THE ABSORPTION OF ANTIBODIES IN VITRO BY MONOCYTES (LARGE MONONUCLEAR LEUCOCYTES).

by

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A Thesis

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INTRODUCTION

In order that the reader may understand the purpose of this work, it is necessary to review briefly the circumstances leading to the present investigation.

The experiments, herein described, were carried out in an attempt to prove, whether or not, monocytes may store antibodies, or at least absorb them, in vitro. This present work is then a step in the determination of the role played by monocytes in the immune response.

In 1952 Girard (54) improved a media to give the optimal conditions of growth for Listeria monocytogenes. From organisms cultured in this medium, he isolated the Monocytosis-Producing Agent. With this agent, he was able to induce and maintain for a period of 20 days a marked monocytosis (1500-3500 cells per c.mm.) in the circulating blood of rabbits. This crude MPA is neither antigenic nor toxic for rabbits. He found that rabbits in this induced state produced antisera against the following antigens: S. typhosa, Staphylococcal toxoid and Horse serum whose titers were twice, 4 or even 8 times greater than the titers of antiserum produced against the same antigens in normal rabbits. He also showed that macrophage exudates, obtained by the intrapleural injections

of a gum-arabic-beef extract mixture, contain antibodies in higher concentration within the exudate cells than in the supernatant fluid in actively or passively immunized rabbits in a state of induced monocytosis. For the active immunization he used the following antigens: S. typhosa, Staphylococcal toxoid, Horse serum and for passive immunization, he used a Listeria monocytogenes rabbit antiserum.

From his results he concluded that antibodies may be formed somewhere in the organism and later on locally concentrated within the monocytes.

Taking into consideration the facts brought to light by the work of Girard, an investigation was undertaken to determine:

a) whether or not antibodies are absorbed by monocytes in vitro and

b) whether or not the absorbed antibodies are concentrated within these cells.

An exudate very rich in macrophages was obtained by intrapleural injection of a mixture of gum-arabic and beef extract in rabbits in a state of monocytosis induced with MPA. The monocytes were washed and resuspended in a preservation media. They were then placed in contact with various dilutions of antiserum, incubated over definite periods of time and titrated to find out whether or not any antibody was absorbed by them. The capacity of monocytes

to concentrate antibodies was determined by breaking these cells by the osmotic pressure method, titrating the supernatant fluid and the residual cell sediment.

HISTORICAL STATEMENT

1 - ORIGIN OF THE MONOCYTE

The origin of the monocyte is, despite many scientific studies, a subject of controversy which has lasted for years and years and which, even in 1953, has not yet been settled. A leading school, holding the Reticulo-Endothelial System origin of the monocyte, is supported by many investigators but there are also other hypotheses concerning the origin. This present discussion chiefly concerns the findings governing the Reticulo-Endothelial System theory, but, nevertheless, the hypotheses concerning the origin of the monocyte will also be discussed, although on a smaller scale.

The extension of the word "monocyte" is limited, for the needs of this thesis, to the monocyte found in the blood and to the wandering macrophages found in the exudate. The reason evoked to name the monocyte of the blood and free macrophage of the exudate "monocyte" is that they are very closely related and, for some authors, the same thing. (34-41-43)

In the course of this present historical survey, if peculiarities or properties special to one of these two cells are discussed, the term "blood monocyte" or "macrophage" will be used according to the case.

As we go along in the review of the literature on the macrophage, we may become confused at times, due to the various names used by the investigators to describe what is thought to be the same cell. What is the reason for so many terms in the designation of what appears to be the same cell? It seems that, at different periods of time, different investigators studied one particular function in detail of that cell and gave to the cell a name describing the function studied.

The macrophage, as named by Metchnikoff and by the Immunologists in general, or the histiocytes as the Histologists named them, were described through the course of years under the name of clasmatocytes by Ranvier to describe their property of shedding, breaking off part of their body. They were also named rhagiocrine cells, histocyte advential cells, Reticulo-Endothelial cells, Maximow's polyblast, Pyrrhol cells or phagocytic mononuclear cells of the blood stream, when the latter is considered to be identical to the macrophage. For Taliafferro the macrophage is "any large mononuclear cell which is not markedly basophile and can become phagocytic without essential change in morphology". (42-46-53-55)

For some authors, the differences between the blood monocyte and the free macrophage are non-existant

non-existant and the ones which are established by some investigators are based upon slight changes in the morphology according to the stimuli involved.

Today, it is generally accepted that the monocyte derives from the Reticulo-Endothelial cell and, consequently, belongs to the Reticulo-Endothelial System. The main points of discussion are the site of origin and the phase at which differentiation of the first free cells becomes sufficiently fixed to allow of nothing but the formation of specific type cells. (64)

This section will include:

a) Concepts of the Reticulo-Endothelial System
b) General view on the embryogenesis of the Reticulo-Endothelial System
c) The situation of the monocyte in the Reticulo-Endothelial System
d) The different theories involved in the formation and maturation of the monocyte.

a) <u>Concepts of the Reticulo-Endothelial System</u>

Oberling considers the Reticulo-Endothelial System as a kind of a wide syncytium that sent its expansions into all the parts of the organisms; circulating blood included. (56)

The members of that system possessing in

common the function of phagocytosis have, as a corollary of that, affinity for acid colloidal dyes. (62)
The affinity for dyes possessed by the members of the system was recognized as early as 1869 by Ponfeck. (61)
It is not necessary, in the course of this study, to give a complete historical survey on the Reticulo-Endothelial System. Nevertheless, it may be mentioned that it was Goldman who tried to group those elements capable of fixing aniline derivatives and pyrrhol blue. The notion of the Reticulo-Endothelial System was proposed by Aschoff and his school to show that the members of that system had physiological functions and potentitalities in commoh.

Later on, other authors brought froward further properties possessed by that system such as its role in the metabolism of the different constituents of the body.

It is not the morphological or histological properties of the members that constitute the unity of the Reticulo-Endothelial System but rather the physiological properties. (55-61)

b) Embryogenesis of the Reticulo-Endothelial System

The Reticulo-Endothelial cells originate from the loose connective tissue and in the embryo from the mesenchymal cells that give rise to the free basophil cells which, in turn, produce all types of blood cells. At this

stage, the controversy upon the origin of the monocyte takes place and gives rise to the different theories. (26-53-57)

c) The Situation of the Monocyte in the Reticulo-Endothelial System

The Ascoff's system may be divided in two sections, the Reticulo-Endothelial System in its broad and in its restricted sense. The monocytes discussed are the tissue histocyte or macrophage and the blood monocyte, both of them belonging to the wandering histocytes, that is, to the Reticulo-Endothelial System in its broad sense, and derives as it has been seen, from the Reticular cells of the connective tissues. (46-57-61)

These cells are also the macrophage of Mechnikoff. (58)

In the lymphoid macrophage system of Taliaferro, the monocytes, although differenciated in the phagocytic direction, are related to thelymphocytes. (37-46-53-61-62)

d) The different theories involved in the formation and maturation of the monocyte.

Most investigators agree that a stem cell gives rise to the monocyte but the discussion takes place when the time comes to establish the steps involved in the maturation of that stem cell. The nature of the stem cell

is also a subject of controversy.

There are two main theories in hematology concerned with the origin of the blood cell: the monophyletic theory and thepolyphyletic theory. Before discussing those, it is interesting to mention that in the early centuries or before, the blood monocyte was thought to be an intermediary form between the lymphatic and myeloid system (Virchow) or to be a transitional form, of myeloid origin, between the agranulocytic and granulocytic elements (Ehrlich). Zielger (1891) and Marchand also thought that the fibroblast was the cell that gives rise to the monocyte. (20)

Then came the monophyletic and polyphyletic theories. In the monophyletic, or unitarian theory, the members believed that the monocyte derived from lymphocytes (Weidenrich). The neo-unitarians with Pappenheim as the leader derived the monocyte from a stem cell, mesenchymal in origin. That stem cell endowed with totopotentialities was named by Ferrata "hemo histioblast" and by Maximow "hemocytoblast". That cell, also named by other investigators, "lymphoidocyte" (Pappenheim) gives rise to various types of blood cells, that is lymphocytes, or granulocytes or monocytes and erythrocytes, the latter through the megaloblast and erythroblast stages. (31-53-5559-62-64).

The polyphyletic theory denied the existance of this hypothetical hemocytoblast. According to the hypo-

thesis that either two or three blast cells are involved in the formation of the three types of leucocytes, it is necessary to distinguish between the Dualists and the Trialists. In both cases, the Dualists and the Trialists recognize the mesenthymal origin of the monocyte and they endow the stem cells with different potentialities.

In the Dualistic theory, some members consider the origin of the monocyte as being myeloid (Naegeli, Turck) some others proclaim a lymphatic origin. (Pappenheim, Maximow, Bloom).

In the Trialistic theory, an individual stem cell and a different site of origin in postnatal life is proclaimed. Here, the monocyte derives from a blast cell called "monoblast", and comes from the connective tissue. In summary, the characteristic of the trialist theory is: three stem cells for the three types of circulating leucocytes the myeloblast, the lymphoblast and the monoblast, the moboblast being produced by the connective tissue. In this theory the individuality of the monocyte is recognized.

All the theories discussed therein are based on debatable arguments but, nevertheless, the Trialistic is the most popular. (20-49-53-55-64). This theory is based upon the morphological, physiological and pathological criteria. (49)

Experiments carried out with dyes will not be described in this study, although an experiment of some interest will be mentioned. This experiment, according to the author, proves the origin of the monocyte from the Reticulo-Endothelial cells.

In 1942, Zen (50) carried out the following experiment which depends also on the fixation capacity of the monocytes. The basis of that experiment is that if the monocyte originates from the Reticulo-Endothelial System the extract of organs rich in monocytes may possess a monocyte producing ability. He obtained with different lipoid solvents extracts of spleen and liver, organs rich in monocytes, and injected these extracts to a group of rabbits. He carried out the same extraction procedure on kidneys, which are poor in monocytes and injected these extracts to a control group of rabbits. In the first case, he got a strong monocytosis. In the second, no action was observed unless an eight fold dose was given. The monocytosis substance is thermostable and resists heating at 100 degrees for one hour. Aquaous extracts of the same organs have no effect in producing a monocytosis. So, it appears that this monocytogenic substance belongs to the lipoid group. This substance was increased when it was extracted from animals that had received one intravenous injection of 3 cc. of India Ink. The animals that received the same injection

over a period of six days, twice a day, gave opposite results. The author concludes to a close relationship and dependence between the variations on the number of the blood monocytes to the Reticulo-Endothelial System.

It is not wise to draw final conclusions concerning the origin of the monocyte and until its origin is definitely proved, we haye to keep in mind the possibility that lymphocytes may give rise to monocytes.

II - FUNCTIONS AND PROPERTIES

A brief review of the different functions of the monocyte will be made without going too deeply into detail. Little is known concerning the functions of the blood monocyte, or macrophage, but it is thought by many investigators that they are capable of phagocytosis and of producing antibodies.

As it was seen, a cell is classified as belonging to the Reticulo-Endothelial System because it shares with the members of this System, a group of common functions and properties which justify the concept of this as an independent system. The monocyte being a member of that System possesses more or less the function to be described. A whole section will be further devoted to one of the most important functions of the monocyte cells, if not the most important, that is: the role in the production of antibodies.

Among the functions and properties which may be attributed to the monocyte belonging, as it does, to the Reticulo-Endothelial System, it is worth-while to mention the following:

1) Phagocytosis
2) Relationship to the metabolism of:
    a) Lipids

b) Proteins

c) Carbohydrates

d) Water

e) Iron and blood pigments

f) Sulfur

g) Calcium

3. Relationship to the endocrine glands

4. Chemotaxis

5. Erythro-leucopoietic or cytopoietic functions

6. Relationship to immunity: a whole chapter will be devoted to that function. (29-37-53-61)

1. PHAGOCYTOSIS

a) Definition

b) Action on different substances

Etymologically, phagocytosis means a "state of eating cells" and may be defined as "ingestion of a particle of foreign material by a living cell". (65) That particle may be inert or alive.

The term "phagocytosis" does not imply anything concerning the fate of the ingested material, that is its digestion, destruction and elimination. Fundamentally, the phagocytic property inherent in all phagocytes is based upon a mechanical activity of engulfing inert or living particles.

Some colloids of the interstial plasma may

penetrate, be digested and eliminated by the monocytes without the usual process of phagocytosis. In these cases, the phagocytic cells are completely passive, no mechanical activity having taken place and the fixation of these colloids in the phagocytic cells is due to the phenomena of adhesion and impregnation. (37)

Microorganisms may be engulfed by the monocytes and proliferate within these cells thus invading the body and causing a partial blockade of the Reticulo-Endothelial System. In such a case, the monocytes appear to play a role in the dissemination of the organisms. (37)

Monocytes engulf not only microorganisms, bacteria or protozoa, but also exert their phagocytic activity on other substances.

They plan a part in the removal of senile or injured red blood cells. Their action on the red blood cells is also connected with the production of blood pigments and with the metabolism of iron, as will be seen later.

They also destroy dead leucocytes, injured tissue cells and, probably, blood platelets. Furthermore, their phagocytic activity is also exerted on inert foreign material present in the circulation such as dyes.

Generally speaking, it seems that the monocytes by their phagocytic function: a) assist the poly-

morphonuclears, which are more sensitive to the microbial leucocidin, in their fight against the infection and b) clean out the infected area by engulfing and removing dead cells, leucocytes or bacteria, in short, all sorts of debris. (17)

2. RELATIONSHIP TO THE METABOLISM OF:

a) Lipids
b) Proteins
c) Carbohydrate
d) Water
e) Iron and blood pigments
f) Sulfur
g) Calcium

The study of the relationship of monocytes to the metabolism of the different biochemical constituents of the body will be briefly summarized; a longer description is outside of the province of this paper.

a) Lipid metabolism

A short study of the metabolism and storage of lipids in relation to the Reticulo-Endothelial System and of course to the monocyte will be described briefly. A few words will be mentioned concerning the connection between vitamins A and D and the Reticulo-Endothelial System.

In all the fields of nutritional exchange,

the Reticulo-Endothelial System shows a physiological activity. It is evident that the capacity of these cells for fixation acts firstly upon susbtances found in the circulatory blood. These substances are taken up by the Reticulo-Endothelial System members and according to the need of the organism are eliminated, diffused or stored. (31-61)

Many investigators have seen evidence of lipids or fat droplets inside of the Reticulo-Endothelial System cells. (53) The Reticulo-Endothelial cells have a place in the transportation of lipids, cholesterol and neutral fats, in the organism. (53-61)

In the liver, the Kupffer cells are concerned in the fixation of cholesterol; a part is esterified and eliminated through the bile, the remainder is kept as such. (53)

A relationship is also believed to exist between the Reticulo-Endothelial System members and the storage of vitamins A and D. If vitamin A is injected into rats in great quantity but without reaching hypervitaminosis, the liver then possesses a greater capacity to take trypen blue. (37)

b) <u>Protein metabolism</u>

The monocytes or any Reticulo-Endothelial System members seem to possess a very elaborate enzymatic

system that makes of the Reticulo-Endothelial System a great apparatus for parenteral digestion of proteins. The enzymatic activity of the Reticulo-Endothelial System members, including the monocyte, upon the proteins is proabbly the link, the bridge that connects the phenomena of immunity, in particular the formation of antibodies to that Reticulo-Endothelial System. (61) The relationship to Protein Metabolism is suggested by the following facts:

This injection of a large dose of India Ink into the rabbit gives rise to an increase in the globulin and urea nitrogen of the blood. (30-55)

The oxidative deamination of the amino acids was thought by some investigators to take place in the Reticulo-Endothelial System cells. (53)

In the splenic pulp the destruction of proteins seem to be active as shown by the abundance of zanthine, creatine and urea present. (61)

Even though the influence of the Reticulo-Endothelial System upon protein metabolism is poorly understood it is possible that the action of serotherapy and vaccinotherapy, may find their applications here.

c) Carbohydrate metabolism

The results obtained are contradictory, negative and positive results being reported. Among the positive results splenectomy, or the blockade of the Re-

ticulo-Endothelial System by dyes, provokes a hyper-glycemia with an increase in the calcium content and a decrease in the potassium content of the blood. (53)

d) Water metabolism

It has been noticed that in blockade experiments, there is decrease in water retention and in protein content of the serum. It seems that the regulation of the osmotic pressure of the blood depends upon the Reticulo-Endothelial System which permits the blood to maintain its ionic equilibrium.

e) Iron metabolism and blood pigments

We cannot separate the activity of the Reticulo-Endothelial System in the metabolism of iron from the destruction of the red blood cells and the production of blood pigments. The sequence of events is as follows: the monocytes phagocytose the red blood cells, these are broken into small pieces, the hemogoblin escapes and the stroma is left in the monocyte. The hemogoblin is broken down and the hemochromogen is separated from the globin. The degradation does further and the hemochromogen gives a water soluble compound containing iron which is stored. (53-61)

f) and g) Sulfur and calcium

Sulfur is stored by the Reticulo-Endothelial System members under the form of glutathions. The Reticulo-Endothelial System is said also to participate in the calcium metabolism.

3. RELATIONSHIP TO THE ENDOCRINE GLANDS

The activity of the monocyte is influenced by the secretion products of the endocrine glands. The removal of the thyroid gland for instance, results in a decrease in the phagocytic power of the monocyte and in the storing function of the Kupffer cells of the liver. The injection of thyroxin quickly restores the normal conditions. Insulin increases the storage of certain substances by the Reticulo-Endothelial System. The sexual hormones have also an effect. (31-57-61)

4. CHEMOTAXIS OF MONOCYTES

Lafargues et al (29) in 1945 were working with cultures of macrophages. Accidentally one of their cultures became contaminated by a Gram-positive Coccus. That misfortune led them to notice that the macrophages in that culture were still alive and had migrated to the bacterial colony and actually phagocytosed it. As a result of that observation they concluded that the macrophages may show chemotaxis that is they may be attracted by the microbes or their constituent.

5. CYTOPOETIC FUNCTION

The first section dealt with the origin of the monocyte. Here, the general cytopoetic function of the

Reticulo-Endothelial System will be narrowed to the cytopoetic potentialities of themonocyte. A wider discussion on the cytopoetic function of the Reticulo-Endothelial System is beyond the scope of this present paper. The study of the cytopoetic function of the monocyte will embrace:

a) cytopoetic function of blood monocyte

b) cytopoetic function of the macrophage

The hypothesis concerning the Reticulo-Endothelial System role in the production of monocytes admitted by most of the authors has been previously studied in this historical survey. But a question arises: are the blood monocytes and the macrophages end-product of cellular development, or are they capable of producing other cells?

a) Cytopoetic function of the blood monocyte

1. In acute tuberculosis the transformation of monocytes into epitheloid cells and into giant cells was demonstrated to take place in monocytes which have ingested bacilli. (11-30)

2. Monocytes may be transformed into macrophages (40) as demonstrated by many investigators and this was confirmed by tissue culture experiments. Abundance of food supply may facilitate that transformation. (15-16-53-57)

3. In vitro experiments have shown that the monocytes may also give rise to fibroblasts. The addition of a filtrate of Rous virus to a culture of monocytes

transforms these cells into fibroblasts. (3-4-52-53)

b) Cytopoetic function of the macrophages

1. They may give rise to blood monocytes, (16-57)

2. To fibroblasts and vice versa, (4-5-6-14-52-57)

3. And to epitheloid cells. (62)

4. They may also fuse to form foreign body giant cells and, thus, be able to interpose a mass of protoplasm between the cells and a body too large to be engulfed. (62)

This is in brief, then, a description of the functions that monocytes possess. The functions and properties of the Reticulo-Endothelial System, as seen, have a direct influence upon our conditions of life.

III - RELATIONSHIP TO IMMUNITY

It seems that there is no doubt that the antibodies are formed by the members of the Reticulo-Endothelial System and in particular by the monocytes. However, this evidence is not supported by all the investigators and the production of antibodies by plasma cells or by lymphocytes is also claimed. (62)

Burnet believes that antibodies are synthetized in the cells by enzymes that have become permanently modified by contact with antigens.

From Heidelberger view point "the presence of antigens in the tissues disturbe the mechanism of globulin synthesis, modifying the method of union or the spatial relationship of the globulin components so that a new globulin, an antibody, is formed which reacts specifically with the antigens by virtue of the distortion by the presence of the antigen at the moment of the synthesis, for if the antigen can only affect aminoacids having affinity for it, these should retain that affinity after their synthesis into globulin".

What is the nature of these cells, mentioned above, which are connected with antibody formation? Are they plasma cells, lymphocytes or monocytes? The possibility that monocyte may form antibodies will chiefly be

discussed in this section, although a brief mention on the possible role of lymphocytes and plasma cells will be given.

The formation of antibodies was related directly or indirectly with the Reticulo-Endothelial System for over half a century.

In 1898, Pffeiffer and Mars stated that antibodies might be present in spleen. Deutsch, one year later, found that splenectomy had an effect on andibody formation. (63) And in 1907 (58) Metchnikoff related the problem of immunity to macrophages.

Splenic extracts of animals immunized to antibodies when injected into another group of the same species of animals gives rise to anitbody formation in the blood serum of the recipients. (47-63)

The macrophage theory of antibody formation is now widely accepted and it seems plausible that the cells which remove and digest the bacteria are concerned in the formation of antibodies. (13-46)

Two main arguments were advanced to support this theory that is: a) the depressant effect upon antibody formation by the blockade of the Reticulo-Endothelial System with particulate matters such as iron-sugar, India Ink, trypan blue, etc. and b) the observation of Sabin in 1939. (13-42)

The blockade seems to interfer with the phagocytic and digestive functions of the monocyte. The monocyte being ingaged in digesting one colloid could not take care of another and, therefore, could not produce antibodies. (13)

The blockading effect is interpreted differently by the various investigators; some support the theory of formation of antibodies by lymphocytes. According to Ehrich, the monocytes break down the antigens and prepare them for proper utilization by the lymphocyte which is considered by this author as being to absorp or adsorp dissolved antigens or split products of antigenic substances.

If such a theory is correct, it is possible that the stimulated monocyte, when submitted to blockade experiments, ingests and destroys the antigen so rapidly that the effective contact with the antibody forming cells is reduced. (13) The adjuvant action of certain oils seems to sustain, in part, that theory. The adjuvants are supposed to slow down the release of antibodies and, consequently, a more effective contact with the antibody forming cells takes place, to result in a greater formation of antibodies. There are two possibilities: a) either that the slow release of antigenic splitted product is absorbed by the lymphocyte which remaining then in

long contact with the antigenic stimulies produces the antibody or b) that the prolonged contact of the monocyte with the antigenic forming substances enables it to produce antibody.

Before leaving the study of the depressing effect of blockade on the antibody formation, it is interesting to mention that the effect is a) independent of the route of administration of the antigen as long as the dose of the antigenis not too large to cause excess of stimulation and b) that the amount of antigen depends upon particulate matter necessary for efficient blockade, vary with the type of antigens and its affinity for the Reticulo-Endothelial System. (48)

Investigators carried out these blockade experiments with sometimes diametrically opposed results. A failure in obtaining positive results may be attributed to:

a) amount of blockading material injected

b) rapidity of regeneration of mesenchymal cells

c) amount of dispersion of materials injected

d) mode of injection of the blockading material

d) period of time over which blockading material was injected

f) number of animals used (2-39-48)

We may also add to these factors the animal species, the age of the animals, the kinds of blocking substances, the number of injections, the combination with splenectomy,

the excretion of blocking substances, the effect of blocking substances on systems other than Reticulo-Endothelial System. Jaffe, 1931, emphasized the importance of injecting the entigen very shortly after the injection of the blocking substances and of producing a new block before each injection of antigens. (54)

The other arguments to support the role of monocyte in producing antibodies, is based upon the observation of Sabin in 1939. Working with a dye coupled to a protein, she noted that after phagocytosis of the dye protein complex by the macrophage, the dye was removed from the dye protein aggregate and that the protein particle disappeared. She interpreted that observation to mean "that the protein has been rendered into soluble form and passed into cytoplasm." The shedding of the cytoplasm by the macrophages and monocytes took place in the appearance of antibodies in the serum coincided with the time when the dye protein complex disappeared. (13) In this phenomena, observed by Sabin, the monocyte acts firstly as a macrophage (big eater) than as clasmatocyte (shedding of the exoplasm). There has been much debate concerning these observations of Sabin. Nevertheless, it would appear that the monocyte plays a role in the antibody formation, especially when we consider the experiments carried out since the last war with fluorescent or isotopic-

labelled antigen or antibody.

In his thesis, Girard summarized under four headings the experiments described in the literature upon the possibility of the Reticulo-Endothelial System producing antibodies.

a) "The use of washed mononuclear phagocytes obtained as exudate cells either to transfer sensitivity or exhibit some degree of cellular immunity".

b) "The use of Reticulo-Endothelial cell blockade with various vital dyes and colloids that depress Reticulo-Endothelial function and along with this the immune response".

c) "The use of non-antigenic lipid material to stimulate and sustain monocyte numbers over a particular immunization period".

d) "The use of tracer antigens, both radioactive or chemical to establish the cell types which phagocytize them and their relation to tissue sources where antibodies appear concentrated". (54) Each of the four going set of experiments may be considered to be approved to support the role of the monocytes in the formation of antibodies.

The literature contains the following experiments that seem to show the relationship between the immunity to macrophages.

a) It was long ago noticed that the presence of

macrophage produced in sterile inflammation increased the resistance of the host tissue towad Streptococci injected at the time the macrophage concentration had reached its maximum. (18)

b) Antibodies were demonstrated in skin tissue where large numbers of macrophages were artificially mobilized by aluminum hydroxide gel, before they were present in the blood serum. (21)

Experiments with antigens or antibodies isotopically labelled or coupled with axo-dyes or fluorescein isocianate bring more support to the possible role of monocyte in the formation of antibodies.

For the last few years, Kruse, McMaster, Coon, and many others, have been studying the cellular localization of soluble Axo-dye coupled with bovine globulin, human serum or albumin after intravenous injections into animals. They found that the tracer antigens were removed and stored in a high concentration in the monocytes and in general in the Reticulo-Endothelial cells where they persisted for a certain time as revealed by the blue coloration or color granules inside of these cells.(7-24-27-32-33).

When the antibody molecule was coupled with fluorescein isocyanate allowed to react with tissue cells containing the homologous antigen, it was found that the antibody precipitated with the antigen in a high coneentration

in the monocytes, as revealed by fluorescent microscopy.(7)

The experiments carried out with protein antigens labelled with I-131 (Bovine gamma globulin) showed that the antigens are removed by the phagocytic cells from the blood and extravascular fluid where they exist in equilibrium. (12)

Haurowitz noticed the appearance of most of the labelled antigens in the mitochondria of cells, but the presence of antigens in the mitochondria does not mean necessarily they are the sites of antibody production as the immunity is never transmitted to descendants. (7-22)

To conclude, the experiments reported by Girard, (54) last year, concerning the effect of the maintained monocytosis in rabbits, may be mentioned. After having produced a monocytosis in rabbits by intravenous injections of M.P.A. (Chloroform extract of listeria monocytogenes), he studied the antibody formation against typhoid, staphylococcus toxoid and horse serum antigens. He found the antibody titers obtained in rabbits submitted to maintained monocytosis are four to eight times greater than those obtained in the control animals. He demonstrated, also, that the antibody content in exudate cells of animals in which a monocytosis is maintained is higher in these cells than in the supe-

natant fluid. The passively introduced antibodies are also in a greater concentration in the exudate cells than in the monocytes of the control animals. The antibodies content in these exudate cells was also found to be in a greater proportion than in the blood serum.

