadaver that in patients with prostatic carcinoma the metastases could reach the sacrum and the spine through the paravertebral plexus of veins instead of passing through the inferior vena cava. Coman and DeLong (1951) confirmed his finding experimentally. They injected Walker carcinoma 256 or the V2 carcinoma into the femoral veins of rats or rabbits and noted that tumours appeared in the vertebral columns of the experimental animals if abdominal pressure was applied so as to reduce the flow along the inferior vena cava. In control animals in which no pressure was applied, tumours appeared only in the lungs. Zeidman (1955) injected V2 carcinoma cells into the thoracic duct of some rabbits and into a vein in others. In both series, tumours developed in the lungs. However, in animals receiving thoracic duct injections, tumours also developed in mediastinal and intercostal lymph nodes. He demonstrated lymphatic connections between the thoracic duct and lymph nodes by injecting dye into the duct. That some of these ducts lead from the thoracic duct to the node was proved by the fact that neoplastic growth occurred in the subscapular lymph sinuses of nodes, where afferent lymphatics empty. Thoracic duct branches were also demonstrated in a human foetus by dye injection. Mediastinal, intercostal and left supraclavicular nodes were stained.

Tumour cells have been found able to pass through the liver, lung and other capillary beds. Zeidman and Buss (1952) injected tumour

cells into the peripheral veins of rats or rabbits and withdrew blood from the aorta immediately afterwards. When the aortic blood was injected intravenously into a second animal of same species, tumours developing in the second animal indicated immediate passage of tumour cells through the lungs of the first animal. Similar studies by Zeidman, Gamble and Cloves (1956) and Korpássy, Kovács and Tibaldi (1954) showed that tumour cells may pass through the liver, kidneys and spleen. And again, tumour cells found in the blood drawn from the antecubital vein of patients with cancer must have passed through lung and the capillaries of the arm, and in some cases through the liver as well (Salgado, Hopkirk, Long, Ritchie, Ritchie and Webster, 1959).

That many of the tumour cells and tumour emboli which reach the blood die without giving rise to metastases was early recognized Gussio (1912), Weil (1913), Iwasaki (1915-16), Takahashi (1915), Pearce and Brown (1923), Tadenuma (1923), Warren and Gates (1936), Goman (1949 & 1951), Crabb (1949), Zeidman, McCutcheon and Goman (1950), Sugarbaker (1952) and Watanabe (1954). Zeidman, McCutcheon and Coman (1950) found only 6% of the tumour cells given intravenously in rabbits grew into tumours. That tumour cells in the blood may fail to give rise to metastases in man has been shown by Engell's (1959) study of 125 patients. Of these 55 (44%) were alive five to nine years after operation although tumour cells had been found in the blood of 28 (51%) of these 55 surviving patients at the time of operation.

Growth of These Cells or Emboli in the Tissues or Organs:

Langenback and Billroth (1879) first asked 'What is that decides what organ will suffer in a case of disseminated cancer?'

Stephen Paget reviewed the problem in 1889. He pointed out that mere mechanical factors cannot explain the distribution of metastases in various organs. In his 735 collected cases of breast cancer, 241 had metastases in the liver, and 17 in the spleen. But in 340 of cases of pyaemia there were 66 cases with abscesses in the liver and 39 with abscesses in the spleen. He argued the spleen, with a rich blood supply, should have more metastases than it does. He offeredthis observation as one reason for stating that some organs form a more favourable site for the growth of metastatic cells. From this time, a number of explanations and hypotheses were proposed to explain the distribution of cancer metastases in the organs. In 1908, Ehrlich postulated his 'athrepsia' hypothesis which suggested the existence of specific growth substances essential to the tumour proliferation, and proposed that these substances were abundant in susceptible organs and scanty in resistant ones. Albrecht (1911) modified this view by saying that the success of the development of metastatic tumour is due to the chemical metabolic conditions of the entire organism. Von Recklinghausen, Lubarsch and Schmidt, on the contrary, considered the main reasons for the difference in the frequency and the localization of the metastatic tumours in different organs to be purely mechanical, differences in the afferent vessels, differences in the morphological structure of the organs, differences in the size of the cancer cells, and difference in capillaries of the organs. Levin (1913) stated that the failure or success of the proliferation of a group of cancer cells transplanted from the primary tumour into a distant organ is a result of the interaction between the character and malignancy of the cancer cell and the general and local susceptibility

of the organism of the host. Warren and Davis (1934) in explaining the scarcity of metastases in the spleen stated that firstly it is due to the absence of the afferent lymphatics in the splenic parenchyma and secondly due to the double blood circulation in the organ. The first circulation goes on continually, the blood passing from the splenic arteries into the sinuses and thence into the splenic veins. The second is intermittent in character, brought about by the physiological contraction and relaxation of the whole organ, and serves to empty into the veins the blood in the pulp spaces which are filled through the lateral channels in the walls of the sinusoids. The second type of circulation might be quite effective in preventing the lodgement of the tumour cells (Kettle, 1912). Levin (1913) showed that when the tumour cells are introduced directly into spleen the tumours grow easily.

The possibility that morphological differences in the various organs might in some part explain the varying frequency of metastases in them will be taken up in more detail subsequently.

CHAPTER VI

HISTOGENESIS OF METASTASIS FORMATION

As already mentioned, in the earlier histological studies Borrel (1903); Bashford, Murray and Cramer (1905), and Haaland (1905) showed that the pulmonary metastases of transplanted carcinomata originated from minute emboli of the terminal branches of the pulmonary artery. These workers described the earliest metastatic deposits to distend the walls of the pulmonary arteries. They also saw isolated cancer cells in the vessels in process of division. In larger arteries, they found emboli which completely occluded the lumen of the vessels, and in places found the continued proliferation of cells lead to the formation of a large growth which extended beyond the limit of the blood vessels and invaded the alveoli of the lungs. Murray (1908) showed also the carcinomatous emboli in the lungs were in the terminal branches of the pulmonary arteries. He described formation of large sausage-shaped masses filling up a considerable part of the pulmonary arterial system. The terminal conditions he described were either healing which occur when the nodules failed to become vascularized and were replaced by sclerotic tissue, or further extension which was initiated when capillaries budded in from the intima of the vessel. The growth, now better nourished, increased rapidly in size, burst through the wall of the vessel, and invaded the lung tissue.

One point which has been discussed is whether metastases arise from lodgement in capillaries or arterioles. Iwasaki (1915) and

Takahashi (1915) were in favour of the arteriolar sources. Takahashi (1915) injected tumour cell suspension into the tail veins of mice. He found many hyaline emboli in the lung vessels in strains in which the tumour failed to grow. Some of these emboli were converted to fibrous remnants which occasionally enclosed a few tumour cells which, though much altered, were still recognizable. The strain producing metastases, showed 24 hours after inoculation tumour emboli lying in but not filling arterioles and surrounded by polymorphonuclear leukocytes. After 3 days, if the emboli were growing, they nearly filled up the vessel lumen. The number of leukocytes was now very low. In cases where the tumour cells were not in contact with vessel wall, the endothelium showed signs of vacuolation. By the 5th day, increasing distension could be made out and capillaries could be seen to enter into the growing tumour from the intima. If this vascularization failed to take place, the tumour cells showed signs of vacuolation. Iwasaki (1915) injected subcutaneously and subsequently intravenously a suspension of Jensen's rat sarcoma into a series of animals and in a second series injected the suspension only intravenously. He described the process of destruction of the tumour cells in the vessels, but noted some of the tumour cells remained and began to grow after a short time. They might extend along the ramifications of the vessels or penetrate out through the wall.

On the other hand, Coman, DeLong and McCutcheon (1951) favoured origin of metastases from capillaries. They injected a suspension of Brown-Pearce rabbit tumour into the heart. The number of single and the number of clumps of tumour cells were counted in several sections from each organ, and their distribution, whether in capillaries

or arterioles was noted. In a second experiment, other rabbits were similarly injected with suspensions of living tumour cells. These animals were sacrificed after 1-3 weeks, and at autopsy the number of visible tumours were counted in the different organs. The number of microscopic tumours were also determined. They compared the number of tumours with the number of emboli in different organs. They found that there was a correlation between the number of emboli lodged in the capillaries and the number of metastases observed. Warren and Gates (1936) also thought most metastases arose from capillaries. They injected a tumour cell suspension into the inferior vena cava just distal to the renal veins. In animals killed immediately, they observed an amorphous debris of pink staining material, filling the majority of the small vessels in the lung. This infiltration diminished after two hours. Intravascular tumour was seen at twelve and 24 hours but extension beyond the vessels did not occur before five days, and then arose from capillaries. At 9 days, hyaline masses suggesting healed thrombi were found. Endothelial swelling or proliferation was absent. Endothelial vacuolization such as recorded by Takahashi was seen occasionally in vessels containing tumour, but was also seen in normal lungs. The tumour foci appeared in vessels of all sizes, localization being thought probably determined by size of the tumour emboli. When ascitic fluid containing tumour cells was injected intravenously, progressive growth of the tumour from such injection was observed. Within $1\frac{1}{2}$ hours the majority of the small vessels and capillaries contained tumour cells, many showing mitotic figures. In some instances necrotic tumour cells were seen. In 12 hours, growth beyond the capillary walls was more universal and

extensive. Hyaline thrombi were numerous. By 24 hours, perivascular growth was well established. The intravascular growth in arterioles showed increasing evidence of necrosis with vacuolization of the cells. Again it was considered that tumour cells lodged in vessels of varying size according to the size of the embolus, usually being found, whether singly or as aggregates at the bifurcation of the vessel. If the tumour lodged in a capillary, growth into the surrounding alveolar wall was early. If the tumour lodged in an arteriole, direct penetration of the wall was infrequent.

Baserga and Saffiotti (1955) reviewed the dispute, and from their experimental studies on the lodgement of metastases in the lung concluded that the metastases may grow from both sites of localization. They transplanted suspension of tumour T 150 in the subcutaneous tissue of the right lumbar region in C 57 black mice and the animals were killed between the 10th and 20th day after implant and studied the lung histologically. In their series, 2/3rds of the metastases grew from capillaries, the other third from the arterioles. Tumour emboli released spontaneously from the primary growth consisted mostly of single or a few cells. These emboli lodged in the vessels either by adhering to the endothelium of arterioles or by plugging the lumen of the capillaries. In arterioles, the spread of tumour cells beyond the endothelial wall appears to be preceded by a phase of intraluminal growth. The deposits in these vessels became covered by thrombus, but in some cases grew to fill the vessels, extending along them as noted by earlier workers. These deposits might break into the substance of the lung by growing down until they reached a capillary, or might penetrate the

arteriolar wall directly. Deposits in the capillaries grew in a similar fashion, often distending the capillary before extending out into the lung. In a few cases regressing and degenerating tumour cell emboli were found in an intravascular position. No instance of regressing or degenerating extravascular growths were recorded. Baserga and Saffiotti also considered that the establishment of metastases depends on the initial vitality of the cells and their ability to establish perivascular growth early, as they apparently were unable to establish permanent growth in the blood vessels.

Saphir (1947) studied twelve lungs of patients dying from carcinoma of various organs, in which carcinoma emboli were encountered on routine histological examination. These emboli were found in smaller and smallest branches of the pulmonary artery, in capillaries and in small pulmonary veins. A small sheath of fibrin and hyaline thrombi almost invariably were encountered adjacent to the cells. The thrombi seemingly caused atrophy of the adjacent tumour cells which were gradually replaced by the growing thrombi. Eventually these thrombi became organized, but clumps of the tumour cells were still recognizable either within the new channels or embedded within the growing connective tissue. Thus, not the tumour emboli but the hyaline thrombus became organized and caused the disappearance of the tumour cells.

Wood (1958) studied in the rabbit's ear chamber the chronology of the localization of the malignant cells in capillaries. In the first stage, tumour cells struck firmly to the capillary endothelium. The cells were then surrounded by a thrombus. After this, a number of tumour cells and leukocytes escaped into the surrounding connective tissue.

This was followed by infiltration with a variety of cells, including tumour cells. Gradually a slow penetration and infiltration by leukocytes and tumour cells occurred through the endothelial defect.

As already described, the initial sticking of the cancer cells to the vascular endothelium is followed after a latent period of a few minutes by the formation of a thrombus and the cancer cells are enmeshed inside the intravascular thrombus. The cells thereafter proliferate into the lumen of the vessels and eventually invade and replace the thrombus (Baserga and Saffiotti, 1955). Thus the blood clot itself does not seem to inhibit the growth of the tumour cells, as was suggested earlier (Haaland, 1905; Iwasaki, 1915-16; Pearce and Brown, 1923; Levin and Sittenfield, 1911). In fact the blood clot provides a good medium for the growth of these cells and is actually induced by the embolus. Lawrence, Bowman, Moore and Bernstein (1953) ascribe it to the thromboplastic activity of the tumour cells. However, recent studies have suggested that the clotting mechanism of the blood may be intimately involved in the sticking of the leukocytes to the vascular endothelium (Allison, Smith and Wood, 1955; Zweifach, 1954), and a similar process may be involved in the sticking of the cancer cells. The early changes in response to the tumour cells are indeed as in acute inflammation, with vasodilatation, changes in the endothelial surface brought on by the injury, and protoplasmic extensions coming out from the endothelial cells (Allison, Smith and Wood, 1955; Nicoll and Webb, 1946; Sawyer and Pate, 1953; Clark and Clark, 1935).

The next event is the penetration of the cancer cells through the vascular endothelium. This is preceded by tissue damage which is

again almost as in inflammatory reactions. Probably the same histamine-like substances (Lewis, 1927), amino acids, and polypeptides (Vasiliev, 1958; Menkin, 1950; Spector, 1951), permeability factors (Miles and Wilhelm, 1955; Milhelm, Miles and Margaret, 1955; Wilhelm, Mill and Miles, 1957; Wilhelm, Mill, Sparrow, McKay and Miles, 1958), and other substances (Rowley and Benditt, 1956; Sparrow and Wilhelm, 1957: Spector, 1957) cause endothelial damage. The endothelial barriers of the arterioles or capillaries offer some resistance to the passage of tumour cells as metastases sometimes extend long distances before breaking through the vessel wall. If the tumour is to escape from the vessel, leukocytes accumulate at the site of endothelial damage, and diapedesis starts. In the defect caused by the passage of a few leukocytes, cancer cells get out of the vessels. Wood (1958) observed cancer cells outside the vessels as early as 3 hours after their adherence to the vascular endothelium. Warren and Gates (1936) reported that six hours after intravenous injection of Walker carcinoma cells, invasion and growth were evident beyond the capillaries. The amoeboid movement of the cancer cells described as early as in 1863 by Virchow may help them escape. Eterline and Coman (1950) demonstrated that V2 carcinoma cells grown in vitro moved at an average rate of approximately 0.7 mu per minute. The relation of leukocytes in general and the lymphocytes in particular to the malignant cells has been discussed. Leukocytes have been suggested to be mobile sources of enzymes or metabolites which are particularly in demand by the actively growing and the dividing cells (Humble, Jayne and Pulvertaft, 1956) and so able to aid the tumour, perhaps especially in its escape from the vessels.

It is important to note that several writers (Schmidt, 1903; Iwasaki, 1915; Saphir, 1943; Wood, 1958) have described small deposits of tumour in the vessel wall and covered only by a thin layer of endothelium and so lying in the intima. This observation is relevant to the work to be described later.

The vascularization of the tumours is also important. Many workers (Algire and Chalkley, 1945; Ide, Baker and Warren, 1939; Lewis, 1927; Goldacre and Sylven, 1959) have shown that vascularization is essential if growth is to continue. Ide, Baker and Warren studied the growth of fragments of the Brown-Pearce carcinoma in the rabbit ear chamber and noted that changes in the blood vessels made their appearance within seventy-two hours of the transplantation. The response consisted in either widening of the pre-existing vascular channels or else a proliferation of small vessels, mostly from the severed ends of the wider parent stems at the margin of the window. Algire and Chalkley studied the vascular reaction to transplanted mammary carcinomata in transparent chambers introduced in the skin of the mice. New capillary sprouts appeared as early as the third day after implantation. They studied new capillary formation in wound healing by the same means and found that proliferation of new capillaries was evident only on the 5th or 6th day. On the other hand, studying the vascularization of omentum transplanted as autografts in a rabbit ear chamber, Williams (1953) observed that new capillary growth from pre-existing blood vessels began within 24 hours which is as early as Wood (1958) found capillary buds arising from the pre-existing vessels and entering the tumour in his experiment with tumour implanted in the rabbit's ear chamber.

Goldacre and Sylven (1959) by injecting a dye which leaked from the vessels and entered the tissues showed that the blood in the interior of many tumours was not in communication with the circulation and that only the peripheral part of the tumours received flowing blood.

CHAPTER VII

FACTORS AFFECTING THE NUMBER AND GROWTH OF METASTATIC TUMOURS

Although much of the material included in this chapter has been previously mentioned, it is thought that in view of the experimental work to be described, it is useful to collect this material here. The effect of cortisone, and of nitrogen mustard on metastases will be reserved for later chapters.

As bacterial infection depends on certain factors, the virulence of the organism, the dose of the infecting agent, the route of entry into the body, the resistance of the host, and last but no least the particular tissue which is infected, similarly the process of metastasis formation is dependent upon a number of factors. As previously mentioned, the earlier workers pointed to three of the fundamental requirements for the occurrence of metastases, the tumour employed for inoculation or transplantation should have exceptional growing energy, the cells must be inoculated in a place unfavourable for rapid growth of the tumour, and the animal inoculated must be one whose organs are adapted for forming metastases (Wrzosek, 1911-12). Since then many other factors have been found to influence the establishment of metastases.

The number of the cells or emboli circulating in the blood is a most important factor in the production of the metastases. Zeidman, McCutcheon and Coman (1950) showed this experimentally by injecting different numbers of cells of a transplantable sarcoma into the tail vein of mice. They found a direct correlation between the number of embolic cells injected and the number of tumours obtained. All the

cells injected did not give rise to metastases, most dying intravascularly.

The size of the tumour emboli is also important, but there is not agreement as to whether larger or smaller emboli are more likely to give rise to metastases. Coman, DeLong and McCutcheon (1951) found that single cells or small clumps were more likely to give rise to metastases than were larger clumps, but Schmidt (1903), Willis (1952), Watanabe (1954), and Weil (1913) all came to the opposite conclusion. Baserga and Saffiotti (1955) found that most pulmonary emboli found in their experiment were of 2 to 4 cells, with a few and as many as seven cells, and some of only one cell.

The greater the duration of the primary tumour, the more numerous are the metastases formed (Falks, 1932; Besredka and Gross, 1936; Zeidman, McCutcheon and Coman, 1950).

The size of the original implant is related less clearly to the number of the metastases formed. Zeidman, McCutcheon and Coman (1950) found that more metastases resulted from implanting a larger fragment of tumour than from implanting a smaller, but there is no demonstrable correlation between the final size of the implanted tumour and the number of metastases formed. They failed to detect any relationship between the size to which the original implant developed and the number of metastases, but in contrast, Wood, Holoyoke, Clason, Sommers and Warren (1954),and Knisely and Mahaley (1958) found that the ultimate size of primary transplant and the number of metastases formed were definitely correlated. One must also remember that as has already been said, several workers have found that excision or partial excision of

the primary implant increases the number or size of the metastases. (Marie and Clunet, 1910; Tadenuma, 1923; Tadenuma and Okonogi, 1923-24).

The proportion of the cells introduced in experimental work which are viable is of obvious importance and depends on the method of preparation of the tumour emulsion or suspension. As previously mentioned, Levin and Sittenfield (1911) failed to get any tumours by injecting suspensions of a spontaneously metastasizing tumour because of faulty technique, and Gross (1932), Besredka and Gross (1933) and Warren and Gates (1936) showed injections of uninjured cells gave earlier and more numerous pulmonary tumours than injections of suspensions in which many tumour cells were injured or dead.

It has been suggested that in any given strain of mice metastatic tumours exhibited a preference for the organs in which the primary tumours of that strain occur. Thus strains of mice which yielded many primary tumours of the liver exhibit also many secondary tumours of the liver. Wells (1919), Slye (1921), Mercier and Gosselin (1931), however, found heredity to have no influence whatsoever on metastasis formation.

The site of inoculation of a transplantable tumour also affects the formation of metastasis. Tumours which do not metastasize when transplanted into the subcutaneous tissue of the trunk may yield high percentage of blood-borne metastases when transplanted into the subcutaneous tissues of the tail. (Baserga and Baum 1955; Bonne, 1925; Levin, 1913; Mercier and Gosselin, 1932; Foulds, 1932). Local factors at the site of lodgement may also be important. As previously mentioned, the emboli or cells reaching the tissue or organ do not all form metastasis, many of the tumour cells die, but others remain latent. It has

been shown that tumour cells may be in the splenic pulp or vessels even though no tumours develop, and that these cells may give rise to tumours when the spleen is inoculated into other tissues in other animals. (Coman, 1951; Zeidman and Buss, 1952; Korpássy, Kovács and Tiboldi, 1954). The failure of the tumours to develop has been attributed to the presence of some inhibitory defensive function of the spleen. Kettle (1912), for instance, considered that physiological rhythmic pulsation of the spleen is the cause of unstabilizing the cancer cells which may be present inside the spleen. The site of lodgement may also affect the rate of growth of metastases. Luckέ, Breedis, Woo, Berwick and Nowell (1952) showed that even after the establishment of the tumours in the liver and the lung, the tumours in the liver grew faster than those in the lung. Therefore liver provides a better environment for the support of the tumour growth. Others have drawn similar conclusions (Levin and Sittenfield, 1913; Willis, 1952).

The duration of a tumour may affect its power to metastasize in another way. Greene (1951) showed that transplantability of spontaneous rabbit tumours varied with their duration. Early tumours could not be transferred to normal animals, whereas tumours obtained after metastases were readily transplantable. By several transplantation experiments he inferred that the ability to survive in the environment of a normal animal is a developmental acquisition and not a property of the tumour from its inception. With this conclusion should be compared the theory of "progression" of tumours elaborated by Foulds (1954) and the evidence that tumours tend to lose antigens (Weiler, 1954).

As previously noted, many other factors may also affect the number or growth of metastases. Massage, operative interference, manipulation of tumours for diagnosis or operation, and perhaps anaesthetics may augment the number or size of metastases. (Marie and Clunet, 1910; Tyzzer, 1913; Gaylord and Simpson, 1916; Wood, 1919; Knox, 1921; Sappington, 1922; Sampson, 1924; Dial, 1930; Hellwig, 1932; Druckery, Hamperl, Herken and Rarei, 1938-39; Cruz, McDonald and Cole, 1956; McDonald, 1957; Cole, Roberts, Watne, McDonald and McGrew, 1958; Schatten, 1958; Schatten and Kramer, 1958). Bleeding may also increase the number of metastases (Gaylord and Simpson, 1916; Tadenuma, 1923; Tadenuma and Okonogi, 1923-24). Roentgen ray radiation has been claimed to increase the number of metastases. In this both the local radiation (Kaplan, 1929; Krebs, 1929; Yamamoto, 1936; Hunter, 1927) and whole body radiation (Cirio and Balestra, 1930; Jenneney, 1930) have been accused. Inoculation of embryo tissues (Ribiger, 1913), of normal tissue or of homologous tissue (Schneyer, 1955), of testicular extracts (Tanzer, 1932), of trypan blue (Foulds, 1932) or of Scharlach R and ether water (Levin, 1912) have also been claimed to help in the establishment of metastases. Pregnancy may also augment the growth of metastases (Greene, 1950; Tyzzer, 1913). Body temperature may be important in frogs. Lucké and Schlumberger (1949) exposed lepord frogs with a kidney carcinoma for 50 days to a constant temperature of 28° C. Fifty-four per cent of the frogs developed metastases, whereas in groups kept at 18°C or at 7°C metastatic dissemination was found in only 6 per cent. They also showed that at the elevated temperature, the metastases were more numerous and more widely distributed.

The effect of artificially induced inflammation on the growth of transplanted tumours is not clear. Some authors observed inhibition of growth (Kubo, 1930; Chambers and Grand, 1937), others found no effect (Molomut, Spain, Kreisler and Warshaw, 1955; Hewitt, 1956), while yet others found stimulation of growth (Selye, 1957; Rous, Murphy, and Tytler, 1912; Levin, 1912; Carrel, 1925; Jones, 1926; Vasiliev, 1958). Mild inflammation has also been reported to favour the metastasis of malignant cells (Hirchfeld, 1919; Zahl and Novac, 1949; Jones, 1926; Selye and Horava, 1952; Selye, 1957; Vasiliev, 1958) and to favour the localization of metastases (Jones and Rous, 1914).

CHAPTER VIII

EFFECT OF CORTISONE ON METASTASIS FORMATION

Greene (1951) discussing tumour autonomy concluded from the results of his experiments with transplants that 'with reference to biological properties, cancers of rabbits, of mice and of man are not sudden transformations in normal cells but on the contrary, represent the final step in a development process. During the greater part of their course, the tumours are dependent in nature, their continued existence being conditioned by factors peculiar to the tumour-bearing individual. Autonomy, or the ability to survive in the absence of such factors, is a late development and is followed by a rapid acceleration in the fatal course of the disease'. He demonstrated in his experiments with mammary and uterine tumours that one great group of factors required by many tumours are endocrinological. The widespread influence of oestrogens was noted. The effect of hormones is also seen in their utilization in the treatment of prostatic and mammary tumours.

Many investigators have studied the effect of cortisone and other similar hormones on tumours, investigating both transplanted and induced neoplasms. The experiments suggest that cortisone has a general inhibitory action on transplantable tumours of the lymphoid series, (Murphy and Strum, 1933; Heilman and Kendall, 1944; Diller, Beck and Blanch, 1948; Emerson, Wurtz and Zanetti, 1950; Higgins and Woods,1950; Storek, 1950; Sugiura, Stock, Dobriner and Rhoads, 1950; Wooley, 1950; Begg, 1951; Donald and Higgins, 1951; Ingle and Nezamis, 1951; Kaplan,

Brown and Marder, 1951; Bloom, 1952; Kottschalk and Grollman, 1952; Monsen, 1952; Antopol, Glabach and Graff, 1954; Iversen, 1957) but there is no agreement as to what its effect is on other transplantable or on induced tumours.

Pomeroy (1954) introduced the tumour cells directly into the left ventricle of mice. With the Krebs II adenocarcinoma he found more tumours in mice treated with cortisone, but with the sarcoma 37 he did not find cortisone had any effect on the number of tumours formed. Eichwald (1952), Foley (1952) and Howes (1951) showed that cortisone promoted the transplantability of the tumours in genetically alien strains.

Zeidman (1957) showed that when the ascites tumour cells are injected intraperitoneally, tumour cells can be seen inside the abdominal organs, but that these never develop into tumours except in animals treated with cortisone. Baserga and Shubik (1954) could not demonstrate by their experiment any difference in the weight and size of the tumours implanted in the subcutaneous tumours induced by injections of methyl-cholanthrene in treated and untreated cases with cortisone.

Agosin, Christen, Badinez, Gasic, Neghme, Pizzaro and Jarpa (1952) described the development of metastases in many organs contemporaneously with the regression of the "primary" mammary tumours transplanted into C3H mice which were kept on injections of cortisone. Molomut, Spain, Gault and Kreisler (1952) observed visceral metastases from scapular implants of sarcoma I in mice treated with cortisone, though no such metastases occurred in mice not given cortisone. Kaliss and Borges (1953) contradicted the finding. Using the same tumour under

similar conditions, they did not get metastases in either cortisone-treated or control mice. Martinez and Bittner (1955) transplanted a mouse adenocarcinoma and observed a decrease in the number of lung metastases as a result of cortisone treatment. Iversen (1957) experimented with five homologous tumour transplants in mice. In only one of the tumours, a chondrosarcoma, could he show that the number of metastases in the lungs was increased by cortisone treatment. With the other four tumours, cortisone did not alter the number of metastases. However, Wood, Holoyoke and Yardley (1956) recorded that mice bearing subcutaneous implants of carcinoma 150 and treated with hydro-cortisone developed an increased number of lung metastases. The growth rate of the primary tumour was not altered in this experiment. They also found that administration of hydro-cortisone prior to the intravenous inoculation of a tumour cell suspension resulted in an increase in the number of lung tumours.

Schatten and Kramer (1958) introduced S91 tumour cells intramuscularly and intravenously in a group of mice which were given three doses of cortisone acetate at daily intervals. A group of these animals was anaesthetized, and another was anaethetized and then subjected to an operation. They found no significant effect of anaesthesia, operation or cortisone administration on the number of pulmonary metastases. Singh, Skoryna, Ritchie and Webster (1957), working at McGill, found in rats that the administration of cortisone increased the proportion of takes when the Walker Carcinoma 256 was grafted into bones. They also noticed a greater tendency for the tumour to spread during the first and second week in the rats given cortisone. Later the

transplants showed necrosis and regression in the cortisone-treated rats. Towbin (1954) studied the effect of cortisone on the transplantation of a mouse carcinoma of the breast to the anterior chamber of the guinea pigs' eye. In the cortisone-treated animals, growth of the transplants occurred earlier, and in greater percentage of the guinea pigs. He also found that with human tumours transplanted into the anterior chamber of the guinea pig's eye, the post-transplantation inflammation was less, and growth occurred in a greater percentage of eyes in the cortisone-treated animals. The growth was also evident earlier, and reached a greater volume than in controls. Selye (1957) prepared 'granuloma pouches' by injecting air subcutaneously in rats. He found that Walker tumours transplanted into a pouch grew faster if an inflammatory reaction was induced in the pouch by injecting croton oil into it, and that cortisone increased the discrepancy between the growth rate of the tumours in pouches with and without croton oil. Selye suggested that this was because cortisone was unable to suppress the inflammatory reaction in the pouches treated with croton oil, though it could in those not so treated.

Baserga and Shubik (1954) and Baker and Whittaker (1949) noted an inhibitory action of cortisone on the induction by 20-methylcholanthrene of skin tumours in mice. Boutwell and Rusch (1953),on the other hand, found that cortisone has no marked influence on the induction of carcinomas in mice by benzpyrene. Ritchie, Shubik, Lane and Leroy (1953) did not find cortisone able to modify the hyperplasia induced by croton oil in the skin of mice. Engelbreth-Holm and Asboe-Hansen (1953) showed that intraperitoneal injection of cortisone markedly inhibited the

development of skin tumours and prolonged the latent period in ST/EH mice painted once on the dorsum with 9,10-dimethyl-1,2-benzanthracene. Zachariae and Asboe-Hansen (1954) showed that if hydro-cortisone was injected topically into the connective tissue of fully developed skin papillomas induced in this way, complete regression resulted in 95%. In a control group of injected with an isotonic saline solution, 48% of the tumours regressed. Zachariae and Asboe-Hansen (1958) showed that if hydro-cortisone acetate was injected once weekly for 16 weeks into the skin carcinomas induced by painting the dorsal skin of mice twice weekly for six weeks with 9,10-dimethyl-1,2-benzanthracene, this resulted in regression of the tumours, which decreased in size or disappeared entirely, but only temporarily for they started growing again and killed their hosts.

Thus survey of the literature on the subject reveals much controversy as to the effect of cortisone on the growth of tumours and on the spread of metastases. The striking effect of cortisone on lymphoid cells is clear, but in all other matters there is confusion.

It could be that cortisone increases the ease with which the tumours penetrate the connective tissue, or give rise to emboli. Cortisone may cause an excretion of calcium (Fischer and Hastrup, 1954). This loss of calcium might decrease the cohesiveness of the tumour cells and so give rise to increase of tumour emboli and so to greater number of metastases. Or it could be as Agosin, Christen, Badinez, Gasic, Pizzaro and Jarpa (1952) suggested that cortisone has a local action on the connective tissue around the primary tumour, leading again to the greater release of the tumour emboli. It has been shown that the

surrounding connective tissue may be greatly altered by cortisone. Cortisone reduces the number of mast cells (Asboe-Hansen, 1955; Cavallero and Braccini, 1951; Seifter, Beader and Begany, 1949), and the mast cells may be largely responsible for the production of the ground substance. Cortisone also changes the physical and chemical qualities of the ground substance, and this in turn may increase the permeability of the connective tissue (Coste and Bourel, 1951). This action is different from that of hyaluronidase (Duran-Reynals, 1942; Meyer, 1947). Indeed, cortisone actually depresses the action of the hyaluronidase (Anderson, Wiesel, Hillman and Stumpe, 1951; Benditt, Schiller, Wong and Dorfman, 1950; Opsahal, 1949; Seifter, Beader and Begany, 1949 & 1953). Cortisone also reduces chondroitin-sulphate synthesis (Layton, 1951), and may reduce fibroplasia, thus again changing the intercellular substance (Dorfman, 1953; Baker and Whittaker, 1950; Ragan, Howes, Meyer and Blunt, 1949; Spain, Molomut and Haber, 1950). Possibly the connective tissue in the walls of the blood and lymph vessels, is similarly affected, so the vessels become more permeable or more easily invaded. The inflammatory response to the tumour or transplant may also be suppressed by cortisone. Cortisone may, however, cause a great exudation of fluid into the stroma and even the formation around the transplant of a degenerate membrane (Iversen, 1957). Capillary proliferation may be much reduced (Taubenhaus and Amromin, 1950; Ducommun and Mach, 1950; Selye, 1949; Spain, Molomut and Haber, 1950;Shapiro, Taylor and Taubenhaus, 1951; Rosenberg and Lieblow, 1954). Unfortunately, just as the effect of cortisone on the tumours is variable and uncertain, so is its effect on connective tissue. As the brief review

shows, different investigators find different actions, and so the relationship of the action of cortisone on connective tissue to its action on the tumours remains abscure.

Schrek (1949) showed that cortisone has a direct cytocidal action on lymphocytes, that it accelerates the normal rate of physiologic ageing and death of these cells, and that it inhibits their mitotic activity. It is not known if it has a similar action on other cells. The phagocytic action of the reticulo-endothelial cells is probably not affected by cortisone (Clawson and Nerenberg, 1953; Fischer and Hastrup, 1954; Gell and Hinde, 1953; Heller, 1953), but perhaps by an action on the reticulo-endothelial system, or by its action on the lymphoid system, it may reduce the ability to form antibodies. The weakening of this antibody response may explain the greater proportion of takes of tumours transplanted into hosts not immunologically identical with the tumour when cortisone is given (Pomeroy, 1954). Singh, Skoryna, Ritchie and Webster (1957), however, thought that the effect of cortisone on the Walker tumour transplanted to the bone was probably in part non-specific, and secondary to other effects, though the suppression of the antigenic reaction was probably of importance.

Finally, it has been suggested that cortisone increases the number of metastases by increasing the survival time of the treated animals. Baserga and Shubik (1955), however, could not demonstrate any difference in survival time in the treated and untreated animals.

CHAPTER IX

EFFECT OF NITROGEN MUSTARD ON METASTASIS FORMATION

Like most of the discoveries in the medical science, the inhibitory action of mustard gas on tumours was hit upon accidentally. Berenblum in 1929 attempted to accelerate the formation of tar-induced tumours in mice. He intended to produce an increased blood supply by inducing mild irritation by mustard gas, thinking this might accelerate the formation of tumours. Instead he found that the mustard gas inhibited tumour formation. The subject was further studied during the war years by Dixon and Needham (1946), Gilman and Philips (1946), Peters (1947) and others. They showed that, after the exposure of tissue to mustard compounds, a portion of the mustard is instantly fixed to the tissue and the remainder is fixed a few minutes later. Mustards have a great affinity for a wide variety of enzymes and other tissue proteins. Rapidly proliferating tissues were found particularly susceptible to damage by mustards, as were the haemopoietic system and the intestinal epithelium (Gaensler, McKay, Ware and Lynch, 1948). In these respects, the action of mustard is similar to that of ionic radiations (Auerbach, 1946; Horowitz, Houlahan, Hungate and Wright, 1946). In experiments on mammalian cornea, nitrogen mustard was able to inhibit almost completely all mitotic activity, even when the substance was used in dilutions of one one-hundredth of the dose which cause signs of intoxication (Friedenwald, Buschke and Scholz, 1947). That the drug was nucleotoxic was demonstrated by the occurrence of the nuclear fragmentation in the basal cell of the cornea.

Gjessing and Chanutin (1946) also showed that nitrogen mustard has definite cytotoxic effects. A similar inhibition of mitosis and a similar nucleotoxic action were demonstrated in the bone marrow, and also in the tissue cultures of a large variety of normal and neoplastic cells (Karnofsky, Burchenal, Ormsbee, Corman and Rhoads, 1947). Gunzman, Grant and Miller (1948) showed that mustard inhibited the respiration of all tissues, but affected the anaerobic glycolysis very little. The respiration of the lymphoid tissues was extremely sensitive to nitrogen mustard. Bodenstein (1947), and Rose, Henry and Walpole (1950) showed that nitrogen mustard affected selectively centres of proliferation and left the differentiating regions unaffected.

In experimental animals, Karnofsky and others observed that 'takes' of mouse leukaemia could be prevented by treating the donor mice with nitrogen mustard. In 1948, Karnofsky, Graff and Smith succeeded in killing with nitrogen mustard sarcoma 180 which was growing on the chorio-allantoic membrane of the chick embryo, without affecting the embryo's growth. Biesele, Philips, Thiersch, Burchenal, Buckley and Stock (1950) found chromosomal alterations in tumours treated with mustard. Sugiura and Stock (1950) studied the effects of nitrogen mustard on different tumours and demonstrated its destructive effects. Cruz, McDonald and Cole (1956) injected Walker carcinoma suspensions into the portal vein of rats and noted nitrogen mustard given into the portal vein, into a systemic vein, or into the peritoneal cavity on the day of injection of cancer cells decreased sharply the number of 'takes'. They also showed that the floating cells were more readily killed by mustard than cells with a 'vascular root'. Similar effects were reported by McDonald,

Livingston, Boyles and Cole (1957), and Cole, Roberts, Watne, McDonald and McGrew (1958).

This is not the place to enter into a discussion of the clinical effects of these drugs.

CHAPTER X

INJECTION OF THE V2 RABBIT CARCINOMA INTO THE PORTAL VEIN AND RENAL ARTERY

Introduction:

From the review of the literature, it is apparent that surprisingly little is known about the factors which determine where and why a metastatic tumour develops. The classical morphological studies suggested the distribution of metastases could not be explained on anatomical factors alone, and to explain the discrepancy it was suggested that some organs afford a particularly favourable environment, so that a tumour metastasis may grow more easily in them than elsewhere (Paget, 1889). Although this theory is widely accepted, few experiments have been undertaken to confirm or extend it (Coman, 1953; Zeidman, 1957), and as has been seen, these experiments do not always agree. It was therfore thought desirable to reinvestigate the factors which determine whether a metastasis develops in an organ into which cells are carried by the blood stream. Luckĕ, Breedis, Woo, Berwick and Nowell (1952) found fewer metastases in the liver when V2 carcinoma was injected into the tributary of the portal vein or into the hepatic artery than were produced in the lung by injecting the carcinoma into the ear vein, but noted that the metastases in the liver grew more rapidly and were on the average larger than those in the lung. In contrast, Pearce and Brown (1923) found that when their tumour was implanted into the testis in rabbits, tumours were more frequent in the liver than the lung, and more frequent in the kidney than either, though they also noted that the liver tumours were bigger than those in the lung. Takahashi (1915) and Sugarbaker (1952) found that

when various kinds of tumours are injected intravenously or intracardially in mice or rats, each type of tumour had its own pattern of metastases, some affecting a certain group of organs, others other groups. Coman, Eisenberg and McCutcheon (1949), Coman and DeLong (1951) also found that when the V2 carcinoma was injected into the left side of the heart metastases were more frequent in some organs than others.

The first stage of the present investigation was based on this work. Aliquots of a suspension of single cells or small clumps of the V2 rabbit carcinoma were injected into the portal vein and the renal artery. After an appropriate delay the rabbits were killed and the number of metastases in the various organs counted.

Method:

The V2 carcinoma, obtained from the University of Pennsylvania by courtesy of Dr. Dale Rex Coman, was maintained in this laboratory by subcutaneous transplantation in white New Zealand male rabbits. A few rabbits of other breeds were used to see if the tumours grew more easily in them but proved of no particular advantage.

Usually, the tumour was transplanted by implanting small fragments. The hair was shaven by means of an electric shaver and the part washed with absolute alcohol and iodine. The donor rabbit was anaesthetized by an injection into the ear veins of 0.5 cc. of 6% Nembutal solution per kg. A portion of healthy tumour was dissected out aseptically, and cut into small pieces by means of a pair of iris scissors. A few small bits were fed into a canula by means of a trocar. The receiving rabbits were also anaesthetized with Nembutal. A small incision was made in the prepared skin and the canula with the trocar

pushed into the subcutaneous space. The pieces of tumour were expressed by pushing the trocar through the canula. The trocar was moved back and forth a few times to ensure that the tumour did not adhere to the needle. The skin on either side of the canula was pressed onto the body and the canula was gently withdrawn. This process prevented escape of any of the inoculated material. Skin edges were apposed by means of Mitchell clips.

Sometimes the tumour was injected as a cell suspension. The cell suspension was made by taking small pieces of healthy tumour tissue from Tyrode's solution and rubbing them lightly on a fine mesh of stainless steel wire having 60 to 80 wires per inch. As the piece of tissue is rubbed on the surface, the screen is washed with Tyrode's or Ringer's solution dropped from a Pasteur pipette, and a suitable suspension of cells results in the washing fluid. A measured amount of the solution was drawn into a tuberculin syringe and was injected subcutaneously into prepared skin by means of a 25 bore needle. The results were much as with the first method but there was less chance of infection.

No difficulty was found in maintaining the tumour by these procedures. In a few animals there was an initial inflammatory reaction at the site of implantation, but this was easily controlled by administration of penicillin and streptomycin (Fortimycin - Ayerst).

Several methods were tried before a similar method was chosen for the preparation of a suspension of tumour cells suitable for intravenous injection. Pieces of tumour were cut out as before and put in Tyrode's or Ringer's solution. Some of the methods tried were:
(i) cutting the pieces into fragments with a fine pointed pair of scissors, suspending the fragments in Ringer's or Tyrode's solution, and taking the

supernatant; (ii) crushing the tissues in a homogenizer, a ground glass test tube with a pestle just fitting it, suspending the homogenate in Ringer's solution and taking the supernatant; (iii) homogenizing the tumour in a Waring Blender, suspending the homogenate in Ringer's solution and taking the supernatant; (iv) the method of Watanabe (1954), brushing well preserved portions of the tumour lightly over a double thickness of wire mesh immersed in physiological saline to which 'Tween 80' was added. None of these methods seemed to offer any advantage over the method with the wire screen already described, and so this method was adopted. The suspension was kept on ice and the larger fragments allowed to settle out.

In order to count the number of viable cells and to estimate the number and size of the cell clumps in the suspension three methods were tried. Krist 3.5 ml. of a solution of safranine 1:4,000 in Tyrode's solution was added to 0.5 ml. of the suspension. A drop of the mixture was placed in a hemocytometer, and the number of stained and unstained cells counted. The number of unstained cells per cubic millimetre was then calculated. Second 3.8 cc. of a 1:2,000 solution of eosin in Tyrode's solution was added to 0.2 cc. of the suspension. The stained and unstained cells were again counted, and their concentration calculated. (Schrek, 1943; 1949). Third, a drop of 1 in 10,000 solution of trypan blue in Tyrode's solution was added to the cell suspension and the number of stained and unstained cells counted in a hemocytometer (Pappenheimer, 1917). The concentration of cells was calculated. This last method was proved to be easier than the previous ones. The number of individual cells and the number and size of the aggregates were counted at the same

time, and their concentration per cubic millimetre calculated. The unstained cells were considered viable, the stained ones dead, as suggested by Schrek (1943; 1949).

White male New Zealand rabbits weighing 2.5 to 4 kg. were used for the experiment. The animals were anaesthetized by injecting 6% Nembutal solution (Abbott Laboratories) 0.5 ml. per kg. into the ear veins. When required, anaesthesia was maintained by open ether given by means of a perforated glass funnel into which ether vapour was pumped from a Wolffe's bottle. The abdomen was opened in the mid-line. The coils of gut were gently shifted and wrapped in warm, sterile gauge. The portal vein and one renal artery, usually the left, were exposed. Using a 27 bore needle the suspension of tumour cells was injected into both vessels. Bleeding was controlled by compressing the vessel between warm saline sponges.

Results:

Twenty-one rabbits were injected in this way. Of these, 4 died in the immediate post-operative period, and 1 died 24 hours after the operation. All the 16 survivors developed tumours. The animals were either killed or allowed to die. The organs were removed immediately and fixed in formol saline. The liver and kidney were cut into slices, the number of tumours counted both macroscopically and by means of a dissecting microscope, and their sizes determined. Table I shows the results and Table II shows the composition of the suspension of tumour cells given each rabbit.

In 15 animals tumours grew in the liver, in 14 in the lungs, in 2 in the spleen, but only in 6 in the kidneys. In 3 cases tumour

grew in the contralateral kidney.

It is apparent that more tumours appeared in the lungs than in either the kidneys or the liver, but that many more tumours appeared in the liver than in the kidney. It is also noteworthy that the tumours in the liver grew much larger than those in the kidney or lung (Fig. I). The relatively large number of tumours found in the spleen is also noteworthy.

Discussion:

It might be held that more tumours appeared in the lung than in the liver or kidney because more tumour cells reached the lung, 0.2 ml. of the tumour suspension being injected into the portal vein and 0.1 ml. into the renal artery. If it be supposed that a major proportion of the tumour cells passed through the liver and kidney unchecked, the lung would receive the cells contained in nearly 0.3 ml. of the suspension. It is known that tumour cells pass through the liver and kidney (Zeidman, 1952; Korpássy, Kovács and Tibaldi, 1953; Zeidman, Gamble and Clovis, 1956), but it is not known what proportion of the cells pass. However, even if the somewhat unlikely possibility that almost all pass is accepted, this still does not explain why there were many more tumours in the lung than would be expected assuming that the relative dosage in lung and liver was 3 to 2, and in the lung and kidney 3 to 1. Another possibility that must be considered is that the lung tumours arose at least in part from emboli released from established hepatic tumours. This would explain both why the lung tumours were more numerous, and why they were smaller than those in the liver. However, it seems unlikely that this factor is of great importance. Rather

does the experiment confirm the conclusion of Lucké, Breedis, Woo, Berwick and Nowell (1952) that given an equal dose of tumour cells, more tumours tend to arise in the lung than in the liver, and shows in addition that given the same dose of tumour cells the kidney will develop even fewer tumours than the liver. It also confirms the earlier finding that though there are fewer tumours in the liver than in the lung, the tumours in the liver grow larger than those in the lung.

The tumours in the spleen may be due to direct inoculation into the splenic vein at the time of injection into the portal vein, with the assumption that the pressure in the portal vein was sufficient to temporarily reverse the flow in the splenic vein.

CHAPTER XI

EFFECT OF CORTISONE ON THE BEHAVIOUR OF SUBCUTANEOUSLY IMPLANTED V2 CARCINOMA

Introduction:

As mentioned earlier, some investigators have found that cortisone increases the frequency of "spontaneous" metastases from implants of a transplantable tumour (Zeidman, 1957; Agosin, Christen, Badinez, Gasic, Neghme, Pizzaro and Jarpa, 1952; Wood, Holoyoke and Yardley, 1956). In the hope of increasing the likelihood of development of relatively "natural" metastases from the V2 carcinoma, rabbits bearing subcutaneous implants of the tumour were treated with cortisone.

