THE INFLUENCE OF HORMONES ON EXPERIMENTAL HEPATIC LESIONS

by

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INTRODUCTION

Fatty infiltration of the liver, though a common pathological finding associated with many diseases and frequently observed in routine autopsies, remains largely an enigma to the clinician and the biochemist. The importance of this lesion is recognized, for fatty infiltration has been shown to result in an impairment of hepatic function (54,188) and, if severe, to terminate in cirrhosis (39,41,46,47). Recent reports in the literature on the use of adrenocorticotropic hormone and cortisone for the treatment of acute and chronic liver disease (26,29,82,44) are somewhat conflicting. The hormones in these cases were used in an empirical manner and little knowledge exists on the effects which these hormones might exert on the various lesions seen in diseased livers.

Portal cirrhosis of the liver and severe fatty infiltration have been shown to follow phosphorus poisoning in man (106,159) and experimental animals (132). It was decided to use phosphorus poisoning as an experimental method to produce fatty infiltration of the liver and alterations in hepatic connective tissue in the rat. Such animals might then be subjected to excesses of adrenal and pituitary hormones, or a corresponding deficiency, to throw light on the effects which these states might have on clinical liver disease.

A complete understanding of factors affecting liver fat and connective tissue necessarily involves a knowledge not only of the effect

of these factors on fat metabolism and connective tissue, but also of their effect on carbohydrate and protein metabolism. To cover the whole of this field would not be feasible within the limits of a single thesis, and the discussion is therefore limited to a brief review of recent knowledge on hormonal influences on collagen tissues, and hormonal and related factors as they might affect liver fat deposition in an experiment such as this.

CHAPTER I : METHODS OF STUDYING THE FAT CONTENT OF THE LIVER

Alterations in the liver lipids may occur by either of two principal processes. The first of these is known as "fatty degeneration" and is a change in the form of protoplasmic fat, rendering visible fat which hitherto was not demonstrable. Thus fatty degeneration actually involves no increase in the amount of lipid present (27,36). This protoplasmic lipid is the "Element Constant" of the French School (51). The second process is known as "fatty infiltration" which represents an increase in the absolute amount of fat contained in the liver. It may represent either an increase in the transfer of fat from the depots, a decrease in hepatic utilization of fat, or an increase in neolipogenesis from other metabolic precursors or a combination of two or more of these situations (185). Fatty infiltration may be studied by any one of a number of methods while fatty degeneration can only be shown by histological techniques.

1. MORPHOLOGICAL

Macroscopic. The normal liver is reddish-brown in colour and presents a mottled, granular appearance. The cut surface is soft, friable and extremely vascular. When marked increases in liver fat content occur, the organ becomes pale, yellow and softer than normal with swollen, rounded edges. The cut surface is rather bloodless and may be greasy (30); small sections of such livers may float when placed in water (160).

Microscopic. Increases in liver fat may be demonstrated by various

histological methods. Sections for fat staining are cut from fresh material by a freeze-drying technique or by formalin fixations. Procedures which involve the use of chloroform, zylol or ether, such as are used when sections are embedded in paraffin or colloidin, cannot be used in fat studies as these fluids are all fat solvents. Sudan III and Scarlet Red are the most commonly used dyes for the staining of lipids. These substances are effective because they are more soluble in fats than in the alcoholic solutions in which they are applied (30). Neutral fat is most intensely stained by these stains, while cholesterol and lecithin have less affinity for the dyes (145). Free fatty acids generally do not take the above stains; however, Jeker obtained some colour with oleic palmitic and stearic acids (103). The fatty acids may be stained by any basic aniline dye. Lipoid substances may be differentiated from neutral fats by the use of Ciaccio's method. The lipoids are rendered insoluble by treatment with potassium bichromate, embedding the tissue in paraffin then dissolving out the neutral fat, after which the lipoids may be stained with Sudan III (30). Lipoids may also be demonstrated by examination of frozen sections with polarized light. Cholesterol and its esters are anisotropic and appear brilliant while neutral fats remain invisible (30).

Normal liver cells contain a small but rather constant proportion of fat. This exists for the most part in an invisible form together with a small part in the form of free fat (51,86). When livers which contain excess fat are sectioned, however, the parenchymal cells are found

to contain fat droplets of variable sizes. These may press the protoplasm and nucleus to one side to give the cell a typical "signet ring" appearance. In some cells one may be unable to see any of the normal cellular constituents (86). In a histological section of a normal liver only the occasional, rare fat droplet may be seen in a field. Mottram (142) studying the effects of starvation on the liver fat of the rabbit and guinea pig, has shown that after a 48-hour fast there may be as many fat droplets in one cell as were visible in a whole field prior to the starvation. This author also pointed out the existence of incompletely stained fat droplets, evidently because the fat is being metabolized, leaving behind non-staining areas in the cell.

The distribution of histological fat is not essentially uniform throughout the liver (142,2). This for some time provided a method of differentiating "fatty degeneration" from "fatty infiltration". A centrilobular distribution of fat droplets was supposed to be indicative of fatty degeneration while, on the other hand, a peripheral distribution within the lobule was thought to represent fatty infiltration (160,30). This classification does not invariably apply as a "fatty degeneration" may occur in the periphery of liver lobules (30). The size of fat droplets was also thought to be a means of differentiating the two conditions, fatty degeneration being characterized by very small droplets (30). In general this may be true, but it is doubtful that droplet size may be used as a means of differentiating the two conditions (30).

As well as variations in lobular distribution of liver fat, there

is considerable data to support the belief that lobar distribution may also vary. This has led to criticism of conclusions which have been based solely on histological evidence (38). It has been found by Chaikoff and Kaplan (38) in depancreatized dogs that the fatty acid concentration of a mixed sample of an entire liver and the amount of fatty acids in sections of its lobes may differ by as much as 86%. Absolute differences of as much as 13% were also found between the six liver lobes. Dowler and Mottram found similar results after fasting (53). These experiments suggest that histological methods may not be entirely valid for quantitative studies on liver fat, and that one cannot justifiably use one lobe or part of a lobe as a control for any other portion of the liver.

II. CHEMICAL

Many varied methods may be found in the literature for the estimation of tissue lipid content. Essentially, they consist of extraction of the tissue with a fat solvent, filtration of the lipid solution, and then determination of the lipids by gravimetric methods.

Although chloroform may be used (64), alcohol appears to be one of the most satisfactory solvents employed as it is directly applicable to the tissues and penetrates rapidly. However, it has several disadvantages in that it removes substances other than neutral fat and, when used with boiling, may chemically alter some of the more labile compounds (89). The first of these difficulties may be overcome by evaporating the alcoholic tissue extract to dryness and resuspending the neutral lipid in ether; the

second is avoided by extraction with cold alcohol. Complete extraction, however, cannot be attained by using cold alcohol and the tissue residue must be further treated with ether. For this reason these two fat solvents are now commonly used in combination for the extraction of lipids in a proportion of three parts alcohol to one part of ether (Bloor's solution) (74,119,72).

Determination of lipid constituents other than neutral fat may be accomplished by partition of the original alcohol-ether extract. Acetone is used to remove the free fat and cholesterol leaving behind the phospholipids. The latter may be taken up in petroleum ether and assayed by oxidation with potassium dichromate and sulphuric acid, by estimation of the phosphoric acid content or by determination of the fatty acids present.

Cholesterol content may be ascertained by digitonin precipitation or by colorimetric estimation. Neither of these methods is specific as the results may be affected to an unknown degree by other tissue constituents. Results using the colorimetric method are usually higher than the digitonin method; the latter is subject to error by decomposition of the digitonin-cholesterol complex or by loss in the wash fluids.

Fatty acids are measured by precipitation with sodium hydroxide, acidification, solution in petroleum ether and then titration of the acids with alkali.

III. PHYSICAL

Chemical methods of study of liver fat can show quantitative changes which may have taken place without regard to the distribution of these

changes in the liver, and qualitative comparisons may be made between liver fats and that found in the diet or in the depots. Histological studies of the liver will demonstrate that "fatty degeneration" or "fatty infiltration" has taken place and possibly may demonstrate the direction from which these processes originated. However, neither of these methods gives any indication of the mechanism by which these changes have occurred. For this purpose various other procedures have been devised in recent years.

Isomerization. The following are several methods which were designed to so alter a natural fatty acid as to render it distinctive and traceable through the normal metabolic reactions. These procedures must give it the ability to withstand the loss of its identity while at the same time giving it some measurable physical property differing it from the natural fatty acids.

A shift in the position of the double bonds of an unsaturated fatty acid may produce an acid which is spectroscopically distinguishable from the normal body fatty acids. Miller, Burr et al. used this method by producing a conjugated isomer of linoleic acid (141).

A second method is isomerization about a double bond. Sinclair (178) has used the trans isomer of oleic acid, elaidic acid as a fatty acid tag. Elaidic acid is readily differentiated from other fatty acids, its concentration in any tissue is readily determined, yet at the same time it is readily absorbed, deposited, mobilized and burned by the animal body (178,177,176).

Isotopic Substances

a) Deuterium. When an animal on a fat-free diet is fed or injected with deuterium water, deuterium is incorporated into a constant proportion of the newly synthesized fatty acid molecules (169). The concentration of deuterium labelled fatty acids reaches a maximum at six to eight days (168) and at this time accurately reflects the rate of synthesis of fatty acid molecules. Schoenheimer and Rittenberg (169) have shown that deuterium uptake is representative of true fatty acid synthesis by demonstrating that it could not occur by saturation and desaturation of existing fatty acids; nor could deuterium enter an already formed fatty acid molecule. Hence the rate of uptake of deuterium into fatty acids is actually the composite rates at which they are formed and destroyed. This method has lent itself to several comprehensive studies of intermediary fat metabolism (167,168), the rates of conversion of carbohydrate to fat (168) and studies on the source of liver fat which accumulates under various conditions.(185).

b) Radioactive isotopes. Radioactive tracers have been used in recent years to study certain aspects of fat metabolism. Phospholipids have been shown to play a prominent role in the intermediary metabolism of fats (27). They undergo a rapid turnover in the liver, new molecules being formed constantly to replace those which have been broken down. If an animal is injected with carrier-free doses of radioactive phosphate, some of the isotopic material will be incorporated into the phospholipid molecule. Since there is a continuous interchange between the lipid and the inorganic phosphorus of the body, the use of radioactive phosphorus will enable one

to determine the rate at which this reaction occurs (178). It has been postulated that the first step in the breakdown of a triglyceride in the liver is the replacement of one of its fatty acidsby a phosphoric-acid-base complex. Thus the radioactivity in the liver of phosphate-injected animals would be at any one time not a measure of the phospholipid present, but of the rate of entry and departure of fat from the organ (40,76).

Adequate methods are available for the qualitative and quantitative study of changes in liver fat. However, adequate techniques for the study of mechanisms governing alterations in fat metabolism and their control are few and yield results which may have equivocal interpretations.

CHAPTER II : FACTORS AFFECTING FAT DEPOSITION IN THE LIVER

A. NON-HORMONAL

1. Damaging agents. It has been stated that toxins and anoxia are two of the major causes producing alterations in liver fat (30,94). However, it is generally believed that toxic agents act by interfering with the proper oxygenation of parenchymal liver cells (30,94,46).

Toxins - the various toxins which affect liver lipids may be divided into organic, using the term in the biological rather than the chemical sense, and inorganic agents. Fatty degeneration of the liver, associated with organic toxins, is a frequent pathological finding associated with various diseases. Among these are typhoid fever (36), septicemia (36), exanthemata (36), pneumonia and other infectious processes which reduce the oxygen content of the blood (36), or which produce a state of partial

starvation (36,94). Accumulation of fat in these conditions is associated with decreased blood and liver phospholipid (140,193) and reduced blood cholesterol and neutral fat (140). An example of the non-specific effect of bacterial toxins is found in the liver of horses which have been subjected to prolonged immunization procedures for the production of immune serum. These animals often show fatty degeneration of the liver, fatty infiltration and, occasionally, changes suggestive of early cirrhosis. The ratio of phospholipid to free cholesterol in the livers of these animals is lowered as their condition becomes more severe (204).

Many inorganic poisons also influence liver fat, among the most important of these are alcohol, chloroform and phosphorus. Autopsy of cases of chronic alcoholism often reveals a pale, swollen liver characteristic of fatty degeneration and infiltration (30,94). When sections of the liver are examined histologically, fat droplets are found in abundance, particularly toward the periphery of the lobule, and cirrhotic changes may be quite marked. Alcohol has been shown to exert a direct effect on liver tissues in vitro (30). However, it is now generally conceded that the effect which alcoholism exerts on the liver is not produced so much by the alcohol per se as by the accompanying malnutrition (30,94,46,47).