From the facts brought forth in this section, it is logical to relate the monocyte to antibody formation. On the other hand, since it is not definitely proven that the monocyte is directly involved in antibody formation, we must continue to take into consideration the theories that implicate the plasma cells and the lymphocytes in antibody formation. However, until proof of the opposite, it is not illogical to believe that more than one type of cells may be involved in the formation of antibodies.

MATERIALS AND METHODS

1. PROCEDURES FOLLOWED IN THE ISOLATION OF MPA (Monocyte-Producing Agent)

   a) Cultivation of Listeria monocytogenes

   b) Isolation of MPA

   c) Concentration of the extract

a) Cultivation of Listeria monocytogenes

The MPA fraction is extracted from Listeria monocytogenes, strain 42 XXVIII, this strain having been tested for purity and found to be pure. The method of cultivation is borrowed from Girard. (54)

The glassware used is washed with a detergent "Orvus", rinced with tap and distilled water and oven-dried before use.

The organisms are grown in a medium consisting of Difco Bacto Tryptose broth to which are added 3.1908 gm of anhydrous Na_2PO_4H per every liter.

Difco Bacto Tryptose brogh formula for one liter of medium:

Bacto-tryptose	27.0 gm
NaCl	5.0 gm
Bacto-dextrose	2.0 gm
Thiamine HCL	0.0005 gm

adjusted to pH 7.2 with the aid of the Becman pH meter.

The organisms are grown in 150 cc of broth for 16 hours; this quantity serves to inoculate the 16 liters of media which are contained in a 20-liter carbuoy. The purity of the inoculum is checked by microscopy. The 16 liters of media are incubated for 48 hours before inoculation, to check the sterility of the medium.

During incubation, the carbuoy is half-immersed in a water-bath at 37oC for 48 hours. Sterile air is passed through the media to provide the necessary oxygen. The carbuoy is fitted with a 3-hole stopper; one serving as an air inlet, one as an air outlet, the third serving to inoculate the flask. The air is Seitz filtered and allowed to pass slowly through the media. The stirring action of the air current combined with the action of the convection current accelerate the growth of the organisms and maintain a constant temperature.

At the end of the 48-hour period of incubation, a viable count is carried out upon a sample according to the method described by Reed and Reed (38) to follow the yield from one batch to another.

The purity of the growth in the carbuoy is verified by plating out a sample on a Blood Agar Plate and also by microscopy. The 16 liters of organisms are then killed by immersing the flask in a water-bath heated at 60-70°C for 1½ hour. Survival is also checked on a

Blood Agar Plate.

A De Laval Separator[x] serves to concentrate the organisms into a paste. The paste after being washed once in physiological saline is poured in a small Erlyn-Meyer flask and subsequently lyophilized[xx] for further use. The dried organisms are kept in a dessicator under vacuum at 4°C until the extraction of the MPA is carried out.

b) Isolation of the MPA

For every extraction 10.6 gm (54) of dried organisms are ground in a mortar in the presence of twice the amount of powdered glass and a few cc. of purified chloroform is added to make a paste. The paste is divided in two portions and poured into 200 cc round bottomed, pyrex, ground glass stoppered flasks to which are added 50 cc of purified chloroform and about the same volume of glass beads. These flasks are fixed to the vibrating machine[xxx] and shaken for four hours or until smears reveal disintegration of the bacteria.

x De Laval Separator: Model MYP 145 type MSO 1152, The De Laval Co., Peterborough, Ont.

xx Centrifugal Freeze Dryer, Model 3 Ps; W. Edwards Co. Ltd., London.

xxx Vibrating Machine: built by Mr. Milos Srb, Optical Engineer, Montreal. (complete description of this machine is given in Girard's thesis, McGill University 1952)

The content of the two flasks after being submitted to the shaking action is poured in a separatory funnel and extracted with 1500 cc of purified chloroform at 4°C for 7 days. The funnel is periodically shaken during the extraction. At the end of the 7-day period the extract is filtered through a 13.5 cm membrane filter under a vacuum of -10 cm Hg to separate the extract from the bacterial debris. This debris is re-extracted as in the above procedure.

The method of the purification of chloroform is that reported by Girard (54)

The powdered glass is prepared from resistant glass (Pyrex) which is ground in a mortar until a fine powder is obtained. The powder is filtered through a screen of 80 meshes fixed to a Seitz filter support. The powdered glass is heated with 1 N HCl and washed with water until the washings gave no positive test for acidity. (25)

The glass beads are heated in the presence of 1 N HCl and washed with water until the washings gave no positive test for acidity.

c) Concentration of the extract

In this present work the vacuum distillation apparatus used previously by Girard (54) has been replaced by a more versatile laboratory concentration apparatus which allows a more rapid recovery of large volume of chloroform

and consequently reduces the time required to concentrate the extract.

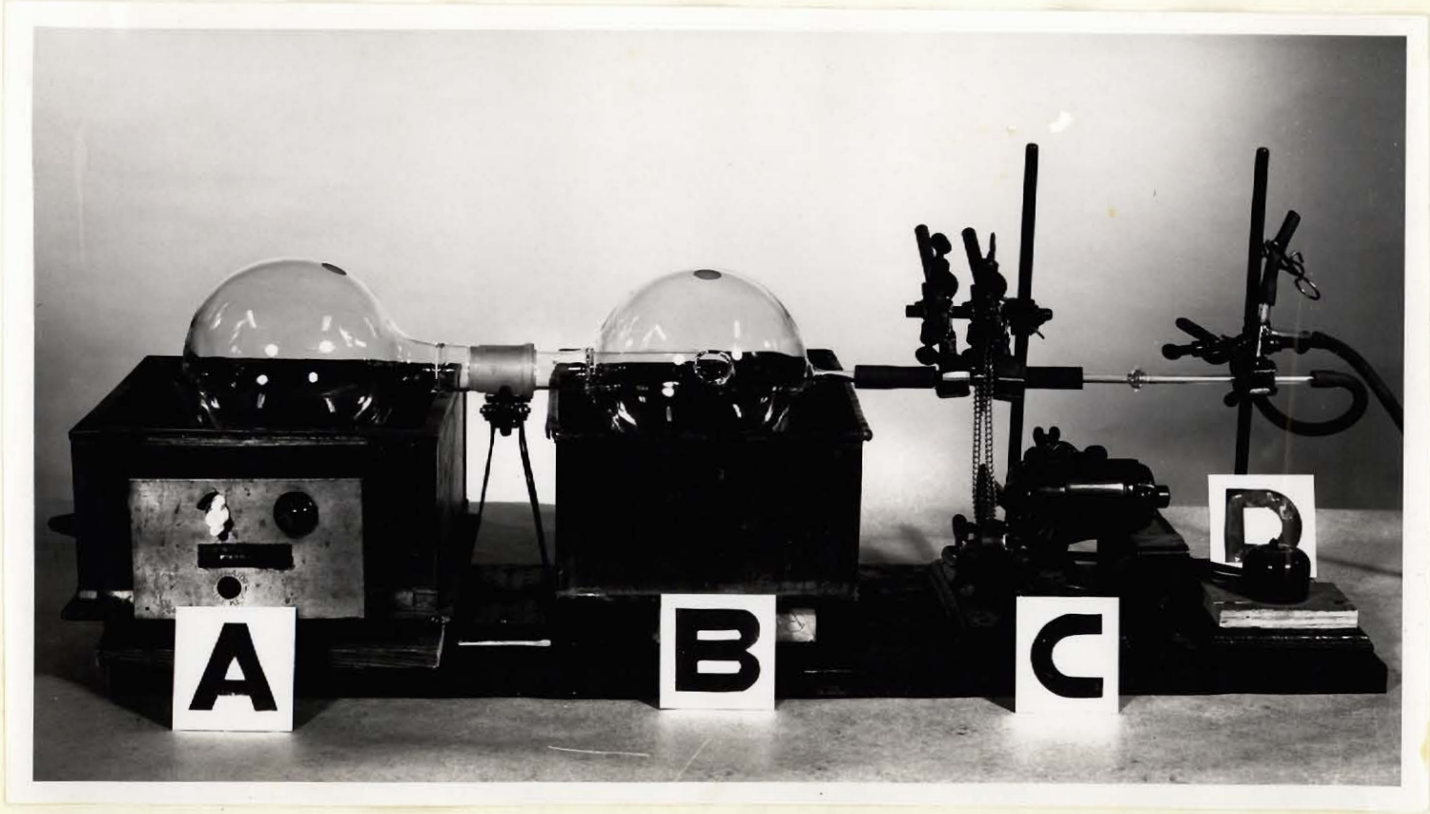
The extract to be concentrated is placed in a round-bottomed flask (A) which rotates in a 37°C water-bath. The condenser is the receiver flask (B) and is joined to (A) by a standard taper joint. The flask (B) is at least as large as or larger than flask (A). Flask (A) may be of three different sizes: large, medium or small. Flask (B) has an inlet tube through which the vapors pass from (A) to (B). Flasks (A) and (B) are supported by two small wheels with solid rubber tires. The flask (B) rotates in a mixture of ice and water contained in a flat pan. The second opening of flaks (B), opposite to flask (A), facing the motor (C), is connected to a short piece of brass tubing. This tubing passes through a pulley as seen in the picture and serves to rotate the two flasks (A) and (B). This pulley is connected by means of a chain drive to a small sewing machine motor (C) whose speed is controlled at will. The brass tubing is connected by a short piece of rubber tubing to a glass tube itself joined to a similar one by a standard universal ball joint. This joint is greased with a heavy stopcock lubricant. To operate, the vacuum is applied and the motor (C) is started at a steady speed so as not to disturb greatly the surface of the fluid in either flasks (A) or (B). A film of liquid is constantly pulled up on the

upper inside wall of flask (A) and a relatively large heated surface is furnished for vaporization. Conversely flask (B) furnished a large cooling surface area for condensation.

The distillation takes place without ebullition as in molecular distillation. There is no tendency for bumping as long as the apparatus is in action when the vacuum is applied at the properly temperature which is, in the course of these experiments, 37°C. This method saves time in concentrating large quantities of extract. (8)

The 1500 cc of extract is divided into 200 cc portions which is about the maximum quantity a large flask (A) may handle at a time. The 1500 cc of extract is concentrated to 10-15 cc in about one hour. When only this quantity of concentrated extract is left, after the last 200 cc portion is concentrated down, the apparatus is stopped. These remaining ? cc of very concentrated extract are poured in a flask. The flask (A) is then washed three consecutive times using 5 cc portions of purified chloroform each time. The washings are then added to the concentrated extract. The concentrated extract and the washings are stored in the refrigerator at 4°C. Before use, the volume of concentrated extract is measured and the quantity of MPA is determined by evaporating, under vacuum, a measured volume of the extract in a dessicator, for 24 hours. The concentration in mgm of MPA per cc of concentrated extract having been thus

VACUUM DISTILLATION SET-UP USED FOR CONCENTRATION OF EXTRACTS



determined, the number of cc of extract calculated to give the amount of MPA desired are placed in a small 12 cc vial in 5 cc amounts and the chloroform evaporated under vacuum. The chloroform-free extract is suspended in 0.3% Sodium Lauryl Sulfate in physiological saline and standardized to give 20 mgm of MPA per cc. Before injection into test animals, the suspension is heated to 37-40°C (44) in order to have an evenly distributed suspension. All the extract thus prepared produced a marked monocytosis in the course of the experiments.

On each batch prepared, Biuret and Sakaguichi test for proteins are carried out (54) No protein has been detected in the course of the experiments.

The first extraction carried out was made according to the method described by Girard (54) with a vacuum distillation apparatus. The second, third, fourth and fifth with the new concentration apparatus. The sixth extraction was also carried out with the new concentration apparatus but in this case a slight modification in the method of growing the organisms was introduced. They were grown for 10 days at 4°C in a carbuoy. Sterile air was passed through the media as in the original method. Listeria has been found to grow very well. This fact suggested the possibility that if the organisms were grown at 4°C, a better yield in MPA might be obtained. An experiment

toward elucidation of this problem was performed and the results obtained recorded as follows:

TABLE NO. 1

METHOD OF EXTRACTION USED	CONCENTRATION APPARATUS USED	EXTRACTION NO.	YIELD FROM 10.6 GM OF DRIED ORGANISMS	%
The method of extraction described in this section is used for the six extractions carried out.	Vacuum distillation apparatus as described by Girard Organisms grown at 37°C for 48 hrs.	1	271 mgm	53.0
	New laboratory concentration apparatus. Organisms grown at 37°C for 48 hrs.	2	378 mgm	73.5
	New laboratory concentration apparatus. Organisms grown at 37°C for 48 hrs.	3	369 mgm	72.0
	New laboratory concentration apparatus. Organisms grown at 37°C for 48 hrs.	4	343 mgm	67.5
	New laboratory concentration apparatus. Organisms grown at 37°C for 48 hrs.	5	418 mgm	81.5
	New laboratory concentration apparatus. Organisms grown at 4°C for 10 days.	6	619 mgm	129.5

N.B. the percentage is calculated on the figures mentioned by Girard (54) in his work: 512 mgm. for every 10.6 gm. of dried organisms used.

The results obtained in this experiment seem to indicate that the extraction of organisms cultured at 4°C for 10 days yields more MPA for the same amount of dried organisms. As seen from Table No. 1, the yield from extraction No. 6 is 29% greater of what obtained by Girard (54) or 50% greater than extraction No. 5 which is the best obtained when growing the organisms at 37°C for 48 hours.

II. METHODS USED FOR THE PRODUCTION OF ANTI-SERA

a) Production of anti-typhoid serum

b) Production of anti-horse serum

c) Production of staphylococcal anti-alpha hemolysin serum

a) Production of anti-typhoid serum

1. PREPARATION OF THE ANTIGENS SUSPENSIONS

The strain of S. typhosa was tested for purity, motility and biochemical reactions on lactose, dextrose, H_2S and urea media. The strain was found to be a pure culture of S. typhosa.

The organisms are grown in stainless steel trays on cellophane, as described by Girard (54). Two

trays have been inoculated and the resultant growth is collected in large centrifuge tubes and washed once with physiological saline. The washed organisms are resuspended in a measured quantity of physiological saline and divided into two 100 cc portions. One portion will serve to prepare the "H" antigens, the other, the "O" antigens.

The "H" antigen is prepared as follows: To one portion, formalin is added to give a final concentration of 0.5%. The organisms are killed by incubation at 40°C for 2 hours, in presence of formalin, and tested for sterility, on Blood Agar Plate. This is the stock "H" antigen suspension. For use in titration tests, the stock "H" suspension is diluted to 500×10^6 organisms/ml at the Evelyn photoelectric colorimeter[x] (transmittance reading 74.5% at 420 millimicron). The relationship between the counting chamber method (Thoma type chamber) and the colorimeter has been determined as follows: Serial dilutions have been made. The density of each dilution has been determined by the counting chamber method using 0.025% carbol fuchsin in 5% carbolic acid (10) as a diluent. Knowing the number of organisms per cc by the counting chamber method in the original dilutions, they were placed in the colorimeter and the corresponding % of transmittance

x Evelyn photoelectric colorimeter, Rubicon Co. Electrical Instrument Maker, 29 North, Sixth St., Philadelphia, Pa.

noted. It has been found that $100x10^6$ organisms/cc corresponds to a transmittance of 58.5% and that $500x10^6$ organisms/cc corresponds to a transmittance of 74.5% at 420 millimicron.

The "O" antigen is prepared as follows: The remaining portion of the washed organisms is heated in the autoclave at 100°C for 2½ hours. Sterility test is carried out on Blood Agar Plate. 0.5% phenol is added as a preservative. The stock "O" suspens on is also diluted to $500x10^6$ organisms/cc when used for titration test.

2. PREPARATION OF THE ANTISERUM

Five rabbits have been inoculated according to the protocol described below but the serum of only one rabbit has been utilized in experiments, that is rabbit 1835, 3.45 kilo. The vaccine used is a dilution $1000x10^6$ organisms/cc of a stock vaccine made up of ½ volume of the concentrated "H" suspension and ½ volume of concentrated "O" suspension.

1st day: intravenous injection of $1000x10^6$ organisms/cc/kilo
i.e. 1 cc of diluted vaccine/kilo of weight.
Weight: 3.45 kilo.

5th day: intravenous injection of 2 cc/kilo of weight of the vaccine ($1000x10^6$ organisms/cc). Weight 3.3 kilo.

7th day: Titration

10th day: intravenous injection of 3 cc/kilo of weight of the vaccine (1000×10^6 organisms/cc).
Weight: 3.40 kilo

12th day: Titration

15th day: Titration

17th and 19th day: bleeding by heart puncture; 50 cc of blood removed each time and the serum of each bleeding pooled and the titre determined.

"O" titre: complete in 1/1280 partial in 1/2560

"H" titre: complete in 1/20480 partial in 1/40960

The titration is carried out at 56°C for 6 hours and read the next morning after being left overnight at room temperature.

b) Production of anti-horse serum

Five rabbits have been used in producing the antiserum but only the antiserum of rabbit 1867 has been used in the experiments. The antiserum has been produced as follows:

1 st. day: intravenous injection of 0.2cc/kilo of weight,
Weight: 2.5 kilo

2 nd. day: intravenous injection of 0.3cc/kilo " "
Weight: 2.6 kilo

10 th. day: intravenous injection of 0.4cc/kilo " "
Weight: 2.7 kilo

15 th. day: intravenous injection of 0.5 cc/kilo of weight

Weight: 2.65 kilo

On the 20th. day, when inoculated with 0.6 cc/kilo 2 rabbits died of anaphylactic shock; the remaining rabbits were bled by heart puncture and the serum collected. 45 cc of blood have been obtained from rabbit No. 1867. The serum of rabbit No. 1867 gives an optimal ratio of 1:39. The titration is carried out at room temperature and the constant antibody optimal ratio is determined.

c) Production of staphylococcal anti-alpha haemolysin serum

1. PREPARATION OF STAPHYLOCCAL TOXIN

The toxin is prepared by a modification of the Burent and Freeman method.

The media used is semi-solid agar, 0.3% containing beef heart extract and 1 per cent proteose peptone, without added salts and from which the phosphates are precipated. It is sterilized by autoclaving at 120 degrees C. at pH 7.2 and for use it is poured into Petri dishes in a layer 1 cm. thick.

The media is inoculated with an 8 hour culture of strain Wood 46 Staphylococcus pyogenes and incubated for a period of 72 hours at 37 deg. C. in an atmosphere of 65% added oxygen and 35% added carbon dioxide.

It is then filtered overnight in the ice box through glass wool to remove the agar, passed through a

Seitz E.K. filter and stored at 5 deg. C. in sealed vials.

2. PREPARATION OF STAPHYLOCOCCAL OF ALPHA HAEMOLYSIN RABBIT ANTISERUM

a) Staphylococcal toxoid is prepared by incubating the staphylococcal toxin (as prepared above) with a concentration of 1% formalin overnight at 37°.

b) The rabbit receives a series of six injections given at one week intervals, subcutaneously in the following doses:

Week	1	2	3	4	5	6
Dose	2 cc	3 cc	5 cc	5 cc	5 cc	5 cc

c) The animal is then bled from the heart one week after the 6th injection for collection of antiserum

d) The serum so collected is passed through a Seitz E.K. filter.

N.B. The toxin and the antiserum used in the experiments have been graciously furnished by Dr. J. Rublee of this Department.

III. METHODS USED FOR THE ABSORPTION OF THE ANTIBODIES BY THE MONOCYTES

a) Treatment of the materials used in the absorption of the antibodies by the monocytes

The glassware used in the manipulation of the monocytes is washed in 33% HNO_3 heated to 90°C for

2 hours and left overnight at room temperature in this solution. Several washings in tap water and in distilled water are carried out until no acidity is detected.

The unused stoppers are boiled in N/2 NaOH, rinsed in tap water, then boiled in N/2 HCl and thoroughly rinsed in tap water and distilled water, oven-dried, placed in Petri-dishes, covered with cheese-cloth and autoclaved for 20 min., 121°C, 15 pds. The used stoppers are washed in warmed water to which is added a detergent (Orvus) and flushed overnight with tap water and the next morning rinsed thoroughly with distilled water.

For incubation the monocytes in contact with the dilutions of antiserum are placed in large 150 x 15 mm tubes.

When antiserum is present in the monocyte preserving media, orginary black No. 1 stoppers are used. In some experiments where synthetic media deprived of antiserum, No. 1 gray-stoppers (36) from the West Co. (Phoenixville, Pa.) stock compound S-124, were utilized.

b) Production of macrophage exudates in the pleural cavity of rabbits

In order to have a rich macrophage exudate, it is produced in rabbits in a state of artificially induced monocytosis. This monocytosis is produced with the aid of the MPA already isolated from Listeria monocytogenes. The

monocytosis is produced in rabbits as follows:

1 st. day: 1. Blood count

2. Injection of 20mgm of MPA intravenously

3 rd. day: 1. Blood count

2. Injection of 20 mgm of MPA intravenously

3. Injection of 6 to 8 cc of a mixture intrapleurally, made up as follows: equal parts of Gum Arabic 40% (U.S.P. G85 Fisher Scientific Co.) and Beef Extract 20% (Difco No. B 126 - Difco Co.) in saline. The solutions of 40% Gum Arabic and Beef Extract 20% in saline are autoclaved separately at 121°C, 15 pds. 20 min.

6 th day: 1. Blood count

2. Removal of the exudate

3. Standardization of the exudate to 15,000 monocytes/c.mm in the preserving media.

The mixture of Gum Arabic and Beef Extract is injected in the right pleural cavity using a 19 gauge adapted as follows: the tip of the needle is cut off with plyers thus effectively sealing the end. The blocked tip is now ground smooth and an opening made into the needle, a few millimeters before the tip, by grinding with the edge of a small emery wheel. This type of needle was found to be more satisfactory for our purposes than one whose point was merely dulled. The layer of skin is perforated with the aid of the sharp end of a small scalpel. Through this

perforation, the needle is passed and the mixture of Gum Arabic and Beef Extract injected into the pleural cavity. Three days later the exudate is removed using the same type of needle after the skin is perforated with the scalpel. A 10 cc syringe is filled up with a balanced salt solution (Tyrode's or Earle's solution) and the pleural cavity washed out with it. The exudate is consequently diluted in this solution and is then readily removed. It may be necessary to enter the pleural cavity several times in order to remove all the diluted exudate.

Rabbits are anesthetized with 1 - 1½ cc of Nembutal (Abbot Co.) when pleural injections are made or when exudate is removed. The exudate is collected in a small Erlyn-Meyer flask containing 1 cc of 2% potassium oxalate, previously oven-dried as recommended by Girard.

c) Preservation media used

As the media studied and used in these experiments were not designed to promote the proliferation of the cells but rather to preserve them over a certain period of time, these media will be termed throughout this work "preservation media" rather than "culture media".

Two principal preservation media were used to keep the monocytes in good conditions during the course of the investigations. In preliminary experiments, Parker's

fluid was used. These experiments were designed to determine:

1. the effect of concentration of monocytes upon the absorption of antibodies by these cells and

2. the absorption of anti-typhoid serum by the standardized quantity of monocytes.

For these two sets of experiments, the tubes containing the various dilutions of antiserum in contact with the constant amount of cells were incubated at 37°C on a roller drum turning at 4 RPM.

After paying a visit to Dr. Parker of the Connaught Laboratories, few modifications were introduced. Earle's Serum solution was found preferrable to Parker's fluid because more absorption of the anti-typhoid serum took place in it. It was also noticed that the cells kept in Earle's Serum solution without signs of degenerescence(?) for a longer period. Consequently, Earle's Serum solution was used throughout the subsequent experiments.

It was thought that the agitating action of the roller drum might have an injurious effect upon the monocytes and hasten their degenerescence. Using the Earle's Serum solution as the diluting and suspending fluid, absorption tests were carried out by placing 2 sets of identical dilutions of anti-typhoid serum in contact with constant amounts of cells at 37°C. One set of dilutions was placed on a roller drum turning at 4 RPM, the other was

laid down on a plane surface. A comparison between the absorption in the 2 sets was made. Since the same amounts of antibodies were found to be absorbed in both sets, incubation on a roller drum was eliminated.

In the course of subsequent investigations, comparison between different media was made and the preservation capacity of each for monocytes over a certain period of time was studied. As will be seen in the experimental part, Earle's Serum solution and Baker's fluid were found to be the best. However, Earle's Serum solution was preferred for its ease of preparation.

1. Parker's fluid: (35)

3 parts of homologous rabbit serum

1 part of isotonic sodium bicarbonate 1.4%

2 parts of Tyrode's solution containing 4 times the usual amount of glucose.

Modified Tyrode's solution formula: (60)

NaCl	7.7 gr.
KCl	.20 gr.
$CaCl_2$	.20 gr.
$MgCl_2$ 6 H_2O	.10 gr.
$NaH_2PO_4H_2O$	.05 gr.
$NaHCO_3$	1.0 gr.
Glucose	4.0 gr.

Distilled water to make 1 liter, pH 7.8

Serum, sodium bicarbonate and freshly prepared Tyrode's solution are mixed in the appropriate proportions and sterilized by filtration through a membrane filter fixed to a Seitz filter support. When Parker's fluid was used in the course of the experiments no antibiotics were added.

The serum used in the preparation of the media is obtained from the individual rabbit that gave the macrophage exudate. The rabbit is bled aseptically by heart puncture after the removal of the exudate. The blood is incubated at 37°C for 1 hour and the serum collected and used in the preparation of the media. The homologous fresh serum when mixed with appropriate balanced salt solution preserved the monocytes for a satisfactory period of time.

2. Earle's Serum solution:

1 part of homologous serum

1 part of Earle's solution

Earle's solution formula: (60)

NaCl	6.80 gr.
KCl	0.40 gr.
$CaCl_2$	0.20 gr.
$MgSO_4 7H_2O$	0.20 gr.
$NaH_2PO_4H_2O$	0.14 gr.
$NaHCO_3$	2.20 gr.
Glucose	1.0 gr.
Water to make 1 liter.	
pH	7.2

The distilled water used in the preparation of this media is redistilled by passing through a glass pyrex column containing a mixture of Amberlites IR-120 and IRA-400 (The Lamotte Products Co., Towson Md. U.S.A.). This frees it from traces of metals known to be toxic for cells kept in media. (23) The freshly prepared mixture of Earle's solution and homologous serum is sterilized through a membrane filter fixed to a porcelain support and used immediately.

Antibiotics are added to the Earle's solution in such a quantity that the desired concentration of antibiotics is obtained when the serum is added to it. The final concentration of antibiotics used in the Earle's serum solution is 1 mgm Penicillin Sodium G per cc of media and 100 mgm Dihydrostreptomycin per cc of media. (Crystalline Penicillin G Sodium, 100,000 units or 60 mgm of crystalline Penicillin G Sodium per vial, Merck Co. Ltd. and Dihydrostreptomycin, 1 gr. per vial, Parke and Davis Co. Ltd., Walkerville, Ont.)

The pH of the media is adjusted with the aid of the Beckman pH meter to 7.2. No phenol red was added to the media as a pH indicator, since this interfers with the reading of results when titrations are carried out upon the tested serum.