Method:

The V2 carcinoma was implanted subcutaneously in 32 white, male New Zealand rabbits of 2 to 4 kg. body weight, either by the trocar and canula method or seeding by injecting suspensions. In 10 of these, cortisone acetate 2 mgm. per kg. body weight was injected subcutaneously daily. Inflammatory reactions if and when they occurred were controlled by Fortimycin injections.

Results:

The results are shown in Table III. Cortisone did not increase the frequency of metastases. Indeed, subcutaneous tumours grew in 20 of the 22 rabbits not given cortisone, and metastases were found in 10 of these, while tumour grew at the site of implantation in only 4 of the 10 rabbits given cortisone, and metastases appeared in only 3

of these. As the administration of cortisone did not increase the frequency of metastases, and actually decreased the frequency with which the tumour grew at the site of implantation, no further experiments of the kind were conducted.

CHAPTER XII

THE ESTABLISHMENT OF METASTASES IN THE MOUSE'S LIVER

Introduction:

At this stage, the work on the rabbit was discontinued and attention was concentrated on the mouse. This was in the main because there is much less antigenic difference between the transplantable tumours available in the pure strains of mouse and their hosts than there is between the rabbits used and the V2 carcinoma. The ideal of studying the metastases of a spontaneous tumour did not seem practicable, as animal has been found to bear commonly a spontaneous tumour which metastasizes in a sufficiently high proportion of cases. Various transplantable tumours which grow in pure strain mice were considered, and the S91 melanoma chosen. This tumour grows at a satisfactory rate, and it was considered that its production of melanin might be useful in making tumour cells and metastases easily recognizable.

The work described in chapter X has confirmed that purely mechanical factors do not seem able to explain the different frequency with which different organs are involved by metastases. The next step was to study the histogenesis of the metastases in the various organs, and to try to determine if the different 'susceptability' of the different organs might be explained by differences in histogenesis. To begin, the histogenesis of 'metastases' in the mouse's liver was studied.

Method:

The S91 melanoma used was obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, was grown in DBA/1 mice, weighing between 15 and 25 grammes, obtained from the same source. The animals were housed in plastic cages and fed Rockland mouse diet and water, both ad libitum. To maintain the melanoma, a portion of healthy tumour was taken from a donor mouse and was transplanted subcutaneously into the recipient mice by putting small pieces of tumour under the skin, or by injecting a suspension of tumour cells, prepared as described in chapter X. Usually 0.2 cc. of a suspension having 200,000 viable cells per c.mm.was injected using a tuberculin syringe and a 25 gauge needle. The tumours appeared 8 to 10 days after the inoculation, developed to a fairly good size by about 21 days and killed the mouse in about 60 days. Metastases were not noted.

In attempts to induce 'natural' metastases, several procedures were tried. As mentioned earlier, several writers found metastases developed more commonly if a tumour was implanted into the tail (Wrzosek, 1911-12; Baserga and Baum, 1955; Bonne, 1925; and others.) Therefore in 10 mice, the melanoma was inoculated into the subcutaneous tissue of the tail. After six months the mice were still alive, with small tumours growing slowly in four. Of these, three had no metastases and one had metastases only in the regional nodes. In a second experiment with another 10 mice, five developed small tumours several months after the inoculation, but no metastases were found in any of them. It was concluded that this method was not useful with the S91 melanoma.

Complete or partial excision is another method reported to augment the number of metastases from transplanted tumours (Tadenuma, 1923; Rousy, Oberling and Guerin, 1936; Druckery, Hamperl, Harken and Rarei, 1938-39; Ketcham, Alfred, Wexler and Mantel, 1959; Prudente, 1959). The melanoma was transplanted subcutaneously in 10 mice, and after the tumour had grown to a reasonable size, the tumour was completely excised from five, and partially from the other five. Three from the former and two from the latter group died in the immediate post-operative period, but in the five survivors no metastases grew.

A more direct method of obtaining "natural" metastases was tried. In a group of 10 mice, a suspension containing 50,000 cells per c.mm. was injected into the tail vein. The apparatus designed by Hubble (1958) proved useful (Figs. 2 and 3). It consists of a plywood box, with a 100 watt bulb inside it for transillumination of the tail, and to provide heat to dilate the tail veins. A toggle switch is mounted on the right hand for turning the light on and off. A slit is cut on the top of the box just over the lamp filaments. The slit is made approximately $1\frac{1}{4}$ in. long, so that injected solution can be visualized as it passes through the vein above the needle. A slit housing is cut from light weight metal and curved to hold the tail. The mouse holder is cut from a household food can and shaped into a conical form as shown in the picture. It is attached to a wooden slide which has its clearences adjusted so that friction will hold the slide at any position. As expected, metastases developed in the lungs, though in only 6 of the 10 mice. No metastases were seen elsewhere. This observation was of little interest. The development of

metastases in the lung has been repeatedly studied, and our purpose was to consider their development in other organs.

Suspensions of the melanoma were injected directly into a mesenteric vein. This method induced tumours in the liver, but also lead to the development of large tumours at the site of injection and sometimes to implants of melanoma throughout the peritoneum. For this reason this method was considered undesirable. So, in order to get the tumours only in the liver, melanoma cells were injected directly into the liver.

A suspension containing 200,000 viable melanoma cells per c.mm. was prepared in the same way as was the suspension of V2 rabbit carcinoma described in chapter X. In the first experiment, 60 mice were anaesthetized by ether and the abdomen opened by a median incision 1-2 cm. long. With a fine pair of forceps, the liver was slightly drawn into the wound, and 0.2 ml. of the tumour cell suspension was injected directly into the substance of the liver using a 27 gauge needle and a tuberculin syringe. The skin was opposed by means of Mitchell's clips. Animals were killed daily. The liver was dissected out and preserved in formol saline. Blocks were taken near and distant from the site of injection, and sections were made for histological studies. Visible tumours appeared between 8 and 17 days after inoculation. A few tumours grew outside the liver, probably because of spillage during the process of inoculation.

The experiment was repeated in 120 mice. Sixty mice were inoculated with 0.2 cc. saline and the remaining 60 with 0.2 cc. of a tumour cell suspension containing 150,000 cells per c.mm. Tumours appeared as in the previous group.

Results:

Specimens of liver were collected daily from 1 day to 21 days and on the 25th and 30th days after the injection of tumour cells. The whole liver was fixed in formalin and blocks taken near and distant from the site of injection of S91 melanoma cell suspension in mice, were processed and cut 3 mu thick, sometimes serially. They were stained routinely with haemalum, phloxine and saffron. Some of the sections were stained by Fontana's silver method, in the hope that the melanoma cells would be easier to detect and counterstained with haemalum, phloxine and saffron. The Fontana's method did not reveal pigment granules in the melanoma cells as was anticipated, so for routine study, we fell back on the haemalum, phloxine and saffron method. Reticulin and P.A.S. stains were also done at times.

Twenty-four to forty-eight hours after the injection, tumour cells were found in 3 sites: (i) around the puncture wound with its associated haemorrhage and blood clot, (ii) under the endothelium of the portal veins in the triads, and (iii) scattered in the sinusoids.

The puncture wound showed scattered tumour cells in the haemorrhagic area round about. Thrombus and a fair number of inflammatory cells were intermixed with the tumour cells. Tumour cells were also found in the adjoining parenchyma (Fig. 4-12). In this region some of the tumour cells were pyknotic and irregular in appearance and were intermixed with moderate number of polymorphs. A few macrophages were seen laden with pigment.

The deposits under the endothelium of the larger portal veins proved more important. They consisted of about 10-20 cells and lay

immediately under intact endothelium. They were found both near to and distant from the puncture wound (Fig. 25-44). In a few of these vessels thrombosis was seen, but in the majority, there was no associated thrombosis. A fair number of inflammatory cells, mostly polymorphs, were present. In serial sections the cells were seen to extend along the portal tracts but maintained that position under the endothelium.

It was, however, deposits in the sinusoids which were the main feature in the early stages. The tumour cells in the sinusoids were mostly single cells or small clumps and were usually but not always associated with thrombosis. These cells or clumps of cells were usually accompanied by inflammatory cells mostly polymorps (Fig.19-21,24). The distribution of the tumour cells in the sinusoids varied from section to section and from case to case. They were sometimes found throughout the liver, but sometimes were localized to some part of it. In some cases they were more common near the site of inoculation but in others were found mainly in distant parts of the liver.

Some of the collections in a sinusoid could be followed in serial sections. They consisted of 6 to 8 cells and extended for about 55 microns. They usually lay midway between the portal tracts and the central vein. Most of the cells were essentially of the same histological pattern. Occasional mitoses were seen in these cells in the sinusoids (Fig. 21). A general congestion of the liver was also apparent.

Up to the 4th day after injection the appearance of the liver was more or less the same as on the 1st day, but by the 4th day the picture had changed rather suddenly. The polymorphonuclear cells had diminished in number, as had the general congestion of the liver.

The clumps of tumour cells in the sinusoids were also becoming less frequent, but many of the deposits in the portal veins were enlarging.

At the site of injection organization was becoming apparent. Small vascular channels were appearing and the tumour cells were becoming established at the periphery of the granulation tissue (Fig.6 & 7). The irregular pyknotic cells seen on the 1st day in the neighbouring tissue had disappeared. However, mitoses were not seen in the tumour cells in this region.

Many of the collection of cells below the endothelium of the portal vessels had now become 2 to 4 layers deep and bulged into the vessels (Fig.27,29). A few clumps were also seen forming in bigger clusters round branches of the portal vessels running into the parenchyma of the liver (Fig.26,28). The accompanying inflammatory cells were now mainly lymphocytes. Others of the portal deposits, however, failed to enlarge, and were either stationary or even perhaps shrunken.

By the 5th day, the sinusoids of the liver had become almost free of tumour cells. The puncture wound showed signs of progressive healing by granulation tissue. The tumour cells in it became increased in number. The deposits in the portal areas continued to grow. Deposits were also seen underneath the capsule of the liver (Fig.13-18), both near the site of injection and distant from it.

By the 7th day and thereafrer, microscopic tumour formation around the granulation tissue of the original puncture wound became obvious. Another common site of tumour formation was underneath the hepatic capsule as described above. The foci beside some portal vessels became more heaped up still and pushed into the parenchyma from the side

of the portal vessels (Fig.35 & 37). The tumour cells had become more numerous and the inflammatory cells around them very much fewer, almost absent. Some of the tumour cells could be seen to develop inside them brownish black granules (Fig. 39). The deposits were now quite conspicuous and could be located even under the low power of the microscope. On the other hand, only occasional collections of tumour cells could be made out inside the sinusoids. It must be stressed again that the number and arrangement of these focal collections varied very much in different animals and in different sections. A few of the sinusoids showed collections of 4 or 5 cells but did not show any sign of growth.

By about the 14th day, the tumours had become established and could be seen by the naked eye. Microscopically, these tumours were found in three places, by the site of inoculation, under the capsule, and from the portal tracts. Scattered tumour cells persisted in the sinusoids, but did not give rise to macroscopically visible tumours, unless perhaps some of the subcapsular tumours which arose from them.

Another feature noted as days advanced was that tumour cells spread out from the portal tracts producing a picture simulating portal cirrhosis, the lobules being prominently demarcated by fibrous tissue and cellular proliferation.

Hereafter the development of any individual tumour followed the usual pattern of direct invasion, with compression of the surrounding parenchyma, invasion of the blood vessels with or without thrombosis, with or without necrosis of the tumour centres and with or without an inflammatory fibroblastic reaction (Figs. 38, 39, 44). This picture of tumour formation was seen in slides taken 21 to 30 days after inoculation.

However, even in these liver sections, small irregular tumour cell collections, single cells or small clumps of 2 to 4 cells were noted in the sinusoids. These clumps showed no signs of activity and but no sign of retrogression. Some of these collections of the cells round the portal vessels had also remained stationary.

Discussion:

Thus it seems that after injection of the melanoma S91 directly into the liver the tumour cells spread out from the primary site, often to involve much of the liver, though in an irregular manner. The tumour cells seemed to localize in four places. At the site of inoculation, many became pyknotic and probably died, but others went on to form visible tumours. In the larger portal veins, small deposits of tumour cells appeared beneath the endothelium, and some of these grew into visible tumours, while others remained stationary or perhaps regressed. In the sinusoids, at first many scattered tumour cells or small clumps of tumour cells were present, but by the fifth day after injection most of these had disappeared, though a few remained throughout, apparently stationary, neither growing nor regressing. No tumours arose from these deposits in the sinusoids. Visible tumours also arose from collections of tumour cells that became evident beneath the capsule, both near and distant from the puncture wound.

CHAPTER XIII

ATTEMPTS TO MODIFY THE ESTABLISHMENT OF METASTASES IN THE MOUSE'S LIVER

Introduction:

Bucher, Scott and Aub (1950), Wenneker and Sussman (1951), and Glinos and Gey (1952) showed that in parabiotic rats after partial hepatectomy in one partner the liver that has not been operated on is stimulated as shown by an increase in weight and an increased frequency of mitoses. Paul, McDonald and Cole (1958) showed that in rats in which the liver was damaged by carbon tetrachloride, the proportion of 'takes' of a suspension of Walker carcinoma given into the portal vein or subcutaneously were increased. Similarly Fisher and Fisher (1959) found that non-specific injury to the liver increased the number of hepatic tumours in their experiment. Rats were injected intra-portally with a small number of tumour cells and then examined them 5 months later for hepatic tumour growth. No tumour was evident. If, however, 3 months after injection the rats were subjected to repeated laparotomy at 7-day intervals, 100% had liver tumours within a few weeks.

In the hope that liver damage might modify the development of the tumours in the liver, the effect of hepatic damage produced by the gastric installation of carbon tetrachloride or by the intrahepatic injection of Escherichia coli on the tumours induced by injection of tumour cells into the liver was studied. The effect of this liver damage on subcutaneous implants of melanoma was also studied.

Effect of Carbon Tetrachloride:

A group of 12 mice were given by stomach tube 0.2 cc. of 32% solution of carbon tetrachloride in liquid paraffin or olive oil (0.005cc. /gm. of body weight) (Fig. 45). Twenty-four hours later, 0.2 ml.of a suspension of tumour cells containing 120,000 viable cells per c. mm. was injected into the liver. Three mice were killed on the 7th, 10th, 14th and 21st days.

The livers showed histologically in a few cases exaggeration of the lobular pattern with centrolobular necrotic areas in the earlier specimens and fatty degeneration in the intermediate zone and reaching almost to the portal areas.

No definite advantage of carbon tetrachloride injury to the liver could be associated with enhancement of the tumour formation in the liver.

Effect of E. coli Injected into the Liver:

To test the effect of the liver damage induced by carbon tetrachloride on the growth of tumour cell inoculated subcutaneously, carbon tetrachloride was introduced into the stomach of 6 mice as before. Twenty-four hours later, a suspension of melanoma cells was injected subcutaneously. In 6 control mice, not given carbon tetrachloride, the same dose of the suspension was given subcutaneously in the same way. Table IV shows the results.

Table IV. Effect of a Single Injection of Carbon Tetrachloride on the Growth of Subcutaneously Implanted Melanoma S91

Number of mice	Carbon Tetrachloride	Number of mice with tumours
18	yes	14
18	no	13

It is apparent that the carbon tetrachloride did not enhance the growth of the subcutaneous implant.

An emulsion was made from a 24-hour culture of E. coli by washing the slant with 10 cc. of sterile saline, and mixing thoroughly in order to make a homogeneous suspension. Three concentrations of the bacilli were made by adding to tubes which contained 10 cc. of sterile nutrient broth 2, 4 or 5 drops of the E. coli emulsion.

Three groups of 8 mice were taken and given an intrahepatic injection of 0.2 ml. of a suspension containing 120,000 viable cells per c.mm. and 0.1 ml. of one of the concentrations of E. coli. A fourth group received only the tumour cell suspension. Animals were killed on the 7th, 10th, 14th and 21st days.

E. coli did not seem to alter the rate or pattern of tumour growth in the liver.

The effect of injecting E. coli into the liver on subcutaneous implants of melanoma was tested by injecting E. coli into the liver as before and giving subcutaneously a suspension of melanoma 24 hours later. Table V shows the findings.

Table V. Effect of Intrahepatic Injection of E. coli on the Subcutaneous Implant of Melanoma S91

Number of mice	E. coli	Number of mice with tumours
18	yes	11
18	no	9

The tumours appeared about the same time in the two groups, and grew to about the same size. Once again, no effect of the liver damage is demonstrated.

The E. coli did not seem to alter the rate or pattern of tumour growth in the liver, or in the subcutaneous tissue.

CHAPTER XIV

HISTOGENESIS OF LIVER TUMOURS IN RABBITS

Introduction:

In order to confirm the findings described in the last chapter, sections were made of the livers of the rabbits in which hepatic tumours were induced by injecting tumour cells directly into the portal vein.

Method:

Specimens of liver were collected from 15 rabbits in which tumour grew by injection of V2 carcinoma cells directly into the portal vein as described in chapter X. Small pieces of liver containing tumours at different stages of growth were cut and fixed in formalin. These were processed and sections were cut at 3 mu., some serially, and were routinely stained by haemalum, phloxine and saffron.

Though these sections were from livers showing visible tumours, pieces were selected to include the smallest tumour, or in some the area adjoining a tumour.

Results:

In all cases, the steps seen in the mouse liver could be traced.

Smaller and bigger collections of extravascular tumour cells were seen in the portal tract or in relation to the endothelium of the portal vein (Fig. 46-49). In some instances many layers of cellular aggregation could be seen extending into the parenchyma (Fig.50 & 51).

The other feature was occasional proliferation of the sinusoidal cells. These were more marked underneath or nearer the capsule

of the liver. In one section 3 to 4 of these individual microscopic foci were seen. In this particular liver several tumours grew macroscopically.

Another feature which was very marked in these sections was the extension of tumour cells from portal tracts so that the liver was often divided by well-formed fibrous bands. The sections examined indicate that most of the tumours grow from proliferation of cellular collections in the portal tracts below the endothelium of the portal veins as evidenced in the mouse melanoma studies. The established tumours outside the endothelium of the portal veins then grow with the same characteristics as in the mice where the course of these has been followed out in more detail. (Fig. 50-57)

Even when well-formed tumours are seen in liver, sinusoidal collections of tumour cells with or without thrombosis and usually associated with inflammatory cells were still noticed (Fig. 49 & 50). Usually the sinusoidal collections were of single cells or of small clumps. They were more common under the capsule. Unlike the findings in the mouse, some of these sinusoidal deposits in the rabbit had proliferated and were forming visible tumours. No capsular deposits of tumour were seen.

Discussion:

Thus the findings in the rabbit confirm those in the mouse. The visible tumours arise in the main from deposits beneath the endothelium of the portal veins. Tumour cells are found also in the sinusoids, and unlike those in the mouse, may occasionally give rise to tumours.

CHAPTER XV

ANATOMY OF THE MOUSE'S MESENTERY

Introduction:

During the previous experiments, it was noted that tumours developed freely on the mesentery. These could be observed from a very early stage to the development of fully formed tumours of different sizes and in varying number. It seemed that the study of the histogenesis of such tumours would be a valuable extensinn of the study. No reports of such work have been found, and the technique of surface staining of the aorta used in this Department (Lautsch, McMillan and Duff, 1953; Duff, McMillan and Ritchie, 1957; Ritchie, Macklem and Weigensberg, 1957) seemed well adapted to the problem. However, first it was necessary to determine the anatomy of the mouse's mesentery.

Method:

The mesentery of normal DBA/1 mice was prepared as a flat preparation. The whole gut was dissected out together with its mesentery and put in normal saline. Great care was taken to avoid damaging the delicate mesothelium. The mesentery was spread out flat on cork and fixed in position by pins (Fig.60 A & B). Usually two such preparations were made from each mesentery. In some cases, with a little care, three were easy to make. Various methods of staining were tried. (Lautsch, McMillan and Duff, 1953; Duff, McMillan and Ritchie, 1957; Poole, Sanders and Florey, 1958). One of the more satisfactory was to immerse the mesentery on its cork in physiological salt solution for half an

hour or more. It was then rinsed in 5% dextrose solution for 5 minutes, using enough dextrose to dilute the sodium chloride ions to an infinitesimal amount. Usually two changes in the dextrose solution were used at this stage. A 0.25% freshly prepared silver nitrate solution was then dropped over the surface of the preparation for 60-90 seconds, the time varying from experiment to experiment. The whole preparation was then immersed in 5% dextrose solution and rinsed by taking in and out of the solution. To ensure that no excess of the silver nitrate solution was left over, it was dipped 3-4 times in another trough containing fresh glucose solution. Next a solution containing 3% cobalt bromide and 1% ammonium bromide was dropped over the preparation from a dropping pipette for a period four times longer than the preparation was exposed to silver nitrate. The specimen was rinsed in 5% dextrose solution as before. It was then immersed in 10% formol saline solution for a period of 30 minutes to one hour (not less than 30 minutes and not more than one hour). Next it was washed in running tap water for 1 hour. The specimen was then taken out and counterstained by dropping on Harris's haematoxylin for 1 minute and 30 seconds, differentiated in 1 in 500 acetic acid, and put back in running water for at least 10 minutes for the development of colour. It was counterstained again with Eosin Y for 5 minutes, and washed in running water. The mesentery was now freed from the gut, floated on cold water, mounted on a glass slide, dehydrated with alcohols, cleared in xylol and mounted with Permount. This method stained the cement between the mesothelial cells as well as tumour and other cells, and fibres.

Another method which proved useful as a modification of that described by Florey, Poole and Meek (1959) for staining endothelium. Freshly spread out mesentery was exposed to the vapour of osmium tetroxide for 10 minutes or longer. Silver nitrate solution was then dropped on as in the previous method. The excess was washed off with water. The specimen was dehydrated, cleared and mounted in Permount. The mesothelial cement lines stained nicely. Some cases were counterstained with hematoxylin and eosin.

To demonstrate collagen and elastic fibres the van Gieson, Gallego and Weigert stains were used. For the van Gieson stain, the mesentery on its cork was fixed in formol saline and rinsed in running water for 1 hour. It was stained by dropping on dilute Harris's hematoxylin for 1 minute, washing in distilled water and counterstained in van Gieson's solution for 1 to 3 minutes. It was then rinsed quickly in distilled water, and the gut was removed. The preparation was dehydrated in 95% alcohol and in 2 changes of absolute alcohol, cleared with 2 changes of xylene and mounted in Permount. The collagen fibres stain red.

For Gallego's iron fuchsin stain, the fixed mesentery was rinsed in running water for 1 hour and stained in Weigert's iron chloride hematoxylin for 6 minutes. It was washed in water, mordanted in iron chloride mordant for 30 seconds, rinsed in water, stained in a fresh dilution of carbol fuchsin for 5 minutes, rinsed in water, retreated with mordant for 2 minutes, rinsed in water, stained in 0.1% aniline blue for 1 minute and rinsed in 0.1% acetic acid. It was then separated from the gut wall, mounted on a glass slide, passed through several

changes of acetone, then changes of equal parts of acetone and xylene, then 2 changes of xylene, and mounted in Permount. By this staining method the collagen and reticulum fibres take a deep blue stain and elastic fibres are purple to red.

For Weigert's resorcin-fuchsin elastic stain, the fixed and rinsed mesentery was stained with Weigert's hematoxylin for 1 to 3 minutes, washed in water, and stained in resorcin-fuchsin solution for 1 to 3 hours. The excess of the stain was washed off in 95% alcohol and the preparation washed in tap water, It was counterstained in van Gieson's solution for 1 minute, separated from the gut, mounted on a glass slide, dehydrated by passing the slide through 95% alcohol, two changes of absolute alcohol, two changes of xylene and mounted in Permount. The elastic fibres are stained blue-black to black by this staining method and collagen red.

To show mast cells, the flat preparation was fixed in formol saline for 1 hour and then washed in running water for 1 hour. Giemsa stain was then dropped on the surface and kept there for 45 minutes. The preparation was rinsed in distilled water, differentiated in acetic acid, rinsed again in distilled water, separated from the gut, mounted on a glass slide, dehydrated in 95% alcohol and then in absolute alcohol, passed through two changes of xylene and mounted in Permount. The granules of the mast cells appeared purple.

Results:

The mesentery of the mouse is a thin transparent membrane composed of loose connective tissue containing many adipose cells, lymphocytes and granular leukocytes and covered on its free surfaces by

mesothelium (Fig.61-63). It contains many blood and lymph vessels. When stretched out flat, it shows a somewhat irregular geometrical pattern of polygonal fields. The sides of these fields are formed by vascular bundles or by the gut. For the purpose of description the surface can be arbitrarily divided into four zones: First, the membranous centre of the polygonal fields; second, the narrow strips of membrane adjoining the fatty layer; third, the fibro-fatty layers which surround the vascular bundles; and fourth, the neuro-vascular bundles.

In the membranous zone, the tissue consists of two layers of flattened mesothelial cells underneath which in most places there was a thin layer of subserosa of varying thickness. The subserosa contains a loose network of irregularly arranged fibres in the interstices of which fibrocytes, lymphocytes and the granular leukocytes are enclosed. The cells of the superficial mesothelium had a polygonal shape with a vesicular nucleus and prominent nucleolus. Their cytoplasm was homogeneous but could not be usually clearly seen.(Fig.65,66). The cement lines were usually fine and well defined. In them were occasional stigmata or stomata similar to those described in endothelial cement lines (references by Altschul, 1954). The stigmata were small nodules on a cement line which appeared solid, the stomata, small spaces enclosed by a cement line (Fig.69-72). The fibres are generally straight but some are wavy and branched. They stain red with van Gieson's stain, blue with Gallego's stain and pink with eosin and so are assumed to be made of collagen. No elastic fibres were demonstrated by Weigert's or Gallego's stains. (Fig. 73)

The fibroblasts were oval, with cigar-like nuclei and cytoplasm which was often clearly defined. Their nuclei were denser than those of the mesothelial cells. Lymphocytes were scattered sparsely. They consisted of dark nuclei and very little blue cytoplasm. Mast cells were occasionally present, and had lobed nuclei and coarse purple granules (as stained with Giemsa) which often hid the nucleus. Plasma cells were rarely seen. These had eccentric nuclei and there was a tendency for the cytoplasm to stain pink. Histiocytes were fairly big cells, either ovoid or irregular, with bean-shaped or folded nuclei. Their cytoplasm usually presented a granular appearance. In addition to the scattered cells, occasionally groups of lymphocytes with histiocytes and other cell types, including a few polymorphonuclear cells were seen. These cellular nodules were scattered randomly across the membrane.

Zone II was not significantly different from zone I, being only distinguished by its proximity to the vascular and fatty bundles. It is distinguished because it showed a somewhat different reaction in the experiments.

Zone III consisted of a varying thickness of fatty tissue disposed between the two layers of mesothelial cells. The fatty layer in general seemed to be more prominent near the larger vessels. In this layer, one could see capillaries around and between the fat cells.

Zone IV consisted of neuro-vascular bundles, in which besides nerves one can see arteries, veins and lymph vessels. The vessels and nerves run parallel and give off few branches as they pass through the mesentery, though both veins and arteries give off branches which

arborize in the fat. The lymphatics give no branches which communicate with capillaries, but do give off branches, some of which re-enter the parent trunk. The nerves give few branches, and these mainly to vessels. The various types of vessel are easily recognized by the structure of their walls. The endothelial cells are stained well with the silver.

There are seen occasional holes in zones I and II. These holes are spaces which in silver-stained preparations have a definite black-stained margin around the periphery. In some cases, cell collections are also noted round these holes (Fig.68,104).

Discussion:

The normal mesentery of the mouse is divided into polygonal fields by vascular bundles. The fields are avascular, being made of two layers of mesothelium between which are straight collagen fibres and a few cells. The vascular bundles consist of arteries, veins and lymphatics and have associated nerves. They are surrounded by fat. Holes were found in the mesentery. Similar holes can be seen in the mesentery of some animals such as frogs (von Möllendorff, 1927). The structures described may be true stomatas. However, they are not shown in every specimen, and the possibility that they may be artefacts cannot be excluded.

CHAPTER XVI

THE FORMATION OF TUMOURS IN THE MOUSE'S MESENTERY

Introduction:

As stated before, following intraperitoneal injection of tumour cells, tumours in the mesentery were observed to develop progressively, and it was considered they offered a fruitful field of study. So the previous method of surface staining method was extended, and applied to the study of the development of metastases in the mesentery.

Method:

The S91 melanoma maintained in DBA/1 mice as described in Chapter XII was dissected out from a donor mouse, and a cell suspension with 200,000 cells per c.mm. made by rubbing pieces of tumour on a monel stainless wire mesh of 60-80 gauge as described in Chapter X. The cells were stored in cold until the time of injection. 0.2 cc. of the homogenized suspension was injected into the peritoneal cavity of DBA/1 hosts by means of a tuberculin syringe and a 25 gauge needle. The mice were housed in plastic cages, 10 in each, and were fed on Rockland mouse diet and water, both ad libitum. Four mice were killed on the 1st, 2nd, 3rd, 5th, 7th, 9th, 12th, 14th, 17th, 21st, 24th, 25th and 30th dyes after injection. Flat preparations of the mesenteries were made and were stained as described in the previous chapter.

Results:

The flat preparations of the mesenteries of mice killed from 24 hours through 30 days after the inoculation of tumour cells were studied.

In them, a gradual change from collections of inflammatory cells to the formation of tumours was seen. The lesions were predominantly inflammatory in the earlier stages, and mostly in the membranous areas (Zones I & II) but after some days they became concentrated in the neuro-vascular and the fibro-fatty areas (Zones III & IV). As days passed, pigment formed in the tumour cells and tumours became visible to the naked eye.

Twenty four hours after injection of tumour cells, a large number of inflammatory foci were scattered over the mesenteric surface. They were approximately of the same size, consisting of 10 to 50 cells. They lay immediately deep to the mesothelium in most, but not all cases. In a few instances they were superficial to it (Fig.64,78). The cells were predominantly polymorphonuclears and lymphocytes, the two cells appearing in almost equal numbers (Fig.79-84). A few macrophages were also present. In many of these foci one or more malignant cells could be found. These had very scanty cytoplasm, which was sometimes eccentric, that is on one side of the nucleus. Sometimes the cytoplasm was seen to bridge over an indentation in the nucleus (Fig. 79,80). Most of the cytoplasm had a brown haze, but in these early stages no granules could be made out. The nuclei could not be seen clearly in some of the cells, but when seen clearly they showed striking variation in size and in shape, but tended to be either round or oval. Nucleoli were prominent and multiple. Some of the tumour cells were bi-nucleate or multi-nucleated. Sometimes it was difficult to distinguish a malignant cell from a histiocyte. Histiocytes usually had a pinkish cytoplasm and an irregular bean-shaped nucleus. They usually had more cytoplasm than tumour cells, and

their nuclei were generally paler, though not vesicular like those of the mesothelial cells. At places fragments of cells were seen and these were probably from disintegrating tumour cells. These inflammatory foci were present in all the four areas described above, in the membranous part of the mesentery (Zone I), in the strips beside the fibro-fatty areas (Zone II), in the fibro-fatty areas (Zone III) and in the neuro-vascular areas (Zone IV). The number of these foci varied considerably from animal to animal, as is seen in Table VI, but at this time they were most common in the membranous areas.

At this stage, the cytoplasm of the fat cells and fibroblasts showed signs of swelling due to the oedema induced by the inflammatory reaction. The blood vessels were congested and dilated.

After 48 hours, the overall picture was almost the same. The inflammatory foci showed polymorphs, lymphocytes and macrophages, some of which contained pigment, though the proportion of polymorphs was now less. In many of them, the tumour cells with large hyperchromatic nuclei were more prominent, and the number of degenerated cells less. The fibro-fatty tissue still showed signs of oedema and the vascular layer signs of congestion.

By the 3rd day, there was a definite change in the arrangement of these cellular collections. Most of them had disappeared from the central membranous area (Zone I). Those that persisted showed a preponderance of lymphocytes. Some contained tumour cells. In contrast, the lesions located in the neuro-v$_a$scular and fibro-fatty areas (Zones IV & III), or in the adjoining strip of the membrane (Zone II) persisted, but they too were now chiefly lymphocytic. Mast cells, not very conspicuous on the first two days, could be seen in fair numbers in the

membranous portion. The fibroblasts around the inflammatory foci showed some mitotic figures. The vessels were less swollen, but some of the branches passing through the inflammatory collections seemed to contain malignant cells in their lumen, or sticking to their walls. (Fig.74-76,85).

By the 5th day, there were still lesions in the membranous portion of the mesentery, but these were small and stationary. The polymorphs had practically disappeared from them and had been replaced by lymphocytes and a few plasma cells. Macrophages were also present, but were few. The tumour cells in the foci which persisted had become still more prominent, and the fibroblasts around them more swollen and bigger.

The collections in the fibro-fatty (Zone III) and in the neurovascular areas (Zone IV) became more condensed, and seemed to have more tumour cells in them. More vessels seemed to be present in the area. Capillaries appeared around the lesions, and seemed to penetrate into them. Small vessels were seen to enter these capillaries from the arteries, and they seemed to drain into similar branches which joined the veins. No lymphatic tributaries or branches from the nerves were seen to approach the lesions. Such branching was not seen in the membranous portion. This vascular proliferation seemed to be important, as it later formed the vascular bed of the growing and developing tumours.

By the 7th day, the few foci of inflammation in the membranous part could be made out to have a granulamatous arrangement, with collections of tumour cells surrounded by inflammatory cells. Fibroblastic cells had proliferated, and intermixed with the cellular collections. The malignant cells became more and more prominent. In the lesions in the fibro-fatty and vascular areas, and in the membranous area adjacent to the vascular zone (Zones IV, III & II), the vascular bed around the lesions became more marked.

On the 9th day, the overall picture remained the same. The persisting inflammatory foci showed malignant cells amongst them. These foci were mainly in the fibro-fatty and vascular zones (Zones III & IV), but could also be seen in the membranous (Zone I) and sub-fatty zones (Zone II) as shown in Table VI. In some foci, malignant cells had proliferated and heaped up. The vascular branches approaching these cell collections from vascular and fibro-fatty areas could be clearly seen. There were relatively few from the arteries, but some of these were sheathed by cuboidal cells which lay outside the endothelium. The venous branches were more numerous but showed no such sheath. The foci in the centre of the membranous areas seemed to have developed a coating of the fibroblasts which surround them.

By the 12th day, the tumour cells had become easily recognizable as they had developed clear pigmented granules. The foci in the vascular, fibro-fatty and the sub-fatty adjacent strip of membrane (Zones II, III & IV) had become well vascularized. The lesions in the membranous portion had still no vascular connections, and were flat, whereas those other areas were rounded. About this time presence of dark spots could be made out without the aid of microscope.

By the 14th day, the lesions had become visible to the naked eye. By this time two types of tumours could be seen. The ones in areas III & IV, near the vascular bundles, formed polyp-like growths, some of them being firmly anchored and sessile, while others were attached to the mesentery by a stalk. Some of the tumours still showed an inflammatory reaction round about, but others did not. The inflammatory cells, if present, were mainly lymphocytes.

By the 21st day, vessels had reached a few of the relatively sluggish tumours in the central membranous areas (Zone I), but by this time the other tumours were too large to permit further examination by this means. The 'ink dots' mentioned in the Table are tumours too dense to see through microscopically. Grossly these tumours closely resembled drops of india ink. Figs. 85-100 depict the gradual development.

Summary:

The histogenesis of the tumour formation in the mouse's mesentery can thus be divided into -

1) an initial stage when there is an inflammatory reaction with collections and cells at places in the mesentery, usually with more on the membranous portion than the vascular regions;

2) an intermediate stage, when the tumour cells become increasingly obvious in these inflammatory foci. Most of the lesions in the membranous part of the mesentery regress while those in the vascular and para-vascular regions grow;

3) the final stage, the proliferation of tumour cells and establishment of a vascular or fibrous bed round them. Two types of tumours form, polypoid ones, in the vascular areas III & IV, and flattened ones, in the membranous areas I & II.

CHAPTER XVII

ATTEMPTS TO MODIFY THE ESTABLISHMENT OF METASTASES IN THE MOUSE'S MESENTERY.

Introduction:

As the mesenteric preparations offered a good way to study the effect of chemotherapeutic and other agents on the growth of the tumours, a preliminary study was undertaken to determine the effect of cortisone and of nitrogen mustard on the development of these tumours. The contradictory literature on the effect of cortisone on various types of tumours has been discussed in earlier chapter. The effect of antitumoral action of drugs like nitrogen mustard is well established. Sugiura and Stock (1950) studied the effects of nitrogen mustard on different tumours and demonstrated its destructive effect. Cruz, McDonald and Cole (1956) showed that if nitrogen mustard was injected on the same day as Walker carcinoma was given, it decreased the number of 'takes' sharply. Similar effects were also shown by McDonald, Livingston, Boyles and Cole (1957) and by Cole, Roberts, Watne, McDonald and McGrew (1958).

Method:

In order to assess the effect of cortisone on tumour formation in the mesentery, 20 DBA/1 mice weighing on the average 22 grammes were injected daily with 1 mgm. of cortisone for 7 consecutive days. On the 8th day 0.2 cc. of a tumour cell suspension containing 200,000 cells per 1 c.mm. was injected into the peritoneal cavity. The cortisone was continued daily.

To test the effect of nitrogen mustard, 20 mice were injected intraperitoneally with an equivalent amount of tumour cell suspension. One hour later, 0.15 cc.(0.015 mgm.) of 'MUSTARGEN' (Chlorohydrate of Mecholrethamine, Merck) solution was injected into the peritoneal cavity.

Another 20 mice were injected with tumour cells along with the other groups, but were on the 15th day injected with the same amount of nitrogen mustard as the previous group. Five of these animals were killed on the 5th day after the mustard inoculation, and another 4 were killed on the 14th day after the inoculation. The remaining mice died inbetween these days.

Twenty control mice were given tumour cells intraperitoneally but no chemotherapy.

All the animals were housed in plastic cages and were fed on Rockland mouse diet and water, both ad libitum. Five animals from each group were killed on the 5th, 10th, 15th and on the 21st days after the injection of the tumour cells. The mesentery was removed and surface preparations were made as described before.

Results: On the 5th day, in the control mice the general features were as described in the previous chapter. Foci of inflammation were seen scattered in different areas. A fair number of these foci contained viable cells. Vascular changes were also noted as described in the last chapter. Table VII shows the findings.

The preparations from the mice treated with cortisone appeared very clear under the microscope. As shown in Table VIII, the paucity of inflammatory foci was striking. The inflammatory cells which were present were scattered over the surface of the preparations, and there were more

pyknotic irregularly staining cells. These were probably the degenerated tumour cells.

In the case treated with nitrogen mustard at the time of injection of the tumour, the picture was like that in cortisone-treated cases. The inflammatory foci were markedly reduced in number and degenerated tumour cells were scattered around without any definite localization. Many macrophages containing brown pigment were seen. (Table IX).

On the 10th day, the mesenteries prepared from the controls showed inflammatory foci with malignant cells amongst them. The foci were mainly in Zones III & IV. Vascularization of these lesions was evident, as in the experiments in the previous chapter. The fibroblastic response was seen round the few lesions in the membranous zone. In few of the preparations, a fair number of foci were seen with cells in which pigment had started to develop. (Table X).

In cortisone-treated cases, the picture was almost the same as on the 5th day. The absence of the inflammatory foci and marked clearing of the fair number of lymphocytes and other cells from the fibro-fatty area was a marked feature. The vessels stood out prominently; but, unlike the controls, no vascular reaction to the tumour was evident. (Table XI).

In the cases given nitrogen mustard at the time of inoculation with tumour, though degenerating tumour cells were scattered on the surface of the leaves, some foci of inflammation were noted, and in some of these viable tumour cells seemed to be present. The reaction was similar to earlier preparations in the controls (Table XII). (Fig. 109-111).

On the15th day, in the control specimens a fair number of visible tumours and microscopic foci of tumour were seen. (Table XIII). In cortisone-treated cases, only a few visible tumours were seen. These were in the fat layer (Table XIV). In cases treated early with nitrogen mustard, the position was intermediate. Besides the tumours shown in the flat preparation, a fair number of tumours developed in the gut and in the abdominal cavity (Table XV), In the cases treated with mustard, and a few in the cortisone group. (Fig. 112-114).

By the 21st day there were many tumours in control cases. These tumours were of different sizes, the biggest being the size of a marble (Table XVI). (Fig.115,120).The cortisone-treated cases showed very few tumours so that on the first sight on opening the abdomen some of the mice almost seemed tumour free. The largest were only 2 mm. in greatest dimension. The preparation again presented a very clear appearance due to paucity of cells in Zones III & IV. (Table XVII). (Fig.116,121) The cases treated with nitrogen mustard at the beginning, presented many black tumours, though fewer than the controls. Scattered pyknotic and irregular cells were still present. The vascular and fatty zones (III & IV) were much less cellular than in the normal controls (Table XVIII) (Fig.117,122),though more cellular than the cortisone group.

In the mice given nitrogen mustard on the fifteenth day after injection of the tumour, the findings were as in the control group (Tables XIX & XX) (Figs.118-119,123-124).

CHAPTER XVIII

D I S C U S S I O N

The differential spread of tumour metastases is a fascinating study. The commonest sites of metastatic tumours in man are the lungs and the liver, as is easily explained by their blood supply, the lung receiving the venous drainage from the caval system and the liver receiving the portal drainage. Less commonly metastatic growths appear in other organs, such as the kidneys, adrenals, spleen and muscles, and here it is difficult to explain the distribution of metastases by difference in blood supply alone. The 'soil hypothesis' of Paget (1889) is one of the most famous attempts to explain the inequalities of their distribution. The experiments with the V2 rabbit carcinoma described in Chapter X showed that the lung and liver were more favourable sites of metastases for the tumour than the kidney. It was also confirmed that metastases in the liver often became larger than those in the lung. As it is clear that the relative paucity of growths in the kidney cannot be explained on mechanical factors alone, attention was fixed on the histogenesis of the growth in the various organs in the hope that this might give some reason for the greater excellence of the 'soil' in some organs than others.

The next portion of the work was therefore the histological study of the formation of metastases in the liver. The metastases were induced in DBA/1 mice by injecting S91 melanoma cells directly into the liver. The tumours developed at the site of inoculation, underneath the capsule both near and far from the site of inoculation, and in the liver substance both near to and far from the site of inoculation.

Histological study revealed that the tumours grew at the site of inoculation in association with the organizing thrombus and later on the granulation tissue formed at that site.

The parenchymal tumour arose from collections of cells initially lying underneath the endothelial lining of the portal veins, and subsequently spreading from the portal tracts into the parenchyma. It was found that after inoculation tumour cells spread out widely and scattered throughout the liver. Besides being at the site of inoculation, and under the endothelial lining of portal veins both near and far away from the inoculation site, the cells lay in the sinusoids throughout the liver. However, in the mice the cells in the sinusoids did not give rise to established tumours.

Study of the tumours arising in the liver of the rabbits in which the V2 carcinoma was injected into the portal vein showed that here too the tumour cells lodged under the endothelium of the portal veins, and that these deposits gave rise to the majority of the tumours. It was also found that tumour cells lay in the sinusoids, and that in a few cases these cells had proliferated to give rise to a tumour.

These observations are in accord with reports scattered in the literature. Wright in 1937 studied human livers containing metastases. He injected coloured gelatin and found it both in sinusoids and larger masses in contact with portal canals. He showed the fibrovascular stroma of the established hepatic metastases were in contact with the portal canals, and that the system of branching arteries, arterioles and capillaries in the neoplasm could in all cases be traced to branches of the hepatic artery, in those digitations of the portal

tracts which entered the tumour. Willis (1930) studied metastases in 8 cases of epidermoid carcinoma of the head and neck. He described that smallest tumours visible to the naked eye appeared to surmount a portal canal, which formed as it were a stalk for the nodule of the growth. His study of the serial sections gave the impression that the emboli arrived by the portal blood. In an experiment with mice, Goldman in 1911 concluded carcinoma cells localized round the bile ducts and branches of the portal vein. In man, neoplastic penetration of the portal vein and proliferation of tumour cells within its lumen have been recorded as a prolific source of embolic dissemination to the whole liver. Virchow (1849) descrived a case of long standing uterine cancer with abundant metastases in the lumbar and upper abdominal lymph glands and in the liver. In the portal vein and its tributaries and branches there was a peculiar thrombus mass containing clumps of elongated and round cells exhibiting fatty degeneration. Willis (1930) thought that there could be no doubt that these were tumour cells introduced by malignant penetration from contiguous lymph nodal deposits, and this accounted for the numerous hepatic metastases present. Spaeth (1866) described a similar case of portal invasion from a carcinoma of the stomach. The dissemination throughout the liver of the tumour cells originating at one spot has also been suggested in man. Venous invasion was recognized as an important property of primary carcinoma of the liver by Eggel (1901), Loehlein (1907), and Ribbert (1909), cited by Willis (1930). Ribbert stressed this feature, and believed that the cases of 'multiple primary hepatoma' are to be explained by venous transport of tumour cells from a single primary growth to give

rise to multiple secondaries in remote parts of the liver. That an identical mechanism frequently underlies the development of multiple secondary neoplasms in this viscus, first impressed itself on Willis (1930) while studying visceral metastases from epidermal cancers of the head and neck. He later confirmed the prevalence and importance of portal vein invasion, both intra- and extra-hepatic, in the development of hepatic metastasis from malignant tumours of various kinds and sources (Willis, 1930). Thus the earlier findings corroborate our observations that tumour cells are scattered throughout the liver, and that metastases arise for the most part in the portal tracts.

To try to understand the distribution of the tumour cells in the liver, it will be necessary to review the hepatic circulation. The liver parenchyma is supplied by both the hepatic artery and the portal vein. Cameron and Mayes (1930) showed that in the rabbit some of the blood from the hepatic artery which reaches the parenchyma has first passed through the capillary networks and venules of the portal tracts into the portal radicles. The hepatic sinusoids also receive blood directly from the terminal branches of the hepatic arteries as well as blood from the portal veins. Most of the portal blood of the lobules is derived from inlet venules, which are short trunks derived from portal veins and entering the lobule through the limiting membrane and then splitting in a tree-like fashion into sinusoids. Two types of sinusoids are described, peripheral sinusoids which run parallel to the limiting membrane and radial sinusoids apparently emerging from the peripheral sinusoids and extending in a rather regular fashion toward the central vein. Extensive communications between the sinusoids in all parts of the lobule obscure the basic pattern of sinusoidal distribution.

The histological observation of Cameron and Mayes (1930) on the effects of experimental ligation of the hepatic artery in animals indicated the existence of communications between the hepatic artery and the portal vein at the arteriolar level in the normal liver, and Wakim and Mann (1942) concluded from observations on the transilluminated liver of living animals, that there were intercommunications occurring between branches of the hepatic artery and the portal vein at sinusoidal and perisinusoidal levels. McFadzean and Grey (1953) injected lipoidol into the hepatic artery and when the liver was radiographed showed lipoidol not only outlined the hepatic arterial tree but also entered large intrahepatic vessels in distribution corresponding to that of the major branches of the portal vein.

The portal tracts, originally called 'interlobular spaces', consist of irregularly arranged collagenous connective tissue bundles, between which some fine fibrillar reticulum fibres are suspended. Between the connective tissue bundles, there are cells, consisting of histiocytes, monocytes, some lymphocytes and plasma cells. The number of these cells varies in normal human beings, and they are seen in mice and guinea pigs after splenectomy, X-ray radiation, intoxications and injection of trypan blue (Popper and Schaffner, 1957).

The liver is drained by lymphatics, which start with their smallest roots in the portal tracts, and possibly even extend to a small way within the lobular parenchyma as well. These empty into the large extrahepatic lymphatic vessels which drain the liver lymph into the cysterna chyli and the thoracic duct. In Glisson's capsule, an extensive intercommunicating network of lymphatics exists, which

communicates with the intrahepatic network and drains into the same extrahepatic trunks.