The profound effects which chloroform exerts on liver fat has led to the recommendation that it be contraindicated as an anaesthetic agent (84). One may expect to find fatty degeneration of the

liver if a patient dies some days after prolonged chloroform anaesthesia (30,94,36). Radvin et al (155) found that during a period of chloroform anaesthesia an inverse ratio existed between liver glycogen and fat. The former steadily decreased over a 24-hour period while liver lipid showed a proportionate increase. MacLachlan (129), however, found no increase in liver fat following chloroform anaesthesia and maintains that a true degeneration is produced.

The administration of phosphorus to an experimental animal characteristically produces an acute necrosis, fatty degeneration and fatty infiltration of the liver (30,94,36). This was first observed by Saikowsky in 1865 in one of the earliest experiments on fat deposition in the liver (161). Rubitsky and Myerson (159) in a recent study of phosphorus poisoning in man, have outlined the extensive morphological and metabolic changes which occur. As well as the histological changes of fatty degeneration and fatty infiltration with cirrhotic changes which have already been described, they noted increases in blood glucose, disappearance of glycogen from the liver and increases in protein catabolism. These alterations are believed to represent glycolysis and inhibition of glycogen synthesis from non-carbohydrate sources. Several other toxic substances, cocaine and arsenic are capable of producing alterations in liver lipids (36,130,50). A detailed description of the alterations they produce and the mechanisms by which they act is not felt to be within the scope of this review.

2. Anoxia. Fatty degeneration of the liver may be encountered in many

conditions associated with poor oxygenation of the tissues, i.e. low atmospheric pressure, anemia, chronic venous congestion or severe hemorrhages. Taurog, Chaikoff and Perlman (192), using radioactive phosphorus as an indicator, have shown that the in vitro formation of phospholipid was inhibited by anaerobic conditions or the respiratory inhibitors, cyanide, hydrogen sulphide or carbon dioxide. This failure to synthesize phospholipid under anoxemic conditions is probably responsible for the fatty changes in anemia (94), and exposure to low barometric pressure (94).

Conclusion

Chronic anoxia and numerous toxic agents have been associated with alterations in hepatic lipids. The exact mechanism by which they cause these changes is unknown; however, there is reason to believe that these agents may act via a common pathway by their effects on respiratory enzyme systems (94).

3. Nutrition. It has been known for almost fifty years that fasting the experimental animal could alter the liver fat content (142). It was first observed in 1914 that the character of the diet could mitigate or exaggerate lesions of the liver due to certain poisons (146). However, it has not been until relatively recent years that certain dietary constituents themselves were found capable of influencing liver fat.

a) Fasting. Mottram in 1909 published the first complete report on the effect of starvation on liver lipids (142). He noted large increases in the liver fat of rabbits and guinea pigs after a 24-hour fast, and in rabbits was able to show that the liver fat was derived from the depots. Although liver fat concentrations were invariably increased in his experiments, absolute increases in lipid were not always the rule. The shrinkage of the liver which occurs during a fast may give a false impression of fatty infiltration.

Similar effects of fasting have been shown for the mouse (131), while in the rat the fasting fatty infiltration of the liver is a much less clearcut phenomenon. Female rats with large fat depots usually respond to some degree, whereas in the male rat a negative response is almost always the rule (24), although the concentration of liver fat may be increased due to shrinkage of the organ. No plausible reason for these species differences is known.

The mechanism of fatty infiltration of the liver in fasting is probably one involving increased mobilization from the depots. As the available carbohydrate stores are exhausted, the animal mobilizes fat at a rate greater than normal to meet its energy requirements. Barrett has shown, using deuterium, that the fat in the liver after fasting is derived from the depots (8).

b) Overfeeding. When an animal is fed an excess of a normal diet over and above its caloric requirements, obesity may be produced, but apparently no relation is seen between this condition and

the fat content of the liver under these circumstances (120). Flock and co-workers, however, were able to produce increases in liver lipids by feeding a high carbohydrate diet to the goose. The increase in liver fat in this instance is chiefly neutral fat and, in the opinion of the authors, represents an increase in fat synthesis from carbohydrate (68).

c) High fat diets. This represents one of the easiest means of increasing liver fat; diets composed of pure cream or fat (126), or fat plus alcohol (68) cause very marked increases in liver fat. This effect may be enhanced by the addition of bile salts to the diet (120).

The amount of protein fed with a high fat diet also influences the degree of fat infiltration of the liver. Li (120) has shown that high fat, low protein diets produce greater infiltration of liver fat than isocaloric diets which contain larger quantities of protein. This effect is probably due to the lipotropic effect of the proteins (120), although Friedman has suggested that the fatty infiltration of the liver may be the result of secondary changes in the pancreas, which shows acinar degeneration on high fat, low protein dietary regimes (73).

d) High cholesterol diets. Chalatow in 1914 was the first worker to produce liver damage with fat infiltration by means of a diet high in cholesterol (42). This has since been reported as a convenient method of increasing liver fat (25,17). Lipid increases after cholesterol feeding are of several types; neutral fat is increased and as well cholesterol and cholesterol ester, the latter showing the greatest

percentage increase (16). Best (25) suggests that the conversion of cholesterol after absorption to its ester may represent an alteration to a less noxious form. The increase in neutral fat in these livers is believed by Himsworth (94) to be the result of mechanical injury by the cholesterol salts to the parenchymal cells. However, Best has found that the neutral fraction responds to the lipotropic action of choline, which might hardly be expected to occur in severely damaged cells (17).

e) Proteins and amino acids. Intensive studies have been carried out during the past 15 years on protein and amino acid factors which influence liver fat. Impetus to these studies was provided by experiments involving fatty infiltration of the liver in depancreatized dogs. Lecithin was found to be capable of inhibiting this increase in liver fat (91,92) and with the discovery that choline was the active agent in the lecithin (13,14) the term "lipotropic factors" was adopted to describe various principles possessing this action. Lipotropic agents are defined as agents which by their absence allow fatty infiltration of the liver to occur and which, when added to the diet, can influence the fatty infiltration due to a variety of agents.

Numerous dietary proteins have been shown to exert a definite lipotropic effect on liver fat (19). The degree of activity shown by a certain protein usually, but not always, parallels its methionine content (180,199). However, a superior effect is produced when methionine is fed in the pure state, rather than in a protein combination

such as casein. This is due to the presence in casein of certain amino acids such as cystine which have an anti-lipotropic action (197).

Best and his group have thoroughly investigated this problem (13,14,16,17,18,19,24). They have demonstrated that the effect methionine has on liver fat is dependent to a large extent on the diet which is fed and the amount of other essential amino acids available (18). Thus it has been postulated that the methionine which acts on the liver in a lipotropic manner is that which is above the amount required for growth (196).

Methionine has a lipotropic action under a variety of experimental conditions (197). However, it has no effect against fatty livers induced by high cholesterol diets, in livers with only a moderate degree of fatty infiltration (17) or after carbon tetrachloride poisoning when the animals are receiving an adequate protein intake (55).

The action of methionine as a lipotropic agent has been clearly shown by Du Vigneaud to be due to its ability to transfer methyl groups for the synthesis of choline (56,57). This reaction may occur under anaerobic conditions which may explain why methionine exerts an effect on fat infiltrations due to anoxia or various toxins. Methionine is thought to have approximately one-fifth the lipotropic activity of choline (43).

Several amino acids, among which are homocystine, homocysteine, cystine and cysteine, have an anti-lipotropic action opposing the action of methionine and in themselves causing an increase in liver fat (179,180).

The mechanism of this anti-lipotropic action is unknown. Present knowledge is of a somewhat confusing nature as Perlman (149) has shown that methionine, cysteine and cystine, in spite of their opposite effects on liver fat, all cause an increase in liver phospholipid turnover.

f) Vitamins.

Vitamin A - Experimental production of vitamin A deficiency has been shown to cause a decrease in liver phospholipids and an increase in blood lipids. This may be due to the inability of the animal to synthesize fatty acids and glycerol to fat or carbohydrate in the absence of vitamin A (121). It has been noted that occasional patients with parenchymal liver damage show signs of vitamin A deficiency, a finding which may be only coincidental (94).

Vitamin D - Sharpe (174) has reported that lower liver and blood phospholipids are a regular concomitant of experimental rickets in the dog. The rachitic puppies also showed a relative decrease in the ratio of liver phospholipid to blood phospholipid. One can only surmise as to the possible relationship of the bone loss of phosphorus in rickets and the decrease of the tissue phospholipids.

Vitamin E - Only a few isolated references may be found in the literature which relate tocopherols to liver fat metabolism. Hove (97) noted that rats maintained on vitamin E deficient diets had lower liver fats than did control animals; this difference could be eliminated if increased protein was fed to the animals. Pappenheimer,

on the other hand, has related vitamin E deficiency to the appearance in the liver of a peculiar lipoid substance known as "ceroid" (148). The significance of these observations is not known; however Schwarz has noted that increases in liver fat resulting from the feeding of alkali-treated casein could be prevented by the addition of vitamin E to the diet and he theorizes that deficiency of tocopherols may sensitize animals to the absence of lipotropic amino acids (170).

Vitamin K - Reports on the activity of vitamin K or its precursor 2-methyl-1,4-naphthaquinone on liver fat are quite controversial. Topelbert has stated that avitaminosis K is accompanied by fatty degeneration of the liver. There is also a report that 2-methyl-1,4-naphthaquinone could replace choline to prevent the fatty infiltration which develops in rats on a choline-free diet (195,96). However, other workers could find no evidence of lipid changes in the livers of chicks maintained on diets free of vitamin K, yet adequate in lipotropic factors (65).

Thiamine - McHenry and his associates (134) have reported that rats kept on a diet deficient in both choline and vitamin B suffer an increase in liver fat but only for a very short time, presumably until the body stores of this vitamin are exhausted. They have also shown that if thiamine is administered to young rats on choline deficient diets, increases in both liver and body fat will be produced (136). Thiamine will also increase the liver and body fat stores when rats are maintained on a high carbohydrate, fat-free diet. This has led McHenry (135) to suggest that thiamine influences the conversion of carbohydrate (possibly

via pyruvate) to fat.

Riboflavin - Dietary deficiencies of this vitamin appear to result in subnormal levels of liver fat (60). Gavin, however, has reported that riboflavin will increase the deposition of fat in the liver provided adequate thiamine is fed (75).

Pyridoxin - Experimental dietary deficiencies of pyridoxin will lead to the development of fatty livers (75) which may be alleviated by the addition of liver preparations containing this vitamin to the diet (83). It has been suggested that this substance acts by the conversion of protein to fat (137).

Biotin - A deposition of fat in the liver, characterized by a high cholesterol content, has been produced by feeding biotin in the presence of other B vitamins. This fatty infiltration may be prevented or cured by feeding inositol (124).

Inositol - Inositol, as has been stated above, inhibits the deposition of fatty acids and cholesterol in the livers of rats fed biotin. Its lipotropic action appears under conditions unfavourable for the action of choline, as when large amounts of cholesterol are present (138). McHenry suggests that inositol, like choline, takes part in the formation of certain phospholipids (138).

Nicotinic acid - Fatty infiltration of the liver is a frequent finding at autopsy of cases of pellagra, which is supposed to be due to nicotinic acid deficiency (77). However, supplementing the diet of infants suffering from pellagra, with vitamins, appears to aggravate

the condition of the liver. For this reason Gillman and Gillman attribute the lipid infiltration to general inanition rather than to any specific vitamin deficiency (77).

Pantothenic acid - Supplementation of a diet containing thiamine, riboflavin, pyridoxin and choline with calcium pantothenate produces a 100% increase in liver fat (60). Like thiamine and riboflavin, pantothenic acid appears to be active in the conversion of carbohydrate to fat (60).

Choline - There is some dispute as to whether choline should be classified with the water-soluble B vitamins. Many workers denote it only as an unclassified lipotropic factor, and with some justification as choline is probably a dietary requisite only when the body supplies of methionine and ethanolamine are markedly restricted (138).

Choline is the most important of the lipotropic agents. These are factors which by their absence allow fatty infiltration to occur and when added to the diet can influence the fatty infiltration due to a variety of agents. Choline is effective in reducing the fatty infiltration resulting from increased dietary intake of cholesterol (20), glycerides (70), starvation (127,128), or carbon tetrachloride poisoning (9). Choline also prevents the fatty infiltration of the liver of depancreatized dogs (15) but has no effect on that produced by the injection of anterior pituitary extracts (143). Choline inhibits the fatty infiltration of the liver produced by a choline-deficient diet at a minimum effective dose of three mg. daily (23) and a maximum dose of 100 mg. (196).