When the cells have been in contact with the

various dilutions of antiserum in the tubes and ready to be incubated, a mixture of CO_2, O_2 and N_2 is blown into the media. This mixture of gases comes from exhaled air which contains about 4% CO_2. This exhaled air is blown through a sterile pipette plugged at the upper end with cotton that serves as a filter. This bubbling of gas is done in order to have the media in the proper range of pH where growth of cells may take place. (60)

The capacity for preserving monocytes of various media was investigated. The pH in these media was adjusted to 7.0-7.2 by bubbling sterile exhaled air through them. The investigated media were sterilized by filtration as previously described and are the following:

1. Serum Ultra Filtrate (Microbiological Associates, Flemington, New Jersey, U.S.A.)

The ultrafiltrate of ox serum contains a substance that seems to be essential for the maintenance of living adult cells. This is the A factor which is an acid with a molecule small enough to pass through the collodium membrane impermeable to proteins. This fraction resists heating to 100°C in a neutral solution but is destroyed by heating in diluted acid or alkali. This serum ultrafiltrate is not species specific because no proteins are present.

2. Earle's Serum solution

This is the preservation media used in the

course of the experiments. This media as has been previously described was found to be the one giving the best results in preserving the cells for the period of time desired.

3. Earle's Ultrafiltrate serum solution

This media consists of equal parts of Earle's solution and serum ultrafiltrate.

4. Simm's ultrafiltrate serum solution

This media consists of equal parts of Simm's solution (60) and serum ultrafiltrate. The formula of the Simm's solution is as follows:

NaCl	8.0 gr.
KCl	0.20 gr.
$CaCl_2 2H_2O$	0.147 gr.
$MgCl_2 6H_2O$	0.203 gr.
$NaHCO_3$	1.01 gr.
Na_2HPO_4	0.213 gr.
Dextrose	1.0 gr.
Phenol red	0.01 gr.

Distilled water to make 1 liter. The Simm's solution has been obtained from the Microbiological Associates.

5. V 614 Solution (Microbiological Associates) (60)

Glucose	2.0 gr.	Fructose diphosphate	0.20 gr.
l-Lysine	0.03 gr.	l-Histidine	0.01 gr.
l-Arginine	0.004 gr.	dl-Valine	0.028 gr
l-Leucine	0.018 gr.	dl-Isoleucine	0.02 gr.
dl-Threonine	0.024 gr.	dl-Phenylalanine	0.014 gr
l-Tryptophane	0.004 gr.	Cystine	0.01 gr.
Glutathione	0.010 gr.	Glutamine	0.25 gr.

distilled water to make 1 liter.

6. V 614 - Serum Ultrafiltrate Solution

This media consists of equal parts of V 614 Solution and serum ultrafiltrate.

7. Baker's fluid (1)

The formula of this media is as follows:

Solution A: 20 cc

Glucose	2000 mgm
NaCl	5810 mgm
KCl	150 mgm
$CaCl_2$	150 mgm
$MgCl_2$ $6H_2O$	75 mgm
$NaH_2PO_4H_2O$	37.5 mgm
$NaHCO_3$	750 mgm
Phenol red	50 mgm

Solution B: 25 cc

Serum	250 cc
Witte's peptone	850 mgm

Solution C: 10 cc

Vit. B1	0.053 units
Vit. B2	0.001 units

Solution D: 10 cc

Vit. C	0.85 mgm
Glutathione	3.4 mgm
Cysteine HCl	11.25 mgm

Solution E: 10 cc

Vit. A	500-1000 units
Vit. D	1-2 units

in 1% Tween 80 solution (10 cc)

Solution F: 10 cc

Insulin	0.12 units

Solution G: 10 cc

Hemin	0.0045 mgm

Solution H: 5 cc

Thyroxine	0.00113 mgm

Metal trace free water to make 1 liter; 100 cc. was prepared.

The Baker's solution is made up by mixing the separately prepared solutions.

8. Baker's - Serum ultrafiltrate solution

This media is made up of equal parts of serum ultrafiltrate and Baker's fluid.

To all these above media, antibiotics have been added to give a final concentration of Penicillin and of Dihydrostreptomycin, as already described.

d) Method of standardization of the monocytes

The exudate, after removal from the pleural cavity is washed once in the balanced salt solution (Tyrode's or Earle's solution), spun down, the supernatant wathings discarded and the cells resuspended in 10 cc of the preservation media used containing the homologous serum. Cell counts are carried out and the number of white cells and monocytes per c.mm. in the 10 cc of the suspension is determined. These 10 cc are then diluted out with the media until the concentration of the monocytes reach 15000 cells/c.mm in the suspension. E.g. 20 cc of exudate is collected. This is washed once and the cells resuspended in 10 cc of media. Cell counts are made and 27,000 white cells per c.mm are found to be present in the 10 cc of suspension. Differential smears are then made and it is found that the monocytes constitute 90% of the exudate, consequently the 10 cc of suspended cells contained 24,300 monocytes per c.mm. The

10 cc of suspended cells are then diluted to:

$\frac{24300 \text{ monocytes/c.mm} \times 10 \text{ cc}}{15000 \text{ monocytes/c.mm.}}$ that is 16.2 cc. These operations are carried out under aseptic conditions. For a cell count an aliquot of the suspended cells is removed aseptically. When the cell suspension has been standardized to 15000 monocytes/c.mm., $\frac{1}{2}$ cc of the standardized suspension is added to each $\frac{1}{2}$ cc of the tested dilutions of antiserum.

White cell count:

The counting chamber used is a Burker type with double Neubauer ruling. The aliquot of cells is drawn to the mark 0.5 of the white cell pipette and the diluting fluid is drawn to mark 11 making a dilution of 1/20. The pipette is shaken for 3 minutes by hand. The cells are placed in the counting chamber and allowed to settle for 3 minutes. They are then examined under the microscope using a 16 mm. objective. The cells are counted in the 4 large corners squares each of which has a volume of 0.1 c.mm. making a total volume of 0.4 c.mm. For routine purpose, the cells in the 4 outside squares are multiplied by 50 when the dilution is 1/20. (55)

Monocyte count:

The monocyte count is carried out in the same type of counting chamber mentioned above, using the same technique except that the diluting fluid is replaced by 1% aqueous neutral red solution (19)

Differential counts of the exudate:

They are made as follows: The Schilling four-field meander method is used when counting the cells on the smears. 200 cells are counted and the % of each type of cells calculated. The staining process of the smears is the following:

Jenner's Giemsa staining method:

$3\frac{1}{2}$ minutes of Jenner's stain
Overlay with distilled water for 1 minute
Rinse in 2 changes of distilled water
$12\frac{1}{2}$ minutes of Giemsa stain (1 drop of Giemsa stain per 1 cc of water)
Rinse in 2 changes of distilled water
Examine under oil immersion

The same techniques were used in examining the blood picture of the rabbits through the course of the experiments.

e) Dilutions of various antisera tested

In the course of these experiments, five sets of dilutions of antiserum are used. The dilutions of various antisera used in the course of these investigations are made using the halving dilution method in $\frac{1}{2}$ cc volume of preserving media. Large 150 x 15 mm. test tubes are used. To each $\frac{1}{2}$ cc of diluted serum, $\frac{1}{2}$ cc of standardized cell suspension (15000 monocytes/c.mm.) is added. Exhaled air

is blown into each tube as already described, the cotton plugs aseptically replaced by sterile rubber stoppers and the tubes incubated at 37°C on a roller drum or laid down on a plane surface depending upon the method used at the time. The time of incubation for each set of dilutions was 4, 8, 16, 24 and 48 hours.

The dilutions of the anti-typhoid serum are set up as follows:

Each set of dilutions of antiserum is prepared the same way.

Dilutions of each set are made in large test tubes numbered 1 to 8.

½ cc of anti-typhoid serum is diluted in 2 cc of the preservation media of choice, to give a diluted antiserum 5 times diluted

½ cc of media is introduced into tubes 2 to 8

½ cc of 5 times diluted antiserum is introduced into tubes 1 and 2

Tube No. 2 is mixed, ½ cc of the mixture transferred into tube No. 3 and so on to tubes 8 and ½ cc of the mixture of tube 8 is discarded. This gives in tubes 1 to 8 halving dilutions of antiserum in ½ cc amounts starting at 1/5 in tube 1.

To tubes 1 to 8, ½ cc of standardized cell suspension is added to give a final dilution of antiserum of

1/10 in tube No. 1 and 1/1280 in tube No. 8.

A control tube is set up by introducing ½ cc of 5 times diluted antiserum and ½ cc of preserving media.

The dilution of antiserum is summarized in the following table:

TABLE NO. 2

TUBES NO.:	1	2	3	4	5	6	7	8	Control
cc of serum 5 times diluted	0.5	0.5							0.5
cc of diluent preservation media added		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Volume of dilution in cc	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.0
Amount of standardized cell suspension added	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
Total volume per tube	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Final dilution of antiserum when in contact with cells	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/10

The dilutions of the anti-horse serum and of the staphylococcal anti-alpha heamolysin are set up as follows:

Five sets of identical series of dilutions are prepared and incubated in contact with monocytes for 4, 8, 16, 24 and 48 hours, respectively.

The dilutions of each set are set up in large test tubes numbered 1 to 5.

½ cc of media is introduced into tubes 2, 3, 4 and 5.

½ cc of antiserum is introduced into tubes 1 and 2

Tube No. 2 is mixed and ½ cc of the mixture transferred to tubes 3 and so on to tube 5, and ½ cc of the mixture in tube 5 is discarded. This gives in tubes 2 to 5 halving dilutions of antiserum in ½ cc amounts starting with non-diluted serum in tube 1.

To tubes 1 to 5, ½ cc of standardized cell suspension is added to give a final dilution of antiserum of ½ in tube 1 and 1/32 in tube No. 8

The dilution of antiserum is summarized in the following table:

TABLE NO. 3

TUBES NO.:	1	2	3	4	5
cc of anti-serum	0.5	0.5			
cc of diluent preservation media		0.5	0.5	0.5	0.5
Volume of dilution in cc	0.5	0.5	0.5	0.5	0.5
Amount of standardized cell suspension	0.5	0.5	0.5	0.5	0.5
Total volume per tube	1.0	1.0	1.0	1.0	1.0
Final dilution of antiserum when in contact with cells	1/2	1/4	1/8	1/16	1/32

The control is made up of an identical set of dilutions of antiserum in which the $\frac{1}{2}$ cc of cells is replaced by $\frac{1}{2}$ cc of media.

f) Titration of the various dilutions of antiserum after being incubated with monocytes

Five sets of a series of 8 dilutions of antiserum were incubated at 37°C for 4, 8, 16, 24 and 48 hours respectively. At the end of each period of incubation, a

set of dilutions of antiserum was removed. Each dilution was then separately titrated to find out whether any reduction in the antiserum titer had taken place as a result of absorption of antibodies by monocytes. Each dilution of each of the five sets of 8 dilutions of antityphoid serum is then titrated as follows:

The final dilutions of the 8 dilutions of antiserum in each series when in contact with monocytes during incubation are then:

1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280

Each dilution of antiserum in contact with monocytes is freed from the cells by centrifugation and the respective sueprnatant removed and placed in the corresponding tube of a series of 8 tubes, numbered 1 to 8.

"O" Titration

For the "O" titration, 0.5 cc of each supernatant is twice diluted by adding 0.5 cc of saline to give a set of 8 tubes. The final dilution of antiserum in the twice diluted supernatant now becomes:

1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280, 1/2560

Each of the latter dilutions is then separately titrated against the "O" antigen as follows:

a) $\frac{1}{2}$ cc of the twice diluted supernatant is put in tubes No. 1 and 2 of a rack containing 11 tubes numbered 1 to 11

b) ½ cc of saline is added to tubes 2 to 11 and mixed with the supernatant antiserum by the halving dilution method

c) ½ cc of "O" antigen (500 x 10^6 organisms/cc) is added.

The 8 sets are incubated for 6 hours at 56°C, left overnight at room temperature and read the following morning.

The titration of each twice diluted supernatant is summarized as follows:

TABLE NO. 4 a)

TUBES NO.:	1	2	3	4	5	6	7	8	9	10	11
Amount of twice diluted supernatant	0.5	0.5									
Amount of saline (0.85%)		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Volume of dilution in cc	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Volume of "O" antigens added 500x10^6org./cc	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Final volume in cc	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

TABLE NO. 4 b)

ilution of ach twice iluted su- ernatant efore titra- ion:	Reciprocal of final dilution for each titrated supernatant after addition of "0" antigens (500×10^6 org./cc) in tubes No.:										
	1	2	3	4	5	6	7	8	9	10	11
1/20	40	80	160	320	640	1280	2560	5120	10240	20480	40960
1/40	80	160	320	640	1280	2560	5120	10240	20480	40960	81920
1/80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840
1/160	320	640	1280	2560	5120	10240	20480	40960	81920	163840	327680
1/320	640	1280	2560	5120	10240	20480	40960	81920	163840	327680	655360
1/640	1280	2560	5120	10240	20480	40960	81920	163840	327680	655360	1310720
1/1280	2560	5120	10240	20480	40960	81920	163840	327680	655360	1310720	2621440
1/2560	5120	10240	20480	40960	81920	163840	327680	655360	1310720	2621440	5242880

The control serum consists of 5 tubes of antiserum 1/10 which are incubated with the tested sera for 4, 8, 16, 24 and 48 hours respectively. At the end of each period of incubation the corresponding control serum is removed and titrated, as previously described, against the "O" antigen. The final dilutions of each control serum in the titration process carried out after "O" antigen is added are the following: 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280, 1/2560, 1/5120, 1/10240, 1/20480.

When the results of agglutination are observed, every dilution of titrated sera, control or test, is read in terms of plusses against "O" gelatin standards.

The gelatin standards for "O" antigens are prepared as described by Bailey (51) and consist of 6 tubes numbered 1 to 6. Tube No. 1 is given a value of 1 plus and shows the least trace of agglutination. Tube No. 6 is given a 6 plus value and shows complete agglutination. The intermediary tubes No. 2, 3, 4, 5 are given respectively values of 2, 3, 4, 5 plusses and show the different stages of agglutination included between "trace of agglutination" and "complete agglutination". After agglutination was completed, each titration dilution is read by comparison with the gelatin standard tubes. The plusses attributed to the different dilutions of the tested serum are added and the

sum compared with the sum of plusses obtained for the corresponding range of dilutions of control serum titrated. A ratio is made between the 2 sums and the percentage of antibody left and absorbed is then calculated.

"H" Titration

All the steps involved in the "O" titration are repeated with the following modifications:

a) 0.25 cc of supernatant of each dilution is diluted with 0.75 cc physiological saline to give a 4 times diluted supernatant instead of a twice diluted one as in the case of the "O" titration. It was not found possible to use 0.5 cc of supernatant for the "H" titration since, although 1cc of supernatant was present at the beginning of each experiment, it must be realized that manipulations of the antisera reduced the amount of supernatant obtained below 1 cc.

b) 13 dilutions of antiserum are made instead of a set of 11 dilutions

c) the final dilutions of antiserum when titrated against "H" antigen are expressed in Table No. 5

TABLE NO. 5 a)

TUBES NO.:	1	2	3	4	5	6	7	8	9	10	11	12	13
Amount of four times diluted supernatant	0.5	0.5											
Amount of saline (0.85%)		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Volume of dilution in cc	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Volume of "H" antigens added 500x10^{6}org./cc	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Final volume in cc	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

TABLE NO. 5 b)

)ilution of :our times lilated su-)ernatant)efore titra- :ion:	Reciprocal of final dilution for each titrated supernatant after addition of "H" antigens (500x10^{6}org./cc) in tubes No.:												
	1	2	3	4	5	6	7	8	9	10	11	12	13
./40	80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840	327680
./80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840	327680	655360
./160	320	640	1280	2560	5120	10240	20480	40960	81920	163840	327680	655360	1310720
./320	640	1280	2560	5120	10240	20480	40960	81920	163840	327680	655360	1310720	2621440
./640	1280	2560	5120	10240	20480	40960	81920	163840	327680	655360	1310720	2621440	5242880
./1280	2560	5120	10240	20480	40960	81920	163840	327680	655360	1310720	2621440	5242880	10485760
./2560	5120	10240	20480	40960	81920	163840	327680	655360	1310720	2621440	5242880	10485760	20971520
./5120	10240	20480	40960	81920	163840	327680	655360	1310720	2621440	5242880	10485760	20971520	41943040

Anti-Horse serum titration

The sets of dilutions of anti-horse serum, incubated for various periods of time are composed of a series of 5 dilutions: 1/2, 1/4, 1/8, 1/16, 1/32, these are the final dilutions when in contact with the cells. At the end of the period of incubation, the cells are separated from the antiserum by centrifugation. The supernatant antiserum is then diluted 8 times as follows: 0.5 cc of each supernatant is placed in the corresponding tube of a set of 5 tubes and 3.5 cc of physiological saline added to each tube. Using the constant antibody-optimal ratio method of titration, different ranges of dilutions of horse serum are used to titrate the corresponding different dilutions of 8 times diluted antiserum, as expressed in Table No. 6

TABLE NO.6 a)

TUBES NO.:	1	2	3	4	5	6	7	8
Amount of diluted horse serum	0.5	0.45	0.40	0.35	0.30	0.25	0.20	0.15
Amount of saline in cc	0.0	0.05	0.10	0.15	0.20	0.25	0.30	0.35
Volume of dilution in cc	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Amount of 8 times diluted supernatant antiserum in cc	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Final volume in cc	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

TABLE NO. 6 b)

Dilution of eight times diluted supernatant antiserum when titrated	Reciprocal of corresponding harmonic dilutions of horse serum in tubes No.							
	1	2	3	4	5	6	7	8
1/16	250	277	312	357	416	500	625	833
1/32	500	555	625	714	833	1000	1250	1666
1/64	1000	1111	1250	1428	1666	2000	2500	3333
1/128	2000	2222	2500	2857	3333	4000	5000	6666
1/256	4000	4444	5000	5714	6666	8000	10000	13333

It was found by the halving dilution method that 50% of antibody is removed from all tested dilutions of antiserum. Harmonic dilutions of horse serum have been set up in order to give a range of dilutions of horse serum sufficiently wide to allow accurate reading.

The control was incubated for 48 hours and is composed of five dilutions as were the tested dilutions of antiserum: 1/2, 1/4, 1/8, 1/16, 1/32. The control serum is then diluted 8 times to give, when titrated, dilutions of 1/16, 1/32, 1/64, 1/128, 1/256.

Each dilution of the control serum is then titrated against the corresponding ranges of harmonic dilutions of horse serum as were the tested antiserum dilutions. The first tube precipitating in the control dilutions was found to be the 7th tube giving an optimal ratio of 1.39.

All titrations of the Horse-anti-horse serum system are carried out at room temperature in 1 cc volume using the constant antibody optimal ratio method of titration.

The dilutions of horse serum precipitating first are noted for each of the tested serum dilutions and for each of the corresponding serum control dilutions. A ratio is made between two corresponding dilutions thus found and the percentage of antibody left and absorbed is then calculated.

Staphylococcal anti-alpha haemolysin titration

Five sets of dilutions of antiserum 1/2, 1/4, 1/8, 1/16, 1/32 were incubated for 4, 8, 16, 24 and 48 hours respectively. At the end of the incubation period, each of the above dilutions is then diluted to 1/400 with saline to give 5 tubes of serum diluted 1/400. Each of these tubes of diluted antiserum is then titrated as follows:

a) harmonic dilution of antiserum made in 1 cc volume

b) to each dilution, 1 cc of 1/450 toxin has been added. That dilution of toxin has been chosen to give 50% hemolysis in a dilution of 1/800 in all dilutions of control serum

c) incubated 1 hour at 37°C

d) at the end of this period, ½ cc of 5% fresh rabbit red blood cells is added to every tube and the series of tubesincubated for 1 hour at 37°C

e) the reading is made in terms of percent hemolysis, 50% hemolysis is chosen as the end point, after the tubes are cooled at 4°C overnight

f) the 50% hemolysis standard is prepared as follows: 1 cc of 1/10 dilution of toxin is diluted with 1 cc of saline, ½ cc of red blood cells is then added and the mixture incubated for 1 hour at

37°C. At the end of the incubation period, 2½ cc of solution showing 100% hemolysin are diluted by addition of an equal amount of physiological saline.

The procedure above described is summarized in the following Table No. 7.

A series of dilutions of control serum, identical with the one of the tested serum, is incubated for 48 hours. At the end of that period, titration is carried out as above. The range of harmonic dilutions of antiserum is so chosen as to have the 50% hemolysis end-point occur in a dilution of 1/800 for the control serum.

The range of harmonic dilutions chosen was such as to be wide enough to note any reduction in the titer of the tested antiserum.

The dilutions of antiserum giving 50% hemolysis are noted for all the antiserum dilutions titrated. The dilutions of the control serum shows a 50% hemolysis end-point for dilution of 1/800. The ratio between the dilution giving 50% hemolysis in the tested serum and the control serum dilution (1/800) giving also 50% is made. Percentage of the antibody left and absorbed is then easily calculated.

IV. ANALYSIS OF THE CONTENT OF MONOCYTES

Anti-typhoid serum dilutions of: 1/10, 1/20,

TABLE NO. 7

TUBES NO.:	1	2	3	4	5	6	7	8	9	10
Amount of 1/400 diluted antiserum in cc	1.0	0.90	0.80	0.70	0.60	0.50	0.40	0.30	0.20	0.10
Amount of saline in cc	0.0	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90
Volume of dilution in cc	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Amount of 1/480 toxin in cc	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Final volume	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Final dilution of antiserum	400	444	500	571	666	800	1000	1333	2000	4000
The tubes are incubated for 1 hour at 37°C										
Amount of 5% fresh rabbit red blood cells added in cc	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Final volume	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5

The tubes are incubated for 1 hour at 37°C,

cooled overnight at 4°C, centrifuged and read.

1/40, 1/80, 1/160, 1/320, 1/640, 1/1280, anti-horse serum and anti-alpha hemolysin serum dilutions of: 1/2, 1/4, 1/8, 1/16, 1/32 are incubated for 8, 16 and 16 hours respectively, in contact with constant amounts of standardized cell suspension. The preparation of the various dilutions of antiserum and the standardization of the cell suspension have been described previously.

The above periods of incubations are used because they were found to be the optimal periods that give the maximum of absorption of antibodies.

At the end of each incubation period, the antiserum dilutions of each set are treated as follows: the cells are separated from the tested antiserum dilutions by centrifugation. The supernatant of each dilution is titrated as previously described. The percentage of antibodies absorbed in each of the 3 sets of antiserum dilutions is compared with that previously found and confirms the experiments carried out on the absorption of antibodies by monocytes.

After the antiserum of each dilution is separated from the monocytes, the cells are then washed 3 consecutive times, using 1 cc of saline (.085%) each time. The washings are titrated separately.

To the washed cells is added 0.5 cc of 9% saline. The cells are then incubated at 37°C for 2 hours. At the end of this period, 4.5 cc of distilled water are

added to give a final concentration of 0.9 in saline and incubated for 2 hours. This procedure lysed the monocytes and is known as the Osmotic Pressure method (54)

The lysed cells thus obtained are centrifuged and the 5 cc of supernatant lysate titrated. The residual sediment of lysed cells is resuspended in 1 cc of saline and titrated. In the horse serum titration, where the constant-antibody optimal ratio is used, the residual sediment of lysed cells is resuspended in 5 cc of physiological saline.

For all the titrations carried out in these investigations, the techniques previously described are used. The results are reported in the experimental part.

EXPERIMENTAL PART

I. INTRODUCTION

The purpose of this work is to determine:

a) whether or not antibodies are absorbed by monocytes in vitro and

b) whether or not the absorbed antibodies are concentrated within these cells.

Preliminary investigations were carried out to provide solutions to the following problems:

a) choice of a medium capable of preserving the monocytes over a certain period of time,

b) determination of the minimum number of monocytes per c.mm. capable of giving a visible absorption of antibodies.

When these two difficulties were overcome, preliminary work on the dilutions and incubation-time relationships to the absorption of antibodies by monocytes was carried out, using anti-typhoid serum, incubated and placed on a roller drum.

At this point it was felt that the preservation medium chosenin a preliminary experiment was not satisfactory, as will be reported later on.

New media were then tested, the Earle's serum solution was chosen and used for subsequent experiments.

Before any experiments were carried out with the chosen medium, it was believed that the agitating action of the roller drum might have an injurious effect on the monocytes and consequently hasten their degene ratio. This possibility was then investigated. The results thus obtained do not differ from those obtained when the tubes were laid flat on a plane surface during incubation. The roller drum action being neither more effective than incubation on a flat surface, nor injurious to monocytes, the tubes containing the cells and the dilutions of antiserum were, in all subsequent experiments, laid flat on a plane shelf in the 37°C incubator.

The absorption of anti-typhoid, anti-horse and staphylococcal anti-alpha hemolysin sera was studied, using the modifications mentioned above (new improved medium, incubation on plane surface).

The antibodies that disappeared from the antiserum as shown by a reduction in titre were believed to be absorbed by the monocytes. As traces of red blood cells were found to be present in all exudates used, it was thought that these cells might absorb the antibodies and thus falsify the results obtained. Controls were then set up and it was proven that the red blood cells did not absorb antibodies.

After absorption of anti-typhoid, anti-horse and staphylococcal anti-alpha hemolysin sera by monocytes

was demonstrated, investigations on the fate of the absorbed antibodies were carried out. As the tested antisera consistently showed a reduction in titre, it was felt that antibodies that had disappeared must have been absorbed or adsorbed by the monocytes.

In a separate experiment, the monocytes remaining after the tested antisera had been incubated, were analysed as follows: the cells were separated from the tested dilutions of antiserum by centrifugation, washed and lysed. The lysates thus obtained from the monocytes and the residual cellular material were titrated.

The results obtained in the experiments briefly reviewed will now be described in detail.

It must be noted that the monocytes were used in all the experiments within the day the exudate was removed.

II. CHOICE OF A MEDIUM CAPABLE OF PRESERVING THE MONOCYTES OVER A CERTAIN PERIOD OF TIME

In a preliminary work, three different media were tested and their capacity for preserving monocytes over a 16-hour period studied.

a) Saline water treated as follows:

The saline water was injected into the peritoneal cavity of a rabbit and removed 4 hours later. The saline water thus treated was then used as a suspending and

preservation medium for monocytes.

b) Tyrode's solution

c) Parker's solution

The monocytes were obtained by intrapleural injection of a mixture of gum-arabic and beef extract solution as previously described. A cell count was carried on the 6 cc of macrophage exudate thus obtained. 85% of the white blood cell types were monocytes, and the monocyte count was 7500 cells/c.mm. of exudate. 2 cc of this exudate were then poured into 3 centrifuge tubes, washed with additional saline and centrifuged. The cell sediments thus obtained in each tube were then resuspended in 1 cc. of the medium to be tested to give 3 tubes containing:

a) 1 cc of a cell suspension standardized to 15000 monocytes/c.mm. in treated saline

b) 1 cc of a cell suspension standardized to 15000 monocytes/c.mm. in Tyrode's solution

c) 1 cc of a cell suspension standardized to 15000 monocytes/c.mm. in Parker's solution

The diluted anti-typhoid serum was obtained from rabbit 1857. The dilution of the antiserum was made as follows:

a) anti-typhoid serum was diluted to 1/1000 in treated saline and 0.5 cc placed in a tube to which was added 0.5 cc of the cell suspension standardized to 15000 monocytes/c.mm.

in treated saline,

b) anti-typhoid serum was diluted in Tyrode's solution and 0.5 cc was placed in a tube to which was added 0.5 cc of the cell suspension standardized to 15000 monocytes/c.mm. in Tyrode's solution,

c) anti-typhoid serum was diluted to 1/1000 in Parker's solution and 0.5 cc was placed in a tube to which was added 0.5 cc of the cell suspension standardized to 15000 monocytes/c.mm. in Parker's solution.