Even so, it is not clear how the cells injected at one place in the liver substance could become so widely disseminated. The picture of dissemination was same in the mice in which tumour cells were injected directly into the liver, as in the rabbits in which the V2 carcinoma was inoculated through the portal vein. It might be assumed that in the latter case the cells took a normal course reaching the portal veins and sinusoids in the natural way, but in the former their distribution cannot be so easily explained. The pressure of the injection or the injury of the needle might have opened up some of the big vessels, but it is hard to think this an adequate explanation. The blood flow within the liver substance may be more complicated than is known. Nor is it clear why the tumour cells localized under the endothelium of the portal veins. It was thought that perhaps these deposits were in tiny branches of the veins, or perhaps in lymphatics. However, serial sections failed to demonstrate any small vessels around region of these deposits, and injections of india ink into mouse liver failed to demonstrate such vessels. It was therefore concluded that the cells lay underneath the endothelium in the wall of the veins. Similar collections of tumour cells beneath the endothelium of moderate-size vessels have been observed in other organs, in the lung by Schmidt (1903), Iwasaki (1915), Saphir (1943) and Wood (1958), and in the spleen by Coman (1953). It thus seems that this site of localization is not peculiar to the liver, though just how the cells reach this site is not clear. In most cases there was no evidence of thrombosis associated with the deposits.

As regards the cells in the hepatic sinusoids, and their failure to develop often into metastases, possibly a comparison can be drawn between the cells in the liver sinusoids and those in the spleen. It has been shown that tumour cells which pass through the spleen produce tumours when injected into other organs, though they failed to do so in the spleen itself (Korpassy, Kovac and Tiboldi, 1954). Thus the spleen is not toxic to tumour cells. The rarity of splenic metastases must be explained otherwise, perhaps by the peculiarities of the blood supply. While all authorities do not agree as to the nature of the splenic circulation, in the spleen two types of circulation seem to take place. The blood may pass from the splenic arteries into the sinuses and thence into the splenic veins, or it may pass from the sinusoids into the pulp. The second circulation is intermittent in character and brought about by the physiological contraction and relaxation of the whole organ. The second type of circulation might be quite effective in preventing the lodgement of tumour cells in the spleen (Warren and Davis, 1934). Kettle (1912) considered that the physiological rhythmic pulsation of the spleen keeps the tumour cells in the organ oscillating and prevents them from becoming anchored to the vascular wall and that a large number of them pass through the organ, while of those that are impacted in the capillaries, only a few obtain anchorage and grow. The hepatic sinusoids receive blood from the terminal branches of the hepatic arteries and also blood from the portal veins. Though these sinusoids are devoid of muscular elements, their structure and the cross current of the arterial and the portal blood which flushes the sinusoids might be responsible for the tumour cells not finding an easy bed in the sinusoids.

However, as in the spleen, some of the tumour cells may gain a footing and develop into tumours, as was seen in the experiments with V2 carcinoma, some of the cells in the hepatic sinusoids may do likewise. Nevertheless, even in the rabbits, majority of the tumour cells in the sinusoids did not grow into tumours. This is in spite of the fact that in some cases tumour cells were present in almost every second or third sinusoid.

Contrarily, the growth of the tumour deposits in the portal tracts could be explained by the presence of connective tissue in these areas. In the studies by Leighton and Kline (1954), Leighton, Kline, Belkin and Tetenbaum (1956) and Leighton (1957) of the interaction of malignant cells (Hela carcinoma D-189 line) and normal tissues in vitro in sponge tissue cultures, it was shown that the Hela cells rapidly invaded those normal tissues of the chik embryo and of the human foetus that gave rise to luxuriant out-growth of connective tissue. It was shown also that malignant cells of the D-189 line and explants of normal connective tissue were attracted by and moved toward one another. When the contact between these two tissues was established, malignant cells began to invade the connective tissue. Stimulation of the growth of connective tissue by tumour explants was observed in the experiments of Santesson (1935) and of Ludford and Barlow (1944). Schleich (1956) showed that the presence of normal connective tissue is necessary for survival of malignant cells of the Yoshida sarcoma in vitro. Powell (1957 & 1958) suggested that monocytic cells contained in the explanted pieces of normal embryonic organs form in vitro some substances which are essential for growth of cells of Ehrlich mouse ascites carcinoma. Connective tissue is present in the liver in the portal tracts, in the

organizing puncture wound, and under the capsule. These are the places where the tumour cells grew into tumours. It may thus be that it is the presence of connective tissue that permits the establishment of tumours in the portal tracts, in the puncture wound, and under the capsule, and which prevents it in the hepatic and for that matter, the splenic sinusoids. Just what the action of the connective tissue is, is not clear. It may, but need not, be of the athreptic or hormonal type suggested above.

A similar explanation of the failure of tumour cells to grow in unfavourable soil such as muscle and spleen is that in these areas a satisfactory blood supply cannot be established (Coman, 1953). As was seen in the experiments with mouse's mesentery, and by Wood (1958) in the rabbit's ear chamber, capillaries grow very soon into metastases. It is clear that such proliferation of capillaries could occur much more easily in the portal tracts than in the hepatic sinusoids, an area devoid of the usual type of the blood vessel. However, in the mouse's mesentery, and in other (Iwąsaki, 1915; Baserga and Saffiotti, 1955) tissues, some growth of tumour cells may occur before vascularization is evident. In the liver sinusoids in the mice, and in most sinusoids in the rabbits, even this limited proliferation of tumour cells did not occur.

Yet another possibility is that it is the endothelium which provides an effective barrier to the growth of metastases. The findings in the liver would be explained by this theory, as the growth into tumours noted in the portal tracts would be because the tumour cells had escaped beyond the endothelium, and their failure to grow in the

sinusoids would be because they fail to escape beyond the endothelium. This theory has been supported in other organs. Takahashi (1915) thought that the obstacle to the establishment of a true metastatic growth in the lung was presented by the endothelial barrier and that once passed this barrier, the tumour cells would grow undisturbed. Willis (1952) describes 'the critical period for embolic tumour cells as the period intervening between lodgement and the attainment of an extravascular position by the proliferating cells. When that position is attained, the cells once again occupy their wanted relation to the connective tissue stroma'. The speed with which the tumour cells in the portal tracts escaped beyond the endothelium is also notable. At the earliest time studied, 24 hours after injection, they were already in the connective tissue. This rapid escape has been noted in other organs too. Warren and Gates (1936) reported that by six hours after intravenous injection of Walker 256 carcinoma cells invasion and growth beyond the capillaries were evident in the lung. Early endothelization of the tumour cells with subendothelial growth and subsequent connective tissue invasion was also clearly shown in the experiment of Wood (1958) in the ear chamber of rabbits. Between 24 and 30 hours after injection of V2 carcinoma into the ear artery, areas of endothelial penetration had occurred and had been covered by endothelial proliferation. It appeared that if attached tumour cells remained within the vessel for 24 hours and failed to penetrate the endothelium, they might be covered by endothelial proliferation.

A striking feature of the mouse livers into which tumour was injected, and more particularly of the livers of the rabbits injected

intraportally with V2 carcinoma, was the cirrhosis which developed. In human beings, the frequent association of cirrhosis and primary carcinoma of the liver, and the increased incidence of primary hepatic carcinoma in cases of portal cirrhosis, would appear to confirm the relationship of tumour to cirrhosis (Srewart, 1931; Hall and Shao-chein, 1951). These studies, and other reports reviewed, indicate that portal cirrhosis is either a precancerous lesion or at least enhances the localization within the liver of a cancer-inducing agent (Greene, 1939; Berman, 1940; Rosenberg and Ochsner, 1948). Our study perhaps suggests an inverse relationship. Tumours growing and extending in the portal tracts may induce cirrhosis. On the other hand, in man secondary tumours of the liver are rare in cases of cirrhosis (Chomet, Valaitis and Pearah,1959).

As regards the localization and growth of tumours in the liver underneath the capsule Cramer (1905) found that when the Jensen tumour was injected intraperitoneally, the growth involved the liver and the small intestine forming small lobulated masses. In the liver the tumour was first seen beneath the peritoneal covering but soon penetrated deeply into the organ destroying liver cells as it advanced. However, in our experiment with V2 carcinoma in the rabbits, no capsular tumours were observed. This result would lend support to the conclusion that the capsular tumours in the mice originated from spillage of tumour cells at the time of inoculation into the coelomic cavity. However, the possibility that the subcapsular tumours in the mice were due to spread by the subcapsular blood vessels or lymphatics, or even by transhepatic passage through the sinusoids, cannot be excluded.

Though tumour cells seen in the mice and rabbits in the sinusoids rarely grew into tumours, they persisted in some of the sinusoids throughout the period of observation. This persistence might be explained in two ways. Firstly, these cells might be dislodged repeatedly from the tumours already formed inside the liver, as some were washed away from the sinusoids, and new ones took their place. The work already cited of Willis (1930) and the writers he quotes confirm this possibility. The other possibility might be that these cells remained from the very beginning, since the time of introduction, lying in the sinusoids neither growing, nor regressing. The experiment of Fisher and Fisher (1959) in which they introduced 50 Walker carcinoma cells into the mesenteric vein in rats, and found no tumours developed if the liver was left undisturbed, but that if after some months repeated laparotomies were performed, tumours appeared. They suggested that these arose from tumour cells which had been dormant in the liver. It may be that the tumour cells in the sinusoids are these dormant cells. On the other hand, cells under the endothelium of the portal veins equally well have been the latent foci of tumour. The evidence available does not permit a conclusion.

In the experimental study of the formation of tumours in the mouse's mesentery following inoculation of a suspension of S91 melanoma cells intra-abdominally in DBA/1 mice, tumours formed freely and followed a more or less a regular course of development. First, there was a stage of inflammation, with focal accumulations of inflammatory cells around living or dead tumour cells. These collections were mostly underneath the mesothelial layer, but a few were on the surface. At first, these foci were as common in the membranous portions (Zones I & II) of

the mesentery as in the fibro-vascular areas (Zones III & IV), but gradually the foci in the membranous areas became fewer while those near the vascular bundles increased in number. From the 5th day onwards, the para-vascular collections predominated.

Gradually the tumour cells proliferated and increased in number. There was also a change in the nature of the inflammatory cells. The polymorphonuclear cells seen in the early stages became fewer, and were replaced by lymphocytes, plasma cells and histiocytes. Another important feature was the development of branches from the vessels in areas III & IV. These branches led to and from capillaries which formed around the tumour nodules in the vascular and para-vascular zones. No lymphatic supply or nerve supply to the tumours was detected. In the membranous portion, there was no formation of vessels around the nodules, but instead a marked fibroblastic reaction.

By about 10 days, the tumour cells were accumulating brownish black granules. They heaped up, obscuring the other cells. These heaps formed visible tumours by 12 - 14 days. By this time, the vascular bed, formed in connection with the tumours developing in the vascular and para-vascular areas was quite apparent. A similar growth of vessels from the adjoining area into some of the tumours arising in the membranous zone could also be seen.

As the tumours became visible, they formed two types of growth. The tumours in the vascular and para-vascular zones formed polyp-like growths, some of them being anchored firmly by a solid base, while others were attached to the mesentery by a thin stalk. The stalks were formed by the vessels entering into the tumour. On the other hand, the growths

on the membranous portion were usually flattened. Penetration of the blood vessels into these flat lesions was very rarely seen.

Another feature not directly connected with these focal lesions was the sticking of the tumour cells on the vessel walls. A few of these cells seemed to be inside the vessel lumen. In some of the autopsies, tumours were seen to have formed on liver and other organs in the abdominal cavity.

By 21 days, the mesentery had become studded with numerous fairly big tumours. Tumours also developed on the gut and abdominal wall, and on the neighbouring viscera.

Although secondary tumours of the serous membranes are common, and usually are caused by liberation of detached tumour cells into a serous cavity, and implantation of these cells to form the metastatic tumours, to our knowledge no histological study of their genesis and growth in experimental animals has been reported. Zeidman (1957) in his review of recent advances in our knowledge of metastasis just mentions 'metastasis by implantation' briefly in passing.

After the injection of the tumour cells into the abdominal cavity, the cells presumably floated about in the peritoneal cavity freely, some living, some dead, some dying. When these cells lodged on the mesentery, they caused inflammatory response which was at first of the acute type, with a fair number of polymorphs, but which gradually changed to a chronic form with lymphocytes, plasma cells and mast cells. The inflammatory response was local.

This inflammatory response is probably a protective measure against the foreign tumour cells which were introduced into the

abdominal cavity. It might be that dead tumour cells are more effective in inducing this reaction than the living cells, because their altered proteins might be more "foreign". This reaction could, however, have helped the establishment of the tumour cells. The inflammatory reaction might have caused local injury and this injury might have helped the establishment of the tumour cells in the fashion described by Jones and Rous (1914), Hertzler (1919) and Sampson (1931) and discussed below, or the inflammatory cells might have supplied some threptic substance to the tumour cells in the way discussed previously.

The rapid penetration of the mesothelium is similar to the rapid penetration of the endothelial vascular lining by the V2 carcinoma cells in the rabbit's liver and the S91 melanoma cells in the mouse's liver. Once again it is not clear, however, why this penetration occurs.

The next step is the establishment and growth of the tumour cells. The reaction was different in the membranous and the fibro-fatty, vascular zones. In the membranous zones, the tumour bed was formed by the proliferation of fibroblasts around the cell collections. In the zones with vessels in the neighbourhood, there was an early vascular proliferation. In this study, these two types of reaction were quite distinct, and the processes could be followed from the initial stage from the licalization of the tumour cells to the final development of visible tumours.

The vascular and fibroblastic responses were associated with different morphological forms of growth of the tumours. Those with a vascular supply, were polypoid growth, with a stalk attached to the mesentery, and those without flat tumours spreading on the surface of the mesentery.

As has been remarked, there is in the literature very little to confirm or extend these conclusions. Hertzler (1919) reviewed the literature on the results of injecting suspensions of pigment into the peritoneal cavity of the lower animals, and included the results of his own studies in this subject. He stated that the foreign bodies float freely until they become anchored to the peritoneum by fibrin, which is in due course transformed into fibrous tissue. Sampson (1931) studied twenty-five cases of peritoneal carcinomatosis associated with ovarian cancer and followed the genesis and growth of the tumours through different stages, the escape of the cancer cells into the peritoneal cavity from the ovarian tumour, the transportation of these cells to their site of implantation and the reaction of the peritoneal tissue injured by the cancer cells lodging on its surface. He, too, stressed the importance of fibrin in the fixation of the tumour cells on the peritoneal surface. In the present study, fibrin seemed of little or no importance.

Jones and Rous (1914) found that whereas intraperitoneal injections of a mouse tumour were seldom successful in producing transplant growth, such growths were obtained in a large proportion of animals which had received previously intraperitoneal injections of lycopodium. These workers suggested that in human carcinomatosis, the irritation excited by dead tumour fragments may facilitate the successful implantation of viable fragments. Sampson (1931) came to a similar conclusion. He thought that some of the floating cancer cells sooner or later lodged on the surface of the peritoneum. Their presence irritated (injured) the peritoneum. This was followed by an exudate, and in places by a denudation of the peritoneum (casting off of the mesothelium). The cause of

the injury, he suggested, might be more than that of an inert foreign body. Possibly there might be a toxin or enzyme present in the cancer cells. In the present work, though possibly a serosal injury caused by dead and dying cancer cells might have helped in the localization of the living cancer cells, there is little or no evidence that this was important.

In the formation of the 'bed' for the tumours, two types of reactions were noted. The first was the early invasion of the cellular masses by vascular branches from the pre-existing vessels. This was quite clear in the fibro-fatty and the vascular areas by about 4 or 5 days after the injection of tumour. This is in accord with other observations. Ide, Baker and Warren (1939) in their study of the growth of fragments of Brown-Pearce carcinoma in the rabbit ear chamber noted blood vessels growing around the tumour transplant as early as 3 days after transplantation. Algire and Chalkley (1945) in their study of tumour transplantation in mouse's skin chambers showed capillary growth into the transplants as early as 3 days after implantation. Williams (1953), studying the vascularization of omentum transplanted as autografts in a rabbit ear chamber, observed new capillary growth from pre-existing blood vessels within 24 hours. Wood (1958), studying the V_2 carcinoma in the rabbit ear chamber, showed capillary buds arising from pre-existing vessels and entering the tumour within 24 hours. In contrast, in the membranous portion fibroplasia formed the stroma of the growing tumours. While it seems that distance from pre-existing vessels determines the occurrence or non-occurrence of the vascular response, it is not clear why so little fibroplasia was seen in the vascularized tumours.

Penetration of tumour cells into the blood vessels and lymphatics has been noted before. When ascites tumour cells were introduced into the peritoneal cavity, some of them soon got into the blood vessels indicating that the peritoneal barrier is not effective against single cells (Zeidman, 1957). (Fig. 101 & 102).

The effect of cortisone on various kinds of tumour under various conditions has been reviewed in Chapter VIII. The reports were confusing and contradictory. In the experiment on the effect of cortisone on the V2 carcinoma of the rabbit, it was seen that cortisone did not increase the frequency of metastases and actually decreased the incidence of growth of the tumour at the site of implantation. These findings did not, however, indicate how cortisone acted. The effect of cortisone on the tumours in the mesentery was more informative. There was a marked reduction in the lymphocytic and other cells in the preparations, an inhibition of the inflammatory and fibroblastic and vascular responses associated with tumour cells, and apparently a cytotoxic action on the tumour cells themselves. Schrek (1949) and many others have shown that cortisone has a direct cytocidal action on lymphocytes, and it is well known that under certain conditions cortisone can inhibit the inflammatory reaction and change the surrounding connective tissue, as has been reviewed in Chapter VIII though just as the effect of cortisone on tumours is variable and uncertain, so is its effect on connective tissue. In the experiments, there was observed a repressive action both on the tumours and the connective tissue proliferation. It may well be that the inhibition of the connective tissue and vascular response was the cause of the diminution of tumour formation. One

cannot exclude a direct cytocidal action of cortisone on the tumour cells, but it is not necessary to postulate one.

The effects of nitrogen mustard were similar to those of cortisone when the mustard was given 1 hour after the inoculation of tumour cells. There was evidence of necrosis of tumour cells, inhibition of the inflammatory reaction, and a general diminution of the number of lymphoid cells. Later however, the tumour seemed to escape, and growth similar in pattern to, though less in degree than that noted in controls, occurred.

The findings are in accordance with the findings recorded in the literature. Nitrogen mustard has cytocidal action on both the tumour cells and on lymphocytes and other cells. The nitrogen mustard was administered as a single small dose. The initial inhibition of inflammatory response and the cytotoxic action on the tumour cells combined together in diminuting the number of tumours. But as the quantity of the drug available dwindled out, the reactions as shown in the control experiments were repeated and tumours formed.

When nitrogen mustard was introduced 15 days after the inoculation of tumour cells, the tumours were already well established and nitrogen mustard had no effect at all. This finding is also in accordance with the previous observations in the literature, as by Cruz, McDonald and Cole (1956) and others.

In summary, in the first step of the experimental study, the differential growth of tumours in rabbit of V2 carcinoma introduced intraportally and into the renal artery was shown. The metastases grew in lung, liver, kidney and in spleen. The number of metastases in the

lungs were more than in the remaining three organs but the metastases in the liver were bigger. This experiment confirmed the view that some organs provide better 'soil' for the growth of metastases.

The work was extended to study the histogenesis of the metastases formed in the mouse liver after injection of S91 melanoma directly into the liver tissue. It was shown that tumours originate at 3 sites, the site of inoculation, from deposits underneath the endothelium of the portal veins, and underneath the capsule. There were though quite a number of cells scattered in sinusoids, but these did not develop into tumours in the mice.

The histological study of the rabbit livers from the first experiment generally confirmed the mode of the formation of hepatic metastases. These mainly arose from the portal veins, and invaded the liver parenchyma only later. However, a few of tumours formed from the proliferation of the sinusoidal cell collections. No capsular tumours were seen in rabbits.

It was brought out that the spread of tumour cells might produce the pattern of hepatic cirrhosis.

Many of the sinusoidal and cell collections underneath the endothelum of the portal veins were seen to remain stationary, it could not be definitely assessed whether these were capable of producing tumours at a later time.

Attempts to modify the tumour formation by means of carbon tetrachloride injury to the liver of by inducing mild inflammation in the liver by injections of E. coli failed to show any conclusive result.

The other main experiment was the study of the establishment of metastases in the mouse mesentery by intraperitoneal injection of S91 melanoma cells. The stages of the formation of tumour were clearly demonstrated from the localization of the tumour cells on the surface of the mesentery to the development of the fully formed tumours by 12-14 days.

The modification of the formation of these mesenteric tumours was studied by treating the mice with cortisone or by challenging the tumour cells with nitrogen mustard. Cortisone markedly diminished the number of metastases. Similarly, nitrogen mustard introduced as a single dose one hour after the tumour was injected inhibited tumour formation in the initial stages, but there was an escape after a certain period, and tumours grew though their number was very much less than in control animals.

When tumour cells were introduced 15 days prior to the challenging dose of nitrogen mustard, the drug did not modify tumour formation.

CHAPTER XIX

GENERAL OBSERVATIONS

In the first experiment, when V_2 carcinoma cells were introduced into the portal vein and into the renal artery of rabbits, many tumours formed in the lungs, fewer in the liver and still fewer in the kidney. However, the lung tumours were smaller than those in the liver. The reason more tumours appeared in the lung might be that more tumour cells passed unarrested through the liver and kidney. That tumour cells do pass through the liver is known already, as cited earlier. Another reason which might explain why there were more tumours in the lung, and why they were smaller than those in the liver, is that tumour emboli might reach the lung from established tumours in the liver. However, both these explanations seem inadequate to explain the discrepancy. Rather it seems that one must adopt at least in part the classical theory of the need for a good 'soil'.

This excellence of the 'soil' has been considered to be due to chemical processes which might be favourable to the growth of the tumour. In the liver, it was thought that the soil was good because of the liver's rich carbohydrate content, poor oxygenation, exceptionally high concentration of the amino acids, and because of the presence of the growth promoting substances.

However, the study of the histogenesis of metastases in the liver of DBA/1 mice in which the S91 melanoma was injected directly into the liver, in rabbits in which the V_2 carcinoma was introduced intraportally showed that tumours developed mostly from cells which localised

underneath the endothelium of the portal veins. The tumours grew in the portal tracts and the tumour cells extended inside the liver through the portal tracts. The localization in this place might be due to the presence of connective tissue, or to the ability to establish early a vascular supply. Thus it can be postulated that tumour cells localize in the liver in the portal tracts in relation to the portal veins because of the presence of connective tissue which supplies a threptic substance, or because these cells can survive easily due to the early availability of a capillary network.

In the early stages, the sinusoids showed great number of tumour cells in them. But as time went by, these collections became rarer. Tumours did not develop from these sinusoidal cells in mice, and very rarely did in rabbits. It is difficult to explain why the tumour cells in the sinusoids did not grow into tumours. Several explanations are possible. Perhaps these cells could not establish themselves because of a peculiarity of the vascular supply to the sinusoids. Currents in the blood stream might dislodge the tumour cells from the sinusoids and wash them away. A second possibility is that the endothelial barrier may retard the formation of tumours. A most critical period in the genesis of a tumour formation is the penetration of the endothelial barrier, and the escape into the surrounding tissues. The endothelium of the sinusoids is of a different character to that of the ordinary capillaries and arterioles. Also next to the endothelial layer of the sinusoids are liver cells, and not a connective tissue. Though it is clear from the work described earlier that tumour cells can easily penetrate the endothelium of the portal veins, it may well be that the sinusoidal endothelium offers a more serious obstacle.

Thirdly, there are in the sinusoids no vessels from which the growing cells could derive the new capillaries needed for their growth and multiplication. Fourthly, it is possible that connective tissue helps the establishmen of tumour cells by supplying a 'threptic' substance to the growing cells, as monocytic cells contained in explanted pieces of normal embryonic organs were considered to form in vitro some substances essential for growth of cells of the Ehrlich mouse ascites carcinoma.

Thus, in summary, among the reasons why the sinusoidal cells did not grow into tumours may be that they might have been washed away into the general circulation; that the structural peculiarity of the sinusoidal endothelium prevented their escape; that a capillary bed could not be established; and that connective tissue which might be essential for the growth of the cancer cells was not available.

These explanations might explain also why more tumours develop in the lungs than in the liver, when an equal dose of tumour cells is injected into the organs. In the liver many of the cells are trapped in the sinusoids where there is little chance of their growing. In the lung they lodge either in the arterioles or the capillaries, but in either case have normal endothelium to penetrate, a ready source of new capillaries, and a ready access to connective tissue.

In this respect the spleen is comparable to the liver. In the spleen, as in the hepatic sinusoids, very few metastases grow. The reason may be that many of the tumour cells are trapped in the sinusoids, and there fail to grow because the cells are washed out of the organ, because they cannot penetrate the sinusoidal endothelium, because there

is no source of capillaries, or because of the lack of connective tissue. Besides, in the spleen, there are no portal tracts. The cells trapped in the arterioles may fail to grow because these vessels may be different.

However, the presence of fibrous tissue and the availability of a new vascular supply cannot always explain the disparity in the frequency of establishment of metastases in the various tissues and organs. For example, in the muscles both these factors are abundantly present but the number of metastases are few.

The common association of connective tissue formation in the form of cirrhosis and primary carcinoma of the liver is a well established fact. This fact lends support to the suggestion that connective tissue and the vessels in it are essential for the establishment and growth of tumours in any organ. It may be that the cirrhotic connective tissue somehow potentiates the growth of the tumours.

Metastasis formation on the mesentery of DBA/1 mice, following injections of S91 melanoma intraperitoneally was comparable to that in the liver or lung. The introduced tumour cells, dead, dying or alive, came in contact with the surface of the mesentery and produced inflammatory changes. The inflammation was probably protective in nature in the early stages, trying to destroy the foreign bodies. Probably some of the tumour cells died, and more leukocytic influx occurred. However, the inflammation might contrariwise have helped the tumour by supplying 'threptic' substances to the viable cells from the dying tumour cells or from the leukocytic concentrations which might be mobile sources of enzymes, as has been discussed earlier. Thereafter, the tumours were established by two kinds of reaction. In the membranous portion fibroblastic proliferation formed the bed for the growing tumour cells. Blood

vessel proliferation was not seen in their neighbourhood. On the contrary, in the vascular and fatty areas, the bed was formed by the proliferation of buds from the existing vessels. No fibroblastic proliferation was seen around these lesions. Thus tumours may develop either by establishing by a young fibroblastic or by a vascular bed. Both are not necessary. These reactions proceeded to form two types of tumours, flat, spreading ones on the membranous portion of the mesentery and round, sessile or polypoid growths, comparatively much bigger on the vascular and fatty areas.

Thus from these experiments and from the reports of previous workers, it may be concluded that the differential growth of tumours in different organs depends on the availability of suitable sites and conditions for the easy establishment of the tumour cells. The nature of these conditions is not clear, though it does seem that either connective tissue must be present, or the tumour must be able to derive new capillaries. However, other conditions must, of course, be met.

CHAPTER XX

S U M M A R Y

A brief review of the concept of tumours in ancient India is given. This is followed by a review of the development of the concept of cancer and of the development of the concept of metastasis.

The experimental work on metastases has been reviewed, with particular reference to the mechanism and the histogenesis of metastasis formation. Factors modifying metastasis formation have also been discussed, and in particular, the effect of cortisone and nitrogen mustard on tumour formation and on metastasis.

Experimentally, an equal number of V_2 carcinoma cells were introduced into the portal vein and into one renal artery in rabbits. More tumours grew in the lungs than in the liver, and more in the liver than in the kidney. However, the tumours in the liver grew larger than those in the lungs.

The histogenesis of metastasis formation following the injection of S91 melanoma into the liver of DBA/1 mice has been studied. Tumours grew at three sites: at the site of inoculation; from collections of tumour cells underneath endothelium of portal veins; and underneath the capsule. Tumour cells were also found scattered through the sinusoids, but no tumours grew from tumour cells in the sinusoids.

In the rabbits injected with V_2 carcinoma intraportally, tumours grew mainly from collections of tumour cells in the portal tracts, though a few tumours grew from sinusoidal collections. No tumours were seen to grow underneath the capsule. The spread of tumour cells in the liver

parenchyma produced a cirrhotic pattern.

Cortisone caused regression of implants of V_2 carcinoma in rabbits, and diminished the number of metastases.

Mouse mesentery was studied by a new surface staining method, establishing its normal anatomy in DBA/1 mice. The establishment and growth of mesenteric metastases was studied by injecting S91 melanoma intraperitoneally. Cancer cells settled in inflammatory foci in the membranous and the fibro-vascular areas of the mesentery and gradually developed into tumours by establishing either a vascular bed (in the fibro-vascular areas), or a fibrous bed (in the membranous portion), so producing two different morphological types of tumours; the former formed polypoid growths with either a solid or a slender stalk, the latter a flat spreading type of growth.

Attempts to modify the establishment of metastases in the mouse's liver by damaging the liver by the administration of carbon tetrachloride introduced into the stomach of the mice or by the intra-hepatic injection of Escherichia coli prior to the introduction of the tumour cells failed to alter the rate or pattern of growth in the liver. A similar attempt to correlate liver injury by either carbon tetrachloride or E. coli with tumour transplants subcutaneously also failed to affect the growth of these implants.

When the mice were treated with cortisone for 7 days prior to the administration of tumour intraperitoneally and the cortisone was continued after inoculation, the number of metastases was considerably diminished. Similarly, nitrogen mustard injected one hour after the inoculation of tumour cells into the peritoneal cavity of the mice greatly diminished the tumour formation in the earlier period, but after

a few days the tumour cells escaped the effect of the drug and formed tumours, though their number was less than in the control cases. When nitrogen mustard was injected 15 days after the administration of the cancer cells, no effect was noted, the tumours developing as in the control animals.

CHAPTER XXI

CLAIM TO ORIGINALITY

The study of the histogenesis of metastases in the liver of animals has not to our knowledge been undertaken previously. The results revealed that in the liver tumours arose from tumour cells which localized underneath the portal endothelium. This observation is original, though the study of human autopsy material indicated that secondary tumours in the liver spread by the portal vein and sometimes grew in relation to them. The results of this study also showed a very close relation between the connective tissue and the tumour formation in the liver.

The study of the mouse mesentery by the new surface staining techniques also to our knowledge has not been reported. The study of the normal anatomy of the mesentery, and of tumour formation from cells seeded on the mesenteric surface to the development of the fully formed tumours is also original.

The effect of cortisone and nitrogen mustard on these tumours was also original and the knowledge can be utilized for application either in therapy of these tumours or for further experimental work.

Lastly, the possibility that the presence of connective tissue might be an important factor in the establishment and growth of tumours in different organs or tissues is suggested.

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A P P E N D I X

Fig. 1 - Photographs of Liver (on the left), Lung (right), Spleen (above) and Kidney (below) from different rabbits showing the relative size of tumours in different organs. Tumour cells were injected into the portal vein and renal artery. The tumours in the liver and spleen are larger.

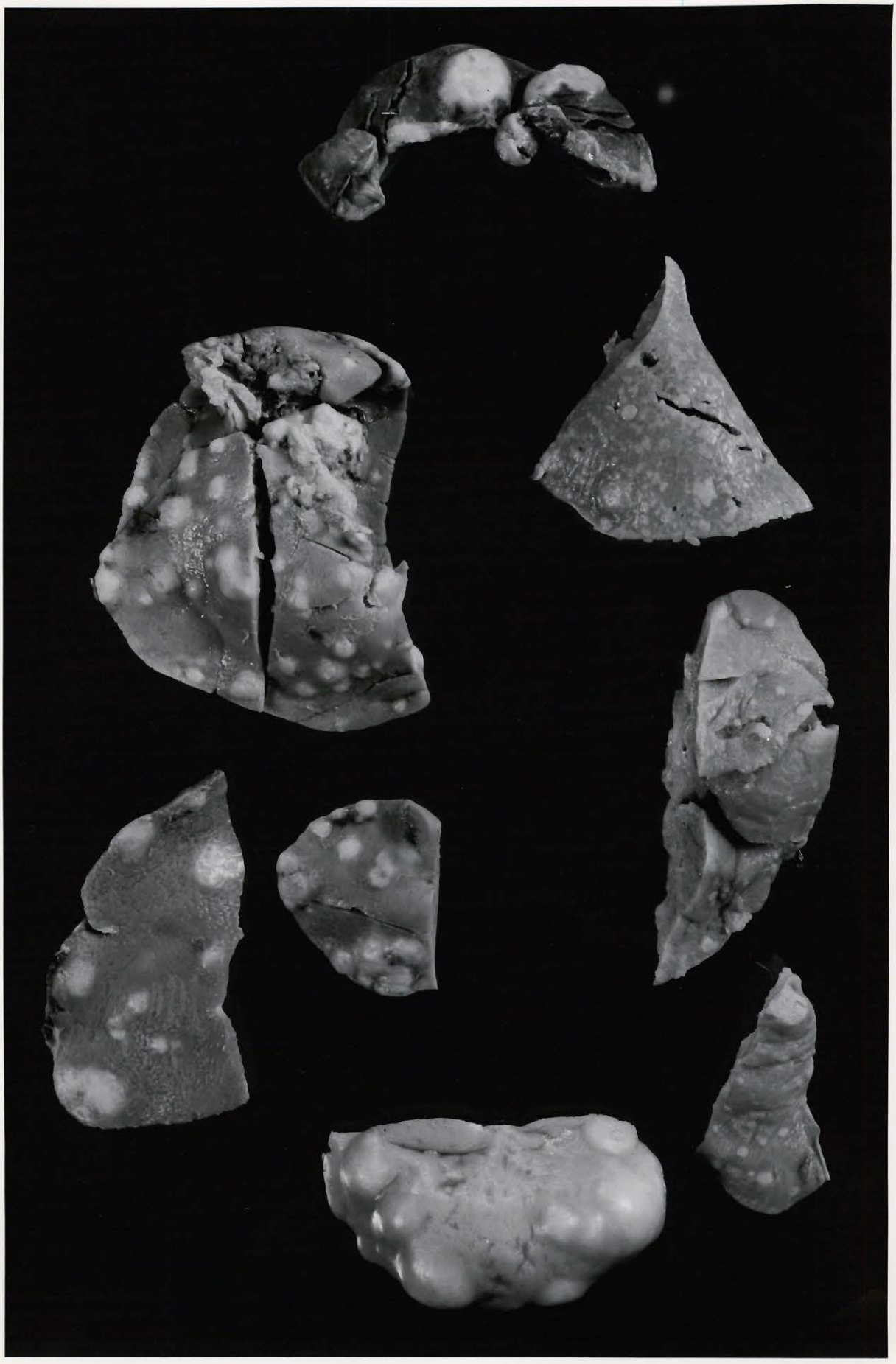
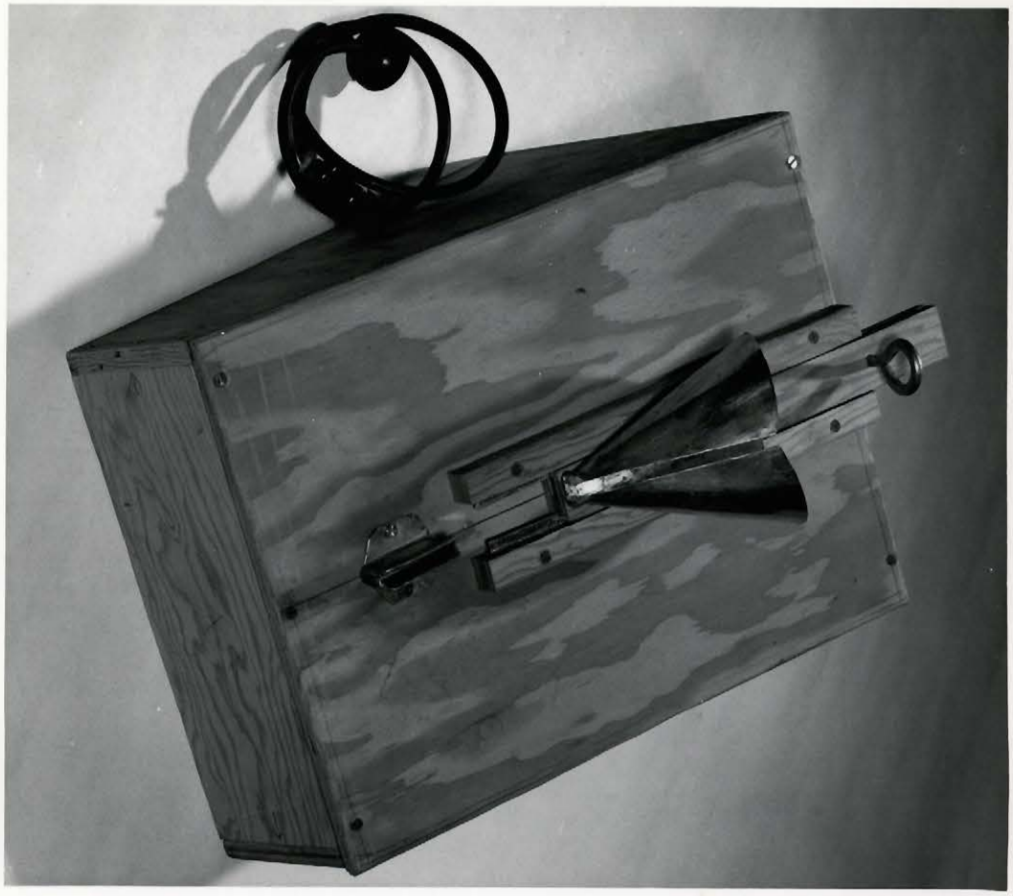


Fig. 2 - Photograph of the apparatus showing the cone-shaped holder, and the slit over which the tail is placed and transilluminated by light from inside the box.

Fig. 3 - The position of the tail at the time of injection.



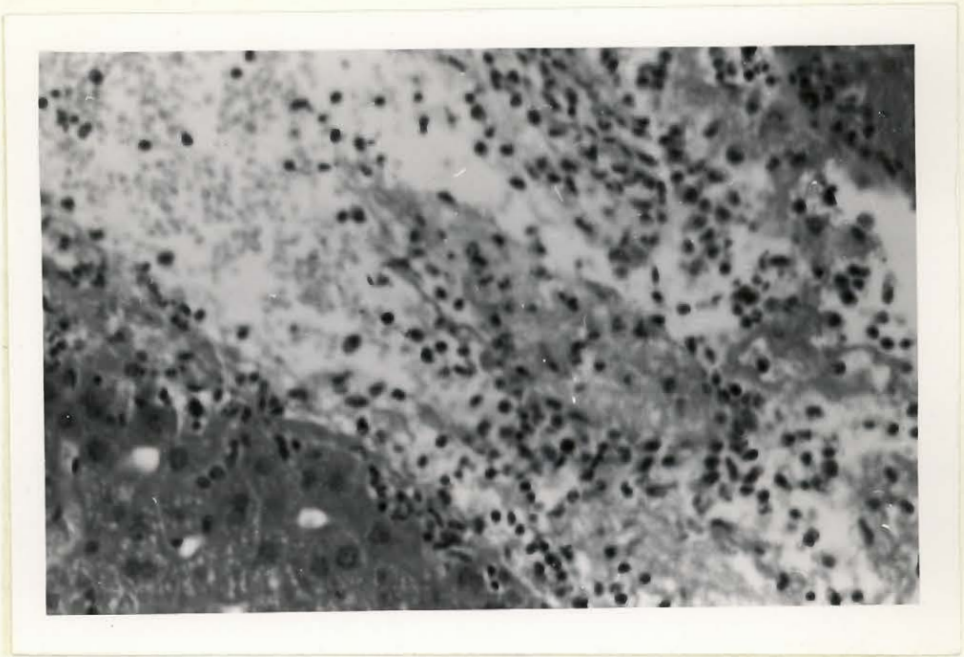


Fig. 4: DBA/1 mice injected with S/91 melanoma into the liver. Puncture wound - 1st day: scattered tumour cells and inflammatory cells in haemorrhagic area.
Haemalum, phloxine and saffron X 200

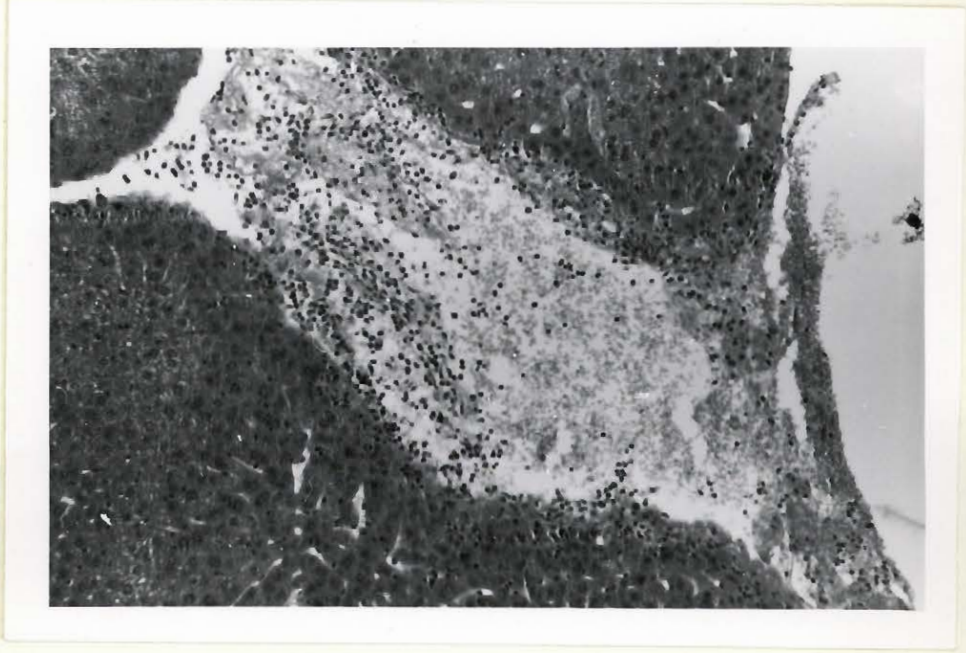


Fig. 5: DBA/1 mice injected with S/91 melanoma into the liver. Puncture wound - 1st day: scattered tumour cells with inflammatory cells and localization of cells at periphery.
Haemalum, phloxine and saffron X 80

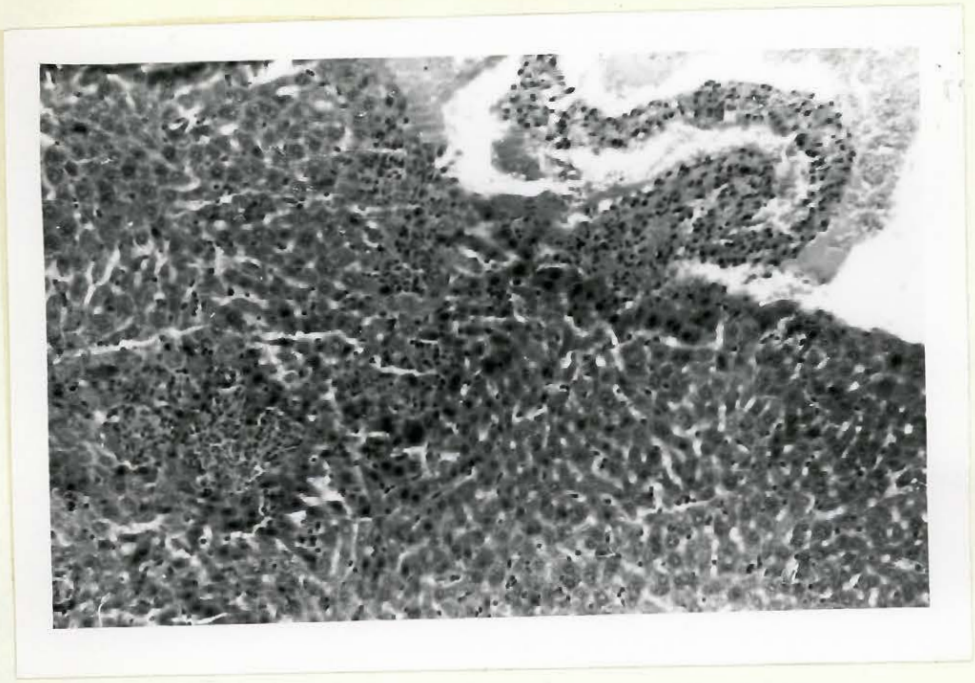


Fig. 6: DBA/1 mice injected with S/91 melanoma into the liver.
Puncture wound - 3rd day: signs of organization.
Haemalum, phloxine and saffron X 80

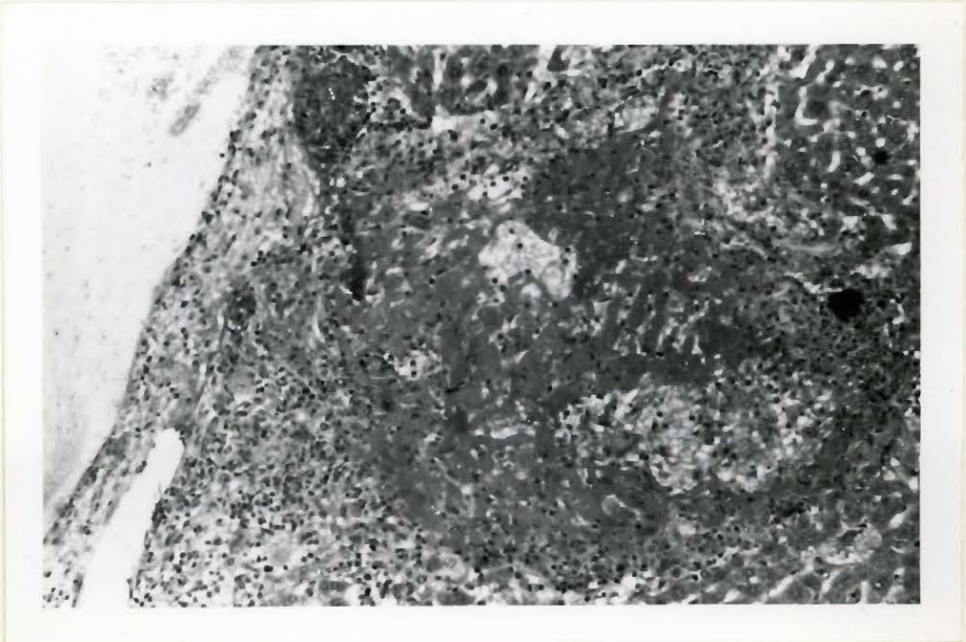


Fig. 7: DBA/1 mice injected with S/91 melanoma into the liver.
Puncture wound - 4th day: signs of organization.
Haemalum, phloxine and saffron X 80.

