A THESIS

ENTITLED

A STUDY BY ANIMAL EXPERIMENT OF THE NATURE AND DISTRIBUTION OF THE PATHOLOGICAL CHANGES PRODUCED IN THE TISSUES BY CORROSIVE SUBLIMATE WITH SPECIAL REFERENCE TO THE EARLY PHASES OF CELL-DEGENERATION AND TO CHANGES IN THE BLOOD-FAT

(Illustrated)

presented by

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F O R E W O R D

Several years ago two cases of mercurial poison-
:ing came to autopsy in the Royal Infirmary, Edinburgh.
Microscopic examination of the liver in each of those
cases revealed the presence of a fairly marked degree
of mitosis. Such mitosis could manifestly be explain-
:ed in either of two ways. On the one hand, it might
be regarded as a direct effect of the mercury whereby
the nuclei of the liver-cells are stimulated to divide.
On the other hand, it might be looked upon as merely
the sequel to a destruction of liver-cells produced by
the toxic action of the mercury: in this case mitosis
would be a purely regenerative phenomenon secondary to
degeneration of liver-tissue.

The immediate aim of this research was to discov-
:er which of these views is the correct one. If it
could be shown that mercury does really exert a spec-
:ific influence on the cells of the liver whereby their
nuclei are stimulated to undergo mitosis, we would,
indeed, be in possession of a fact of prime importance.
For then we would be in a position to say that in mer-
:cury we had a drug capable of controlling the growth
of liver-cells in so far as by administering it we
could at will produce their division and proliferation.

While/

While working out the answer to this question I simultaneously made an investigation into the nature and distribution of the pathological changes produced by mercury in most of the other organs, kidney, heart, spleen, intestine, etc. Particular attention has been paid to the earliest phases of degeneration. For example, a special endeavour was made to define the relation between the various kinds of granules which appear in the cells of the kidney when that org-:an is subjected to intoxication - the granules of ordinary cloudy swelling, the mitochondrial granules and those of fatty degeneration. Such an investigat-:ion is timely, for as Lorrain Smith states - "It is clear that the interrelations of the granules which appear in degenerative conditions of the protoplasm, to each other, and to the normal structure, must be the subject of much more investigation before we can reach a conception of their relation to those changes in metabolism from which degeneration results." Fur-:ther, wherever phenomena have been encountered in-:volving fundamental pathological principles an attempt has been made to interpret the phenomena observed with a view to increasing our knowledge of these principles. This is exemplified in the case of certain changes in the spleen.

As/

As a result, moreover, of observations made in the earlier stages of the research my attention was ultimately directed to certain alterations produced by mercury in the amount of fat in the circulating blood. The findings hereafter recorded in this connection con-:stitute, perhaps, the most interesting of my contrib-:utions to the pathology of mercurial intoxication. I have discussed the etiology of these changes in blood-fat so far as is possible and have indicated lines along which further research may with advantage be directed.

Corrosive sublimate, being one of the best known mercurial compounds and one of the easiest to admin-:ister, was chosen as the drug whose effects were to be investigated.

. . . . . .

This research was originally undertaken upon the recommendation and advice of the late Professor Lorr-:ain Smith, my first and very highly esteemed Chief. This work has, indeed, the distinction of being the last investigation which to my knowledge was set afoot by him and it is my disappointment that he is not now here to see and appraise its results. For the in-:valuable aid on many points of difficulty and for the continual encouragement he gave me during the greater part/

part of the time over which this research was conducted I am his lasting debtor. In equal measure am I indebted to Dr Theodore Rettie who never failed to give me the most generous advantage of his wide research experience and scientific knowledge, especially regarding fat metabolism and the technique of various methods I employed: to him I tender my sincerest and most heartfelt thanks. My thanks are also due to Dr James Davidson and Dr W.G. Millar of the Pathology Department and to Dr C.P. Stewart of the Medical Chemistry Department for the assistance they frequently extended to me in elucidating various problems and for the interest they kindly took in this work. I would also express my sincere indebtedness to Dr R. Gaddie who very generously carried out for me a series of blood-fat estimations, and lastly to Mr T. Dodds for the valuable aid he gave me in the preparation of the microphotographs.

During the first year of the period over which this work extended I held a Carnegie Research Scholarship. For such invaluable and gracious assistance I take this opportunity of expressing to the Carnegie Trust my deep indebtedness and sincerest thanks.

Finally, to the Committee of the Moray Endowment Fund I would express my gratitude for their generous financial aid toward the defraying of my research expenses.

C O N T E N T S

E X P E R I M E N T S

To each of a series of rabbits various doses and strengths of corrosive sublimate solution were admin-:istered at intervals. As will be seen below the duration of the experiments varied greatly - from 4 hours to 90 days. Two routes of administration were employed - intra-oral and intramuscular.

25:11:29. Experiment 1:- Rabbit of 1.5 kilos. received intra-orally 60 mgms. corrosive sublimate per kilo body weight in 1 in 500 solution.

After this dose the animal was dull and inactive for the remainder of the day. Next day the animal was very weak and had diarrhoea. It died 27:11:29.

Urine taken post-mortem from the bladder showed albumen ++, blood ++, no casts.

26:11:29. Experiment 2:- Rabbit of 1.7 kilos. received intra-orally 15 mgms. per kilo.(1 in 500 sol-:ution) on 4 successive days. Animal was killed accidentally on 5th day.

Urine at autopsy showed albumen +, blood+, no casts.

28:11:29. Experiment 3:- Rabbit of 1.8 kilos. received intra-orally 30 mgms. per kilo.(1 in 500 sol-:ution).

For two days subsequent to the administration of the/

the drug the animal ate almost nothing and was less lively than usual. Subsequently it recovered its nor-:mal vitality and ate well. But on the 6th day it again began to grow dull and listless. It ceased to eat and became rapidly weaker to die on the 7th day.

Urine at autopsy showed albumen+, blood+, no casts.

16:1:30. Experiment 4:- Rabbit of 2.5 kilos. received intra-orally 30 mgms. per kilo.(1 in 500 sol-:ution).

The animal gradually became more and more lethar-:gic and died 4 hours after receiving the drug.

Urine not examined.

3:3:30. Experiment 5:- Rabbit of 2.05 kilos. received intra-orally 30 mgms. per kilo.(1 in 500 sol-:ution).

Animal was killed after the lapse of 8 hours.

4:3:30. Experiment 6:- Rabbit of 1.82 kilos. received intra-orally 15 mgms. per kilo.(1 in 500 sol-:ution) once weekly for 4 weeks.

Animal was killed at end of 4th week.

Urine showed albumen++, no blood, no casts.

18:3:30. Experiment 7:- Rabbit of 2.4 kilos. received intra-orally 15 mgms. per kilo.(1 in 500 sol-:ution) daily for 5 days.

Animal/

Animal became rapidly weaker, developed diarrhoea on 6th day, and died 23:3:30.

Urine showed albumen + (Esbach ½ gram per litre), no blood, granular casts +++, hyaline casts +.

24:3:30. Experiment 8:- Rabbit of 2 kilos. received intramuscularly 15 mgms. per kilo.(½ per cent. solution).

On the following day animal was lethargic. Urine showed albumen ++, no blood, no casts. On third and fourth days animal passed no urine. On fifth day animal passed a little urine. Died same day.

Urine at autopsy showed albumen (4 grams per litre), blood+, hyaline casts +.

21:5:30. Experiment 9:- Rabbit of 2.3 kilos. received intra-orally 60 mgms. per kilo.(1 in 500 sol-:ution).

Animal dead in 12 hours.

Urine at autopsy showed albumen +++, no blood, no casts.

8:9:30. Experiment 10:- Rabbit of 2 kilos.re-:ceived intramuscularly 1 mgm. (1 in 500 solution) for 1 month; then 2 mgms. (1 in 500 solution) for second month; then 3 mgms. (1 in 500 solution) for third month (except last week).

Animal/

Animal lived three months all but one week. To-:ward end animal began to develop sores on its legs and was killed on 2:12:30. Animal showed marked emaciation and loss of strength, but continued through-:out experiment to eat fairly well.

9:9:30. Experiment 11:- Rabbit of 1.7 kilos. received intra-orally 60 mgms. per kilo. (1 in 500 sol-:ution).

Animal gradually became weaker and toward the end very drowsy. Died 4 days after administration of dose. During these 4 days animal ate no food.

Urine at autopsy showed albumen+++, no blood, granular casts ++ .

15:9:30. Experiment 12:- Rabbit of 1.5 kilos. re-:ceived intra-orally 60 mgms. per kilo. (1 in 500 sol-:ution).

Animal dead in 7 hours.

1:10:30. Experiment 13:- Similar to Experiment 12.

Animal dead in 8 hours.

11:11:30. Experiment 14:- Rabbit of 1.8 kilos. received intramuscularly 14 mgms. per kilo. (½ per cent. solution). On 25:11:30, 3:12:30, and 9:12:30 animal weighed on each occasion 1.36 kilos. and received 10 mgms. per kilo. (½ per cent solution). On 30:12:30 animal/

animal weighed 0.9 kilo. and received 5 mgms. per kilo. Animal died 31:12:30.

27:11:30. Experiment 15:- Rabbit received intramuscularly 5 mgms. (½ per cent. solution) daily until 20:12:30 when animal died.

3:2:31. Experiment 16:- Rabbit of 1.9 kilos. received intramuscularly 2½ mgms. (½ per cent. solut-:ion) daily.

11:2:31 weight - 2.05 kilos. 19:2:31 weight- 2.2 kilos. 28:2:31 dose increased to 5 mgms. daily. 11:3:31 weight - 1.8 kilos. 9:5:31 weight - 1.7 kilos. 29:5:31 weight - 1.5 kilos. 9:6:31 weight - 1.2 kilos.

Animal gradually weakened during 2 - 3 weeks prior to 11:6:31 when animal died.

Urine at autopsy showed albumen++, no blood, and no casts.

URINE AT AUTOPSY

In nine of the above experiments the urine found post-mortem in the bladder was examined for the pres-:ence of albumen, blood and casts. Albumen was pres-:ent in all cases - in most of them in large quantities. The urine in each of four cases (1, 2, 3, 8) gave a positive guaiac test for blood. In all the urine was examined microscopically for casts, but these were ob-:served in only three instances (7, 8, 11). The casts were mostly granular in character, but a few were hyaline.

METHODS OF INVESTIGATION

Blocks of tissue from the various organs (kidney, liver, spleen, heart, lungs, intestine, suprarenals, lymph glands, and bone marrow) were fixed immediately after death in 10 per cent. formalin. Paraffin sect-:ions were prepared and stained haematoxylin and eos-:in - or in the case of the bone marrow, eosin and methylene blue. In particular instances other stain-:ing methods were employed, e.g. the Prussian Blue Reaction for the demonstration of iron pigment, and alizarin sulphate for the detection of calcium.

Frozen sections of kidney, liver, and heart-muscle were stained with Sudan III or by the Aldol Method[1] for the presence of fat. The Aldol Method I have found to be of unquestionably great service in giving a clear definition of the earliest and minutest granules of fat.

Considerable attention has been paid to the mito-:chondria of the kidney and liver. In the study of the changes exhibited by these structures the Bi-:chromate Method described by Lorrain Smith and Rettie[2] was employed. In this case the tissues were fixed in 20 per cent. formalin neutralised by magnes-:ium/

magnesium carbonate. Further reference to this meth-:od will be made below.

In a series of experiments an investigation was made into the changes produced by corrosive sublimate in the total amount of fat in the blood. The blood-fat estimations were performed by Dr R. Gaddie of the Medical Chemistry Department, Edinburgh University, to whom I am deeply indebted. In these estimations Dr Gaddie employed a modification of a method origin-:ally described by Stewart and White in 1925[3].

K I D N E Y

LITERATURE

In 1860 Pavy[4] studied the pathological changes induced experimentally by white precipitate in the kidneys of animals. He observed that the Malpighian bodies escaped injury, but that the tubules were necrotic and the seat of calcification. These calcified tubules could be made out as white columns radiating through the cortex from the base of the medulla to the surface of the organ. These results were confirmed a few years later by Salkowsky (1866)[5].

The exact mode of production of the pathological lesions found in the kidney (and colon) and the selective character of their distribution have been the subject of much experimentation and speculation. The oldest and most popular theory generally accepted since the time of Virchow[6] is that of elimination. This assumes a direct destructive action on the epithelium of the kidney and bowel during the course of elimination - an explanation which is sponsored in textbooks such as those of Schmeideberg and Kobert. In an attempt to refute this theory it has been argued (see Kaufmann below) that in its elimination from the body and in its concentration in the blood mercury is probably/

probably never present in sufficient quantity to do the damage by direct action that we subsequently find at necropsy.

In 1881 von Mehring[7] considered the toxic action of mercuric chloride on the kidney and intestine to be due to its inducing a general vasomotor paralysis; this results in such an extreme fall in blood-pressure as to interfere with the nutrition of the organs men-:tioned and in this way degenerative changes are pro-:duced. But in experimental poisoning with mercury Kolb (1903)[8] has shown that during the first few days of the intoxication there may be no fall in general blood-pressure - more often, indeed, a rise is observ-:ed - and during these early days gross lesions may undoubtedly be produced. Giesboeck (1905)[9] confirms Kolb's clinical findings.

Elbe (1905)[10] put forward the theory that the anat-:omical changes are due not to a general but to a local blood-pressure disturbance induced by the mercury in the blood having a selective action on the vasomotor mechanism of the arterioles of the kidney and large bowel. This action is to cause a marked constriction of these vessels whereby the inflow of blood into the capillary system is interfered with and there result in consequence areas of anaemic infarction in the kid-:ney and haemorrhagic infarction in the large bowel.

Recent/

Recent experiments by Weiler (1913)[11] would seem to confirm in part the conclusions of Elbe. In this connection it was shown by Rosenheim (1888)[12] who perfused dog-kidneys with defibrinated blood containing mercurial salts that at first one obtains a vasodilatation which is soon followed by a marked and progressively increasing vasoconstriction. Natus (1910)[13] confirmed this vasoconstrictor action of mercury. Again, Strake (1920)[14] observed in rabbit's kidneys exposed and examined in the living condition under the binocular microscope a distension of the vessels and a slowing of the current until stasis occurred. In the course of this slowing red corpuscles passed out of the glomerular capillaries into the tubules. There the corpuscles broke down and the products of their disintegration acted upon and damaged the lining cells of the tubules.

Heineke (1888)[15] and Kaufmann (1889)[16] described the formation in the capillaries of thrombi produced by fusion of red corpuscles into homogeneous masses. Kaufmann attributes this thrombus-formation to direct injury of the red cells by the mercury or to its inducing the liberation of fibrin-ferment whereby coagulation of blood occurs in the minutest capillaries. He thus regards the necrosis affecting the kidney and intestine as an anaemic necrosis. This theory would seem/

seem to be thoroughly eliminated by Lyon (1904)[17] and Priebatsch (1910)[18] who have shown that no thrombi are to be found in the affected areas. Again, Kohan (1909)[19] and Sievert (1910)[20] showed that when clotting of the blood is prevented no variations occur in the histological changes typical of mercurial poisoning.

Virchow (6) maintained that the lime was deposited first in the lumina of the kidney tubules and then sec-:ondarily in the epithelium. He believed that mercur-:ial salts induced a direct decalcification of the bones and regarded the occurrence of calcification in the kidney as analogous to the phenomenon of calcareous metastasis which occurs when tumours or destructive processes in bone lead to an overloading of the blood with lime salts. Klemperer (1889)[21] agreed with Vir-:chow's conclusions. On the other hand, Kaufmann, Prévost (1882)[22] and Lyon find deposition of calcium salts only in necrotic cells. Leutert (1895)[23] and Karvonen (1898)[24] recognise two distinct processes of calcification. They find that lime salts are depos-:ited in injured but still functioning cells and con-:sider it an abnormal secretion process; they also describe calcification of totally necrosed cells and of casts produced by their fusion. They thus dis-:tinguish an active separation of lime salts by still functioning cells and a passive deposit in necrosed cells/

cells.

Harnack and Kustermann (1898)[25] describe in detail diffuse fatty changes in the renal epithelium of the cat, but as the kidney of this animal normally infil-:trates fat their results are valueless.

Karvonen in acute mercurial poisoning in rabbits found that a perivascular infiltration with mononuc-:lear leucocytes was almost constantly present after the second day. He described the accumulation of these cells around necrotic and calcified tubules and even their emigration into some of the tubules. Ac-:cording to his observations these cells in cases of chronic intoxication become transformed into fibro-:blasts and so proceed to the formation of connective tissue which replaces the atrophic tubules. Leutert, Klemperer, and Harnack and Kustermann also encountered an interstitial infiltration with small round cells. In acute cases of poisoning Karvonen observed congest-:ion of the glomerular tufts and the passage of album-:en, blood and leucocytes into the capsular space. In cases of subacute poisoning he found the glomerular and capsular epithelium often desquamated, many glomer-:uli devoid of nuclei, emigration of leucocytes into the capsular space, and in chronic cases an increase of connective tissue in the glomerular tufts. He al-:so/

also found areas of necrosis in the tubules.

In 1904 Lyon[17] endeavouring to determine the sequence of pathological changes in acute, subacute and chronic nephritis employed mercuric chloride experimentally in rabbits. He was unable to obtain any characteristic changes in the glomeruli. In acute cases of intoxication he found congestion of the glomerular tufts, but no thrombi in the vessels and no desquamation of epithelium, either glomerular or capsular. He described an irregular affection of the secreting tubules: some appeared normal; others showed either of two types of necrosis; the most severely affected cells underwent a change resembling Weigert's coagulative necrosis; the cells of the remaining tubules underwent intense swelling and granular degeneration. The coagulated cells calcified rapidly. There were no interstitial changes.

In 1907 Vlisenger[26] found that the severity of the kidney lesions is influenced to a greater degree by the size of the dose than it is by the duration of the intoxication. In the liver, on the other hand, he found the duration of the intoxication of greater importance than the intensity of dosage.

In 1912 Aschoff[27] described the renal pathology in mercurial poisoning as consisting of swelling of the cells/

cells, hyaline vacuolation and necrosis. Burmeister and McNally (1917)[28] more or less agree with Aschoff's findings, but since in all their experiments they gave preliminary doses of chloretone which in some cases was supplemented by ether anaesthesia, their conclusions are valueless.

In 1918 W.de B. McNider[29] administered corrosive sublimate in solution to a series of dogs by the stom-:ach tube. His results enabled him to divide the animals into three main groups:- (1) Animals which de-:velop an intense gastro-enteritis and die from shock within 48 hours before sufficient time has elapsed for the development of a serious renal injury. (2) Ani-:mals which show mercury in the urine and develop a Weigert-like necrosis of the tubular epithelium which is likely due to the mercury acting as such. (3) Ani-:mals which develop a delayed acute renal injury when the urine may have been free from mercury for several days. Such animals have a severe type of acid-intox-:ication and exhibit intense swelling of the renal epithelium followed by necrosis. It would appear that this delayed type of renal injury is not due to the mercury, but is in some way dependent upon the disturb-:ance in the acid-base balance of the blood which does not primarily originate as a retention phenomenon.

The/

The glomerular changes consist in intense congestion with or without an exudate of serum and fibrin into the subcapsular spaces.

In man mercurial poisoning produces lesions sim-:ilar to those in experimental animals. The glomeruli are rarely affected and the lesions have been found chiefly in the secreting tubules of the cortex. But according to Kaufmann and others (Campbell (1917)[30], Miller (1926)[31], Harmon (1928)[32]) it is particularly the convoluted tubules which are affected in man, whereas in rabbits the ascending limbs of Henle suffer more than the convoluted tubules. Calcification of necrotic tubules and interstitial changes have also been described in man (Prévost)[22].

Regeneration of the tubular epithelium has been described in rabbits by Lyon, and in man by Campbell, Miller and Harmon.

PERSONAL/

PERSONAL OBSERVATIONS

Macroscopic Appearances.

In cases of acute intoxication the kidneys are swollen, sometimes markedly so, tense and pale. Their shape is sometimes more globular than actual kidney-shape. The capsule always strips easily leaving a smooth, pale surface over which are scattered numerous minute patches of congestion so that the organ has often a definitely mottled appearance. On section the cortex is found to be increased in depth and abnormally pale with numerous streaks of reddish colour radiating from the medullary border to the periphery: these reddish streaks represent congested interlobular arterioles and the subcapsular areas of congestion are seen to be situated at the superficial extremities of these vessels. Scattered throughout the cortex there are, besides, numerous pin-point red dots representing congested glomeruli. The medulla is likewise the seat of marked congestion, the latter being always most severe in the boundary zone where the tissue is inevitably of a deep, reddish-purple colour.

In subacute and chronic cases of poisoning these changes - swelling, pallor and congestion - are less in/

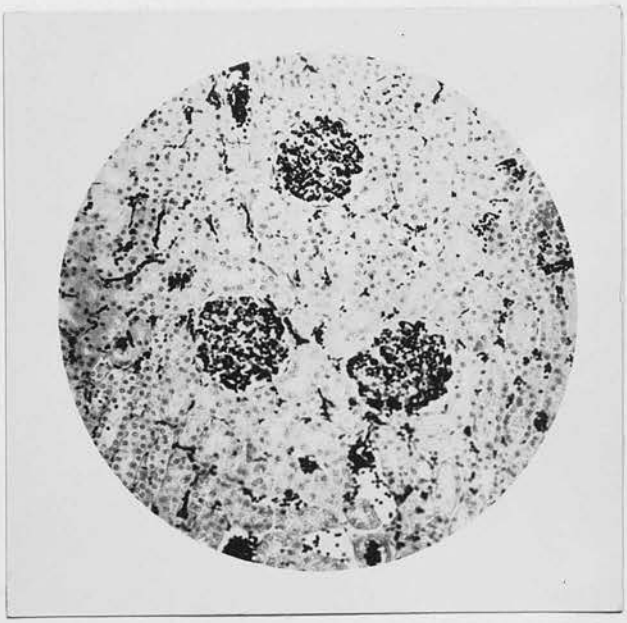


Fig. 1. Glomeruli showing marked congestion and swelling of their tufts. (Haem. and Eos.) x 100.