These tubes containing the diluted antiserum and the cells were then incubated at 37oC for 16 hours, on a roller drum. At the end of this period, the ability of the three media to preserve the monocytes was tested by microscopical and absorption tests. The absorption tests were carried out because the absorption of antibodies by the monocytes depends on their physiological state.

At the end of the incubation period, the ability of each medium to preserve the monocytes was studied by smearing aliquots of each medium and the state of degeneration of the cells in each of the tested media observed. The smears were stained by Jenner's Giemsa method. A complete lysis of the monocytes was found to have taken place in the tube containing the cells suspended in treated saline. Of the suspended cells in Tyrode's solution, a moderate number was lysed while those in the Parker's solution showed

only a few degenerated cells.

The cells were then separated from the diluted antiserum by centrifugation, the supernatant titrated and the results compared with those obtained from the control antiserum. The titrations on each supernatant of the 3 dilutions of antiserum were carried out as follows:

The antiserum diluted 1/1000 was halved and became 1/2000 after the addition of 0.5 cc of standardized cell suspension.

The control tube was a 1/2000 dilution of antiserum in 1 cc. 0.5 cc of the supernatant from each tube was diluted to 1/4000 with 0.5 cc of saline, and titrated.

TABLE NO. 8 a)

TUBES NO.:	1	2	3	4	5	6	7	8	9	10
Amount of 1/4000 antiserum in cc	0.5	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10	0.05
Amount of saline added in cc	0.0	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45
Dilution volume in cc	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Amount of "H" antigens (500×10^6 org./cc added in cc	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Final volume	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Final dilution	8000	8888	10000	11428	13333	16000	20000	26666	40000	80000

TABLE NO. 8 b)

	Reciprocal of final dilution of dilutions of antiserum after "H" antigen is added										Total of plusses in tested serum	Total of plusses in control serum	Ratio	% Left	% Absorbed
sted media	8000	8888	10000	11428	13333	16000	20000	26666	40000	80000					
ontrol anti-erum diluted n treated aline	6	6	6	6	6	6	6	4	3	2		51			
ested anti-erum diluted n treated aline	6	6	6	6	6	6	5	3	2	1	47		47/51	91.5	8.5
ontrol anti-erum diluted n Tyrode's olution	6	6	6	6	6	6	6	4	3	2		51			
ested anti-erum diluted n Tyrode's olution	6	6	6	6	6	5	4	3	2	1	45		45/51	88.0	12.0
ontrol anti-erum diluted n Parker's olution	6	6	6	6	6	6	6	4	3	2		51			
ested anti	6	6	5	4	4	3	2	2	1		33		33/51	65.0	35.0

It is concluded that Parker's fluid was the most efficient of the 3 tested media, since the cells suspended in it were not lysed after a period of 16 hours and since a greater degree of absorption of antibodies was obtained compared with the other two media.

III. EFFECT OF THE CONCENTRATION OF MONOCYTES ON THE ABSORPTION OF ANTIBODIES

Before studying the relationships between the absorption of antibodies by monocytes to:

a) various dilutions of antiserum and

b) to the time of incubation,

an experiment was carried out in order to find out whether or not the concentration of monocytes exerts a marked effect on the absorption of antibodies.

A rabbit was used to produce the macrophage exudate. After the exudate had been removed, the rabbit was bled by heart puncture, the serum collected and used in the preparation of Parker's solution.

The exudate is produced as follows:

1st. day:

a) blood picture:

white blood cells: 12500 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	1	30	61	8	1000cells/c.mm.

b) injection of 20 mgm. of MPA intravenously

3rd. day:

a) blood picture

white blood cells: 11100 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	65	19	16	1760

b) injection of 20 mgm. of MPA intravenously

c) injection of 8 cc of a mixture of gum-arabic and beef extract intrapleurally

6th. day:

a) blood picture:

white blood cells: 10500 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	41	42	17	1785

b) removal of exudate

c) rabbit was bled by heart puncture and serum collected from the 50 cc of blood obtained and used in the preparation of Parker's solution

d) picture of the exudate: 25 cc of exudate were obtained after the pleural cavity was opened,

white blood cells: 42000 cells/c.mm.

red blood cells: 36000 cells/c.mm.

differential count: polymorphoneuclears: 3%

lymphocytes: 7%

macrophages: 90%

This exudate was washed once with 25 cc phy-

siological saline. 2.5 cc, 6.6 cc and 13.2 cc of the exudate were placed in 3 centrifuge tubes, centrifuged and the cells in each tube resuspended in 5 cc of Parker's solution to give 3 tubes containing: 15000 monocytes/c.mm., 50000 monocytes/c.mm. and 100000 monocytes/c.mm. respectively. Three sets of identical series of 8 dilutions of anti-typhoid serum (obtained from rabbit No. 1835) were prepared in 0.5 cc of Parker's solution to give the following dilutions: 1/5, 1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640. To the tubes of each set was added 0.5 cc of the standardized cell suspension to be tested. This gave 3 sets of identical series of dilutions of antiserum having a final dilution after addition of the cells of: 1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280. The dilutions of each set were in contact with: 15000 monocytes/c.mm., 50000 monocytes/c.mm. and 100000 monocytes/c.mm., respectively. These operations are summarized in Table No. 9.

At the end of the 16-hour period of incubation, the cells are centrifuged and the supernatant antiserum thus obtained from each dilution, titrated against "H" antigen and the results compared with those found after titration of a control antiserum diluted to 1/10 in 1 cc of the Parker's solution. For titration, 0.25 cc of each supernatant was diluted with 0.75 cc physiological saline and each of the diluted supernatants then titrated. The results obtained are reported in Table No. 10 and Fig. No. 1.

TABLE NO. 9

TUBE NO.	1	2	3	4	5	6	7	8
Dilution of antiserum in 0.5 cc volume	1/5	1/10	1/20	1/40	1/80	1/160	1/320	1/640
Amount of cells added in set No.:								
1. 0.5 cc of cell suspension standardized to 15000 monocytes/c.mm. in each tube								
2. 0.5 cc of cell suspension standardized to 50000 monocytes/c.mm. in each tube								
3. 0.5 cc of cell suspension standardized to 100000 monocytes/c.mm. in each tube								
Final volume	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Final dilution after cells added	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280

The smallest concentration of monocytes (15000 cells/c.mm.) was used in all subsequent experiments for reason of economy, since it was felt that the increased absorption of antibodies obtained with the two other concentrations tested (50000 and 100000 cells/c.mm.) was not pronounced enough to warrant the use of concentrations larger than 15000 monocytes/ c.mm.

TABLE NO. 10

EFFECT OF CONCENTRATION OF MONOCYTES ON ABSORPTION OF ANTIBODIES
(15,000 cells/ccm.)

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated													No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840	327680					
3 hrs.	1/10	6	6	6	6	6	6	6	5	3	2	1			53	63	53/63	83.5	16.5
	1/20		6	6	6	6	6	6	6	5	2	1			50	57	50/57	83.5	16.5
	1/40			6	6	6	6	6	4	4	2	1	1		42	51	42/51	81.5	18.5
	1/80				6	6	6	6	5	4	2	1	1		37	45	37/45	81.0	19.0
	1/160					6	6	6	5	4	2	1	1		31	39	31/39	80.5	19.5
	1/320						6	6	5	4	3	1	1		26	33	26/33	80.0	20.0
	1/640							6	6	4	3	2	1		22	27	22/27	79.0	21.0
	1/1280								6	5	3	1	1		16	21	16/21	78.0	22.0
	Control	6	6	6	6	6	6	6	6	6	3	3	2	1				100.0	0.0

TABLE NO. 10 (CONT'D)

EFFECT OF CONCENTRATION OF MONOCYTES ON ABSORPTION OF ANTIBODIES
(50,000 cells/ccm.)

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated													No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840	327680					
6 hrs.	1/10	6	6	6	6	6	6	6	5	3	1	1			52	63	52/63	82.0	18.0
	1/20		6	6	6	6	6	6	5	3	2	1			47	57	47/57	81.5	18.5
	1/40			6	6	6	6	6	5	4	2	1			42	51	42/51	81.5	18.5
	1/80				6	6	6	6	5	3	2	1			35	45	35/45	78.0	22.0
	1/160					6	6	6	5	3	2	1			29	39	29/39	75.0	25.0
	1/320						6	6	5	4	3	1			25	33	25/33	74.5	25.5
	1/640							6	5	3	2	1	1		19	27	19/27	70.5	29.5
	1/1280								5	4	3	2	1		15	21	15/21	72.0	28.0
	Control	6	6	6	6	6	6	6	6	6	3	3	2	1				100.0	0.0

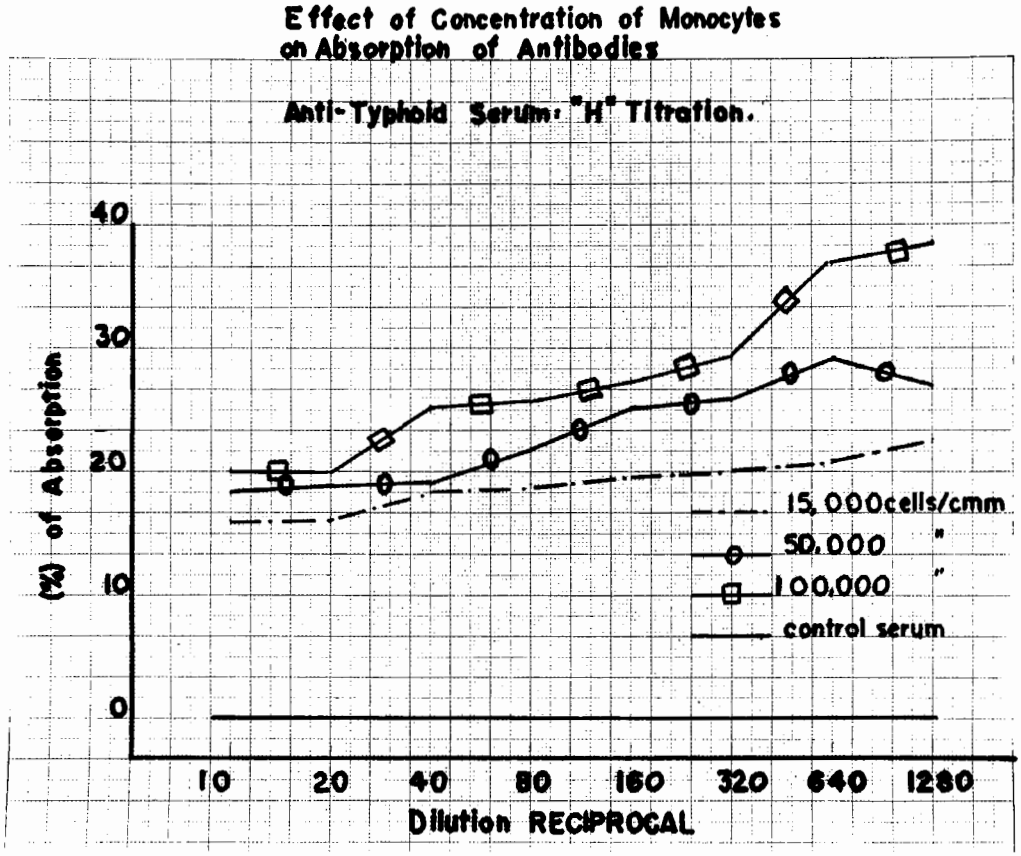
TABLE NO.10 (CONT'D)

EFFECT OF CONCENTRATION OF MONOCYTES ON ABSORPTION OF ANTIBODIES
(100,000 cells/ccm.)

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated													No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840	327680					
.6 hrs.	1/10	6	6	6	6	6	6	6	4	2	1	1			50	63	50/63	80.0	20.0
	1/20		6	6	6	6	6	6	4	3	1	1			45	57	45/57	80.0	20.0
	1/40			6	6	6	6	6	4	2	1	1			38	51	38/51	75.5	24.5
	1/80				6	6	6	6	4	3	1	1			33	45	33/45	73.5	26.5
	1/160					6	6	6	5	3	1	1			28	39	28/39	72.0	28.0
	1/320						6	6	6	3	2	1			24	33	24/33	72.0	28.0
	1/640							6	5	3	2	1			17	27	17/27	62.5	37.5
	1/1280								5	3	3	2			13	21	13/21	61.5	38.5
	Control	6	6	6	6	6	6	6	6	6	3	3	2	1				100.0	0.0

FIG. NO. 1


Effect of Concentration of Monocytes
on Absorption of Antibodies
Anti-Typhoid Serum: "H" Titration.
40
30
20
10
0
(%) of Absorption
15,000cells/cmm
50,000 "
100,000 "
control serum
10
20
40
80
160
320
640
1280
Dilution RECIPROCAL

IV. ABSORPTION OF THE ANTI-TYPHOID SERUM (diluent: Parker's solution)

In this experiment, the relationships between the absorption of antibodies by monocytes to a) various dilutions of anti-typhoid serum and b) periods of incubation were studied.

Five identical sets of dilutions of anti-typhoid serum were set up in 0.5 cc volume and to each dilution was added 0.5 cc of standardized cell suspension to give dilutions of antibodies from 1/10 to 1/1280 in 1 cc volume. Each set was incubated for 4, 8, 16, 24 and 48 hours, respectively. At the end of each incubation period, titrations against "O" and "H" antigens were carried out as previously described and the results reported in Table No. 11.

The antiserum used was obtained from rabbit No. 1835.

This experiment was carried out in two parts:

a) 2 sets of identical series of dilutions were incubated for 4 and 8 hours, respectively, in contact with monocytes obtained from rabbit No. 1862

b) 3 sets of identical series of dilutions were incubated for 16, 24 and 48 hours, respectively, in contact with monocytes obtained from rabbit No. 1861

The exudate in rabbit No. 1862 is produced as follows:

1st. day:

a) blood picture:

white blood cells: 10200 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	1	30	63	6	612

b) injection of 20 mgm. of MPA intravenously

3rd. day:

a) blood picture:

white blood cells: 11000 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	25	60	15	1650

b) injection of 20 mgm. of MPA intravenously

c) injection of 8 cc of a mixture of gum-arabic and beef extract intrapleurally

6th. day:

a) blood picture:

white blood cells: 11500 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	23	60	17	1975

b) removal of the exudate

c) rabbit was bled by heart puncture and serum collected from the 50 cc of blood obtained and used in the preparation of Parker's solution

d) picture of the exudate: 10 cc of exudate were collected. White blood cells: 15000 cells/c.mm.

red blood cells: 50000 cells/c.mm.

differential count: polymorphoneuclears: 9%

lymphocytes: 12%

macrophages: 79%

8 cc of cell suspension standardized to 15000 monocytes/c.mm. are obtained.

The exudate in rabbit No. 1861 is produced as follows:

1st. day:

a) blood picture:

white blood cells: 9150 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	39	64	4	366

b) injection of 20 mgm. of MPA intravenously

3rd. day:

a) blood picture:

white blood cells: 7800 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	15	71	14	1092

b) injection of 20 mgm. of MPA intravenously

c) injection of 8 cc of a mixture of gum-arabic and beef extract intrapleurally

6th. day:

a) blood picture:

white blood cells: 9850 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	49	42	13	1015

b) removal of the exudate

c) rabbit was bled by heart puncture and serum collected from the 50 cc of blood obtained and used in the preparation of Parker's solution

d) picture of the exudate: 21 cc of exudate were collected.

White cells: 10000 cells/c.mm.

red cells: 50000 cells/c.mm.

differential count: polymorphoneuclears: 7%

lymphocytes: 14%

macrophages: 79%

13 cc of cell suspension standardized to 15000 monocytes/c.mm. are obtained.

From the results reported in Table No. 11 and Fig. No. 2-3-4-5, it appears that the proportion of antibodies absorbed by monocytes increases as the dilution of the antiserum increases. Maximum of absorption took place after 16-hour period of incubation for the anti-typhoid "O" serum and after 24-hour period of incubation for the anti-typhoid "H" serum.

At this point it was felt that the preservation medium used might be improved because after 48 hours of incubation a high percentage of monocytes were lysed. Simul-

taneously it was observed that only a very low degree of absorption of antibodies took place. It was felt that the low degree of absorption of antibodies occurring after 48 hours of incubation might be due to the possibility that monocytes in the medium used degenerate after 24 hours and, consequently, liberate the absorbed antibodies. This degeneration of monocytes might be due to the poor preserving properties of the medium or to the agitating action of the roller drum.

TABLE NO. 11

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated												No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840					
hrs.	1/10	6	6	6	6	6	6	6	6	6	3	1	1	59	59	59/59	100.0	0.0
	1/20		6	6	6	6	6	6	6	6	3	1	1	53	53	53/53	100.0	0.0
	1/40			6	6	6	6	6	6	6	3	1	1	47	47	47/47	100.0	0.0
	1/80				6	6	6	6	6	4	3	1	1	39	41	39/41	95.0	5.0
	1/160					6	6	6	6	5	3	1	1	34	35	34/35	97.0	3.0
	1/320						6	6	6	5	3	1	1	28	29	28/29	97.0	3.0
	1/640							6	6	5	3	1	1	22	23	22/23	95.5	4.5
	1/1280								6	5	3	1	1	16	17	16/17	94.0	6.0
	Control	6	6	6	6	6	6	6	6	6	3	1	1				100.0	0.0

TABLE NO. 11 (CONT'D)

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated												No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840					
hrs.	1/10	6	6	6	6	6	6	6	6	5	4	1	1	59	60	59/60	98.5	1.5
	1/20		6	6	6	6	6	6	6	5	3	1	1	52	54	52/54	96.5	3.5
	1/40			6	6	6	6	6	6	5	3	1	1	46	48	46/48	96.0	4.0
	1/80				6	6	6	6	6	5	3	1	1	40	42	40/42	95.5	4.5
	1/160					6	6	6	6	5	3	1	1	34	36	34/36	94.5	5.5
	1/320						6	6	6	4	3	1	1	27	30	27/30	90.0	10.0
	1/640							6	6	5	3	1	1	22	24	22/24	91.5	8.5
	1/1280								6	5	3	1	1	16	18	16/18	89.0	11.0
	Control	6	6	6	6	6	6	6	6	6	4	1	1				100.0	0.0

TABLE NO. 11 (CONT'D)

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated												No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840					
6 hrs.	1/10	6	6	6	6	6	6	6	6	5	4	2	1	60	62	60/62	97.0	3.0
	1/20		6	6	6	6	6	6	6	5	4	2	1	54	56	54/56	96.5	3.5
	1/40			6	6	6	6	6	6	5	4	2	1	48	50	48/50	96.0	4.0
	1/80				6	6	6	6	6	5	4	2	1	42	44	42/44	95.5	4.5
	1/160					6	6	6	6	5	4	2	1	36	38	36/38	95.0	5.0
	1/320						6	6	5	4	3	2	1	27	32	27/32	84.5	15.5
	1/640							6	5	3	3	2	1	20	26	20/26	77.5	22.5
	1/1280								5	4	3	2	1	15	19	15/19	79.0	21.0
	Control	6	6	6	6	6	6	6	6	6	4	3	1				100.0	0.0

TABLE NO. 11 (CONT'D)

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated												No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840					
24 hrs.	1/10	6	6	6	6	6	6	6	6	5	4	2		59	64	59/64	92.5	7.5
	1/20		6	6	6	6	6	6	6	5	4	2		53	58	53/58	91.5	8.5
	1/40			6	6	6	6	6	6	5	4	2		47	52	47/52	90.5	9.5
	1/80				6	6	6	6	6	5	4	2		41	46	41/46	89.5	10.5
	1/160					6	6	6	6	5	4	2		35	40	35/40	87.5	12.5
	1/320						6	6	4	4	3	1		24	34	24/34	70.5	29.5
	1/640							6	6	4	3	1		20	28	20/28	71.5	28.5
	1/1280								6	4	3	1	1	15	22	15/22	68.5	31.5
	Control	6	6	6	6	6	6	6	6	6	4	4	2				100.0	0.0

TABLE NO. 11 (CONT'D)

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated												No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840					
8 hrs.	1/10	6	6	6	6	6	6	6	6	6	4	2		60	63	60/63	95.0	5.0
	1/20		6	6	6	6	6	6	6	6	4	2		54	57	54/57	95.0	5.0
	1/40			6	6	6	6	6	6	6	4	2		48	51	48/51	94.0	6.0
	1/80				6	6	6	6	6	6	4	2	1	43	45	43/45	94.5	4.5
	1/160					6	6	6	6	6	4	2	1	37	39	37/39	95.0	5.0
	1/320						6	6	6	6	4	2	1	31	33	31/33	94.0	6.0
	1/640							6	6	6	4	2	1	25	27	25/27	92.5	7.5
	1/1280								6	6	4	3	1	20	21	20/21	95.5	4.5
	Control	6	6	6	6	6	6	6	6	6	4	3	2				100.0	0.0

FIG. NO. 2

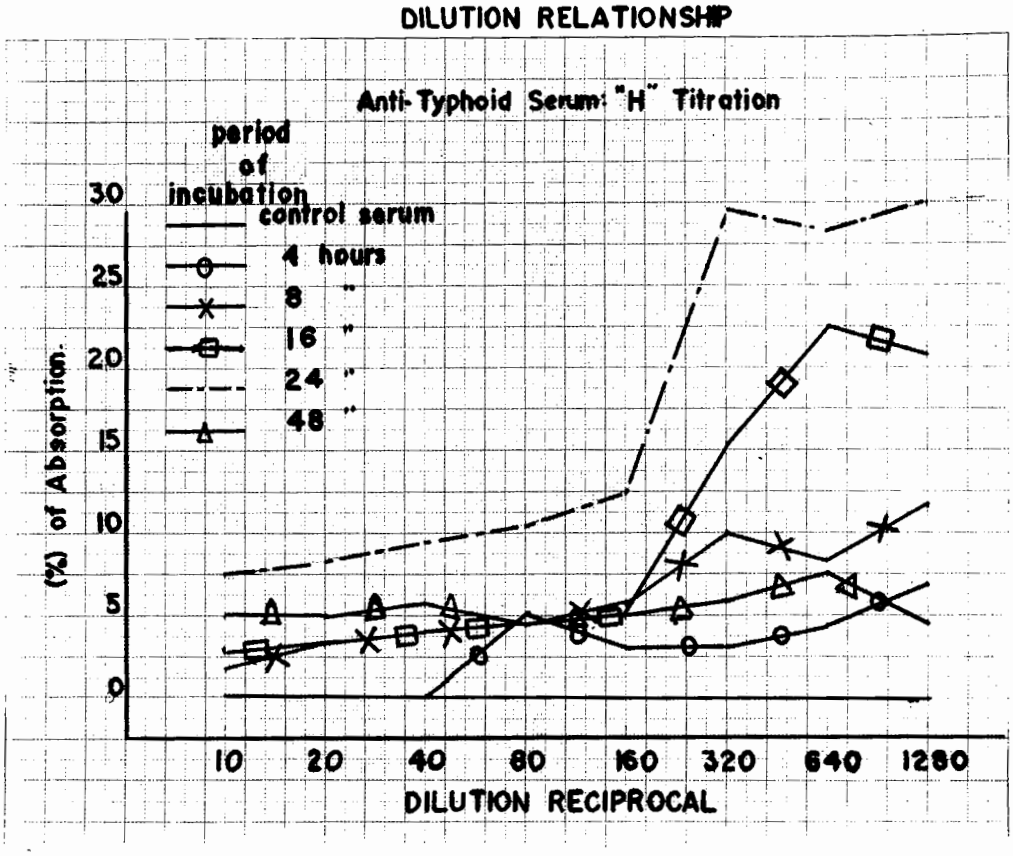

DILUTION RELATIONSHIP
Anti-Typhoid Serum "H" Titration
period of incubation
control serum
4 hours
8 "
16 "
24 "
48 "
(%) of Absorption
30
25
20
15
10
5
0
10
20
40
80
160
320
640
1280
DILUTION RECIPROCAL

FIG. NO. 3

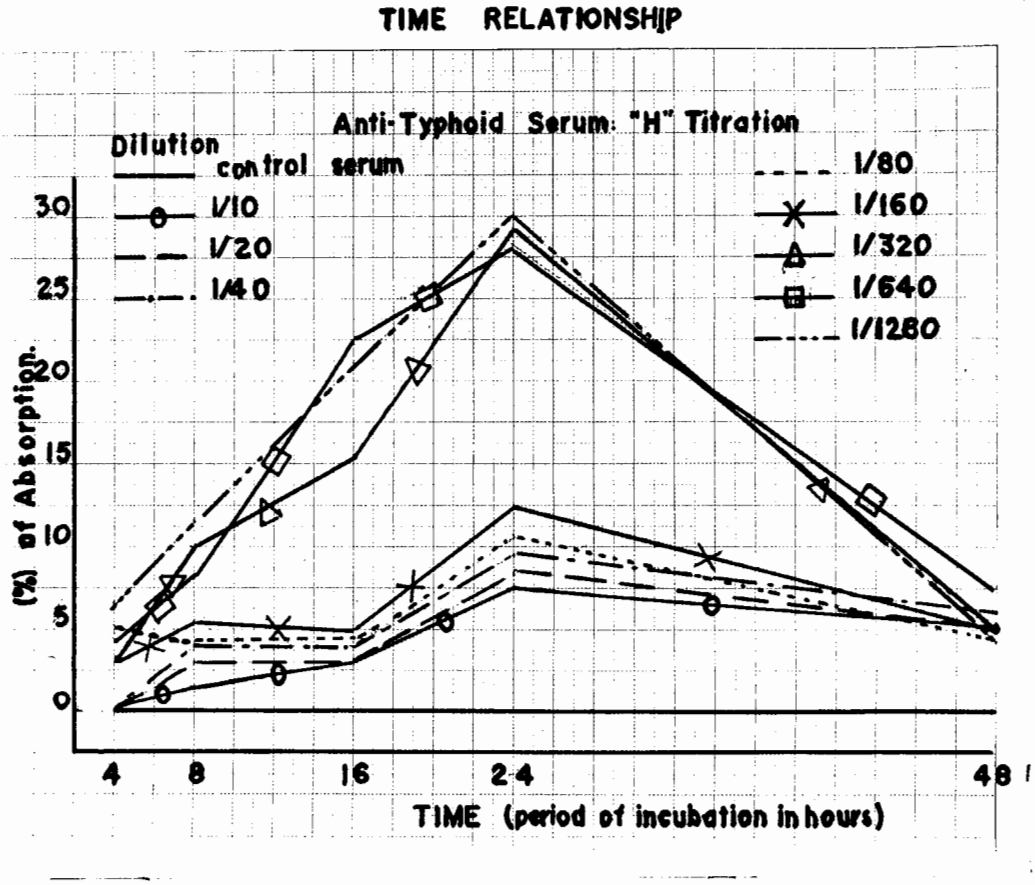

TIME RELATIONSHIP
Anti-Typhoid Serum: "H" Titration
Dilution
control serum
1/10
1/20
1/40
1/80
1/160
1/320
1/640
1/1280
(%) of Absorption.
30
25
20
15
10
5
0
4
8
16
24
48
TIME (period of incubation in hours)