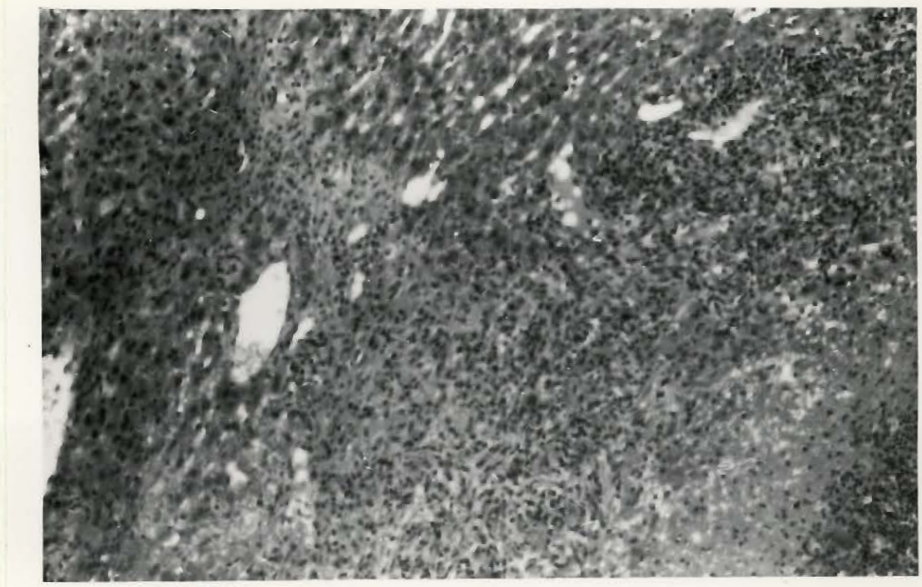


Fig. 8: DBA/1 mice injected with S/91 melanoma into the liver. Puncture wound: - 5th day; progressive healing with increasing number of tumour cells.
Haemalum, plexine and saffron X 80

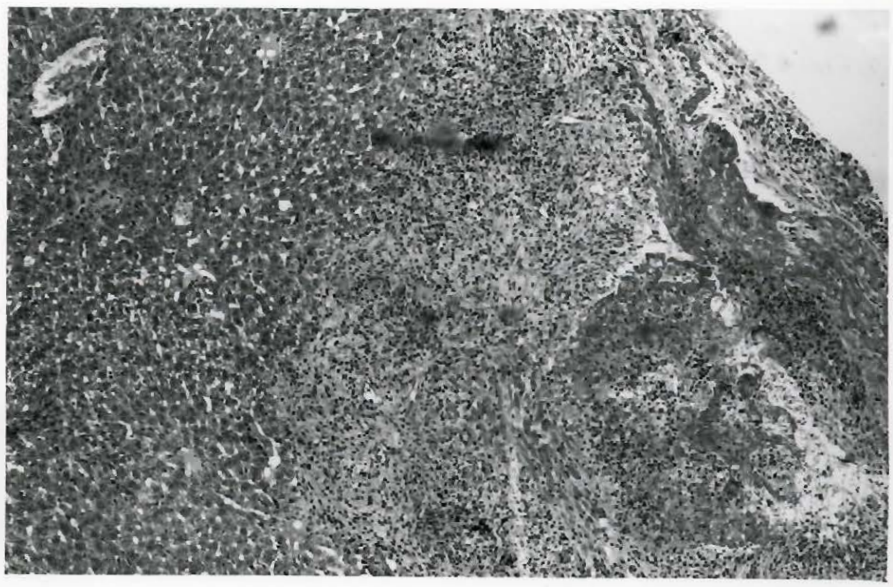


Fig. 9: DBA/1 mice injected with S/91 melanoma into the liver. Puncture wound - 7th day: tumour cells scattered in granulation tissue.
Haemalum, phloxine and saffron X 50

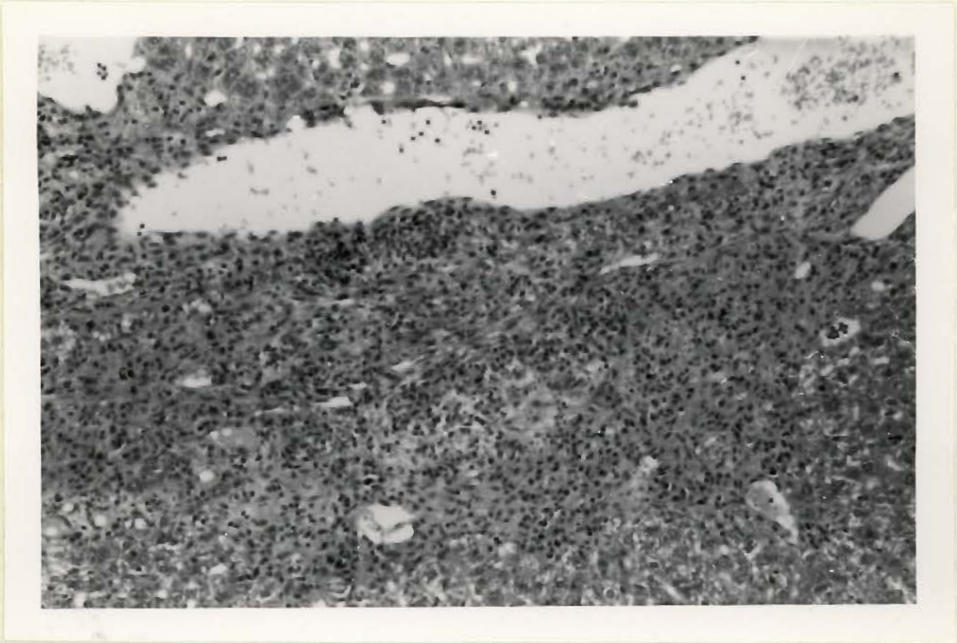


Fig. 10: DBA/1 mice injected with S/91 melanoma into the liver. Puncture wound - 9th day: increase in the number of tumour cells.
Haemalum, phloxine and saffron X 80

Fig. 11: DBA/1 mice injected with S/91 melanoma into the liver. Puncture wound - 14th day: formation of easily visible tumour.
Haemalum, phloxine and saffron X 50

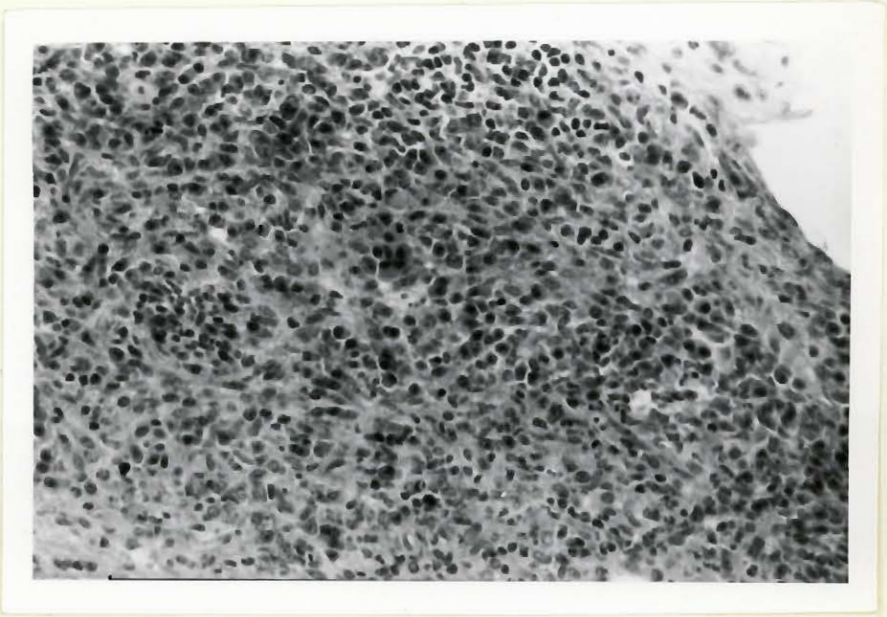


Fig. 12: DBA/1 mice injected with S/91 melanoma into the liver. Puncture wound - 21st day: Visible tumour with pigmented cells.
Haemalum, phloxine and saffron X 200

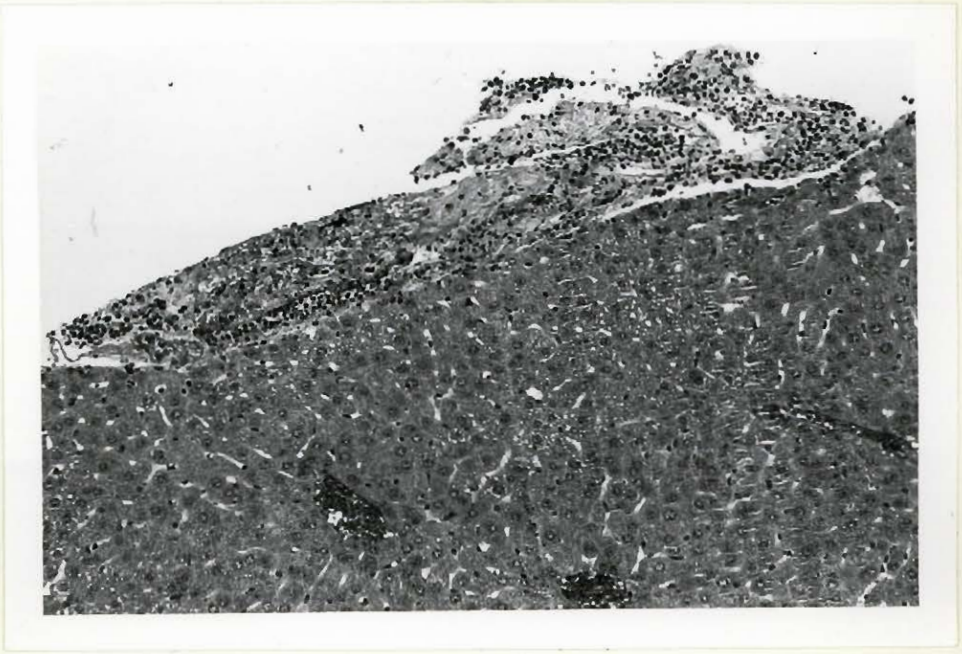


Fig. 13: DBA/1 mice injected with S/91 melanoma into the liver. 1st day: localization of tumour and inflammatory cells under the capsule.
Haemalum, phloxine and saffron X 80

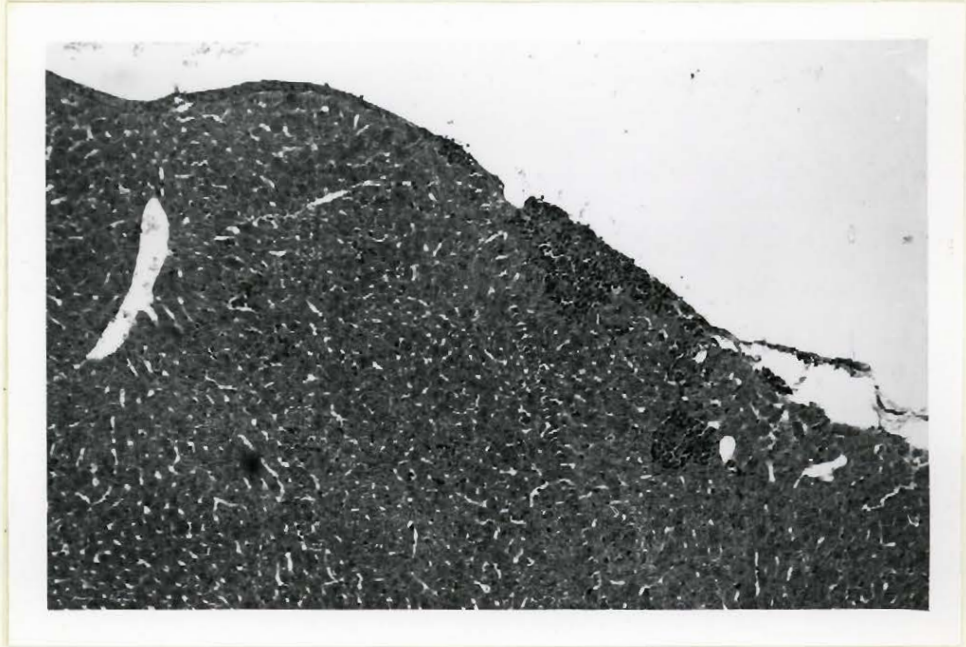


Fig. 14: DBA/1 mice injected with S/91 melanoma into the liver 7th Day: proliferation of tumour cells under the capsule.
Haemalum, phloxine and saffron X 50

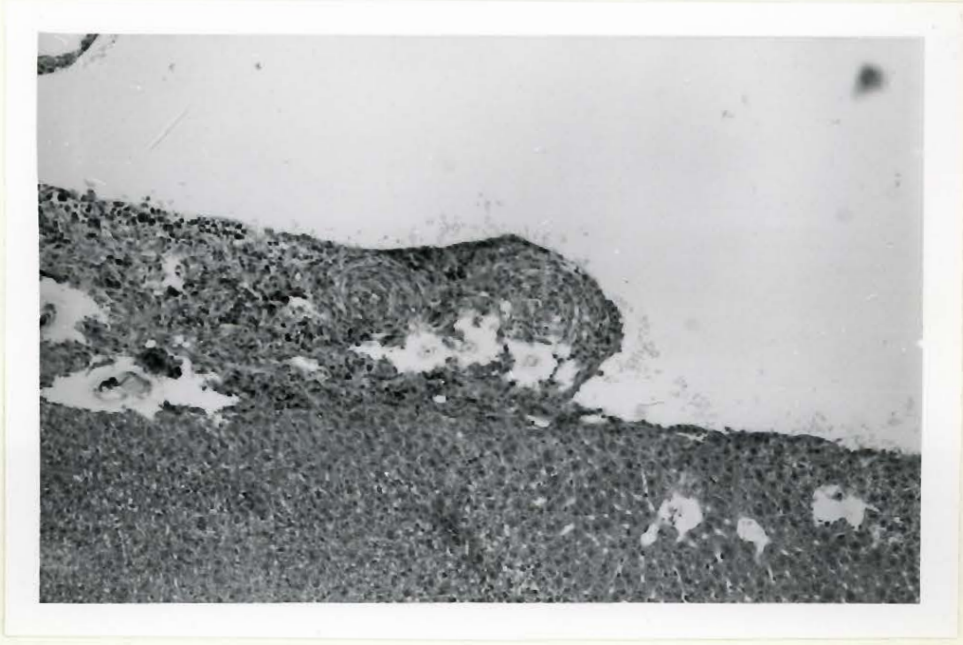


Fig. 15: DBA/1 mice injected with S/91 melanoma into the liver. 9th day: formation of tumour under the capsule.
Haemalum, phloxine and saffron X 50

Fig. 16: DBA/1 mice injected with S/91 melanoma into the liver.
12th day: visible tumour under the capsule.
Haemalum, phloxine and saffron X 50

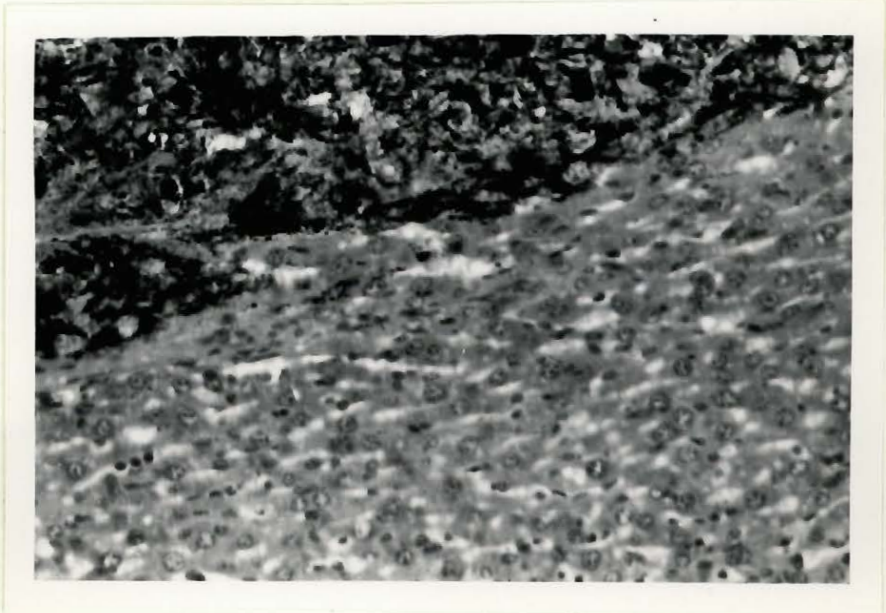


Fig. 17: DBA/1 mice injected with S/91 melanoma into the liver.
21st day: tumour with well developed pigment under the capsule and pushing into the liver substance.
Haemalum, phloxine and saffron X 200

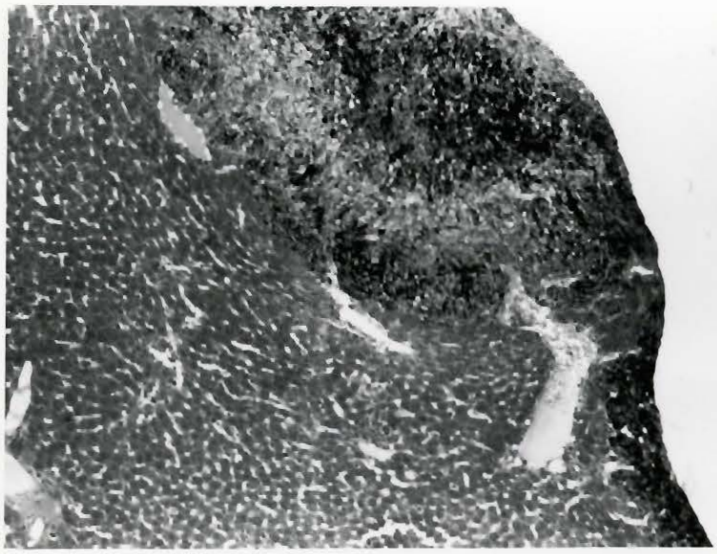


Fig. 18: DBA/1 mice injected with S/91 melanoma into the liver.
1 month: tumour under capsule with compression of liver tissue.
Haemalum, phloxine and saffron X 50

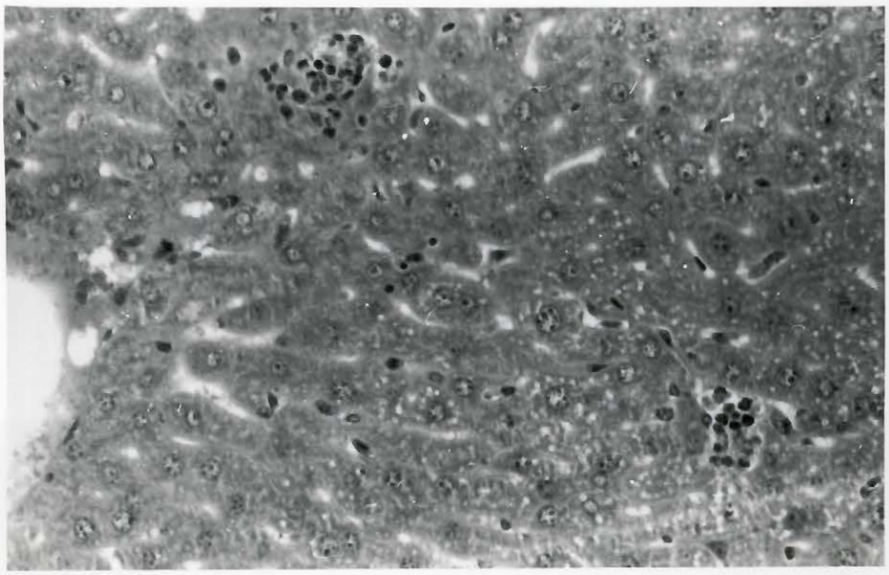


Fig. 19: DBA/1 mice injected with S/91 melanoma into the liver.
1st day: tumour cells scattered in the sinusoids.
Haemalum, phloxine and saffron X 200

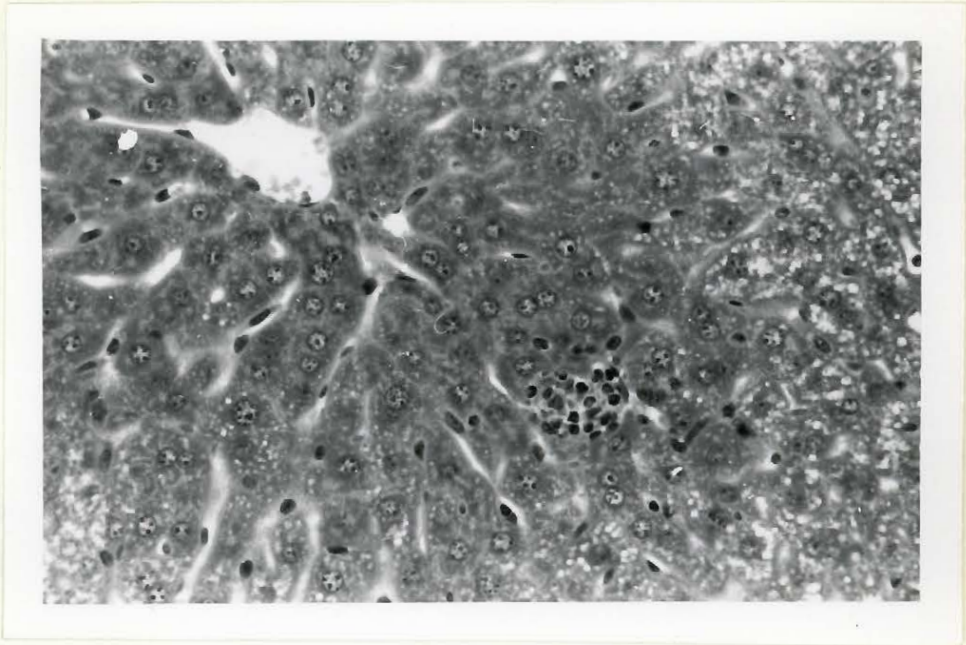


Fig. 20: DBA/1 mice injected with S/91 melanoma into the liver. 1st day; tumour cells in sinusoid in relation to central vein
Haemalum, phloxine and saffron X 200

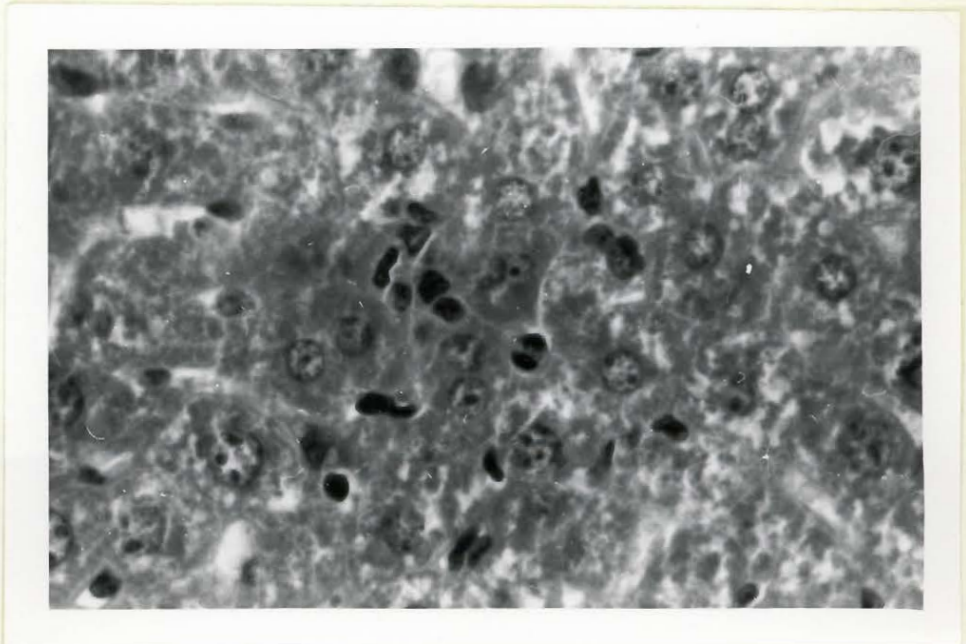


Fig. 21: DBA/1 mice injected with S/91 melanoma into the liver. 2nd day: tumour cell in the sinusoid showing mitosis
Haemalum, phloxine and saffron X 500

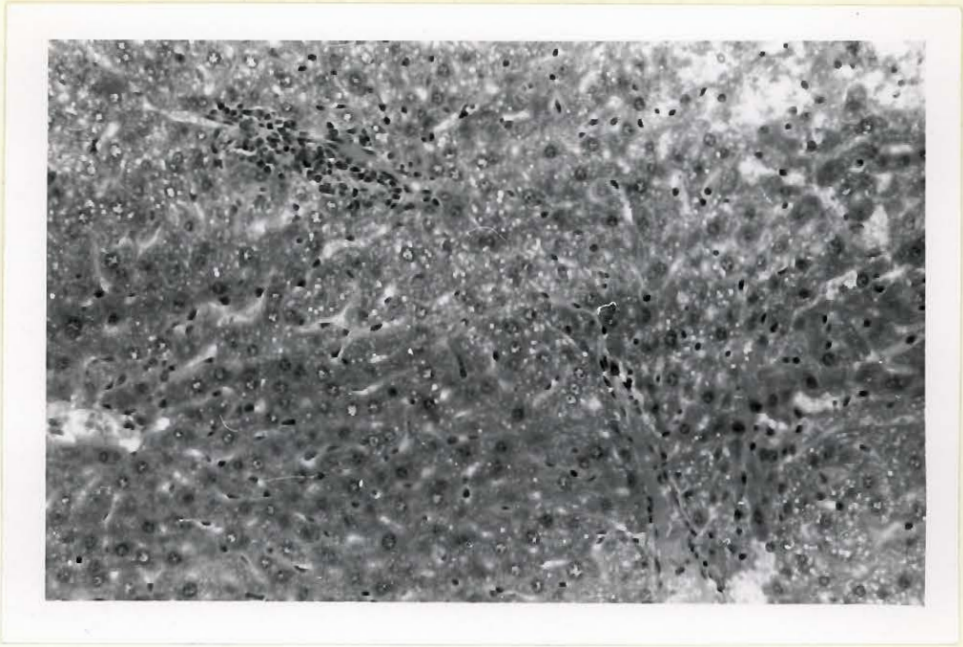


Fig. 22: DBA/1 mice injected with S/91 melanoma into the liver. 1st day: tumour cells underneath endothelium of portal vein.
Haemalum, phloxine and saffron X 80

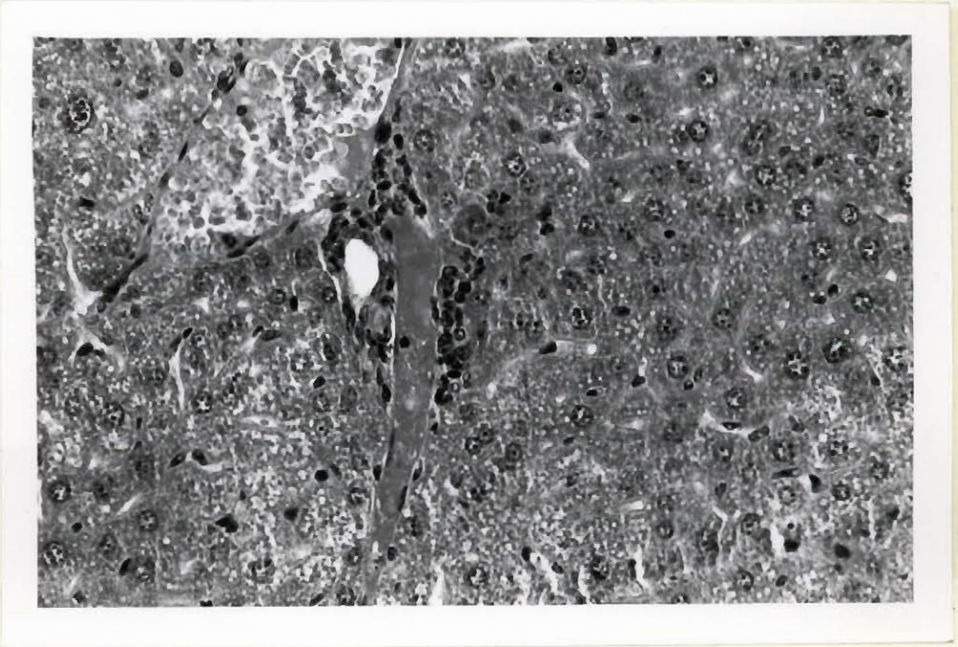


Fig. 23: DBA/1 mice injected with S/91 melanoma into the liver 1st day: tumour cells underneath the endothelium of the portal vein.
Haemalum, phloxine and saffron X 200

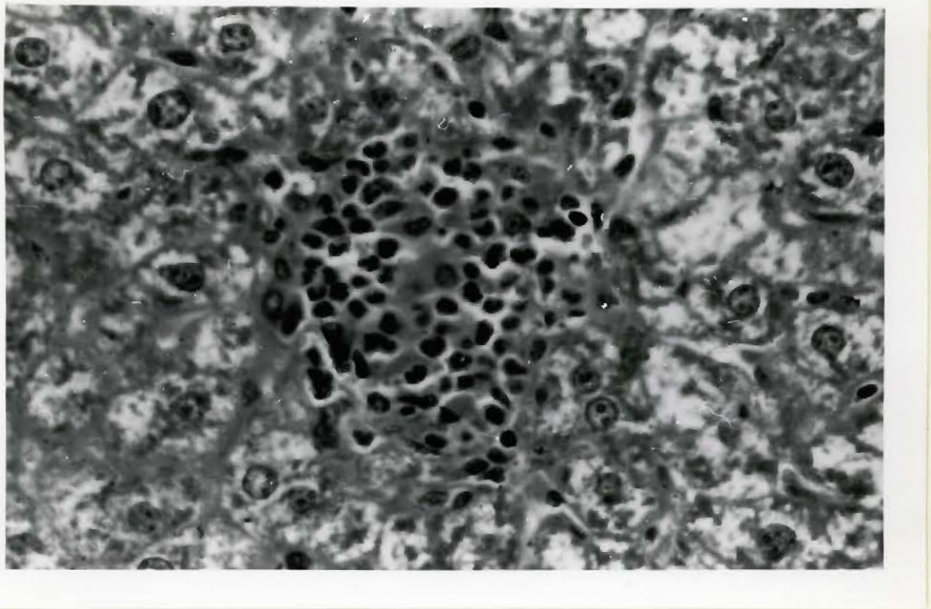


Fig. 24: DBA/1 mice injected with S/91 melanoma into the liver. 2nd day: tumour cells in sinusoid; note fair number of polymorphs.
Haemalum, phloxine and saffron X 320

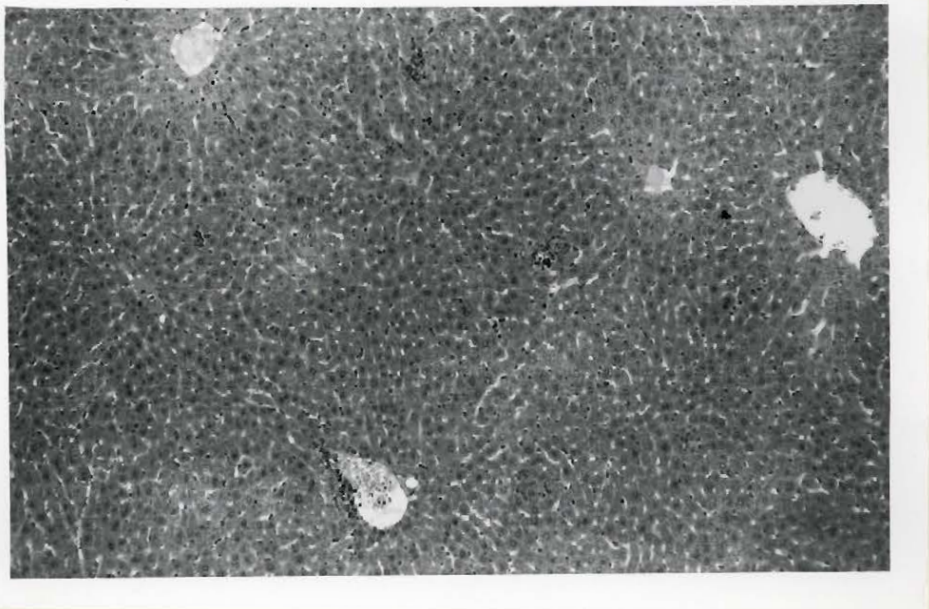


Fig. 25: DBA/1 mice injected with S/91 melanoma into the liver. 5th day: general view of liver showing tumour cell collections in sinusoids and underneath the endothelium of portal vein.
Haemalum, phloxine and saffron X 50

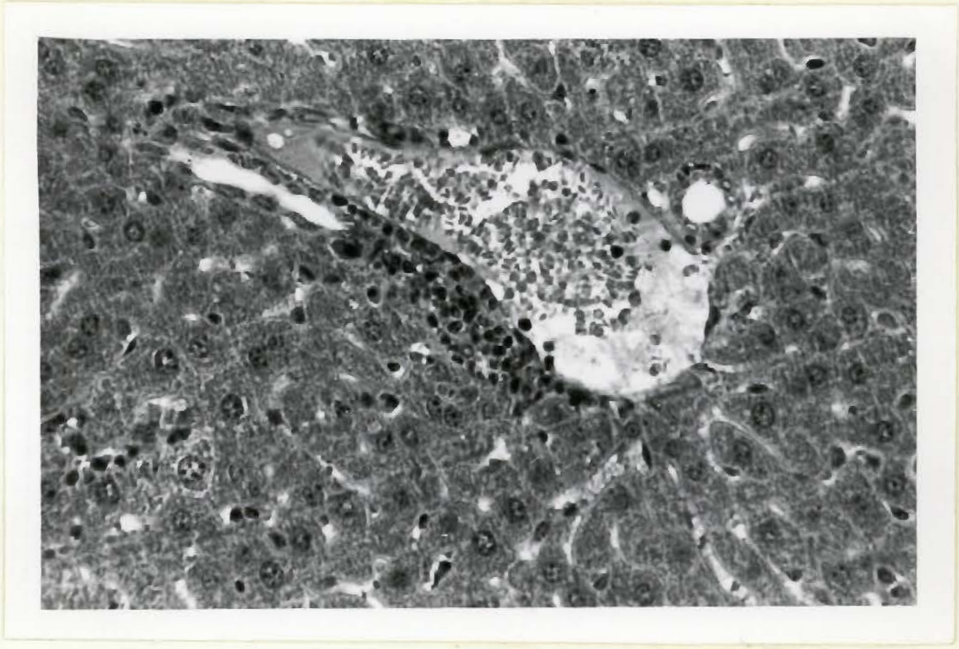


Fig. 26: DBA/1 mice injected with S/91 melanoma into the liver. 3rd day: tumour cells localized under endothelium of portal vein.
Haemalum, phloxine and saffron X 200

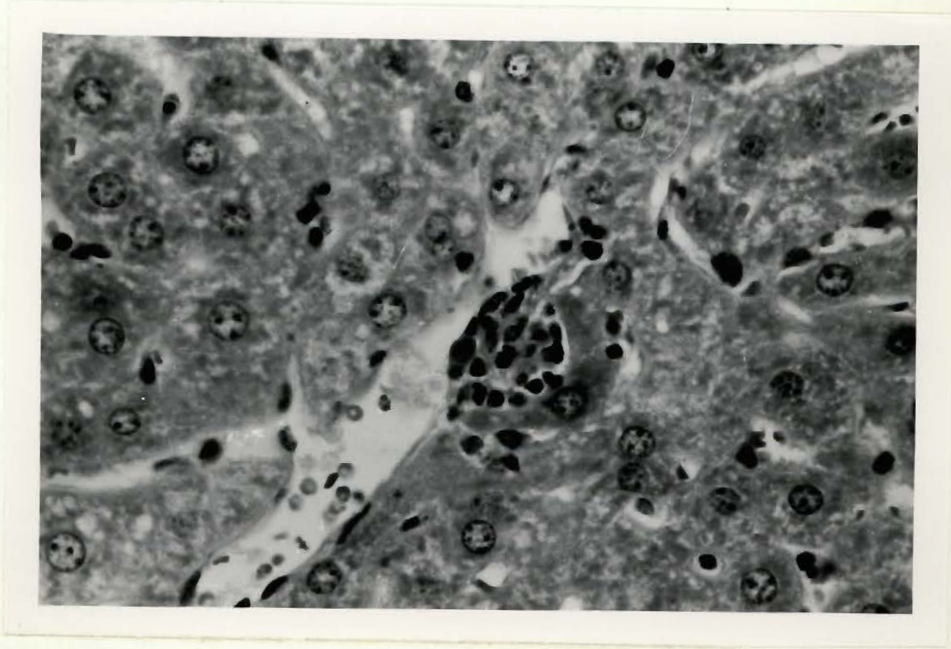


Fig. 27: DBA/1 mice injected with S/91 melanoma into the liver. 4th day: tumour cells under endothelium bulging into vessels.
Haemalum, phloxine and saffron X 320

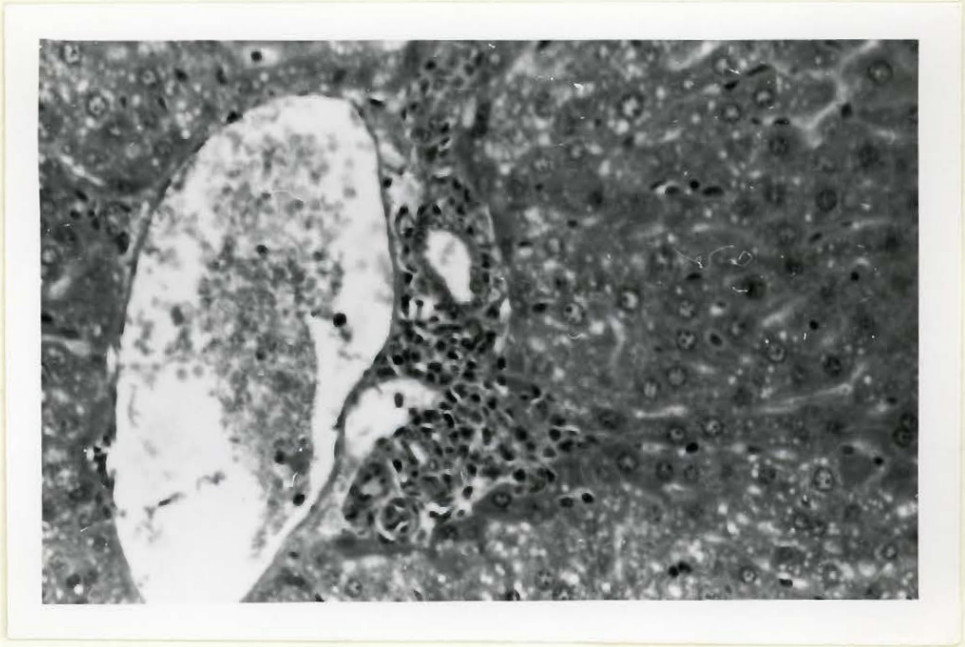


Fig. 28: DBA/1 mice injected with S/91 melanoma into the liver. 4th day: tumour cells under the endothelium of portal vein.
Haemalum, phloxine and saffron X 200

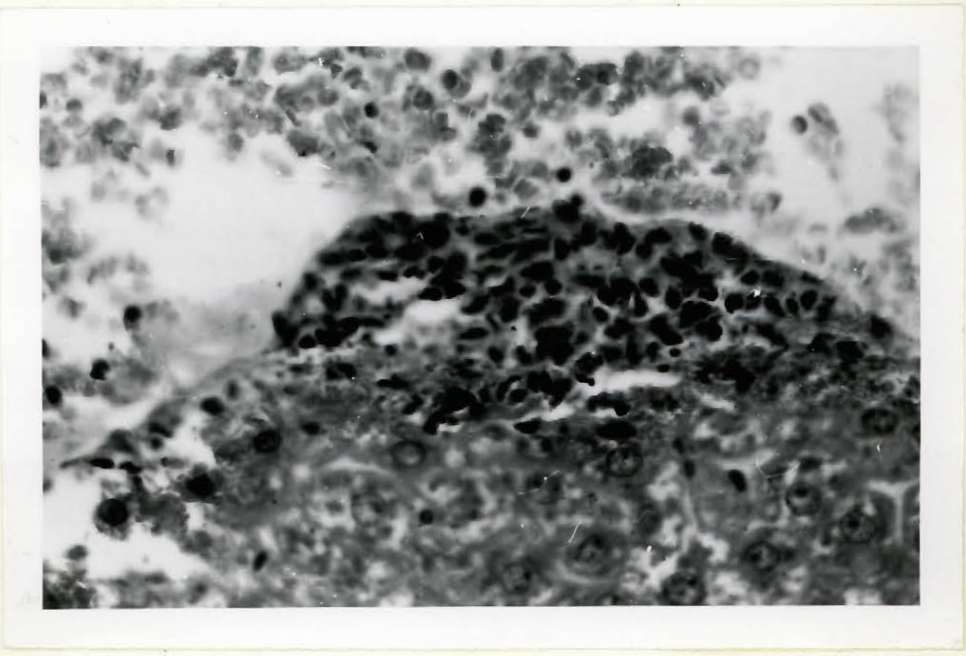


Fig. 29: DBA/1 mice injected with S/91 melanoma into the liver. 4th day: tumour cells underneath the endothelium pushing into the portal vein.
Haemalum, phloxine and saffron X 320

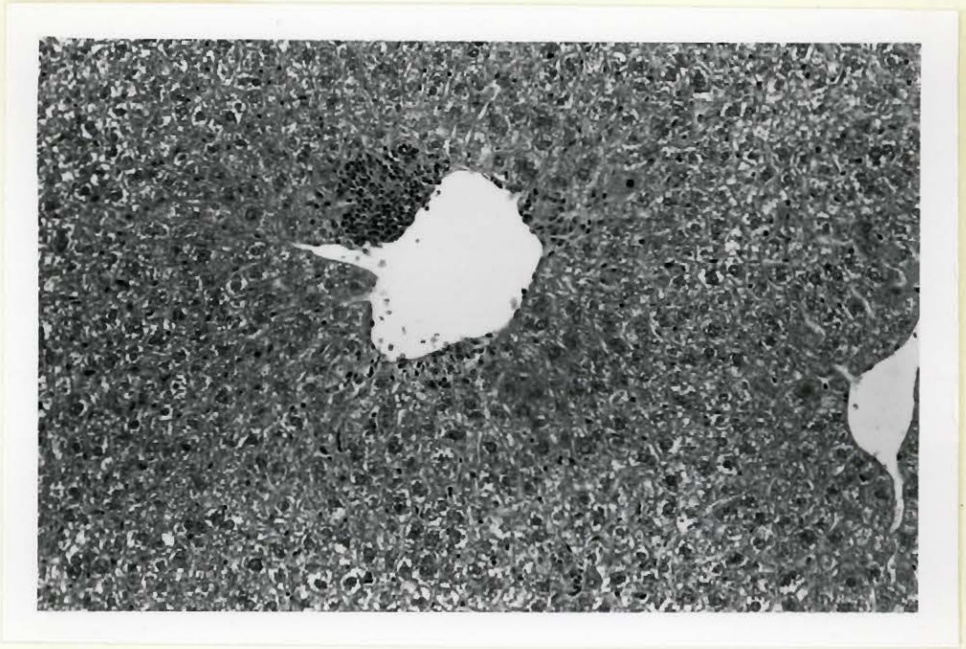


Fig. 30: DBA/1 mice injected with S/91 melanoma into the liver. 5th day: tumour cells localized mostly in relation to portal vein.
Haemalum, phloxine and saffron X 80

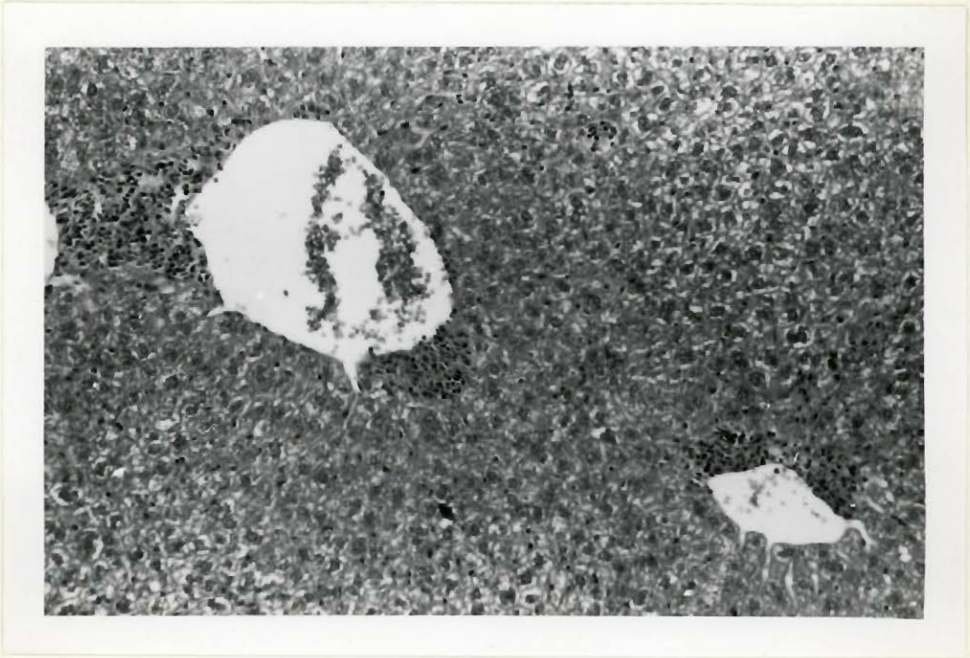


Fig. 31: DBA/1 mice injected with S/91 melanoma into the liver. 5th day: tumour cells in relation to portal vessels.
Haemalum, phloxine and saffron X 80

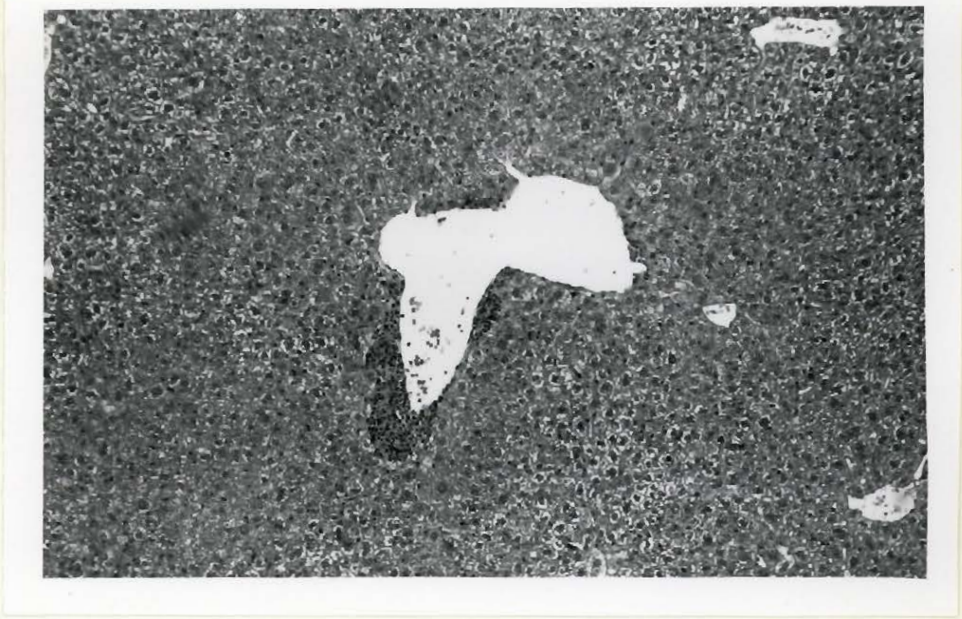


Fig. 32: DBA/1 mice injected with S/91 melanoma into the liver. 5th day: tumour cells proliferation round portal vessel.
Haemalum, phloxine and saffron X 50