However, a dose response relationship exists between the dose of choline and its lipotropic effect only at low dosages approaching the minimum effective dose (206).

The exact role which choline plays in intermediary metabolism to bring about its lipotropic effect is unknown. Its site of action has been shown to be on the formation and removal of phospholipids (150,151). Choline greatly increases the phospholipid turnover of the liver, which has led to two schools of thought on its possible action.

Labelling the choline molecule with arsenic (205), with radioactive phosphorus (in the lecithin) (150), or with heavy nitrogen (184) has shown that the whole molecule is incorporated into the phospholipids. Thus it may act as a material directly essential to phospholipid synthesis, which appears to be its most probable role.

A second possibility is that choline acts in a catalytic manner on liver fat oxidations. Trowell has studied the effect of choline in vitro on the respiration of liver fat slices (198) and found that it increases the oxygen consumption in direct proportion to the amount of choline present. However, it also decreases the rate of acetoacetic acid production (198) which has led to the belief that the increased oxygen consumption is due to oxidation of choline itself.

More investigation on these two possibilities will be necessary to definitely resolve the problem; at present the former appears to be the more likely.

Vitamin C - Guinea pigs maintained on a scorbutogenic diet

develop a fatty infiltration of the liver (183). There is no proof that this is due to any specific avitaminosis and it might well be attributed to the anemia and loss of appetite which were admittedly present in these animals (183).

4. Miscellaneous. Though some of the following factors may act in manners which have already been described and therefore such division as has been made is necessarily an arbitrary one, it is felt that separation as follows will add to the clarity of this discussion.

Neural factors. The importance of neural mechanisms in fat metabolism has been recognized since it was shown by Wertheimer in 1926 that section of the upper portion of the spinal cord prevented the accumulation of fat in the liver which occurs following phlorhizin poisoning (208). This inhibition he felt was the result of inability of those animals to mobilize depot fat. Various cerebral lesions may also influence liver fat and lipid mobilization - puncture of the floor of the fourth ventricle (Claude Bernard's sugar puncture) causes a fatty infiltration of the liver in rabbits which is concurrent with a decrease in liver glycogen. Division of the splanchnic nerves or denervation of the adrenal prevents the fatty infiltration (11). Obesity has been produced in the dog by partial destruction of the paraventricular hypothalamic nuclei (90), and by similar lesions in the rat (93). Graef et al found that hypothalamic injury following hypophysectomy in the dog was often associated with marked obesity (81). It is also reported that many cases of human obesity ascribed to pituitary

dysfunction are found to have damaged areas in the surrounding brain tissue (111).

These findings are important not only for their suggestion of neural agencies affecting fat metabolism, but also for the bearing that such lesions may have on function of the pituitary gland, and thus indirect effects on fat metabolism. The exclusion of hypothalamic injury would seem to be an important measure in the evaluation of experimentation involving hypophysectomy.

Non-specific stressors. Radical changes in total body metabolism may result in physiological changes in liver fat. Excessive exercise (104) and a number of other non-specific damaging agents (107, 69,113) have been shown to cause fatty infiltration of the liver. This has led Leblond et al to include it in a description of the typical Alarm Reaction (107), although Selye (171) did not find it as a constant occurrence in this reaction. The fatty infiltration which follows non-specific stress is probably a reflection of the increased need of the body for energy in these situations. This view is borne out by the fact that exercise produces a greater increase in liver fat than the other agents and that fasting further increases the fat deposition in the exercised rat.

X-ray irradiation of the mouse (58) and the rat (108) has also been shown to cause increases in sudanophile fat in the liver. The mechanism by which this agent increases liver fat is unknown. It has been suggested, however, that x-ray irradiation causes release of histamine

which acts in a toxic manner on the liver (58).

Partial hepatectomy. Removal of part of the liver of the dog, rabbit and cat has been shown to result in marked fatty infiltration of the hepatic remnant (45), especially if the animal is fasted. Choline has been shown to have no effect on the increased lipid (45). It is hardly likely that this operation per se causes an increased mobilization of depot fat but rather that the fraction of liver remaining in the animal is unable to dispose of normal amounts of fat which are brought to it.

Pathological alterations in liver fat. A number of so-called lipoid storage diseases have been described which are characterized by disturbances in fat metabolism. In Gaucher's disease, which affects the reticulo-endothelial system, large pale cells filled with a cerebroside appear in the liver, spleen, lymph nodes and generally throughout the reticulo-endothelial system. Niemann-Pick's disease manifests itself by a similar histological picture with a similar distribution except that the cells are filled with phosphatide. Schuller-Christian's syndrome is a deposition of cholesterol or its esters in the white fibrous tissues of the body (27,30).

In each of these conditions deposition of a specific lipid substance occurs, but in each case the lipids are different. Although the etiologies of these diseases are obscure, they probably represent defects in the intermediary metabolism or the excretory mechanism of the individual lipids concerned.

B. HORMONAL

1. Adrenal Cortex

Adrenalectomy per se has been shown to result in changes in body lipid constituents which would suggest that hormones from this gland play a distinct role in fat metabolism. Welt and Wilhelmi (207), McKay (126), Szego (189) and others (114) have shown that adrenalectomy results in a decrease in liver lipids. In the rat this decrease in liver lipids is more apparent in the male than in the female (175). The low liver fat seen in these animals is also accompanied by decreases in carcass fat (165,166,187) which is independent of the nature of the diet fed. In all cases adrenalectomized animals have lower peripheral fat reserves than do normal paired fed controls. No constant change is seen in the blood lipids of adrenalectomized animals (85) although decreases in phospholipid and fatty acid content have been reported in adrenalectomized cats (209).

In normal rats fatty livers can be produced by a variety of methods, but in adrenalectomized animals the fatty infiltration of the liver is inhibited. Verzar and Laszt noted that fatty livers normally produced by poisoning with yellow phosphorus do not occur after adrenalectomy unless the animals are treated with cortical extract (202). McKay and Barnes (125) and Fry (74) similarly observed that adrenalectomized animals do not develop fatty livers following the administration of anterior pituitary extract. This effect is not seen when the adrenal

medulla alone is removed.

McKay (126) extended these observations to show that adrenalectomy reduces the fatty infiltration of the liver, following the feeding of pure cream or fat, during the alkalosis produced by feeding sodium bicarbonate or as a result of epinephrine injections. The high concentrations of fat in the livers of rats fed high fat diets is reduced more quickly if such animals are adrenalectomized than in the normal controls. Normally the female rat has more fat in the liver than does the male; adrenalectomy tends to abolish this difference. McKay and Carne (127) and later Berman (12) studied the fatty infiltration which occurs in the liver remnant following partial hepatectomy; the extent of the fat deposition was decreased following adrenalectomy. The reduction of fat in these livers was limited to the neutral fraction.

Replacement therapy after adrenalectomy with either adrenal cortical extract (202), 17-hydroxycorticosterone, 11-dehydrocorticosterone or 11-desoxycorticosterone (114) enables one to provoke a response by an agent which increases liver fat, similar to that seen in the normal animal. However, no adrenal cortical hormone in the form of a crude extract or as a pure steroid has been shown by itself to be capable of altering liver fat content in either the normal or adrenalectomized animal fed a normal diet.

Similarly, cortisone (187) and to a lesser extent desoxycorticosterone acetate and adrenal cortical extract (166) have been shown to be capable of preventing the loss of peripheral fat reserves which otherwise

occurs in adrenalectomized rats. Thus far no investigator has reported the production of obesity in an experimental animal treated with a pure adrenal steroid.

Goldzieher has claimed to have been able to cause fixation of blood fat in the tissues by means of an adrenal extract which he has prepared (78). Ingle (99) does not recognise extracts prepared in the manner so described as possessing any adrenal cortical activity. McKinley and Fischer (139) and Fieschi (66) have reported that their experimental animals gained in weight when fed dried adrenal cortex tissue. Such preparations are without adrenal cortical activity (99).

Hartman and Brownell (87,88) claimed to have isolated a new adrenal hormone from cortical tissue by chromatographic adsorption which they termed "fat factor". This substance is thought to be capable of increasing liver fat of adrenalectomized animals following a fast. Methods of preparation of this substance have not been published, nor has the original work been verified by any other group.

The exact mechanism by which adrenalectomy modifies fat deposition in the liver is not fully clarified. It is felt that this impairment may be one of the primary changes of cortical insufficiency for, as may be seen in the following experimental data, it persists in adrenalectomized animals maintained in a state of apparent good health on a high intake of sodium chloride.

The available experimental data show that in adrenalectomized animals fatty livers are produced much less readily, if at all, by any of

the experimental methods used. Three possible mechanisms for such a result are postulated - the adrenalectomized animal may have inadequate mobilization of depot fat, a high rate of removal of fat from the liver, faulty deposition of fat in the liver, or a combination of these.

Fat Mobilization from the Depots

Barnes (7) and Levin (114) feel that the failure of adrenalectomized animals to develop fatty livers may be accounted for by a decreased ability of these animals to release neutral lipid from their depots and thereby transport it to the liver. Barnes (7) and later Chaikoff (40) found that adrenalectomy impedes the rate of entrance of tagged fatty acids into the liver neutral fat.

However, such experiments by themselves cannot be taken as irrefutable evidence that adrenalectomy inhibits fat mobilization. Tagged fatty acids may become lost in the liver through changes wrought in their structure by this organ. Experiments in which quantitative studies of peripheral fat depots after adrenalectomy were done (165,166, 187) have shown that these animals have, if anything, an increased ability to mobilize depot fat.

In adrenal cortical insufficiency a rapid loss of adipose tissue occurs (194) which can be restored to normal by the administration of potent adrenal cortical extracts (95). Similarly, Addison's disease is generally characterized by a loss of body weight and fat (99). However, nutritional as well as hormonal factors must play an important role in

these disease states, and evaluation of the relative importance of each is difficult.

In adrenal cortical hyperfunction, on the other hand, an increased deposition of peripheral fat occurs; tumours of the adrenal cortex (95) are usually associated with obesity of the face, trunk and abdomen - the so-called "buffalo" obesity. When such tumours are removed, the obesity disappears (99,79). Ingle (99) has indicated that the fat which accumulates with adrenal cortical tumours is true adipose tissue and is not due to an increase in tissue fluids.

Fat Metabolism in the Liver

The failure of the adrenalectomized rat to deposit fat in its liver has been attributed by Verzar to a failure in its ability to phosphorylate fats (202). His claim that phosphorylation was decreased after adrenal extirpation was based on the observation that adrenalectomized rats and dogs absorbed fat at a far slower rate than normal (100) and that this rate returned to normal if the animals were treated with a preparation of the adrenal cortex (201). Verzar did not attempt to correlate his findings with other of his observations - that the absorption velocity of other foodstuffs was also decreased following adrenalectomy (203).

It has now been established that if adrenalectomized animals are maintained in good condition on salt therapy, the rates of absorption of most fats is identical with that in the normal animal (6,10). As

well, in contradiction to Verzar, phosphorylation has been shown to proceed normally in adrenalectomized animals. Barnes et al have shown that the phospholipid content of the liver of adrenalectomized animals is normal (7). Chaikoff and co-workers have shown that adrenalectomy has no effect on the incorporation of radioactive phosphorus into liver phospholipid, which is further evidence that no impediment to phosphorylation exists in these animals (40).

Recent experiments by Welt and Wilhelmi may help to throw some light on the problem of liver metabolism of fats following adrenalectomy (207). These workers measured the deuterium uptake by liver and carcass fats of female rats on a high carbohydrate, fat-free diet. Adrenalectomized animals in this experiment had a significantly greater uptake of D_2O into the liver and carcass fat than did the normal animal, and it is concluded that the adrenalectomized animal tends to utilize a greater proportion of carbohydrate over the pathway of fat synthesis and oxidation (207). The demonstration that the oxidation of fat is not impaired following adrenalectomy suggests that the low liver fat in these animals may be accompanied by normal or increased oxidation of fats.

Conclusions

The failure to produce changes in liver fat by overdosage with adrenal cortical principles in normal animals leaves unanswered the question as to whether the effects of the adrenal on fat metabolism are direct or are mediated by changes in carbohydrate and protein metabolism. The

adiposity associated with adrenal cortical hyperfunction suggests that adrenal hormones may stimulate the metabolism of carbohydrate and protein and thereby suppress the catabolism of fat. The loss of depot fat and the reduced neutral fat in the blood following adrenalectomy indicates an increase in fat catabolism, to supply energy in the presence of failure of other forms of metabolism. This view does not exclude the evidence that the adrenalectomized animal is unable to attain a high fat concentration in the liver, for if the adrenalectomized animal is dependent on fat for a large part of its energy requirements, the phospholipid turnover in the liver would be increased and fat would be withdrawn from the blood and catabolized at a rapid rate.