Fig. 2. Marked congestion of the Arterial Rectae in the boundary zone of the renal medulla. (Haem. and Eos.) x 100.

in evidence, and in mild cases of intoxication the kid-:neys may show little departure from the normal. When calcification of the tubules has occurred delicate streaks of whitish colour are to be seen running through the cortex in the line of the medullary rays.

The renal pelves are always healthy.

Microscopic Appearances.

Vascular System.

In all my experiments but one the kidney tissue is the seat of congestion. This hyperaemia is most marked in acute cases and less severe in subacute and chronic cases. In one experiment, viz. 10, where the intoxication was of a very mild and chronic character congestion is absent. The congestion involves the whole arterial system - interlobular arterioles, glom-:eruli, intertubular capillary plexus and arteriae rectae. The interlobular arterioles may be widely dilated and form conspicuous columns in the cortex, but in the majority of cases the congestion affects the glomeruli and the arteriae rectae of the medulla most severely. The congestion of these structures may be extreme (Figs. 1 and 2). Occasionally the glomeruli are affected in greater measure than the arteriae rect-:ae; occasionally the reverse is the case; but in most instances/

instances they are more or less equally involved. In spite of the excessively congested state of the vessels no haemorrhage into the glomeruli, tubules or inter-:stitial tissue has ever been observed.

Glomeruli.

As already stated the glomeruli in all but the mildest cases of intoxication are the seat of congest-:ion. They may be all affected more or less uniformly, but in the majority of cases they exhibit variations in the degree to which they are involved. Even in an acute case such as Experiment 11 some glomeruli are scarcely more vascular than normal while others are markedly hyperaemic and appear almost converted into simple balls of red blood cells. Such congestion may tend to emphasise the natural lobulation of the tufts. Extreme hyperaemia may be accompanied by varying deg-:rees of swelling of the tufts so that the space en-:closed by Bowman's capsule may be almost or altogether obliterated. Homogeneous thrombi produced by fusion of red cells such as have been described by Kaufmann I never observed either in the glomerular capillaries, in the afferent arterioles, or, indeed, in any of the renal vessels.

The cells of the capillary endothelium are always well-preserved and their nuclei well stained. In

most/

most cases the capsular epithelium and that covering the tufts show no obvious departure from the normal. Very occasionally the epithelial cells covering the tufts and the cells of the subcapsular epithelium are abnormally swollen and prominent, but they have never been observed to undergo desquamation or proliferation. I never noted any accumulation of leucocytes in the capillaries of the glomerular tufts or emigration of such cells into the subcapsular spaces. Neither have I ever detected any atrophy or fibrous transformation of the Malpighian corpuscles. In several respects, therefore, my findings differ from those of Karvonen.

In some of my experiments the glomeruli frequent-:ly show accumulations of finely-granular, almost homogeneous, pink-staining material. The latter is usually deposited in Bowman's space in the form of small round or oval masses variable in size and fre-:quently quite well defined. Sometimes, however, the granular material is scattered with no such struct-:ural formation. These granular bodies are placed as a rule just inside the capsular epithelium; only rare-:ly have I observed infiltration of granular débris far into the space or between the lobules of the tufts. These granular bodies may be distributed in varying number more or less irregularly round the space just inside/

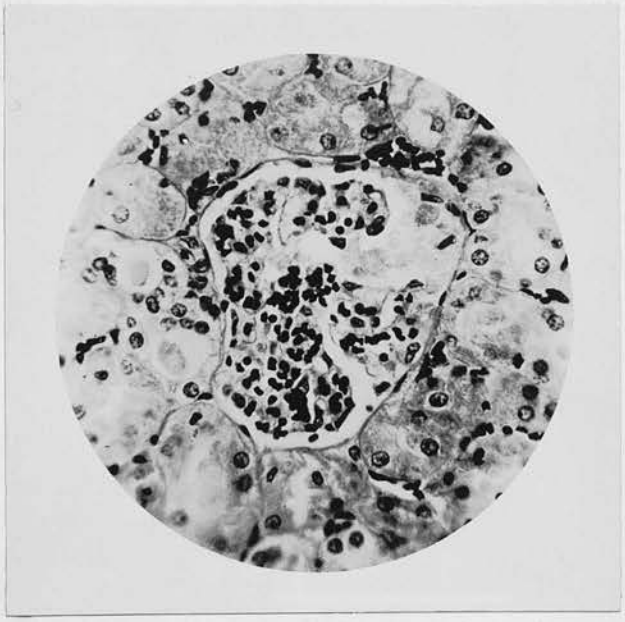


Fig. 3. Glomerulus showing an accumulation of granular material in its capsular space. The mat-:erial is deposited in the form of small masses localised at one point. (Haem. and Eos.) x 360.

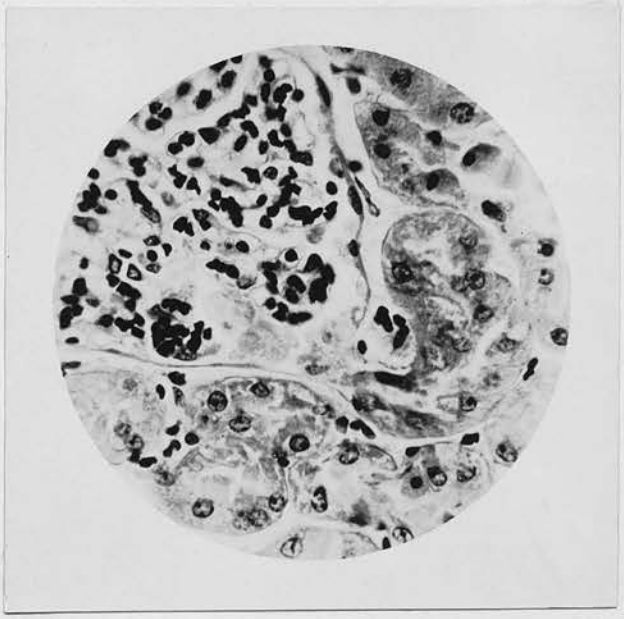


Fig. 4. Glomerulus showing the relation of the granular débris in the capsular space to the commencement of the first convol-:uted tubule. Note the nuclear remains in the débris. (Haem. and Eos.) x 540.

inside the capsular epithelium, but as a rule are gathered mainly at one point (Fig. 3). Sometimes the remains of degenerated nuclei can be discerned amongst this granular material; this observation suggests that the material is cellular in origin. Further inform-:ation is afforded regarding the source of this mater-:ial at points where the glomerulus and the commence-:ment of the first convoluted tubule are cut in the same plane (Fig. 4). Here it is seen that the neck of the tubule is filled with a granular substance sometimes forming round or oval bodies containing de-:generated nuclei similar to the structures described above. The appearance, indeed, is very much as though the granular material in the glomeruli were derived from the commencement of the tubule; the cytoplasm of this region of the tubules would seem first to have undergone degeneration and subsequently fragments of disintegrated cytoplasm with nuclear remains have drift-:ed backward into the glomerular space. The frequent-:ly localised position of the granular bodies within the glomerular spaces, the presence of degenerated nuclei and the condition of the commencement of the first convoluted tubules certainly support such a con-:clusion.

Convoluted/

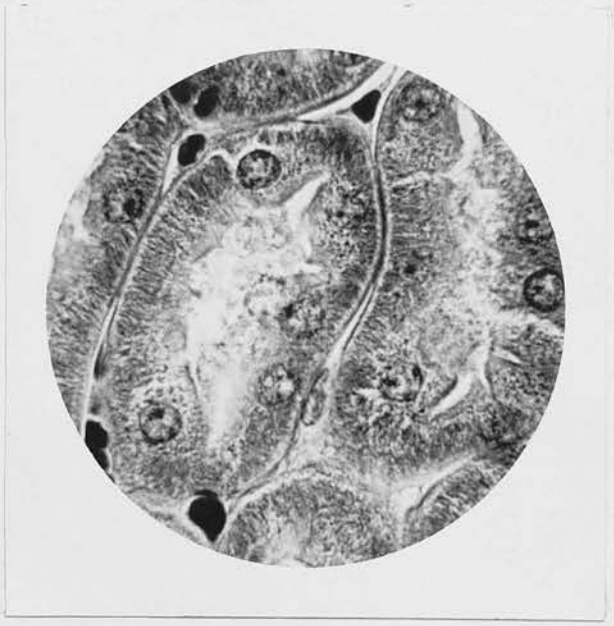


Fig. 5. Convoluted tubules showing normal arrangement of cytoplasmic rods in the basal zone. The rods are placed in close parallel formation normal to the basement membrane. A clear zone exists between tips of rods and lumen. cf. Figs. 20 and 21. (Haem. and Eos.) x 900.

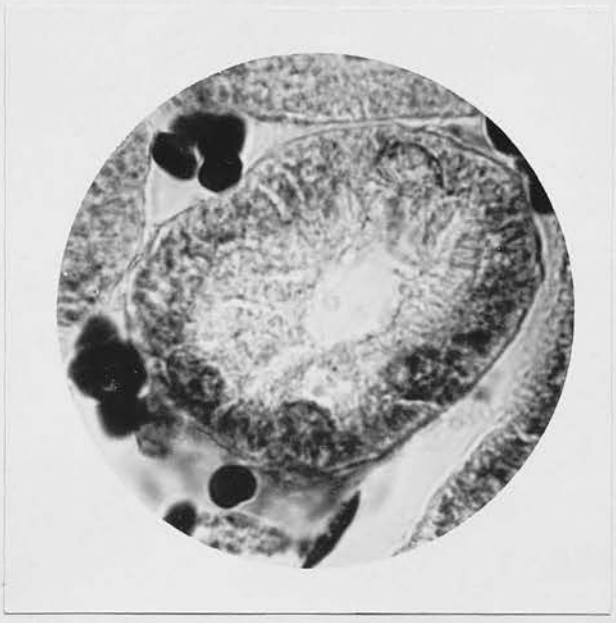


Fig. 6. Convoluted tubules showing the earliest phases of cloudy swelling. The basal rods have undergone fragmentation with the production of many granules which are distributed through the zone normally occupied by the rods. cf. Fig. 22. (Haem. and Eos.) x 900.

Convoluted Tubules and Henle's Loops.

Haematoxylin and Eosin. Careful examination of the cells lining the convoluted tubules of normal kidney tissue reveals the fact that their cytoplasm in the region of the basement membrane has a striated appear-:ance (Fig. 5). This can be seen to be due to the presence in this zone of short, slender rods or fila-:ments placed in close parallel formation normal to the basement membrane. These rods extend half or two-thirds of the way toward the lumen, their tips embrac-:ing the nuclei. A clear, very finely granular zone exists between the tips of the filaments and the lumen. The filaments are slightly more eosinophilic than the clear zone around the lumen.

The very earliest change I have observed in the convoluted tubules consists in the fragmentation of these rods with the production of a great host of min-:ute granules (Fig. 6). The latter may occasionally be seen to lie in rows in the position of the normal filaments at right-angles to the basement membrane. But as a rule they have lost this row-formation and are scattered uniformly through the zone of cytoplasm adjacent to the basement membrane in which the rods ordinarily lie. At this stage there may be no swell-:ing of the cells and the clear zone around the lumen of each tubule still remains devoid of granules.

At/

At a very early stage, however, the lining cells of the tubules undergo swelling. Such swelling may occur without any affection of the inner cell-membrane so that the lumina of the tubules though narrowed con-:tinue to be well-defined. But where it is at all marked swelling is almost invariably accompanied by disintegration of the inner cell-membrane and by drift-:ing into the lumen of material from the superficial parts of the cells. This may be so marked that the lumen becomes almost or altogether occluded by débris.

On desquamation into the lumen the cytoplasm fre-:quently shapes itself into a number of small, round or oval bodies. These bodies may exhibit the remains of degenerated nuclei and are identical with those some-:times observed inside the glomerular spaces. Some-:times the cytoplasm which is shed into the lumina is distributed there in the form of an intricate and delicate, granular network (Fig. 9). In proportion as desquamation takes place the cells lining the base-:ment membrane undergo progressive thinning; in con-:sequence what remains of the cells may be no deeper than the diameter of the nuclei and the most extreme stage is reached in the case of tubules whose basement membranes have in part been denuded of cytoplasm.

Meanwhile the granules in the basement zone of the cells undergo changes in distribution. At an early/

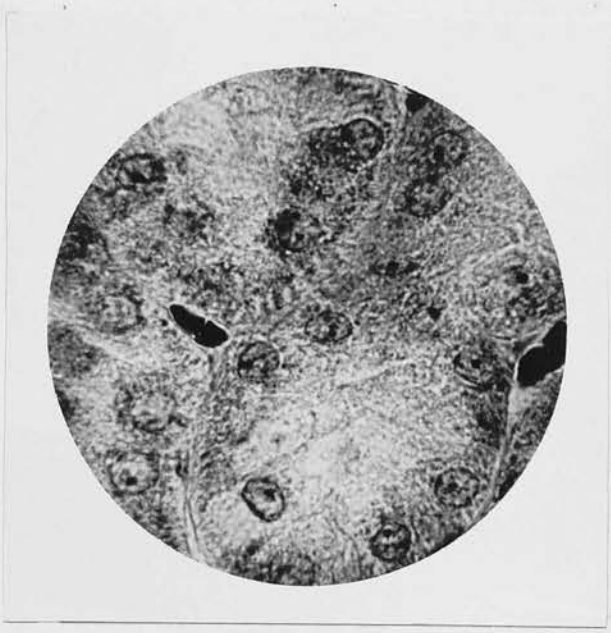


Fig. 7. x 900.

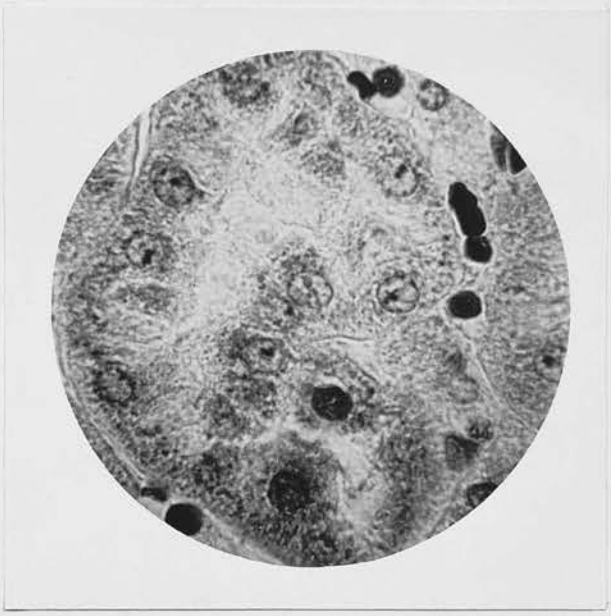


Fig. 7a. x 900.

Figs. 7 and 7a. Convoluted tubules showing more advanced cloudy swelling. The lining cells are greatly swollen and the lumina much narrowed. The granules are still gathered most densely in the region of the basement membrane, but the superficial granules are showing a definite tendency to drift toward the lumen. cf. Figs. 23 and 24. (Haem. and Eos.)

early stage the superficial granules begin to drift in-
:to the clear zone around the lumen. And subsequent-
:ly strands of granules can be seen flowing out toward the lumen in prolongations of the swollen cytoplasm. Nevertheless, the granules for a time continue to be most densely gathered in the basement zone and this I have found to be the commonest distribution of the gran-
:ules in cases of acute intoxication (Figs. 7 and 7a). Not infrequently, however, many of the granules in the region of the basement membrane also begin to drift to-
:ward the lumen, the result in such cases being that the granules ultimately come to be distributed more or less uniformly throughout the cytoplasm of the swollen cells. The granules thus show a tendency more and more to de-
:sert their normal situation in the region of the basement membrane.

In acute cases of intoxication the granules, al-
:though varying a little in size, remain uniformly minute. In cases of subacute intoxication, on the other hand, the granules increase considerably in size and many of them reach fairly large dimensions. There is at the same time a definite reduction in the number of granules and their distribution, moreover, becomes very irregular. Sometimes they are gathered in groups placed deeply in the cells, but most often they show a distinct tendency to be distributed around, between and internal to the nuclei (Figs. 8 and 8a). These

large/

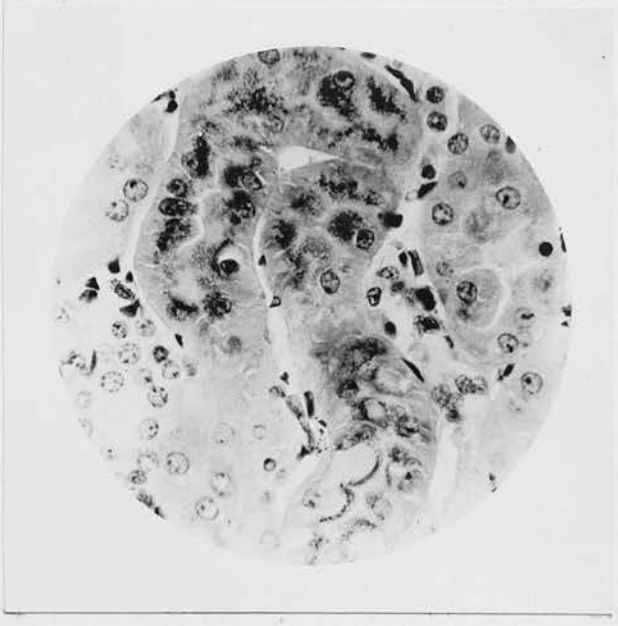


Fig. 8 x 500.

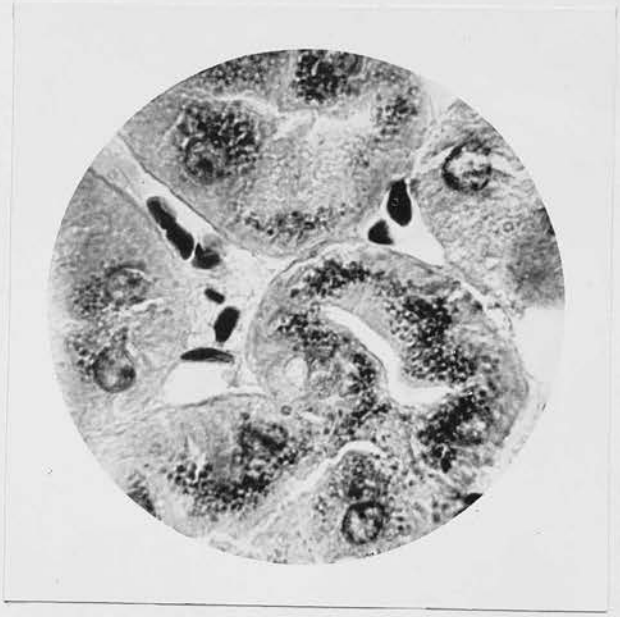


Fig. 8a. x 900.

Figs. 8 and 8a. Convoluted tubules showing coarse cyto-:plasmic granules arranged mainly around and between the nuclei. These granules are hyaline in character and strongly eosinophilic. cf. Figs. 32 and 33.
(Haem. and Eos.)

large granules are strikingly hyaline in character and stain brilliantly with eosin; in consequence they stand out as very conspicuous structures in the cyto-:plasm. The large size of many of the granules, the reduction in the number of granules and their occur-:rence in groups are indications that in all probabil-:ity the larger granules are produced by coalescence of the smaller ones.

The nuclei show various degenerative changes. In the mildest cases of poisoning they may show no change, but nuclear degeneration becomes more and more marked as the cytoplasm undergoes disintegration. Not in-:frequently they become swollen and vesicular, some-:times markedly so; nuclei showing such changes sub-:sequently often show signs of fading out of existence altogether. In Experiment 3 disappearance of the nuclei is a conspicuous feature; many tubules in this experiment have lost all but one or two of their nuc-:lei and occasionally a tubule exhibits no nuclei whatsoever. As will be mentioned below disappearance and loss of nuclei is more commonly a feature of the ascending limbs of Henle's loops. Sometimes the nuclei show pyknosis, but this form of degeneration is less commonly encountered than karyolysis. The changes described above are those characteristic of cloudy/

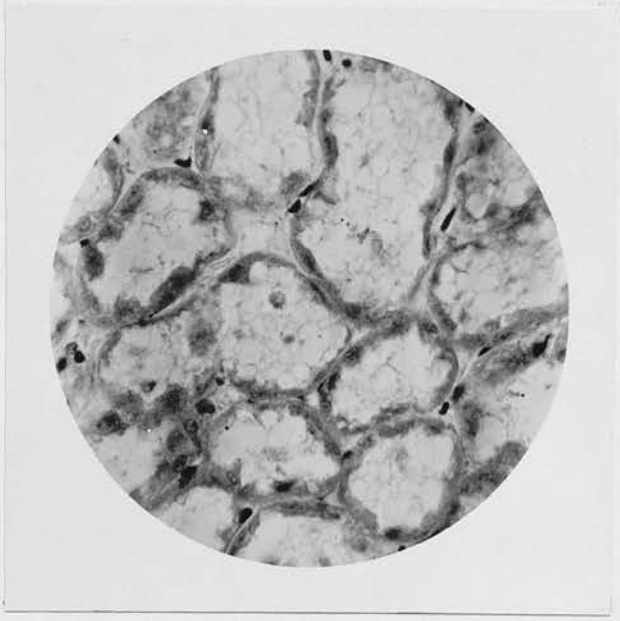


Fig. 9. Convoluted tubules show-:ing cloudy swelling and necrosis. Note disappearance of nuclei and the delicate network formed by the cytoplasm shed into each lumen. Basement membranes are at points denuded of cytoplasm. (Haem. and Eos.) x 500.