TABLE NO. 11 (CONT'D)

ANTI-TYPHOID SERUM: "O" TITRATION

Period of incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated											No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		40	80	160	320	640	1280	2560	5120	10240	20480	40960					
hrs.	1/10	6	6	6	6	6	6	5	4	3	2	1	51	51	51/51	100.0	0.0
	1/20		6	6	6	6	6	5	4	3	2	1	45	45	45/45	100.0	0.0
	1/40			6	6	6	6	5	4	3	2	1	39	39	39/39	100.0	0.0
	1/80				6	6	6	5	4	3	2	1	33	33	33/33	100.0	0.0
	1/160					6	6	5	4	3	2	1	27	27	27/27	100.0	0.0
	1/320						6	5	4	3	2	1	21	21	21/21	100.0	0.0
	1/640							5	4	3	2	1	15	15	15/15	100.0	0.0
	1/1280								4	3	2	1	10	10	10/10	100.0	0.0
	Control	6	6	6	6	6	6	5	4	3	2	1				100.0	0.0

TABLE NO. 11 (CONT'D)

ANTI-TYPHOID SERUM: "O" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated										No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		40	80	160	320	640	1280	2560	5120	10240	20480 40960					
hrs.	1/10	6	6	6	6	6	6	5	4	1	1	47	48	47/48	98.0	2.0
	1/20		6	6	6	6	6	5	4	1	1	41	42	41/42	98.0	2.0
	1/40			6	6	6	5	5	4	1	1	34	36	34/36	94.5	5.5
	1/80				6	6	5	5	4	1	1	28	30	28/30	93.5	6.5
	1/160					6	6	5	4	1		22	24	22/24	92.0	8.0
	1/320						6	5	4	1	1	17	18	17/18	94.5	5.5
	1/640							5	4	1	1	11	12	11/12	92.0	8.0
	1/1280								4	2	1	7	7	7/7	100.0	0.0
	Control	6	6	6	6	6	6	5	4	2	1				100.0	0.0

TABLE NO. 11 (CONT'D)

ANTI-TYPHOID SERUM: "O" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated										No. of plusses in tested serum dilution	No. of plusses in Corresponding control serum dilution	Ratio	% Left	% Absorbed
		40	80	160	320	640	1280	2560	5120	10240	20480 40960					
6 hrs.	1/10	6	6	6	6	6	6	5	4	3	1	49	50	49/50	98.0	2.0
	1/20		6	6	6	6	6	5	4	2	1	42	44	39/41	95.5	4.5
	1/40			6	6	6	6	5	4	3	1	37	38	37/38	95.0	5.0
	1/80				6	6	5	4	4	3	1	29	32	29/32	91.0	9.0
	1/160					6	5	4	4	3	1	23	26	23/26	89.0	11.0
	1/320						5	4	4	3	1	17	20	17/20	85.0	15.0
	1/640							4	4	3	1	12	14	12/14	86.0	14.0
	1/1280								4	3	1	8	9	8/9	89.0	11.0
	Control	6	6	6	6	6	6	5	5	3	1				100.0	0.0

TABLE NO. 11 (CONT'D)

ANTI-TYPHOID SERUM: "O" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated											No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		40	80	160	320	640	1280	2560	5120	10240	20480	40960					
4 hrs.	1/10	6	6	6	6	6	6	5	4	3	2		50	51	50/51	98.0	2.0
	1/20		6	6	6	6	6	5	4	3	2		44	45	44/45	98.0	2.0
	1/40			6	6	6	6	5	4	3	2		38	39	38/39	97.5	2.5
	1/80				6	6	6	5	4	2	1		30	33	30/33	91.0	9.0
	1/160					6	6	5	4	2	1		24	27	24/27	89.0	11.0
	1/320						6	5	4	3	1		19	21	19/21	90.5	9.5
	1/640							5	4	3	2		14	15	14/15	93.5	6.5
	1/1280								4	3	2		9	9	9/9	100.0	0.0
	Control	6	6	6	6	6	6	5	4	3	2	1				100.0	0.0

TABLE NO. 11 (CONT'D)

ANTI-TYPHOID SERUM: "O" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated											No. of plusses in tested serum Dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		40	80	160	320	640	1280	2560	5120	10240	20480	40960					
3 hrs.	1/10	6	6	6	6	6	6	5	5	4	2	1	53	53	53/53	100.0	0.0
	1/20		6	6	6	6	6	5	5	4	2	1	47	47	47/47	100.0	0.0
	1/40			6	6	6	6	5	5	4	2	1	41	41	41/41	100.0	0.0
	1/80				6	6	6	5	5	4	2	1	35	35	35/35	100.0	0.0
	1/160					6	6	5	5	4	2	1	29	29	29/29	100.0	0.0
	1/320						6	5	5	4	2	1	23	23	23/23	100.0	0.0
	1/640							5	5	4	2	1	17	17	17/17	100.0	0.0
	1/1280								5	4	2	1	12	12	12/12	100.0	0.0
	Control	6	6	6	6	6	6	5	5	4	2	1				100.0	0.0

FIG. NO. 4

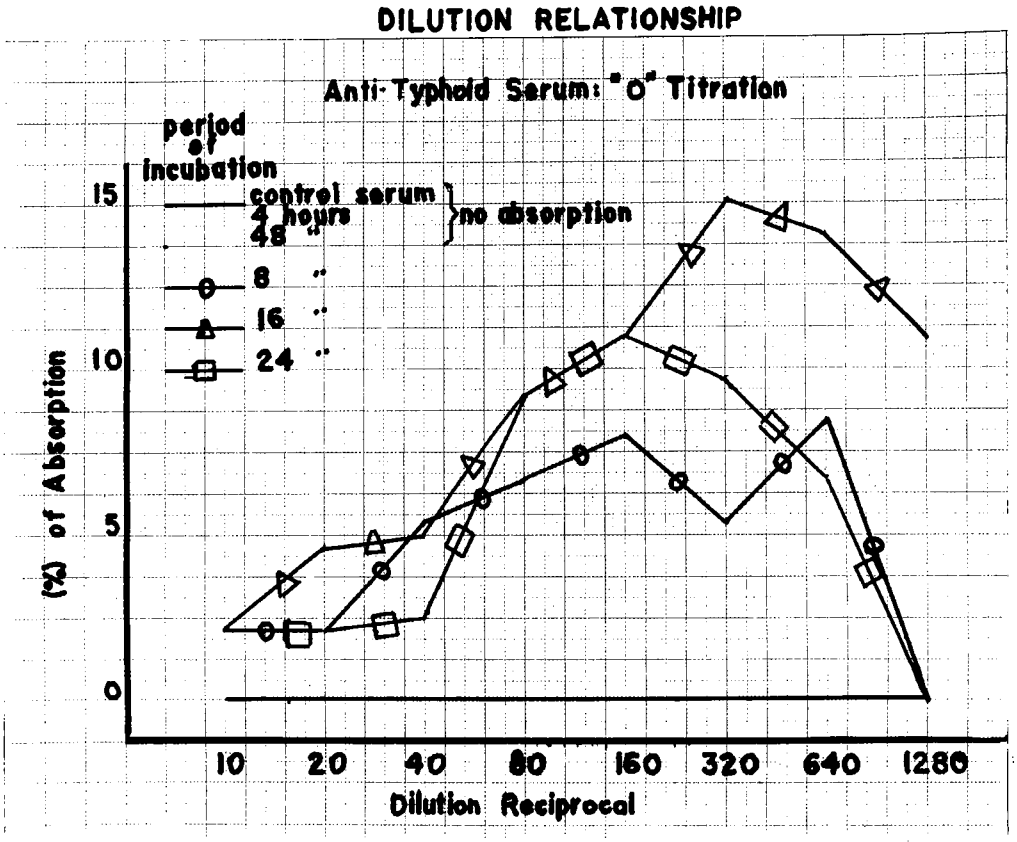
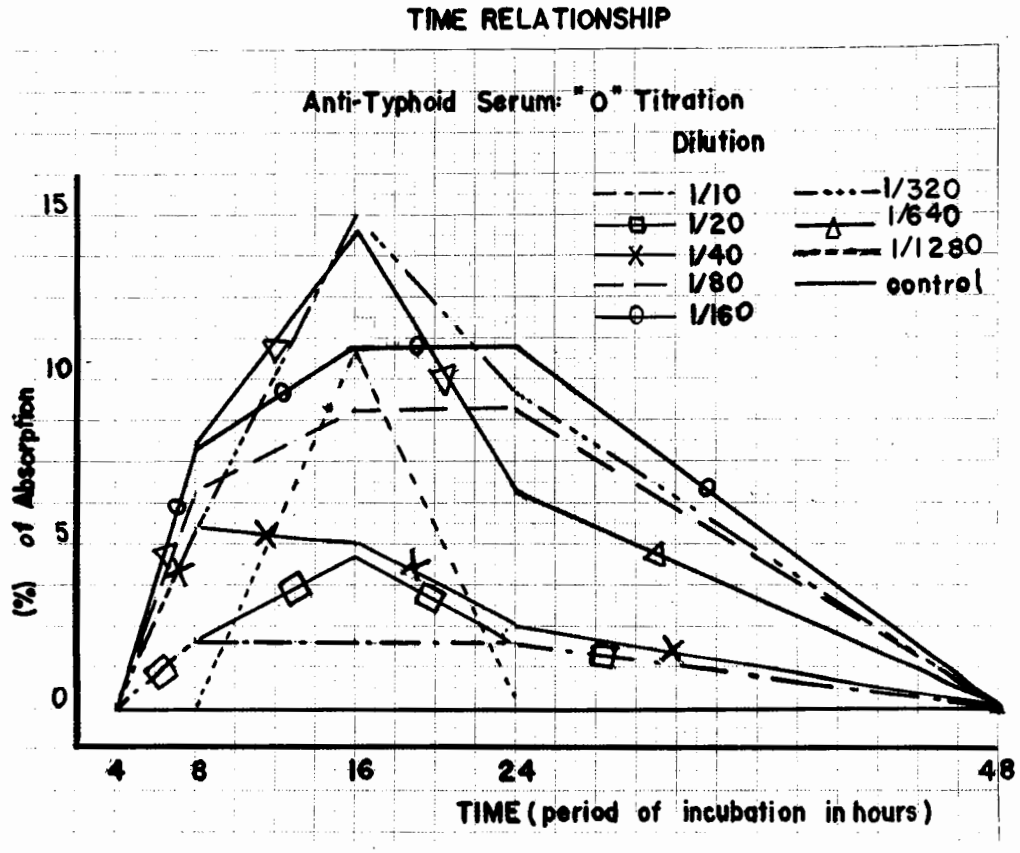

DILUTION RELATIONSHIP
Anti-Typhoid Serum: "O" Titration
period of incubation
control serum
4 hours
48 "
no absorption
8 "
16 "
24 "
(%) of Absorption
15
10
5
0
10
20
40
80
160
320
640
1280
Dilution Reciprocal

FIG. NO. 5


TIME RELATIONSHIP
Anti-Typhoid Serum: "O" Titration
Dilution
1/10
1/20
1/40
1/80
1/160
1/320
1/640
1/1280
control
15
10
5
0
(%) of Absorption
4
8
16
24
48
TIME (period of incubation in hours)

V. CHOICE OF A MEDIUM

In order to obtain a medium which will preserve the monocytes for at least 48 hours, serum ultrafiltrate, rabbit serum and synthetic media were tested. It was hoped that the synthetic media would prove to be the best since no serum other than the tested antiserum would be present. The monocytes were obtained from rabbit No. 1898 treated as follows:

1st. day:

a) blood picture

white blood cells: 8000 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	1	1	28	65	5	400

b) injection of 20 mgm. of MPA

c) injection of 8 cc of a mixture of gum-arabic and beef extract intrapleurally

3rd. day:

a) blood picture:

white blood cells: 14200 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	21	64	15	1300

b) removal of the exudate

c) rabbit was bled by heart puncture and serum collected from the 50 cc of blood obtained and used in the preparation of Earle's serum solution.

d) pciture of the exudate: 10 cc of exudate were collected.

White cells: 12000 cells/c.mm.

red cells: 10000 cells/c.mm.

differential count: polymorphoneuclears: 13%

lymphocytes: 5%

macrophages: 82%

8 cc of cell suspension standardized to 7500 monocytes/c.mm. are obtained.

1 cc of standardized cell suspension 7500 monocytes/c.mm. in saline was added to each of 8 tubes and centrifuged. The cells were resuspended in 1 cc of each of the following media:

Medium No. 1: serum ultrafiltrate solution

" No. 2: Earle's serum solution

" No. 3: Earle's ultrafiltrate solution

" No. 4: Simm's ultrafiltrate solution

" No. 5: V-614 solution

" No. 6: V-614 ultrafiltrate solution

" No. 7: Baker's solution

" No. 8: Baker's ultrafiltrate solution

The tubes were laid flat during the 48-hour incubation. During the incubation, the monocytes settle along the wall of the tubes and adhere to the wall if not agitated.

At the end of 24 hours, smears from the above

media were made and stained by Jenner's Giemsa method. Cells suspended in media No. 2, 7, 8 showed no degeneration. Cells suspended in media No. 1, 3, 4, 5 and 6 showed varying degrees of lysis. At the end of 48 hours, the appearance of the cells suspended in the different media was as follows:

Medium No.	Density of growth obtained on wall of tubes after 48 hours	Monocyte Count at 48 hours	Jenner's Giemsa staining at 48 hours
1	+	4000 m./c.mm.	Few monocytes Few red blood cells Much debris
2	+++	6000 m./c.mm.	Large monocytes without signs of lysis. Red blood cells still present in a normal state.
3	+	Negligible	Few monocytes. No red blood cells visible. Much debris.
4	+	Negligible	No monocytes seen. No red blood cells visible. Much debris
5	+	Negligible	Few monocytes. Few red blood cells. Debris.
6	+	Negligible	Few monocytes. Few red blood cells. Debris.
7	++++	8000 m/c.mm.	Monocytes show no lysis, some are dividing. Red blood cells still present in a normal state.
8	+++	5600 m/c.mm.	Idem.

Media No. 2, 7 and 8 preserve monocytes without lysis for at least 48 hours. The preiod is sufficient to carry out absorption tests since the maximum absorption appears to take place within this period.

Earle's serum solution, which gives as good results as the Baker's solution, is preferred because of its ease of preparation and is, consequently, used in all subsequent experiments.

VI. EFFECT OF AGITATION UPON ABSORPTION OF ANTIBODIES

Two sets of identical series of dilutions were prepared, using anti-typhoid serum. One set was placed on the roller drum at 37°C, the other was laid flat in the 37°C incubator.

It was found that the agitating action of the roller drum did not bring about degeneration of the monocytes after 16 hours of incubation.

The percentage of absorption in the two sets of antiserum dilutions is approximately the same. Consequently, in all the other subsequent experiments, the tubes were not agitated, but were laid flat in the incubator.

The cells used for this experiment were obtained from rabbit No. 1868 treated as follows:

1st. day:

a) blood picture:

white blood cells: 12000 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	20	76	4	480

b) injection of 20 mgm. of MPA intravenously

3rd. day:

a) blood picture

white blood cells: 16600 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	2	0	27	57	16	2656

b) injection of 20 mgm. of MPA intravenously

c) injection of 8 cc of a mixture of gum-arabic and beef extract intrapleurally

6th day:

a) blood picture:

white blood cells: 16600 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	1	28	56	15	2490

b) removal of exudate

c) rabbit was bled by heart puncture and serum collected from the 50 cc of blood obtained and used in the preparation of Earle's serum solution

d) picture of the exudate: 10 cc of exudate were obtained.

White blood cells: 27000 cells/c.mm.

red blood cells: 44000 cells/c.mm.

differential count: polymorphoneuclears: 5%

lymphocytes: 2%

macrophages: 93%

17 cc of cell suspension standardized to 15000 monocytes/c.mm. in Earle's serum solution are obtained.

5 cc of this cell suspension were used as follows and the 12 cc remaining were kept for an other experiment carried out the same day:

The two sets of identical dilutions of anti-typhoid serum (from rabbit No. 1835) were prepared to give the following final dilutions of antiserum when in contact with cells: 1/160, 1/320, 1/640, 1/1280, 1/2560.

The dilutions of antiserum were made in 0.5 cc of diluent (Earle's serum solution). To each dilution was added 0.5 cc of standardized cell suspension. The first set of tubes was incubated on the roller drum, the second incubated in an horizontal position.

At the end of 16-hour incubation period, the supernatant of each dilution, from both sets, was separated from the cells by centrifugation and titrated. The results are reported in Table No.12a and Fig. No. 6 .

TABLE NO. 12a

EFFECT OF CIRCULATING FLUID MEDIA ON ABSORPTION OF ANTIBODIES

ANTI-TYPHOID SERUM: "H" TITRATION

(TUBES PLACED ON A ROLLER DRUM)

Period of incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated: 1280	2560	5120	10240	20480	40960	81920	163840	327640	No. of plusses in tested serum dilution	No. of plusses in corresponding serum dilution	Ratio	% Left	% Absorbed
16 hrs.	1/80	6	6	6	6	4	3	2	1		34	40	34/40	85.0	15.0
	1/160		6	6	6	4	3	2	1		28	34	28/34	82.0	18.0
	1/320			6	6	4	3	2	1		22	28	22/28	79.0	21.0
	1/640				6	4	3	2	1		16	22	16/22	72.0	28.0
	1/1280					4	4	2	1		11	16	11/16	68.0	32.0
	Control (placed on a roller drum)	6	6	6	6	6	4	3	2	1				100.0	0.0

(TUBES PLACED ON A PLANE SURFACE)

16 hrs.	1/80	6	6	6	6	4	3	2	1		34	40	34/40	85.0	15.0
	1/160		6	6	6	4	3	2	1		28	34	28/34	82.0	18.0
	1/320			6	6	4	3	2	1		22	28	22/28	79.0	21.0
	1/640				6	3	3	1	1		14	22	14/22	62.5	37.5
	1/1280					4	4	2	1		11	16	11/16	68.0	32.0
	Control (placed on a plane surface)	6	6	6	6	6	4	3	2	1				100.0	0.0

TABLE NO. 12a

EFFECT OF CIRCULATING FLUID MEDIA ON ABSORPTION OF ANTIBODIES

ANTI-TYPHOID SERUM: "O" TITRATION

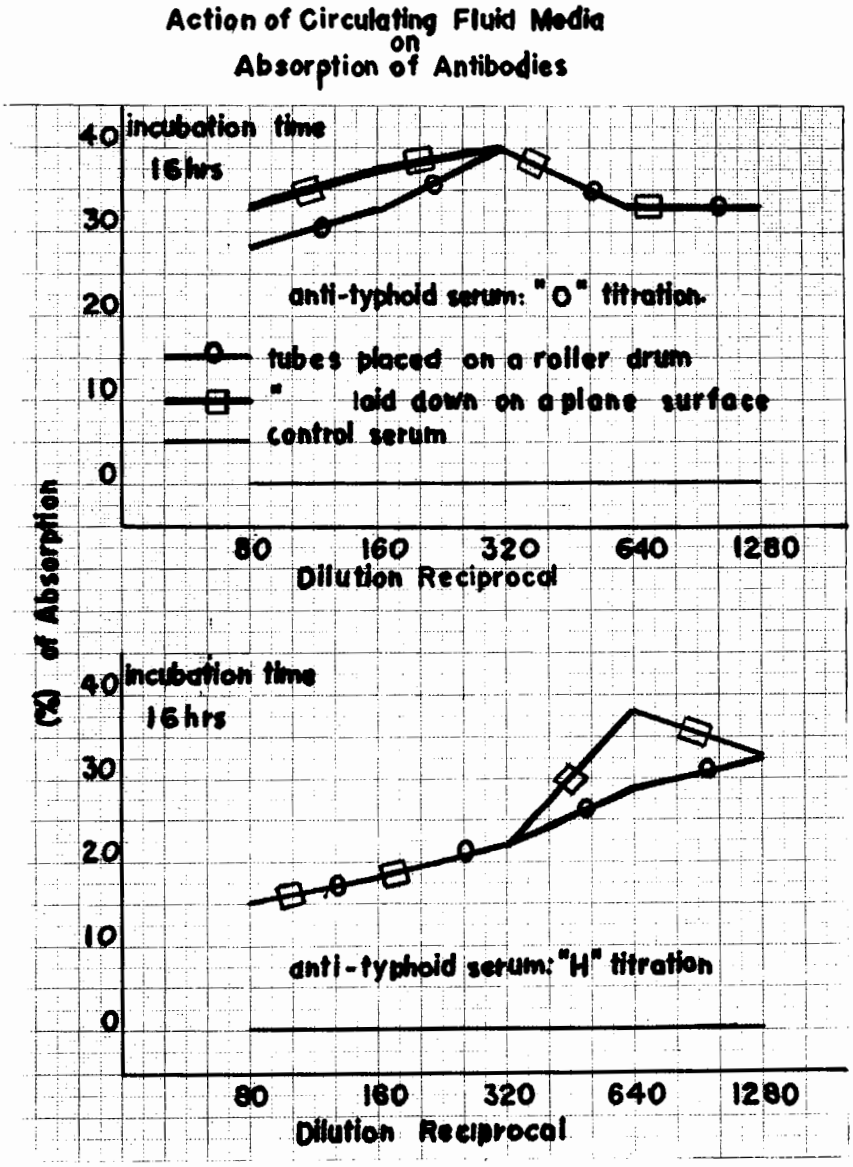
(TUBES PLACED ON A ROLLER DRUM)

Period of incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated						No. of plusses in tested serum dilution	No. of plusses in corresponding serum dilution	Ratio	% Left	% Absorbed
		1280	2560	5120	10240	20480	40960					
16 hrs.	1/80	5	4	3	2	1		15	21	15/21	72.0	28.0
	1/160		4	3	2	1		10	15	10/15	67.0	33.0
	1/320			3	2	1		6	10	6/10	60.0	40.0
	1/640				2	1	1	4	6	4/6	67.0	33.0
	1/1280					1	1	2	3	2/3	67.0	33.0
	Control (placed on a roller drum)	6	5	4	3	2	1				100.0	0.0

(TUBES PLACED ON A PLANE SURFACE)

16 hrs.	1/80	5	4	3	1	1		14	21	14/21	67.0	33.0
	1/160		4	3	1	1		9	15	9/15	60.0	40.0
	1/320			3	2	1		6	10	6/10	60.0	40.0
	1/640				2	1	1	4	6	4/6	67.0	33.0
	1/1280				2	1	1	2	3	2/3	67.0	33.0
	Control (placed on a plane surface)	6	5	4	3	2	1				100.0	0.0

FIG NO. 6


Action of Circulating Fluid Media
on
Absorption of Antibodies
incubation time
16 hrs
40
30
20
10
0
anti-typhoid serum: "O" titration.
tubes placed on a roller drum
" laid down on a plane surface
control serum
80
160
320
640
1280
Dilution Reciprocal
(%) of Absorption
incubation time
16 hrs
40
30
20
10
0
anti-typhoid serum: "H" titration
80
160
320
640
1280
Dilution Reciprocal

VII. ABSORPTION OF ANTI-TYPHOID SERUM (diluent: Earle's serum solution)

Antiserum from rabbit No. 1835 was diluted in Earle's serum solution in 0.5 cc volume to give 5 sets of identical series of dilutions. To each dilution was added 0.5 cc of standardized cell suspension and each incubated for 4, 8, 16, 24 and 48 hours, respectively. The monocytes used for the 4 and 8-hour periods of incubation were obtained from rabbit No. 1899; those used for periods of incubation of 16, 24 and 48 hours were obtained from rabbit 1868 (the 12 cc remaining from the experiment carried out on the effect of agitation upon the absorption of antibodies).

Rabbit No. 1868 was treated as described on page 111. Rabbit No. 1899 was treated as follows:

1st. day:

a) blood picture

white blood cells: 8650 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	27	71	2	173

b) injection of 20 mgm. of MPA intravenously

3rd. day:

a) blood picture

white blood cells: 20700 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	33	42	25	5175

b) injection of 20 mgm. of MPA intravenously

c) injection of 8 cc of a mixture of gum-arabic and beef extract intrapleurally

6th. day:

a) blood picture:

white blood cells: 12250 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	44	39	17	2082

b) removal of exudate

c) rabbit was bled by heart puncture and serum collected from the 50 cc of blood obtained and used in the preparation of Earle's serum solution

d) picture of the exudate: 15 cc of exudate were obtained.

White blood cells: 19600 cells/c.mm.

red blood cells: 18000 cells/c.mm.

differential count: polymorphoneuclears: 10%

lymphocytes: 5%

macrophages: 85%

16.6 cc of cell suspension standardized to 15000 monocytes/c.mm. in Earle's serum solution are obtained.

The results obtained are reported in Table No. 13 and Fig. No. 7-8-9-10.

It appears that the degree of absorption of antibodies is increased with increasing dilutions of antiserum. The optimum time for maximal absorption of anti-typhoid "O" serum is 16 hours and for anti-typhoid "H" serum is 8 hours

In this experiment, using Earle's serum solution as a suspending fluid for the monocytes, a higher degree of absorption of antibodies was obtained than in the corresponding experiment carried out with the Parker's solution as the suspending medium. The optimum time of incubation for maximal absorption is shorter when Earle's serum solution is used than when Parker's fluid is used. That would appear to indicate that the monocytes are in a more active state in the Earle's serum solution.

When using Earle's serum solution, only a slight proportion of monocytes was lysed after 48 hours of incubation as compared with Parker's solution. It was also found that the degree of absorption after 48 hours of incubation was approximately the same as that obtained after 24 hours of incubation. This was not so when Parker's solution was used as reported in a previous experiment. Consequently, it would appear that the Earle's serum solution is the most efficient medium for this work.