2. The Anterior Pituitary

There is abundant evidence in the literature supporting the thesis that the hypophysis exerts marked effects on fat metabolism. The relationship, as with all metabolic processes affected by the pituitary, is extremely complex. Nearly all the pituitary principles from the anterior and posterior lobes have at one time or another been implicated in fat metabolism. This discussion will be limited to several anterior pituitary factors which in some manner exert their action via the adrenal gland or its hormones. However, by limiting the scope of this review in this manner the author does not suggest that these factors are more important than, or are not affected by other pituitary hormones. For more detailed information on hormones from the

hypophysis not included here, the reader is referred to comprehensive reviews on the subject (101,102,154).

Hypofunction

Clinical. Pituitary hypofunction in man may be associated with obesity (80,172), normal energy metabolism (172,71) or excessive loss of fat reserves (172). It is difficult if not impossible to draw any conclusion as to the effect of clinical pituitary hypofunction on fat metabolism.

Simmonds' disease is characterized by almost complete depletion of body fat depots (172), but certainly these cases are complicated by the extreme degree of anorexia which exists.

In basophilic adenomas of the pituitary the obesity which occurs is believed to be of non-pituitary origin (79) as such adenomas are usually too small to cause hypofunction by interference with the rest of the parenchyma of the gland.

It would appear likely that changes in fat metabolism occurring in conjunction with pituitary hypofunction in man might be attributed not only to metabolic and disgestive disturbances, but also to pressure changes in the hypothalamus.

Experimental. The total body fat content of hypophysectomized animals shows a considerable reduction from the normal if the animals are allowed to eat according to their appetite (110,158). If, however, groups of hypophysectomized and paired-fed control animals are force-fed isocaloric

diets by means of a stomach tube, the hypophysectomized animals contain as much body fat as the control animals, but concentrated more in the periphery and less in the liver (162,163,164). Samuels and co-workers found this effect to be particularly marked in rats fed a high carbohydrate diet (163). They concluded that the fundamental disturbance after hypophysectomy was an inability to mobilize endogenous stores of both protein and fat; these animals were still able to burn exogenous fat, and carbohydrate was readily converted into fat.

The discrepancies between these data can be resolved if the differences in dietary intake are recognized. In Samuel's experiments the rats were force-fed amounts greater than they would otherwise consume (162), and greater than the requirements of the decreased metabolism following hypophysectomy. Lee (110), however, in allowing his animals to feed ad libitum, was probably not supplying their entire caloric requirements. It does not seem surprising that in the former case larger amounts of depot fat should be found than in the latter. Reiss (158) has shown that if hypophysectomized rats are maintained for long periods, their food consumption will, in 6-8 weeks, approach the normal and the body fat which they had previously lost is completely regained.

The liver fat of hypophysectomized animals is invariably low (162,163,164,61,112), and techniques which cause a fatty infiltration of the liver in normal animals fail to do so in the absence of the hypophysis. Removal of the pituitary prevents the fatty infiltration of the liver of

rats under the influence of carbon tetrachloride (100), phosphorus (100) or a diet low in lipotropic factors (175). These effects may be mediated by the adrenal as Verzar (100) has shown that when adrenal cortical extract is given simultaneously, fat deposition occurs to about the same degree as in the normal. The effect of hypophysectomy on fatty hepatic infiltration following pancreatectomy is uncertain, since Long (122) found it exerted an inhibiting effect in the cat, while in the dog it does not (37).

Anterior Pituitary Fractions

a) Anterior pituitary extracts. Crude extracts of the anterior pituitary gland have been known for many years to be capable of causing increases in liver fat (1,21,22,98,144,113). Best and Campbell (21) noted an increase of 137% in the total liver fat of fasted white mice; maximal effect of a single injection was observed in 24 hours. Daily injections in the rat cause a maximal result on the third day, while in guinea pigs the greatest effect is seen somewhat earlier (22). Species differences occur in the response to pituitary extracts, guinea pigs are more sensitive than rats (21,22) while mice are the most sensitive of the three (21). Campbell found that female mice were more responsive than males (33). Butts, however, (32) could find no sex difference in response of rats or between castrates and normals. This action of anterior pituitary extracts is related to the functions of the adrenal cortex as it does not occur following adrenalectomy (74,125).

The increase in liver lipids following injections of anterior pituitary extracts is confined to the glyceride fraction (34) and is associated with a decrease in the total quantity of fat in the body (34). Barrett (8) and, later, Stetten (185) had shown previously, using deuterium, that the augmentation in liver lipid was due to transfer of neutral fats from the depots.

b) Adrenocorticotropic principle. A survey of the literature on the effect of ACTH on liver fat shows that there is little agreement on this point. This factor has been shown to cause an increase in hepatic lipids in the rabbit (52), mouse (114), in the force-fed rat (117) or in the rat maintained on a high carbohydrate diet (4,118). Jeffries found that ACTH did not cause an increase in liver lipids in the guinea pig (101). In each of these experiments relatively large amounts of the adrenocorticotropic factor have been used, and in some cases doses of ACTH greater than that of crude anterior pituitary extract have been found necessary to produce an effect (116). Levin, in a series of experiments with various ACTH preparations, found that only a few of them were effective in causing an increase in liver fat, that large amounts were required and that the effectiveness of a particular preparation in producing increased hepatic fat bore no relation to its adrenal ascorbic acid depleting ability (116). These results lead one to believe that a contaminant present in the adrenocorticotropic preparation used may be responsible for its activity in increasing liver lipids.

c) Growth principle. There is evidence in the literature to suggest a relationship between the growth promoting hormone of the anterior pituitary and the factor responsible for causing increased fat deposition in the liver and mobilization of fat from the depots. Evans and Long concluded from experiments in 1922 that increased storage of fat was a factor in the increase of weight by rats receiving growth promoting extracts (62). Lee and Schaffer, working with a more purified growth promoting pituitary extract (109), have shown that precisely the opposite is the case; increases in weight in animals injected with growth hormone is due mainly to a retention of nitrogen with synthesis and deposition of protein, accompanied by a high body water content and low reserves of depot fat. It is implied in these experiments that injected rats oxidize more fat than untreated controls, although Evans was not able to influence the obesity of rats subjected to hypothalamic injury by repeated injections of growth hormone (63). Li, Simpson and Evans, using relatively pure somatotropic hormones (119), have confirmed the ability of this factor to decrease peripheral fat depots, whether their rats were maintained on a restricted diet or fed ad libitum.

The effect of growth hormone on liver fat deposition has also been studied; Li (118), using large amounts of growth hormone (5 mg.) given in one injection to fasted mice, was able to show an increase of almost 100% in liver lipids. If this treatment is continued a decrease in hepatic fat is seen which Li attributes to an increased utilization of fat under the influence of the hormone (118).

Szego and White (189) were able to demonstrate an increase in the liver fat of the fasted mouse treated with growth hormone above that seen in fasted animals. This effect, they maintain, is not inhibited in the absence of the adrenals (189). Other workers (147, 115,116) have not been able to confirm these results. Payne (147) has tested numerous preparations for their ability to cause increases in hepatic fat. This property he has found to be common to several pituitary principles - somatotropic, adrenocorticotropic and thyrotropic hormones - but only when used in large doses and in the case of the latter two, not co-related with their more specific metabolic activities.

Levin has proposed a theory to explain these conflicting data (196). He believes that somatotropic hormone is responsible for the fat mobilizing properties of anterior pituitary extracts, but that the action of growth hormone is dependent on an adequate supply of circulating adrenal cortical hormones. Final judgment on this thesis must be reserved until similar results are obtained using amounts of the injected hormone which are more reasonable according to the size of the animal. The large amounts of hormone used in these experiments (2.5-5 mg. to 20 gm. mice) do not obviate the possibility of impurities being responsible for activities attributed to the hormone.

Conclusions

The exact nature of factors from the anterior pituitary which influence fat metabolism and the mechanism by which they produce this

action is still obscure. They have not been isolated and attempts at identification with other known principles have been without success.

CHAPTER III : HORMONAL EFFECTS ON CONNECTIVE TISSUE

The profound alterations in connective tissue in clinical hyperadrenocorticalism - Cushing's Syndrome - have been observed since this disease was first described. The striae formation, increased fragility of blood vessels and poor healing of wounds are believed to be the result of adrenal cortex hormonal activity on the connective tissues of the body (173). With the advent of adrenocorticotropic hormone and cortisone it has been found that therapy with these substances was capable of producing similar effects. Ragan (156) first reported poor wound healing with ACTH in man, and found that cortisone was capable of producing similar effects in the rabbit and, less markedly, in the rat (49,153).

Numerous workers, using various methods of producing alterations in connective tissue, testify to the profound effects of adrenal and several anterior pituitary hormones on this body constituent. Taubenhaus (190,191), in an extensive series of experiments, demonstrated by studying the connective tissue surrounding artificially produced turpentine abscesses, that adrenal and hypophyseal hormones could markedly influence the connective tissue reaction. He found that abscesses in either adrenalectomized or hypophysectomized rats were surrounded by much

less fibrous tissue than normal control animals; fibroblasts in the former were small and flat while the collagen present was sparse and coarsely clumped. Treatment of hypophysectomized animals with growth hormone greatly increased the fibroblastic response and granulation tissue. Marked stimulation to fibrosis was the result of treatment of either normal or adrenalectomized rats with desoxycorticosterone acetate. In keeping with the opposing actions of the glucocorticoid and mineralocorticoid elements (173), Taubenhaus found that either ACTH or corticosterone inhibited the proliferating fibroblasts and decreased the amount of granulation tissue found in these abscesses.

Several theories have been advanced as to the possible mechanism of these hormonal actions. Ragan (156) noticed that cortisone inhibited wound healing in rabbits, associated with low tissue ascorbic acid levels. It has been shown that guinea pigs maintained on a scorbutic diet suffer decreases in the collagen content of the lungs, liver and kidney (59) and that the adrenal hormones are concerned in as yet a poorly defined manner with ascorbic acid metabolism (173). It is possible to theorize that some part of the action of ACTH and Cortisone on mesenchymal tissue may be by a depression of tissue ascorbic acid. However, Spain was unable to overcome cortisone inhibition to wound healing in mice by the oral administration of 10 mg. amounts of ascorbic acid daily (182).

A second possible role the hyperadrenal state may play in connective tissue reactions is a depressive effect on host reactivity (152)

to any traumatizing agent. It has also been proposed that the action of adrenal hormones on local wound healing may be through their more general, catabolic or anti-anabolic effect on protein metabolism (157). This action cannot be excluded, as protein deficiency has been shown experimentally to result in poor wound healing with decreased fibroplasia and lowered cellular response (105).

Local hormonal action on the cellular elements in the immediate area of experimental wounds has been suggested as a mode of inhibition by cortisone. Baker (5) found that inunction of an area of skin with cortisone or adrenal cortical extract followed by removal of a circular piece of treated skin and continued application of the hormones resulted in retarded wound healing. He suggests that these hormones have a direct influence on the cellular elements or interstitial matrix of the connective tissues. In vitro evidence for such a hypothesis is contained in the work of Porter (153), who demonstrated a complete inhibition of collagen fibre formation when cortisone was added to the medium of fibroblast tissue cultures.

Only a very few reports may be found in the literature concerning the effects of cortisone or ACTH treatment on the connective tissue elements of the liver. This may be due to the inherent dangers of serial liver biopsies in man and the difficulties associated with chemical assay of collagen and elastin in tissue samples. Aterman has described studies of this nature in the rat (3). When these animals are injected with small doses of carbon tetrachloride over periods lasting several weeks,

precirrhotic changes occur in the non-parenchymal elements of the liver. These consist of increased cellularity of the stroma, appearance of coarse collagenous bundles and reticular fibres and, in some cases, marked structural alterations of the lobules. Cortisone, when administered concurrently with the carbon tetrachloride, caused a reduction in the connective tissue response. Treated livers contained less collagen and elastin and less round cell invasion was seen than in the untreated controls. Unfortunately, the cortisone dosage schedule in these experiments was of such an order (10-15 mg. per day to 125 gm. rats) as to make the interpretation of these experiments very difficult. Cavallero (35) conducted a similar series of experiments using carbon tetrachloride as the hepatotoxin and cortisone at a somewhat lower dose level (1 mg. daily per rat). He observed, as did Aterman, that this hormonal treatment inhibited the development of increased collagen and reticular fibres which was shown by the controls. Cortisone did not appear to alter the fatty degeneration which occurs following carbon tetrachloride poisoning in the rat (35,3).