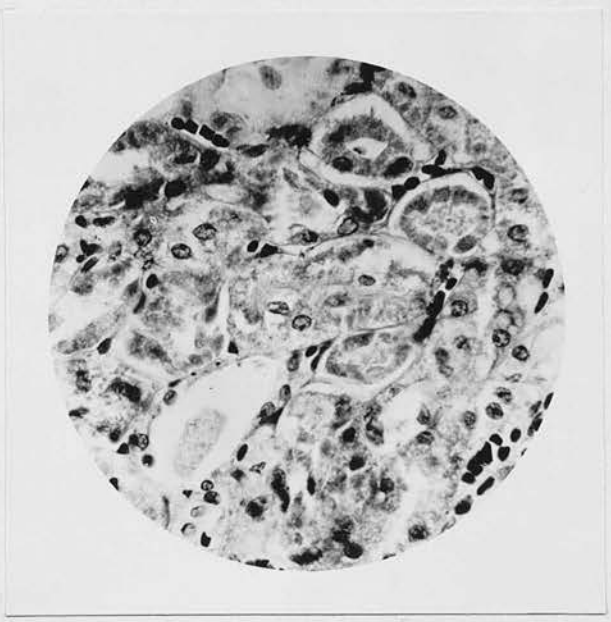


Fig. 10. Convoluted tubules show-:ing coagulative necrosis. The necrotic tubules are hyaline in character and deeply stained with eosin. The remains of some pyk-:notic nuclei are visible. (Haem. and Eos.) x 400.

cloudy swelling and necrosis.

Again, in one or two of my experiments the cells lining some of the convoluted tubules do not exhibit any swelling and in a few instances, indeed, would appear to have undergone shrinkage (Fig. 10). The cytoplasm of these cells is non-granular, hyaline and deeply-stained with eosin. In most cases the nuclei of these cells have disappeared altogether. Here and there the remains of degenerated nuclei can be made out; as they undergo degeneration the nuclei first become shrunken and intensely pyknotic and then gradually fade out of existence; this process of disappearance may not occur uniformly throughout a nucleus for frequently a few minute granules represent the remains of one. These cells have clearly undergone a coagulative, hyaline change, cellular and nuclear structure being destroyed and the new material being hyaline and acid-:ophile in character. The coagulated cells frequently show a tendency to crack and split in the process of fixing and staining as if they were unduly brittle. These tubules have undoubtedly undergone the change commonly known as coagulative necrosis (Weigert).

. . . . . .

The/

The descending limbs of Henle's loops never show any pathological changes. Frequently, however, their lumina exhibit casts of various kinds. These will be more fully described below.

A conspicuous feature in all my experiments is the degree to which the ascending limbs of Henle's loops have been involved. The measure of degeneration sustained by them I have found to be constantly greater than that exhibited by the convoluted tubules. This selective affection of the ascending limbs is made still more manifest by frozen sections stained sudan III or aldol as will be described below.

The ascending limbs exhibit various degrees of degeneration according to the severity of the intoxic-:ation. Most often their lining cells have undergone a marked degree of swelling and the inner cell-membran-:es having disintegrated the cellular débris has drfited into the lumina of the tubules to their com-:plete obliteration in almost every case.

At the same time the basal filaments (which are similar to those of the convoluted tubules, but short-:er and more delicate) undergo disintegration with the production of a great host of small granules. These may occasionally be seen as in Experiment 4 where the lining cells show little or no swelling to be restrict-:ed/

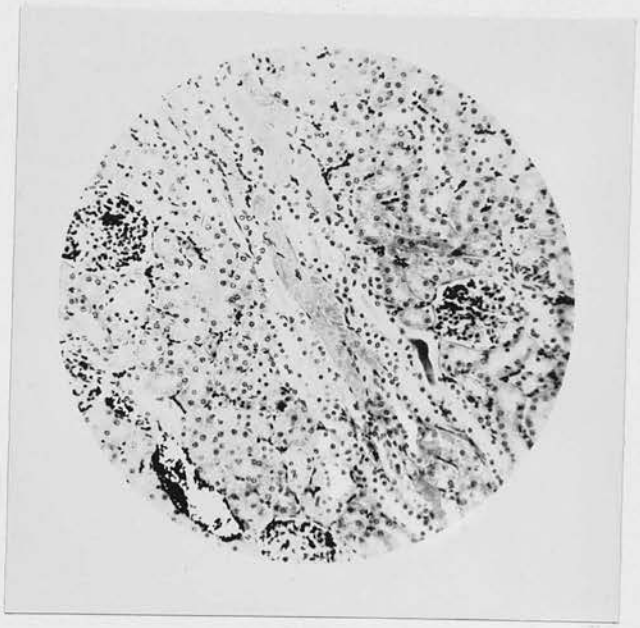


Fig. 11. x 110.

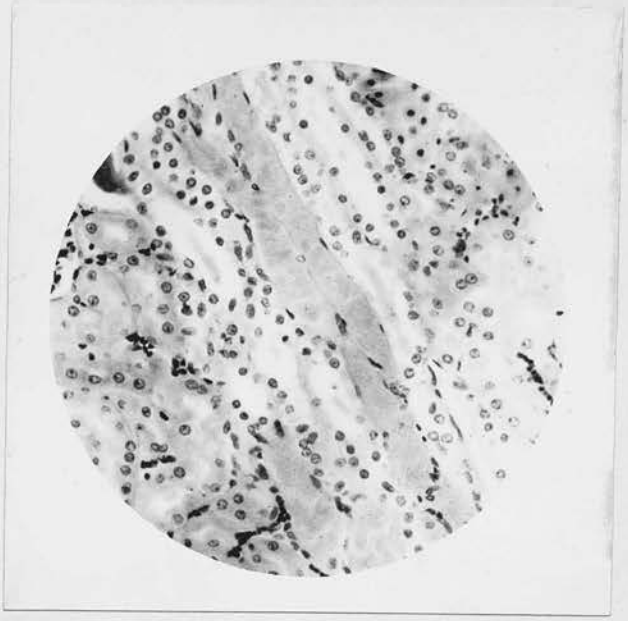


Fig. 11A. x 160.

Figs. 11 and 11A. Ascending limbs of Henle showing cloudy swelling and necrosis. The tubules merely consist of columns of finely granular, almost homogeneous material devoid of nuclei. Note that the adjacent collecting tubules are healthy. (Haem. and Eos.)

restricted to the zone normally occupied by the fila-
:ments or distributed through the entire depth of the
cells but most densely in the region of the basement
membranes. Wherever the cells show any degree of
swelling the granules, however, tend to be disseminat-
:ed uniformly or irregularly throughout the cytoplasm
of the swollen cells, and this I have found to be the
commonest distribution of the granules in cases of ac-
:ute poisoning. In all such acute cases, moreover,
the granules remain minute. In one case where the in-
:toxication was subacute large, eosinophilic granules
similar in character to those described in the con-
:voluted tubules are visible.

A noteworthy feature is the rapidity with which
the nuclei degenerate and disappear. Thus, nuclei are
often seen in advanced stages of karyolysis; then,
occasionally, short strips of the tubules are observed
to have lost their nuclei; finally and most frequent-
:ly of all tubules may exhibit no nuclei whatever.
When this state of affairs is reached each tubule con-
:sists merely of two basement membranes enclosing a
dense column of granular débris (Figs. 11 and 11a).
The poison would thus appear to be particularly injur-
:ious toward the nuclei of the ascending limbs. A
further stage in the process of degeneration is seen
where/

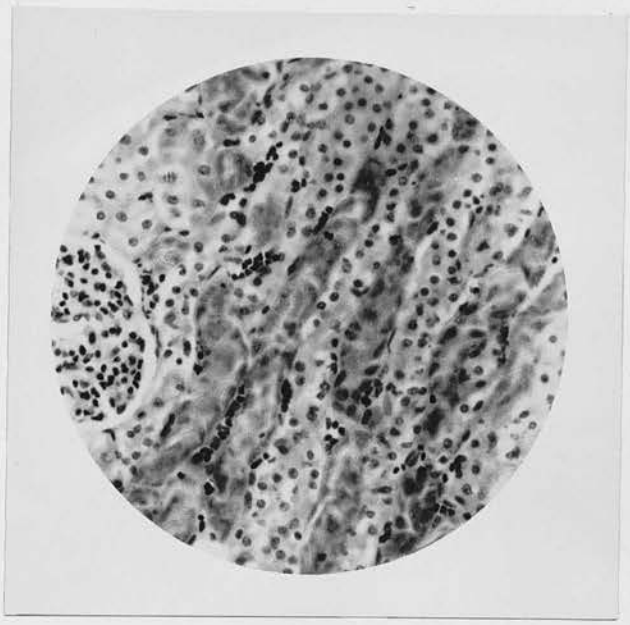


Fig. 12. Ascending limbs of Henle showing coagulative necrosis. The cells lining these tubules are of normal dimensions, but their nuclei have disappeared and their cytoplasm is markedly hyaline and acidophile in character. (Haem. and Eos.) x 200.

where the débris derived from the swollen cells be-
:comes homogeneous and hyaline in appearance and stains brilliantly with eosin. All the above-described chang-
:es are characteristic of cloudy swelling and necrosis.

The severest degree of injury exhibited by the ascending limbs is observed in cases (Experiments 1 and 10) where the lining cells have undergone coagulative necrosis (Fig. 12). These cells do not show any swell-
:ing, but are of normal dimensions, while their cyto-
:plasm is markedly hyaline and acidophile. With the occurrence of this change the majority of the nuclei disappear: an examination of the few remaining shows that the nuclei first become intensely pyknotic and sometimes shrunken before they fade away.

Sometimes each ascending limb seems more or less uniformly affected throughout its entire length, but in several experiments it can be clearly demonstrated that the segments of the tubules situated in the bound-
:ary zone of the medulla have sustained a proportion-
:ately greater degree of damage. The reason for this will be discussed later.

Sudan III and Aldol. In the milder cases of intoxic-
:ation no fat can be detected in either the convoluted tubules or the loops of Henle though haematoxylin and eosin may reveal the presence of degenerative changes.

In/

In the severer cases of poisoning fat granules are first discernible in the ascending limbs of Henle's loop. Thus, in Experiment 4 I have been able by means of the aldol method to demonstrate the presence of fat granules in the ascending limbs after a period of 4 hours' intoxication, but at this stage no fat is discernible in the convoluted tubules. In still more severe cases of poisoning fat appears in a certain num-:ber of convoluted tubules. But at best it is only a small proportion of these tubules that is affected. The vast majority even in the worst cases fail to ex-:hibit the presence of any fat. This absence of fat in the convoluted tubules is rather remarkable consider-:ing the degree of degeneration frequently exhibited by them and is further to be contrasted with the state of the mitochondria which is to be described. When it appears fat is most commonly to be found distributed in little groups of globules adjacent to the basement membrane. Two or three such groups may be observed at various points round the tubule and the cytoplasm be-:tween may be devoid of fat. Each group comprises several globules, some fairly large, others minute. In other cases not only are such accumulations present, but the cytoplasm between the latter and that encroach-:ing upon or actually occluding the lumen may be ir-:regularly/

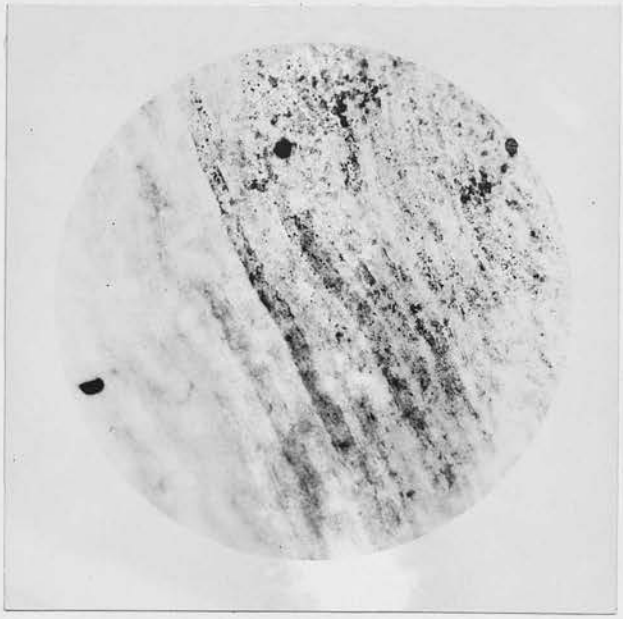


Fig. 13. Ascending limbs of Henle showing fatty degeneration. The fat is distributed irregularly throughout the tubules in the form of minute granules. (Aldol). x 120.

Fig. 14. Ascending limbs of Henle in the boundary zone of the medulla showing an advanced degree of fatty change. The fat is deposited as large globules which occupy the entire depth of the lining cells. (Sudan III and Haem.) x 380.

irregularly showered over with minute fatty granules. From the manner in which they are grouped the large globules would appear to be formed by the coalescence of numerous smaller ones.

As already stated the ascending limbs of Henle are the first tubules to exhibit the presence of fat. Ex-:periment 4 demonstrates fat-granules in the cells of these tubules after the lapse of only 4 hours. In an early case such as this the granules take the form of spherules showing considerable variation in size: many are extremely minute, others somewhat larger. They tend to be accumulated in greatest numbers in the immediate vicinity of the basement membrane and to be-:come scantier toward the lumen. Sometimes the area of cytoplasm around the lumen is free of granules and in this region at best they do not exceed more than two or three isolated granules. In severer cases where the lumen becomes more or less obliterated by infil-:trated cellular débris the fat granules are scattered sometimes more or less uniformly, sometimes irregular-:ly, throughout the tubule (Fig. 13). In the latter instance the granules tend to be more densely grouped together at various points and not infrequently the granules in these clumps are all fairly large; some-:times, indeed, very big globules of fat occur in such focal/

focal accumulations. Such accumulations may be found either adjacent to the basement membrane or in the centre of the tubule.

In any one experiment all the ascending limbs are affected in about the same measure. Further, in most cases the deposit of fat occurs more or less uniformly throughout the entire length of the tubule, no one segment being more heavily affected than another. But occasionally the fat is clearly deposited in largest amount in the boundary zone of the medulla. Thus in Experiment 15 a very marked degree of fatty change - the most marked degree observed throughout my series of experiments - occurs in the region of the boundary zone (Fig. 14). The fat in this case can be seen to be deposited in very large, round or oval, globules occupying the entire depth of the cytoplasm lining the tubules. The origin of such localised deposits of fat will be discussed later.

Collecting Tubules.

The cells lining these tubules are almost invariably healthy. In only one of my experiments, viz. 8, in which the intoxication was fairly severe, do they exhibit any signs of degeneration. Even in this instance the majority of the collecting tubes fail to show/

show any obvious departure from the normal. But the cells of some, especially in the deeper part of the cor-:tex and boundary zone of the medulla, exhibit varying degrees of karyolysis of their nuclei. Marked karyo-:lytic changes in the nuclei are accompanied by dis-:integration of the cytoplasm and filtering of the latter in the form of granular débris into the lumen. These are the only changes I have observed in the coll-:ecting tubules. Actual desquamation of cells I have never seen nor have I ever been able to demonstrate by aldol or sudan III the presence of fat granules in their cytoplasm.

In the majority of my experiments the collecting tubes contain casts of various kinds. These will be described below.

Calcification.

In three of my experiments (7, 11, 14) I am able to demonstrate the occurrence of calcification. In Experiments 7 and 11 the deposit of calcium has occur-:red in association with tubules, in Experiment 14 with casts.

In both Experiments 7 and 11 calcification is lim-:ited to the ascending limbs of Henle and the convolut-:ed tubules have escaped entirely. In Experiment 7 calcification/

calcification has just begun and is very limited in its extent. The ascending limbs in this case are re-:presented in haematoxylin and eosin section by columns of granular material devoid of nuclei and small seg-:ments of this are beginning to stain deeply with haematoxylin. In Experiment 11 calcification is an extensive and outstanding phenomenon. The cells lining the ascending limbs are in this case sharply defined. They show no swelling whatever and would not infrequent-:ly appear to have undergone a degree of shrinkage and narrowing. They have lost their characteristic gran-:ular appearance and are seen now to be composed of homogeneous hyaline material which has stained intense-:ly with haematoxylin. In the majority of cases the nuclei have disappeared entirely. Here and there the shrunken remains of nuclei showing karyorrhexis can be seen rather faintly lying in the blue-staining cyto-:plasm, but the number of nuclei so discernible is small. The hyaline cytoplasm shows a great tendency to crack and split in the course of preparation. Both sudan III and aldol fail to reveal the presence of any fat granules in the lining cells of these tubules.

The fact that these cells have stained with haema-:toxylin (Fig. 15) suggests alone that they are the seat of calcification. To confirm this observation paraffin/

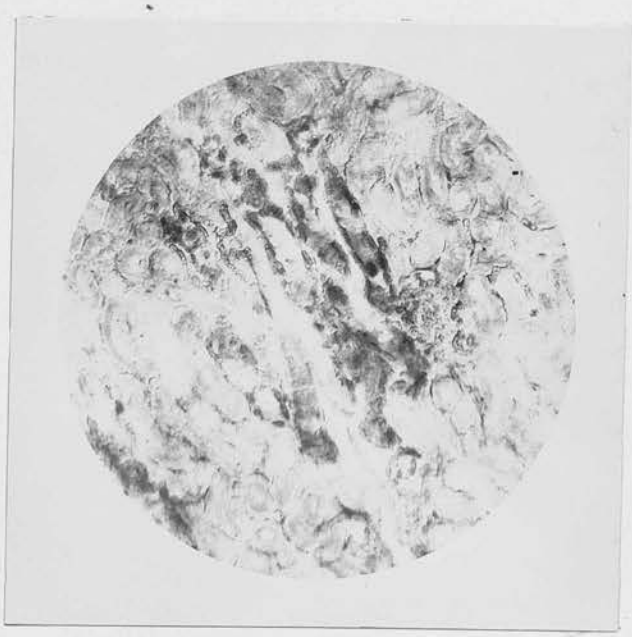


Fig. 15. Calcification of the ascending limbs of Henle. The calcified tubules have stained intensely with haematoxylin. (Sudan III and Haem.) x 100.

Fig. 16. Calcification of the ascending limbs of Henle. The calcified tubules have stained black with silver nitrate. (von Kossa's method). x 40.

paraffin sections were subjected to the action of a 1 per cent. solution of silver nitrate (von Kossa's method). So treated, all the ascending limbs without exception stain black (Fig. 16). Here and there a tubule less heavily affected is seen and in the init-:ial stages of their deposit the calcium salts can there be observed to take the form of coarse irregular granules deposited uniformly throughout the depth of the cells. No part of any cell appears more affected than another. These granules gradually become more numerous and larger until finally the cell gives a uni-:form black staining. Thus stained the ascending limbs of Henle stand out conspicuously as dense black columns running in the medullary rays. Prior to staining with silver nitrate the tissue was treated with dilute HCl: the result was that the ascending limbs failed to give a positive staining reaction. The tissue had undoubtedly been decalcified by the acid.

Of all stains for calcium the most specific is alizarin sulphate (Cameron)[33] which stains only calcium, strontium and iron irrespective of the character of their anion. Calcium and strontium are stained red, iron blackish-purple. After treatment of sections with a 1 per cent. aqueous solution of alizarin sul-:phate/

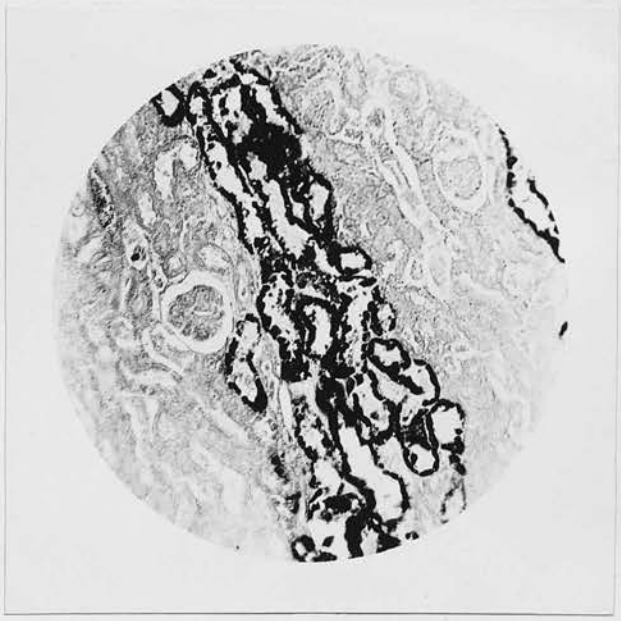


Fig. 17. Calcification of the ascending limbs of Henle. The calcified tubules have stained bright red with alizarin sul-:phate. x 100.

sulphate the cells of the ascending limbs stain a bright red colour even after decolourisation of the remainder of the tissue (Fig. 17).

After obtaining positive results with these sever-:al tests I am in a position to state with confidence that the ascending tubules are the seat of calcificat-:ion. All the calcium is, moreover, according to my observations definitely intracellular in position. Un-:like Virchow I have not observed any deposit of cal-:cium in the lumina of the ascending tubules. The pathology of this phenomenon will be discussed later.

Cast-Formation.

Attention has already been drawn to the occurrence of small masses of finely granular, almost homogeneous material in the convoluted tubules. Long columns com-:posed of similar material are often to be found in the descending limbs of Henle's loop, but the structure and development of the various kinds of casts can be followed best in the collecting tubes where they are frequently to be observed in considerable numbers.

In a fairly severe case of intoxication some collecting tubules may show short narrow streaks of loose, granular, faintly pink-staining material. Others contain longer and denser columns of such dé-:bris/

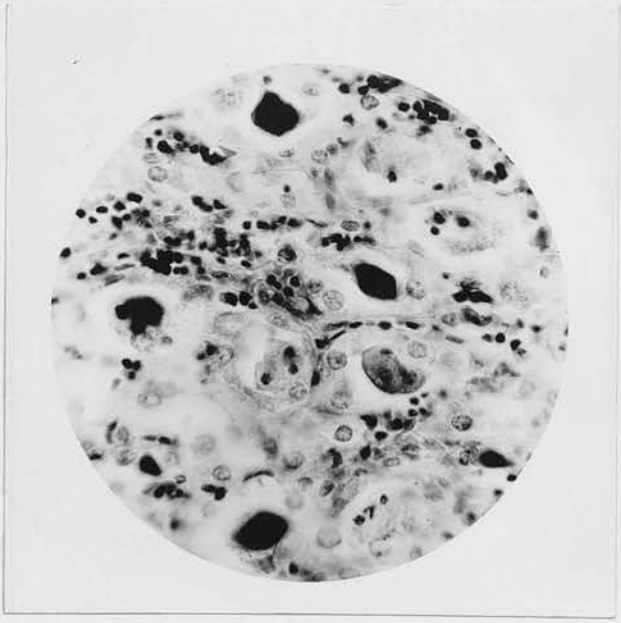


Fig. 18. Cast-formation in the collecting tubules. The faintly-stained casts consist of finely-granular débris with the remains of degenerated nuclei. The dark-:ly stained casts are composed of hyaline, acidophile substance. (Haem. and Eos.) x 200.