FIG. NO. 7

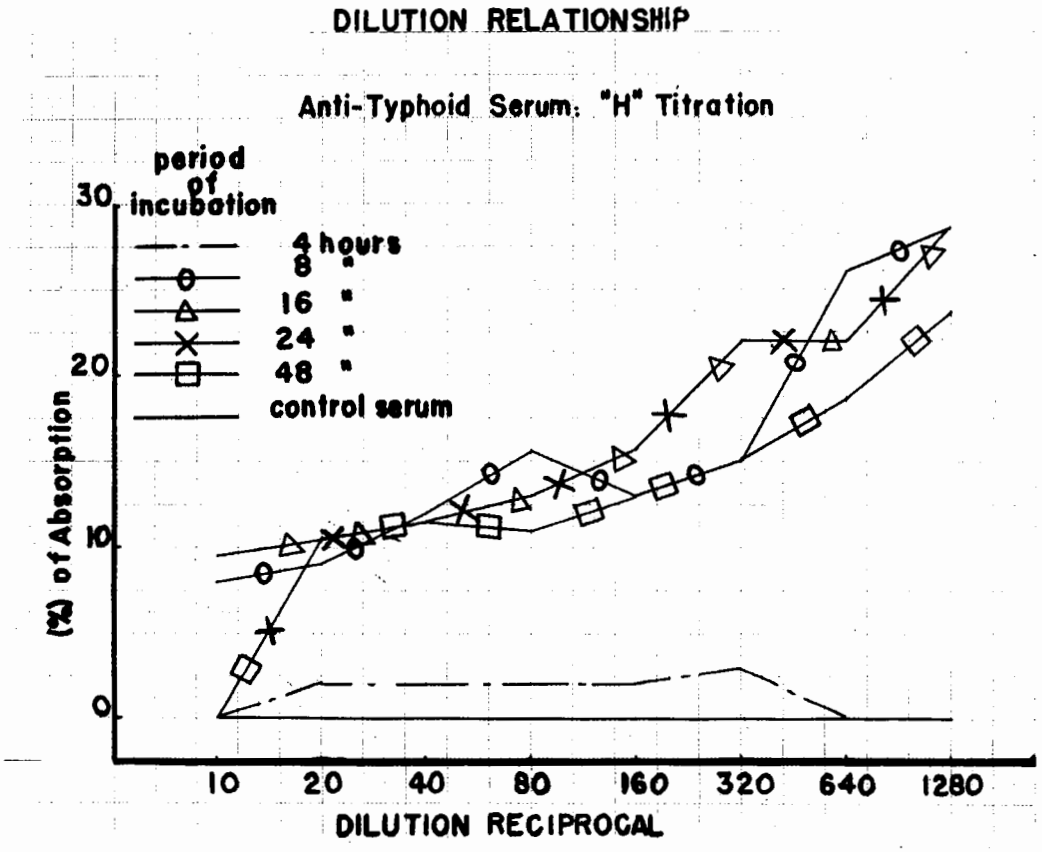

DILUTION RELATIONSHIP
Anti-Typhoid Serum: "H" Titration
period of incubation
4 hours
8 "
16 "
24 "
48 "
control serum
(%) of Absorption
30
20
10
0
10
20
40
80
160
320
640
1280
DILUTION RECIPROCAL

FIG. NO. 8

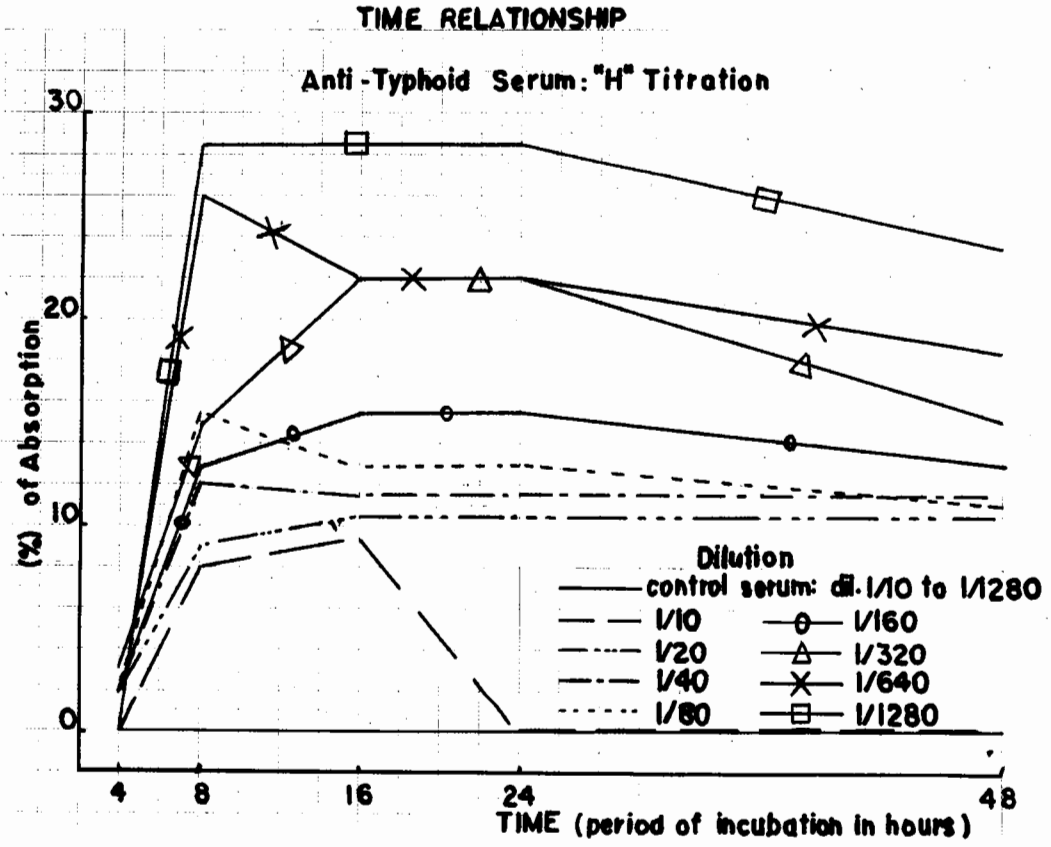

TIME RELATIONSHIP
Anti-Typhoid Serum: "H" Titration
30
20
10
0
(%) of Absorption
4
8
16
24
48
TIME (period of incubation in hours)
Dilution
control serum: dil. 1/10 to 1/1280
1/10
1/20
1/40
1/80
1/160
1/320
1/640
1/1280

TABLE NO. 12

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated													No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840	327680					
hrs.	1/10	6	6	6	6	6	6	6	6	6	4	3	1	1	63	63	63/63	100.0	0.0
	1/20		6	6	6	6	6	6	6	5	4	3	1	1	56	57	56/57	98.0	2.0
	1/40			6	6	6	6	6	6	5	4	3	1	1	50	51	50/51	98.0	2.0
	1/80				6	6	6	6	6	5	4	3	1	1	44	45	44/45	98.0	2.0
	1/160					6	6	6	6	5	4	3	1	1	38	39	38/39	98.0	2.0
	1/320						6	6	6	5	4	3	1	1	32	33	32/33	97.0	3.0
	1/640							6	6	6	4	3	1	1	27	27	27/27	100.0	0.0
	1/1280								6	6	4	3	1	1	21	21	21/21	100.0	0.0
	Control	6	6	6	6	6	6	6	6	6	4	3	1	1				100.0	0.0

TABLE NO. 12 (CONT'D)

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated													No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840	327680					
hrs.	1/10	6	6	6	6	6	6	6	5	5	3	2	1	1	59	64	59/64	92.0	8.0
	1/20		6	6	6	6	6	6	5	5	3	2	1	1	53	58	53/58	91.0	9.0
	1/40			6	6	6	6	6	5	4	3	2	1	1	46	52	46/52	88.5	11.5
	1/80				6	6	6	5	5	4	3	2	1	1	39	46	39/46	85.0	15.0
	1/160					6	6	6	5	5	3	2	1	1	35	40	35/40	87.5	12.5
	1/320						6	6	5	5	3	2	1	1	29	34	29/34	85.0	15.0
	1/640							5	5	4	3	2	1	1	21	28	21/28	75.0	25.0
	1/1280								5	4	3	2	1	1	16	22	16/22	72.0	28.0
	Control	6	6	6	6	6	6	6	6	6	4	3	2	1				100.0	0.0

TABLE NO. 12 (CONT'D)

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated													No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	31920	163840	327680					
5 hrs.	1/10	6	6	6	6	6	6	6	6	4	3	2	1		58	64	58/64	91.5	9.5
	1/20		6	6	6	6	6	6	6	4	3	2	1		52	58	52/58	89.5	10.5
	1/40			6	6	6	6	6	6	4	3	2	1		46	52	46/52	88.5	11.5
	1/80				6	6	6	6	6	4	3	2	1		40	46	40/46	87.0	13.0
	1/160					6	6	6	6	4	3	2	1		34	40	34/40	85.0	15.0
	1/320						6	6	5	5	3	2			27	34	27/34	79.0	21.0
	1/640							6	5	5	3	2	1		22	28	22/28	79.0	21.0
	1/1280								5	5	3	2	1		16	22	16/22	72.0	28.0
	Control	6	6	6	6	6	6	6	6	6	4	3	2	1				100.0	0.0

TABLE NO. 12 (CONT'D)

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated													No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	31920	163840	327680					
4 hrs.	1/10	6	6	6	6	6	6	6	6	6	4	3	2	1	64	64	64/64	100.0	0.0
	1/20		6	6	6	6	6	6	6	4	3	2	1		52	58	52/58	89.5	10.5
	1/40			6	6	6	6	6	6	4	3	2	1		46	52	46/52	88.5	11.5
	1/80				6	6	6	6	6	4	3	2	1		40	46	40/46	87.0	13.0
	1/160					6	6	6	6	4	3	2	1		34	40	34/40	85.0	15.0
	1/320						6	6	5	4	3	2	1		27	34	27/34	79.0	21.0
	1/640							6	6	4	3	2	1		22	28	22/28	78.0	22.0
	1/1280								6	4	3	2	1		15	22	15/22	72.0	28.0
	Control	6	6	6	6	6	6	6	6	6	4	3	2	1				100.0	0.0

TABLE NO. 12 (CONT'D)

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated													No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840	327680					
8 hrs.	1/10	6	6	6	6	6	6	6	6	6	4	3	2	1	64	64	64/64	100.0	0.0
	1/20		6	6	6	6	6	6	6	5	3	2	1		52	58	52/58	89.5	10.5
	1/40			6	6	6	6	6	6	4	3	2	1		46	52	46/52	88.0	12.0
	1/80				6	6	6	6	6	5	3	2	1		41	46	41/46	89.0	11.0
	1/160					6	6	6	6	5	3	2	1		35	40	35/40	87.0	13.0
	1/320						6	6	6	5	3	2	1		29	34	29/34	85.0	15.0
	1/640							6	6	5	3	2	1		23	28	23/28	81.5	18.5
	1/1280								6	5	3	2	1		17	22	17/22	77.0	23.0
	Control	6	6	6	6	6	6	6	6	6	4	3	2	1				100.0	0.0

TABLE NO. 12 (CONT'D)

ANTI-TYPHOID SERUM: "O" TITRATION

Period of incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated										No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		40	80	160	320	640	1280	2560	5120	10240	20480 40960					
hrs.	1/10	6	6	6	6	6	6	4	3	2	1	46	46	46/46	100.0	0.0
	1/20		6	6	6	6	5	4	3	2		38	40	38/40	95.0	5.0
	1/40			6	6	6	6	4	3	2		33	34	33/34	97.0	3.0
	1/80				6	6	5	4	3	2		26	28	26/28	92.5	7.5
	1/160					6	5	4	3	2		20	22	20/22	91.0	9.0
	1/320						5	4	3	2		14	16	14/16	87.5	12.5
	1/640							4	3	1	1	9	10	9/10	90.0	10.0
	1/1280								3	2	1	6	6	6/6	100.0	0.0
	Control	6	6	6	6	6	6	4	3	2	1				100.0	0.0

TABLE NO. 12 (CONT'D)

ANTI-TYPHOID SERUM: "O" TITRATION

Period of incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated											No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		40	80	160	320	640	1280	2560	5120	10240	20480	40960					
3 hrs.	1/10	6	6	6	6	6	5	4	3	3	2	1	48	50	48/50	96.0	4.0
	1/20		6	6	6	6	4	4	3	2	1		38	44	38/44	87.0	13.0
	1/40			6	6	6	4	4	2	2	1		31	38	31/38	82.0	18.0
	1/80				6	6	4	4	3	2	1		26	32	26/32	81.5	18.5
	1/160					6	4	4	3	2	1		21	26	21/26	76.5	23.5
	1/320						5	4	3	2	1		16	20	16/20	80.0	20.0
	1/640							4	3	2	1		10	14	10/14	71.0	29.0
	1/1280								3	2	1		6	9	6/9	67.0	33.0
	Control	6	6	6	6	6	6	5	3	3	2	1				100.0	0.0

TABLE NO. 12 (CONT'D)

ANTI-TYPHOID SERUM: "O" TITRATION

Period of incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated											No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		40	80	160	320	640	1280	2560	5120	10240	20480	40960					
.6 hrs.	1/10	6	6	6	6	5	4	3	3	2	1		42	51	42/51	82.5	17.5
	1/20		6	6	6	5	4	3	2	1	1		34	45	34/45	75.5	24.5
	1/40			6	6	5	3	2	1	1			24	39	24/39	62.0	38.0
	1/80				6	5	3	2	2	1			19	33	19/33	57.5	42.5
	1/160					5	4	2	2	1			14	27	14/27	52.0	48.0
	1/320						5	3	2	1	1		12	21	12/21	58.0	42.0
	1/640							4	3	1	1		9	15	9/15	60.0	40.0
	1/1280								3	1	1		5	10	5/10	50.0	50.0
	Control	6	6	6	6	6	6	5	4	3	2	1				100.0	0.0

TABLE NO. 12 (CONT'D)

ANTI-TYPHOID SERUM: "O" TITRATION

Period of incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated											No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		40	80	160	320	640	1280	2560	5120	10240	20480	40960					
24 hrs.	1/10	6	6	6	6	6	5	3	2	1			41	49	41/49	84.0	16.0
	1/20		6	6	6	6	5	3	2	1			35	43	35/43	81.5	18.5
	1/40			6	6	6	4	3	2	1			28	37	28/37	76.0	24.0
	1/80				6	6	5	3	2	1			23	31	23/31	75.0	25.0
	1/160					6	4	3	2	1			16	25	16/25	64.0	36.0
	1/320						5	4	3	2	1		15	19	15/19	79.0	21.0
	1/640							3	2	2	1		8	13	8/13	61.0	39.0
	1/1280								2	1			3	9	3/9	33.0	66.0
	Control	6	6	6	6	6	6	4	3	3	2	1				100.0	0.0

TABLE NO. 12 (CONT'D)

ANTI-TYPHOID SERUM: "O" TITRATION

Period of incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated											No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		40	80	160	320	640	1280	2560	5120	10240	20480	40960					
18 hrs.	1/10	6	6	6	6	6	5	2	2	1			40	48	40/48	83.0	17.0
	1/20		6	6	6	6	5	2	1	1			33	42	33/42	79.0	21.0
	1/40			6	6	6	5	2	1	1			27	36	27/36	75.5	24.5
	1/80				6	6	4	2	2	1			21	30	21/30	70.0	30.0
	1/160					6	4	2	1	1			14	24	14/24	58.5	41.5
	1/320						4	4	2	1			11	17	11/17	62.0	38.0
	1/640							2	1	1			4	12	4/12	67.0	33.0
	1/1280								3	2	1	1	7	7	7/7	100.0	0.0
	Control	6	6	6	6	6	6	5	3	2	1	1				100.0	0.0

FIG. NO. 9

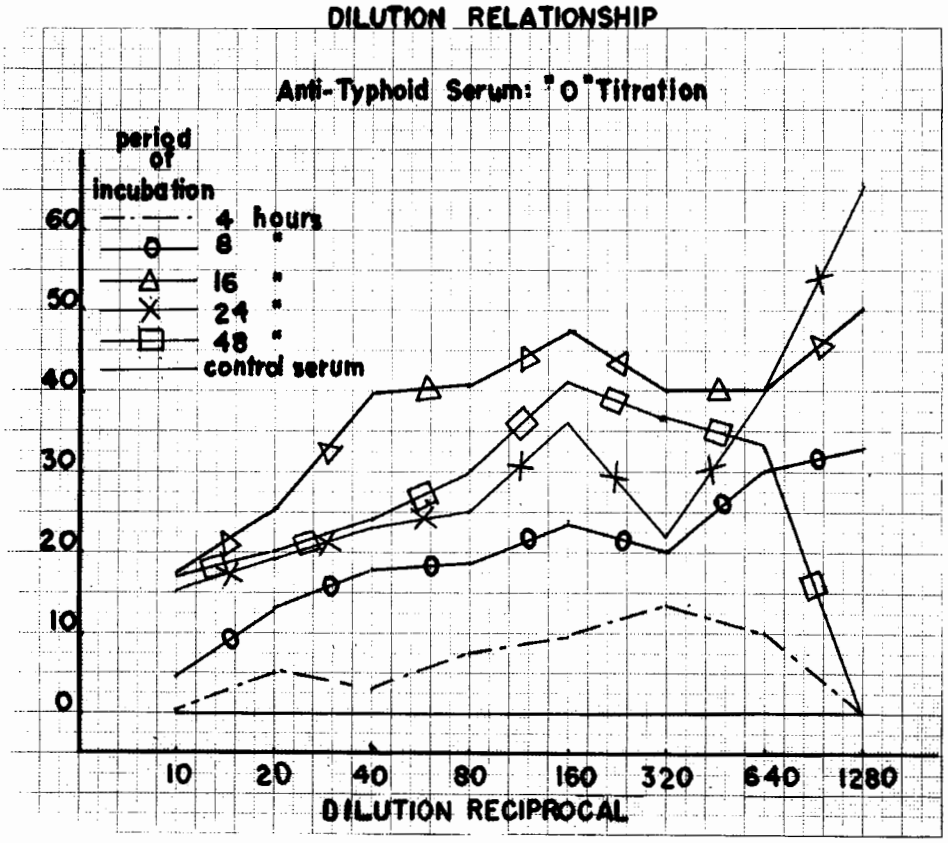
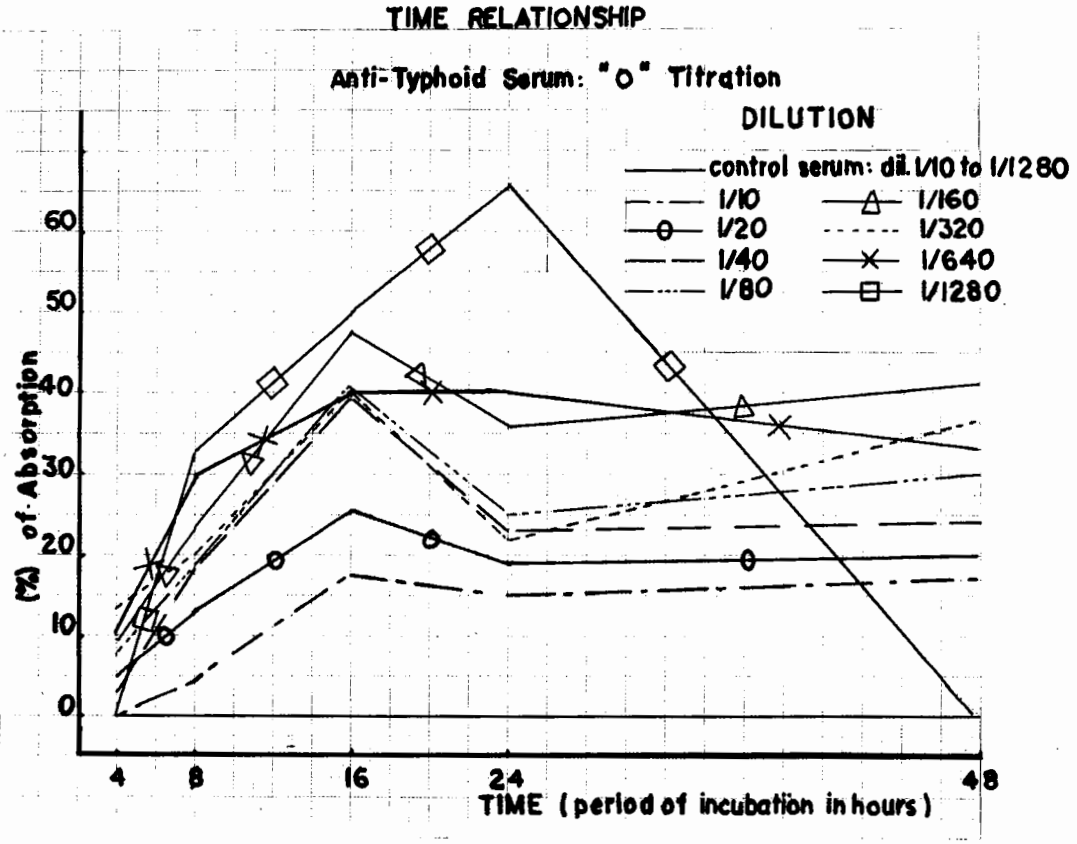

DILUTION RELATIONSHIP
Anti-Typhoid Serum: "O" Titration
period of incubation
4 hours
8 "
16 "
24 "
48 "
control serum
60
50
40
30
20
10
0
10
20
40
80
160
320
640
1280
DILUTION RECIPROCAL

FIG. NO. 10


TIME RELATIONSHIP
Anti-Typhoid Serum: "O" Titration
DILUTION
control serum: dil. 1/10 to 1/1280
1/10
1/20
1/40
1/80
1/160
1/320
1/640
1/1280
(%) of Absorption
0
10
20
30
40
50
60
4
8
16
24
48
TIME (period of incubation in hours)

VIII. ABSORPTION OF ANTI-HORSE SERUM

Five identical sets of a series of 5 dilutions of antiserum were incubated for 4, 8, 16, 24 and 48 hours, respectively. The antiserum used in this experiment was obtained from rabbit No. 1867. To each dilution of antiserum was added 0.5 cc of standardized cell suspension and incubated. This experiment was carried out in two steps:

a) In the tests carried out for 16, 24 and 48 hours, exudate obtained from rabbit No. 1871 was used.

b) In the tests carried out for 4 and 8 hours, exudate obtained from rabbit No. 1899 was used.

To produce the exudate, rabbit No. 1899 was treated as reported in a previous experiment, page 116.

Rabbit No. 1871 was treated as follows:

1st. day:

a) blood picture

white blood cells: 11050 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	28	66	6	663

b) injection of 20 mgm. of MPA intravenously

3rd. day:

a) blood picture

white blood cells: 16000 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	48	34	18	2880

b) injection of 20 mgm. of MPA intravenously

c) injection of 8 cc of a mixture of gum-arabic and beef extract intrapleurally

6th day:

a) blood picture:

white blood cells: 15500 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	44	40	16	2480

b) removal of exudate

c) rabbit was bled by heart puncture and serum collected from the 50 cc of blood obtained and used in the preparation of Earle's serum solution

d) picture of the exudate: 15 cc of exudate were obtained.

White blood cells: 16000 cells/c.mm.

red blood cells: 20000 cells/c.mm.

differential count: polymorphoneuclears: 7%

lymphocytes: 5%

macrophages: 88%

14 cc of cell suspension standardized to 15000 monocytes/c.mm. in Earle's serum solution are obtained.

The results obtained are reported in Table No. 13 and Fig. No. 11 - 12.

It would not appear, in this case, that a higher degree of absorption takes place in a higher dilution of antiserum. The degree of absorption obtained in all of the tested

dilutions varied within a range of 43 to 55%. The optimal time of incubation for maximal absorption of antibodies is 8 hours.

TABLE NO. 13

ANTI-HORSE SERUM TITRATION

Period of Incubation	Dilution of anti-serum when in contact with monocytes	Dilution of anti-serum when titrated	Reciprocal of Dilution of Horse-serum when Titrated								Dilution of tested serum giving first flocculation	Dilution of control serum giving first flocculation	Ratio	% Left	% Absorbed
			250	277	312	357	416	500	625	833					
hrs.	1/2	1/16	p	p	p	F	p	p	p	p	357	625	357/625	57.0	43.0
hrs.	1/2	1/16	p	p	p	F	p	p	p	p	357	625	357/625	57.0	43.0
6 hrs.	1/2	1/16	p	p	p	F	p	p	p	p	357	625	357/625	57.0	43.0
4 hrs.	1/2	1/16	p	p	p	F	p	p	p	p	357	625	357/625	57.0	43.0
8 hrs.	1/2	1/16	p	p	p	F	p	p	p	p	357	625	357/625	57.0	43.0
ontrol	1/2	1/16	p	p	p	p	p	p	F	p		625		100.0	0.0
			500	555	625	714	833	1000	1250	1666					
- hrs.	1/4	1/32	p	p	F	p	p	p	p	p	625	1250	625/1250	50.0	50.0
3 hrs.	1/4	1/32	p	p	F	p	p	p	p	p	625	1250	625/1250	50.0	50.0
16 hrs.	1/4	1/32	p	p	p	F	p	p	p	p	714	1250	714/1250	57.0	43.0
24 hrs.	1/4	1/32	p	p	p	F	p	p	p	p	714	1250	714/1250	57.0	43.0
48 hrs.	1/4	1/32	p	p	p	F	p	p	p	p	714	1250	714/1250	57.0	43.0
ontrol	1/4	1/32	p	p	p	p	p	p	F	p		1250		100.0	0.0

TABLE NO. 13 (CONT'D)

ANTI-HORSE SERUM TITRATION

Period of incubation	Dilution of anti-serum when in contact with monocytes	Dilution of anti-serum when titrated	Reciprocal of Dilution of Horse-serum when Titrated								Dilution of tested serum giving first flocculation	Dilution of control serum giving first flocculation	Ratio	% Left	% Absorbed
			1000	1111	1250	1428	1666	2000	2500	3333					
+ hrs.	1/8	1/64	p	p	F	p	p	p	p	p	1250	2500	1250/2500	50.0	50.0
3 hrs.	1/8	1/64	p	F	p	p	p	p	p	p	1111	2500	1111/2500	45.0	55.0
16 hrs.	1/8	1/64	p	p	F	p	p	p	p	p	1250	2500	1250/2500	50.0	50.0
24 hrs.	1/8	1/64	p	p	p	F	p	p	p	p	1428	2500	1428/2500	57.0	43.0
+8 hrs.	1/8	1/64	p	p	p	F	p	p	p	p	1428	2500	1428/2500	57.0	43.0
Control	1/8	1/64	p	p	p	p	p	p	F	p		2500		100.0	0.0
			2000	2222	2500	2857	3333	4000	5000	6666					
+ hrs.	1/16	1/128	p	p	F	p	p	p	p	p	2500	5000	2500/5000	50.0	50.0
3 hrs.	1/16	1/128	p	F	p	p	p	p	p	p	2222	5000	2222/5000	45.0	55.0
16 hrs.	1/16	1/128	p	p	p	F	p	p	p	p	2857	5000	2857/5000	57.0	43.0
24 hrs.	1/16	1/128	p	p	p	F	p	p	p	p	2857	5000	2857/5000	57.0	43.0
+8 hrs.	1/16	1/128	p	p	p	F	p	p	p	p	2857	5000	2857/5000	57.0	43.0
Control	1/16	1/128	p	p	p	p	p	p	F	p		5000		100.0	0.0

TABLE NO. 13 (CONT'D)

ANTI-HORSE SERUM TITRATION

Period of Incubation	Dilution of anti-serum when in contact with monocytes	Dilution of anti-serum when titrated	Reciprocal of Dilution of Horse-serum when Titrated								Dilution of tested serum giving first flocculation	Dilution of control serum giving first flocculation	Ratio	% Left	% Absorbed
			4000	4444	5000	5714	6666	8000	10000	13333					
hrs.	1/32	1/256	p	p	F	p	p	p	p	p	5000	10000	5000/10000	50.0	50.0
hrs.	1/32	1/256	p	p	F	p	p	p	p	p	5000	10000	5000/10000	50.0	50.0
.6 hrs.	1/32	1/256	p	p	p	F	p	p	p	p	5714	10000	5714/10000	57.0	43.0
4 hrs.	1/32	1/256	p	p	p	F	p	p	p	p	5714	10000	5714/10000	57.0	43.0
8 hrs.	1/32	1/256	p	p	p	F	p	p	p	p	5714	10000	5714/10000	57.0	43.0
ontrol	1/32	1/256	p	p	p	p	p	p	F	p		10000		100.0	0.0