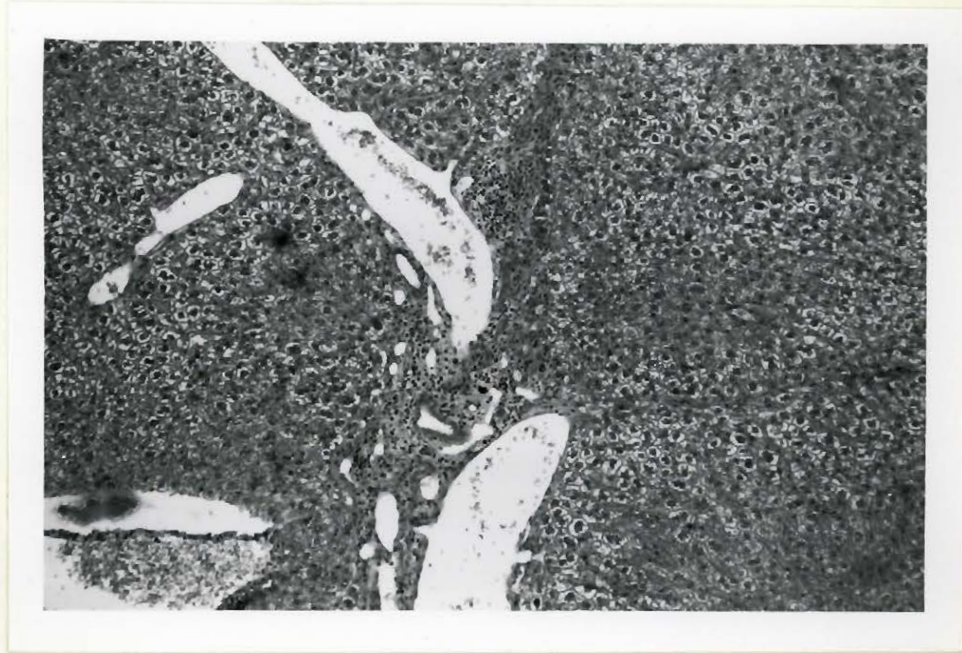


Fig. 33: DBA/1 mice injected with S/91 melanoma into the liver. 5th day: tumour cells proliferating in portal tract.
Haemalum, phloxine and saffron X 50

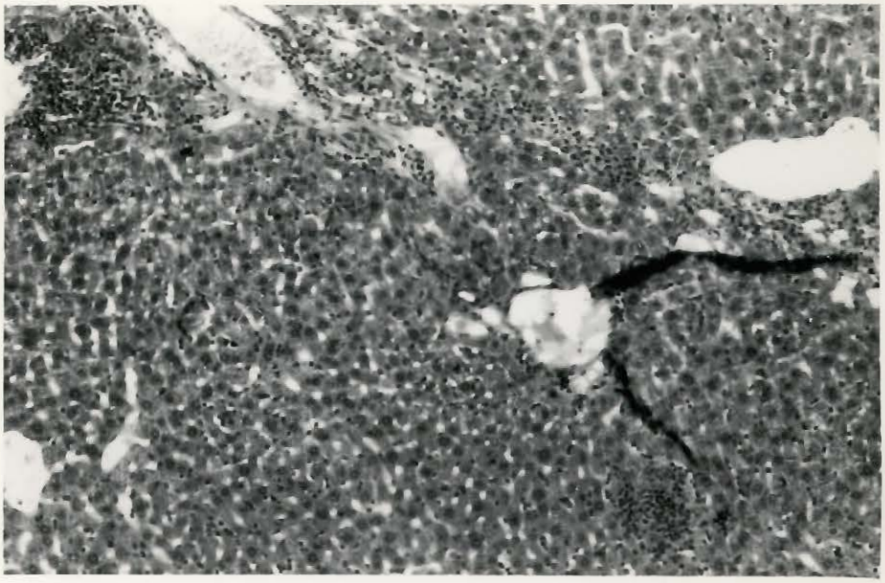


Fig. 34: DBA/1 mice injected with S/91 melanoma into the liver. 7th day: general view of liver showing proliferation of tumour cells in relation to portal tract.
Haemalum, phloxine and saffron X 80

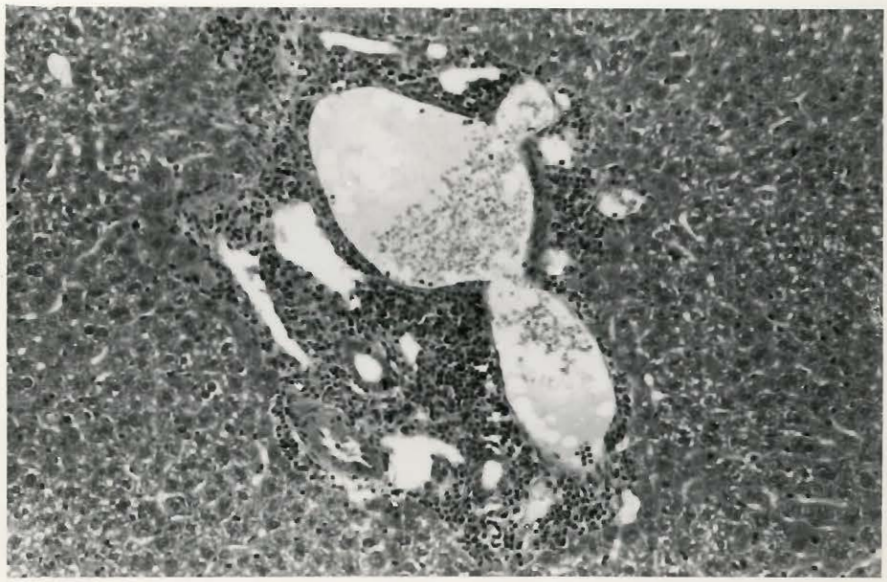


Fig. 35: DBA/1 mice injected with S/91 melanoma into the liver 7th day: tumour cell proliferation in portal tract.
Haemalum, phloxine and saffron X 80

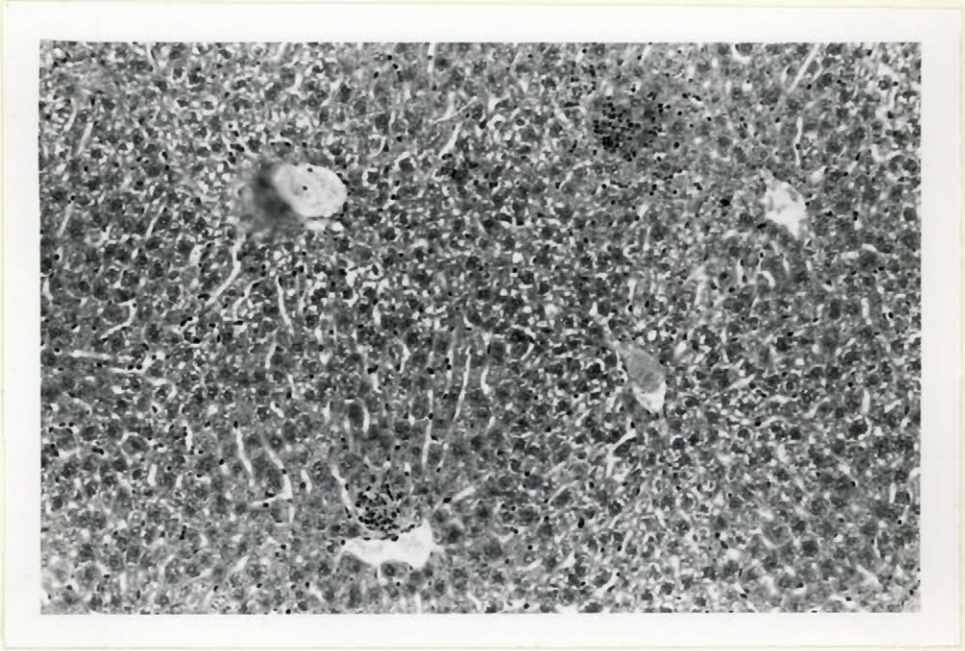


Fig. 36: DBA/1 mice injected with S/91 melanoma into the liver. 7th day: tumour cells remaining stationary in sinusoid and in relation to portal veins.
Haemalum, phloxine and saffron X 80

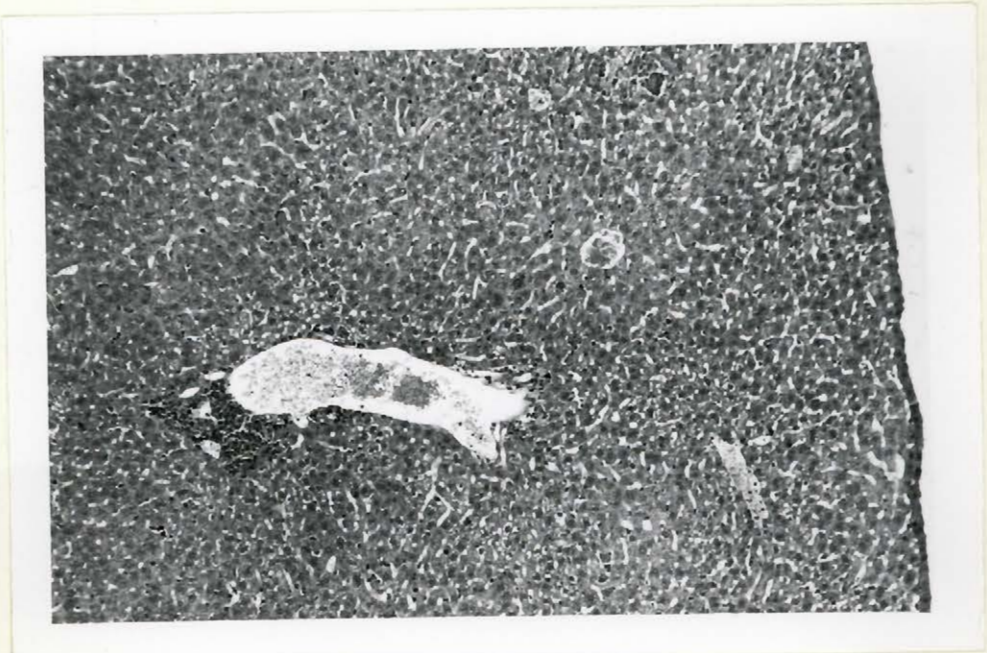


Fig. 37: DBA/1 mice injected withS/91 melanoma into the liver. 7th day: tumour cells multiplying around portal vein.
Haemalum, phloxine and saffron X 50

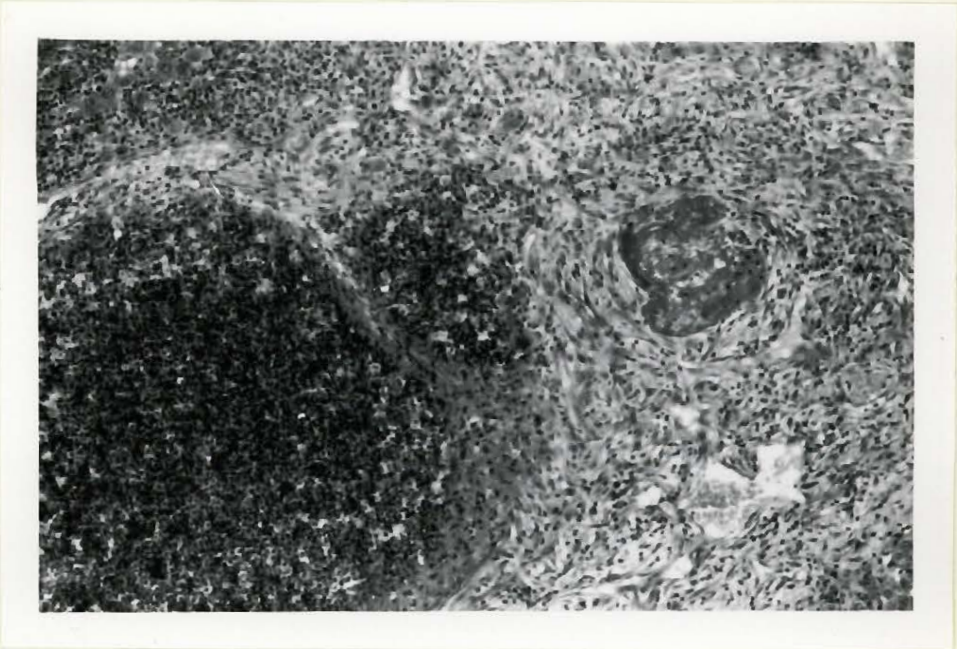


Fig. 38: DBA/1 mice injected with S/91 melanoma into the liver. 7th day: from portal tract - formation of microscopic tumour.
Haemalum, phloxine and saffron X 80

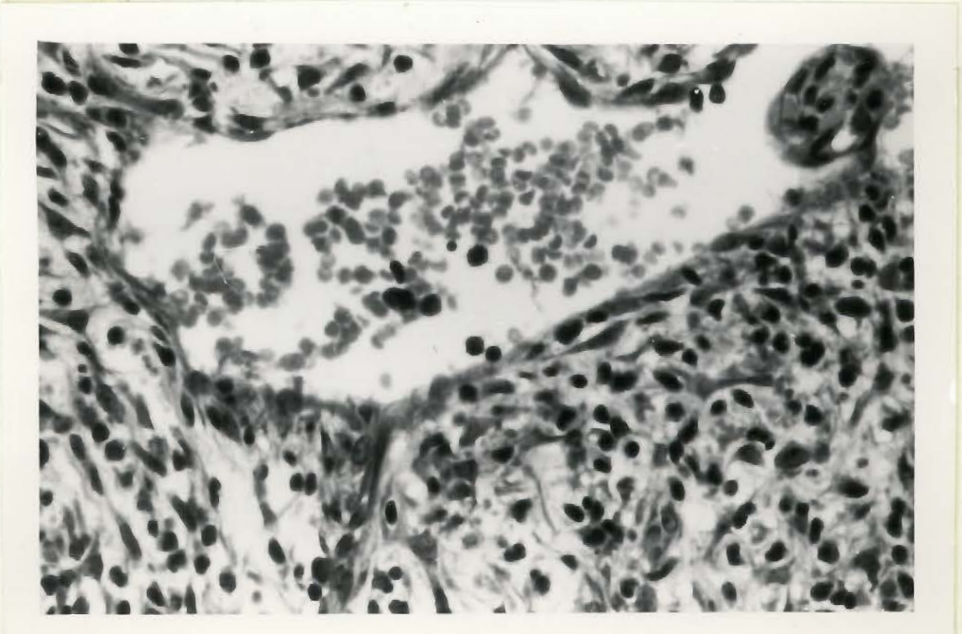


Fig. 39: DBA/1 mice injected with S/91 melanoma intothe liver. 7th day: tumour cells from a tumour under the endothelium of portal vein.
Haemalum, phloxine and saffron X 320

Fig. 40: DBA/1 mice injected with S/91 melanoma into the liver. 9th day: tumour cells - proliferation and extension in portal tract.
Haemalum, phloxine and saffron X 50

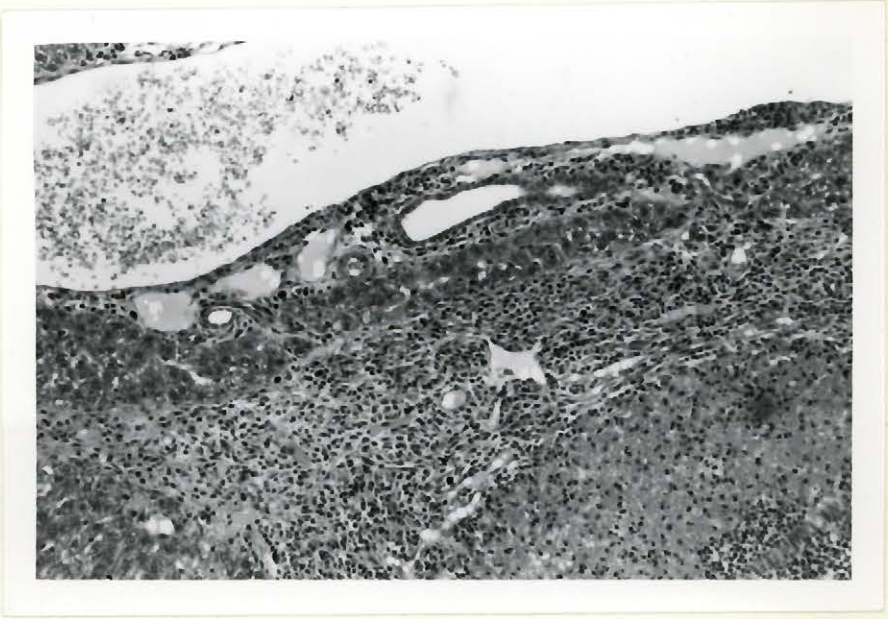


Fig. 41: DBA/1 mice injected with S/91 melanoma into the liver. 9th day: tumour cell proliferation in portal tract.
Haemalum, phloxine and saffron X 80

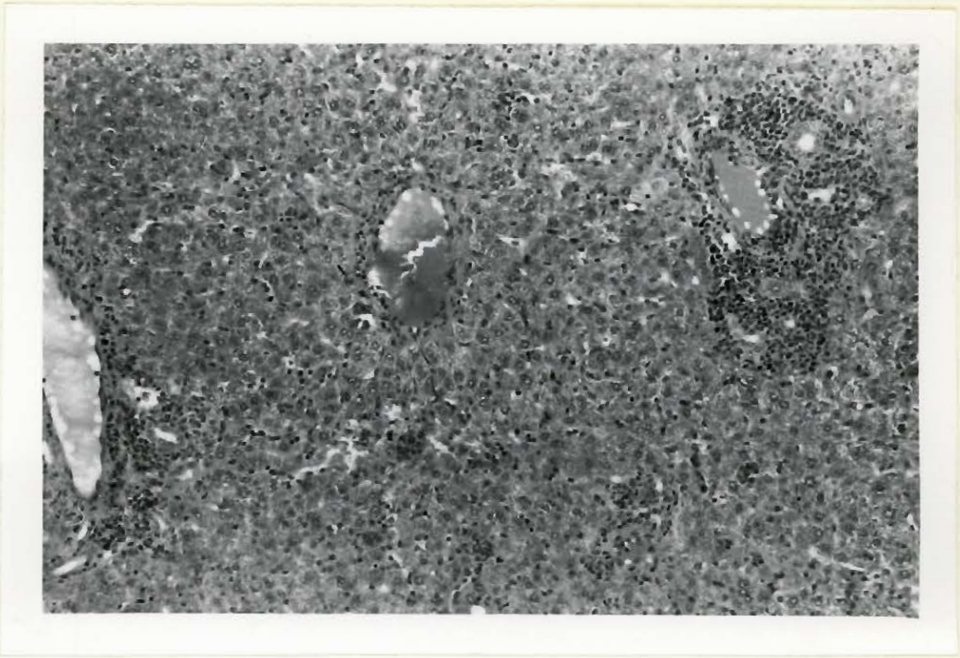


Fig. 42: DBA/1 mice injected with S/91 melanoma into the liver.
10th day: developing tumour in portal tract.
Haemalum, phloxine and saffron X 80

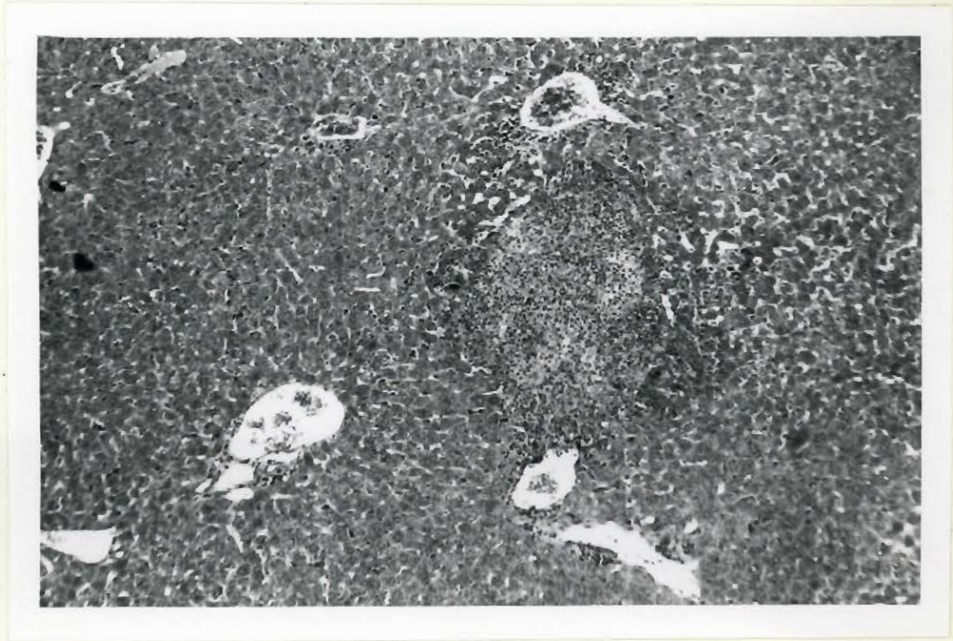


Fig. 43: DBA/1 mice injected with S/91 melanoma into the liver.
12th day: tumour formation.
Haemalum, phloxine and saffron X 50

Fig. 44: DBA/1 mice injected with S/91 melanoma into the liver.
21st day: tumour formation with central degeneration.
Haemalum, phloxine and saffron X 50

Fig. 45.

A hypodermic needle modified to introduce the carbon tetrachloride directly into the stomach of the mouse is shown just about to be introduced.

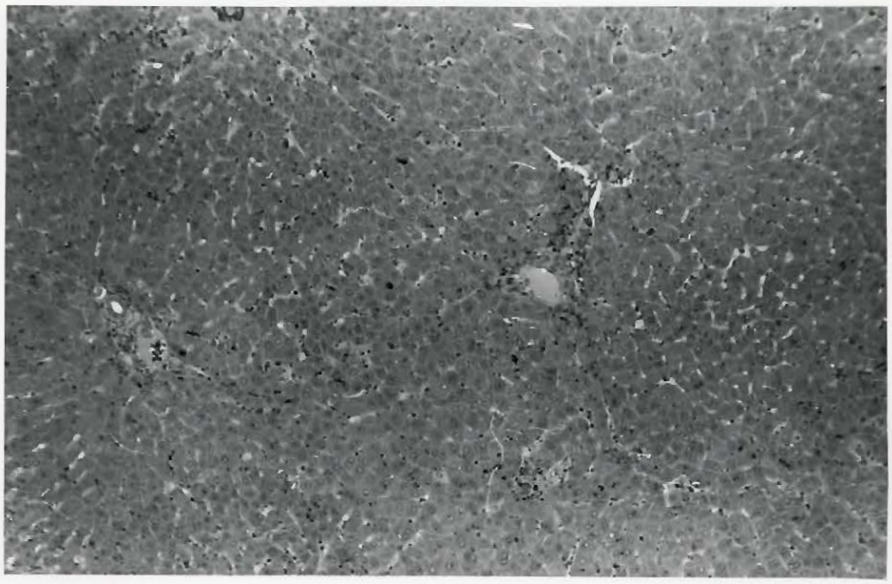


Fig. 46: V_2 carcinoma intraportally into rabbits. Scattered tumour cells collected mostly around portal vein.
Haemalum, phloxine and saffron X 50

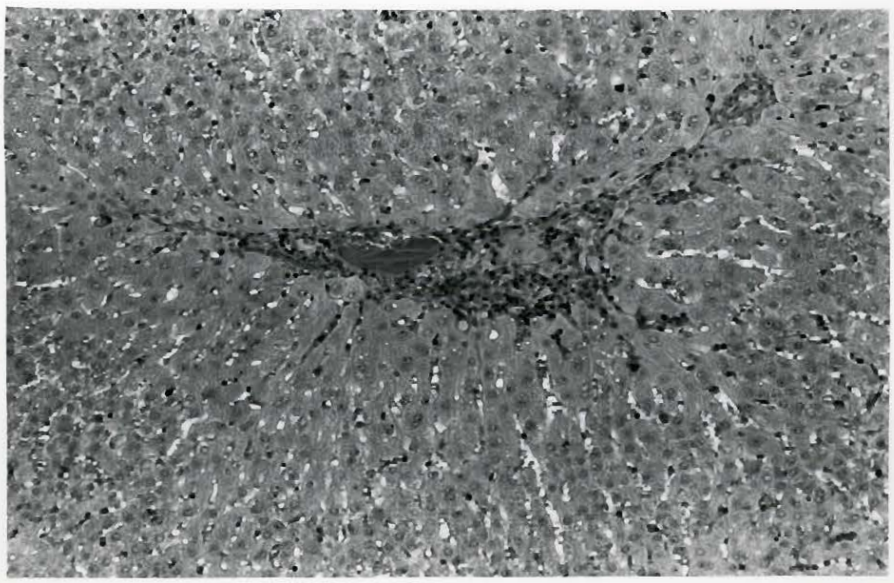


Fig. 47: V_2 carcinoma intraportally into rabbits. Localization and proliferation of tumour cells in portal tract.
Haemalum, phloxine and saffron X 80

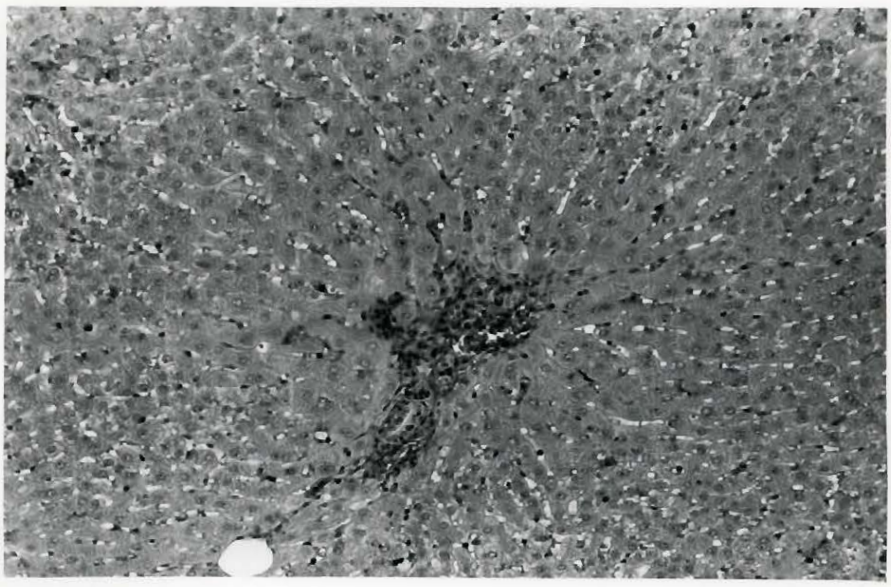


Fig. 48: V_2 carcinoma intraportally into rabbits.
Collection and proliferation of tumour cells around portal vein.
Haemalum, phloxine and saffron X 80

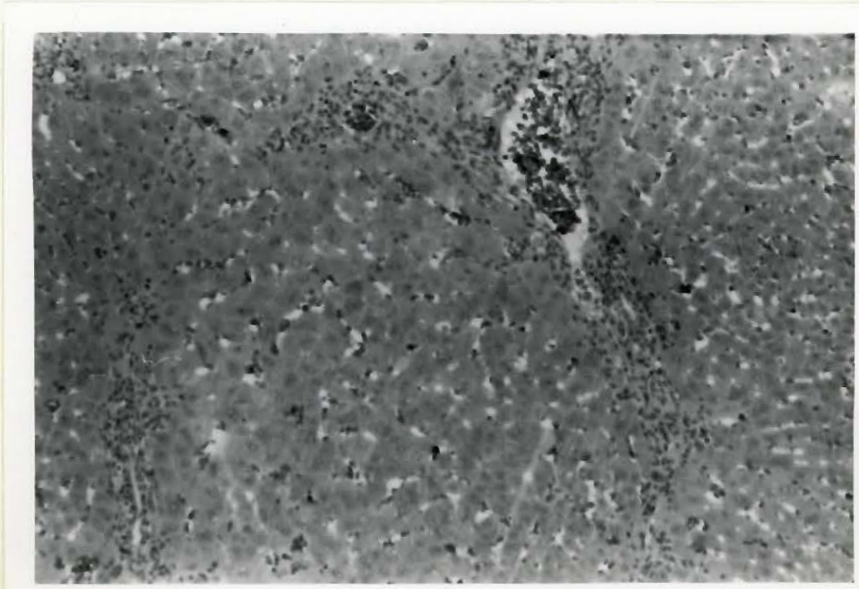


Fig. 49: V_2 carcinoma intraportally into rabbits.
Extension of tumour cells along portal tracts.
Haemalum, phloxine and saffron X 80

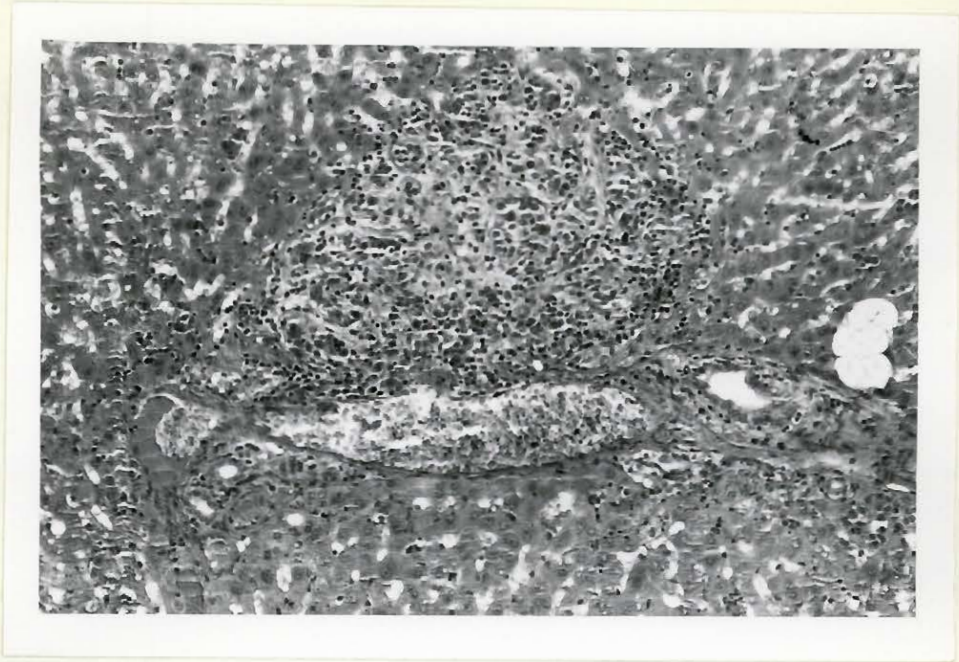


Fig. 50: V_2 carcinoma intraportally into rabbits.
Tumour formation from cells underneath portal vein.
Haemalum, phloxine and saffron X 80

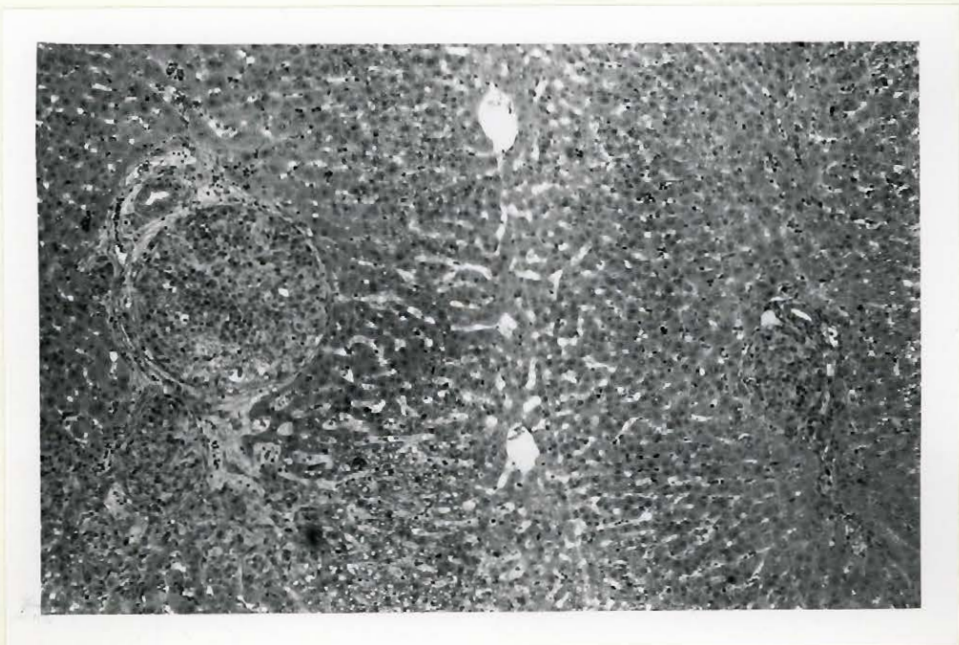


Fig. 51: V_2 carcinoma intraportally into rabbits.
Tumour formation in portal tract.
Haemalum, phloxine and saffron X 50

Fig. 52: V_2 carcinoma intraportally into rabbits.
Multiple tumour formation in liver parenchyma.
Haemalum, phloxine and saffron X 20

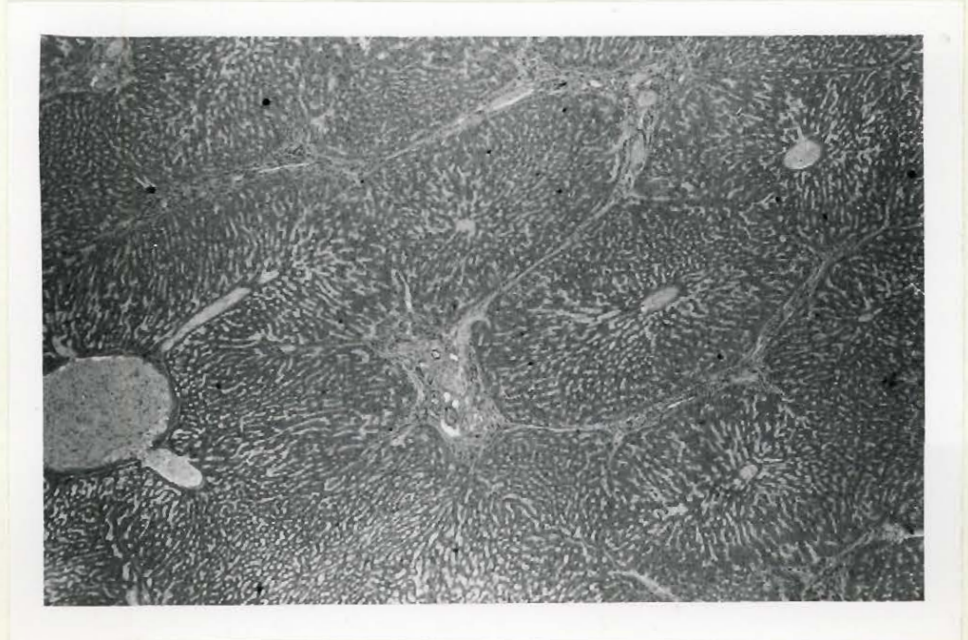


Fig. 53: V_2 carcinoma intraportally into rabbits.
Extension with cirrhosis along portal tract.
Haemalum, phloxine and saffron X 20

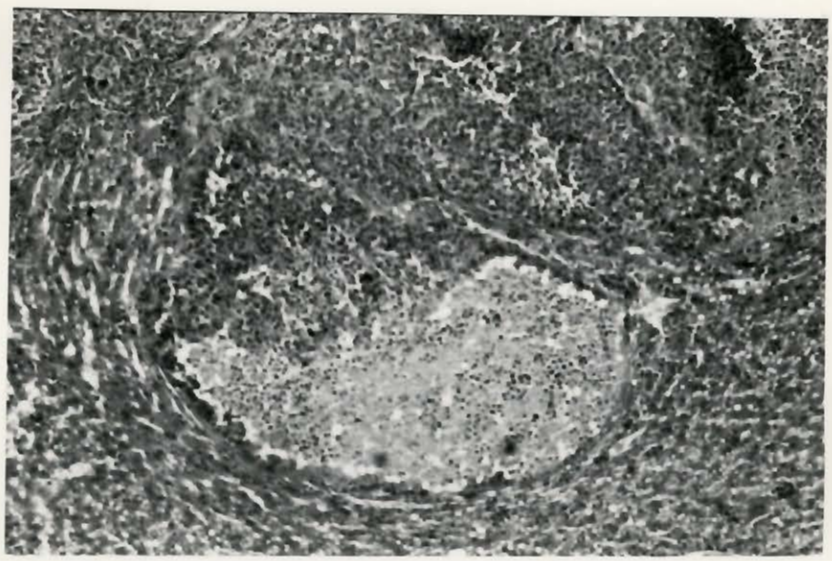


Fig. 54: V_2 carcinoma intraportally into rabbits.
Extension of tumour with compression of liver parenchyma.
Haemalum, phloxine and saffron X 50

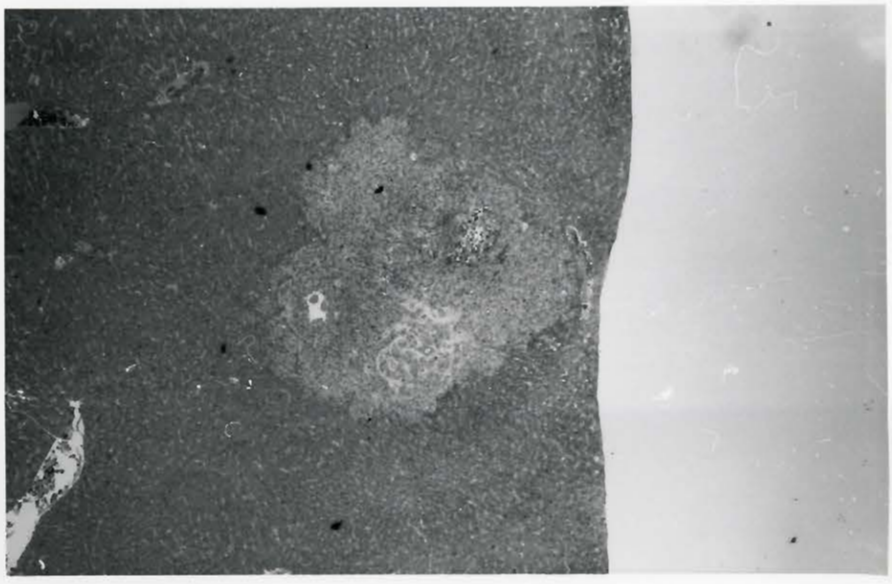


Fig. 55: V_2 carcinoma intraportally into rabbits.
A fairly big tumour.
Haemalum, phloxine and saffron X 20

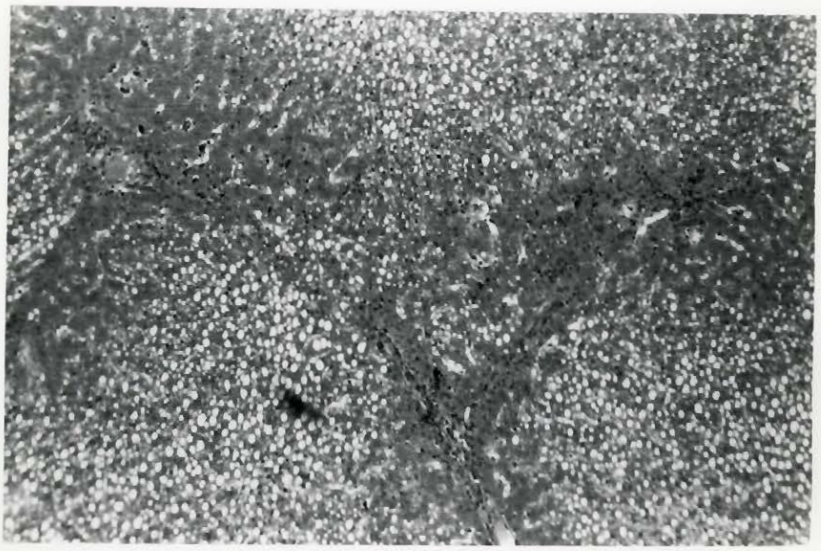


Fig. 56: V_2 carcinoma intraportally into rabbits.
Cirrhotic pattern with marked fatty degeneration of adjoining liver cells.
Haemalum, phloxine and saffron X 50

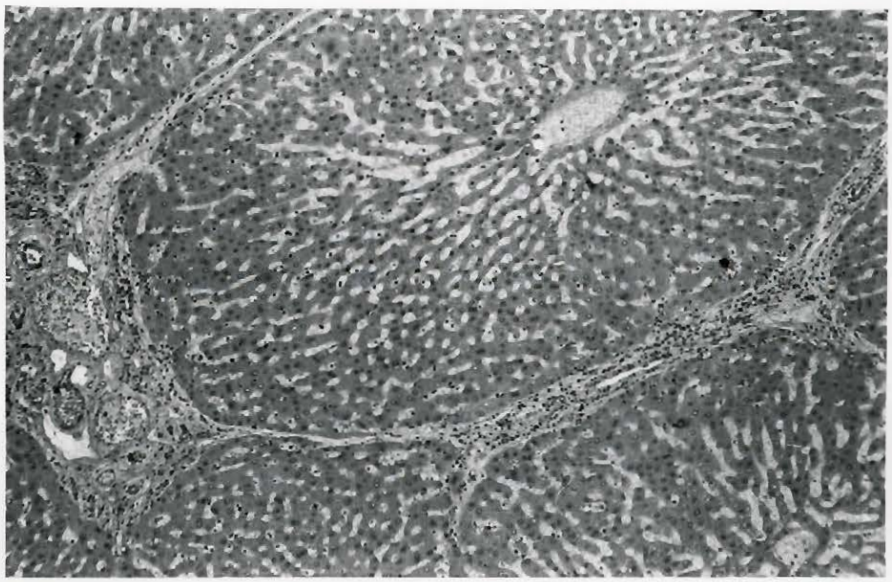


Fig. 57: V_2 carcinoma intraportally into rabbits.
Marked cirrhosis, periportal in distribution.
Central vein is seen on the right upper part.
Haemalum, phloxine and saffron X 50.

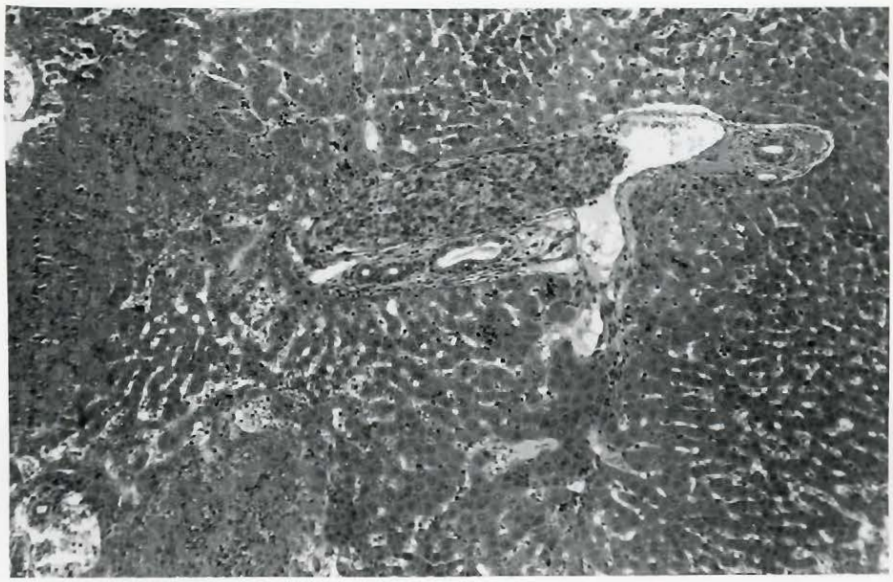


Fig. 58: V_2 carcinoma intraportally into rabbits.
Invasion of portal vein by tumour cells.
Haemalum, phloxine and saffron X 50

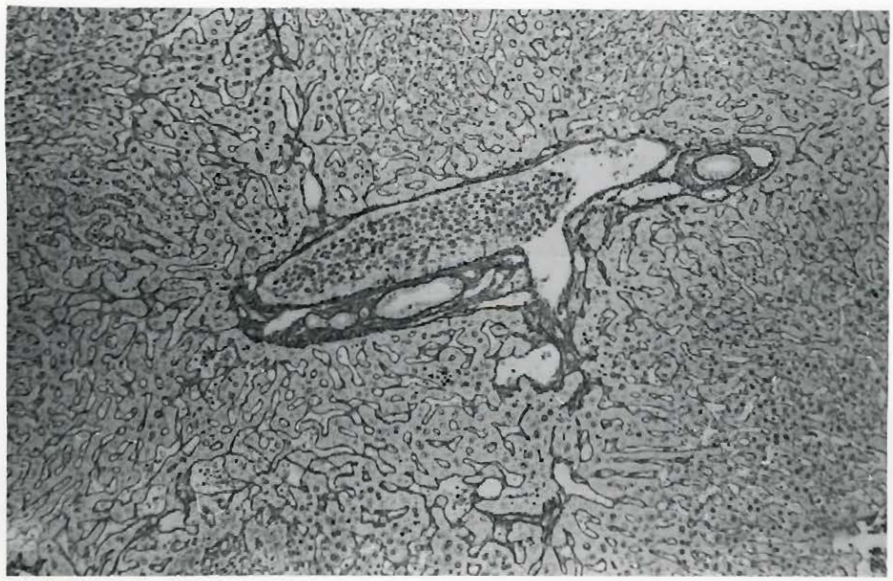


Fig. 59: V_2 carcinoma intraportally into rabbits.
Invasion of portal vein by tumour cells.
Reticulin stain X 50

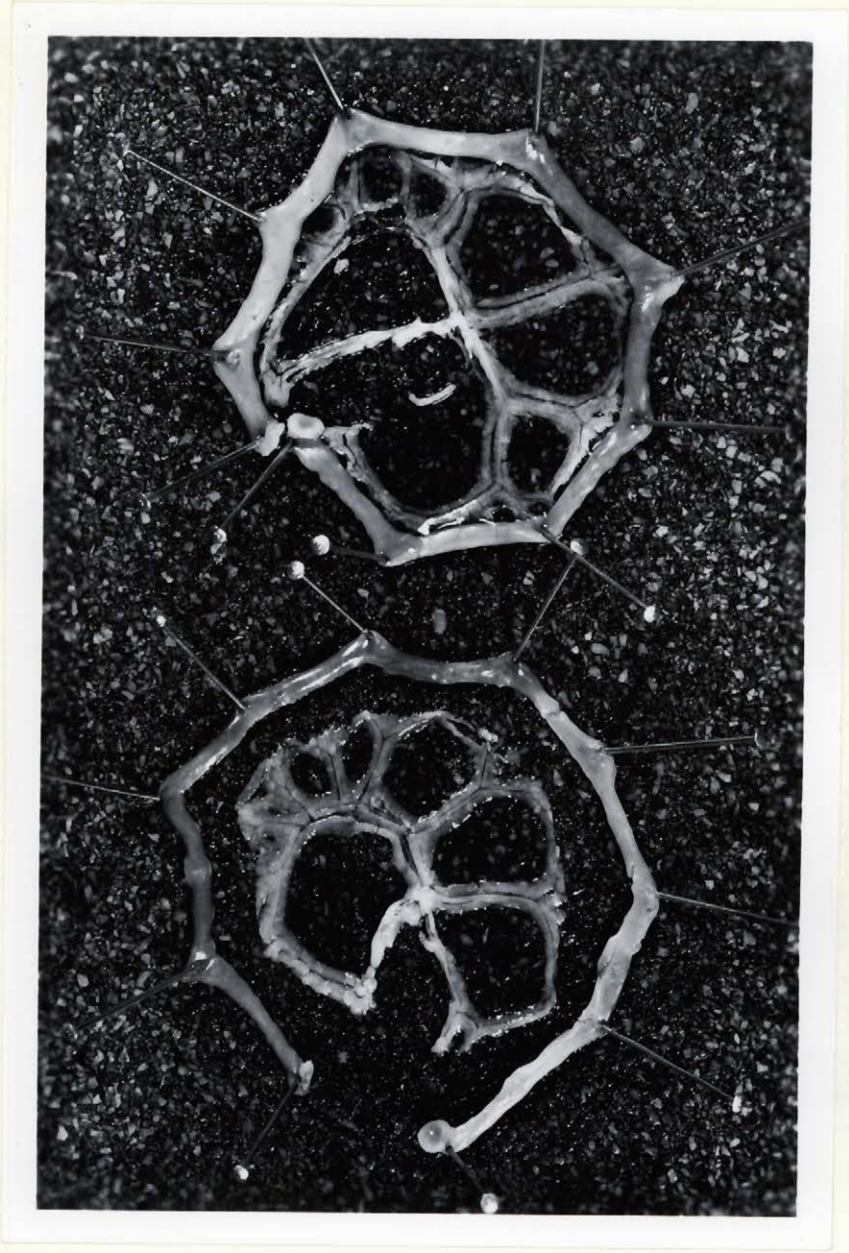


Fig. 60.