EXPERIMENTAL

SECTION ONE - METHODS

1. Care and Feeding of Animals

All animals used in these experiments were immature male rats of the black and white "hooded" strain from a stock which has been inbred in this laboratory for many years. These animals were raised and maintained on a diet consisting solely of "purina" fox chow. Rats were removed from stock cages several days prior to the initiation of an experiment and placed in individual metabolic cages. These were designed so that the dietary intake of each animal could be accurately measured for paired-feeding.

Room temperature could not be controlled in these experiments. However, if variations in temperature occurred during experiments which resulted in mortality in groups of hypophysectomized or adrenalectomized animals, these experiments were discontinued.

In the majority of the following experiments paired-feeding technique is used. For this purpose the standard chow pellets were pulverized and daily rations were weighed in individual glass beakers on a triple beam balance.

At the termination of experimental periods the animals were killed by the intraperitoneal injection of 0.25 c.c. of sodium pentabarbitol.

2. Operative Procedures

In the experiments to be described adrenalectomies, hypophysectomies and sham variations of these operations were employed. All operations were carried out under ether anaesthesia. Operative sites were prepared by clipping away the hair and swabbing the area with 70% alcohol. All wounds were closed with interrupted sutures of cotton thread; after adrenal intervention both muscle and skin layers were included in the sutures, while after hypophysectomies the thread was used to oppose the skin only. All animals were allowed to recover consciousness before returning them to their cages, recovery usually requiring less than a minute after the completion of an operation.

a) Adrenalectomy

The two adrenals were removed by separate incisions into the peritoneal cavity. Celiotomies were performed with scissors, the incision bissecting the angle formed by the vertebral column and the costal margin. Using one pair of forceps as retractors, the adrenal was exposed and, with a second pair of forceps, the adrenal was elevated by grasping the fat and connective tissue between it and the kidney. The anterior attachments of the adrenal were then severed with a pair of iris scissors, after which the adrenal and the entire fat body were dissected away from the renal capsule and removed. The incision was then closed and the operation repeated on the opposite side. All adrenals were examined for signs of trauma to the capsule. If the

capsule was incomplete or broken the animal from which the gland was removed was discarded. Adrenalectomized rats were maintained on 1% sodium chloride in the drinking water.

Sham Adrenalectomy

The procedure for these operations was similar to that described for adrenalectomy, with the exception that the adrenal was left untouched.

b) Hypophysectomy

This operation was performed by conventional methods (172) which have been improved by several minor modifications (181).

The operative site was approached by the para-pharyngeal route, an incision being made in the median line starting from the submental papilla and directed caudad about 2 cm. By blunt dissection the angle formed by the sternohyoid, thyrohyoid and digastricus muscles was exposed. Two Merriams' forceps were then introduced at this angle, the muscles separated and the forceps manipulated below the thyroid artery anteriorly and cranially to the spheno-occipital synchondrosis. The forceps were firmly placed and taken over by an assistant to serve as retractors. The sphenoid bone was now cleared of tissue with a cotton pledget and drilled with a dental burr of an appropriate size.

The dura mater was torn with a fine dental pick, which exposes the hypophysis. The gland was then aspirated with a suction cannula. A small pledget of fibrin foam was placed in the drill cavity to promote coagulation of blood and to seal the hole. After removal of the

retracting forceps the animals were resuscitated by artificial respiration. No more than 40 seconds were required from the time the forceps were placed to the time of their removal.

The completeness of hypophysectomies was checked by examination of the sella turcica at autopsy. Excluded from the data were animals incompletely hypophysectomized or animals in which the diaphragm had been ruptured during the operation, resulting in damage to the base of the brain.

All hypophysectomized animals were given 10% glucose in their drinking water.

Sham Hypophysectomy

This operation was performed in a manner similar to that for hypophysectomy, except that after drilling the sphenoid bone with the dental burr, the dura was not torn and the hypophysis was left intact.

3. Chemical Procedures

a) Liver lipids

Lipids were estimated in these experiments by determining the total ether soluble fraction of freshly ground liver aliquots. Livers removed at autopsy were quickly blotted dry with filter paper and weighed. They were then homogenated by Potter-Eveljehm grinders in an ice bath. Duplicate aliquots of the liver homogenate weighing between 0.5 and 1.5 gms. were then transferred directly into Soxhlet thimbles which had been weighed previously and the exact weight of the liver tissue

was recorded. The liver lipids were extracted by means of 100 cc of freshly made up Bloor's solution (3 parts 95% ethyl alcohol to 1 part chemical ether, by volume), using a technique similar to that described by Li (119). The extraction with boiling Bloor's solution was allowed to proceed for a period of 4 hours. Repeated extraction of liver samples after this period with fresh solvent revealed no trace of lipid substances. The Bloor's solution was evaporated from the lipids under vacuum at 40-50°C. The neutral fats were dissolved in 10 cc of ether and filtered through #1 Whatman filter papers into 125 cc Erlenmeyer flasks. After rinsing the Soxhlet flask with three successive portions of 5 cc of ether, the combined filtrates were slowly evaporated by placing the Erlenmeyer flasks in warm water (35-45°C.) in a fume cupboard.

The flasks containing the lipid were then placed in a dessicator for 24 hours, after which the neutral fat was determined by weighing. On the same sample of tissue the variation in values obtained by duplicate analysis was not greater than ten percent.

b) Carcass lipids

To determine carcass fat a method similar to that for liver lipids was used. After removal of the liver the entire carcass was cut into small pieces with a pair of large scissors and placed in a Waring Blender, 100 cc of water was then added and the carcass was ground until it formed an even mixture. The homogenate was dried in an oven at approximately 70°C. to constant weight. The entire dried residue was then transferred to a large Soxhlet thimble and extracted with boiling

Bloor's solution for 24 hours. At the end of this period renewed extraction of the tissues with fresh solvent did not yield any additional lipid substances. The solvent containing the carcass lipid was processed according to the method described for liver fat and the total carcass fat determined by gravimetric methods.

4. Histological Technique

The cutting and staining of all sections for microscopic examination was done by the Department of Pathology of the Royal Victoria Hospital.

At autopsy wedge-shaped slices were removed from each of the principal liver lobes and placed in a solution of Bouin's fluid 4 parts, to 1 part of 40% neutral formalin. The liver slices were fixed in this solution for 12 to 18 hours and then transferred to a bath of running tap water for 24 hours. After washing the sections were placed in an iodine-alcohol solution (0.5% iodine in 95% ethanol) for storage prior to cutting.

Each liver section was stained with haematoxylin eosin and Masson's trichrome (133). The former was used to study intracellular changes, the trichrome stain is specific for collagen fibres which it stains a bright blue colour.

5. Phosphorus Poisoning Technique

Yellow phosphorus was used in a number of these experiments as a means of producing fatty infiltration of the liver and alterations in

hepatic connective tissue.

A 1% solution of yellow phosphorus was made up by adding 1 gm. of yellow phosphorus, which had been weighed under water, to 100 cc of oil of sweet almonds. This solution served as a stock solution, from which dilutions for injection were made up prior to each experiment. Ten cc of the above solution was diluted to 100 cc with almond oil so that an injection of 0.5 cc of this solution was equivalent to 0.5 mg. of yellow phosphorus. This dose of yellow phosphorus was found to be capable of producing a significant degree of fatty infiltration in the normal rat (see page 57) without being fatal to an adrenalectomized rat.

Tuberculin syringes of 1 cc with one-inch 20 gauge needles were used to administer the phosphorus. Injections were made over the gluteal region and in each case the needle was passed through the skin and the muscle before depositing the oil in the subcutaneous tissues. This procedure was carried out to prevent part of the injected fluid from leaking through the hole in the skin made by the needle.

The use of a parenteral route to administer the phosphorus was somewhat novel. It was hoped by so doing to avoid errors in dosage which are inherent, due to variations in absorption, when such materials are given orally. It was also hoped that a uniform portal cirrhosis might be produced by parenteral administration of the hepatotoxin. This had not been found possible when the phosphorus was administered orally (2).

SECTION TWO - EXPERIMENTAL RESULTS

Experiment 1

This experiment was an attempt to produce a portal cirrhosis in the rat, similar to that which has been described for the guinea pig and the rabbit (132), but if possible more uniform. If this method had been successful quantitative differences in liver collagen under various experimental conditions were to be studied using the methods of Lowry and Katersky (123).

A group of 20 rats weighing between 150 and 200 grams were injected every fourth day with 0.5 mg. of yellow phosphorus until a total of ten injections had been given. At weekly intervals after the completion of the injections, five animals were autopsied and samples of the livers removed for histological sectioning.

Results

In none of the animals was a uniform portal cirrhosis produced. Liver damage was observed in all the animals, fatty infiltration being evident on gross examination. However, cirrhotic changes were seen in only the caudate and accessory liver lobes.

Examples of these results are shown in Figs. 1,2,3,4.

Though the black and white reproductions do not illustrate cellular detail or fine differences between the sections, nevertheless they are presented for the marked alterations in parts of the livers which they demonstrate quite clearly.

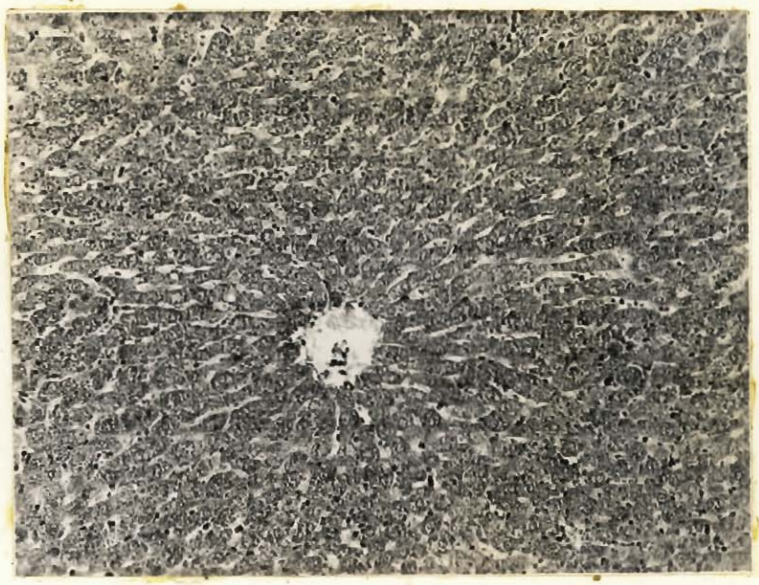

Fig. 1

Normal rat liver, Masson's trichrome, x 160.

Fig. 2

Liver from a rat 24 hours after having received a single subcutaneous injection of 0.5 mg. of yellow phosphorus, Masson's trichrome, x 160.

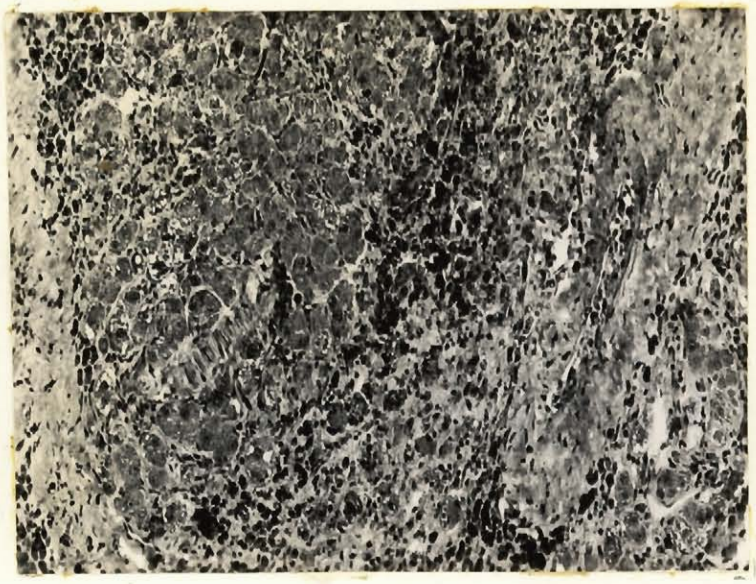

Fig. 3.

Cirrhotic changes in the right accessory lobe of a rat liver 54 days after commencing a series of 10 injections of 0.5mg. each of yellow phosphorus, Masson's trichrome, x 160.