: particulation

: flocculation

FIG. NO. 11

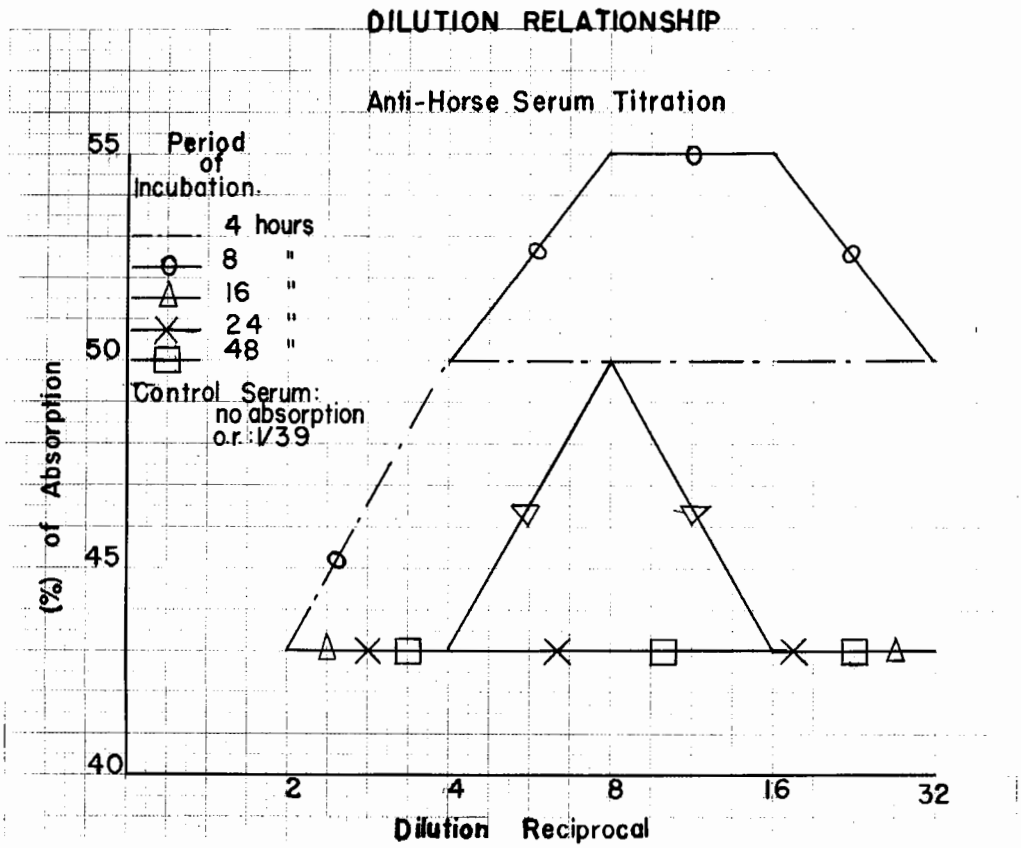
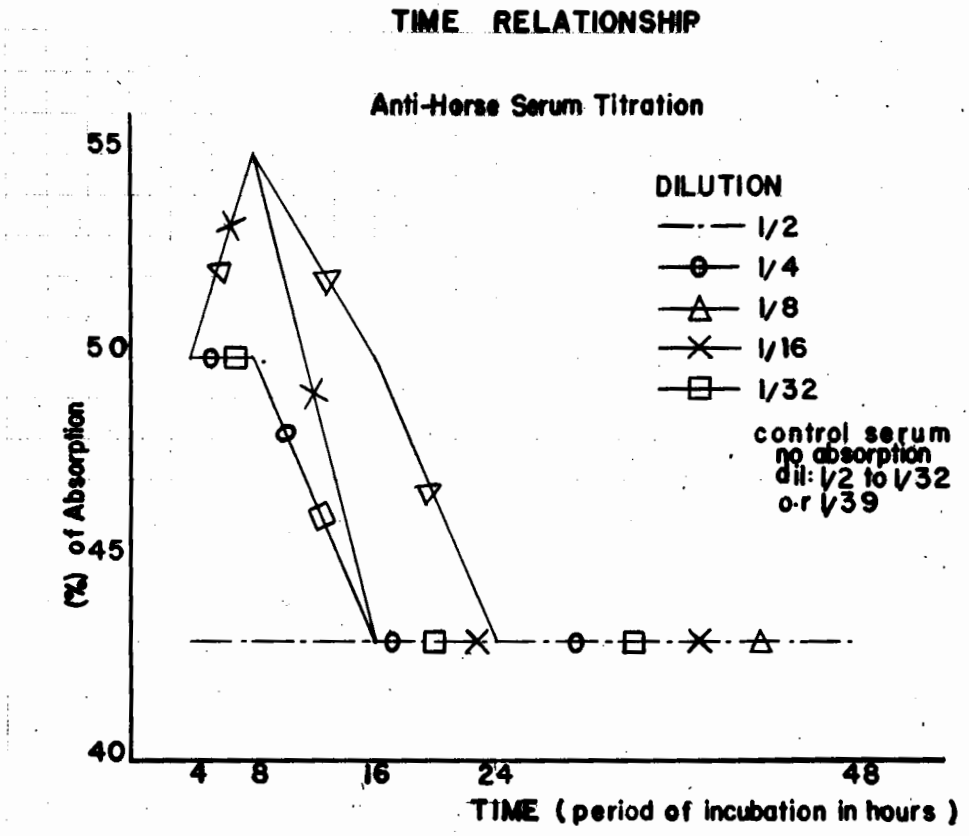

DILUTION RELATIONSHIP
Anti-Horse Serum Titration
Period of Incubation.
4 hours
8 "
16 "
24 "
48 "
Control Serum: no absorption o.r. 1/39
(%) of Absorption
55
50
45
40
2
4
8
16
32
Dilution Reciprocal

FIG. NO. 12


TIME RELATIONSHIP
Anti-Horse Serum Titration
55
50
45
40
(%) of Absorption
DILUTION
1/2
1/4
1/8
1/16
1/32
control serum
no absorption
dil: 1/2 to 1/32
o-r 1/39
4
8
16
24
48
TIME (period of incubation in hours)

IX. ABSORPTION OF STAPHYLOCOCCAL ANTI-ALPHA HEMOLYSIN SERUM

This experiment was carried out in two steps:

a) In the tests carried out for 24 and 48 hours, remaining exudate from rabbit No. 1871 was used. To produce this exudate, rabbit No. 1871 was treated as reported in a previous experiment, page 129.

b) In the tests carried out for 4, 8 and 16 hours, the exudate obtained from rabbit No. 1863 was used. To produce the exudate, rabbit No. 1863 was treated as follows:

1st. day:

a) blood picture

white blood cells: 7500 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	29	66	5	375

b) injection of 20 mgm. of MPA intravenously

3rd. day:

a) blood picture

white blood cells: 10000 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	25	56	19	1900

b) injection of 20 mgm. of MPA intravenously

c) injection of 8 cc of a mixture of gum-arabic and beef extract intrapleurally

6th. day:

a) blood picture:

white blood cells: 12500 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	40	43	17	2125

b) removal of exudate

c) rabbit was bled by heart puncture and serum collected from the 50 cc of blood obtained and used in the preparation of Earle's serum solution

d) picture of the exudate: 10 cc of exudate were obtained.
White cells: 16000 cells/c.mm.
red cells: 30000 cells/c.mm.
differential count: polymorphoneuclears: 2%
lymphocytes: 8%
macrophages: 90%

16.8 cc of cell suspension standardized to 15000 monocytes/c.mm. in Earle's serum solution are obtained.

Five identical sets of series of 5 dilutions of antiserum (obtained from Dr. J. Rublee and numbered 25/IV/17/53) was used and incubated for 4, 8, 16, 24 and 48 hours, respectively.

The toxin (obtained from Dr. J. Rublee and numbered L1/46) contained 2228 B.U.

In the titration of the antiserum, the toxin was diluted to 1/450 in order to bring the end point chosen (50% hemolysis) in the dilution 1/800 of the control serum.

The results are reported in Table No. 14 and Fig. No. 13-14.

The percentage of absorption of antibodies was almost nil for the first period of incubation. For the other periods tested, it would not appear that a higher degree of absorption takes place in a higher dilution of antiserum. The degree of absorption obtained in the dilutions of antiserum incubated for 8, 16, 24 and 48 hours varied within a range of 17.5 to 28.5%. The optimal time of incubation for maximal absorption of antibodies is 16 hours.

TABLE NO. 14

TITRATION OF ANTIALPHA HEMOLYSIN SERUM

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Dilution of antiserum when titrated										Dilution giving 50% hemolysis in tested serum	Dilution giving 50% hemolysis in control serum	Ratio	% Left	% Absorbed
		400	444	500	571	666	800	1000	1333	2000	4000					
hrs.	1/2	0	0	sl	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
	1/4	0	0	sl	-25	-50	≠50	≠50	≠50	≠50	≠50	733	800	733/800	91.5	8.5
	1/8	0	0	sl	-25	-50	≠50	≠50	≠50	≠50	≠50	733	800	733/800	91.5	8.5
	1/16	0	0	sl	-25	25	≠50	≠50	≠50	≠50	≠50	800	800	800/800	100.0	0.0
	1/32	0	0	0	sl	-50	≠50	≠50	≠50	≠50	≠50	733	800	733/800	91.5	8.5
hrs.	1/2	0	sl	-25	-50	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
	1/4	0	sl	-25	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
	1/8	0	0	sl	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
	1/16	0	0	sl	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
	1/32	0	0	sl	-25	-50	≠50	≠50	≠50	≠50	≠50	733	800	733/800	91.5	8.5

TABLE NO. 14 (CONT'D.)

TITRATION OF ANTIALPHA HEMOLYSIN SERUM

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Dilution of antiserum when titrated										Dilution giving 50% hemolysis in tested serum	Dilution giving 50% hemolysis in control serum	Ratio	% Left	% Absorbed
		400	444	500	571	666	800	1000	1333	2000	4000					
6 hrs.	1/2	0	sl	-25	-50	≠50	≠50	≠50	≠50	≠50	≠50	618	800	618/800	77.5	22.5
	1/4	sl	-25	25	50	≠50	≠50	≠50	≠50	≠50	≠50	571	800	571/800	71.5	28.5
	1/8	0	0	sl	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
	1/16	0	0	sl	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
	1/32	0	0	sl	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
4 hrs.	1/2	0	sl	-25	-50	≠50	≠50	≠50	≠50	≠50	≠50	618	800	618/800	77.5	22.5
	1/4	sl	-25	25	50	≠50	≠50	≠50	≠50	≠50	≠50	571	800	571/800	71.5	28.5
	1/8	0	0	sl	25	-50	≠50	≠50	≠50	≠50	≠50	618	800	618/800	77.5	22.5
	1/16	0	0	sl	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
	1/32	0	sl	-25	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0

TABLE NO. 14 (CONT'D)

TITRATION OF ANTIALPHA HEMOLYSIN SERUM

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Dilution of antiserum when titrated										Dilution giving 50% hemolysis in tested serum	Dilution giving 50% hemolysis in control serum	Ratio	% Left	% Absorbed
		400	444	500	571	666	800	1000	1333	2000	4000					
8 hrs.	1/2	0	sl	-25	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
	1/4	0	sl	-25	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
	1/8	0	0	-25	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
	1/16	0	0	sl	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
	1/32	0	0	-25	25	50	≠50	≠50	≠50	≠50	≠50	666	800	666/800	83.0	17.0
ontrol	1/2	0	0	sl	-25	25	50	≠50	≠50	≠50	≠50		800		100.0	0.0
	1/4	0	0	0	-25	25	50	≠50	≠50	≠50	≠50		800		100.0	0.0
	1/8	0	0	0	-25	25	50	≠50	≠50	≠50	≠50		800		100.0	0.0
	1/16	0	0	0	-25	25	50	≠50	≠50	≠50	≠50		800		100.0	0.0
	1/32	0	0	0	-25	25	50	≠50	≠50	≠50	≠50		800		100.0	0.0

sl: very slight trace of hemolysis
-50: less than 50, more than 25
-25: less than 25, more than slight trace
≠50: over 50

FIG. NO. 13

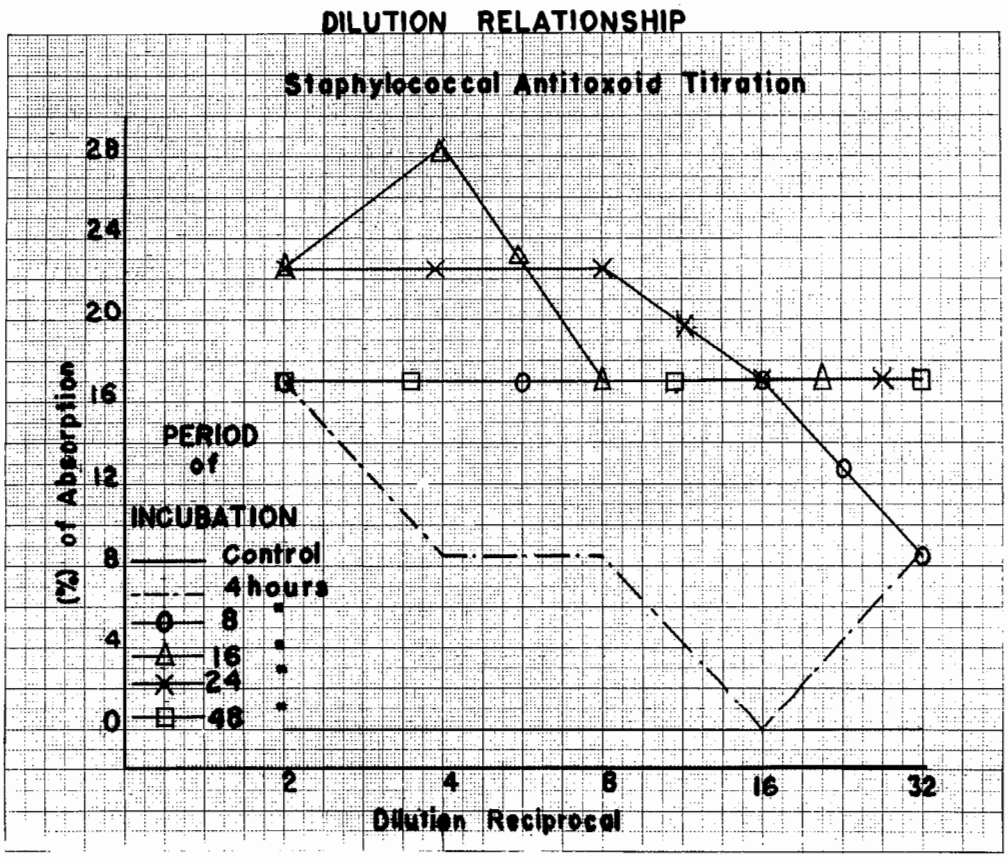
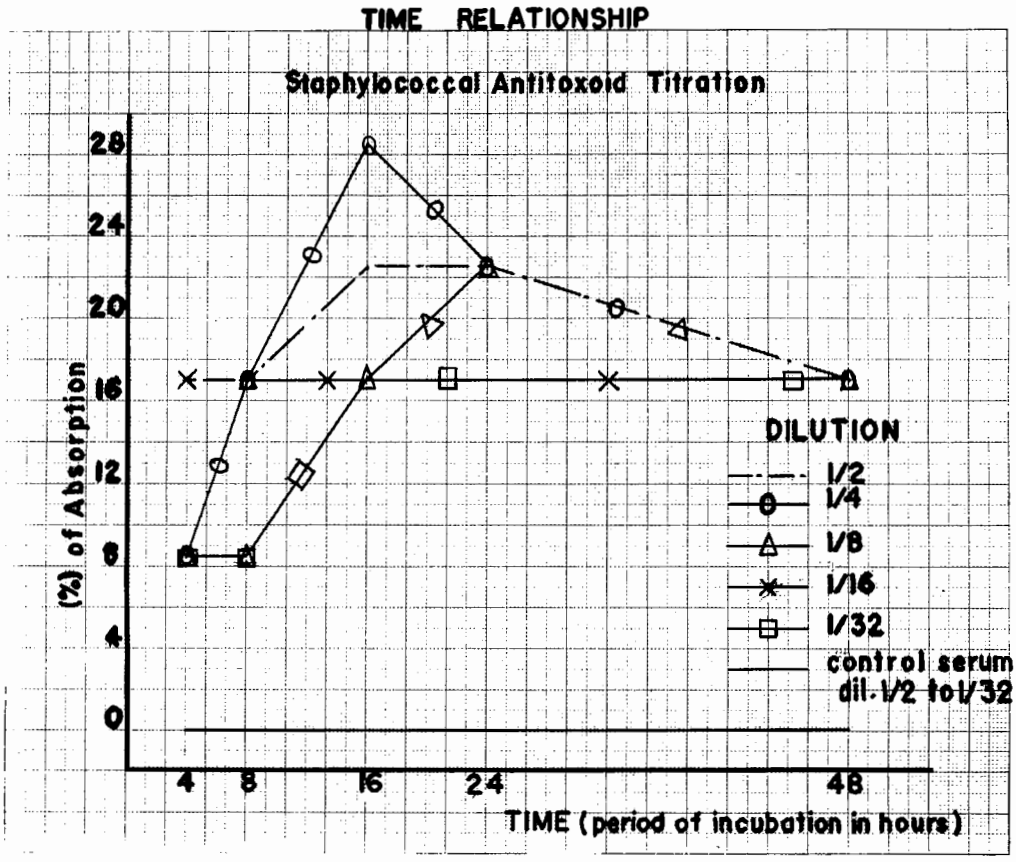

DILUTION RELATIONSHIP
Staphylococcal Antitoxoid Titration
(%) of Absorption
28
24
20
16
12
8
4
0
PERIOD of INCUBATION
Control
4 hours
8
16
24
48
2
4
8
16
32
Dilution Reciprocal

FIG. NO. 14


TIME RELATIONSHIP
Staphylococcal Antitoxoid Titration
(%) of Absorption
28
24
20
16
12
8
4
0
4
8
16
24
48
TIME (period of incubation in hours)
DILUTION
1/2
1/4
1/8
1/16
1/32
control serum
dil. 1/2 to 1/32

X. ABILITY OF ERYTHROCYTES TO ADSORB ANTIBODIES

As traces of erythrocytes were consistently present in the exudates, the ability of these cells to adsorb antibody was tested as follows:

The blood was obtained by heart puncture from rabbit No. 1871 and clotting prevented by addition of sodium citrate. The erythrocyte count was 4,500,000 cells/c.mm. This blood was diluted to 50,000 erythrocytes/c.mm. which corresponded to the highest number of erythorcytes found in any of the exudates used through out this work. The blood picture was as follows:

white cells: 11500

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential	0	0	40	54	6	630

After dilution to 50000 erythrocytes/c.mm. the absolute number of monocytes was $\frac{630}{90}$ or 7 monocytes/c.mm. This quantity of monocytes will not interfere in this experiment.

The 3 antisera previously used were tested against erythrocytes. For each antiserum, one set of dilutions was carried out. The technique used was the same as in previous experiments except the standardized monocytes suspension was replaced by standardized erythrocytes suspension (50000 cells/c.mm.). The incubation time was 16 hours for each antiserum tested. No antibodies were found

to be removed in any of the three antisera. Consequently, the traces of blood present in the exudates used did not play any part in the removal of antibodies by monocytes.

The negative results obtained are reported in Tables No. 15 - 16 - 17.

TABLE NO. 15

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated												No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840					
6 hrs..	1/10	6	6	6	6	6	6	6	6	6	4	2	1	61	61	61/61	100.0	0.0
	1/20		6	6	6	6	6	6	6	6	4	2	1	55	55	55/55	100.0	0.0
	1/40			6	6	6	6	6	6	6	4	2	1	49	49	49/49	100.0	0.0
	1/80				6	6	6	6	6	6	4	2	1	43	43	43/43	100.0	0.0
	1/160					6	6	6	6	6	4	2	1	37	37	37/37	100.0	0.0
	1/320						6	6	6	6	4	2	1	31	31	31/31	100.0	0.0
	1/640							6	6	6	4	2	1	25	25	25/25	100.0	0.0
	1/1280								6	6	4	2	1	19	19	19/19	100.0	0.0
	Control	6	6	6	6	6	6	6	6	6	4	2	1				100.0	0.0

TABLE NO. 15 (CONT'D)

ANTI-TYPHOID SERUM:"O" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated											No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840					
5 hrs.	1/10	6	6	6	6	6	5	4	3	2	1			45	45	45/45	100.0	0.0
	1/20		6	6	6	6	5	4	3	2	1			39	39	39/39	100.0	0.0
	1/40			6	6	6	5	4	3	2	1			33	33	33/33	100.0	0.0
	1/80				6	6	5	4	3	2	1			27	27	27/27	100.0	0.0
	1/160					6	5	4	3	2	1			21	21	21/21	100.0	0.0
	1/320						5	4	3	2	1			15	15	15/15	100.0	0.0
	1/640							4	3	2	1			10	10	10/10	100.0	0.0
	1/1280								3	2	1			6	6	6/6	100.0	0.0
	Control	6	6	6	6	6	5	4	3	2	1						100.0	0.0

TABLE NO. 16

ANTI-HORSE SERUM TITRATION

Period of Incubation	Dilution of anti-serum when in contact with monocytes	Dilution of anti-serum when titrated		Reciprocal of Dilution of Horse-serum when Titrated								Dilution of tested serum giving first flocculation	Dilution of control serum giving first flocculation	Ratio	% Left	% Absorbed
				250	277	312	357	416	500	625	833					
5 hrs.	1/2	1/16	T.	p	p	p	p	p	p	F	p	625		625/625	100.0	0.0
			C.	p	p	p	p	p	p	F	p		625			
				500	555	625	714	833	1000	1250	1666					
	1/4	1/32	T.	p	p	p	p	p	p	F	p	1250		1250/1250	100.0	0.0
			C.	p	p	p	p	p	p	F	p		1250			
				1000	1111	1250	1428	1666	2000	2500	3333					
	1/8	1/64	T.	p	p	p	p	p	p	F	p	2500		2500/2500	100.0	0.0
			C.	p	p	p	p	p	p	F	p		2500			
				2000	2222	2500	2857	3333	4000	5000	6666					
	1/16	1/128	T.	p	p	p	p	p	p	F	p	5000		5000/5000	100.0	0.0
			C.	p	p	p	p	p	p	F	p		5000			

TABLE NO. 16 (CONT'D)

ANTI-HORSE SERUM TITRATION

Period of Incubation	Dilution of anti-serum when in contact with monocytes	Dilution of anti-serum when titrated		Reciprocal of Dilution of Horse-serum when Titrated								Dilution of tested serum giving first flocculation	Dilution of control serum giving first flocculation	Ratio	% Left	% Absorbed
.6 hrs.	1/32	1/256	T.	p	p	p	p	p	p	F	p	10000	10000	10000/10000	100.0	0.0
			C.	p	p	p	p	p	p	F	p					

T.: tested serum

C.: control serum

p: particulate

F: flocculate

TABLE NO. 17

TITRATION OF ANTI-ALPHA HEMOLYSIN SERUM

Period of Incubation	Original dilution of antiserum when in contact with monocytes		Reciprocal of Dilution of antiserum when titrated										Dilution giving 50% hemolysis in tested serum	Dilution giving 50% hemolysis in control serum	Ratio	% Left	% Absorbed
			400	444	500	571	666	800	1000	1333	2000	4000					
hrs.	1/2	T.	0	0	0	-25	25	50	≠50	≠50	≠50	≠50	800		800/800	100.0	0.0
		C.	0	0	sl	-25	-25	50	≠50	≠50	≠50	≠50		800			
	1/4	T.	0	0	0	-25	25	50	≠50	≠50	≠50	≠50	800		800/800	100.0	0.0
		C.	0	0	0	-25	25	50	≠50	≠50	≠50	≠50		800			
	1/8	T.	0	0	0	0	25	50	≠50	≠50	≠50	≠50	800		800/800	100.0	0.0
		C.	0	0	0	-25	25	50	≠50	≠50	≠50	≠50		800			
	1/16	T.	0	0	sl	-25	25	50	≠50	≠50	≠50	≠50	800		800/800	100.0	0.0
		C.	0	0	0	-25	25	50	≠50	≠50	≠50	≠50		800			
	1/32	T.	0	0	0	-25	25	50	≠50	≠50	≠50	≠50	800		800/800	100.0	0.0
		C.	0	0	0	-25	25	50	≠50	≠50	≠50	≠50		800			

sl: very slight trace of hemolysis
-25: less than 25, more than slight trace
≠50: over 50

T.: tested serum
C.: control serum

XI. FATE OF THE ABSORBED ANTIBODIES

In these following experiments, an attempt was made to determine the fate of the antibodies absorbed by monocytes. Serial of dilutions was set up for each of the three antisera and incubated with standardized monocyte suspension for 16 hours. The dilutions used were identical with those used in previous work.

At the end of the incubation period, the antiserum was separated from the cells by centrifugation and titrated separately. The monocytes remaining in each tube were washed three times, using 1 cc of saline each time and the washings were titrated separately. The washed cells were lysed and the sepernatant lysate obtained after centrifugation from each tube were titrated. The residual cellular material was resuspended in 1 cc normal saline and titrated.

The cell lysates obtained from monocytes which had been in contact with anti-horse serum were found to contain traces of antibody. The cell lysates obtained from monocytes which had been in contact with the 2 other antisera (anti-typhoid serum and staphylococcal anti-alpha hemolysin serum) were found to contain no significant trace of antibody.

No antibodies could be demonstrated in the residual cellular material obtained from monocytes which

had been in contact with anti-typhoid serum or staphylococcal anti-alpha hemolysin serum, while antibodies were demonstrated as traces from the residual cellular material obtained from monocytes that had been in contact with anti-horse serum.

For those experiments exudates from rabbits No. 1900 and 1901 were pooled, washed and standardized to contain 15000 monocytes/c.mm. The rabbits used to produce the exudates were treated as follows:

Rabbit No. 1900

1st. day:

a) blood picture

white blood cells: 9000 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	22	74	4	360

b) injection of 20 mgm. of MPA intravenously

3rd. day:

a) blood picture

white blood cells: 12650 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	27	55	18	2250

b) injection of 20 mgm. of MPA intravenously

c) injection of 8 cc of a mixture of gum-arabic and beef extract intrapleurally

6th. day:

a) blood picture:

white blood cells: 20750 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	20	61	19	3942

b) removal of exudate

c) rabbit was bled by heart pucnture and serum collected from the 50 cc of blood obtained and used in the preparation of Earle's serum solution

d) 18 cc of exudate were obtained.

Rabbit No. 1901

1st. day:

a) blood picture

white blood cells: 14450 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	37	59	6	867

b) injection of 20 mgm. of MPA intravenously

3rd. day:

a) blood picture

white blood cells: 16700 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	23	58	19	3173

b) injection of 20 mgm. of MPA intravenously

c) injection of 8 cc of a mixture of gum-arabic and beef ectract intrapleurally

6th.day:

a) blood picture:

white blood cells: 14800 cells/c.mm.

	E%	B%	P%	L%	M%	Absolute No. Monocytes
differential count:	0	0	29	55	16	2368

b) removal of exudate

c) rabbit was bled by heart puncture and serum collected from the 50 cc of blood obtained and used in the preparation of Earle's serum solution

d) 12 cc of exudate were obtained.