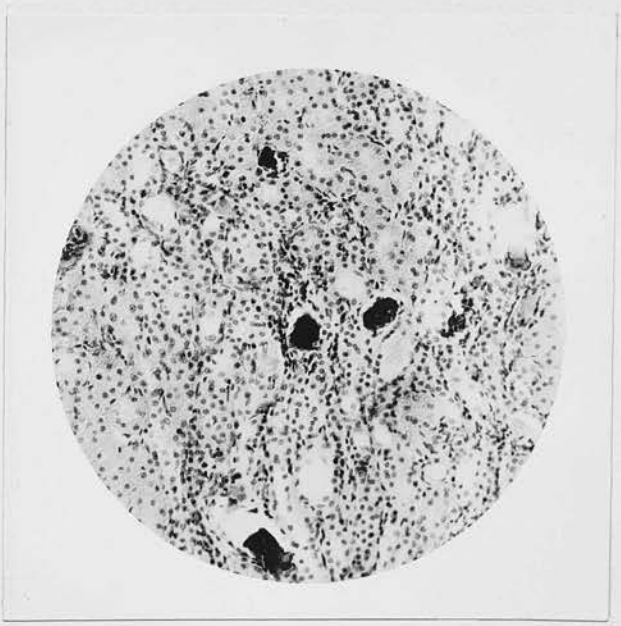


Fig. 19. Calcified casts in the collecting tubules of the cortex. The casts have stained deeply with haematoxylin. (Haem. and Eos.) x 160.

débris. Many other tubules exhibit long columns of dense, hyaline, refractile substance intensely stained with eosin. The intensity with which these structures stain with eosin often renders them very conspicuous. (Fig. 18). Frequently throughout the substance of these columns are scattered cells or fragments of cells with nuclei showing pyknosis or karyorrhexis. Sudan III may reveal the presence of small fatty globules in these cells. In the case of the faintly-staining casts the outlines of the cells are often still dis-:cernible, but not infrequently their cytoplasm has fused with the granular material around— and such fus-:ion is always present in the case of the deeply-staining eosinophilic casts. In the latter, too, many of the nuclei are fading out of existence; oc-:casionally they are fragmented. Casts intermediate in their staining properties between the finely-granul-:ar, faintly-staining type and those which are homo-:geneous and strongly eosinophilic can also be made out.

The casts in the earliest stages of their format-:ion thus consist of faintly-staining granular mater-:ial mixed, it may be, with variously-shaped fragments of cells. These elements are undoubtedly carried down from the convoluted tubules above and represent in/

in the main the products of disintegration of the cells lining these tubules. Granular material and cell-fragments then undergo a hyaline, basic change into homogeneous eosinophilic substance, the last structures to disappear being the degenerated nuclei. The end-result is thus the hyaline, eosinophilic cast. Scat-:tered through such a cast may sometimes be seen numbers of minute vacuoles - no doubt representing fat-globules. These various kinds of casts are present in greatest numbers in the deeper part of the cortex and boundary zone of the medulla. They may also be found in the superficial cortex and lower medulla, but in these reg-:ions, especially the latter, they are as a rule much less in evidence.

In Experiment 17 several of the collecting tubules in the cortex exhibit homogeneous casts which have stained intensely blue with haematoxylin (Fig. 19). These casts also stain black with silver nitrate and can, therefore, be regarded as the seat of calcareous deposit. The tubules containing them are in no way dilated, but the lining epithelial cells show in places a tendency to undergo flattening.

Interstitial/

Interstitial Tissue.

The congestion of the intertubular capillary plex-:us and the arteriae rectae of the medulla has already been remarked upon. This hyperaemia in its varying degrees is the one and only change I have ever noted in the interstitium of the kidney. In presence of its severer grades I would not have been surprised if I had discovered haemorrhage occurring from the dilated cap-:illaries, but this I never observed. Lyon describes some oedema of the stroma, but I cannot confirm this ob-:servation. Nor can I support Karvonen's statement that a perivascular infiltration with mononuclear leu-:cocytes is an almost constantly recurring phenomenon after the second day, for such never occurred in my series of experiments. Nor have I ever observed ac-: cumulation of such cells around necrotic and calcif-:ied tubules nor their emigration into such tubules as Karvonen describes. In like manner my experiments have never revealed any increase of stroma even in the most chronic cases of intoxication.

Beyond congestion which, however, is a constantly recurring and often very severe phenomenon the inter-:stitial tissue does not exhibit any pathological ab-:normalities in my experiments.

Mitochondria.

Introductory. With a view to studying the earliest and minutest changes produced by corrosive sublimate in the cells of the kidney tubules I determined to make observations on the mitochondria. The investig-:ation into the changes which these fascinating struct-:ures undergo has constituted a large and most interest-:ing part of this research.

Mitochondria were first brought into prominence by Altmann[68] in 1894 as the result of a brilliant series of investigations. But consequent upon the bizarre speculations which he made regarding their or-:igin and character his work fell into disrepute. He thought that the granules were elementary organisms which existed in the form of colonies in all cells. In the light of modern research such an idea is unten-:able and at the present moment it is generally thought that they afford a new and delicate criterion of cell-activity and metabolism. That in all probability they do play a rôle in the metabolism of the cell is evident from Champy's statement (1911)[34] that "he would not re-:gard as living a cytoplasm which does not contain mitochondria", and from Cowdry's observation (1916)[35] that "mitochondria are as characteristic of the cell as/

as chromatin is of the nucleus". Again, Romeis (1912)[36] has described an increase of mitochondria in the cells of regenerating tissues. Goetsch (1916)[37] has discov-:ered that increased functional activity on the part of the thyroid entails an increase of mitochondria in the epithelial cells of that gland. And Lewis and Lewis (1915)[38] have given an exhaustive account of the extremely pleomorphic character of mitochondria in liv-:ing cells; they carried out their investigations on tissue cultures and were actually able to observe the mitochondria continually bending and changing in shape. In view of all the findings we are, therefore, justif-:ied in concluding that in all probability mitochondria are not only constant structures in the cytoplasm of all cells, but that there can be assigned to them a function of some essential importance. What this function may be, however, is still beyond our know-:ledge.

Of late years an endeavour has been made to inter-:pret the earliest phases of cell degeneration in terms of changes in the mitochondria. Thus, Cowdry[35] states that "variation in the size and shape of the mito-:chondria offers the most delicate criterion of cell-injury"; and more recent experimental results obtain-:ed/

obtained by Lorrain Smith and Rettie (1925)[2] lead them to make the statement that "cloudy swelling and fatty degeneration (in the kidney) begin in damage to the mitochondria". Gough (1931)[39] substantiates these statements. Thus it was with a view to determining the earliest changes in the kidney cells that the foll-:owing observations were made on their mitochondria. These observations will be found to throw some light on (1) the relative effects of corrosive sublimate on the cytoplasm and nuclei of the renal cells; (2) the variety of changes which the mitochondria of the renal cells may exhibit during the early phases of cell-in-:jury (cloudy swelling and fatty degeneration); and (3) the relations that exist between the granules of cloudy swelling, those of mitochondrial disintegration and the earliest globules of fat to appear in degener-:ated kidney-cells.

In the study of these changes I have employed as stated in the paragraph on "Methods of Investigation" the Bichromate Method described by Lorrain Smith and Rettie in 1925[2]. This method is a modification of that originally devised by Weigert for the staining of the myelin sheath of medullated nerves and an applic-:ation of this modification to the staining of the lipoid-protein compound of mitochondria. It may here be stated that several investigators (Regaud[40], Fauré/

Fauré-Fremiet[41], Löwschin[42]) have separately come to the conclusion that mitochondria are made up of a combination of lipoid and albumin. As a result of an exhaustive series of experiments Lorrain Smith and Mair (1909)[43] advanced the theory that the staining of lipoid by haematoxylin after mordanting with bichromate is due to the presence of unsaturated groupings. At a certain stage in the process of oxidation of these unsaturated elements a lipoid-chromium link develops which is capable of forming a lake with haematoxylin and thus staining follows. As the process of oxidation continues the unsaturated elements are ultimately completely oxidised, the lipoid chromium link is no longer possible and the tissue in consequence ceases to stain. Thus if chroming be carried out for a sufficient period the myelin sheath will cease to stain. Similarly, also, but more slowly fat globules lose their power of staining.

Literature. The only reference I can find regarding the changes which the mitochondria of the kidney tubules undergo in corrosive sublimate poisoning is contained in a statement by Morel, Mourigaud and Policard (1912)[44] - "Transformation granuleuse des bâtonnets mitochondriaux".

Personal/

Fig. 20. x 440.

Fig. 21. x 1000.

Figs. 20 and 21. Convoluted tubules showing normal structure and arrangement of mitochondria. They take the form of slender filaments placed in close parallel formation normal to the basement membrane with their tips embracing the nuclei. A clear cytoplasmic zone exists between the tips of the fila-:ments and the lumen of each tubule. (Bichromate).

Personal Observations.

Normal Structure and Arrangement. As demonstrated by the Bichromate Method the mitochondria of the first and second convoluted tubules take the form of slender filaments placed in close parallel formation at right angles to the basement membrane (Figs. 20 and 21). From the latter they extend about half or two thirds of the distance toward the lumen, the nuclei as a rule being enclosed by their tips. A clear cytoplasmic zone devoid of mitochondria thus exists between the tips of the filaments and the lumen of the tubules. Occasionally it may be seen that the mitochondria take the form of discrete granules arranged in rows. The cells of the descending limb of Henle's loop contain only one or two short filaments. The cells of the ascending limb on the other hand exhibit numerous filamentous mitochondria arranged like those of the convoluted tubules in close parallel formation normal to the basement membrane; the filaments of the ascending limb are, however, shorter and more delicate than those of the convoluted tubules. The cells of the collecting tubes exhibit only a few scattered granules.

Pathological Findings. (1) Convoluted Tubules. - The earliest indication that the mitochondria of these tubules/

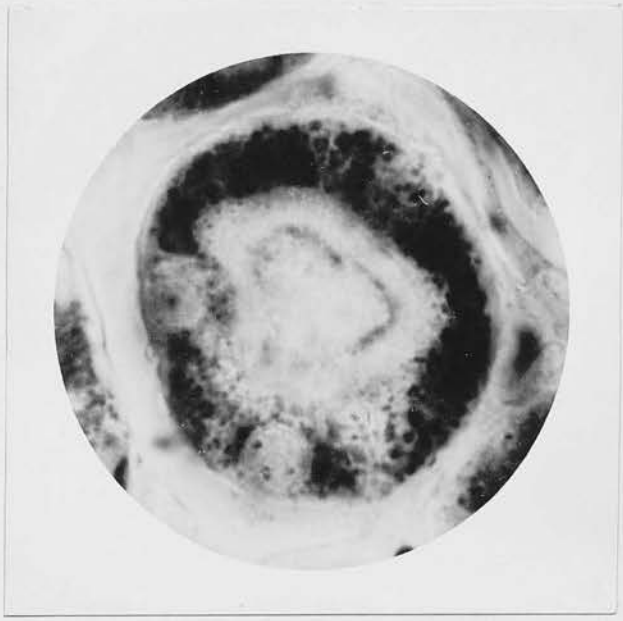


Fig. 22. Convoluted tubule showing the earliest stages in the process of mitochondrial disintegration. The filaments have undergone fragmentation with the production of a great host of granules which are distributed throughout the area normally occupied by the mitochondria. Here and there the mitochondria are disposed in rows in the position of the normal filaments. Note the clear zone around the lumen. (Bichromate). x 2000.

tubules have been injured is loss of their regular filamentous arrangement round the basement membrane. Briefly, it is seen that the filaments first of all undergo fragmentation more or less in situ with the production of a great host of granules. These are spherical or oval in shape and although all very small vary somewhat in size between minute pencil-points and globules of fair dimensions. Considered together, however, the granules are wonderfully uniform in size. Sometimes they are seen to lie more or less in rows in the position of the normal filaments. This observation would indicate that the first stage in the process of fragmentation consists in the breaking up of the filaments into several small granules which continue to occupy the position of the normal threads. But as a rule the granules are seen to have lost this row-formation and to be scattered more or less irregularly through the zone normally occupied by the mitochondria. So distributed prolongations of granules extend inward to embrace the nuclei. The clear cytoplasmic zone which occurs normally between the tips of the mitochondria and the lumen of the tubule is still observed at this stage to be devoid of granules (Fig. 22). It should be stated, however, that although they are to be observed clearly enough these early phases in the/

the process of mitochondrial degeneration are encount-:ered rather rarely. They are to be seen in the case of tubules whose lining cells are still of normal dim-:ensions, but immediately the latter show any indicat-:ion of swelling and enlargement the granules in their basal zones begin simultaneously to undergo changes in distribution. It can, therefore, be understood how very readily these early phases pass into those about to be described.

Following upon granular disintegration of the fila-:ments in situ and pari passu with the swelling of the cells the superficial granules become disseminated through the cytoplasm and so come to occupy in greater or less measure the clear zone around the lumen which as stated above is normally devoid of granules. In addition many of the tubules at this stage exhibit strands of granules which have flowed out with prolong-:ations of the cytoplasm into the lumina. Not in-:frequently such strands become detached and then irregular groups of granules are to be seen lying free in the lumina of the tubules. These groups of gran-:ules correspond with the small, round or oval casts described above as observed in the convoluted tubules in sections stained haematoxylin and eosin. This process of granular infiltration leads gradually to the/

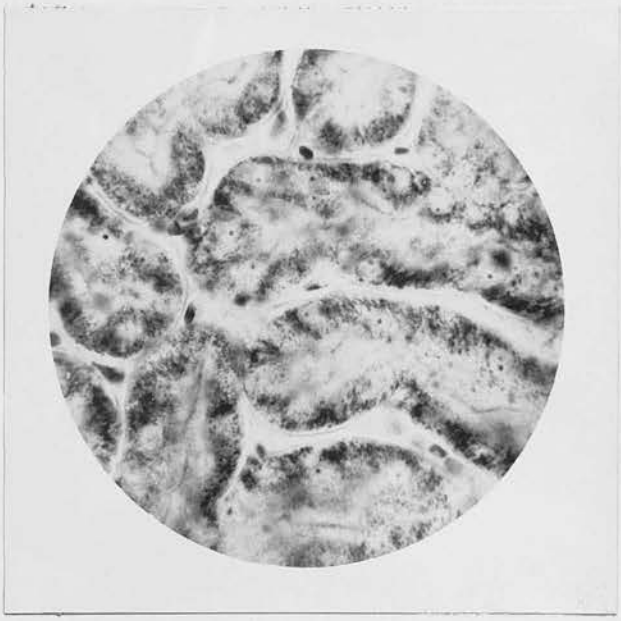


Fig. 23. x 600.

Fig. 24. x 900.

Figs. 23 and 24. Convoluted tubules showing the mitochondrial granules still gathered most densely in the region of the base-:ment membranes, but ~~note~~ how in many places the superficial granules have drifted with prolongations of the cytoplasm into the lumina. (Bichromate).

the stage where granules in varying numbers are to be found scattered throughout the cytoplasm occluding the lumen. Throughout the phases of mitochondrial dis-:integration so far described it is merely the more superficial granules that drift toward the lumen. Consequently the granules still continue to be accum-:ulated in greatest numbers and density in the region of the basement membrane; as they approach the centre of the tubule they become less numerous and more wide-:ly spaced. To such a distribution of the mitochond-:rial granules I have for the sake of brevity applied the term "basal accumulation". Such basal accumulat-:ion I have found to be the most commonly recurring mode of distribution of the granules in conditions of acute intoxication (Figs. 23 and 24).

A further advance upon basal accumulation is sometimes to be observed in tubules which have been severely injured. In these tubules the granules tend more and more to desert the basement membrane and to become scattered more and more uniformly through the swollen cytoplasm. In these instances a lumen may or may not be discernible. If not then the cytoplasm filling the tubule is evenly showered over with gran-:ules. There is at this final stage no tendency to basal accumulation (Figs. 25 - 27).

Although/

Fig. 25. x 600.

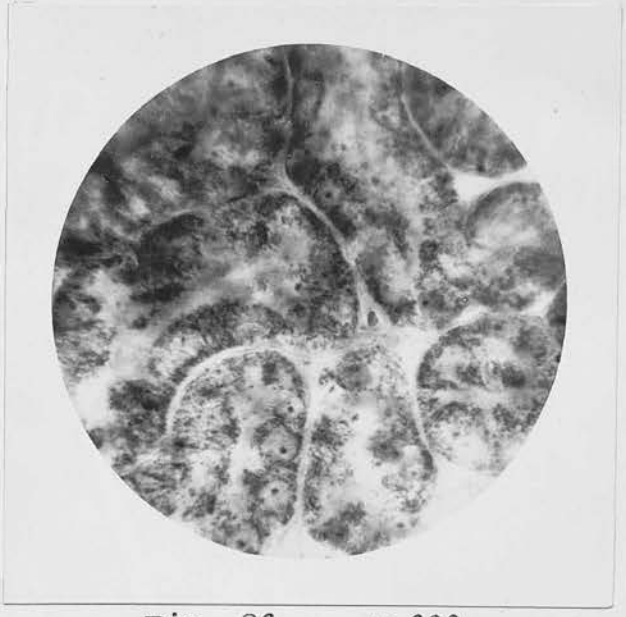


Fig. 26. x 600.

Figs. 25 and 26. Convoluted tubules showing the mitochondrial granules distributed uniformly throughout the cytoplasm of their swollen cells. All the granules are more or less equal in size and uniformly minute. (Bichromate).

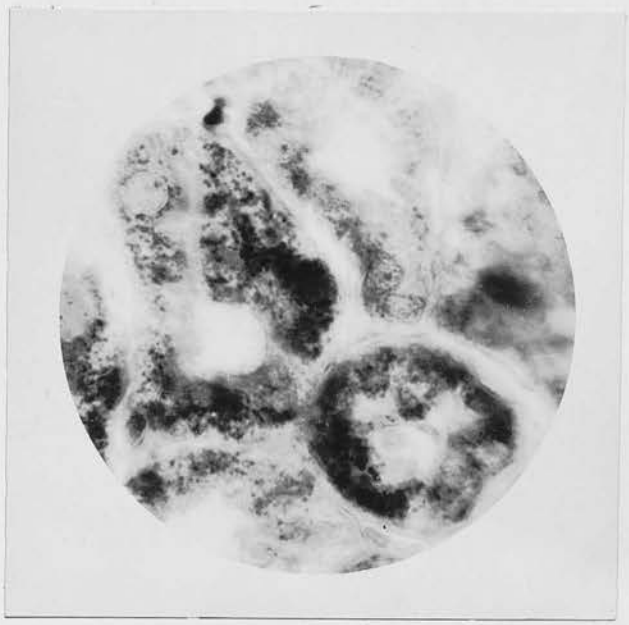


Fig. 27. Convoluted tubules showing a more or less uniform distribution of their mitochond-:rial granules. (Bichromate). x 900.

Although these various phases in the distribution of the mitochondria have been more or less separated one from another it must be understood that any such sharp distinction does not in reality occur. Although one stage usually predominates it is customary to find all stages between the earliest and the most advanced represented in varying degrees in every case. From this I deduce that the convoluted tubules are not all affected in equal degree.

Even as the mitochondria are undergoing the changes described above as occurring in acute condit-:ions of poisoning it may occasionally be noticed, especially in less acute intoxication, that a convol-:uted tubule here and there exhibits one or two groups of granules which are considerably above the average size. This increase in size of the granules becomes an outstanding feature in conditions of subacute and chronic intoxication as exemplified by Experiments 3, 10 and 14. Experiment 3 may be taken as an example. The mitochondrial granules are in this case scattered more or less <u>irregularly</u> throughout the cytoplasm of the swollen cells and show no tendency whatever to basal accumulation (Figs. 28 and 29). Indeed, in some tubules the mitochondria would appear to have altogeth-:er deserted the cytoplasm in the immediate neighbour-:hood/

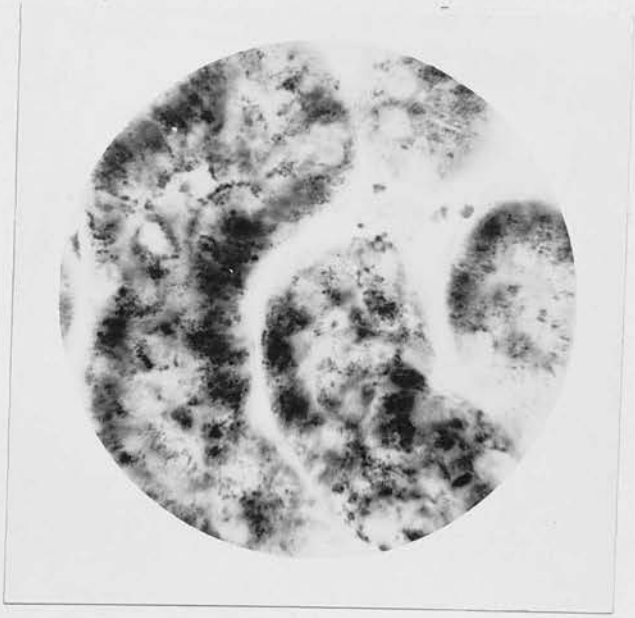


Fig. 28. x 900.

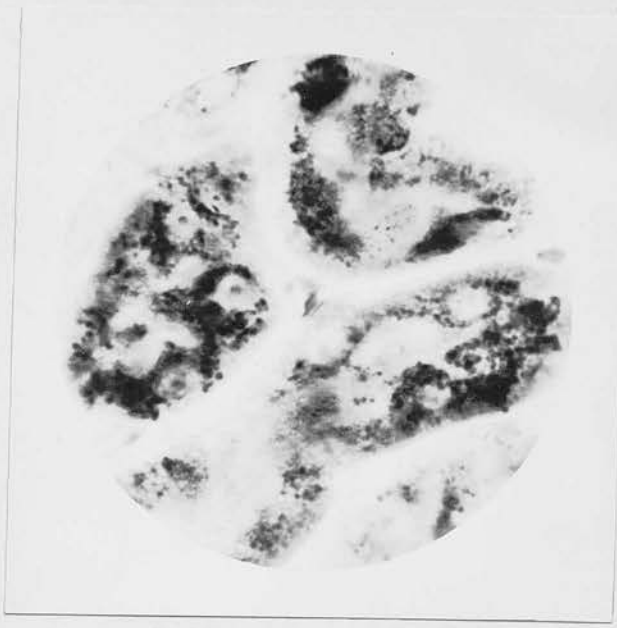


Fig. 29. x 900.