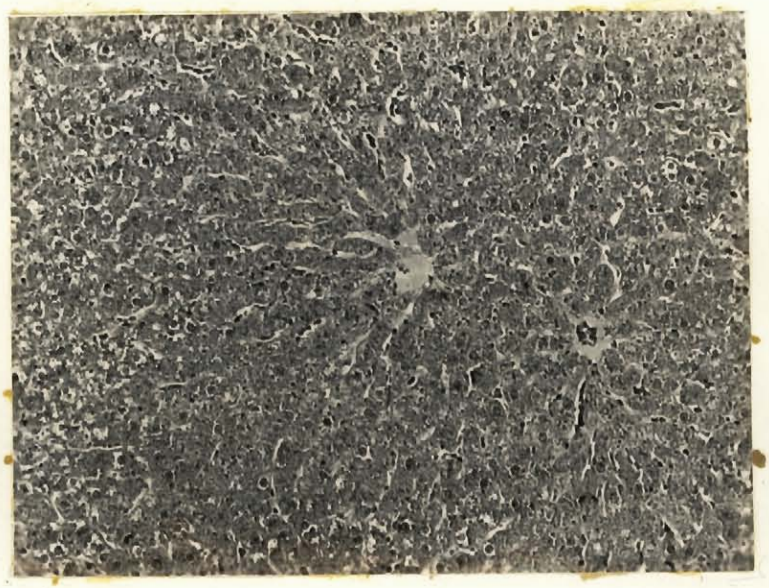

Fig. 4

Section from the main lobe of the same liver as Fig. 3. Residual damage is apparent in the periportal areas. Cirrhotic changes are absent.

Fig. 1 : Rat. Normal liver.

Fig. 2 : This is a section of liver from a rat which had received a single injection of 0.5 mg. of yellow phosphorus. Swelling of the parnechymal cells is evident, obliterating the sinusoids and making the normal lobular pattern difficult to discern.

Fatty degeneration may be seen affecting nearly all the liver cells. Cytoplasmic degeneration is marked, especially in periportal areas. The nuclei of the liver cells are not so severely affected as the cytoplasm; however, some karyorrhectic necrosis can be found.

Fig. 3 : This preparation is from the right accessory lobe of a rat fourteen days after termination of the phosphorus injections. The section shows evidence of advanced portal cirrhosis. Normal hepatic architecture is completely lost; most of the parenchymal tissue has been replaced by collagen fibres, leaving only isolated islands of hepatic cells. Numerous mitosis could be seen in the liver cells. There is evidence of bile duct proliferation in this section but this may be only a condensation phenomenon as the lobe was markedly atrophic.

Fig. 4 : This section is from the left main lobe of the same liver as Fig. 3. There is evidence of residual damage to hepatic cells as is seen in Section B, affecting chiefly the cytoplasm. Fatty infiltration with chiefly a periportal distribution is evident. However, there is no histological evidence of an increase in collagenous tissues, and normal hepatic structure is maintained.

Discussion

It was found impossible to produce a uniform portal cirrhosis with yellow phosphorus in the rat using the methods outlined in this experiment. These results are in agreement with similar work in the guinea pig (2). Further studies are necessary to explain this lack of uniformity; the possibility is advanced by Ashburn (2) that the focal lesions in his experiments might be due to uneven distribution of the hepatotoxin in the portal blood when the phosphorus is administered orally. This criticism cannot be applied to this experiment since the phosphorus was administered parenterally and would be expected to be evenly distributed in the blood when it reached the liver. It seems illogical to assume that the parenchymal cells in one part of the liver are inherently more susceptible to injury by phosphorus than those in another. A possible explanation might be variations in the relative capacity of liver lobes to regenerate following necrosis of hepatic cells. Anatomical locations, pressures and variations in arterial blood and portal blood reaching the lobes (94) may be factors influencing regenerative capacity.

Experiment 2

This experiment was undertaken to establish normal liver lipid levels using the method of fat extraction described previously.

Ten male rats were killed immediately upon removal from the stock colony. No attempt was made to fast the animals prior to autopsy. The concentration and absolute values of liver fat were then determined.

Results of this experiment are summarized in Table I, the mean liver fat percentage using this method is 4.06 with a Standard Deviation of ±.712. These values are in substantial agreement with others reported in the literature - Best and Campbell (21) found a mean value of 3.8% for normal fed rats. Levin reports slightly higher normals of 5.23% (112). Slight differences such as these may be accounted for by variations between strains of animals used and diets which are fed.

Experiment 3 - Effect of Adrenalectomy on Liver Fat Deposition.

Verzar (202) was the first worker to report that adrenalectomy inhibited the fat deposition in the liver which occurs as the result of poisoning by yellow phosphorus. There are several valid criticisms of his experiments. Firstly, the mortality in his experiments was high. This may have been due to electrolyte depletion in his rats as they were not maintained on sodium chloride. It must be assumed that animals which survived if properly adrenalectomized were also suffering from low serum and tissue levels of sodium chloride. Secondly, control animals in Verzar's experiments were not paired fed. The abundant literature reported earlier on the effects of nutrition on liver fat would seem to render paired feeding obligatory in any experiment of this type.

Experiment 3A is composed of eight adrenalectomized rats and eight paired fed sham-operated control rats. The animals in this experiment

TABLE I

LIVER FAT OF NORMAL FATS IN THE FED STATE

Number	Treatment	Body Weight gms.	Weight gms.	Liver Fat gms.%	Total Fat gms.
N-1	none	190	10.3286	3.06	.3160
N-2	"	190	10.2471	3.28	.3361
N-3	"	199	10.1413	4.41	.4472
N-4	"	200	11.0467	4.45	.4915
N-5	"	198	9.1747	4.53	.4156
N-6	"	195	8.7738	5.21	.4571
N-7	"	200	9.9755	4.54	.4528
N-8	"	198	8.3750	4.21	.3525
N-9	"	192	8.4654	3.15	.2666
N-10	"	150	9.2955	3.81	.3541

were males weighing between 180 and 205 grams. The standard dose of phosphorus (0.5 mg.) used in these experiments was administered on three successive days commencing on the same day as the animals were operated. On the fourth day post-operatively, the rats were killed and the livers removed for determination of lipids.

The results of this experiment are contained in Table II. The mean liver fat percentage of the adrenalectomized series is 4.94 with a standard deviation of $\pm$.506.

The control animals have a mean liver fat percentage of 6.41 with a standard deviation of $\pm$.758. These results are significant when analysed by Fisher's method (66A) for paired fed animals ($t = 5.20$; $P = .01$).

No constant difference in total liver weight may be seen between the two groups of animals. However, the differences in liver fat concentration between the adrenalectomized and control series are reflected by similar differences in absolute values for liver fat.

The possibility cannot be excluded in this experiment that the results were influenced by residual levels of circulating corticoids at the time when the first injection of phosphorus was given. Tyslowitz and Astwood (200) have shown that maximal sensitivity to cold was not reached in the case of adrenalectomized rats until 24 hours after the operation and for hypophysectomized rats until four days post-operatively.

In order to obviate the above possibility and also to design an experiment which might be compared later with a hypophysectomized series,

TABLE II

EXPERIMENT 3A - LIVER FAT OF ADRENALECTOMIZED AND PAIRED-FED SHAM-OPERATED CONTROL RATS GIVEN 0.5 mg. OF YELLOW PHOSPHORUS ON THREE SUCCESSIVE DAYS COMMENCING THE SAME DAY AS THE OPERATIONS.

Animal	Treatment	Body weight gm. Initial	Final	Loss	Weight gm.	Liver Fat gms%	Total fat gm.
A-1	Adrenal-X	200	192	8	7.1775	5.37	.3854
A-2	"	180	168	12	7.0048	4.99	.3495
A-3	"	193	170	23	7.5685	4.21	.3186
A-4	"	204	193	11	7.8016	4.61	.3596
A-5	"	196	174	22	7.6490	5.00	.3824
A-6	"	194	179	15	8.3979	5.85	.4912
A-7	"	214	195	19	8.1951	4.98	.4081
A-8	"	204	190	14	8.3794	4.58	.3837
C-1	Sham Adrenal-X	190	148	42	7.7360	6.98	.5399
C-2	"	195	150	45	7.7787	5.11	.3974
C-3	"	197	173	24	8.1443	7.08	.5766
C-4	"	210	180	30	8.5650	5.98	.5121
C-5	"	194	148	46	6.7502	7.38	.4981
C-6	"	196	150	46	7.0425	6.75	.4753
C-7	"	190	158	32	9.1507	6.00	.5490
C-8	"	188	164	24	7.3688	6.03	.4443

this experiment using adrenalectomized animals was repeated.

Experiment 3B was similar to 3A with the exception that all animals were maintained for four days on a paired-feeding schedule after their operations before the series of three phosphorus injections was started.

The results of this experiment, Table III, confirm those from experiment 3A. The mean liver fat percentage of the adrenalectomized group is 4.73% as compared to 7.37% for the control series. These differences are highly significant ($t = 5.78$; $P = .001$) and are reflected by similar differences in absolute values of liver fat.

The small differences between the mean liver fat concentrations of the adrenalectomized animals in series 3A and 3B are not significant. It would seem therefore that in series 3A any adrenal cortical hormone in the adrenalectomized animals at the time of the first injection of phosphorus was not sufficient to alter the inhibition to liver lipid deposition which is present.

Discussion. It is evident from these experiments that adrenalectomy inhibits the fatty infiltration of the rat liver which occurs after poisoning with yellow phosphorus. This data lends support to the theory that the impairment in deposition of liver fat is one of the primary changes of cortical insufficiency (99), since it persists in animals maintained in a state of apparent good health on a high sodium chloride intake. The results are in agreement with those of other workers (202) although the increment in liver fat concentration in control animals in this experiment, 7.37%, is

TABLE III

EXPERIMENT 3B - LIVER FAT OF ADRENALECTOMIZED AND SHAM-OPERATED RATS MAINTAINED ON 1% SODIUM CHLORIDE AND FED AD LIBITUM FOR 4 DAYS BEFORE BEGINNING A SERIES OF 3 DAILY INJECTIONS OF 0.5mg. OF PHOSPHORUS

Animal	Treatment	Body Weight Gm. Initial	Final	Loss	Liver Weight gms.	Fat gm%	Total Fat gms.
A-1	Adrenal-X	214	185	29	7.2185	7.39	.5334
A-2	"	195	165	30	7.4706	5.77	.4310
A-3	"	208	178	30	8.0912	7.05	.5704
A-4	"	198	165	33	6.5512	3.09	.2024
A-5	"	194	184	10	9.0850	5.07	.4606
A-6	"	190	138	52	5.6959	3.33	.1896
A-7	"	196	170	26	7.8823	4.34	.3420
A-8	"	194	168	26	7.3357	4.47	.3297
A-9	"	201	168	33	6.9059	3.17	.2189
A-10	"	198	182	16	8.4364	3.90	.3290
A-11	"	222	196	26	9.2200	4.72	.4351
A-12	"	215	194	21	8.1605	4.49	.3664
C-1	Sham Adrenal-X	200	153	47	7.9513	9.86	.7839
C-2	"	190	167	23	9.9035	11.82	1.1705
C-3	"	190	174	16	9.5134	8.71	.8286
C-4	"	195	158	37	9.6276	8.48	.8164
C-5	"	190	160	30	7.8990	7.96	.6287
C-6	"	189	146	43	8.2000	5.33	.4370
C-7	"	185	155	30	7.2376	7.53	.5449
C-8	"	190	158	32	7.7971	6.37	.4966
C-9	"	206	163	43	7.9655	4.84	.3855
C-10	"	201	161	40	8.5891	4.86	.4174
C-11	"	194	158	36	8.5136	7.07	.6019
C-12	"	224	178	46	9.4676	5.65	.5349

somewhat less than the 11% which was observed by Verzar (202). The failure of control animals in this experiment to attain greater increases in liver lipid may be attributable to the smaller amounts of phosphorus used.

An interesting difference between the adrenalectomized and sham-operated animals in experiment 3A is seen in the data on weight loss. The adrenalectomized animals lost significantly less weight than the controls during the four day experimental period; the mean weight loss of the control group is more than double that of the adrenalectomized series. No studies were done to determine the composition of the weight loss. The differences in this regard are less striking in experiment 3B.

There are several possible explanations for this difference in weight loss. Adrenalectomized animals have decreased absorption of carbohydrate and protein (203) and may suffer dehydration (172) which would tend to make their weight loss greater than that of paired fed controls. However, they are alleged to be less able to utilize endogenous fats (7,40), protein (172) and have lower metabolic rates (172). It is felt that a combination of these factors may explain the difference between the weight loss of adrenalectomized and normal paired fed animals. Szego was unable to find any difference in the relative composition of the carcass of mice after adrenalectomy.