The exudates were pooled together to give 30 cc of standardized monocyte suspension 15000 monocytes/c.mm. The picture of the exudate was:

white cells: 16500 cells/c.mm.

red cells: 20000 cells/c.mm.

differential count: polymorphoneuclears: 5%

lymphocytes: 3%

macrophages: 92%

1. <u>Anti-typhoid "H" serum titration</u>

a) Titration of the supernatant.

As in previous work, a set of a series of 8 dilutions was set up. Each supernatant obtained after centrifugation from each dilution of antiserum was titrated and the results reported in Table 18. Fig. 15

The results obtained approximate those obtained in previous experiments and appear to confirm them.

TABLE NO. 18

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated												No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840					
6 hrs.	1/10 T.	6	6	6	6	6	6	6	6	4	2	0		54		54/60	90.0	10.0
	C.	6	6	6	6	6	6	6	6	6	3	2	1		60		100.0	0.0
	1/20 T.		6	6	6	6	6	6	6	4	2	0		48		48/54	88.0	12.0
	C.		6	6	6	6	6	6	6	6	3	2	1		54		100.0	0.0
	1/40 T.			6	6	6	6	6	4	3	2			39		39/48	81.0	19.0
	C.			6	6	6	6	6	6	6	3	2	1		48		100.0	0.0
	1/80 T.				6	6	6	6	5	3	2	1		35		35/42	83.0	17.0
	C.				6	6	6	6	6	6	3	2	1		42		100.0	0.0
	1/160 T.					6	6	6	6	4	3	1		32		32/36	88.0	12.0
	C.					6	6	6	6	6	3	2	1		36		100.0	0.0

TABLE NO. 18 (CONT'D)

ANTI-TYPHOID SERUM: "H" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated												No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		80	160	320	640	1280	2560	5120	10240	20480	40960	81920	163840					
6 hrs.	1/320 T.						6	6	6	4	2	1		25		25/30	83.0	17.0
	C.						6	6	6	6	3	2	1		30		100.0	0.0
	1/640 T.							6	6	4	2	1		19		19/24	79.0	21.0
	C.							6	6	6	3	2	1		24		100.0	0.0
	1/1280 T.								4	3	2			9		9/18	50.0	50.0
	C.								6	6	3	2	1		18		100.0	0.0

b) Titration of washings

The 1st, 2nd and 3rd washings obtained from the cells from each tube of a series of 8 dilutions of antiserum were titrated separately. From each of the washings to be titrated, halving dilutions were carried out in 0.5 cc volume and 0.5 cc of "H" antigen added to each of dilutions thus obtained. The results obtained are reported in Table No. 19.

TABLE NO. 19

Washings No. 1

Tubes No.	Final dilution of washings after "H" antigen is added (Reciprocal of)											
	4	8	16	32	64	128	256	512	1024	2048	4096	8192
1	6	6	6	6	6	6	4	3	1			
2	6	6	6	6	4	2	1					
3	6	6	6	4	1							
4	6	6	4	2								
5	5	2	1									
6	4	2	1									
7	2	1										
8	2	1										

Washings No. 2

1	6	6	4	2	1
2	5	3	2	1	
3	2	2	1		
4	2	1			

Tubes No. 5, 6, 7 and 8 show negative results

Washings No. 3

All negative

c) Titration of lysates

After the cells were lysed by the osmotic pressure method, 5 cc of lysate were obtained by centrifugation.

The lysate from each tube was titrated againts "H" antigen as follows: halving dilutions of lysate were prepared in 0.5 cc normal saline. To each of the dilutions was added 0.5 cc of "H" antigen. The results obtained are reported in Table No. 20.

TABLE NO. 20

Tubes No.	Reciprocal of final dilution of lysate after "H" antigen is added.									
	2	4	8	16	32	64	128	256	512	1024
1	4	3	2	1	1					
2	3	3	2	1						
3	2	2	1	1						
4	3	2	1							
5	2	2	1							
6	3	2	1	1						
7	2	2	1							
8	2	2	1							
Control	3	2	2	1						

The control consists of lysate obtained after lysis of normal monocytes that were never in contact with the antiserum tested.

No traces of antibodies appear to be present in this lysate.

d) Titration of sediments

The cellular material remaining from each tube was resuspended in 1 cc of normal saline and titrated as follows. From each suspension halving dilutions were carried out in 0.5 cc of normal saline. 0.5 cc of "H" antigen was added to each dilution. The results obtained for each sediment titrated are reported in Table No. 21.

TABLE NO. 21

Sediment from tube No.	Reciprocal of final dilution of sediment after "H" antigen is added									
	4	8	16	32	64	128	256	512	1024	2048
1	4	2	1							
2	3	2	1							
3	2	1								
4	3	2	1							
5	2	2	1							
6	3	2	1							
7	3	2	1							
8	2	2	1							
Control	3	2	1							

The control consists of the residual cellular material obtained after lysis of normal monocytes that were never in contact with the tested antiserum.

From the above test, no significant amount of antibodies appear to be attached to the debris.

2. Anti-typhoid "O" serum titration

a) Titration of the supernatant.

The supernatants obtained from each of the eight dilutions were titrated. The titration was carried out as described previously, when titrating the supernatants against "H" antigen. The results are reported in Table No. 22. Fig. 15.

In this case, the results obtained confirm those found in previous experiments.

b) Titration of washings

The washings were titrated using the method described previously when titrating the washings against "H" antigen. The results are reported in Table No. 23.

TABLE NO. 23

Washings No. 1

Tube No.	Final dilution of washings after "O" antigen is added (Reciprocal of)									
	4	8	16	32	64	128	256	512	1024	2048
1	6	6	6	5	3	2	1			
2	6	5	4	2	1					
3	5	5	4	2	1					
4	5	4	3	2	1					
5	3	3	2	1						
6	2	2	1							
7	2	2	1							
8	2	1								

TABLE NO. 22

ANTI-TYPHOID SERUM: "O" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated											No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		40	80	160	320	640	1280	2560	5120	10240	20480	40960					
16 hrs.	1/10 T.	6	6	6	6	6	6	4	3	2	1		46		46/47	98.0	2.0
	C.	6	6	6	6	6	6	5	3	2	1			47		100.0	0.0
	1/20 T.		6	6	6	6	5	4	2	1			36		36/41	87.0	13.0
	C.		6	6	6	6	6	5	3	2	1			41		100.0	0.0
	1/40 T.			6	6	6	5	4	2	1			30		30/35	86.0	14.0
	C.			6	6	6	6	5	3	2	1			35		100.0	0.0
	1/80 T.				6	6	5	3	2	1			23		23/29	79.0	21.0
	C.				6	6	6	5	3	2	1			29		100.0	0.0
	1/160 T.					6	4	3	2	1			16		16/23	70.0	30.0
	C.					6	6	5	3	2	1			23		100.0	0.0

TABLE NO. 22 (CONT'D)

ANTI-TYPHOID SERUM: "O" TITRATION

Period of Incubation	Original dilution of antiserum when in contact with monocytes	Reciprocal of Dilution of Antiserum When Titrated											No. of plusses in tested serum dilution	No. of plusses in corresponding control serum dilution	Ratio	% Left	% Absorbed
		40	80	160	320	640	1280	2560	5120	10240	20480	40960					
6 hrs.	1/320 T.						4	3	2	1			10		10/17	59.0	41.0
	C.						6	5	3	2	1			17		100.0	0.0
	1/640 T.							3	2	1			6		6/11	54.0	46.0
	C.							5	3	2	1			11		100.0	0.0
	1/1280 T.								2	1			3		3/6	50.0	0.0
	C.								3	2	1			6		100.0	0.0

FIG. NO. 15

TITRATION of the SUPERNATANT SERUM

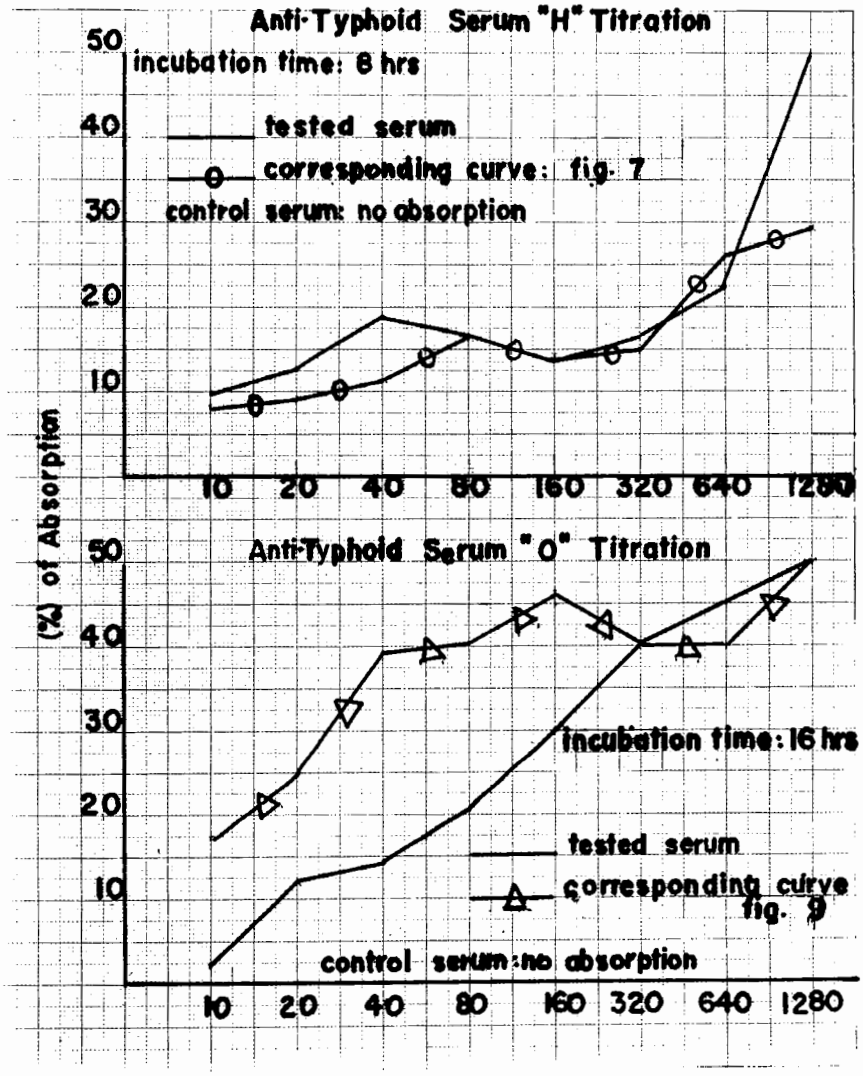


TABLE NO. 23 (CONT'D)

Washings No. 2

Tube No.	Final dilution of washings after "O" antigen is added (Reciprocal of)									
	4	8	16	32	64	128	256	512	1024	2048
1	6	5	2	1						
2	3	3	2	1						
3	3	2	1							
4	2	2	1							
5	2	1								

Tubes No. 6, 7 and 8 show negative results

Washings No. 3

All negative

c) Titration of lysates

Each of the lysates obtained from the monocytes that were in contact with the dilutions of antiserum is titrated using procedures employed in the "H" titration of the lysates. The results are reported in Table No. 24.

TABLE NO. 24

Tube No.	Reciprocal of final dilution of lysate after "O" antigen is added.									
	2	4	8	16	32	64	128	256	512	1024
1	3	2	1	1						
2	2	2	1	1						
3	2	2	1	1						
4	3	2	1							
5	2	2	1	1						
6	2	1	1							

TABLE NO. 24 (CONT'D)

Tube No.	Reciprocal of final dilution of lysate after "O" antigen is added.									
	2	4	8	16	32	64	128	256	512	1024
7	2	1	1							
8	2	1	1							
Control	3	2	2	1	1					

The control was a lysate from monocytes that had not been in contact with antiserum.

No traces of antibodies appear to be present in this lysate.

d) Titration of sediments

To titrate the residual cellular material procedures employed for "H" titration of the sediment were used. Results are reported in Table No. 25.

TABLE NO. 25

Sediment from tube No.	Reciprocal of final dilution of sediment after "O" antigen is added									
	4	8	16	32	64	128	256	512	1024	2048
1	3	2	2	1						
2	3	2	2	1						
3	2	2	1							
4	3	2	1							
5	2	2	1							
6	3	2	2	1						

TABLE NO. 25 (CONT'D)

Sediment from tube No.	Reciprocal of final dilution of sediment after "O" antigen is added									
	4	8	16	32	64	128	256	512	1024	2048
7	3	2	1							
8	2	2	1							
Control	2	2	2	1						

The control was the residual cellular material obtained after lysis of monocytes which had not been in contact with antiserum.

3. Horse-Serum titration

a) Titration of the supernatant

The antiserum was separated by centrifugation from the cells in each of the five dilutions. The supernatant thus obtained was treated as previously described by diluting each supernatant 1:8 and the constant-antibody optimal ratio method of titration was used. The results obtained are reported in Table No. 26, Fig. 16.

The results approximate those obtained in previous work and appear to confirm them.

b) Titration of the washings

The cells remaining after antiserum was removed were washed three times, using 1 cc of normal saline each time, and titrated by the constant-antibody optimal ratio method. Each washing was diluted 1:8 to give 4 cc of solution. The results are reported in Table No. 27.

TABLE NO. 26

ANTI-HORSE SERUM TITRATION

Period of Incubation	Dilution of anti-serum when in contact with monocytes	Dilution of anti-serum when titrated		Reciprocal of Dilution of Horse-serum when Titrated								Dilution of tested serum giving first flocculation	Dilution of control serum giving first flocculation	Ratio	% Left	% Absorbed
				250	277	312	357	416	500	625	833					
8 hrs.	1/2	1/16	T.:	p	F	p	p	p	p	p	p	277		277/625	45.0	55.0
			C.	p	p	p	p	p	p	F	p		625		100.0	0.0
				500	555	625	714	833	1000	1250	1666					
	1/4	1/32	T.	p	F	p	p	p	p	p	p	555		555/1250	45.0	55.0
			C.	p	p	p	p	p	p	F	p		1250		100.0	0.0
				1000	1111	1250	1428	1666	2000	2500	3333					
	1/8	1/64	T.	p	p	p	F	p	p	p	p	1428		1428/2500	57.0	43.0
			C.	p	p	p	p	p	p	F	p		2500		100.0	0.0
				2000	2222	2500	2857	3333	4000	5000	6666					
	1/16	1/128	T.	p	p	F	p	p	p	p	p	2500		2500/5000	50.0	50.0
			C.	p	p	p	p	p	p	F	p		5000		100.0	0.0
				4000	4444	5000	5715	6666	8000	10000	13333					
	1/32	1/256	T.	p	p	F	p	p	p	p	p	5000		5000/10000	50.0	50.0
			C.	p	p	p	p	p	p	F	p		10000		100.0	0.0

T.: antiserum tested
C.: control antiserum

p: particulate
F: flocculate

TABLE NO. 27

Washings No. 1

Tube No.	Reciprocal of final dilution of washings when titrated							
	2	4	8	16	32	64	128	256
1	F	F	p	p	op.	op.		
2	p	op.	op.	op.				
3	op.	op.						

Tubes No. 4 and 5 are negative

Washings No. 2

Tube No.	Reciprocal of final dilution of washings when titrated							
	2	4	8	16	32	64	128	256
1	p	op.	op.					
2	op.							

Tubes No. 3, 4 and 5 are negative

Washings No. 3

Tube No.	Reciprocal of final dilution of washings when titrated							
	2	4	8	16	32	64	128	256
1	op.	op.						

Tubes No. 2, 3, 4 and 5 are negative

c) Titration of lysates

The 5 cc of lysate obtained were titrated using the constant-antibody optimal ratio method of titration and the results reported in Tabel No. 28.

The final results were obtained after 4 days of incubation at room temperature. Compared with the control,

the lysate obtained from monocytes exposed previously to dilutions of antiserum showed a higher degree of precipitation although the difference was not very marked. Consequently, it is suggested that only traces of antibody were present within the monocytes at the time the titrations were carried out.

TABLE NO. 28

Tube No.	Reciprocal of final dilution of lysates when titrated							
	2	4	8	16	32	64	128	256
1	F	F	F	F	p	p	p	op.
2	F	F	F	F	p	p	p	op.
3	F	F	F	p	p	op.	op.	op.
4	F	F	F	p	p	op.	op.	op.
5	p	p	p	p	op.	op.	op.	
Control	p	p	p	p	op.	op.		

The control was a lysate obtained from normal monocytes which had been in contact with the preserving media alone, containing no horse antiserum.

d) Titration of the sediments

The cellular material remaining in each tube after lysis was resuspended in 4 cc of normal saline in order to have enough material to carry out a precipitation test using the constant-antibody optimal ratio method of titration. The results are reported in Table No. 29.

TABLE NO. 29

Tube No.	Reciprocal of final dilution of sediment suspension when titrated										
	2	4	8	16	32	64	128	256	512	1024	2048
1	F	F	F	F	p	p	p	op.	op.		
2	F	F	F	F	p	p	p	op.	op.		
3	F	F	F	p	p	op.	op.	op.			
4	F	F	F	p	p	op.	op.				
5	p	p	p	F	p	p	op.	op.			
Control	p	p	p	p	op.	op.	op.				

F: flocculate
p: precipitate
op: opalescence

Having only 4 cc of sediment suspension, titrations were then carried out from dilutions 1:2 to 1:512.

The control was residual cellular material obtained fron normal lysed monocytes and diluted in 4 cc of normal saline.

The residual cellular materials obtained from monocytes that had been in contact with antiserum showed a higher degree of precipitation than the control.

4. Staphylococcal anti-alpha hemolysin titration

The antiserum of each of the five dilutions were separated from the monocytes by centrifugation and the supernatant thus obtained diluted 1:400 and titrated. The results obtained are reported in Table No. 30, Fig. 16.

TABLE NO. 30

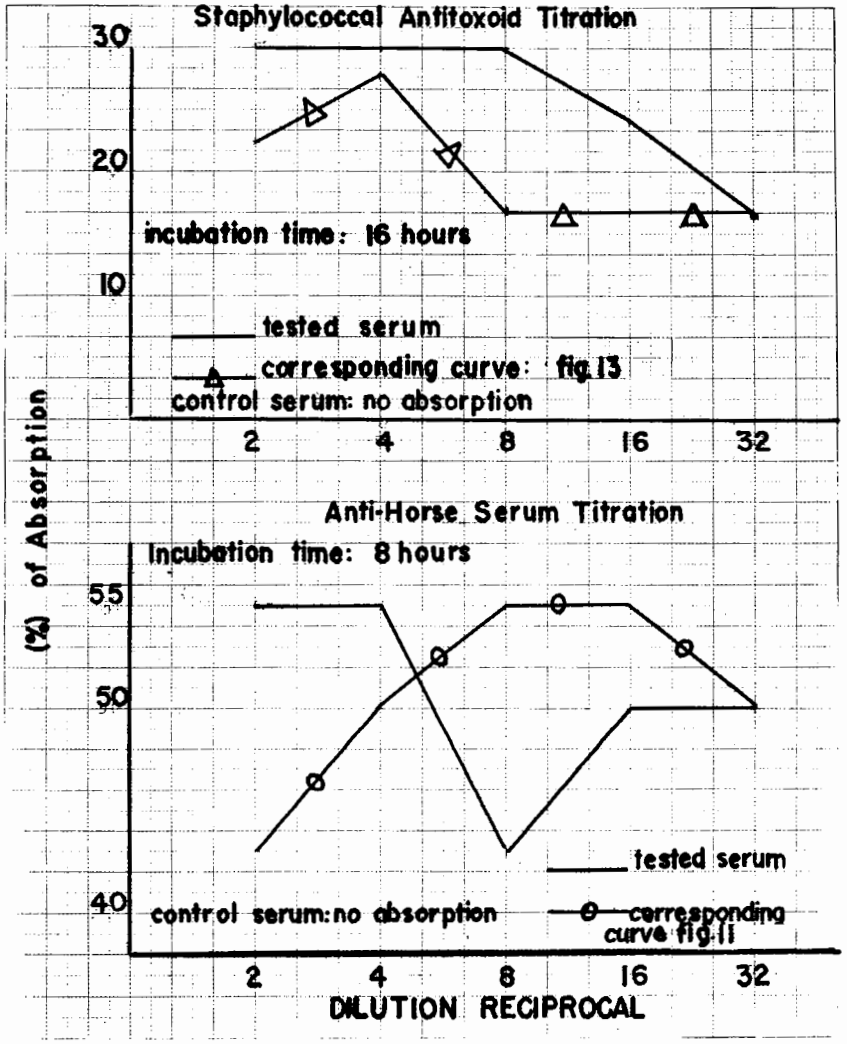
TITRATION OF ANTI-ALPHA HEMOLYSIN SERUM

Period of Incubation	Original dilution of antiserum when in contact with monocytes		Reciprocal of Dilution of antiserum when titrated: 400	444	500	571	666	800	1000	1303	2000	4000	Dilution giving 50% hemolysis in tested serum	Dilution giving 50% hemolysis in control serum	Ratio	% Left	% Absorbed
5 hrs.	1/2	T.	25	-50	-50	50	≠50	≠50	≠50	≠50	≠50	≠50	571		571/800	70.0	30.0
		C.	sl.	-25	25	-50	-50	50	≠50	≠50	≠50	≠50		800		100.0	0.0
	1/4	T.	-25	25	-50	50	≠50	≠50	≠50	≠50	≠50	≠50	571		571/800	70.0	30.0
		C.	sl.	-25	25	-50	-50	50	≠50	≠50	≠50	≠50		800		100.0	0.0
	1/8	T.	-25	25	-50	50	≠50	≠50	≠50	≠50	≠50	≠50	571		571/800	70.0	30.0
		C.	sl.	-25	25	-50	-50	50	≠50	≠50	≠50	≠50		800		100.0	0.0
	1/16	T.	-25	25	25	-50	≠50	≠50	≠50	≠50	≠50	≠50	618		618/800	77.0	23.0
		C.	sl.	-25	25	-50	-50	50	≠50	≠50	≠50	≠50		800		100.0	0.0
	1/32	T.	-25	25	25	-50	50	≠50	≠50	≠50	≠50	≠50	666		666/800	83.0	17.0
		C.	sl.	-25	25	-50	-50	50	≠50	≠50	≠50	≠50		800		100.0	0.0

sl: very slight trace of hemolysis
-50: less than 50, more than 25
-25: less than 25, more than slight trace
≠50: over 50

FIG. NO. 16

TITRATION of the SUPERNATANT SERUM



The results found confirmed those obtained in previous work.

b) Titration of the washings

Each cc of the washings obtained from each tube was titrated and the results reported in Table No. 31.

TABLE NO. 31

Washings No. 1

Tube No.	Reciprocal of final dilution of washings when titrated									
	2	4	8	16	32	64	128	256	512	1024
1	sl	-25	25	50	≠50	≠50	≠50	≠50	≠50	≠50
2	-25	25	≠50	≠50	≠50	≠50	≠50	≠50	≠50	≠50
3	-25	25	50	≠50	≠50	≠50	≠50	≠50	≠50	≠50
4	≠50	≠50	≠50	≠50	≠50	≠50	≠50	≠50	≠50	≠50
5	≠50	≠50	≠50	≠50	≠50	≠50	≠50	≠50	≠50	≠50

Washings No. 2 and washings No. 3 show no traces of antibodies.

c) Titration of the lysates

The lysates obtained from monocytes which had been in contact with the various dilutions of antiserum were titrated separately against toxin diluted 1:450. The results obtained are reported in Table No. 32.

No traces of antibody were found in the lysates.

TABLE NO. 32

Tube No.	Reciprocal of final dilutionsof lysates when titrated									
	2	4	8	16	32	64	128	256	512	1024
1	≠50	and followings								
2	≠50	"	"							
3	≠50	"	"							
4	≠50	"	"							
5	≠50	"	"							

d) Titration of the sediments

The sediments obtained from the lysed monocytes were titrated separately against toxin 1:450. The results are reported in Table No. 33. No traces of antinodies were found in the cellular sediments.

TABLE NO. 33

Tube No.	Reciprocal of final dilutions of sediment suspension when titrated									
	2	4	8	16	32	64	128	256	512	1024
1	≠50	and followings								
2	≠50	"	"							
3	≠50	"	"							
4	≠50	"	"							
5	≠50	"	"							

SUMMARY and CONCLUSIONS

Girard (54) found that rabbits in a state of induced monocytosis produced antisera of higher titre than normal rabbits. He also demonstrated that during active or passive immunization, the antibodies were concentrated in the monocytes. These findings suggest that monocytes may play a part in the production of antibodies or merely store them.

Exudates rich in monocytes were obtained from rabbits in a state of artificially induced monocytosis. The monocytosis was induced by injections of MPA (Monocytosis-producing agent) which was prepared from an extract of L. monocytogenes.

To demonstrate whether or not monocytes may absorb and store antibodies in vitro, the following investigation was undertaken. Serial dilutions of anti-typhoid, anti-horse and staphylococcal anti-alpha hemolysin sera were placed in contact with monocytes suspended in an improved preservation medium. Absorption of antibodies by monocytes was studied after 4, 8, 16, 24 and 48 hours incubation at 37°C.

Maximal absorption of antibody was found to have taken place after 8 hours incubation in the case of the anti-horse serum and after 16 hours incubation in the case of anti-typhoid and staphylococcal anti-alpha hemolysin

sera when the optimal conditions for preservation of monocytes were applied.

The monocytes showed a higher degree of absorption for anti-horse serum than for anti-typhoid or staphylococcal anti-alpha sera. The difference between the absorbed antibody for each system may be attributed to the different nature of antibodies.

In a second series of experiments the monocytes contents were determined after incubation with the various dilutions of antisera over the period of time found to give maximum absorption for each system. The monocytes after having been separated from the antiserum, were washed, lysed and the resultant supernatants, washings, lysates and residual cellular sediments thus obtained were titrated.

Although the absorption of antibody by monocytes has been proven for the 3 systems studied, traces of antibody were found only in monocytes which had been in contact with anti-horse serum for 8 hours while no trace of antibody was found in monocytes which had been in contact for 16 hours with either anti-typhoid or staphylococcal sera.

These experiments reported in this work are not strictly quantitative.

The absorption of antibody by monocytes may then be demonstrated to occur <u>in vitro</u> as proven by this present work as well as in <u>in vivo</u> as reported by Girard (54) and

appears to be true for one of the 3 systems studied in the *in vitro* experiments.

The finding that no antibody could be demonstrated in the monocytes which had been in contact with the 2 other antisera (anti-typhoid serum and staphylococcal anti-alpha hemolysin serum) might be attributed to the fact that monocytes were incubated for a longer period, 16 hours rather than 8 hours as in the case of the anti-horse serum. This time interval, though giving maximum of absorption of antibodies may be sufficient to bring about a destruction of the absorbed antibodies due possibly to the action of the proteolytic enzymes of the monocytes. The rapid disappearance of antibodies may be attributed to the artificial environment to which the monocytes are exposed.

The ability of monocytes to absorb and store antibodies as shown by the *in vivo* and the *in vitro* experiments may suggest that the monocytes play a part in the process of immunity by absorbing and storing antibodies. It may also be suggested that monocytes by their absorbing and storing functions destroy antibodies slowly after active immunization and more rapidly after passive immunization.

No definite conclusion concerning the part played by monocytes in the process of immunity may be drawn.

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