Figs. 28 and 29. Convoluted tubules in which the mitochondrial granules are showing a marked tendency to clump together in groups. Note also that granules of fairly large dimensions are beginning to make their appearance. (Bichromate).

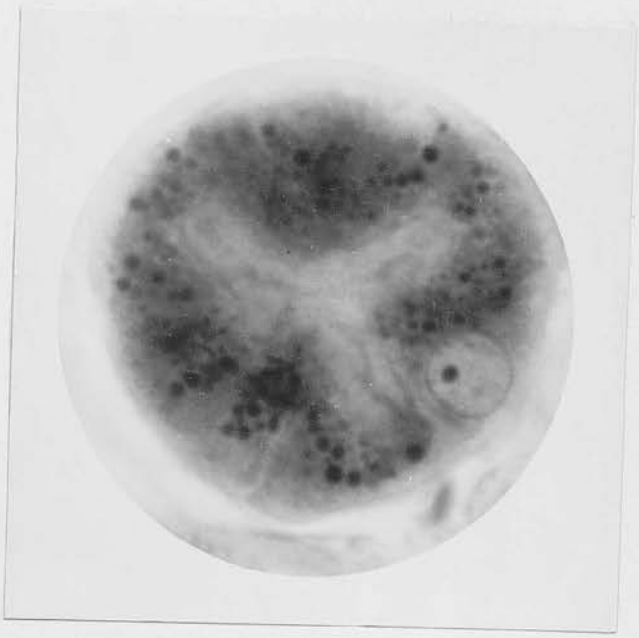


Fig. 30. x 2000.

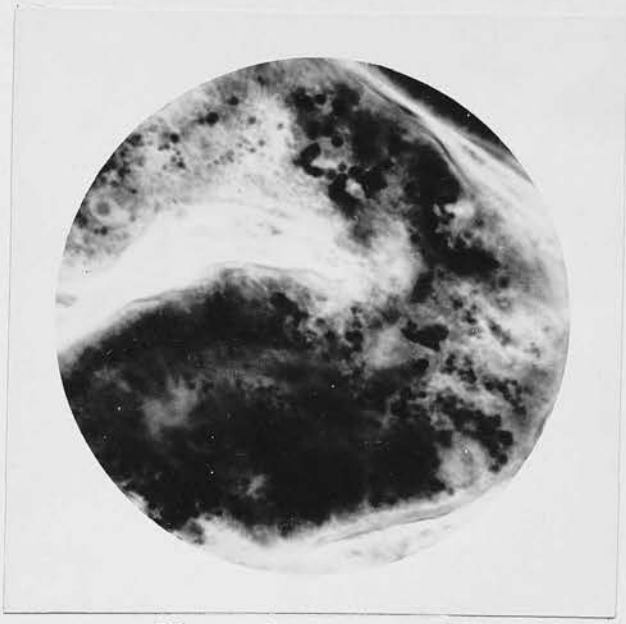


Fig. 31. x 2000.

Figs. 30 and 31. Convoluted tubules showing mitochondrial granules of large size. The distribution of the granules is very irregular and their total number is reduced. Note their tendency to desert the basement membranes. (Bichromate).

neighbourhood of the basement membrane and to be scattered between and internal to the nuclei. Many of the granules are minute, but many others are very large and between those extremes every variation can be made out (Figs. 30 and 31). The irregularity in the distribution of the granules is explained by the fact that at points here and there they are clumped to-:gether more densely than elsewhere, but even in such clumps the individual mitochondria are easily dis-:tinguishable. Sometimes the granules in such clumps show a tendency to be ringed around the nuclei which show as clear spaces (Figs. 32 and 33). Again, a large globule may occasionally be observed to be sur-:rounded by numerous smaller ones. And sometimes the larger globules are placed in rows running at right angles to the basement membrane. As stated above the striking feature is the remarkable size of many of the globules and accompanying this phenomenon there is a definite reduction in the number of granules. These findings - clumping together of the smaller granules, the presence of strikingly large globules and a reduct-:ion in their numbers - afford sufficient proof to justify the statement that the smaller granules in all probability undergo a process of coalescence with the production of larger globules and a consequent dim-:inution/

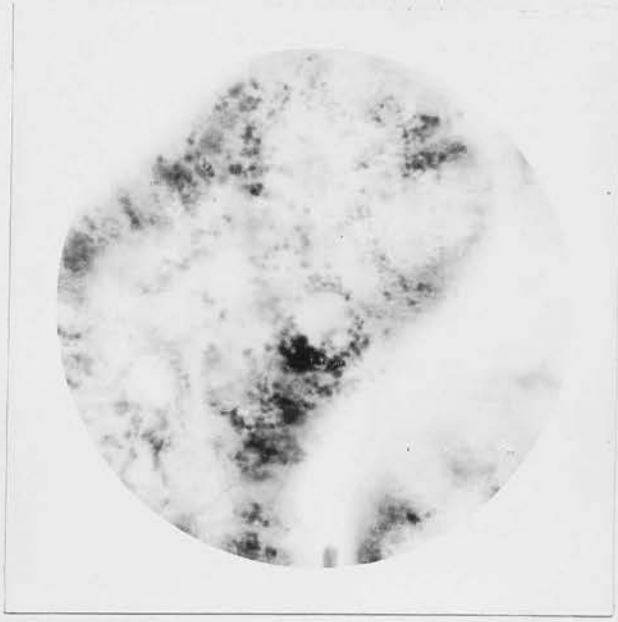


Fig. 32. x 2000.

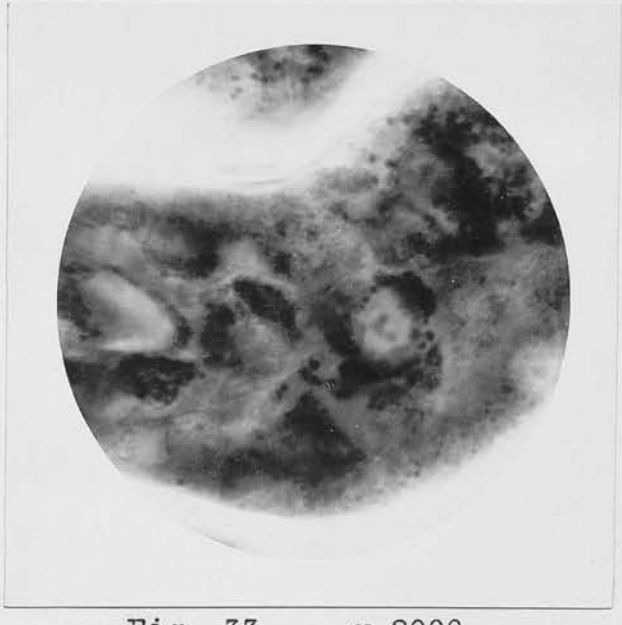


Fig. 33. x 2000.

Figs. 32 and 33. Convoluted tubules showing the mitochondrial granules arranged around the nuclei which show as clear spaces. c.f. distribution of eosinophile granules in Figs. 7 and 8. (Bichromate).

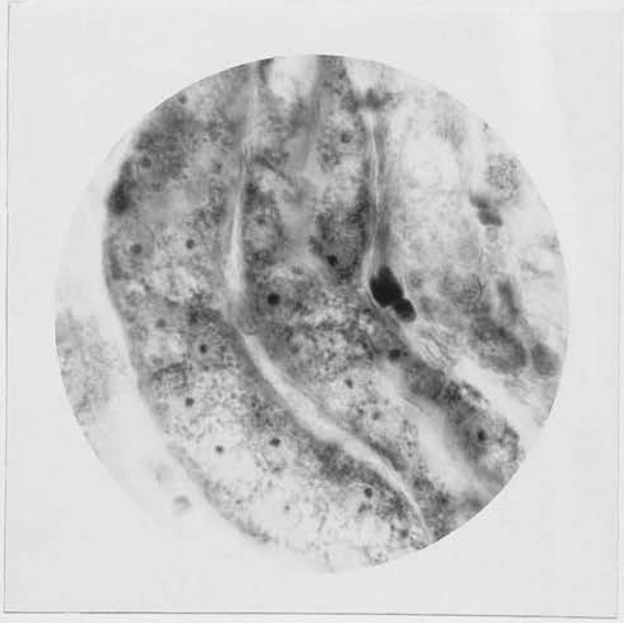


Fig. 34. x 900.

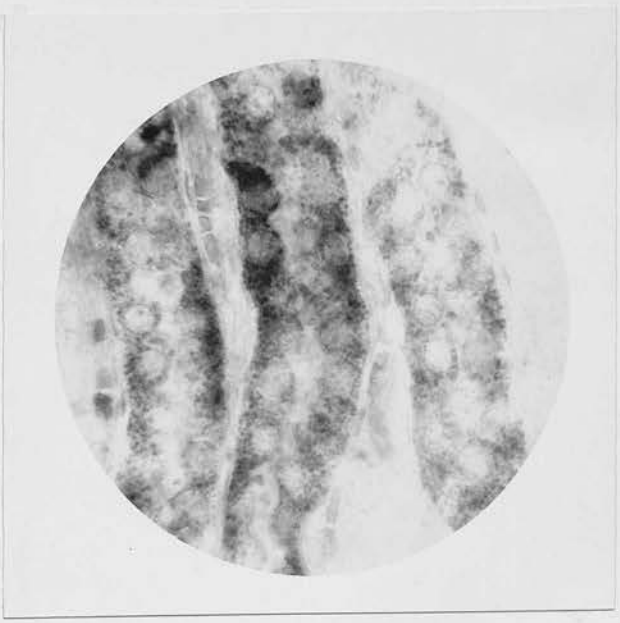


Fig. 35. x 900.

Figs. 34 and 35. Ascending limbs of Henle showing fragmentation of their mitochondrial filaments. Note the minute size and uniform distribution of the granules. (Bichromate).

diminution in number.

(11) Ascending Limbs of Henle's Loops. - Again the bichromate method reveals universal disintegration of the mitochondrial filaments with the production as in the case of the convoluted tubules of many granules. These are at an early stage distributed throughout the cytoplasm mainly in the region of the basement membrane. In some places they are restricted to the zone normally occupied by the filaments. In others the superficial granules have in addition drifted into the clear zone around the lumen. Such basal accumulation of the gran-:ules is met with only in the very earliest phases of cloudy swelling and since as has already been mention-:ed the ascending limbs are commonly the seat of severe degenerative change such a distribution of the granules is encountered but rarely.

In most of my experiments the ascending limbs ex-:hibit more or less severe grades of cloudy swelling and the bichromate method demonstrates that in such conditions the mitochondria rarely show basal accumul-:ation. Most often I have found them distributed sometimes irregularly, sometimes more or less uniform-:ly throughout the cytoplasm occluding the tubules (Figs. 34 and 35). In the former instance the gran-:ules are sometimes clumped at points here and there around/

around clear, circular spaces which undoubtedly re-:present nuclei - an appearance which suggests that cells or portions of cells and their nuclei have be-:come detached from the basement membrane. In cases of such severity the majority of the granules are ob-:served to be very minute, sometimes scarcely discern-:ible and definitely smaller on the whole than those of the convoluted tubules. They form, indeed, what might almost be described as a fine dust throughout the tubule. The presence of this dust explains a phenomenon observed when the tissue is subjected to prolonged chroming as will be referred to below. When the ascending limbs have undergone coagulative necrosis the mitochondrial granules are seen to be uniformly distributed through the cytoplasm of their lining cells.

In one experiment only (No. 3) have I observed the occurrence in the ascending limbs of mitochondrial granules of large size. Experiment 3 it will be re-:membered was a case of subacute intoxication and as the condition of the globules is exactly comparable with that of the granules in the convoluted tubules already described there is no necessity for repetition.

I have noticed that occasionally when the ascend-:ing limbs of Henle are the seat of fatty degeneration the mitochondrial granules may require a longer period of/

Fig. 36. x 120.

Fig. 37. x 80.

Figs. 36 and 37. Ascending limbs of Henle - the seat of diffuse fatty degeneration - uniformly and deeply stained with haema-:toxylin after 86 days' mordanting with bichromate.

of chroming than the usual 2 - 4 days. Thus a period of 7 - 14 days may be necessary in order that the gran-:ules be definitely stained. This is easily explained on the grounds as shown by Lorrain Smith and Rettie[2] that globules of degenerated fat require a longer per-:iod of mordanting than normal lipoidal structures.

I have observed also that when the ascending limbs of Henle show diffuse fatty degeneration progressive mordanting with bichromate for a period of 35 days and upwards leads to these tubules being stained uniformly and deeply with haematoxylin. They thus come to be represented by intensely stained, dark columns running through the cortex and medulla (Figs. 36 and 37). Such uniform staining is probably due as explained ab-:ove to the fact that the mitochondrial granules now reduced to simpler fatty globules are distributed in the form of a very fine dust throughout the cytoplasm of the tubules and to the fact that the prolonged per-:iod of mordanting allows of the staining of every particle of lipoid or fatty substance no matter how de-:generated it may be. The phenomenon is, indeed, a striking one.

Phenomena observed on Progressive Overdifferentiation. Frozen sections exhibiting various stages in the pro-:cess of mitochondrial disintegration were subjected to/

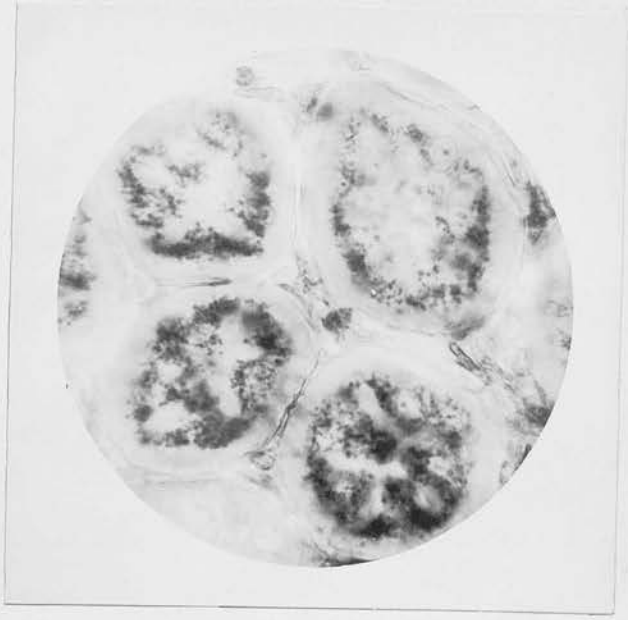


Fig. 38. x 900.

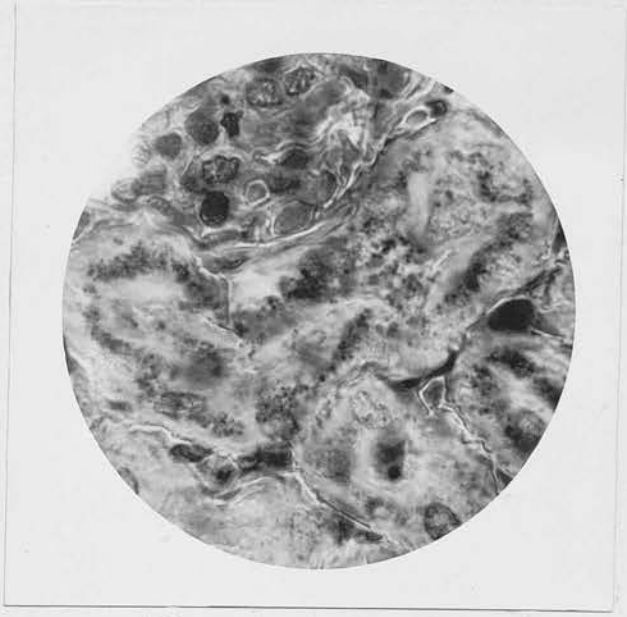


Fig. 39. x 1000.

Figs. 38 and 39. Convoluted tubules with mitochondrial granules showing the effect of progressive overdifferentiation. The granules adjacent to the basement membranes have been decolourised, while those around the lumina, having so far resisted decolourisation, are still stained with haematoxylin. (Bichromate).

to gradual overdifferentiation with borax-ferricyanide solution. It was then discovered that when the mitochondrial granules of the convoluted tubules were distributed widely throughout the swollen cytoplasm decolourisation of the granules proceeded by gradual stages from the basement membrane toward the lumen. A zone of clear cytoplasm devoid of stained granules appears adjacent to the basement membrane and this widens by degrees until ultimately only a narrow ring of granules appears stained round the lumen (Figs. 38 and 39). Finally, even the innermost or most centrally placed granules are decolourised. This phenomenon of "peripheral decolourisation" is observed only in those phases of cloudy swelling where as stated above the mitochondrial granules are widely distributed through the cytoplasm. It is not exhibited by normal mitochondrial filaments and is not observed in those cases where the granules of mitochondrial disintegration occupy the normal site of the filaments. In these instances all the granules undergo simultaneous decolourisation.

Peripheral decolourisation may be observed when the tissue is mordanted for the usual period of 2 - 4 days, but the process of overdifferentiation is apt to proceed too rapidly for the phenomenon to be seen properly/

properly. It can be demonstrated much more clearly and convincingly when the tissue is first subjected to a prolonged period of chroming, e.g. 35 days or more, in which case a strong (undiluted) solution of Weig-:ert's borax-ferricyanide must be employed if differ-:entiation is to be secured within a reasonable space of time. The prolonged period of oxidation apparent-:ly leads to the development in some way of a strong link between the lipoid and haematoxylin which is only broken down slowly and with difficulty. Under these circumstances I have found peripheral decolourisation to be a constantly recurring phenomenon.

It may be mentioned here that the uniformly and intensely stained ascending limbs of Henle above de-:scribed are among the last structures to resist de-:colourisation. Thus I have observed convoluted tubules showing advanced degrees of peripheral decol-:ourisation placed side by side with ascending limbs still deeply stained.

The significance of these phenomena will be dis-:cussed in the next chapter.

DISCUSSION/

DISCUSSION.

Microscopic examination of the kidney reveals it constantly to be the seat of two phenomena - congestion and degeneration. The first of these is the normal response of the capillary system to the presence of a poison in the blood and is manifest in all toxaemic states. The second is the result of the injurious in-:fluence of the drug on the delicate epithelium of the kidney tubules. Both phenomena have two points in common. First, they vary in degree according to the severity of the intoxication, and second, both are selective in distribution, some parts of the vascular system and certain segments of the renal units being more severely involved than the others.

Thus although the congestion is generalised the glomeruli and arteriae rectae of the medulla are us-:ually most markedly involved and sometimes the degree of hyperaemia exhibited by these structures is extreme. It is interesting, moreover, to note that in any case of intoxication the glomeruli may vary greatly in the extent to which they are involved in the congestive process. This variation may be due to the fact that the poison is being distributed unequally to the glom-:eruli/

glomeruli, or possibly it can be explained on the grounds of biological variation in the degree of re-:sistance or reactionary power possessed by the differ-:ent glomerular units. Recently, Moore and Hellman (1931)[45] carried out a large series of experiments to prove that the anuria which frequently occurs in mer-:curial poisoning is not due to any stasis of the cir-:culation through the glomerular tufts. Microscopic examination of the renal tissue alone affords proof that far from stasis being present in the glomerular capillaries the circulation through the latter is un-:duly active.

It is an interesting and remarkable fact that the glomeruli in my experiments exhibit no constant path-:ological change of note. My observations, therefore, more or less coincide with those of Lyon, but differ in several ways from those of Karvonen who, as I have already indicated, describes such phenomena as prolif-:eration of the capsular epithelium and emigration of red and white cells into the glomerular spaces. In the literature, moreover, I have drawn attention to the fact that Heineke and Kaufmann independently de-:scribe the occurrence in the glomerular capillaries of homogeneous thrombi produced by fusion of red cells and to the fact, further, that Kaufmann lays great stress on the occurrence of these thrombi as a factor in/

in the causation of the degenerative changes in the kidney. Naturally, therefore, since I have altogether failed to observe the formation of any such red-cell thrombi I cannot agree with Kaufmann's explanation of the way in which the renal lesions are produced. In acute cases of poisoning I have described the not in-:frequent occurrence of granular material in the glom-:erular spaces. With Lyon's conclusion that this material is not exudative in character, but cellular in origin and derived from the commencement of the first convoluted tubules I agree entirely.

The poison it has already been stated is peculiar-:ly selective in its action on certain parts of the renal structure. On the one hand, the glomeruli, the descending limbs of Henle's loops and the collecting tubules escape almost entirely, while, on the other, both sets of convoluted tubules and the ascending limbs of Henle are constantly involved in greater or less degree. Nor are the two series of tubules last mentioned equally involved, for in any one experiment I have invariably found the ascending limbs to be the seat of a more advanced degree of degeneration than the convoluted tubules. This selective action of the drug on certain parts of the kidney is no doubt to be associated/

associated with differences in the function and chem-:ical structure of its various segments, but in the ambiguous light of our present knowledge regarding the physiology of these segments it is impossible at this date to be more explicit. Corrosive sublimate, how-:ever, is not unique in this way for I have remarked that in toxaemias of various kinds the ascending limbs of Henle are not infrequently the most severely damaged elements in the kidney. The question is rendered still more complicated by the observation that while in rab-:bits the ascending limbs of Henle exhibit the most advanced degenerative changes, in human corrosive sub-:limate poisoning, on the other hand, it is the convol-:uted tubules that are most profoundly affected.

I have also indicated that not infrequently it is that segment of the ascending limbs which is situated in the boundary zone of the medulla which is most grossly involved. This is I am sure to be correlated with the markedly hyperaemic state of the arteriae rectae in the boundary zone - an observation which sug-:gests that those segments of tubules situated in the boundary zone are being subjected to a proportionate-:ly greater degree of intoxication. Hence the pec-:uliar affection of this segment of the ascending tubules.