A - A mesentery being prepared for the surface staining method.

Shown fixed on a cork sheet.

B - The same being separated from the gut wall.

Fig. 61: Normal DBA/1 mouse
Mesentery - mesothelial lining.
Silver nitrate (cobalt and ammonium bromide),
haematoxylin and eosin X 80

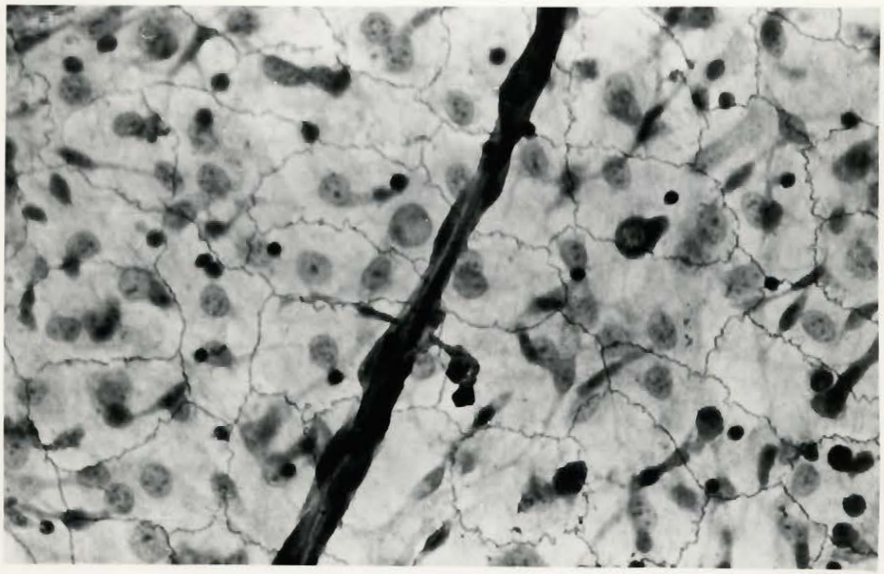


Fig. 62: Normal DBA/1 mouse
Mesentery - mesothelial lining.
Silver nitrate (cobalt and ammonium bromide),
haematoxylin and eosin X 200

Fig. 63: Normal DBA/1 mouse
Mesentery - mesothelial lining over zones III & IV.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 200

Fig. 64: DBA/1 mice injected with S/91 melanoma intraperitoneally. 5th day; mesentery - cell collection underneath the mesothelial lining.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 200.

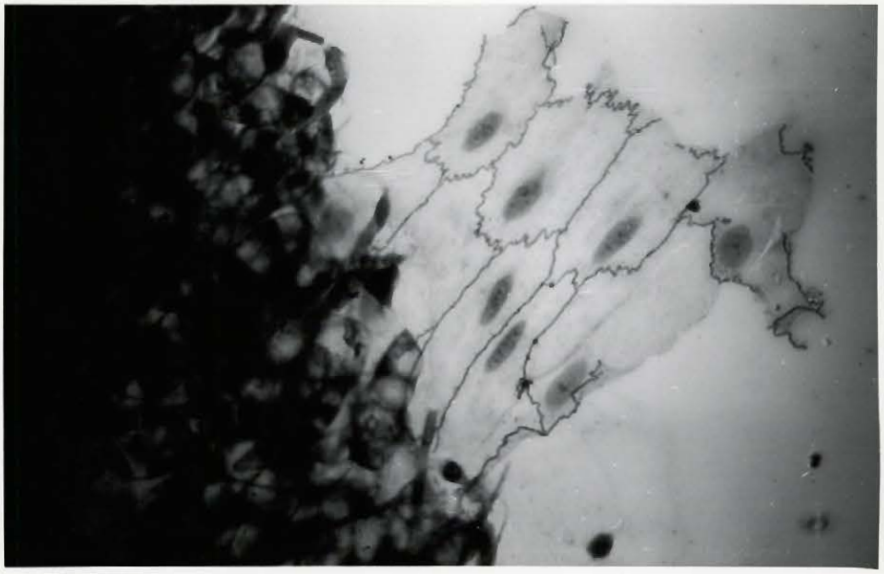


Fig. 65: Normal DBA/1 mouse
Mesentery - mesothelial cells.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 200

Fig. 66: Normal DBA/1 mouse
Mesentery - mesothelial cells
Silver nitrate (osmium tetroxide), haematoxylin and eosin X 200

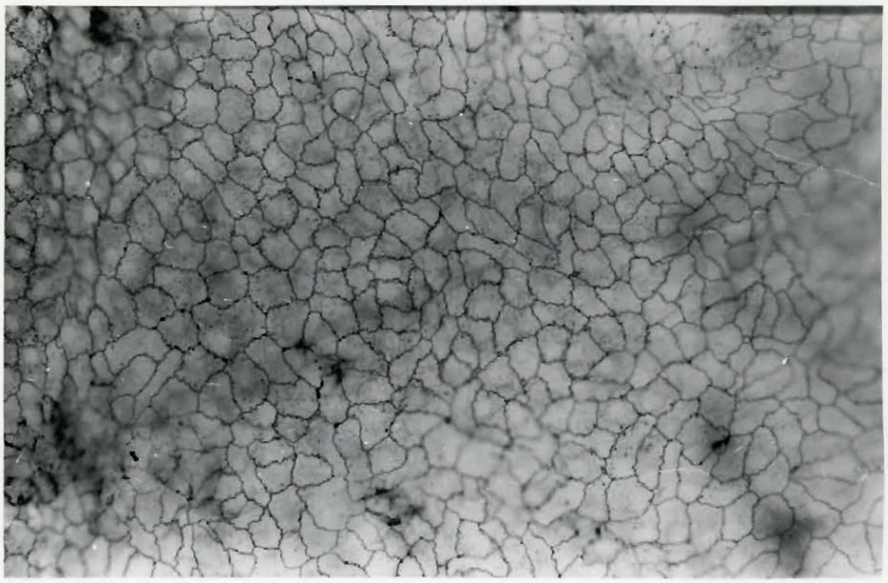


Fig. 67: Normal DBA/1 mouse
Mesentery - mesothelial lining
Silver nitrate (osmium tetroxide) X 80

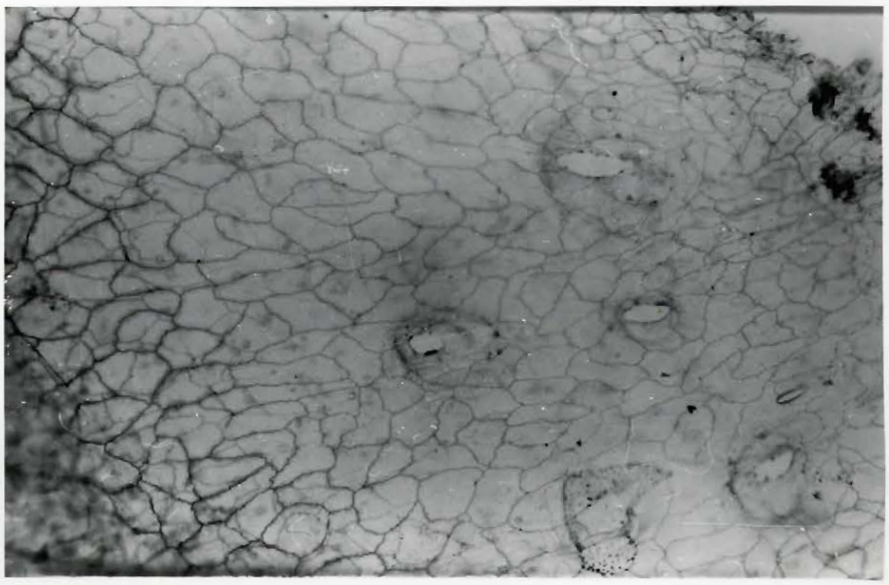


Fig. 68: Normal DBA/1 mouse
Mesentery showing holes on surface.
Silver nitrate (cobalt and ammonium bromide) X 100

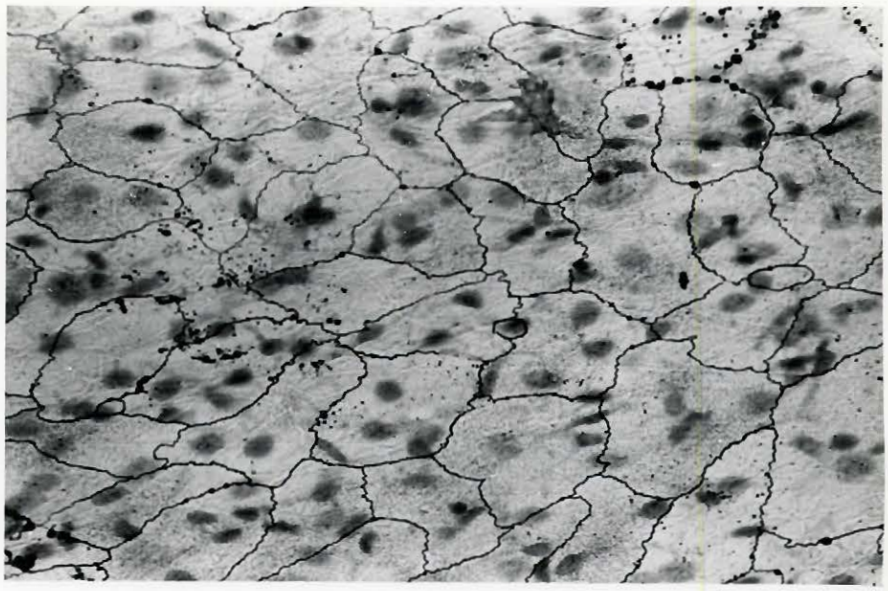


Fig. 69: Normal DBA/1 mouse
Mesentery - stomata in mesothelial lining.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 200

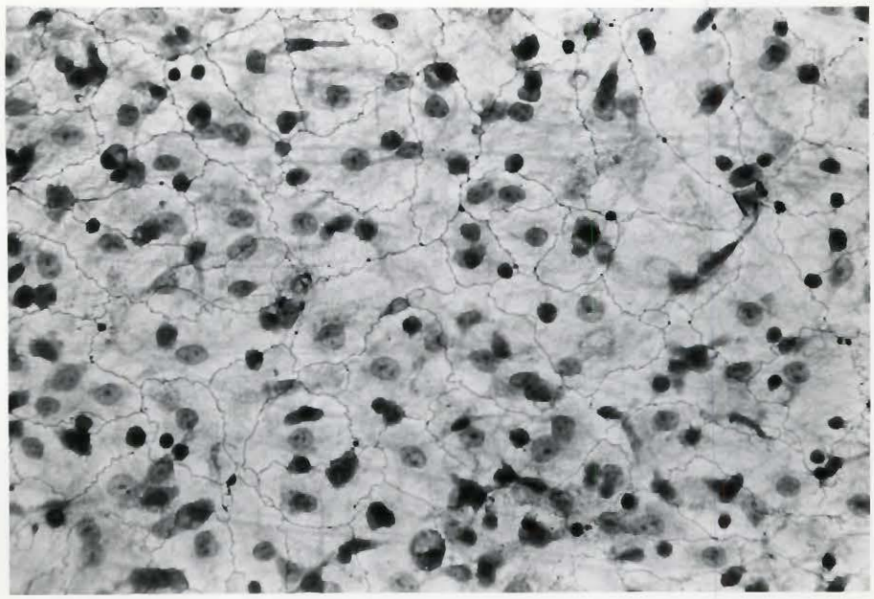


Fig. 70: Normal DBA/1 mouse
Mesentery - stomata in mesothelial lining.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 200

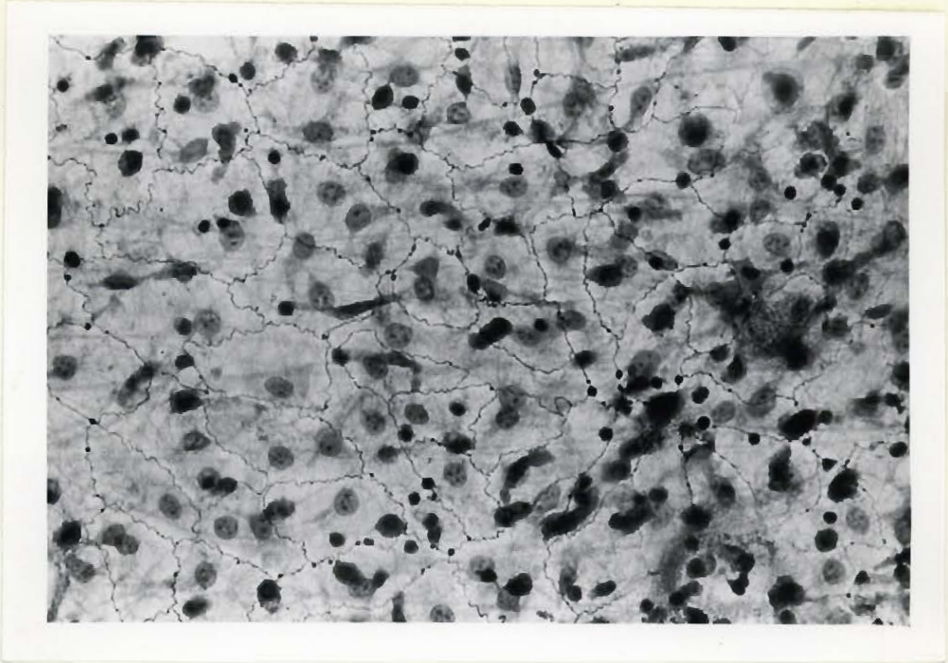


Fig. 71: Normal DBA/1 mouse
Mesentery - stigmata in mesothelial lining.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 200

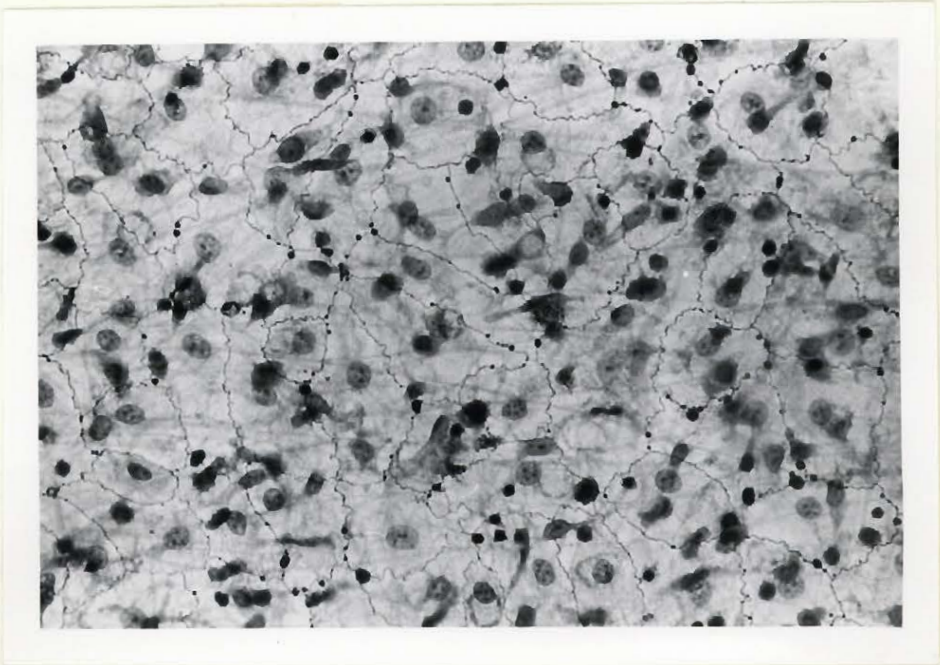


Fig. 72: Normal DBA/1 mouse
Mesentery - stigmata in mesothelial lining
Silver nitrate (cobalt and ammonium bromide) haematoxylin and eosin X 200.

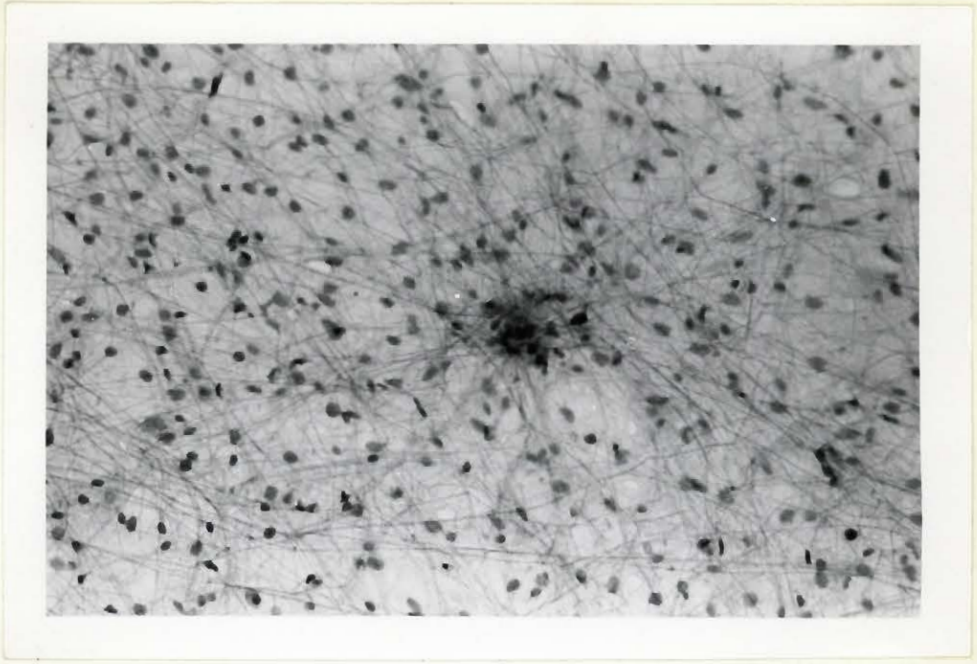


Fig. 73: Normal DBA/1 mouse
Mesentery - cells and fibres.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 80

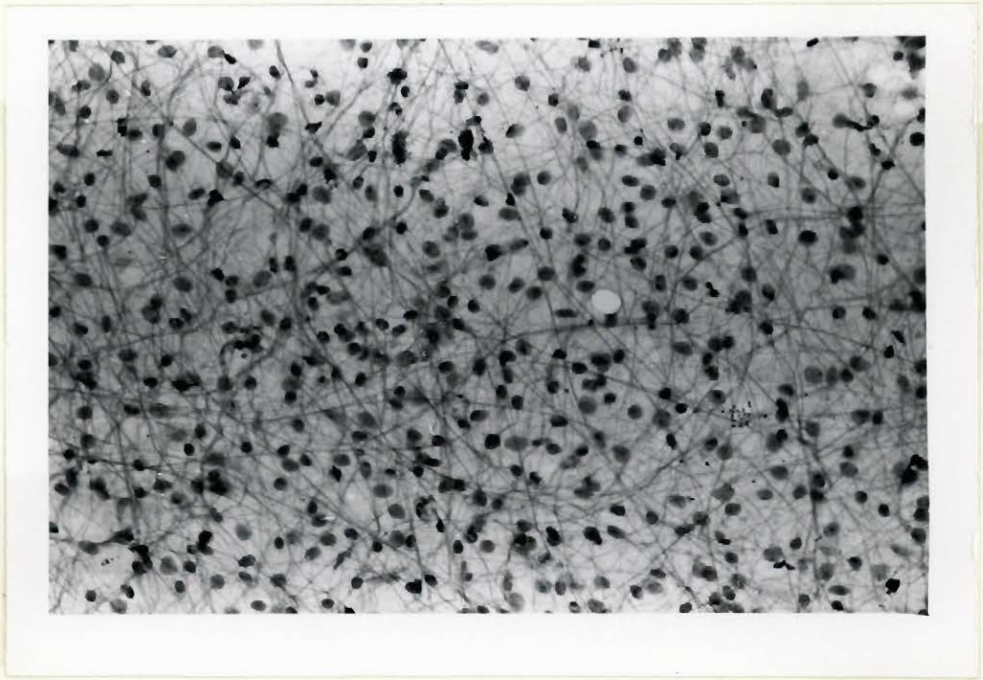


Fig. 74: DBA/1 mice injected with S/91 melanoma intraperitoneally.
Mesentery - cells and fibres; scattered mast cells.
Silver nitrate (cobalt and ammonium bromide)
haematoxylin and eosin X 80

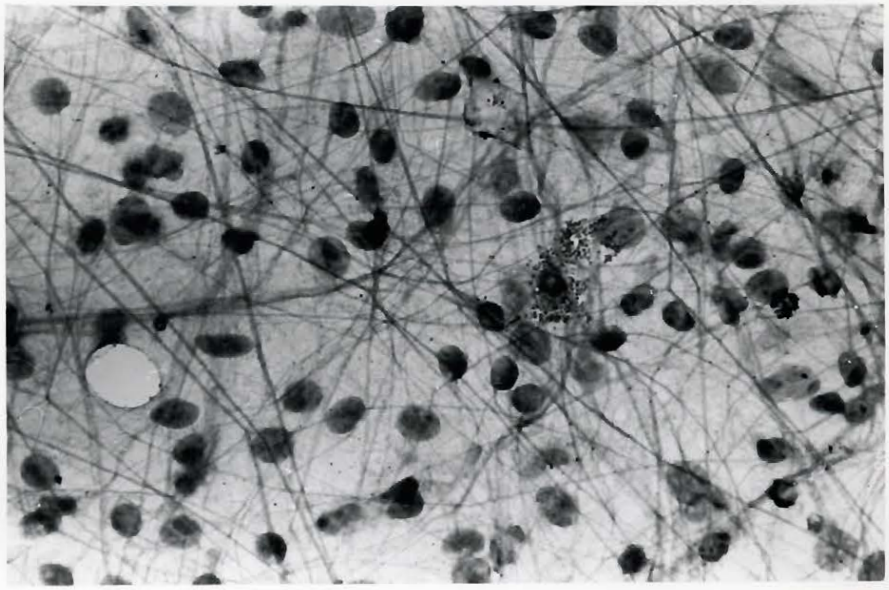


Fig. 75: DBA/1 mice injected with S/91 melanoma intraperitoneally.
Mesentery - fibres and mast cell.
Giemsa X 200

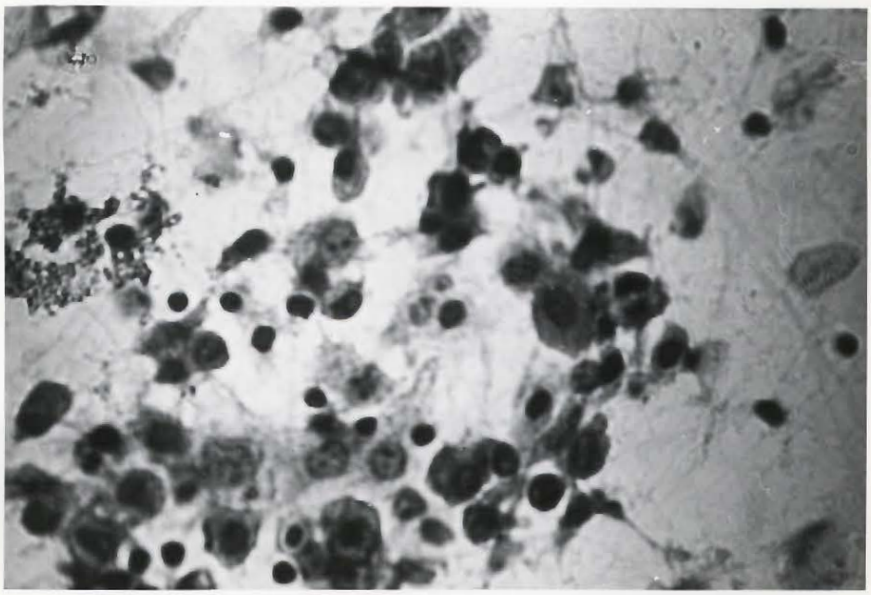


Fig. 76: DBA/1 mice injected with S/91 melanoma intraperitoneally.
Mesentery - collection of different cells, including a mast cell.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 320

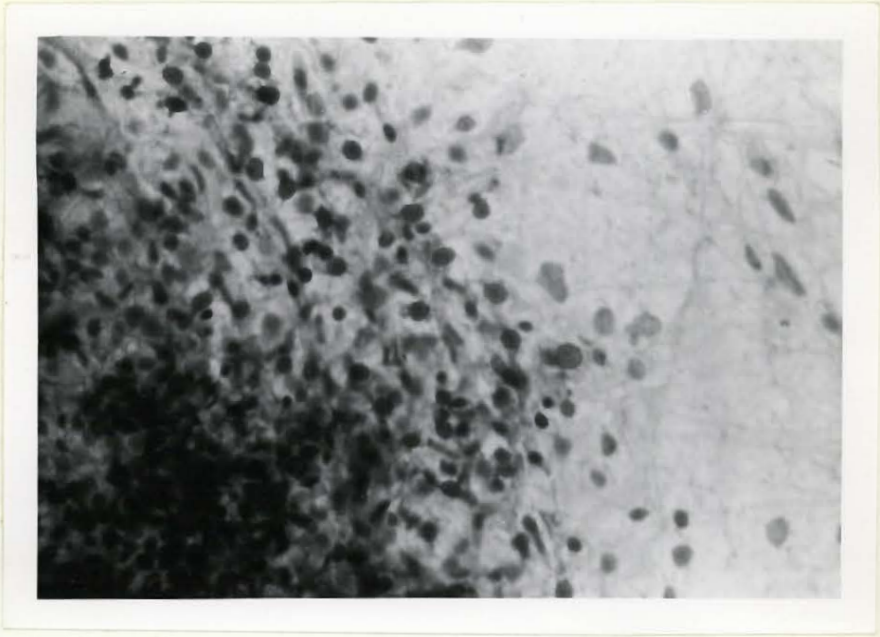


Fig. 77: Normal DBA/1 mouse
Mesentery - collection of cells in zone I, mostly lymphocytes.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 200

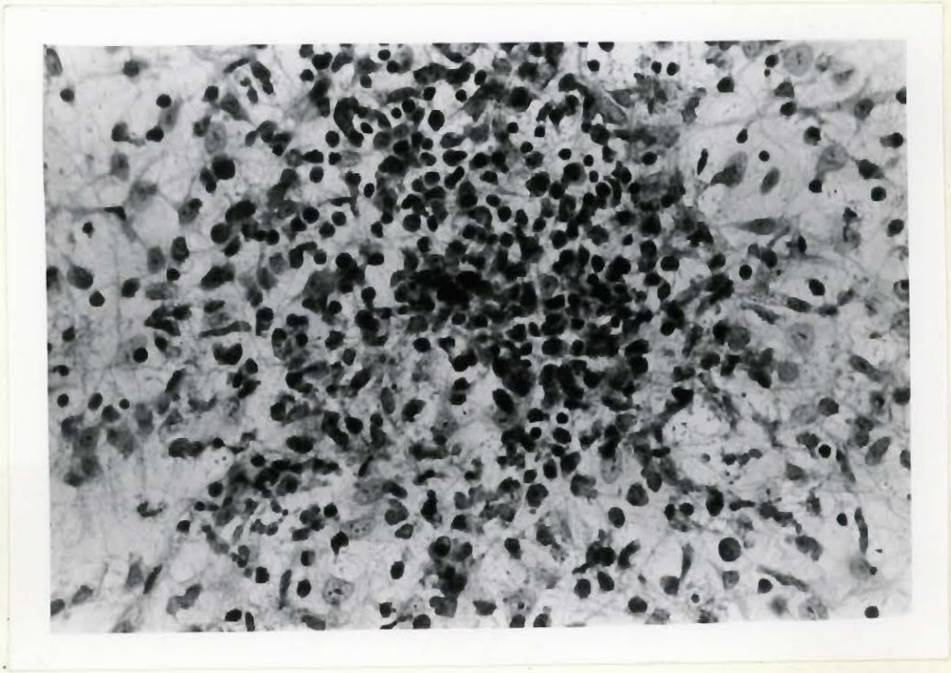


Fig. 78: DBA/1 mice injected with S/91 melanoma intraperitoneally. 1st day: mesentery - collection of cells with fibroblasts at periphery.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 200

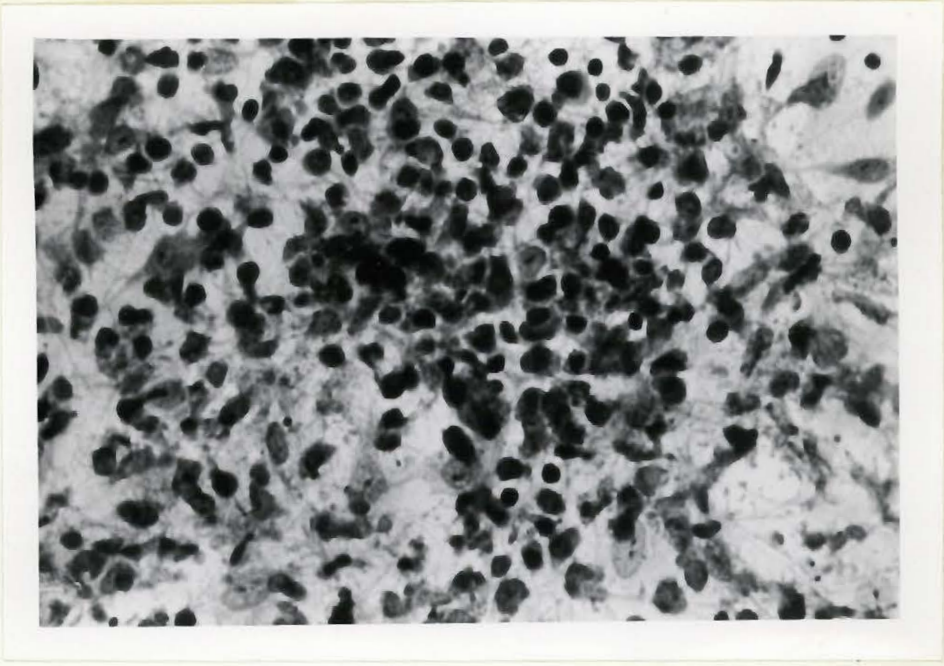


Fig. 79: DBA/1 mice injected with S/91 melanoma intraperitoneally 2nd day: mesentery - collection of inflammatory cells with tumour cells.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 200

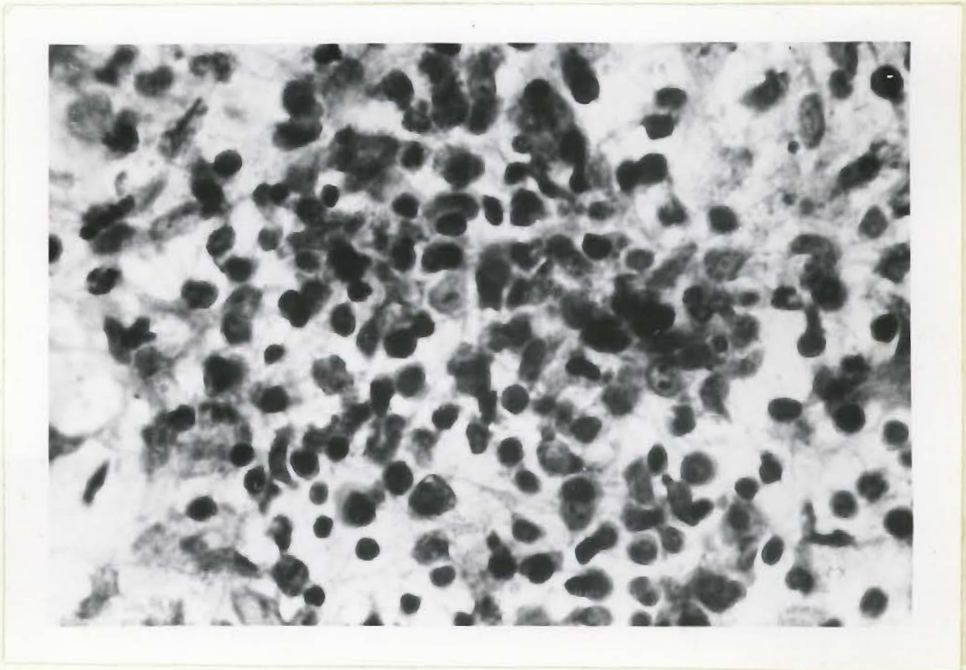


Fig. 80: DBA/1 mice injected with S/91 melanoma intraperitoneally 2nd day: mesentery - collection of inflammatory and tumour cells.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 320

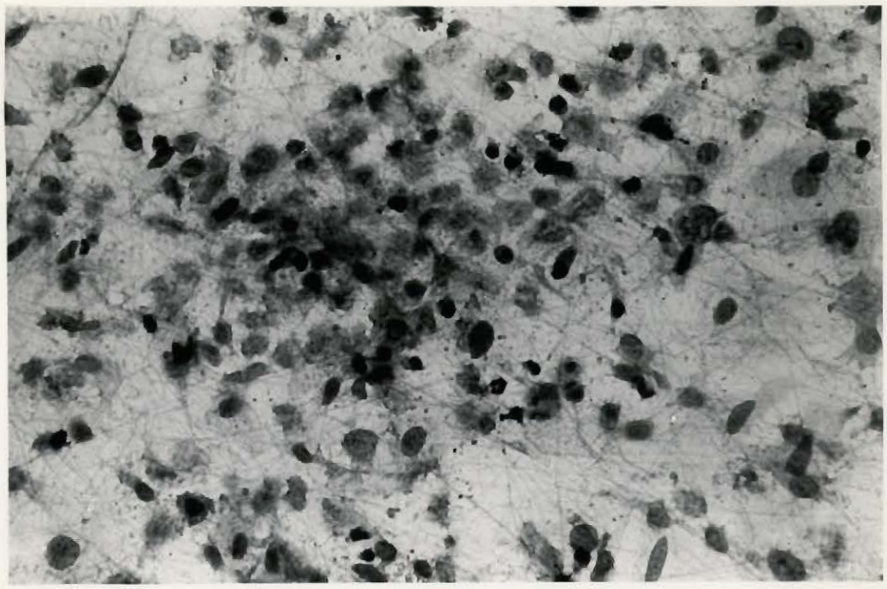


Fig. 81: DBA/1 mice injected with S/91 melanoma intraperitoneally 2nd day: mesentery - collection of inflammatory cells with fair number of polymorphs.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 200

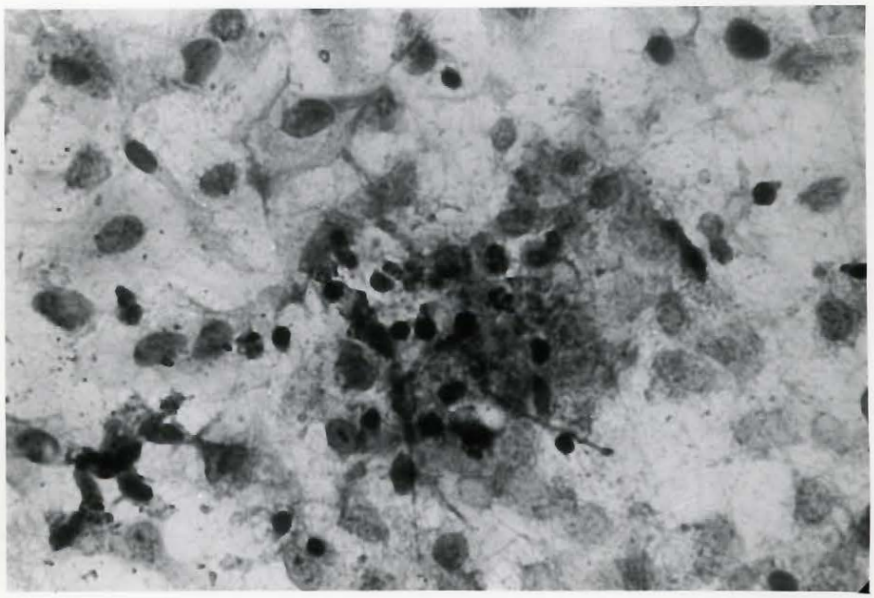


Fig. 82: DBA/1 mice injected with S/91 melanoma intraperitoneally. 3rd day: mesentery - collection of inflammatory and tumour cells with fair number of polymorphs.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 320

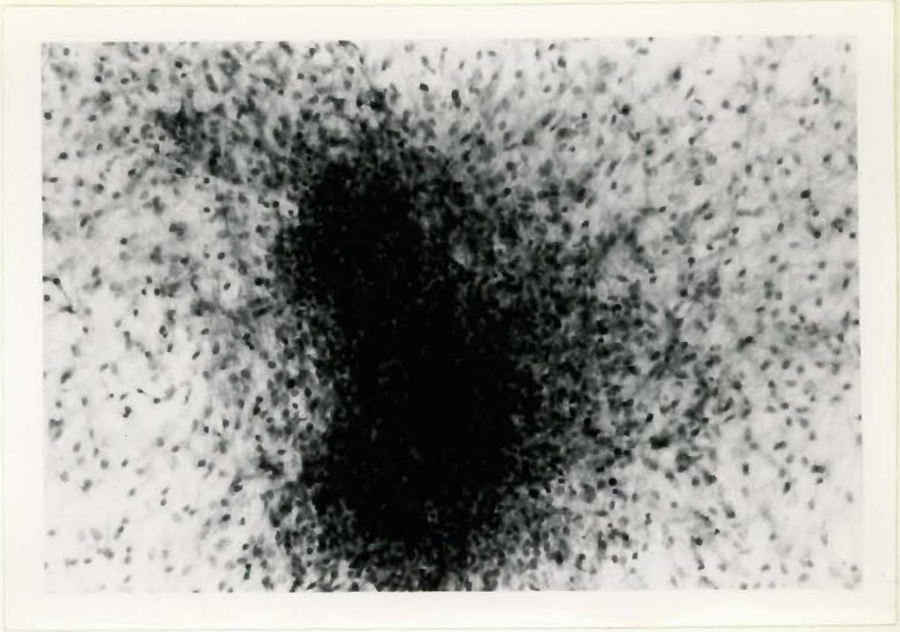


Fig. 83: DBA/1 mice injected with S/91 melanoma intraperitoneally 4th day: mesentery - collection of cells, mostly polymorphs. Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 50

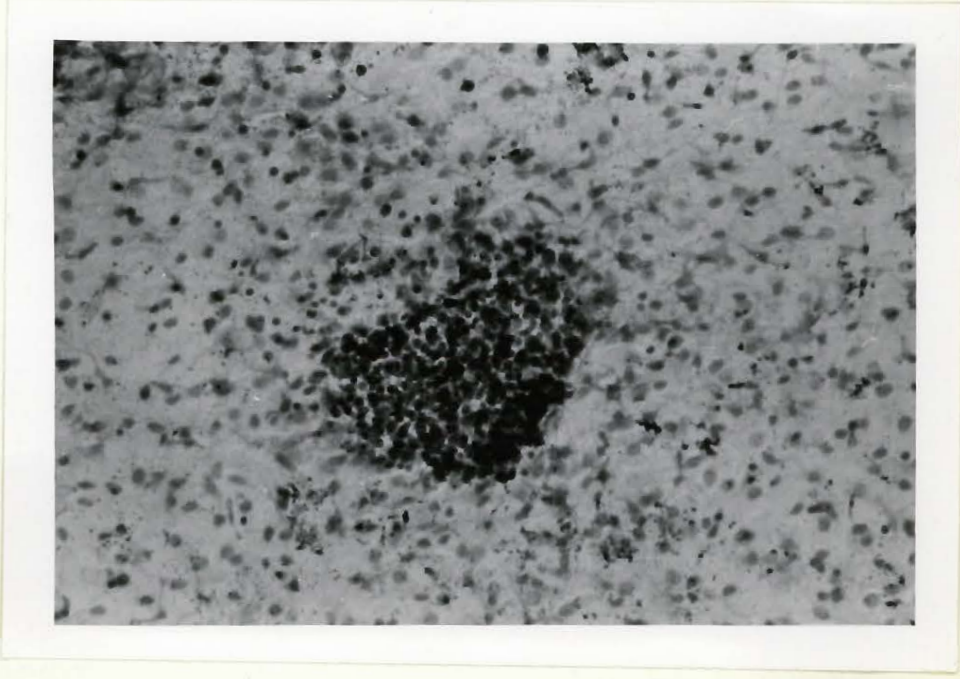


Fig. 84: DBA/1 mice injected with S/91 melanoma intraperitoneally 5th day: mesentery - collection of cells with growing tumour cells.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 80

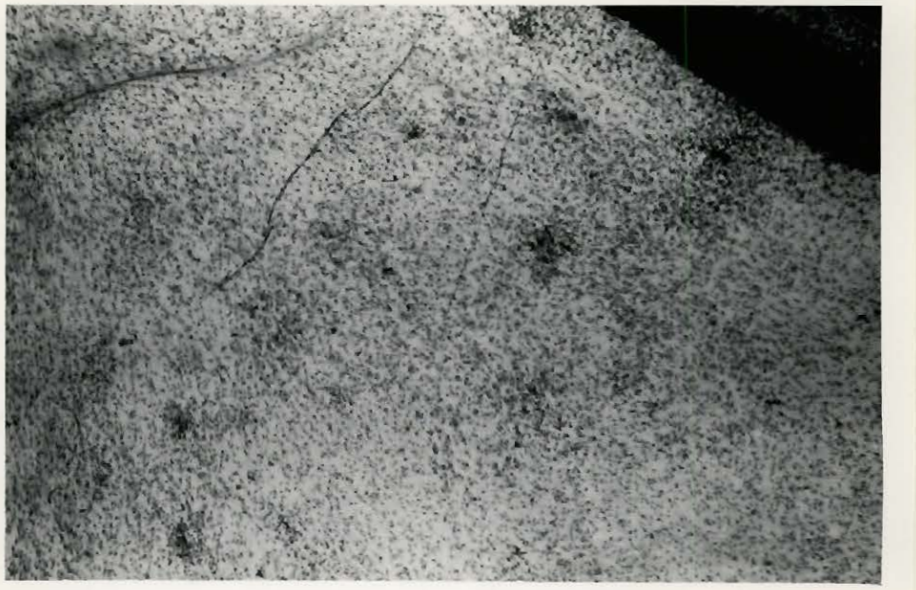


Fig. 85: DBA/1 mice injected with S/91 melanoma intraperitoneally. 3rd day: mesentery - scattered inflammatory foci in zones I & II.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

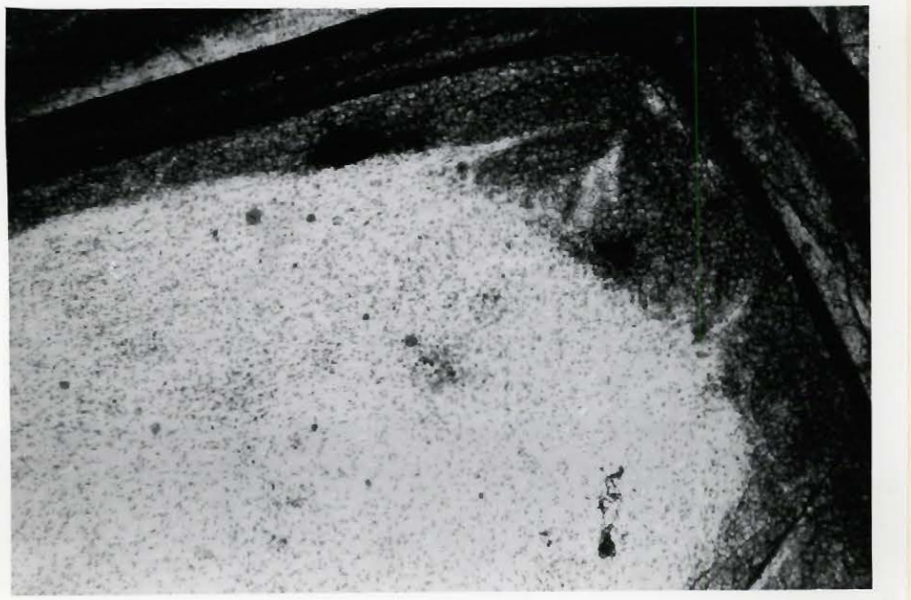


Fig. 86: DBA/1 mice injected with S/91 melanoma intraperitoneally 5th day: mesentery - clearing of zones I & II and concentration of foci in zones III & IV.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

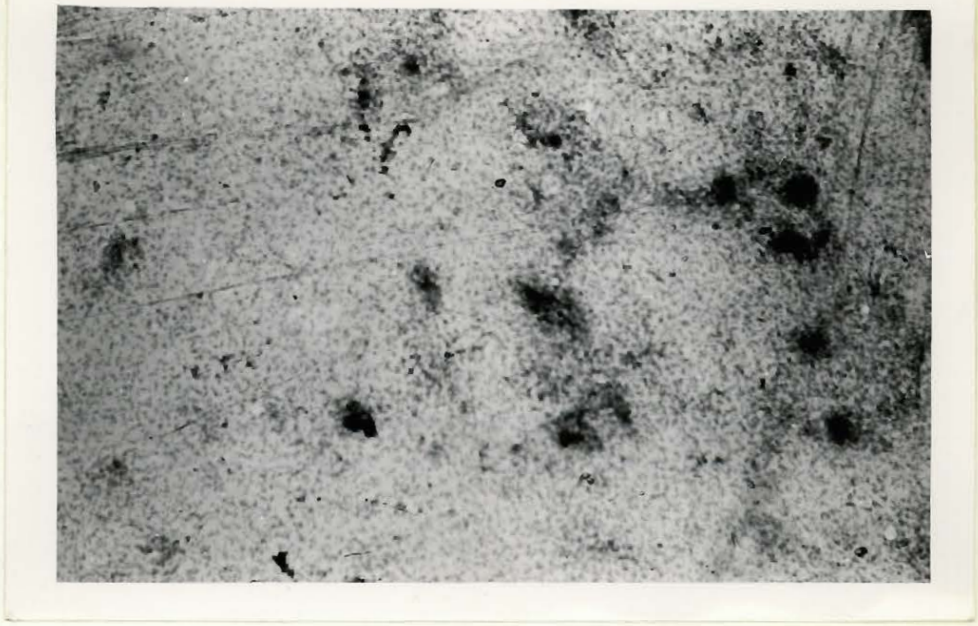


Fig. 87: DBA/1 mice injected with S/91 melanoma intraperitoneally 7th day: mesentery - scattered foci with tumour cells in zones I & II.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