Experiment 4

This experiment was planned to parallel experiment 3B, with the exception that hypophysectomized instead of adrenalectomized animals were

used. The animals in this series are divided into three groups. Group A is made up of hypophysectomized animals maintained for four days on 10% glucose drinking water and a ground chow diet before the start of a series of three daily injections of 0.5 mg. of yellow phosphorus. Group B is composed of sham-operated, paired-fed control rats receiving the same diet and similarly treated with phosphorus. Group C animals were hypophysectomized and paired fed with those in Group A, but unlike those in the latter group these animals received no injections of yellow phosphorus. The additional control group in this series, Group C, was undertaken in an endeavour to ascertain whether hypophysectomized animals were capable of any response by way of increases in liver fat, after poisoning with phosphorus, as compared to hypophysectomized animals simply maintained on a paired-feeding schedule.

Results The hypophysectomized rats in Group A were unable to increase their liver fat depots in response to phosphorus poisoning. The mean liver fat concentration and absolute liver fat values in this group were 3.39% and .2329 grams respectively.

This is compared with corresponding values of 6.62% and .4628 grams in the sham-operated control animals of Group B. These differences are statistically highly significant with a t value of 11.506 and P = .001. The second control group in this experiment, which were hypophysectomized but received no phosphorus in oil, had a mean liver fat concentration of 3.02% and a mean absolute liver fat value of .1808 gms. The differences between Groups A and C in this experiment are not statistically

TABLE IV

EXPERIMENT 4 - LIVER FAT OF HYPOPHYSECTOMIZED AND SHAM-HYPOPHYSECTOMIZED RATS MAINTAINED ON 10% GLUCOSE AND FED AD LIBITUM FOR 4 DAYS BEFORE BEGINNING A SERIES OF 3 DAILY INJECTIONS OF PHOSPHORUS (0.5 mg.)

Animal	Treatment	Body Weight gm.			Liver		
		Initial	Final	Loss	Weight gm.	Fat gm%	Total Fat gm.
A-1	HypoX & Phos.	220	173	47	7.0431	3.55	.2500
A-2	"	215	184	31	7.9174	4.08	.3230
A-3	"	200	164	36	6.1023	2.60	.1586
A-4	"	224	183	41	6.7135	3.42	.2296
A-5	"	194	164	30	6.8589	3.38	.2661
A-6	"	218	170	48	6.5884	3.37	.2220
A-7	"	214	184	30	6.0970	3.28	.1999
A-8	"	194	157	37	6.3040	3.33	.2099
A-9	"	229	179	50	7.9336	2.99	.2372
	Sham						
B-1	HypoX & Phos.	190	154	36	5.7961	6.38	.3697
B-2	"	194	170	24	6.7572	7.13	.4817
B-3	"	214	186	28	6.0218	5.61	.3378
B-4	"	210	180	30	7.1571	6.78	.4852
B-5	"	210	180	30	6.3067	7.77	.4900
B-6	"	220	174	46	7.1754	8.23	.5905
B-7	"	209	158	51	8.0855	5.40	.4366
B-8	"	202	164	38	7.7875	5.62	.4376
B-9	"	201	152	49	8.0513	6.66	.5362
C-1	HypoX only	194	176	18	6.0263	2.78	.1675
C-2	"	207	158	49	6.0098	3.37	.2025
C-3	"	190	155	35	5.5453	3.39	.1879
C-4	"	185	144	41	5.7968	2.82	.1634
C-5	"	190	146	44	6.4669	2.80	.1810
C-6	"	182	130	52	4.2402	2.80	.1187
C-7	"	188	160	28	7.0395	3.33	.2344
C-8	"	170	143	27	5.5913	2.91	.1627
C-9	"	184	170	14	7.2311	3.03	.2191

significant.

Discussion These results are in complete agreement with the findings of other workers. They confirm the experiments of Issekutz and Verzar (100) who have also used phosphorus in a study of liver fat in the hypophysectomized rat, and parallel the results these workers got from similar experiments using carbon tetrachloride as the hepatotoxin.

Data such as these have led numerous workers to postulate that the hypophysectomized animal has an inability to mobilize its peripheral fat depots, in response to an agent such as phosphorus, which in the normal animal causes an increase in liver lipids (100,116). On this premise these workers term any agent capable of causing an increase in liver lipids a "fat mobilizing factor" (116). However, when the hypophysectomized animals in these experiments came to autopsy it was noticed that their peripheral fat depots - retroperitoneal, mesenteric, perirenal and testicular - had all but disappeared. It can also be seen from Table IV that there is little difference between the weight loss of animals in the three groups. For these reasons the author is loath to suggest that the results of these experiments can be interpreted as an inability of the hypophysectomized animals to mobilize their peripheral fat depots. It would also seem that postulates of other authors who interpret low liver lipid levels as reflecting poor fat mobilizing ability must be accepted with reservation.

The second control group in this experiment - group C - shows that the phosphorus and the almond oil in group A actually exert no effect on the liver lipids of hypophysectomized animals.

Experiment 5

These experiments were designed to study the effects which cortisone or adrenocorticotropic hormone might have on the fatty infiltration of the liver in normal rats after phosphorus poisoning. There are numerous clinical reports on the use of these hormones in liver diseases such as cirrhosis (26,29,44,82) when fatty infiltration might be expected to be one aspect of the pathological lesion (44). However, no information on the effects of these hormones on the fatty infiltration of the liver caused by phosphorus poisoning could be found.

Experiment 5A is composed of eighteen normal male rats. Nine of these animals received 0.5 mg. of yellow phosphorus daily and 0.5 mg. of cortisone twice daily for three successive days. The nine paired-fed control animals received only the treatment with yellow phosphorus.

Results The data of this experiment are compiled in Table V. No significant differences in either liver fat concentration or absolute amounts of liver fat were observed between the cortisone treated and the control group.

Experiment 5B was carried out in a manner similar to that of experiment 5A. A total of twelve normal male rats were used. Six of these animals received 0.5 mg. of yellow phosphorus daily and 0.5 mg. of adrenocorticotropic hormone (Armour Lot. No. R-J17409) twice daily for three consecutive days. The control group which was paired-fed with the hormone treated series, received only the injections of yellow phosphorus.

Results The results of this experiment, Table VI, are similar to those of

TABLE V

EXPERIMENT 5A - THE EFFECT OF 1mg. OF CORTISONE DAILY ON THE LIVER FAT OF NORMAL RATS RECEIVING YELLOW PHOSPHORUS, COMPARED WITH RATS RECEIVING PHOSPHORUS ALONE

Animal	Treatment	Body Weight gm. Initial	Final	Difference	Liver Weight gms.	Fat gm%	Total Fat gm.
N-1	Cortisone & Phos.	189	182	7	8.2431	6.94	.5720
N-2	"	194	195	1	9.1187	8.29	.7559
N-3	"	190	183	7	9.3347	6.92	.6459
N-4	"	180	190	10	8.9688	6.80	.6098
N-5	"	200	200	0	9.6791	9.06	.8769
N-6	"	190	198	8	9.3620	6.56	.6141
N-7	"	203	190	13	9.7152	5.48	.5323
N-8	"	190	165	25	8.3005	7.26	.6026
N-9	"	200	178	22	8.2711	6.05	.5004
C-1	Phos.only	196	200	4	7.6895	6.56	.5044
C-2	"	195	201	6	9.6090	6.04	.5803
C-3	"	190	200	10	10.5140	8.43	.8863
C-4	"	188	199	11	10.0412	6.23	.6255
C-5	"	190	179	11	8.9870	7.22	.6488
C-6	"	189	183	6	10.0495	6.82	.6853
C-7	"	188	176	12	10.1419	6.11	.6196
C-8	"	204	204	0	9.7756	6.60	.6451
C-9	"	198	188	10	9.9980	6.92	.6918

TABLE VI

EXPERIMENT 5B - THE EFFECT OF 1mg. OF ACTH DAILY ON THE LIVER FAT OF NORMAL RATS RECEIVING PHOSPHORUS AS COMPARED TO RATS RECEIVING YELLOW PHOSPHORUS ALONE

Animal	Treatment	Body Weight gms. Initial	Final	Difference	Liver Weight gms.	Liver Fat gm%	Total Fat gms.
D-1	ACTH & Phos.	208	195	13	7.9600	6.35	.5054
D-2	"	198	186	12	8.7197	6.82	.5946
D-3	"	192	175	17	9.4362	6.82	.6435
D-4	"	190	170	20	8.1400	6.77	.5510
D-5	"	197	190	7	9.0340	6.33	.5718
D-6	"	198	175	23	7.7392	6.52	.5045
C-1	Phos. only	190	178	12	8.3888	6.84	.5735
C-2	"	190	180	10	7.9543	6.68	.5313
C-3	"	195	176	19	9.1553	7.18	.6573
C-4	"	204	200	4	9.7521	6.29	.6134
C-5	"	197	185	12	10.1729	6.60	.6714
C-6	"	200	187	13	9.0023	6.97	.6274

experiment 5A. Increases in liver lipid concentration and absolute values of liver fat are seen in both the adrenocorticotropic treated and the control group. However, treatment with the hormone in the manner described did not appear to influence the liver infiltration with fat in any way.

Discussion In this experiment neither cortisone nor adrenocorticotropic hormone appeared to exert an effect on the fatty infiltration of the liver resulting from phosphorus poisoning in the rat.

This is in accordance with the experimental findings of Cavellero (35) who observed that doses of 1 mg. of cortisone daily to rats had no effect on the lipid infiltration of the liver following carbon tetrachloride poisoning. Similarly, it has been found impossible by other workers to alter liver lipid concentrations of normal fasted animals by means of these hormones (116,99).

It has been reported that improvements have followed the use of these hormones in various types of chronic liver disease in man (26,29,44). A rather remarkable response followed the treatment of a case of acute phosphorus poisoning in man (31). The results of these experiments and similar data in the literature would suggest that it is unlikely that the value of adrenal cortex hormones in the treatment of various liver diseases lies in any effect which these hormones may exert on the liver lipids.

Experiment 6

It can be seen from experiments 3 and 4 that adrenalectomy or hypophysectomy prevents the fatty infiltration of the liver following

phosphorus poisoning in the rat. The low liver fats in animals subjected to these operations and their failure to respond to agents which in the normal animal cause increases in liver lipids is believed by many workers to be the result of inability of these animals to mobilize lipids from their peripheral depots (115,116,147,189).

It was observed in experiment 4 that the hypophysectomized animals in series A and C appeared to have very small reserves of lipids in their peripheral depots, even though the concentration of lipids in their livers was very low. Experiment 6 was designed to measure and compare the liver and depot fats of hypophysectomized and normal animals after a 7-day experimental period. The hypophysectomized rats were fed the regular diet ad libitum and given 10% glucose to drink during this period. The normal control animals were paired-fed the same diet and also given the same amount of 10% glucose to drink. No difficulty was encountered in getting the normal animals to consume the same amount of 10% glucose as the hypophysectomized groups.

At the termination of the experimental period the animals were killed and their carcasses photographed. Testicular weight in the groups was taken to serve with visual examination of the sella as additional evidence of complete hypophysectomy. Total carcass fat in these experiments represents the ether soluble portion of the entire carcass with the exception of the liver.

Results In Fig. 5-10 the gross differences between these animals can be seen. Figs. 5 and 6 are animals from the control group; the size and

distribution of the mesenteric, peripheral and retroperitoneal fat depots can be seen. In the hypophysectomized animals, Fig. 7 and 8, a marked reduction in the peripheral depots may be seen. The perirenal fat bodies which in the normal animal almost completely hide the adrenal, have all but disappeared, leaving the adrenal gland quite exposed. Figs. 9 and 10 are sham-operated control animals - again a reduction in peripheral fat reserves is seen. It was difficult to detect any gross difference between the hypophysectomized animals and their controls.

The results of this experiment are summarized in Table VII.

No significant difference was observed in the liver fat concentrations of the three groups. The mean concentrations of the three groups are - stock control animals 3.88%; hypophysectomized animals 3.89; paired-fed controls 3.47. The absolute value of liver fat and the liver weight are decreased in the hypophysectomized series.

The mean total body fats, expressed in grams per 100 gms. body weight of the three groups are as follows - stock control animals 4.82 $\pm$.625; hypophysectomized animals 1.91 $\pm$.685; paired-fed control animals 2.76 $\pm$.540. The differences between total body lipids of animals in groups A and B are significant ($t = 12.02$; $P < .01$) and the differences between groups A and C are significant ($t = 11.73$; $P < .01$). The smaller differences between groups C and B are not significant ($t = 2.24$; $P > .2 < .1$).