The/

The convoluted tubules and the ascending limbs of Henle we have seen show varying degrees of degenerat-:ion according to the severity of the intoxication. During the early phases of degeneration through which these tubules pass interest naturally centres round the granules which appear in the cytoplasm of their lining cells. We have observed how in sections stain-:ed haematoxylin and eosin very definite granules ap-:pear in the cells of these tubules when they undergo cloudy swelling: how simultaneously the bichromate method reveals that their mitochondria undergo frag-:mentation with the production of a great host of granules: and, lastly, in the case of the ascending limbs how aldol has not infrequently demonstrated the presence therein of minute fatty granules. We natur-:ally enquire - Is it possible to relate these various types of granule one to the other? What is the relat-:ion, for example, between the granules of ordinary cloudy swelling and those of mitochondrial origin? And what is the connection between the latter and the granules of fatty nature revealed by aldol?

I shall discuss these questions in relation first to the convoluted tubules and begin by considering the changes to which the mitochondrial filaments of these tubules are subjected. It will be remembered that the earliest/

earliest change which these filaments undergo is fragmentation more or less in situ with the production of many granules which are at first restricted to the area normally occupied by the filaments. With the development of the severer grades of cloudy swelling, however, the granules become scattered widely and ultimately more or less uniformly throughout the cytoplasm of the swollen cells. And occasionally they may, as I have demonstrated, show a tendency actually to desert the region of the basement membrane.

Thus, as intoxication proceeds and cloudy swelling becomes more and more an established phenomenon it is clear that the granules produced by mitochondrial fragmentation are pari passu removed more and more widely from their normal location in the basal zone. And the question naturally arises - what is the reason for the granules undergoing such wide dissemination? The answer to this question it seems to me is but part of the larger problem as to the causation of cell-enlargement in cloudy swelling. In this state the increase in size which a cell undergoes is generally considered to be due to an imbibition of fluid, which is in its turn effected by an alteration of the intracellular osmotic tension. Now in the case of the convoluted tubules this fluid will be derived from the blood in the/

the capillaries which are situated subjacent to the basement membrane of the tubules. The fluid will thus enter the cells at their base and pass through the cytoplasm toward the lumen of the tubule. As it trav-:els in this direction the fluid will naturally tend to carry with it the granules derived from the broken-down mitochondrial filaments. So the granules will be driven from the region of the basement membrane and become disseminated more and more widely throughout the cytoplasm of the swollen cells until their distrib-:ution is practically uniform. Such reasoning serves, further, to explain why occasionally the granules show a tendency actually to desert the basal zone of the cells.

Moreover, so long as the intoxication is acute in character and the convoluted tubules are in consequence the seat of well-marked cloudy swelling these widely disseminated granules remain minute and show little or no tendency to undergo coalescence. When, how-:ever, the intoxication is less severe and the nephritis in consequence more subacute in character it is interesting to note that the granules show a greater/

greater or less tendency to run together - a phenomen-:on which is in all probability to be related again to some change in intracellular osmotic tension. This we have seen leads to the formation of globules of comparatively large size and to a definite reduction in the number of granules. The fact that the large granules so produced tend as I have shown to take up a position between and internal to the nuclei, i.e. in the inner or luminal zone of the cells, is in all probability due to their being carried thither by the absorbed fluid in the manner above described.

Now I have described how haematoxylin and eosin demonstrates the presence in the convoluted tubules of certain rod-like structures identical in shape, size and distribution with the mitochondrial filaments in frozen sections of normal kidney stained by the bi-:chromate method. Moreover, I have shown how the phenomenon of cloudy swelling is initiated by fragment-:ation of these rods into granules which subsequently during the more advanced stages of cloudy swelling be-:come widely disseminated through the swollen cytoplasm. Indeed, these granules according to my observations undergo changes in shape, size and distribution in all respects/

respects similar to those exhibited by the mitochond-
:rial granules. This is borne out particularly well in cases of subacute nephritis where the mitochondrial granules as demonstrated by the bichromate method are seen to have undergone an increase in size and a re-
:duction in number and to be distributed irregularly in relation to the nuclei, while haematoxylin and eos-
:in demonstrates granular changes of an exactly paral-
:lel character. The similarity, on the one hand, of the rods in haematoxylin and eosin sections to the mitochondrial filaments demonstrated by the bichromate method in frozen sections, and on the other, the par-
:allel changes in shape, size and distribution exhib-
:ited by the granules derived from these two sets of structures lead me to the conclusion that the rods and granules visible in haematoxylin and eosin sections and the mitochondrial rods and granules demonstrable by the bichromate method are in all respects identical. In other words, the rods visible in haematoxylin and eosin sections are really normal mitochondrial fila-
:ments and the granules characteristic of cloudy swell-
:ing in haematoxylin and eosin sections are in reality mitochondrial granules.

I would like at this juncture to emphasise the fact/

fact that the earliest phase in cloudy swelling to be observed both in sections stained haematoxylin and eos-:in and in frozen sections stained by the bichromate method is fragmentation in situ of the basal rods. This phenomenon is to be observed in the absence of any nuclear change and even before the cell shows any sign of swelling or enlargement. I, therefore, agree with Lorrain Smith and Rettie's statement that "cloudy swell-:ing begins in damage to the mitochondria". These seem to be the most delicate and, therefore, the most easily injured structures in the cytoplasm.

In the case of the ascending limbs of Henle the identity of the granules characteristic of cloudy swell-:ing and those of mitochondrial origin again in my es-:timation holds good. These granules undergo the same changes in size, shape and distribution as those of the convoluted tubules, but in the majority of cases the granules in the ascending limbs tend to be scattered more or less uniformly throughout the swollen cells whereas as will be remembered the granules of the con-:voluted tubules tend mostly to show basal accumulation. The wide dissemination of the granules is in my opinion an indication that the cells of the ascending limbs are more severely injured than those of the convoluted tub-:ules - a statement at which we have already arrived

from a consideration of other findings.

Moreover, I have shown that not infrequently the ascending limbs are the seat of fatty deposit. Ald-:ol, further, reveals that the fatty granules which make their appearance in the ascending limbs are id-:entical in shape, size and distribution with both the granules of cloudy swelling and those of mitochondrial origin. These observations justify the statement that the first granules of fat deposited in the ascending limbs are derived from constituents of the cytoplasm, viz. the mitochondria. In other words, the appearance of fat in the kidney tubules is due primarily to fatty degeneration and not infiltration. It follows also that as they fragment the mitochondrial filaments must simultaneously undergo a chemical change. Mitochond-:rial substance as stated above is thought to be of a protein-lipoid character, the fatty element in which is normally unstainable by ordinary fat-stains such as sudan though stainable by haematoxylin after mordant-:ing with bichromate. Apparently the chemical change which mitochondrial substance undergoes when it is sub-:jected to toxic influences consists in cleavage of this protein-lipoid compound as a result of which the lipoid moiety thus liberated is rendered stainable by sudan and aldol.

In/

In contrast with the ascending limbs the convol-:uted tubules only occasionally exhibit very small quantities of fat. Yet the mitochondria of these tub-:ules constantly show advanced fragmentation and dis-:semination. This means that although they are in-:jured to a degree they are not damaged so severely as to lead to dissociation of their chemical structure. The mitochondria of the convoluted tubules are, there-:fore, less severely damaged than those of the ascend-:ing limbs.

The cells of the convoluted tubules and of the ascending limbs after passing through the phase of cloudy swelling may proceed to complete necrosis. It follows from my observations above that the convoluted tubules reach the stage of necrosis without, as a rule, exhibiting any intermediate phase of fatty degenerat-:ion. The ascending limbs, on the other hand, not infrequently pass through the phase of fatty degener-:ation before becoming necrotic. Fatty degeneration, therefore, need not necessarily occur as a stage in the degeneration of cells as they pass from the phase of cloudy swelling to that of necrosis. Whether or no fatty degeneration occurs as an intermediate stage would appear to depend on the degree to which or upon the manner in which the mitochondria are injured. It is/

is, indeed, remarkable to find the poison damaging the mitochondria of one series of tubules in such a way as to free their lipoid moiety, while the mitochondria of a neighbouring series of tubules subjected apparently to an equal measure of poisoning are injured, but not not so severely as to cause dissociation of their chem-:ical structure and liberation of their fat.

In one case extremely large globules of fat are visible in the ascending limbs in the boundary zone. These globules occupy the whole depth of the lining cells and could not in my estimation be derived by a simple process of fatty degeneration from the mito-:chondria of the tubules. A considerable proportion of the fat in these tubules consists in my opinion of infiltrated fat - fat brought to the cells as food by the blood-stream and deposited in the cells because they are too injured to metabolise and mask it. In this case, therefore, the fatty deposits are due to combined fatty degeneration and fatty infiltration. The occurrence of such extensive fatty change in that segment of the ascending limbs which is situated in the boundary zone is probably to be related as suggest-:ed above to the extreme degree of hyperaemia exhibit-:ed by the arteriæ rectae.

Attention has already been drawn to the fact that in subacute conditions of poisoning granules of large size/

size, hyaline in character and strongly eosinophilic may make their appearance in the cells of the convol-:uted tubules. That such oxyphile granules may occur in conditions of subacute and chronic nephritis has long been recognised and their exact nature and origin have given rise to much speculation. As Lorrain Smith in a classical discussion on the subject of degenerat-:ion (1913)[46] states - "whether this (the formation of these granules) is to be classed with proteid-hyaline or acidophile-lipoid degeneration has not been deter-:mined".

In elucidating the nature of these eosinophilic granules I would recall in the first place my conclus-:ion above regarding the identity of the granules of ordinary cloudy swelling and those of mitochondrial origin, and in the second place the statement made in my descriptions to the effect that transitional stages can be traced between the granules of ordinary cloudy swelling and the eosinophilic granules under discussion. A consideration of these facts justifies the conclusion that the large, eosinophilic granules are in reality merely large mitochondrial granules. A comparison of sections stained on the one hand haematoxylin and eos-:in and on the other by the bichromate method certainly demonstrates without dubiety that these two types of granule - eosinophilic and mitochondrial - are absolute-:ly identical in shape, size and distribution.

But/

But wherefore the oxyphile character of the gran-:ules? It has already been seen how fragmentation of the mitochondrial filaments is accompanied by a chem-:ical dissociation of the proteid and lipoidal elements of their substance and how this proceeds until the lip-:oid moiety is liberated. Now in the convoluted tub-:ules showing these eosinophilic granules no fat has as yet appeared which means that chemical dissociation of mitochondrial structure is just in progress. To ex-:plain the phenomenon under consideration I would there-:fore suggest that as it is being dissociated the pro-:tein part of the granules simultaneously undergoes some kind of chemical change whereby basic elements are produced. In this way the granules would be rendered abnormally acidophile in their staining reaction.

The phenomenon which I have termed "peripheral de-:colourisation" is at once interesting and arresting. It will be remembered that the phenomenon is witnessed only when the mitochondrial granules are disseminated beyond their normal zone of distribution and that al-:though it occurs after only a few days' mordanting, it may be demonstrated much more convincingly after a prolonged period of chroming, e.g. 35 days. I sup-:pose that this long period of oxidation leads to the development of a stronger lipoid-haematoxylin link than is formed after only a few days' chroming. By reason of this strong link differentiation naturally

proceeds/

proceeds more slowly and the phenomenon of peripheral decolourisation is in consequence more easily observed. The suggestion that a strong lipoid-haematoxylin link develops is supported by the practical finding that a full-strength borax-ferricyanide solution is necessary in order to effect differentiation within a reasonable space of time.

What is the explanation of this phenomenon? A clue to the discovery of its meaning might be found in the observation that the ascending limb of Henle when full of aldol-stainable fat granules is the last structure to resist decolourisation. Such a tubule it will be remembered after 35 days' chroming stains uniformly and intensely with haematoxylin. And it has already been demonstrated that ascending limbs still staining solidly may exist side by side with convoluted tubules exhibiting advanced degrees of peripheral decolourisation or in which all the granules have been decolourised. From the fact that the fatty ascending limb is the last structure to resist differentiation I might conclude that the most degenerated structure is the most difficult to decolourise. Applying this deduction to the mitochondria of the convoluted tubes I am justified in saying that the mitochondria which are situated in the neighbourhood of the lumen and which most/

most resist decolourisation are more degenerated than those nearer the basement membrane which decolourise first. Unfortunately this conclusion cannot be entertained, for it means that those granules gathered round the lumen being most degenerated ought to be the first to free their lipoid moiety and so give rise to fat granules stainable by sudan.

But it is well-known that the first fat granules to appear do so in the immediate neighbourhood of the basement membrane. My reasoning, therefore, cannot be correct. Indeed, I may say that in spite of having devoted considerable thought to the elucidation of this phenomenon I have found it impossible to arrive at a satisfactory conclusion. It may be that after all it is simply a mechanical change.

Calcification of tubules has occurred in two of my experiments and in both cases its incidence is confined to the ascending limbs of Henle. In one case calcification has just begun and has been preceded by cloudy swelling and necrosis of the lining cells; in the other calcification is advanced and has been preceded by coagulative necrosis of the cells. Basing my evidence on these cases I cannot agree with either Leutert or Karvonen who independently describe the deposition of calcium salts in injured but <u>still functioning</u> cells. All the calcified cells in my experiments are obviously necrotic and functionless. Nor

can I confirm Virchow's statement that the lime salts are first deposited in the lumina of the tubules and then secondarily in the epithelium. All the calcium so far as my observations go is definitely intracell-:ular in position, excepting, of course, the calcium which has been deposited in association with casts. In both these points I am in agreement with Kaufmann, Prévost, and Lyon.

The fact that the collecting tubules are almost invariably healthy has already been remarked upon. But they afford an excellent opportunity for studying the development of casts in their various forms. The material composing these casts is derived in the main from the disintegrated lining cells of the convoluted and straight tubules above. In the earliest stages of their formation they consist of faintly staining, granular material mixed with fragments of cells con-:taining fat globules: granular material and cells then undergo a hyaline, basic change into homogeneous, eosinophilic substance, the last structures to dis-:appear being the degenerated nuclei. Hyaline mater-:ial as is well-known frequently forms a basis for the deposit of calcium salts and it is, therefore, not surprising to find that the casts in one case are cal-:cified.

It/

It is somewhat remarkable to find that beyond con-:gestion of its capillaries the interstitial tissue fails even in the most chronic of my cases to reveal any pathological abnormality. Lyon in a series of experiments extending over varying periods of time en-:deavoured by means of corrosive sublimate to produce the various glomerular, tubular and interstitial phen-:omena characteristic of acute, subacute and chronic nephritis. But his attempt proved futile, for although he was able to produce a variety of tubular changes, the glomeruli and the intertubular stroma remained to all intents and purposes passive and unaffected. In finding an absence of gross changes in the stroma I, therefore, agree with him in entirety.

CONCLUSIONS.

In corrosive sublimate poisoning:-

1. The kidney is the seat of congestion varying in degree according to the severity of the intoxication and affecting particularly the glomeruli and arteriae rectae.

2. The glomeruli beyond this congestion exhibit no con-:stant pathological change of note. But frequently granular material is observed in the glomerular spaces. This material is derived from the epithelial cells lin-:ing/

lining the commencement of the first convoluted tub-
:ules.

3. The convoluted tubules according to the intensity of the intoxication exhibit various degrees of cloudy swelling which may proceed in severe cases to complete necrosis. Severe poisoning occasionally results in coagulative necrosis.

4. The ascending limbs of Henle's loops undergo the same pathological changes, but they always exhibit a more profound degree of degeneration than the convolut-
:ed tubules. That segment which is situated in the boundary zone of the medulla is not infrequently the most severely injured part of the ascending limbs.

5. During the various phases of cloudy swelling the mitochondrial filaments of the convoluted tubules and ascending limbs undergo the following changes:-

(i) The earliest sign of degeneration is frag-
:mentation of the filaments in situ. This leads to the production of a great host of granules which are at first restricted to the zone normally occupied by the rods. Cloudy swelling thus begins in damage to the mitochondria.

(ii) The superficial granules then drift with the swollen cytoplasm toward the lumen while the more deep-
:ly placed granules constituting the majority still remain/

remain densely accumulated in the region of the base-:ment membrane. Such "basal accumulation" I have found to be the most constantly recurring mode of dis-:tribution of the granules in conditions of acute in-:toxication.

(iii) The granules then become disseminated uni-:formly throughout the swollen cytoplasm and may occasionally, indeed, show a tendency to desert the region of the basement membrane. The granules are thus removed more and more from their normal location.

(iv) In cases of acute poisoning the granules re-:main small, but in conditions of subacute and chronic intoxication they become increased in size, apparently by coalescence of smaller granules, reduced in number, and scattered irregularly throughout the cytoplasm. Under these circumstances the granules not infrequently show a distinct tendency to be distributed around and between the nuclei.

5. Haematoxylin and eosin demonstrates the presence in the convoluted tubules and ascending limbs of cyto-:plasmic rods which are similar in shape, size, and distribution to the mitochondrial filaments in frozen sections stained by the bichromate method. During the various phases of cloudy swelling these cytoplasmic rods are subjected to fragmentation with the production of/

of many granules which undergo changes in shape, size and distribution similar in all respects to those exhibited by the granules derived from the mitochondrial filaments. These observations are highly suggestive of the conclusion that the granules characteristic of cloudy swelling and those of mitochondrial origin are identical structures.

6. In cases of subacute and chronic intoxication large, hyaline, eosinophilic granules appear in the cells of the convoluted tubules. It follows from my conclusion above that these are in reality merely large mitochondrial granules. Their intensely oxyphile character is perhaps due to the fact that the proteid moiety of their structure has developed strongly basic properties.

7. Fat appears only rarely and in small quantities in the cells of the convoluted tubules. The ascending limbs of Henle, on the other hand, exhibit the presence of fat more frequently and in larger amounts.

8. The first granules of fat to appear in the convoluted tubules and in the ascending limbs of Henle are derived from the mitochondria. In the majority of my experiments all the fat observed in these tubules is derived from this source and has, therefore, been produced by a process of simple fatty degeneration.

Occasionally/

Occasionally, the amount of fat present in the cells of the ascending limbs is so great as strongly to suggest that a considerable part of it is infiltrated fat.

9. The cells of the convoluted tubules and ascending limbs after passing through the phase of cloudy swelling may proceed to complete necrosis. The convoluted tubules reach the stage of necrosis without exhibiting any fatty degeneration. The ascending limbs, on the other hand, pass through the phase of fatty degeneration. Fatty degeneration, therefore, need not occur as a stage in the degeneration of cells as they pass from the phase of cloudy swelling to that of necrosis.

Whether or no fatty degeneration occurs as an intermediate stage appears to depend on the degree of injury to which the mitochondria are subjected.

10. When the convoluted tubules exhibiting widely scattered mitochondrial granules are subjected to overdifferentiation, decolourisation of the granules proceeds from the basement membrane toward the lumen. This phenomenon which I have designated "peripheral decolourisation" is best observed when the tissue is subjected to a prolonged period of chroming, e.g. 35 days/

days or more. Its significance is obscure.

11. Calcification of tubules has occurred in two of my experiments. It is confined to the ascending limbs of Henle and has in both cases been preceded by necrosis of the affected cells. No extra-cellular deposition of calcium (except in association with casts) is to be observed.

12. The descending limbs of Henle's loops never show any pathological changes. The collecting tubules are likewise almost invariably healthy. Both types of tubule frequently exhibit various kinds of casts the material composing which is derived from the degener-:ated lining cells of the convoluted and ascending tubules. The casts occasionally become calcified.

13. Beyond congestion the interstitial tissue fails to reveal any pathological abnormalities. Even in the most chronic cases of intoxication I have never ob-:served any increase of fibrous tissue or any cellular infiltration of the stroma.

L I V E R

LITERATURE

Pilliet and Cathelineau (1892)[47] describe swelling and necrosis of the liver-cells followed by their ab-:sorption. Large spaces thus come to be present in the tissue which acquires a lacework appearance. Nuclear division and cell-multiplication are also seen to be in progress in the neighbourhood of the vessels (the latter receive no name).

Fiessinger (1907)[48] investigating the effects of corrosive sublimate experimentally states that after one hour's intoxication the liver-cells show cloudy swelling. After three hours certain cells show fat-laden vacuoles. These lesions predominate in the central zone of the lobules and their severity depends more upon the duration of the intoxication than the in-:tensity of dosage.

Burmeister and McNally (1917)[28] administered sub-:limate intra-orally to dogs. They describe marked degeneration of the cells of the central zone of the liver-lobules; this degeneration sometimes extends out to the periphery, but as a rule the outermost zone is/

is less affected and is not infrequently intact. On examination of sections stained sudan III they found that the degeneration did not involve the deposition of fat. But as stated in the literature-section on kid-:ney they employed chloretone to produce anaesthesia before administering the corrosive sublimate and some-:times supplemented this by ether. Their results are therefore more or less valueless.

Heitzmann (1918)[49] found in the liver of a person who died from mercurial poisoning on the fifth day zones of degeneration accompanied by great cellular proliferation, nuclear division taking place either by mitosis or amitosis.

McNider (1918)[29] as described under the section on kidney divided his animals into three groups. In the first two groups the animals of which died as a result of the direct action of the poison "the changes in the liver consist first in a deposition of fat in the cells surrounding the central vein of the lobule. The sev-:erer changes consist in cloudy swelling and necrosis of these cells and an extension of the process to the periphery of the lobule". This surely is an unusual sequence of events in the process of cell-degeneration. In the third group the animals of which develop an acid/

acid intoxication the liver shows "two types of change. First, evidence of repair of some previous injury as evidenced by the presence of mitotic figures and occasionally large cells with more than one nucleus"; and, second, acute degenerative changes most marked in the mid-zone and periphery of the lobule and consisting in "an acute necrosis preceded by fatty infiltration and oedema. In the areas of necrosis the sinusoids are large and distended with blood".

Miller (1926)[31] described a case of human corrosive sublimate poisoning. The liver in this instance was pale and soft and microscopically showed evidence of "profound toxic change". But in this case a degree of peritonitis was also present.

Harmon (1928)[32] reports four cases of human corrosive sublimate poisoning by intravenous injection. In each of these cases he observed a uniformly mild grade of parenchymatous degeneration of the liver with no necrosis and no selective localisation to any part of the lobules.