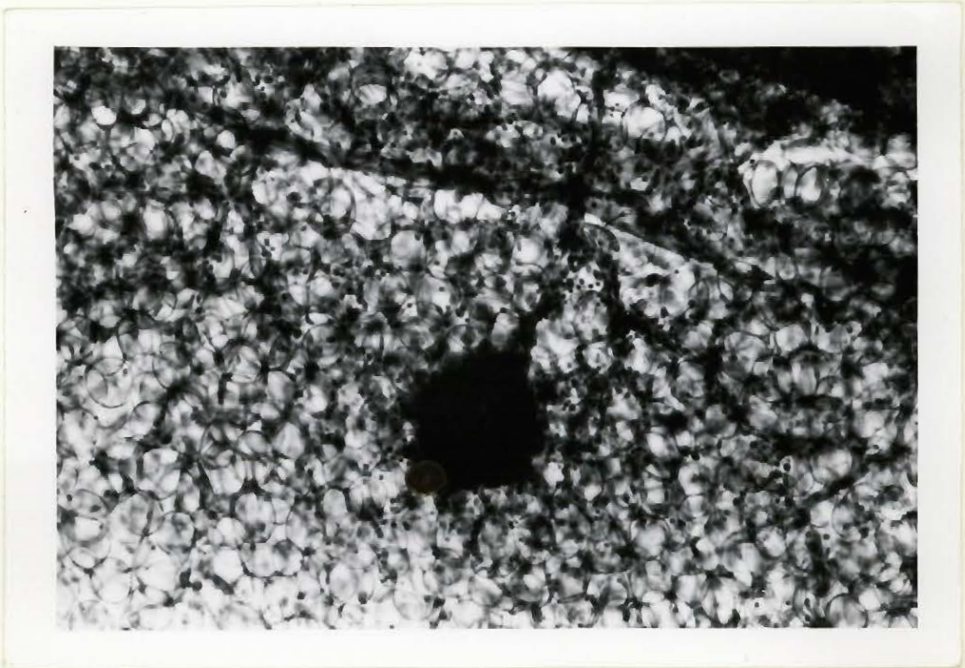


Fig. 88: DBA/1 mice injected with S/91 melanoma intraperitoneally 7th day: mesentery - a focus growing in zone III. Note vascular ingrowth.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

Fig. 89: DBA/1 mice injected with S/91 melanoma intraperitoneally 9th day: mesentery - a growing focus.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 40

Fig. 90: DBA/1 mice injected with S/91 melanoma intraperitoneally 10th day: mesentery - growing tumour foci with vascularization in zones II & III, avascular in zone I.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

Fig. 91: DBA/1 mice injected with S/91 melanoma intraperitoneally 12th day: mesentery - a tumour with vascular bed and pigment formation in zone III.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

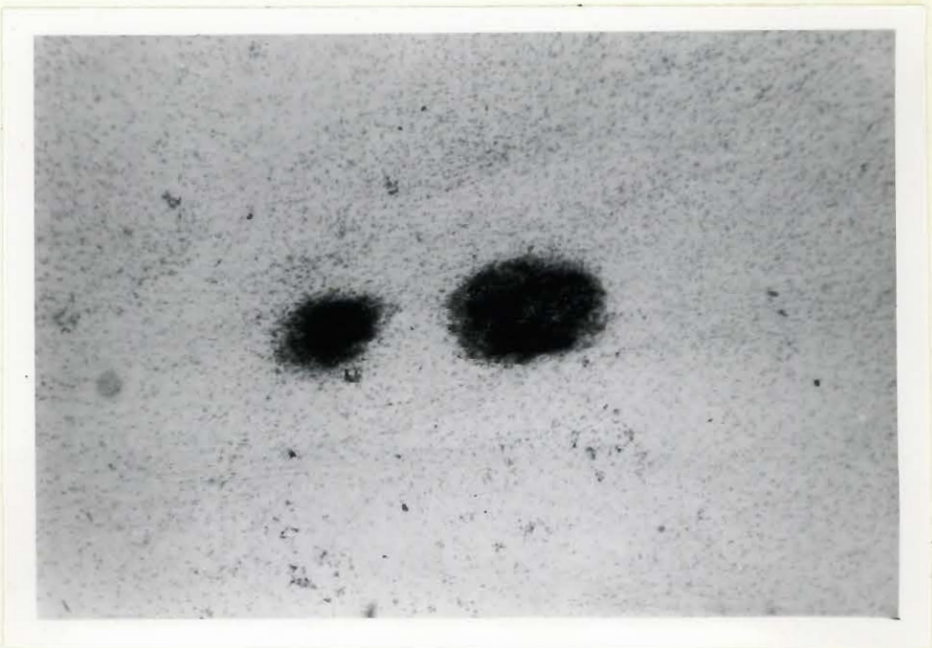


Fig. 92: DBA/1 mice injected with S/91 melanoma intraperitoneally 12th day: mesentery - two foci with formation of pigment in the tumour cells in zone I.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

Fig. 93: DBA/1 mice injected with S/91 melanoma intraperitoneally 12th day: mesentery - a fully formed round tumour in zone III.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 50

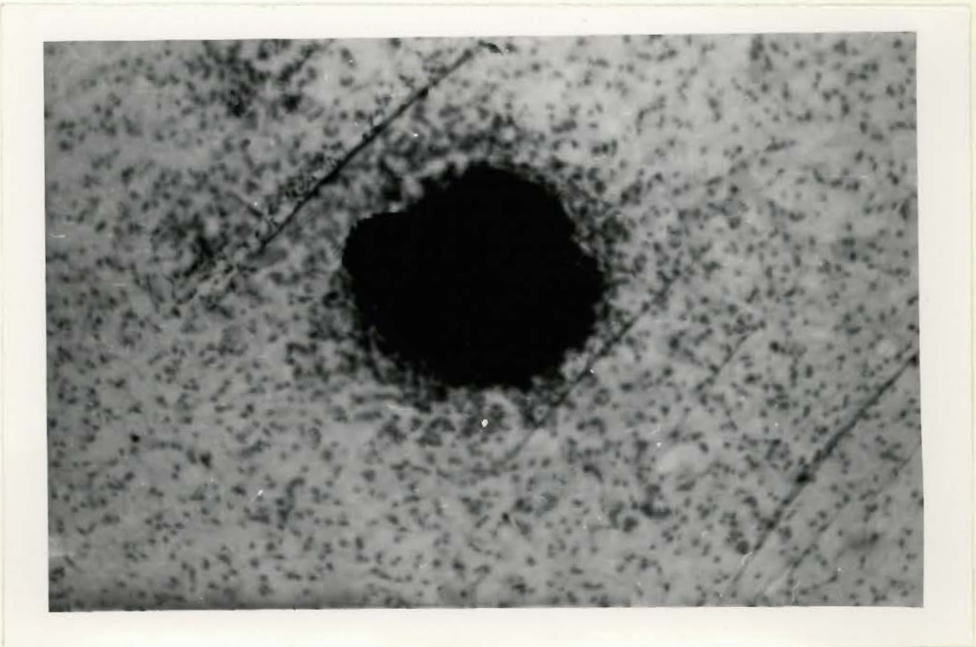


Fig. 94: DBA/1 mice injected with S/91 melanoma intraperitoneally 14th day: mesentery - a flat spreading tumour in zone I.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 80

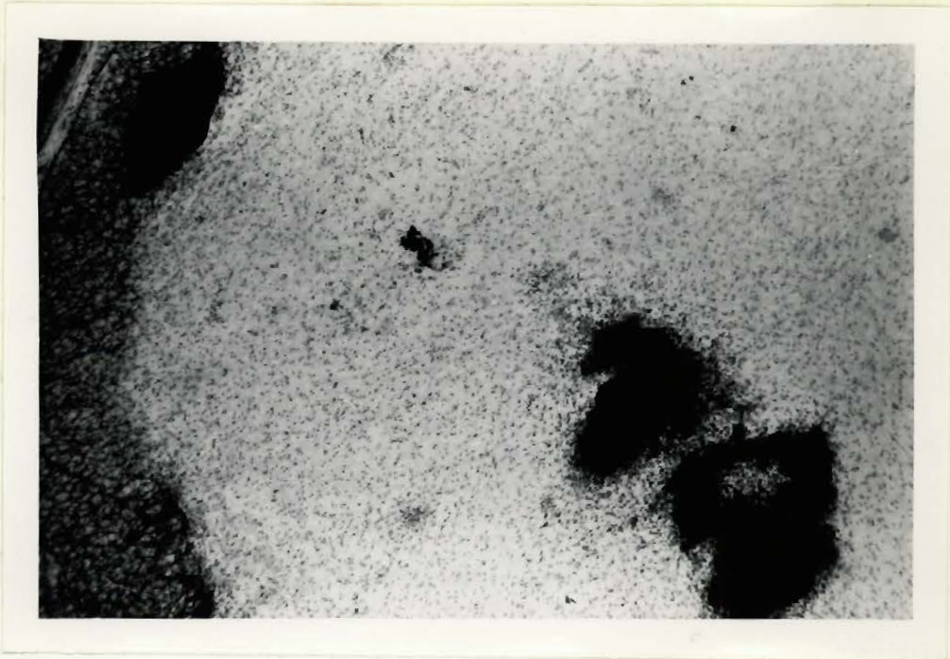


Fig. 95: DBA/1 mice injected with S/91 melanoma intraperitoneally 15th day: mesentery - flat, avascular tumour in zone I. Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

Fig. 96: DBA/1 mice injected with S/91 melanoma intraperitoneally 15th day: mesentery - tumour formation in zone II with vascularization from adjacent zone III. Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

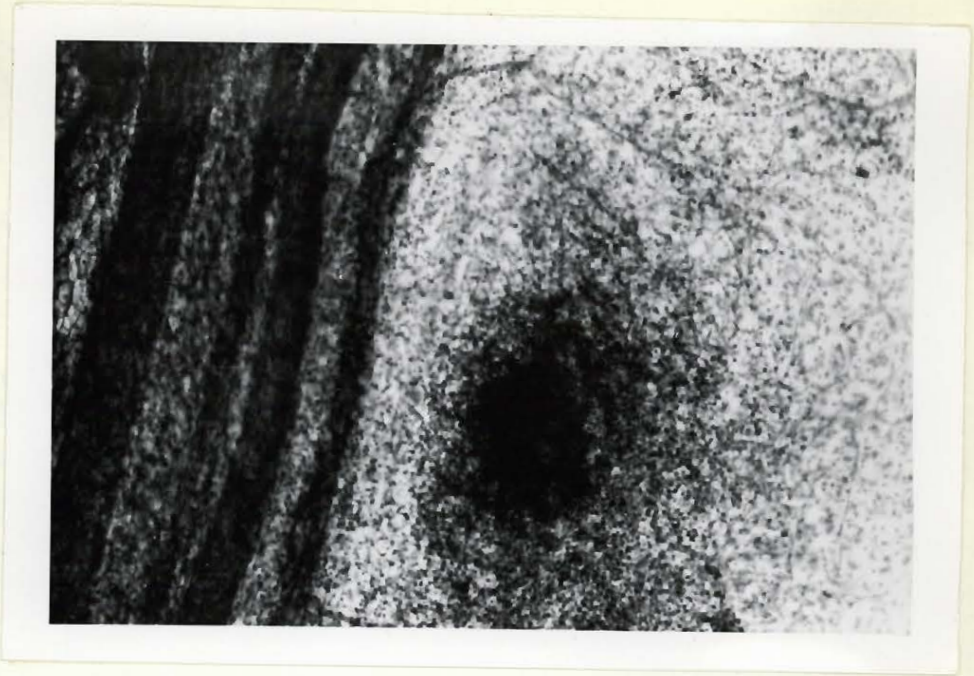


Fig. 97: DBA/1 mice injected with S/91 melanoma intraperitoneally 10th day: mesentery - tumour formation with inflammatory reaction around.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

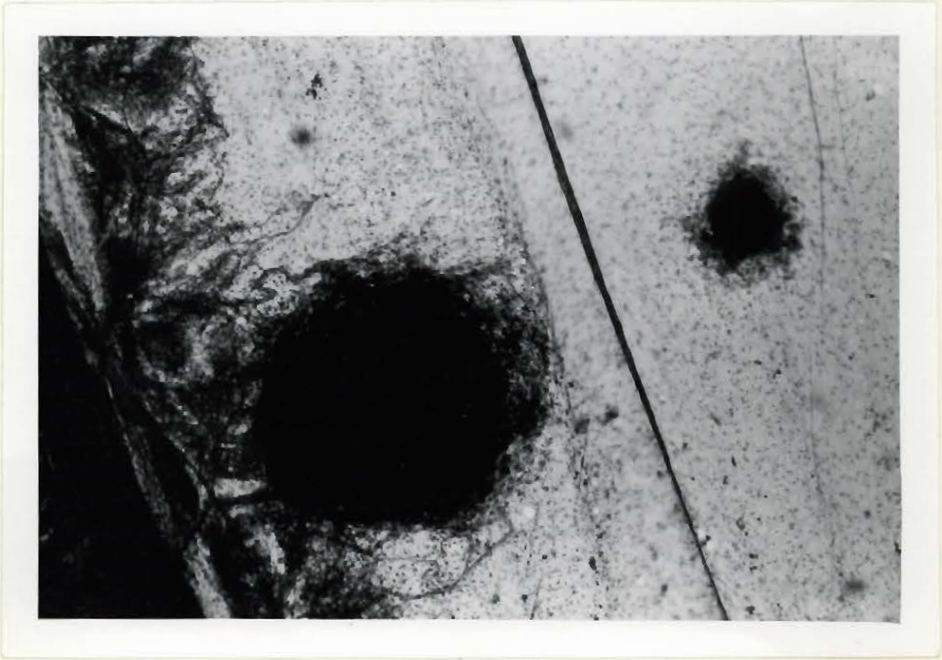


Fig. 98: DBA/1 mice injected with S/91 melanoma intraperitoneally 15th day: mesentery - tumour development with vascular and avascular bed in adjacent zones.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

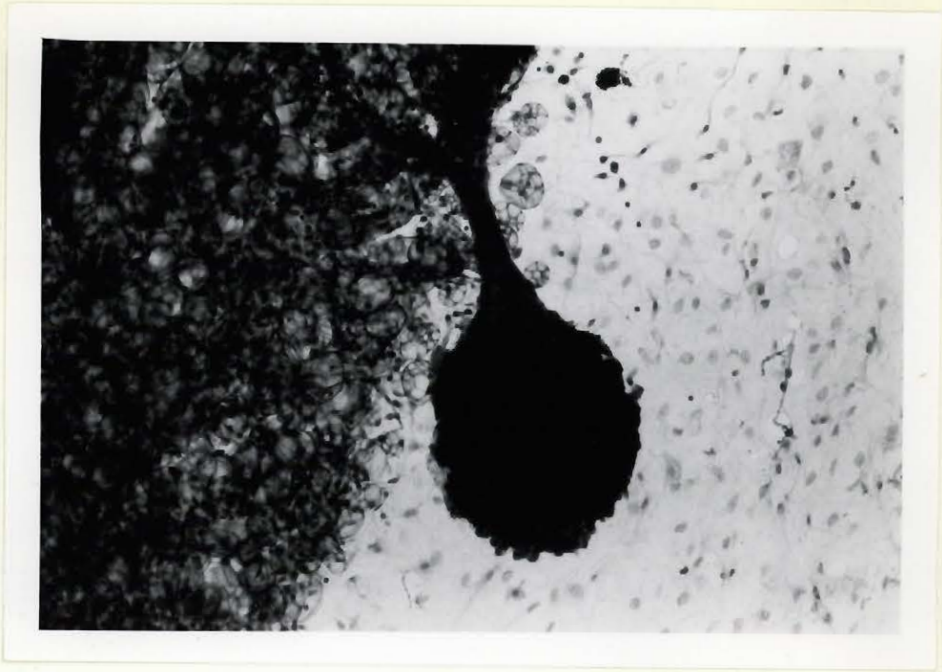


Fig. 99: DBA/1 mice injected with S/91 melanoma intraperitoneally 21st day: mesentery - a polypoid tumour with the vessels forming the stalk.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 80

Fig. 100: DBA/1 mice injected with S/91 melanoma intraperitoneally 21st day: mesentery - a sessile, round tumour in zone IV.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

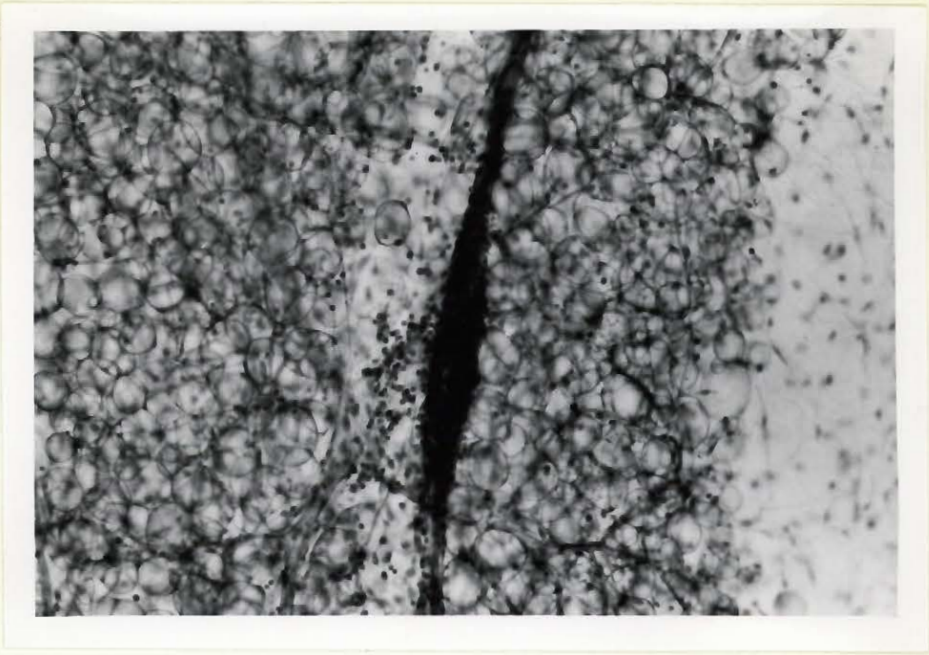


Fig. 101: DBA/1 mice injected with S/91 melanoma intraperitoneally
Mesentery - tumour cells in relation to the blood vessel.
Silver nitrate (cobalt and ammonium bromide),
haematoxylin and eosin X 80

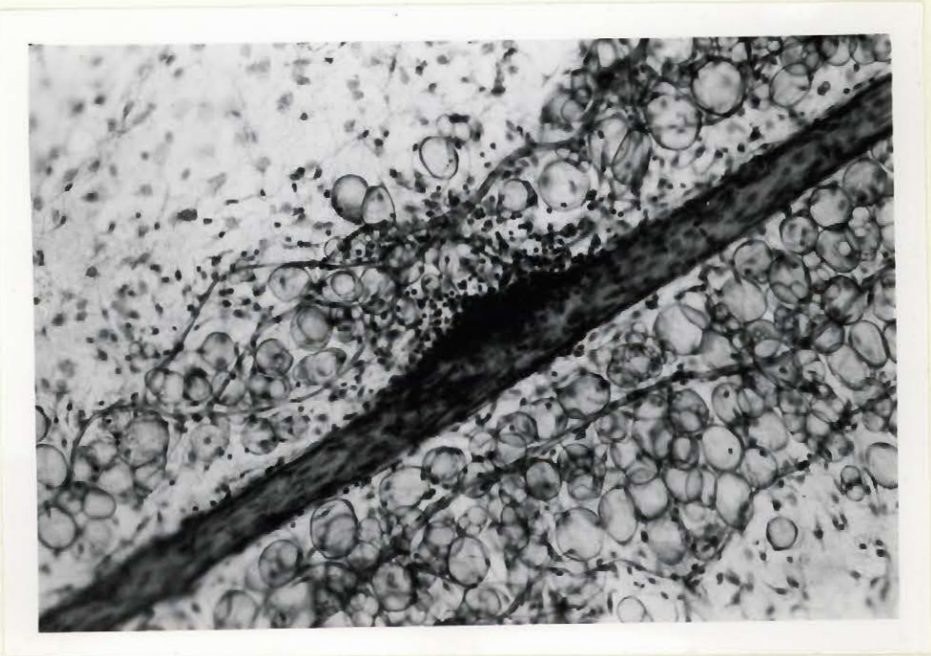


Fig. 102: DBA/1 mice injected with S/91 melanoma intraperitoneally
Mesentery - tumour cells sticking to the vessel wall and inside lumen.
Silver nitrate (cobalt and ammonium bromide),
haematoxylin and eosin X 80

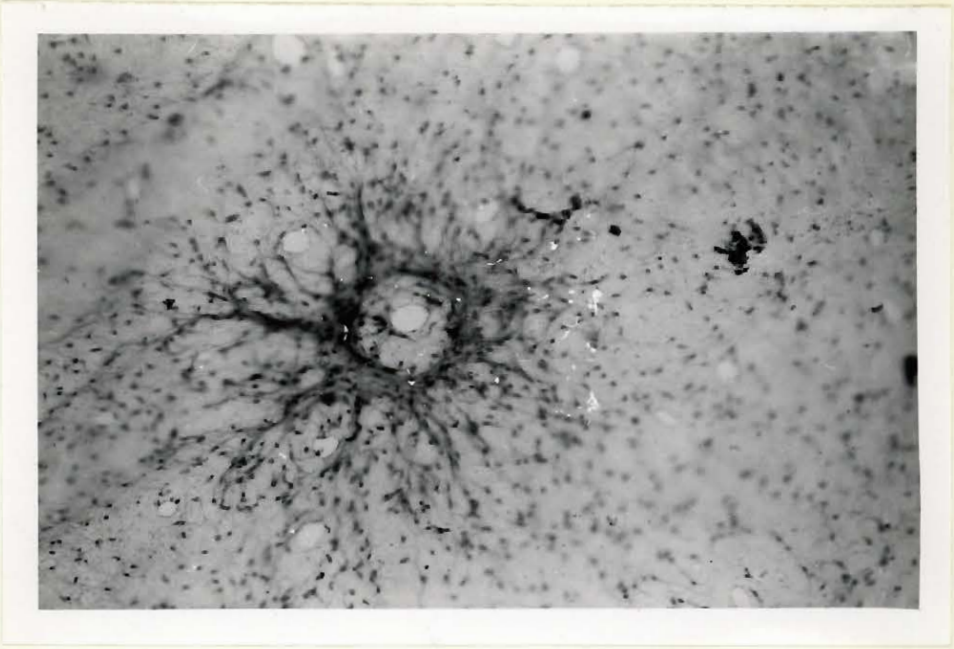


Fig. 103: DBA/1 mice injected with S/91 melanoma intraperitoneally
Mesentery - proliferation of fibroblasts around a 'hole'.
Silver nitrate (cobalt and ammonium bromide),
haematoxylin and eosin X 50

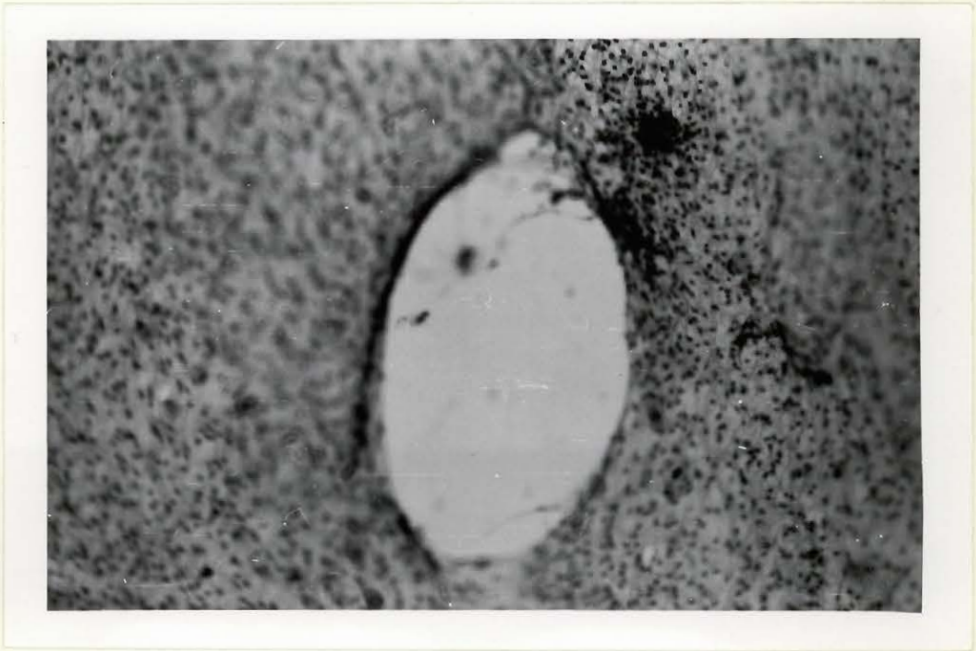


Fig. 104: Normal DBA/1 mouse
Aggregation of inflammatory cells around a 'hole'.
Silver nitrate (cobalt and ammonium bromide),
haematoxylin and eosin X 80

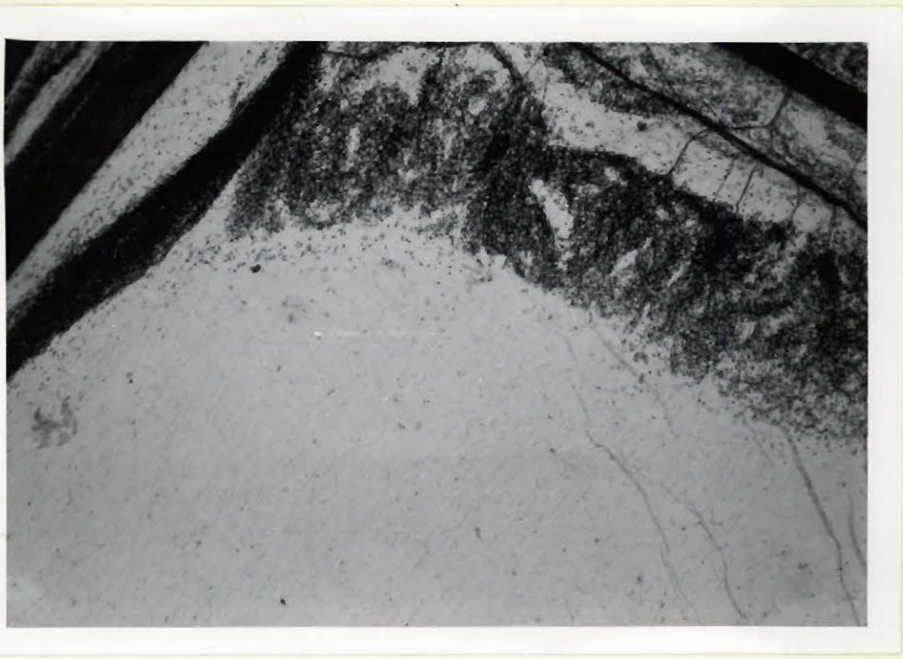


Fig. 105: DBA/1 mice injected with S/91 melanoma intraperitoneally, treated with cortisone.
Mesentery - marked clearing of the cells in all zones.
Treated case.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

Fig. 106: DBA/1 mice injected with S/91 melanoma intraperitonaelly, treated with cortisone
Mesentery - note absence of cells in zones III & IV; producing a marked clearing effect.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 20

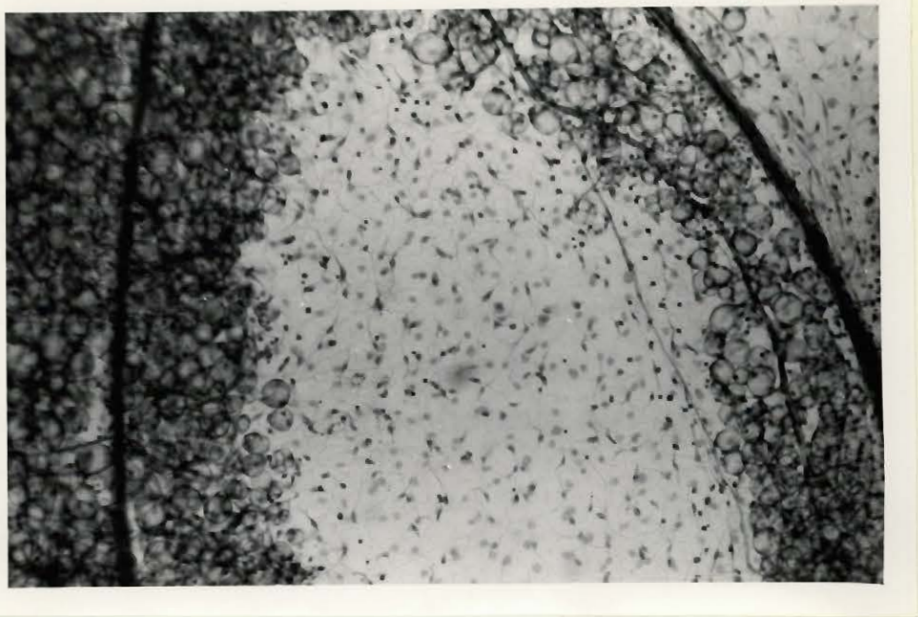


Fig. 107: DBA/1 mice injected with S/91 melanoma intraperitoneally, treated with nitrogen mustard.
Mesentery - marked clearing of all zones in early cases.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 50

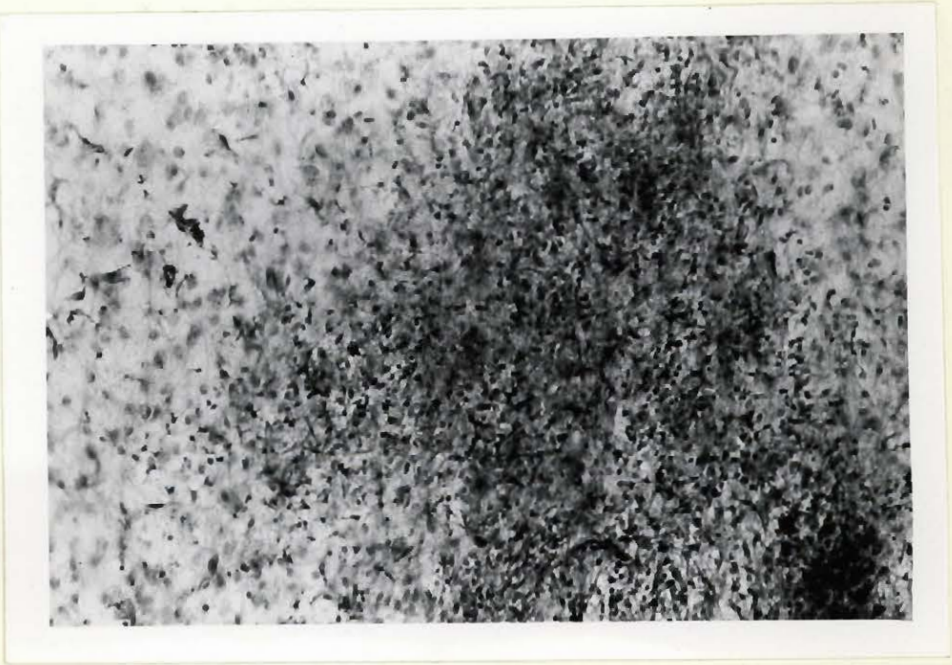
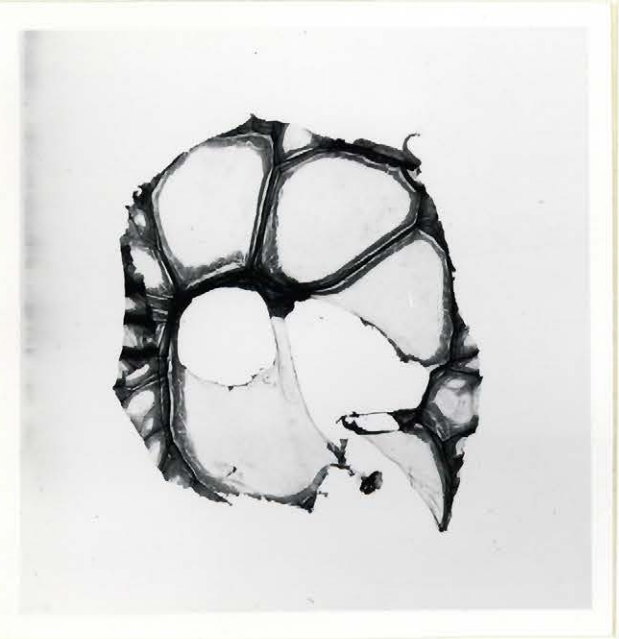


Fig. 108: DBA/1 mice injected with S/91 melanoma intraperitoneally, treated with nitrogen mustard.
Mesentery - collection of irregular and pyknotic cells on the leaflets.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin X 80

Fig: 109: DBA/1 mice injected with S/91 melanoma intraperitoneally
10th day: mesentery - no visible tumour.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin.

Fig. 110: DBA/1 mice injected with S/91 melanoma intraperitoneally and treated with cortisone.
10th day: mesentery - no visible tumours. A very clear appearance of the septa.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin.

Fig. 111: DBA/1 mice treated with nitrogen mustard 1 hour after injection with S/91 melanoma intraperitoneally.
10th day: mesentery - no visible tumours.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin.



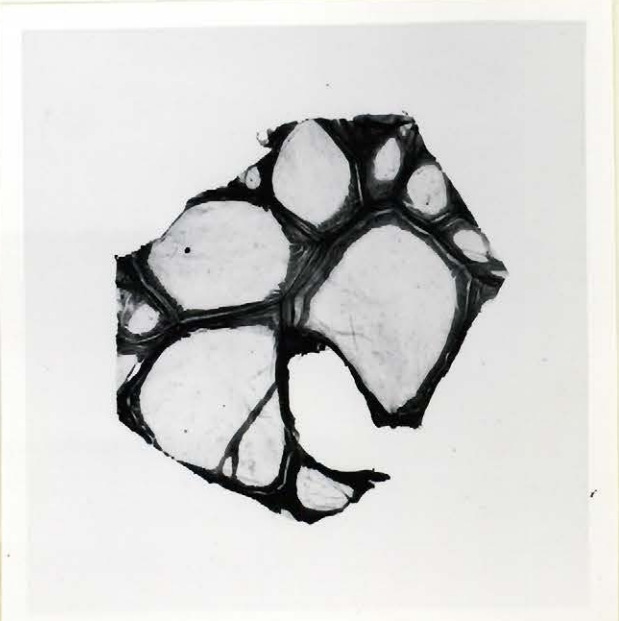
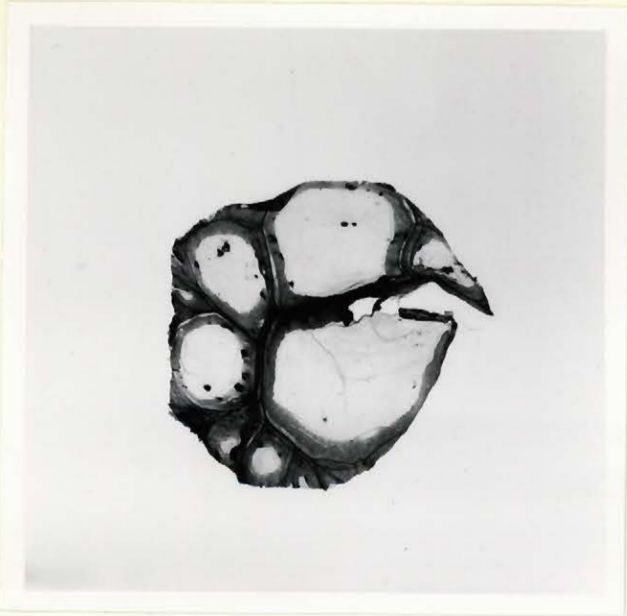
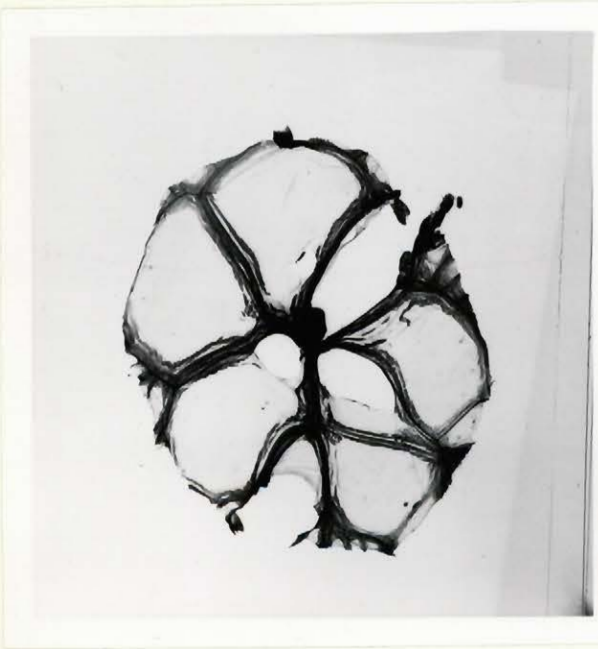


Fig. 112: DBA/1 mice injected with S/91 melanoma intraperitoneally.
15th day: mesentery - a few tumours, mostly in zones III & IV.
Silver nitrate (cobalt and ammonium bromide),
haematoxylin and eosin.

Fig. 113: DBA/1 mice injected with S/91 melanoma intraperitoneally and treated with cortisone.
15th day: mesentery - no visible tumour. Clear appearance of septa.
Silver nitrate (cobalt and ammonium bromide),
haematoxylin and eosin.

Fig. 114: DBA/1 mice treated with nitrogen mustard 1 hour after injection with S/91 melanoma intraperitoneally.
15th day: mesentery - a few visible tumours, mostly in zones III & IV. Less than in control.
Silver nitrate (cobalt and ammonium bromide),
haematoxylin and eosin.





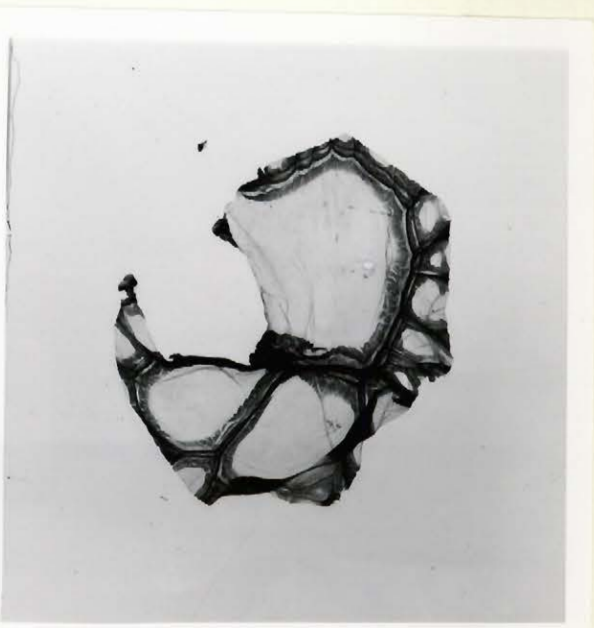
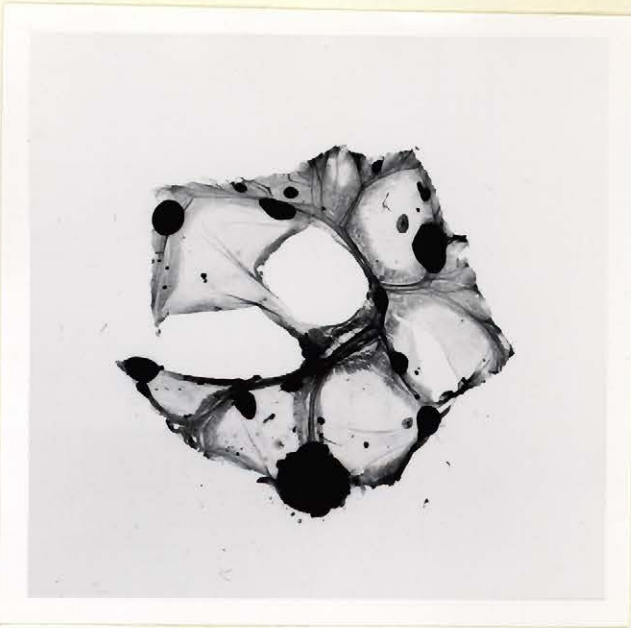
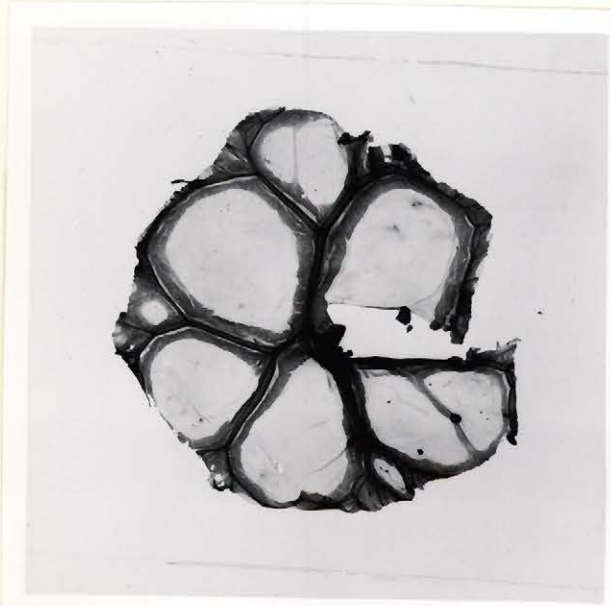


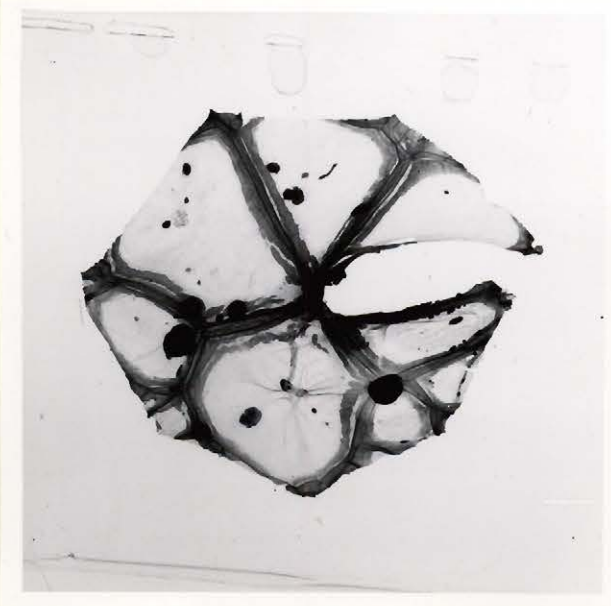
Fig. 115: DBA/1 mice injected with S/91 melanoma intraperitoneally. 21st day: mesentery - several tumours, still mostly in zones III & IV, and a few in zones I & II.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin.

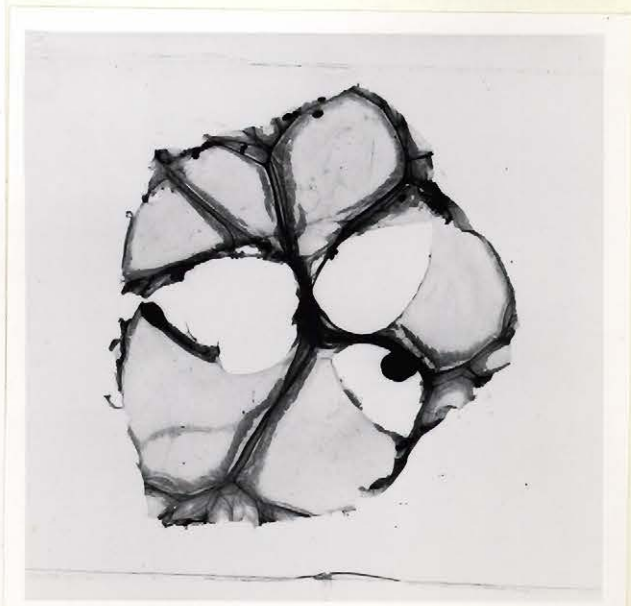
Fig. 116: DBA/1 mice injected with S/91 melanoma intraperitoneally and treated with cortisone.
21st day: mesentery - occasional tumour. Clear appearance of septa.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin.

Fig. 117: DBA/1 mice treated with nitrogen mustart 1 hour after injection with S/91 melanoma intraperitoneally.
21st day: mesentery - visible tumours, mostly in zones III & IV. Less than in control.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin.









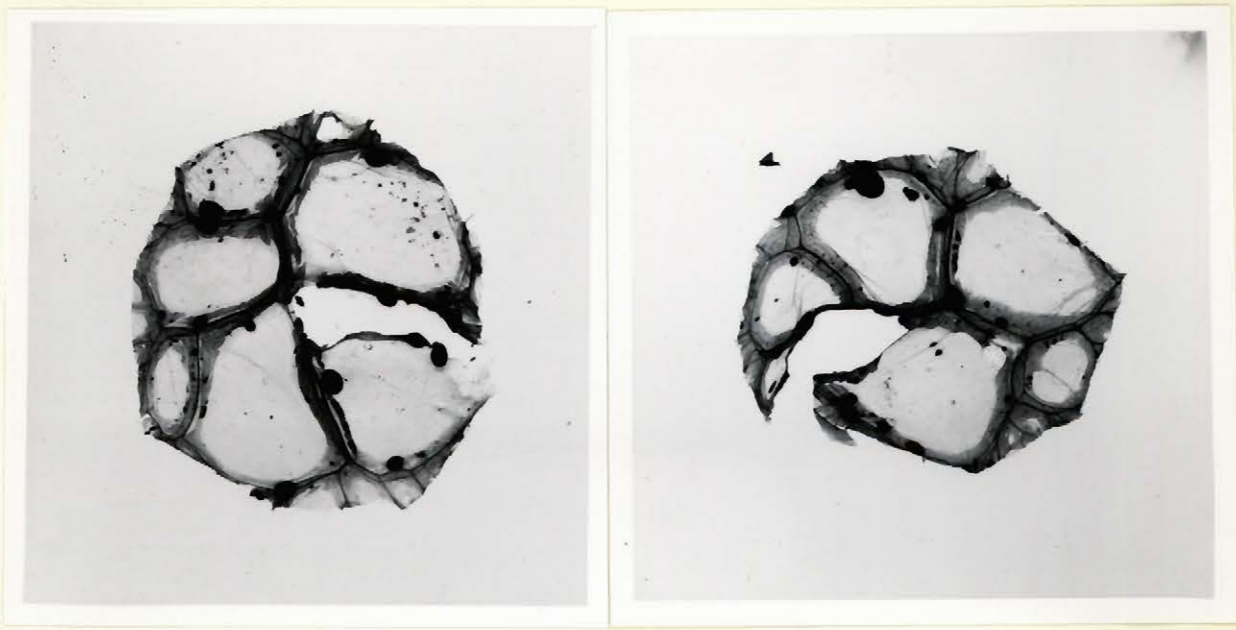


Fig. 118: DBA/1 mice injected with nitrogen mustard 15 days after inoculation with S/91 melanoma intraperitoneally.
Mesentery - 5 days after nitrogen mustard: tumours visible as in control.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin.