Discussion The conclusions which may be drawn from this experiment are limited somewhat by the relatively small number of animals in each group. Time did not permit the enlargement of the series. In spite of this limit-

ation the results of the total body fat determinations are statistically significant and worthy of comment.

It is immediately apparent that in spite of the low levels of fat in the livers of the hypophysectomized and paired-fed control groups, both of these groups lost considerable body weight and utilized a large proportion of their peripheral fat reserves. Hence it must be concluded that in the absence of the hypophysis an extra-pituitary mechanism exists which allows the animal to utilize its peripheral fat depots. An enlargement of this work is planned in which larger groups of animals will be used. By sacrificing the animals at different time intervals it is hoped to gain some knowledge of the comparative rates of depot fat mobilization in hypophysectomized animals and paired-fed controls.

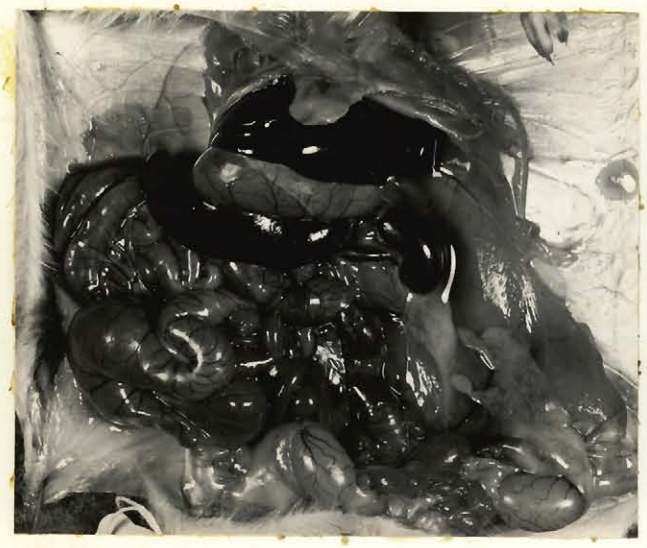

Fig. 5

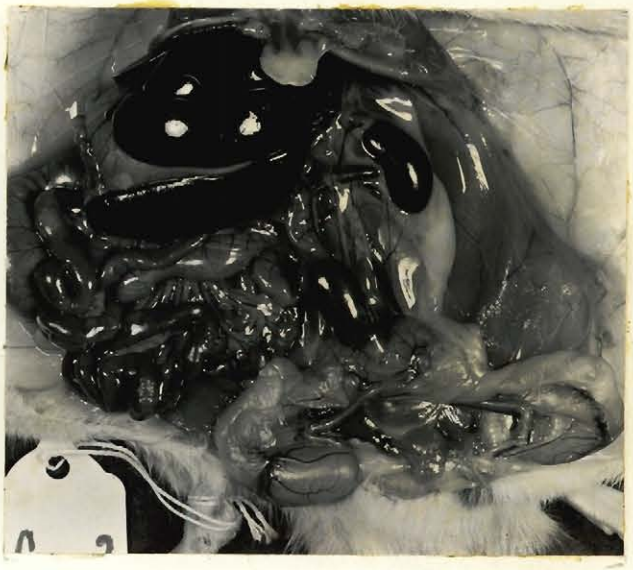

Fig. 6

The distribution of depot fat in the carcass of normal rats from the colony

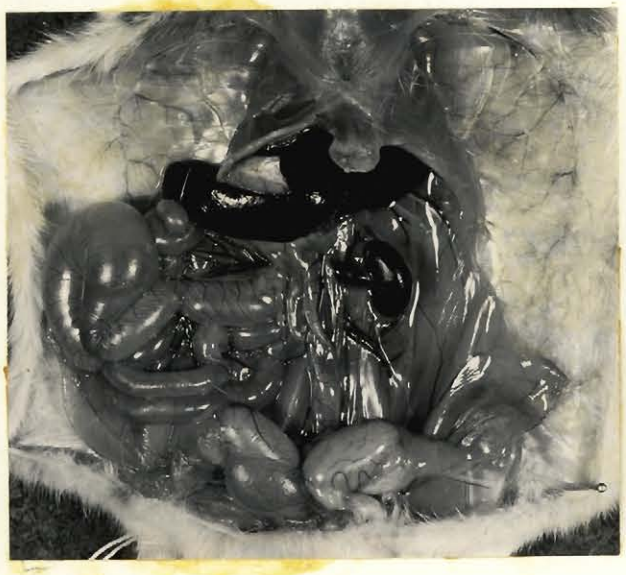

Fig. 7

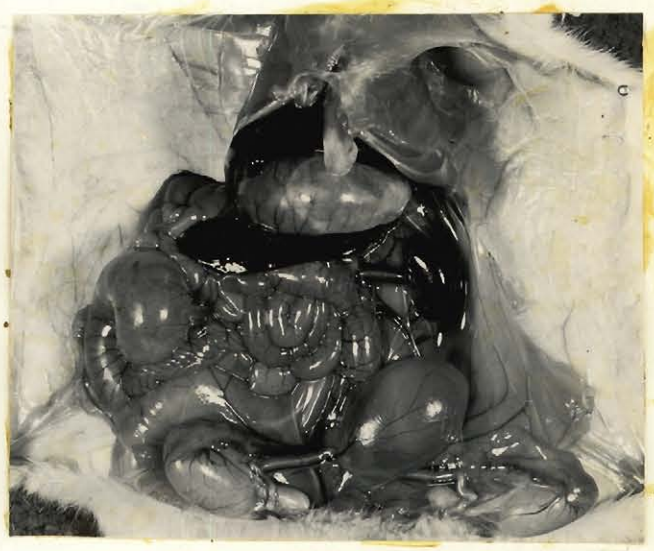

Fig. 8

Carcass depot fat of two hypophysectomized rats 7 days post-operatively. Animals fed ad libitum on the standard chow diet.

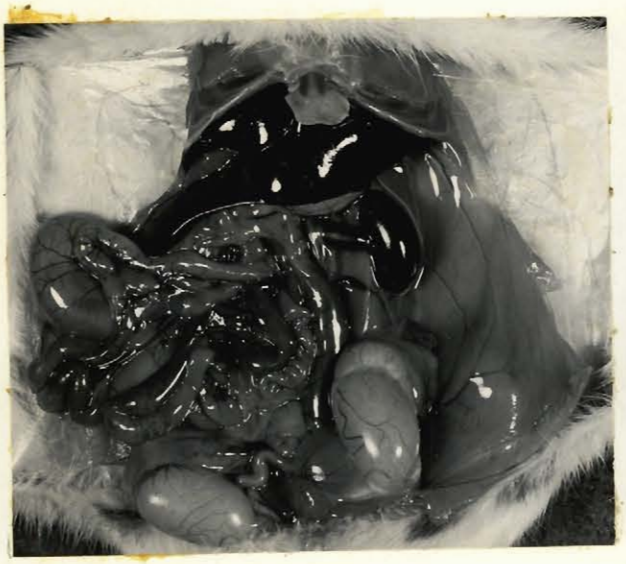

Fig. 9

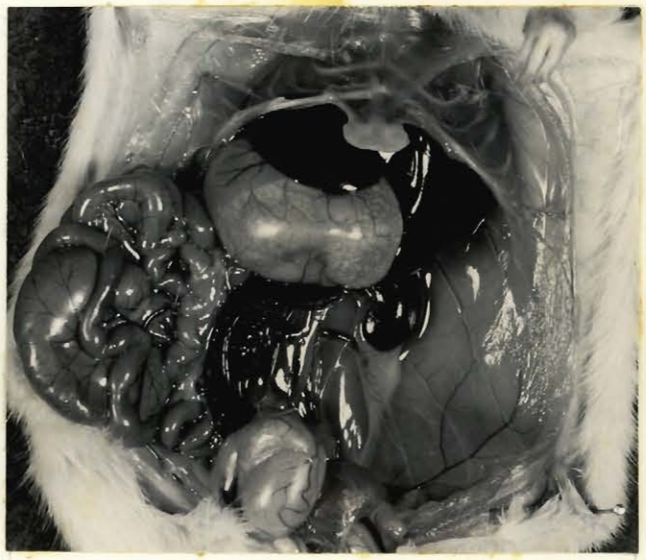

Fig. 10

Carcass depot fat of sham-hypophysectomized control rats, paired-fed with the hypophysectomized rats of group B.

TABLE VII

EXPERIMENT 6 - A COMPARISON OF THE LIVER AND CARCASS FAT OF HYPOPHYSECTOMIZED AND SHAM-OPERATED RATS PAIRED-FED DURING A SEVEN-DAY EXPERIMENTAL PERIOD. PRE-EXPERIMENTAL VALUES ARE GIVEN FOR STOCK ANIMALS

Animal	Treatment	Body Weight gms.			Dry Weight	Testicular Weight gms.	Liver			Total Body Fat gm/100gm. B.W.
		Initial	Final	Loss			Weight gms.	Fat gm%	Total fat gms.	
A-1	Normal	152	-	-	40.56	2.23	9.1686	3.18	.2915	4.20
A-2	"	160	-	-	44.30	2.80	7.8961	3.63	.2866	4.83
A-3	"	154	-	-	44.44	2.24	8.7410	3.88	.3391	5.45
B-1	Hypo-X	157	114	43	29.26	1.88	4.4370	3.67	.1628	2.61
B-2	"	144	118	26	29.46	1.91	4.6740	3.93	.1832	1.89
B-3	"	165	116	49	29.99	1.92	4.5863	3.90	.1788	1.24
C-1	Sham Hypo/x	150	113	37	30.44	2.23	5.6373	3.92	.2209	2.22
C-2	"	160	126	34	34.21	2.00	7.4464	3.24	.2412	2.76
C-3	"	164	127	37	33.36	2.45	7.7184	3.25	.2508	3.30

General Discussion

It has been shown in these experiments that adrenalectomy and hypophysectomy prevent the fatty infiltration of the liver which occurs after phosphorus poisoning. Also it would seem that neither cortisone nor adrenocorticotropic hormone affects phosphorus-induced fat infiltration in the liver of normal rats. These results are in substantial agreement with the bulk of the evidence in the literature. However, the demonstration that hypophysectomized animals may mobilize large quantities of fat from their depots yet still have low concentrations and absolute values of liver fat, throws new light on the problem.

It has been stated that depot fat mobilization is inhibited by adrenalectomy or hypophysectomy (201,202), the postulate being based on the low liver fat levels seen in such animals. Efforts have been directed at finding a hormone from the adrenal (87,88) or from the hypophysis (147,189, 116) capable of increasing the liver lipids in animals from which these endocrine glands have been removed. The assumption is made (116) that such an agent will act by stimulating the mobilization of peripheral depots.

However, adrenalectomized (166) and hypophysectomized (158) animals have been shown to be capable of losing their peripheral fat depots, and in the case of hypophysectomized animals this experiment has shown that depot fat loss can occur in the presence of low liver lipid levels.

It might then be postulated that the action of a hormone affecting this aspect of fat metabolism would be on the liver rather than the fat depots. Acting in some manner to allow the liver to achieve increased

levels of neutral lipids. Stetten and Salcedo have summarized the various factors which control liver fat levels (185). A hormone which increases liver lipids must act by a) increasing the mobilization of depot fats; b) decreasing the degradation of liver lipids; c) increasing the absorption of dietary fat or d) increasing the synthesis of fats from non-lipid substances. It has been shown (page 30) that hormones play little part in dietary fat absorption, but the part which endocrine factors play in reactions a), b) and d) is far from clear. Future experiments should be designed to simultaneously study the relative importance of these factors under various experimental endocrine conditions.

Summary and Conclusions

A survey has been made of the literature upon non-hormonal and hormonal influence on liver fat deposition, with the purpose of distinguishing those effects which are directly due to hormones and emphasizing the importance of diet in experiments of this nature.

It had been reported that adrenalectomy and hypophysectomy inhibit the fatty infiltration of the liver subsequent to phosphorus poisoning. These observations have been confirmed in experiments where animals were maintained in good condition and in which comparisons have been made with animals sham-operated and maintained on strict paired-feeding schedules. Neither cortisone nor adrenocorticotropic hormone appear to exert an effect on liver fat infiltration after phosphorus poisoning under these experimental conditions.

It was observed in experiments with hypophysectomized animals that

peripheral fat depots were decreased during the experimental period. These observations have been confirmed in a study of the quantitative differences of depot and liver fats of hypophysectomized and control rats. Decreases in peripheral fat depots have been demonstrated in the presence of low liver fat levels.

Efforts to produce a uniform cirrhosis of the liver in rats using yellow phosphorus as the hepatotoxin were unsuccessful. Experiments on this aspect of the problem are outlined and the possible reasons for their failure discussed.

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