PERSONAL/

PERSONAL OBSERVATIONS.

Haematoxylin and Eosin. In severe cases of intoxic-:ation the liver is the seat of generalised, moderate-:ly intense hyperaemia. Portal vessels, hepatic capillaries and intralobular veins all share in the congestion. No matter whether the intra-oral or intra-:muscular method of administration be employed this congestion is as a rule uniform throughout each lobule, all its zones being equally affected. One exception to this statement will be described below. In less severe cases of poisoning the congestion of the tissue is less in evidence and may, indeed, be slight or ab-:sent.

In the majority of my experiments the parenchyma of the liver-lobules shows remarkably slight departures from the normal even in cases of severe acute intoxicat-:ion. At most I have found the liver-cells somewhat swollen and their cytoplasm the seat of an abnormal granularity. Their nuclei are not infrequently swol-:len, sometimes remarkably so, and although occasional-:ly these swollen nuclei tend to stain more deeply than usual with haematoxylin, they are as a rule ab-:normally vesicular. Some of the vesicular nuclei may have almost faded out of existence altogether. These are/

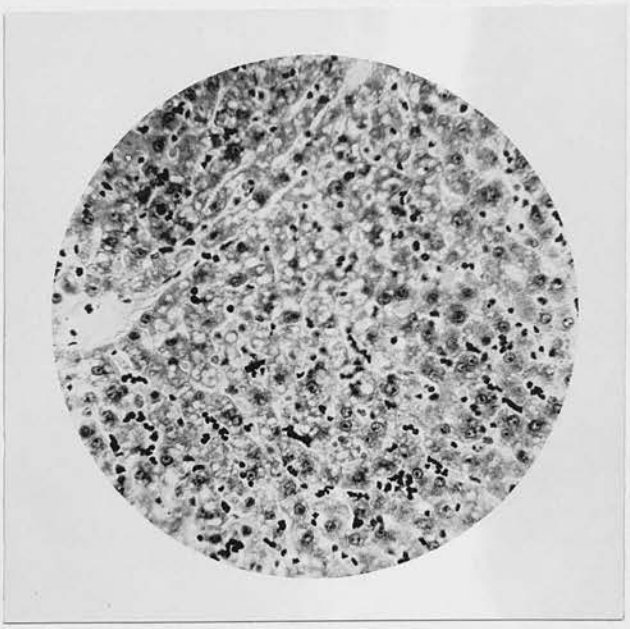


Fig. 40. Hydropic Degeneration of liver. The degeneration is most marked in the neighbourhood of the intralobular vein which is seen toward the left. (Haem. and Eos.) x 180.

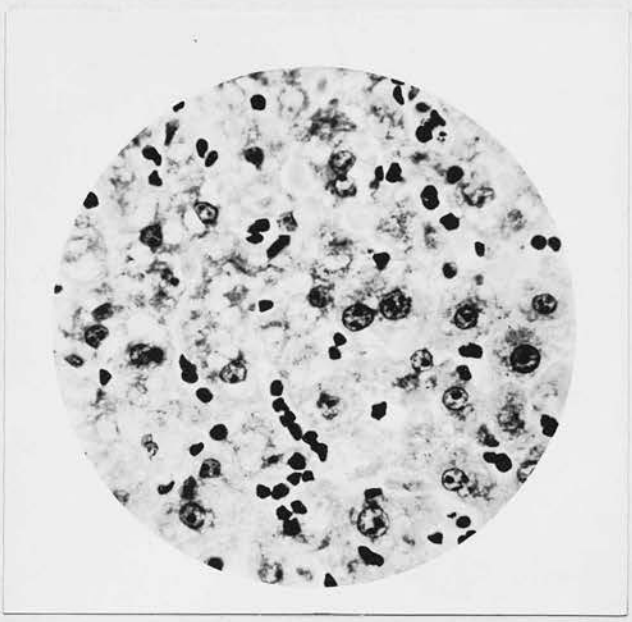


Fig. 41. Hydropic Degeneration of liver. Note the extreme vacuolation of the cells, degen-:eration and disappearance of some of the nuclei, and loss of regular trabecular structure. (Haem. and Eos.) x 500.

are the changes characteristic of cloudy swelling and like the congestion they affect each lobule uniformly no matter the method of administration of the drug.

Another feature which I have observed in two or three cases is a certain inequality or irregularity in the staining property of the tissue. In these cases numerous areas of the parenchyma, some small, some large, stain much more intensely with eosin than the tissue around. Individual cells are thus sometimes deeply stained, sometimes several cells in a trabecula, sometimes several trabeculae or portions of trabeculae. Areas so staining are not infrequently found in the im-:mediate neighbourhood of the portal tracts, but their distribution may be more or less irregular.

In three of my experiments (Nos. 3, 6, and 11) cloudy swelling of the liver-parenchyma is accompanied by another phenomenon of considerable interest. Round many of the intralobular veins in these cases the liver-cells are seen to contain clear vacuoles (Figs. 40 and 41). The latter are round or irregularly oval in shape and vary in number and size in each cell. Some-:times there is only one very large vacuole which dis-:tends the cell; sometimes a fairly large central vacuole is surrounded by two or three smaller ones, these/

these being separated by only a thin line of cytoplasm. Frequently adjacent vacuoles are seen in process of coalescing. Often these vacuoles are placed close a-:gainst the nucleus which may actually be indented at one side. Occasionally the nucleus is completely sur-:rounded by vacuoles. A striking feature in such areas is the degree to which many of the nuclei have undergone swelling and these swollen nuclei are as a rule pale and vesicular. Very occasionally a vac-:uole is visible inside a nucleus which thus acquires a signet-ring appearance. In markedly degenerated areas many of the cells are devoid of nuclei and reg-:ular trabecular structure may, indeed, be more or less destroyed. This phenomenon of vacuolation diminishes toward the periphery, but may be evident to some extent round the portal tracts. The lobules thus vary in the extent to which they are affected. In frozen sections the content of these vacuoles fails to be stained by sudan III; it cannot therefore be of a fatty nature. In the absence of fatty change the appearances are highly suggestive of Hydropic Degeneration. In one case it is a noticeable feature that the areas of par-:enchyma which are the seat of degeneration are defin-:itely more congested than the tissue elsewhere.

Throughout/

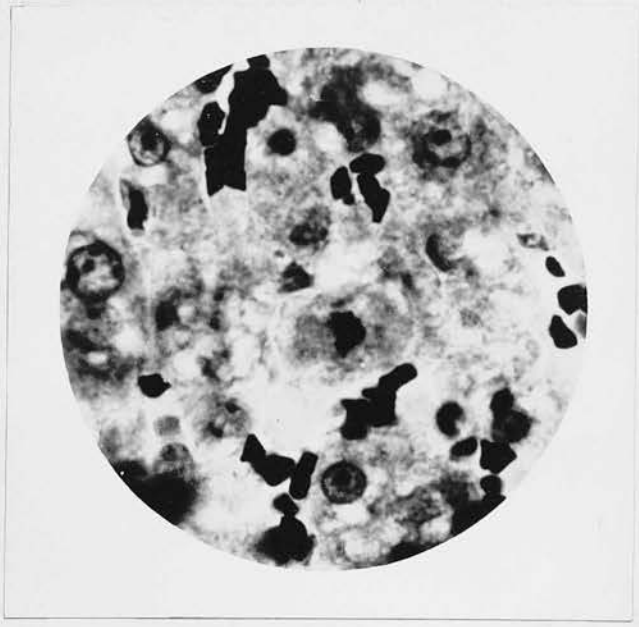


Fig. 42. Liver-cell showing mitosis. Note that the liver-cells around are the seat of hydropic degeneration. (Haem. and Eos.) x 800.

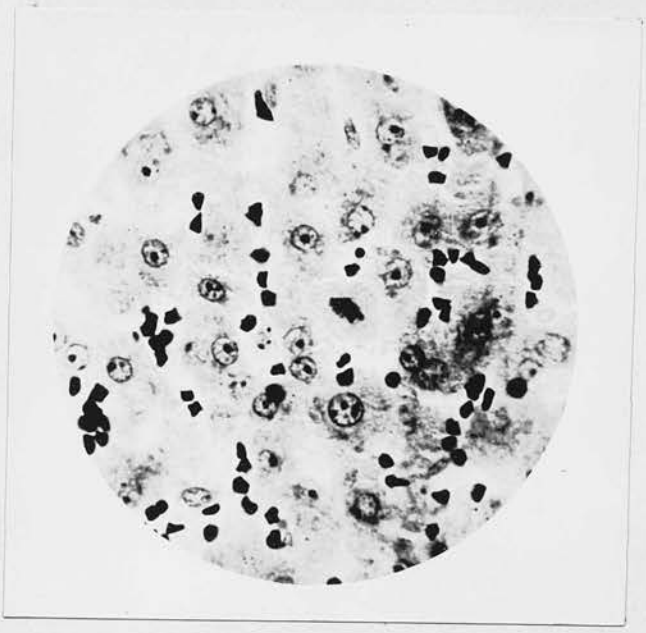


Fig. 43. Liver-cell showing mitosis. This cell is situat-:ed on the margin of an area showing hydropic degeneration. (Haem. and Eos.) x 500.

Throughout the tissue of one of the livers show-:ing hydropic degeneration numerous mitotic figures are discernible (Figs. 42 to 45). And an important observation is that each dividing cell is situated either in the centre or on the verge of an area of de-:generating cells. No mitosis is to be seen in areas showing merely the changes characteristic of cloudy swelling. Moreover, the cytoplasm of cells showing mitosis is always much healthier-looking than that of the vacuolated cells around. The figures represent all stages in the process of mitotic division of the nucleus.

In several of my experiments the Küpffer cells throughout the liver-lobules are heavily laden with granules of golden-yellow pigment and in consequence are markedly swollen. Occasionally the shrunken re-:mains of a red-cell can be seen within a Küpffer cell and sometimes a number of eosinophilic or reddish-brown granules newly derived apparently from disintegrated red-cells are to be observed in the midst of golden-yellow granules. This granular content of the Küpffer cells gives a positive Prussian Blue Reaction and is stained black by ammonium sulphide (Figs.46,47). From this observation and their association with red-cells we/

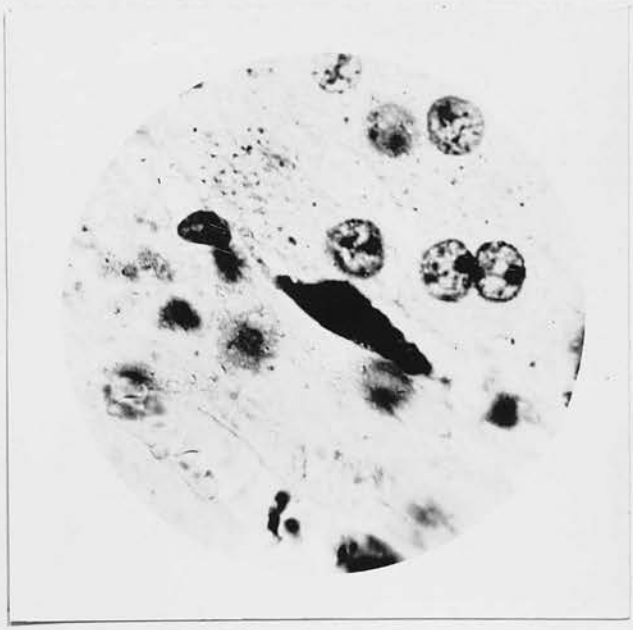


Fig. 46. x 1100.

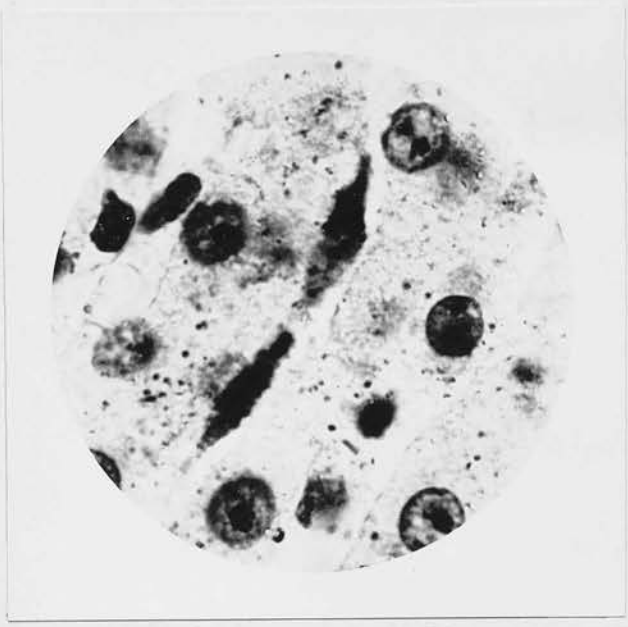


Fig. 47. x 1160.

Figs. 46 and 47. Küpffer cells heavily laden with iron-pigment. The pigment has stained black with ammonium sulphide.

Fig. 48. x 600.

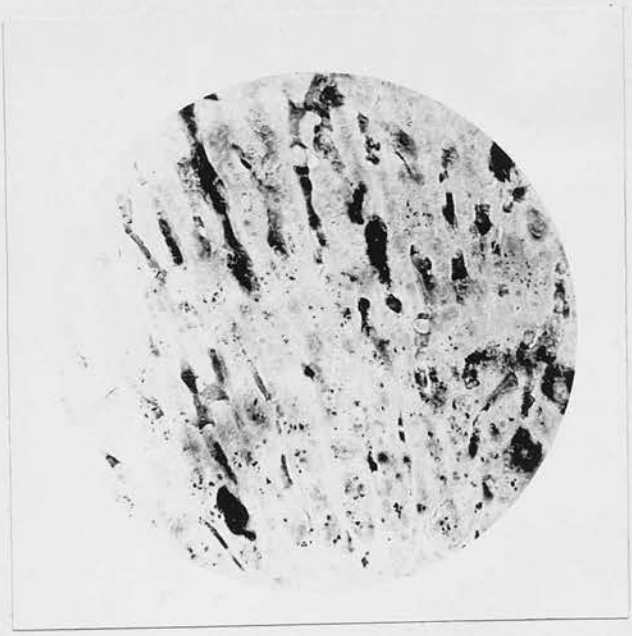


Fig. 49. x 360.

Figs. 48 and 49. Kupffer cells laden with fat-globules. (Sudan III and Haem.)

we deduce that the golden-yellow granules are of the nature of haemosiderin.

Sudan III and Aldol. In all my experiments I have made careful investigations into the presence or absence of fat in the liver-cells. But in no case have I ever observed a deposit of fat in them - an observation which is all the more striking considering the severity of the intoxication in some cases.

But an outstanding feature of my experiments has been the constancy with which the Küpffer cells have exhibited a content of fat (Figs. 48 and 49). The latter is laid down within the cells in the form of granules or globules of varying size. I have observed fat granules in the cytoplasm of these cells after a period of four hours' intoxication. As their content of fat increases the Küpffer cells become swollen, sometimes greatly so and much elongated. The affected Küpffer cells are scattered irregularly throughout the liver-lobules and never show any localisation to a particular zone. In frozen-sections the fat-laden Küpffer cells thus come to be conspicuous features of the parenchyma.

I have on several occasions also observed fat-laden cells - apparently polymorph leucocytes - among the red blood corpuscles of the portal vessels (Fig.50).

A/

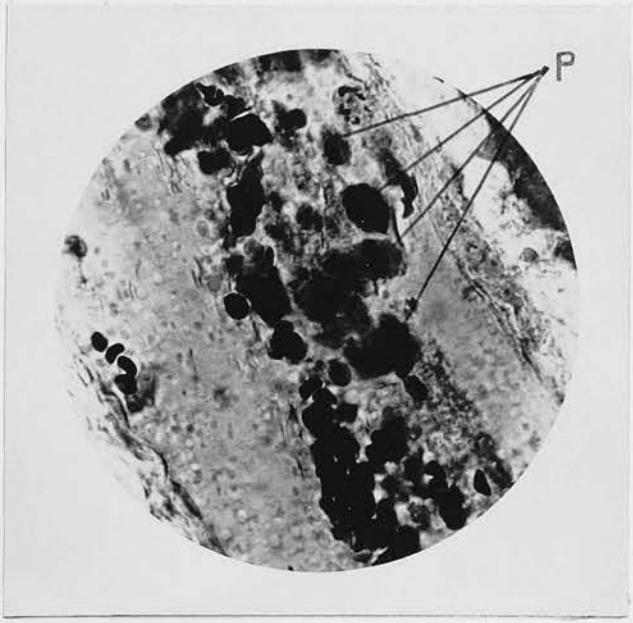


Fig. 50. A portal blood-vessel showing several polymorphonuclear leucocytes (P) laden with fat. (Sudan III and Haem.) x 840.

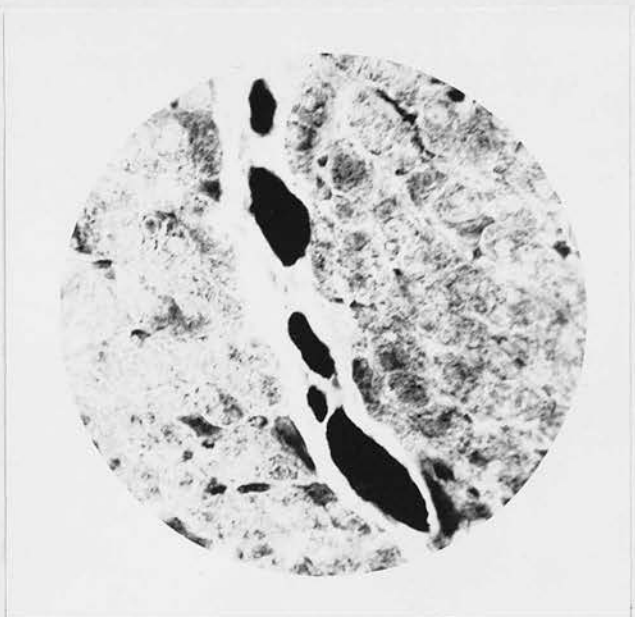


Fig. 51. Portal lymphatic vessels heavily laden with fat. (Sudan III and Haem.) x 400.

A phenomenon to be correlated with the foregoing statements is the observation that in several of my experiments the lymphatics of the portal tracts are more heavily laden than usual with fat (Fig. 51). Indeed, the extent to which these lymph-channels are swollen with fat-globules is sometimes remarkable.

The significance of these various phenomena will be discussed below.

Bichromate Method.

Normal Structure and Arrangement of Mitochondria. In their normal state the mitochondria of the liver-cells can be definitely stained after two days' mordanting (Fig. 52).

As demonstrated by this method they take the form of small, round or oval granules exhibiting a little variation in size. They are distributed uniformly throughout the cell and are set fairly closely so that each cell possesses a considerable number of them. The granules stain simultaneously and uniformly throughout each lobule.

The nucleus centrally placed is as a rule represented by a clear, circular space with the nucleolus showing up very faintly in the centre; sometimes the nucleus is stained dark gray with the nucleolus showing black.

Pathological/

Fig. 52. Liver-cells showing the normal structure and arrangement of their mitochondria which take the form of small, round or oval granules distributed uniformly through the cells and set fairly closely so that each cell possess-:es a considerable number of them. (Bichromate). x 1100.

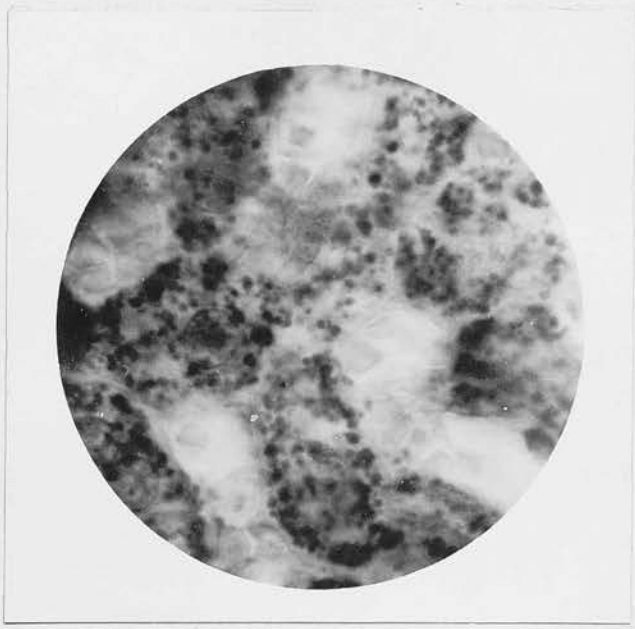


Fig. 53. x 1200.

Fig. 54. x 1100.

Figs. 53 and 54. Note (1) the increase in size of many of the mitochondrial granules: (2) the irregular distribution of the granules: and (3) the reduction in the number of granules in each cell. (Bichromate).

Pathological Findings. In all my experiments the mitochondria of the liver-cells as in the case of the normal granules have been well stained after two days' mordanting. This is true even of cases where the mito-:chondria show considerable departure from the normal.

In a case of mild intoxication the mitochondria of the liver-cells may show no departure from the normal. In severer cases while some cells continue to exhibit a normal state of their mitochondria others pre-:sent slight abnormalities. The mitochondria of these cells show a greater variation in size than is normal-:ly detectable. This is due to the appearance of granules somewhat larger than usual. Several such granules may have made their appearance. In still severer cases of poisoning all the liver-cells come to exhibit this change and the variation in size of the granules becomes much more marked (Figs. 53 and 54). Thus many of the granules may be of a size several times greater than the normal average. The granules, further, become more irregularly distributed through-:out the cells and the greater the number of large granules the more irregular is their distribution. The granules thus come to be more widely spaced than usual, though in places they may tend to be gathered together in little groups.

Further/

Further, coincident with the increase in size of the granules there is a reduction in the number of mitochondria in each affected cell. When large gran-:ules are few the diminution in the number of mito-:chondria is scarcely perceptible, but as the number of large granules increases the reduction in the total number of mitochondria becomes a clearly established phenomenon.

Thus reduced in number and many of them increased in size the mitochondria may be scattered irregularly throughout the cytoplasm of the cell and show no spec-:ial localisation, but not infrequently the granules tend to be distributed more densely round the periphery of the cell though even in such instances granules are always to be seen at the centre. Sometimes they are distributed fairly regularly round the margin, but not infrequently they tend to be accumulated here and there at the periphery in little groups.