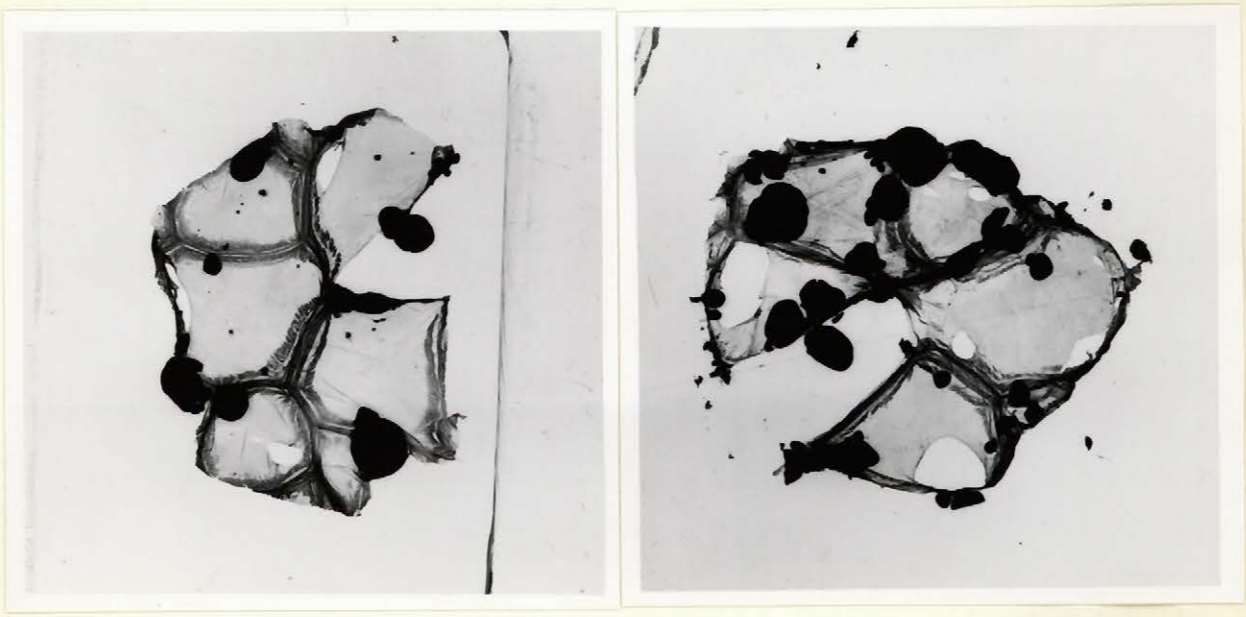


Fig. 119: DBA/1 mice injected with nitrogen mustard 15 days after inoculation with S/91 melanoma intraperitoneally.
Mesentery - 14 days after nitrogen mustard. Heavy growth of tumours as in control.
Silver nitrate (cobalt and ammonium bromide), haematoxylin and eosin.

Fig. 120: DBA/1 mice injected with S/91 melanoma intraperitoneally. 21 days - tumours of varying sizes growing on the gut, mesentery and abdominal wall.

Fig. 121: DBA/1 mice treated with cortisone for 7 days. On the 8th day injected with S/91 melanoma intraperitoneally. Cortisone continued 21 days. Only a few tumours. Gut is clear apparently.

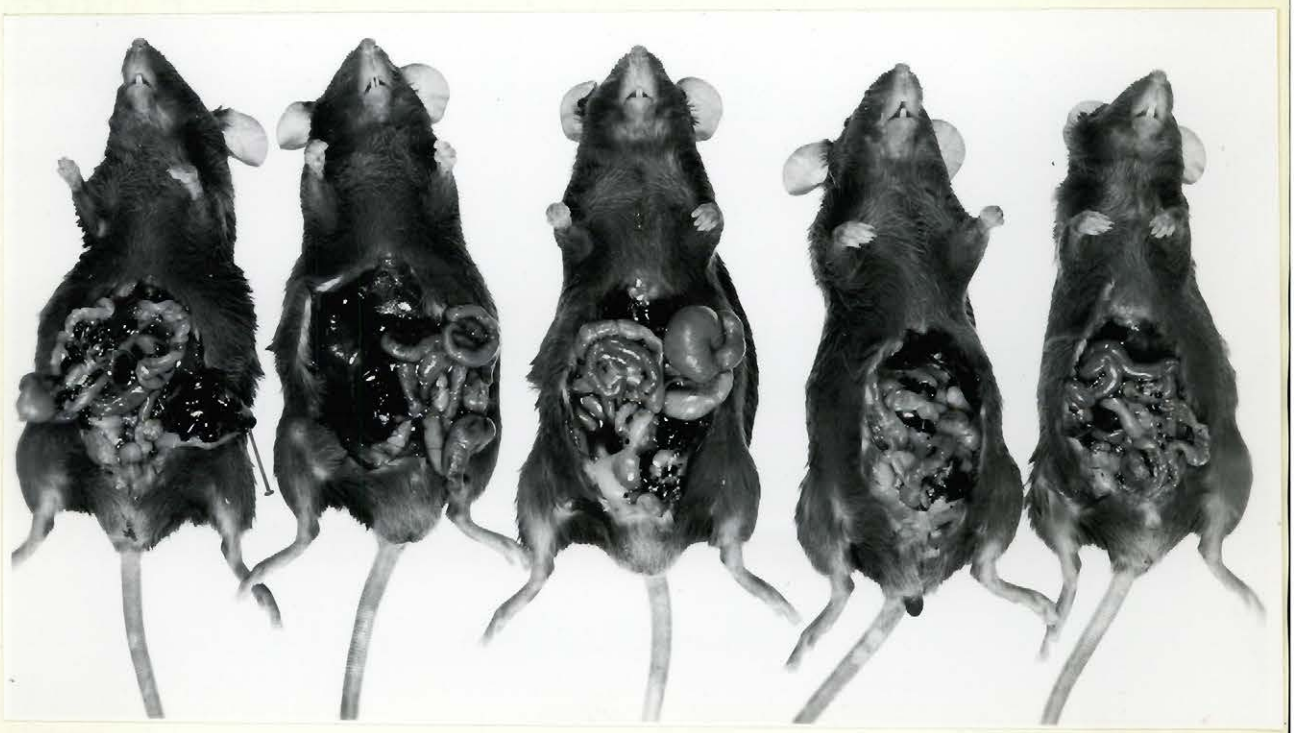


Fig. 122: DBA/1 mice treated with nitrogen mustard 1 hour after inoculation with S/91 melanoma intraperitoneally. 21 days - tumours grew but number fewer than in control.

Fig. 123: DBA/1 mice treated with nitrogen mustard 15 days after inoculation with S/91 melanoma intraperitoneally. Killed 5 days after nitrogen mustard. Growth of tumours as in normal.

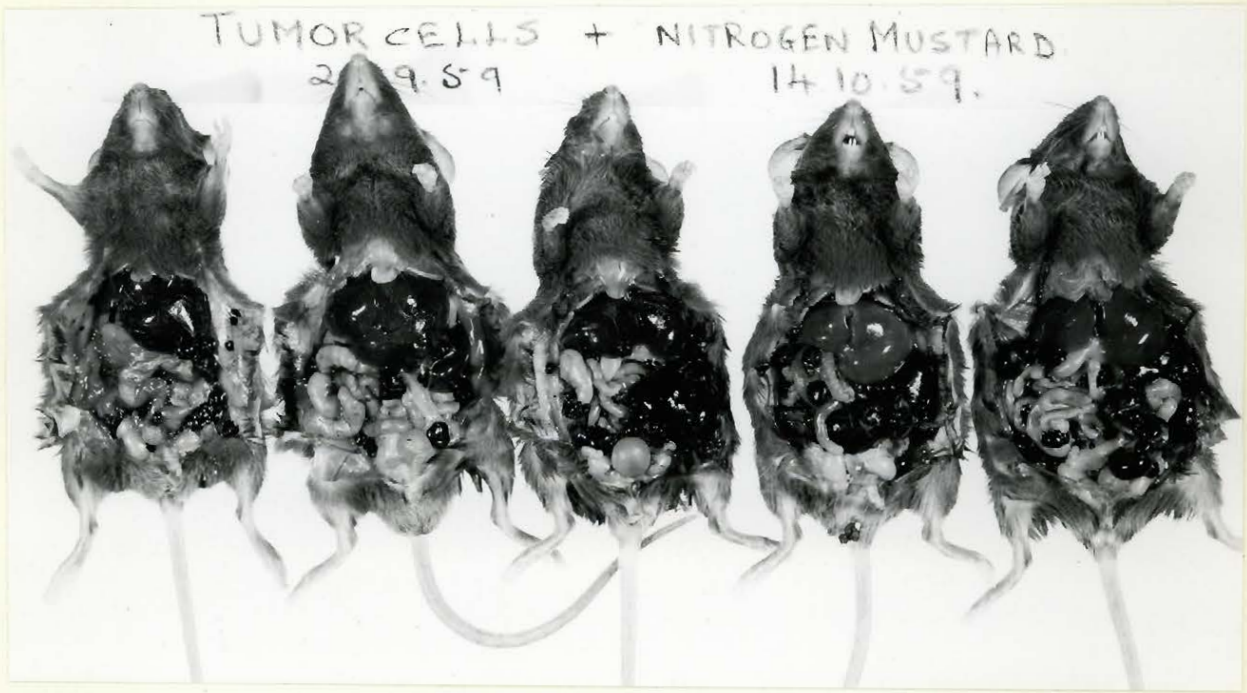


Fig. 124: DBA/1 mice treated with nitrogen mustard 15 days after inoculation with S/91 melanoma intraperitoneally. Killed 14 days after nitrogen mustard. Heavy growth of tumours as in control.

Table I: Tumours found in Rabbits following Injection of a Suspension of the Rabbit Carcinoma into the Portal Vein and Renal Artery

Rabbit	Survival in days	Volume injected into portal vein	Volume injected into renal artery	Number and size of metastases in Liver	Lungs	Spleen	Kidney side injected	Kidney other side	Remarks
39S	25	0.2 ml.	0.1 ml.	11 pin heads	-	-	-	-	
45S	23	"	"	21 23 mm	11 pin heads	-	-	-	
R3	67	"	"	8 12 mm	numerous - pin heads	-	4 2 mm	-	
R 9	46	"	"	5 2mm	numerous pin heads	-	-	-	
R15	90	"	"	3 1.5 mm	35 2.5 mm	-	-	-	
R16	22	"	"	riddled with tumours of diff. sizes biggest-28mm.	40 2.5 mm	3 25 mm	5 2.5 mm	-	
R32	27	"	"	*	30 2.5 mm	-	+	-	* A mass at site of injection + A mass size of hen's egg - due to leakage from injection
R33	60	"	"	-	23 4.5 mm.	-	-	-	

Table I - Cont'd

R36	26	0.2 ml.	0.1 ml.	67 2.5 mm	numerous pin heads	-	-	-
R36A	32	"	"	4 1.5 mm.	-	-	-	-
R38	45	"	"	-	-	2 2.5 mm	10 2 mm	4 1.5 mm
R40	70	"	"	4 pin head	numerous pin heads	-	1 big mass	9 2 mm
R41	30	"	"	5 pin head	numerous pin heads	-	-	-
R42	15	"	"	2 pin head	10 1-2.5 mm	-	-	-
R44	20	"	"	3 1-2 mm	9 1-2 mm	-	-	-
R47	45	"	"	148 1.5-2.5 mm	45 1.3 mm	-	22 2 mm	3 1.5 mm
R52	95	"	"	11 2.2 mm	numerous pin heads	-	-	-

Note: The tumours were mostly spherical. Where a figure is given as to the tumour size, it is the length of a diameter. Where only one figure is given, the tumours were all about the same size. Where there were too many tumours to count accurately, 'numerous' is noted.

Table II: Composition of Tumour Cell Suspension injected into the Rabbits. Cell Counts per cmm.

Rabbit	Single cells per c. mm.		Clumps per c. mm.	
	Viable	Non-viable	Small	Large
39S	1,000,000	5,000	1,000	200
45S	1,500,000	4,500	800	-
R3	1,250,000	3,000	1,200	200
R9	1,480,000	3,600	600	-
R15	1,000,000	2,200	600	200
R32	800,000	800	2,200	600
R33	800,000	800	2,200	600
R36	960,000	1,000	400	-
R36A	960,000	1,000	400	-
R38	400,000	600	200	-
R40	1,500,000	1,000	400	-
R41	1,000,000	800	600	200
R42	1,000,000	800	600	200
R44	900,000	4,200	460	200
R47	1,500,000	1,300	240	-
R52	1,800,000	2,200	580	100

Table III. Effect of Cortisone Acetate on Subcutaneous Implants of the V2 Rabbit Carcinoma

Rabbit	Strain	Weight	Method of Transplant	Cortisone	Survival	Findings at site of Inoculation	Metastases
R2	N.Z.	3.25 kg.	Trocar	nil	24 days	S.C. tumour, size of small hen's egg. Lung riddled with tumours	In lungs
R4	Grey-white	2.00 "	Trocar, L side Cells, R side	nil	30 "	S.C. tumour - 35 mm.,both sides	nil
R5	Grey-white	3.40 "	Trocar	nil	16 "	S.C. tumour - 25 mm.	nil
R6	N.Z.	2.95 "	Trocar, L side Cells, R side	nil	20 "	S.C. tumour - 28 mm.,both sides	nil
R7	N.Z.	3.86 "	Trocar, both sides	nil	15 "	S.C. tumour - 20 mm. rt. side 25 mm. lt. side	nil
R10	N.Z.	3.80 "	Trocar, L side Cells, R side	nil	16 "	S.C. tumour - 25 mm. rt. side 27 mm. lt. side	nil
R11	N.Z.	3.10 "	Trocar	nil	14 "	S.C. tumour - 10 mm.	nil
R 12	N.Z.	3.94 "	Trocar, L side Cells, R side	nil	21 "	Initially accompanied by inflammation. Controlled by pehicillin & streptomycin. Tumour - 30 mm. rt. side 20 mm. lt. side	nil
R13	N.Z.	4.17 "	Trocar	nil	13 "	Initially accompanied by inflammation. Controlled as before. Tumour - 15 mm.	nil
R17	N.Z.	3.85 "	Trocar, L side Cells, R side	nil	33 "	Tumour - 25 mm. left side, just palpable swelling on rt.	In lungs
R18	Grey-white	2.99 "	Trocar	nil	10 "	S.C. tumour felt Slight inflammatory reaction initially, controlled as before	nil

Table III - Cont'd

R30	N.Z.	4.70 kg.	Trocar, both sides	nil	105 days	Sloughing tumour on both sides	In liver & lungs
R33	N.Z.	1.70 "	Trocar, both sides	nil	120 "	Small inappreciable swelling on both sides	In lungs
R34	N.Z.	2.95 "	Trocar - L side Cells - R side	nil	120 "	Two tumours - size of cricket ball on either side. Left side slightly bigger than the right. Intrapelvic tumour on rt. side, size of a small tennis ball	In lungs
R35	N.Z.	2.90 "	Trocar, L side Cells, R side	nil	64 "	Sloughing tumours on both sides	In lungs
R48	N.Z.	2.15 "	Trocar	nil	60 "	Sloughing tumours on both sides	In lungs
R49	N.Z.	2.50 "	Trocar, L side Cells, R side	nil	6 months	Initial swelling regressed Animal died.	nil
R50	N.Z.	2.77 "	Trocar, L side Cells, R side	nil	6 "	Sloughing tumour on either side	In lungs
R51	N.Z.	2.88 "	Trocar, L side Cells, R side	nil	36 days	S.C. tumours - 35 mm. on right side 28 mm. on left side	In lungs and liver
R54	N.Z.	2.85 "	Trocar, L side Cells, R side	nil	32 "	Tumour size of 5 mm. on right side. Sloughing tumour on left side	In lungs
R57	N.Z.	3.25 "	Trocar, L side Cells, R side	nil	30 "	Sloughing tumour on both sides	

Table III - Cont'd

SR3	N.Z.	3.20 kg.	Trocar	2 mgm/kg. 3 weeks. daily	30 days	Two cystic swellings. 80 mm. on right side, and 65 mm. on left side. Dilated veins on the surface. Nodular feeling at places. Filled with thick porridgy fluid	In lungs
SR9	N.Z.	3.75 "	Trocar, L side Cells, R side	3 weeks daily	56 "	Autopsy - no tumour	
R28	N.Z.	2.56 "	Trocar both sides	3 weeks daily	25 "	No tumour	
R45	N.Z.	2.46 "	Trocar, L side Cells, R. side	3 weeks daily	31 "	No tumour	
R53	N.Z.	3.30 "	Cells,both sides	3 weeks daily	28 "	No tumour	
R8	N.Z.	3.85 "	Cells, both sides	3 weeks daily	60 "	No tumour	
R14	N.Z.	3.82 "	Trocar, L side Cells, R side	3 weeks daily	45 "	Right - 10 mm., Left - 8 mm.	In lungs, spleen, kidney
R21	N.Z.	1.75 "	Trocar, L side Cells, R side	3 weeks daily	120 "	A small swelling appeared on 7th day, subsided on giving penicillin & streptomycin injection.	In lungs
R29	N.Z.	3.45 "	Trocar, L Side Cells, R side	3 weeks daily	120 "	Sloughing tumours on both side. A tumour on right foreleg - 35 mm.	
R 46	N.Z.	2.70 "	Trocar, L side Cells, R side	3 weeks daily	120 "	No tumour	

Table VI. Number of Inflammatory Foci and Tumours in the Various Areas of the Mesentery at Various Times

Day	Mouse	Total No. of Leaflets	Inflamm. Foci without Tumour cells — Zones I	II	III	IV	Inflammatory Foci with Tumour Cells — Viable — Zones I	II	III	IV	Degenerated — Zones I	II	III	IV	Established Tumours — With Inflammat. — Zones I	II	III	IV	Without Inflammat. — Zones I	II	III	IV	Ink Dots — Zones I	II	III	IV
1	1	5	29	15	7	3					14	6	4	-												
	2	4	38	14	8	5					10	5	3	-												
	3	4	2	1	-	-					-	-	-	-												
	4	4	3	3	2	-					2	1	2	-												
2	5	4	34	9	4	-	1	1	-	-	8	-	-	-												
	6	3	3	1	-	-	-	-	-	-	-	-	-	-												
	7	5	44	12	12	-	3	-	-	-	3	2	2	-												
	8	4	3	3	-	-	-	-	-	-	1	-	-	-												
3	9	5	15	8	10	-	-	-	10	-	6	-	-	-												
	10	3	2	3	4	-	-	-	-	-	2	2	2	-												
	11	4	7	5	4	-	2	1	4	-	2	2	1	-												
	12	5	8	3	13	-	-	-	2	-	1	-	-	-												
5	13	9	6	3	7	3	-	-	4	-	1	1	-	-												
	14	5	27	10	3	-	2	3	3	-	2	3	-	-												
	15	6	10	2	4	1	-	-	4	1	1	1	-	-												
	16	5	15	5	7	2	2	1	2	1	1	3	-	-												

Table VI - Cont'd

			I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
7	17	4	16	9	3	-	-	-	3	-	6	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	6	1	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	19	8	32	36	20	3	-	25	20	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20	6	37	16	16	3	1	9	-	-	5	3	-	-	-	-	4	2	-	-	3	-	-	-	-	-
9	21	7	6	5	7	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	5	-	-	-	2	-
	22	7	4	3	13	1	-	-	-	-	2	2	-	-	-	-	10	-	-	-	3	1	-	-	1	-
	23	6	5	3	7	1	2	3	3	-	-	-	-	-	2	1	5	2	-	1	2	2	-	-	-	-
	24	7	3	3	5	-	-	-	2	1	-	-	-	-	4	2	7	1	1	-	3	2	-	-	-	-
12	25	5	6	7	17	4	-	2	-	-	-	-	-	-	-	4	15	-	-	-	-	-	-	-	-	-
	26	6	3	11	14	2	-	2	9	-	-	-	-	-	-	-	5	-	-	-	-	1	-	-	1	1
	27	6	5	7	7	1	-	-	-	-	-	-	-	-	3	2	7	2	2	3	2	1	-	1	3	1
	28	4	1	3	2	-	-	-	-	-	-	-	-	-	5	3	5	1	1	2	7	2	-	-	2	2
14	29	7	5	3	7	1	6	13	16	-	-	-	-	-	-	2	20	5	-	-	-	1	3	2	9	7
	30	8	7	5	3	-	-	-	8	-	-	-	-	-	-	2	16	-	2	1	-	3	1	-	3	1
	31	9	6	3	9	2	-	-	-	-	-	-	-	-	3	1	9	3	-	2	5	2	3	2	2	2
	32	5	9	3	7	3	2	5	5	1	-	-	-	-	-	2	5	2	1	3	7	3	-	3	3	-
17	33	9	5	3	-	-	4	2	2	-	1	4	-	-	-	1	2	-	6	3	30	9	-	-	19	31
	34	7	-	-	-	-	2	3	-	-	-	-	-	-	2	2	-	-	11	8	15	6	1	3	10	5
	35	8	-	-	-	-	-	-	2	-	-	-	-	-	5	3	-	-	6	5	22	8	3	3	13	9
	36	5	2	2	-	-	3	-	-	-	-	-	-	-	7	3	-	-	17	6	29	31	3	3	9	11

Table VI - Cont'd

			I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
21	37	5	-	-	-	-	1	-	-	-	-	2	-	-	-	-	1	-	8	5	12	3	3	6	12	5
	38	6	-	-	-	-	-	-	-	-	4	2	-	-	-	-	-	-	25	11	9	-	7	5	11	13
	39	8	-	-	-	-	3	1	-	-	-	-	-	-	2	-	-	-	10	13	12	5	7	4	7	15
	40	5	4	3	-	-	-	-	2	-	3	1	-	-	-	2	2	-	9	5	17	6	5	3	23	27
24	41	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	2	5	3	7	3	2	3	11	9
	42	6	-	-	-	-	2	3	-	-	1	2	-	-	1	-	-	-	7	5	5	5	1	-	7	10
	43	4	2	5	-	-	1	-	3	-	-	-	-	-	2	1	-	-	-	2	11	7	3	-	8	7
	44	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	5	18	21	-	4	30	45
25	45	7	10	7	2	1	3	-	-	-	-	-	-	-	3	2	-	-	7	11	15	13	3	3	17	14
	46	5	-	-	-	-	2	-	-	-	-	-	-	-	5	2	-	-	-	3	13	9	5	2	7	8
	47	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	2	3	4	23	7	1	3	14	29
	48	5	-	-	-	-	5	2	-	-	-	2	-	-	1	1	-	-	5	-	11	5	7	4	11	5
30	49	5	2	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	5	5	13	8	-	-	14	11
	50	4	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	3	2	3	2	2	-	31	34
	51	5	-	-	-	-	-	-	-	-	-	-	-	-	3	1	2	2	11	6	9	10	5	3	17	43
	52	5	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	1	9	2	18	11	11	2	36	29

Table VII. CONTROLS injected Intraperitoneally with 200,000 Tumour Cells per c.mm. - killed on 5th Day.

Mouse No.	Total No. of Leaflets	Inflammatory foci without tumour cells — Zones I	II	III	IV	Inflammatory foci with tumour cells — Viable Zones I	II	III	IV	Degenerated Zones I	II	III	IV	Established Tumours with Inflammation Zones I	II	III	IV	without Inflammation Zones I	II	III	IV	Ink Dots Zones I	II	III	IV
301	(7	21	9	-	-	7	9	7	-	23	13	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	(7	12	8	-	-	14	21	9	-	21	11	2	-	-	1	-	-	-	-	-	-	-	-	-	-
302	(8	19	13	2	-	6	12	11	1	24	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(7	79	49	16	3	34	46	40	18	61	38	8	-	-	-	-	-	-	-	-	-	-	-	-	-
303	(6	33	22	5	-	11	12	14	1	35	12	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	(6	41	19	3	-	11	14	15	5	34	25	2	-	-	-	-	-	-	-	-	-	-	-	-	-
304	(8	7	2	1	-	1	1	12	2	37	23	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	(7	7	4	1	-	1	7	29	4	24	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
305	(6	5	7	2	-	6	5	11	3	7	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(5	4	8	3	1	7	3	6	2	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table VIII. Cortisone 1 mgm. Subcutaneously for 7 Days - on the 8th Day Tumour Cells 200,000 per c. mm. Injected Intraperitoneally and Cortisone Continued Daily - killed on the 5th Day

Mouse No.	Total No. of Leaflets	Inflammatory foci without tumour cells Zones I	II	III	IV	Inflammatory foci with tumour cells Viable Zones I	II	III	IV	Degenerated Zones I	II	III	IV	Established Tumours with Inflammation Zones I	II	III	IV	without Inflammation Zones I	II	III	IV	Ink Dots Zones I	II	III	IV
306	6	10	6	3	-	1	1	1	-	26	18	11	-	-	-	-	-	-	-	-	-	-	-	-	-
	8	7	5	2	-	-	-	-	-	9	13	13	-	-	-	-	-	-	-	-	-	-	-	-	-
307	6	-	-	-	-	1	-	2	-	7	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	1	-	7	5	30	-	-	-	-	-	-	-	-	-	-	-	-	-
308	7	-	-	-	-	1	-	2	-	9	1	9	1	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	1	-	1	1	7	1	-	-	-	-	-	-	-	-	-	-	-	-
309	8	-	-	-	-	-	-	-	-	2	5	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	2	1	6	1	-	-	-	-	-	-	-	-	-	-	-	-
310	11	-	-	-	-	-	-	-	-	4	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-

REMARKS: Weight at the beginning of the experiment: average 22 gms.; after one week of cortisone - average weight 21.5 gms.

Table IX. Injected Intraperitoneally with 200,000 Tumour Cells per c.mm. - 1 Hour Later Injected with 'Mustargen' 0.5 mgm. per kg. of body weight - killed on the 5th Day.

Mouse No	Total No. of Leaflets	Inflammatory foci without tumour cells Zones I	II	III	IV	Inflammatory foci with tumour cells Viable Zones I	II	III	IV	Degenerated Zones I	II	III	IV	Established Tumours with Inflammation Zones I	II	III	IV	without Inflammation Zones I	II	III	IV	Ink Dots Zones I	II	III	IV
311	(8	-	-	-	-	-	-	-	-	1	4	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	(9	-	-	-	-	-	-	-	-	40	10	4	-	-	-	-	-	-	-	-	-	-	-	-	-
312	(12	-	-	-	-	-	-	-	-	16	10	7	1	-	-	-	-	-	-	-	-	-	-	-	-
	(6	-	-	-	-	-	-	-	-	29	6	2	-	-	-	-	-	-	-	-	-	-	-	-	-
313	(6	-	-	-	-	-	-	-	-	16	10	8	-	-	-	-	-	-	-	-	-	-	-	-	-
	(7	-	-	-	-	-	-	-	-	12	6	5	-	-	-	-	-	-	-	-	-	-	-	-	-
314	(9	-	-	-	-	-	-	-	-	11	3	9	-	-	-	-	-	-	-	-	-	-	-	-	-
	(6	-	-	-	-	-	-	-	-	1	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-
315	(9	-	-	-	-	-	-	-	-	19	6	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	(8	-	-	-	-	-	-	-	-	14	3	4	-	-	-	-	-	-	-	-	-	-	-	-	-

REMARKS: In most of the places there is no inflammatory focus; degenerated tumour cells are scattered around. There are many macrophages. No loss in weight.

Table X. Controls Injected Intraperitoneally with 200,000 Tumour Cells per c.mm. Killed on the 10th Day

Mouse No.	Total No. of Leaflets	Inflammatory foci without tumour cells Zones I	II	III	IV	Inflammatory foci with tumour cells Viable Zones I	II	III	IV	Degenerated Zones I	II	III	IV	Established Tumours with Inflammation Zones I	II	III	IV	without Inflammation Zones I	II	III	IV	Ink Dots Zones I	II	III	IV
316	6	13	9	-	-	25	16	4	1	4	-	-	-	10	9	7	2	2	2	-	-	-	-	-	-
	7	11	5	2	-	10	17	15	3	6	4	-	-	7	8	5	4	2	-	-	-	-	-	-	-
317	7	8	6	-	-	6	7	7	-	2	1	-	-	7	5	6	1	-	-	1	-	-	-	-	-
	5	-	1	-	-	-	3	4	-	2	-	-	-	3	3	3	-	1	1	1	-	-	1	-	-
318	7	21	20	1	-	36	41	19	3	1	-	-	-	6	10	5	1	-	1	-	-	-	1	1	-
	7	17	14	2	-	27	40	22	10	-	-	-	-	4	17	7	1	-	-	-	-	1	-	-	-
319	9	3	1	-	-	14	5	8	2	-	-	-	-	19	6	9	1	8	1	-	-	-	-	1	-
	7	1	1	-	-	2	2	6	-	1	-	-	-	4	4	3	1	2	3	4	3	-	-	-	-
320	8	-	-	-	-	8	3	6	-	8	2	-	-	14	3	13	2	15	6	6	5	-	-	-	-
	7	-	-	-	-	2	3	3	-	2	6	-	-	-	1	3	1	-	-	2	1	-	-	-	-

REMARKS: A few black tumours were present on the fat and on the intestines.

Table XI. Cortisone 1 mgm. Subcutaneously for 7 Days - on the 8th Day Tumour cells 200,000 per c.mm. Injected Intraperitoneally and Cortisone Continued Daily. Killed on the 10th Day

Mouse No.	Total No.of Leaf-lets	Inflammatory foci without tumour cells Zones I	II	III	IV	Inflammatory foci with tumour cells Viable Zones I	II	III	IV	Degenerated Zones I	II	III	IV	Established Tumours with Inflammation Zones I	II	III	IV	without Inflammation Zones I	II	III	IV	Ink Dots Zones I	II	III	IV
321	(7	-	-	-	-	1	-	-	-	4	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	(10	-	-	-	-	-	-	1	-	4	5	2	-	-	-	-	-	-	-	-	-	-	-	-	-
322	(10	-	-	-	-	-	-	-	-	7	7	2	-	-	-	-	-	-	-	-	-	-	-	-	-
	(8	-	-	-	-	-	-	-	-	9	4	7	1	-	-	-	-	-	-	-	-	-	-	-	-
323	(10	-	-	-	-	2	-	-	-	10	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(10	-	-	-	-	-	1	-	-	7	4	6	1	-	-	1	-	-	-	7	-	-	-	-	-
324	(4	-	-	-	-	-	-	-	-	9	11	5	-	-	-	-	-	-	-	-	-	-	-	-	-
	(6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
325	(9	-	-	-	-	-	-	-	-	4	6	1	-	-	-	-	-	-	-	3	-	-	-	-	-
	(10	-	-	-	-	-	-	-	-	7	3	6	-	-	-	-	-	-	1	5	1	-	-	1	-

REMARKS: Besides these localized lesions, degenerating tumour cells were seen scattered on the surface of all areas.
Average weight - 21 gms.

Table XII. Injected Intraperitoneally with 200,000 Tumour Cells per c. mm. – 1 Hour later injected with 'Mustargen' 0.5 mgm. per kg. of Body Weight. Killed on the 10th Day

Mouse No.	Total No. of Leaflets	Inflammatory foci without tumour cells Zones I	II	III	IV	Inflammatory foci with tumour cells Viable Zones I	II	III	IV	Degenerated Zones I	II	III	IV	Established Tumours with Inflammation Zones I	II	III	IV	without Inflammation Zones I	II	III	IV	Ink Dots Zones I	II	III	IV
326	(11	5	1	–	–	5	7	3	–	37	14	7	2	–	–	–	–	–	–	1	–	–	–	–	–
	(8	7	1	–	–	4	–	3	–	13	2	6	–	–	–	–	–	–	–	–	–	–	–	–	–
327	(8	–	–	–	–	1	–	5	–	4	1	10	–	–	–	–	–	–	–	1	–	–	–	–	–
	(6	11	4	–	–	3	1	4	–	7	3	6	–	–	–	–	–	–	–	–	–	–	–	–	–
328	(9	13	7	–	–	5	2	1	–	11	4	3	–	–	–	–	–	–	–	–	–	–	–	–	–
	(5	21	6	–	–	2	2	–	–	4	3	1	–	–	–	–	–	–	–	–	–	–	–	–	–
329	(9	13	11	–	–	8	5	2	–	31	20	4	–	2	–	–	–	–	–	–	–	–	–	–	–
	(8	–	1	–	–	3	2	2	–	7	4	2	–	–	–	–	–	–	–	–	–	–	–	–	–
330	(11	–	–	–	–	1	4	3	–	5	3	2	1	–	–	–	–	–	–	–	–	–	–	–	–
	(7	–	–	–	–	5	4	4	–	6	7	4	1	–	–	–	–	–	–	4	–	–	–	–	–

REMARKS: Besides these lesions, degenerating tumour cells were seen scattered on the surfaces of the leaves. Some lesions were seen round vacuolated areas, and dense, coarse pink staining material was noted in some places.

Table XIII. Controls Injected Intraperitoneally with 200,000 Tumour Cells per c. mm. Killed on the 15th Day

Mouse No.	Total No. of Leaflets	Inflammatory foci without tumour cells, Zones I	II	III	IV	Inflammatory foci with tumour cells, Viable, Zones I	II	III	IV	Degenerated, Zones I	II	III	IV	Established Tumours with Inflammation, Zones I	II	III	IV	without Inflammation, Zones I	II	III	IV	Ink Dots, Zones I	II	III	IV
331	(9	9	2	-	-	4	2	2	-	1	4	-	-	-	1	2	-	6	3	30	11	-	-	21	36
	(8	2	2	-	-	2	2	-	-	1	-	-	-	1	3	-	-	2	11	36	16	-	6	23	34
	(5	2	1	-	-	1	-	-	-	-	-	-	-	4	3	-	-	-	6	17	10	1	-	9	12
332	(10	-	-	-	-	3	2	-	-	10	2	-	-	10	3	-	-	18	19	59	13	4	8	29	25
	(8	-	-	-	-	4	7	-	-	1	1	-	-	-	1	-	-	6	3	31	6	3	3	8	8
333	(8	-	-	-	-	3	-	-	-	-	-	-	-	7	2	-	-	17	8	29	33	12	9	15	11
	(6	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	2	4	-	5	2	5	2
	(9	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	4	2	7	-	5	1	15	11
334	(7	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	4	1	1	1	-	-	4	9
	(8	-	-	-	-	2	1	-	-	3	-	-	-	2	2	-	-	14	14	15	6	8	4	17	20
335	(7	-	-	-	-	1	-	-	-	3	-	-	-	4	2	-	-	11	8	7	1	1	-	10	2
	(7	9	4	-	-	3	3	-	-	1	-	-	-	1	3	-	-	4	7	22	6	3	3	16	2
	(7	-	-	-	-	3	2	1	-	2	1	-	-	6	9	10	2	-	1	12	4	-	1	2	-

REMARKS: Numerous tumours of different sizes almost filling the whole abdomen, also on inner side of abdominal wall. Several on guts. In 335 a big mass on the left side of the abdomen. Many scattered ones but comparatively fewer than others. Only 2 tumours on the gut.

Table XIV. Cortisone 1 mgm. Subcutaneously for 7 Days - on the 8th Day Tumour Cells 200,000 per c. mm. Injected Intraperitoneally and Cortisone Continued Daily. Killed on the 15th Day.

Mouse No.	Total No. of Leaflets	Inflammatory foci without tumour cells Zones I	II	III	IV	Inflammatory foci with tumour cells Viable Zones I	II	III	IV	Degenerated Zones I	II	III	IV	Established Tumours with Inflammation Zones I	II	III	IV	without Inflammation Zones I	II	III	IV	Ink Dots Zones I	II	III	IV
336	7	-	-	-	-	1	-	2	-	3	5	1	-	-	-	-	-	-	-	1	-	-	-	-	-
	11	-	-	-	-	1	-	1	-	6	2	2	-	1	-	-	-	-	-	-	-	-	-	-	-
337	8	-	-	-	-	1	-	1	-	6	1	5	-	-	-	3	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
338	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
339	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	-	-	-	2	-	7	-	-	3	5	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	1	-	3	-	10	4	2	-	-	-	-	-	-	-	-	-	1	-	3	-	-	-	-	-

REMARKS: Cells diffusely distributed and clumping of cells
Very few cells on the leaves. Marked rarefaction in the Zones III & IV.
Degenerating tumour cells scattered diffusely
In 4 mice 11 small tumours on the fat and intestine
One mouse died.
Average Weight - 20.5 gms.

Table XV. Injected Intraperitoneally with 200,000 Tumour Cells per c. mm. - 1 Hour later Injected with 'Mustargen' 0.5 mgm. per kg. of Body Weight. Killed on the 15th Day

Mouse No.	Total No. of Leaflets	Inflammatory foci without tumour cells Zones I	II	III	IV	Inflammatory foci with tumour cells Viable Zones I	II	III	IV	Degenerated Zones I	II	III	IV	Established Tumours with Inflammation Zones I	II	III	IV	without Inflammation Zones I	II	III	IV	Ink Dots Zones I	II	III	IV
341	(10	-	-	-	-	9	5	3	-	15	9	4	-	15	14	9	3	3	1	-	-	-	-	-	-
	(11	-	-	-	-	10	6	2	-	16	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
342	(8	-	-	-	-	1	-	-	-	5	1	1	-	-	-	2	-	3	1	-	1	-	-	-	-
	(9	1	1	-	-	1	-	-	-	1	1	-	-	-	-	-	-	-	-	3	2	3	1	1	-
343	(7	11	6	1	-	-	1	9	-	6	4	1	-	-	-	-	-	1	-	-	-	-	-	-	-
	(8	29	9	-	-	-	-	-	1	4	3	1	-	-	-	3	-	-	-	2	-	-	-	-	-
	(6	-	-	-	-	1	-	-	-	1	1	-	-	-	-	6	5	-	-	1	-	-	-	-	-
344	(6	9	3	-	-	-	2	1	-	5	2	1	-	1	-	8	1	-	-	9	-	-	-	4	-
	(7	3	1	-	-	-	1	-	-	1	1	5	-	-	-	5	-	-	-	-	-	-	-	1	-
345	(4	-	1	1	-	-	2	-	-	3	3	2	-	-	-	2	-	2	-	2	-	-	-	3	1
	(7	-	-	-	-	1	-	1	-	9	3	3	-	-	-	2	-	2	-	5	2	1	-	1	1
	(8	-	-	-	-	-	-	-	-	3	3	-	-	1	-	1	-	1	-	5	5	1	-	8	3

REMARKS: Degenerating tumour cells scattered without proper localization
In all the mice tumours present in the abdomen.
In 341: a tumour on large gut, size 2 x 3 mm.
In 342: around spleen 3, stomach 2. Gut wall 12, of different sizes, biggest pea size.
In 343: spleen and gut wall - 14. Large gut - 5.
In 344: perisplenic tissue - 12. Gut wall - 8. Mesentery - 10. Rectal wall - 2.
In 345: A big one size 10x5 mm.in between the loops of upper gut,around spleen 5,gut wall 11,mesentery 5.

Table XVI. Controls Injected Intraperitoneally with 200,000 Tumour Cells per c. mm. Killed on the 21st Day

Mouse No.	Total No. of Leaflets	Inflammatory foci without tumour cells Zones I	II	III	IV	Inflammatory foci with tumour cells Viable Zones I	II	III	IV	Degenerated Zones I	II	III	IV	Established Tumours with Inflammation Zones I	II	III	IV	without Inflammation Zones I	II	III	IV	Ink Dots Zones I	II	III	IV
346	8	-	-	-	-	-	-	-	-	3	2	-	-	-	-	1	-	5	5	12	1	4	6	3	3
	7	-	-	-	-	-	-	-	-	3	1	-	-	1	-	-	-	8	8	5	1	3	4	12	44
347	7	-	-	-	-	-	-	-	-	3	1	-	-	-	-	-	-	10	4	5	4	2	2	15	30
	10	-	-	-	-	-	-	-	-	10	3	-	-	1	1	-	-	30	14	30	11	7	14	80	61
348	8	-	-	-	-	-	-	-	-	4	2	-	-	-	-	-	-	27	10	12	5	27	11	47	65
	7	-	-	-	-	-	-	-	-	9	6	2	-	-	-	-	-	7	1	6	-	3	5	4	13
349	6	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	6	3	5	-	2	1	18	23
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	5	1	-	7	5	7	15
350	6	-	-	-	-	-	-	-	-	5	2	--	-	-	-	-	-	13	10	13	3	12	5	28	31
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	2	-	-	1	-	8	17

REMARKS: Numerous black tumours were present in the abdominal cavity - tumours were of different sizes, biggest being the size of a marble. (Fig. 120).

Table XVII. Cortisone 1 mgm. Subcutaneously for 7 Days - on the 8th Day Tumour Cells 200,000 per c.mm. Injected Intraperitoneally and Cortisone Continued Daily. Killed on the 21st Day

Mouse No.	Total No. of Leaflets	Inflammatory foci without tumour cells Zones I	II	III	IV	Inflammatory foci with tumour cells Viable Zones I	II	III	IV	Degenerated Zones I	II	III	IV	Established Tumours with Inflammation Zones I	II	III	IV	without Inflammation Zones I	II	III	IV	Ink Dots Zones I	II	III	IV
356	(10	-	-	-	-	-	-	-	-	16	4	3	-	-	-	-	-	6	1	6	2	-	-	8	9
	(9	1	-	-	-	-	-	-	-	17	5	5	-	-	1	-	-	3	1	2	-	-	-	1	-
357	(8	-	-	-	-	-	-	-	-	13	6	3	-	-	-	1	-	1	1	3	2	-	-	3	4
	(7	-	-	-	-	-	-	-	-	21	6	6	3	-	-	-	-	1	-	11	1	-	-	1	-
358	(9	-	-	-	-	-	-	-	-	17	3	10	5	-	-	-	-	2	3	22	8	-	-	-	1
	(12*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
359	(6	-	-	-	-	-	-	-	-	5	3	-	-	-	-	-	-	2	1	5	1	-	-	6	2
	(8	-	-	-	-	-	-	-	-	13	4	-	-	-	-	-	-	1	-	1	-	-	-	1	-
360	(7	1	-	-	-	-	-	-	-	11	2	-	-	1	1	-	-	3	-	-	-	4	-	-	2
	(10	-	-	-	-	-	-	-	-	39	7	6	-	-	-	-	-	4	-	1	-	-	-	3	1

REMARKS: *In this slide multiple degenerated cells scattered all over. No localization. Preparations very clear; surface of the leaflets much less cellular than in controls, especially in areas III & IV. Very few tumours in the abdominal cavity; on first sight at opening some mice appeared almost tumour-free. Most of the gut coils free of apparent tumours. Largest tumour pea sized (Fig. 121).

Table XVIII. Injected Intraperitoneally with 200,000 Tumour Cells per c. mm. - 1 Hour later Injected with 'Mustargen' 0.5 mgm.per kg. of Body Weight. Killed on the 21st Day

Mouse No.	Total No.of Leaf-Lets.	Inflammatory foci without tumour cells Zones I	II	III	IV	Inflammatory foci with tumour cells Viable Zones I	II	III	IV	Degenerated Zones I	II	III	IV	Established Tumours with Inflammation Zones I	II	III	IV	without Inflammation Zones I	II	III	IV	Ink Dots Zones I	II	III	IV
351	(6	1	-	-	-	1	1	1	-	15	8	3	-	1	1	4	-	-	2	1	-	1	2	2	1
	(6	1	-	-	-	-	-	-	-	3	-	1	-	-	-	-	-	9	5	7	1	3	1	13	10
352	(9	1	-	-	-	-	-	-	-	9	5	-	1	-	-	2	-	3	-	-	-	5	1	2	2
	(6	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	5	3	2	-	2	2	15	40
353	(5	-	-	-	-	-	-	-	-	26	10	6	-	7	1	-	-	7	7	19	3	1	2	14	11
	(11	1	-	3	-	-	-	-	-	7	1	5	1	-	-	1	-	1	-	5	-	-	1	3	-
354	(8	-	-	-	-	-	-	-	-	8	5	1	-	-	-	-	-	1	1	-	-	-	2	12	25
	(9	-	-	-	-	-	-	-	-	4	3	3	2	-	-	-	-	2	-	5	-	1	-	3	12
355	(8	-	-	-	-	-	-	-	-	16	4	3	-	1	-	-	-	3	1	7	7	2	4	14	23
	(5	-	-	-	-	-	-	-	-	5	2	3	1	-	-	2	-	2	1	4	3	3	2	11	17

REMARKS: Vascularization around developing tumours very marked. Black tumours filling the open surface of the abdomen. They were quite numerous, but were less so than in the controls and were smaller. Scattered highly pigmented, irregular cells on the surface of the leaflets and in irregular clumps. Areas III & IV very much less cellular than in controls (Fig. 122).

Table XIX. Injected Intraperitoneally with 200,000 Tumour Cells per c. mm. - Challenged with 'Mustargen' 0.5 mgm. per Kg. of Body Weight on the 15th Day. Killed 5 Days after Injection of Mustard

Mouse No.	Total No. of Leaflets	Inflammatory foci without tumour cells Zones I	II	III	IV	Inflammatory foci with tumour cells Viable Zones I	II	III	IV	Degenerated Zones I	II	III	IV	Established Tumours with Inflammation Zones I	II	III	IV	without Inflammation Zones I	II	III	IV	Ink Dots Zones I	II	III	IV
361	(7	-	-	-	-	-	-	-	-	1	-	-	-	7	1	7	-	33	5	41	30	2	1	20	9
	(8	-	-	-	-	-	-	-	-	4	2	-	-	-	-	-	-	35	13	12	4	7	5	48	23
	(6	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	18	10	11	3	4	5	45	28
362	(7	9	-	-	-	2	-	-	-	-	-	-	-	4	-	-	-	13	7	32	14	3	2	36	29
	(5	-	-	-	-	-	-	-	-	5	2	9	7	-	-	-	-	4	6	-	1	1	1	9	13
	(9	-	1	-	-	-	-	-	-	7	-	-	-	1	1	1	-	-	-	-	-	-	-	1	-
363	(11	6	2	2	-	-	-	-	-	6	-	1	-	5	-	-	-	-	-	2	1	-	-	11	-
	(7	-	-	-	-	-	-	-	-	15	8	16	12	1	-	-	-	6	2	4	-	1	3	19	27
	(5	-	-	-	-	-	-	-	-	8	2	-	-	-	-	-	-	3	7	3	1	-	1	14	37
364	(8	-	-	-	-	-	-	-	-	21	2	12	8	1	1	-	-	4	2	28	41	2	-	17	19
	(8	3	1	-	-	2	-	-	-	10	3	-	2	-	3	-	-	12	11	18	21	-	4	30	103
365	(6	-	-	-	-	-	-	-	-	7	2	-	-	-	-	-	-	12	7	3	-	11	3	31	34
	(7	-	-	-	-	-	-	-	-	21	6	-	-	-	-	-	-	9	6	14	11	3	2	15	32

REMARKS; Abdomen nearly filled with black tumours. The picture almost same as that of a control of same date (Fig. 123).

Table XX. Injected Intraperitoneally with 200,000 Tumour Cells per c. mm. - Challenged with 'Mustargen' 0.5 mgm. per kg. of Body Weight on the 15th Day. Killed 14 Days after Injection of Mustard

Mouse No.	Total No. of Leaflets	Inflammatory foci without tumour cells Zones I	II	III	IV	Inflammatory foci with tumour cells Viable Zones I	II	III	IV	Degenerated Zones I	II	III	IV	Established Tumours with Inflammation Zones I	II	III	IV	without Inflammation Zones I	II	III	IV	Ink Dots Zones I	II	III	IV
366	(8	4	5	-	-	30	24	4	-	18	11	2	-	20	12	11	-	4	2	5	1	-	-	2	2
	(7	-	-	-	-	21	9	1	-	13	8	-	-	19	14	4	-	24	12	10	2	3	-	1	2
367	(8	-	-	-	-	-	-	-	-	12	6	2	1	1	2	-	-	9	9	11	-	5	4	6	5
	(9	-	-	-	-	-	-	-	-	3	6	5	-	1	-	-	-	4	9	9	-	1	2	10	7
368	(9	-	-	-	-	-	-	-	-	30	12	17	-	-	-	-	-	44	19	8	1	14	9	43	73
	(7	-	-	-	-	-	-	-	-	5	4	-	-	-	-	-	-	33	13	-	-	2	5	19	60
369	(7	-	-	-	-	-	-	-	-	6	3	-	-	-	-	-	-	7	5	2	-	-	-	4	27
	(6	-	-	-	-	-	-	-	-	10	9	3	1	5	-	-	-	1	2	12	-	-	-	17	67

REMARKS: Pictures depict the heavy growth of the tumours (Fig. 124).

The remaining mice died.