The enlarged granules vary somewhat in shape: the majority are round, others are oval, some pear-shaped.

The above is an account of the mitochondrial changes when the liver-cells exhibit the phenomena of ordinary cloudy swelling as demonstrated by haematoxy-:lin. When the liver-cells are the seat of hydropic degeneration/

degeneration the mitochondrial granules also exhibit these changes in varying degree and are seen, moreover, to be distributed in groups particularly around the vacuoles in the cytoplasm.

The Liver in Two Cases of Human Corrosive Sub-:limate Poisoning. (Haematoxylin and Eosin).

Case 1. The liver-tissue in this case is the seat of moderately severe congestion uniform in its distribution and showing no localisation to any part-:icular zone of the liver-lobules. The cells them-:selves are the seat of slight cloudy swelling and in many cases exhibit vesicular nuclei. In addition the cells of the central zones of the liver-lobules are the seat of vacuolation. The vacuoles vary greatly in number and size in different cells. Many of the vacuoles are very large. In relation to these vacuol-:ated areas a nucleus undergoing mitotic division is occasionally to be observed.

Case 2. The tissue in this case is likewise the seat of moderately severe congestion uniform in dis-:tribution. The parenchyma shows the phenomena characteristic of early cloudy swelling with vesicular-:ity of the nuclei. Vacuolation of the cells in this case/

case is confined strictly to the peripheral zone of the liver-lobules and on the whole the vacuoles are much smaller than in the previous case. In relation to the degenerated areas numerous mitotic figures can be discerned.

Unfortunately in neither case have I been able to secure fresh tissue. It has, therefore, been impossible for me to investigate the nature of the content of the vacuoles. But certainly the resemblance between Case 1 and the three cases of hydropic degeneration which I myself produced is striking.

DISCUSSION.

In the majority of my experiments the liver exhibits the changes characteristic of simple cloudy swelling. My observations in these cases are thus in agreement with Harmon's findings in four cases of human corrosive sublimate poisoning: the liver in each of these cases was the seat of a mild grade of parenchymatous degeneration. Nevertheless, after such severe intoxication it is surprising that I have never been able to detect the presence of fat in the liver-cells even employing a staining technique so delicate as the aldol method. Considering this matter we naturally/

naturally turn to the mitochondria and enquire into their condition since it is from them that the first granules of fat are derived. There we find that in the milder cases of intoxication the mitochondrial granules show no change, but that in cases of severer poisoning they undergo a variety of changes - an in-:crease in size accompanied by a reduction in number and a change in distribution. These changes are dis-:tinct enough, but considering the severity of the in-:toxication in some cases it is remarkable that they are not of a more outstanding and drastic character. Moreover, from the observation that no fat appears in the liver-cells we can deduce that the mitochondria of these cells are not subjected to a degree of chemical dissociation sufficient to liberate their lipoid moiety. In this respect the mitochondria of the liver-cells are more or less comparable with those of the convoluted tubules of the kidney: it will be remember-:ed that in the majority of cases these tubules failed to exhibit any content of fat.

In three of my experiments (all intra-oral cases) the cells in the central zone of the liver-lobules show a high degree of vacuolation. As already stated the content of these vacuoles is not fatty in nature. This/

This fact together with the general appearance of the affected zones prompted a diagnosis of hydropic degen-:eration. Several authors also describe degeneration in the central zone of the lobules, e.g. Fiessinger, McNider, Burmeister and McNally, and possibly Pilliet and Cathelineau. Of these Fiessinger and McNider state independently that the degeneration is fatty in character, but they do not say that they stained and examined frozen sections specifically for the presence of fat. On the other hand, Burmeister and McNally state clearly that on the examination of frozen sect-:ions stained sudan III the degeneration does <u>not</u> in-:volve the deposition of fat in the central zones of the lobules. Of the two cases of human corrosive sub-:limate poisoning which I described above one liver shows vacuolation of the central zone, the other of the peripheral zone, but regarding the nature of the content of the vacuoles I am not in a position to say definitely.

Correlating the literature with observations of my own I would conclude that the degeneration affect-:ing the liver-parenchyma varies in character. Mild intoxication results in simple cloudy swelling without the deposition of fat. Severer poisoning causes vac-:uolation/

vacuolation of the liver-cells, sometimes of the cent-
:ral zone, sometimes of the peripheral zone of the
lobules. This vacuolation so far as my experiments
go does not involve the deposition of fat and would
appear to be of the nature of hydropic degeneration.

Nuclear division and its relation to the action
of corrosive sublimate was one of the points principal-
:ly to be determined by this series of experiments.
I have observed it in only one of my experiments al-
:though in this case it is an outstanding phenomenon.
As already stated all the mitotic figures are very de-
:finitely related to areas of degeneration. Both
cases of human corrosive sublimate poisoning described
above show evidence of nuclear division, one of them
particularly, and in both cases every mitotic figure
lies clearly in relation to an area of degeneration.
Again, Pilliet and Cathelineau, McNider, and Heitzmann
report mitosis in the liver and each describes it in
association with degeneration. My own observations
and those of other authors would thus make nuclear
division primarily dependent on parenchymatous degen-
:eration. Nuclear division and cell-proliferation
are, in other words, clearly secondary to degeneration
and are, therefore, with a view to repairing damage
caused/

caused by the action of the mercurial poison. Mitos-:is is thus purely a reparative phenomenon and is not the result of a direct, stimulative action of the drug.

The Küpffer cells of the liver-lobules exhibit their function of phagocytosis in two directions - toward (1) erythrocytes and (2) fat. The Küpffer cells, of course, constitute part of the reticulo-endo-:thelial system and it will, therefore, be convenient to discuss the phenomenon of red-cell phagocytosis after reviewing the action of other elements in this system, e.g. the spleen and lymph glands.

The constancy with which fat granules have been observed in the Küpffer cells is striking. Even in my earliest experiments I remarked their presence and their origin puzzled me. They could manifestly be derived either from the Küpffer cells themselves by a process of fatty degeneration or from the blood by phagocytosis. The idea of the Küpffer cells under-:going fatty degeneration in the absence of any simil-:ar change occurring in the liver-cells themselves was far from entertaining. And yet why the Küpffer cells should begin to absorb fat from the blood-stream and deposit it in granular form in their cytoplasm was likewise far from clear. Two views were possible - either/

either (1) mercury stimulated these cells to absorb fat from the blood-stream; or (2) these cells absorbed fat from the blood because the circulating blood-fat was abnormally increased; phagocytosis would then be an attempt on the part of these cells to remove the excess from the blood. The last-mentioned idea was supported by the fact that the portal lymphatics in some cases exhibited an increased content of fat, the natural deduction being that this was due to an excess of fat in the circulating blood. The results of my early experiments only enabled me vaguely to postulate such a statement. But later as I shall show below I was able to prove that this early supposition is, in-:deed, fact and that corrosive sublimate intoxication does produce a true lipaemia. The fat granules in the Kupffer cells, then, in all probability represent fat phagocyted from the blood.

Mayer, Rathery and Schaeffer[50] have investigated the influence of many kinds of substances (acids, al-:kalies, anaesthetics, toxic and therapeutic agents) on the mitochondria of the liver-cells. According to their observations the reaction of the mitochondria always follows one or other of two courses which they have termed (1) "cytolyse et chondriolyse", and (2)/

(2) "Homogénéisation et chondriomégalie". During the phemonemon of "chondriolyse" the mitochondrial granules gradually diminish in size and number without in the early stages undergoing any coalescence so that large clear spaces are formed; in the final stages the re-:maining granules coalesce to form small irregular masses and very large empty spaces are in consequence produced. "Chondriomégalie" is essentially charact-:erised by progressive fusion of the mitochondrial granules so that they become larger and less numerous; these granules may be distributed throughout the whole cell or be gathered especially round the nucleus.

Our observations would thus justify the inclusion of corrosive sublimate in the catalogue of substances which cause the mitochondria of the liver-cells to undergo "homogénéisation". The measure to which this change is produced, however, is only moderately well marked.

CONCLUSIONS.

1. In severe cases of intoxication the liver is the seat of congestion moderately intense in its degree and uniform in its distribution throughout each lobule. In less severe cases of poisoning the hyperaemia is less/

less in evidence. The method of administration of the drug - intra-oral or intramuscular - does not influence the distribution of the congestion.

2. In the majority of my experiments the parenchyma of the liver-lobules shows the changes characteristic of simple cloudy swelling. Like the congestion these changes affect each lobule uniformly no matter the method of administration of the drug. I have never detected the presence of fat in the liver-cells.

3. Three cases exhibit hydropic degeneration of the liver-parenchyma. This is most marked around the intralobular veins and diminishes toward the portal tracts.

One of the these livers presents numerous mitotic figures all in relation to zones of degeneration. Two livers from cases of human corrosive sublimate poisoning also reveal mitosis occurring in relation to areas of degeneration. A study of these cases proves that mitosis is a reparative process secondary to degeneration and that it is not the result of a direct stimulative action of the drug.

4. The Kupffer cells constantly show a granular content of fat phagocyted from the blood-stream. In severe cases of intoxication these cells sometimes exhibit/

exhibit a content of erythrocytes and haemosiderin.

5. The mitochondria of the liver-cells fail to exhibit any visible changes in the milder grades of intoxication. When the intoxication is more severe the mitochondrial granules become increased in size, reduced in number and distributed more irregularly. Not infrequently they take up a position round the periphery of the cell. Corrosive sublimate is thus a member of that group of substances which produce the series of phenomena designated "chondriomégalie" by Mayer, Rathery and Schaeffer.

S P L E E N

LITERATURE

In acute experimental poisoning with corrosive sublimate Lyon (1904)[17] states that there is always con-:gestion of the pulp varying in intensity in different cases. The Malpighian bodies show an absence of pathological changes. They may be compressed by the dilated sinuses, their capillaries particularly at the periphery may be congested, and at most a few of their lymphoid cells may show necrotic changes. The large endothelial cells of the Malpighian bodies arefrequent-:ly swollen and the number of cells with large vesicul-:ar nuclei is often increased. He describes also an extensive proliferation of the endothelial cells of the pulp and a marked phagocytosis by them of red blood cells. The amount of pigment in the pulp increases with the chronicity of the intoxication.

No other references to the spleen in sublimate poisoning can be found.

PERSONAL OBSERVATIONS.

In all my experiments the splenic pulp is the seat of congestion varying in degree with the severity of the poisoning. In very acute cases the congestion may be extremely severe, the architecture of the tissue be-:ing obscured by dense accumulations of red cells throughout the sinuses. In subacute and chronic cases of intoxication congestion is always moderate in degree. As a rule the pulp is uniformly congested; occasional-:ly the congestion is patchy and irregular in distrib-:ution.

Not infrequently the dilatation of the splenic sinuses tends to round off and demarcate the Malpig-:hian bodies more clearly than usual and occasionally in the presence of extreme congestion of the pulp the Malpighian bodies are so compressed that they undergo a diminution in size, the lymphoid cells becoming more compactly set (Fig. 55). More often than not the central arterioles of the Malpighian corpuscles are dilated and there is almost invariably congestion of the capillaries supplying the lymphoid tissue. The congestion varies in degree being sometimes so severe as to lead to extravasation of blood into the Malpighian/

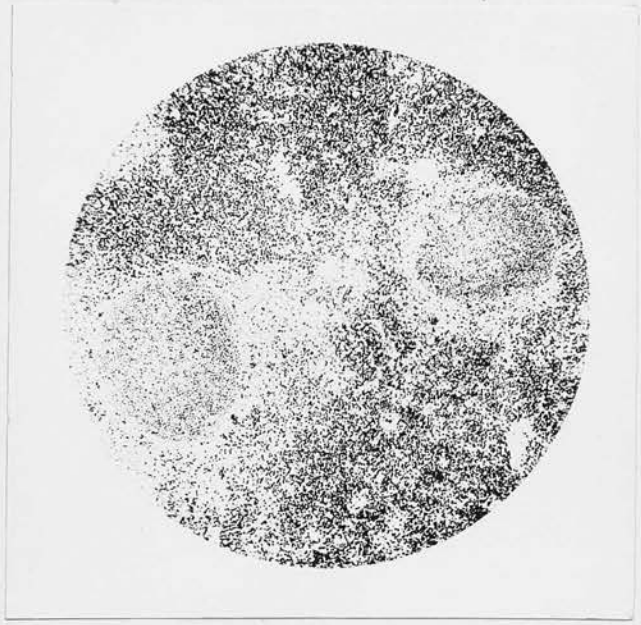


Fig. 55. Spleen showing marked congestion of pulp and demarcat-:ion of Malpighian Bodies. (Haem. and Eos.) x 80.

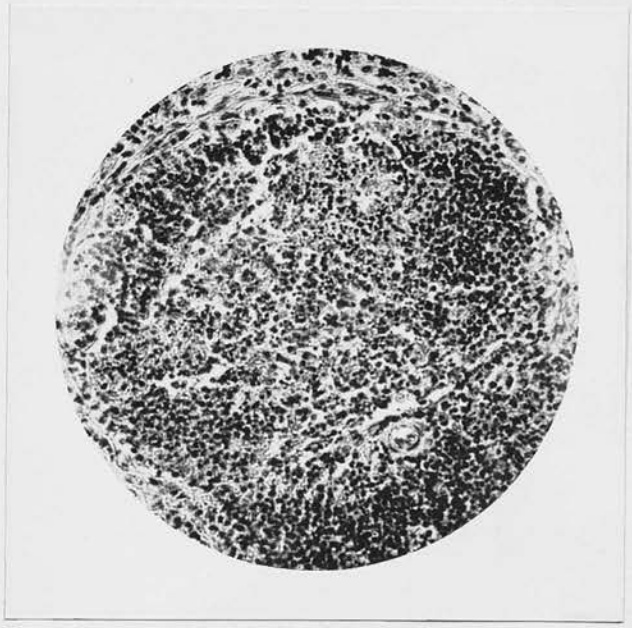


Fig. 56. Malpighian Body showing extensive necrosis and disin-:tegration of its lymphocytes. (Haem. and Eos.) x 280.

Malpighian body. The congestion is never localised to any particular zone of the Malpighian corpuscles.

In addition to congestion the Malpighian bodies in a case of severe acute poisoning may show necrosis and disintegration of their lymphoid cells (Fig. 56). In its mildest degrees such necrosis affects a few groups of one or two lymphocytes and as a result of the disintegration of these cells small accumulations of nuclear débris are to be seen here and there in the affected Malpighian bodies. The débris consists of strongly basophilic granules of various shapes and sizes. Other Malpighian bodies show necrosis of a larger number of their lymphocytes and ultimately a Malpighian body may exhibit comparatively few healthy cells. When the lymphocytes of a Malpighian body are extensively destroyed in this way pyknotic granules are to be found scattered in large numbers throughout the corpuscle. In some Malpighian bodies the lympho-:cytes of one segment may be more affected than those of the remaining zones. Infiltration of red cells among the degenerated lymphocytes may at times be very marked.

Such destruction of lymphocytes may be followed by one of two phenomena:-

(1)/

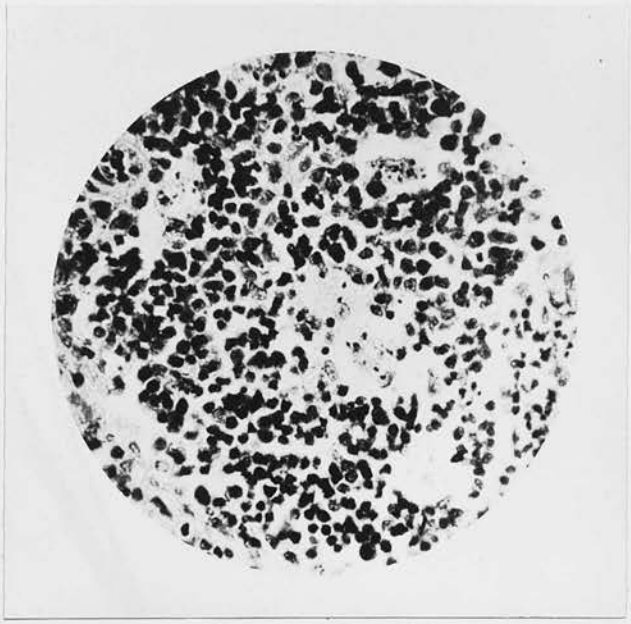


Fig. 57. x 500.

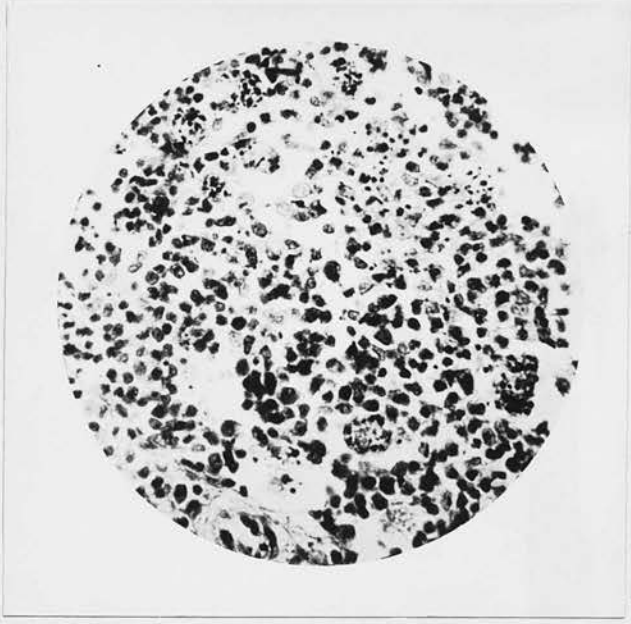


Fig. 58. x 500.

Figs. 57 and 58. Malpighian Bodies in which mononuclear leucocytes are seen acting as phagocytes toward the gran-:ular débris of necrotic lymphocytes. Some of the phagocytes are heavily laden with granules. (Haem. and Eos.)

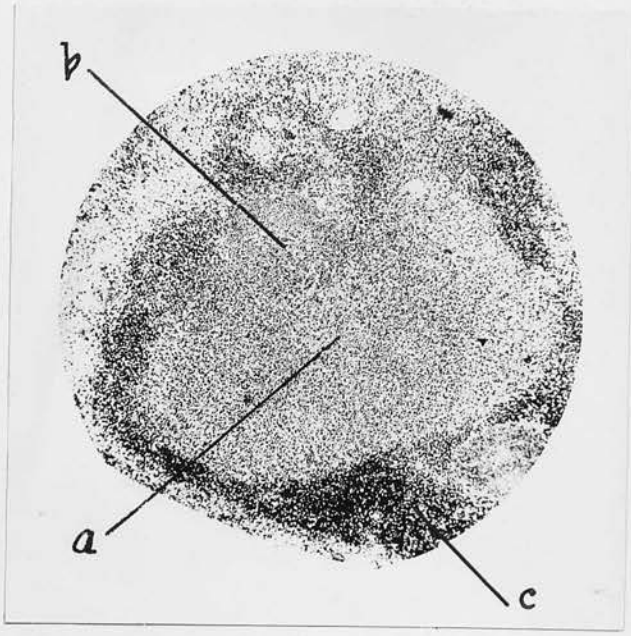


Fig. 59. x 60.

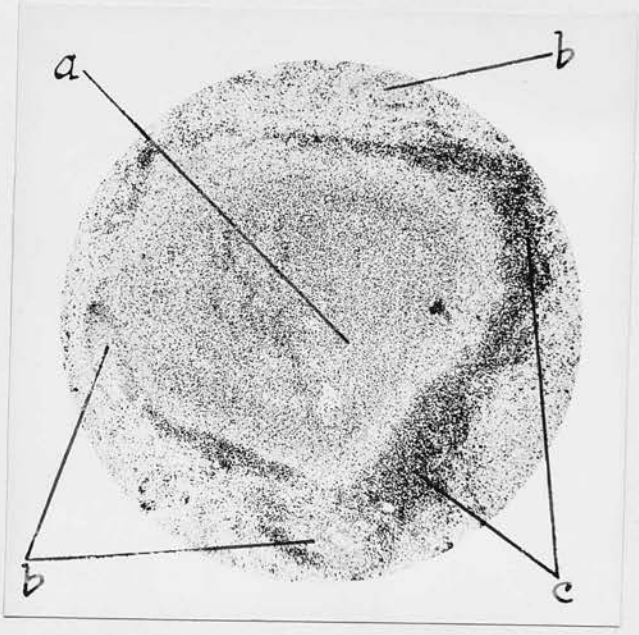


Fig. 60. x 40.

Figs. 59 and 60. (a) Vast accumulation of polymorph leucocytes in the pulp in the neighbourhood of (b) Malpighian Bodies whose lymphoid cells are extensively necrosed (see Figs. 57 and 58). (c) Marked congestion of the pulp-sinuses around zones of leucocytic infiltrat-:ion. (Haem. and Eos.)

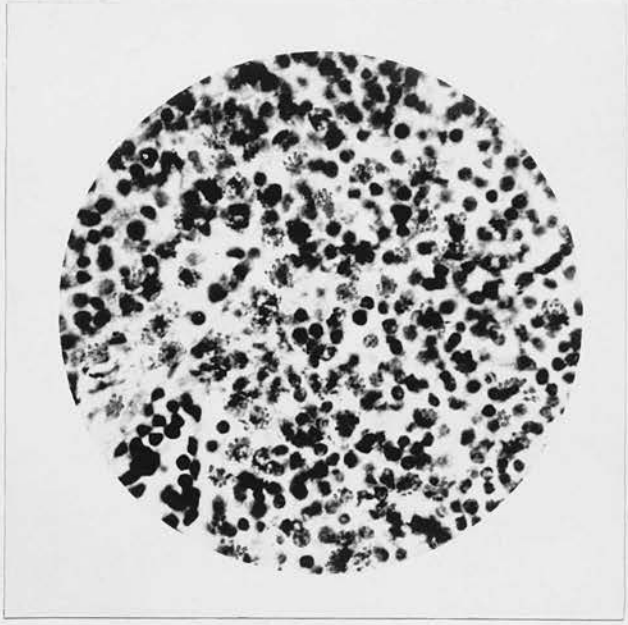


Fig. 61. x 600.

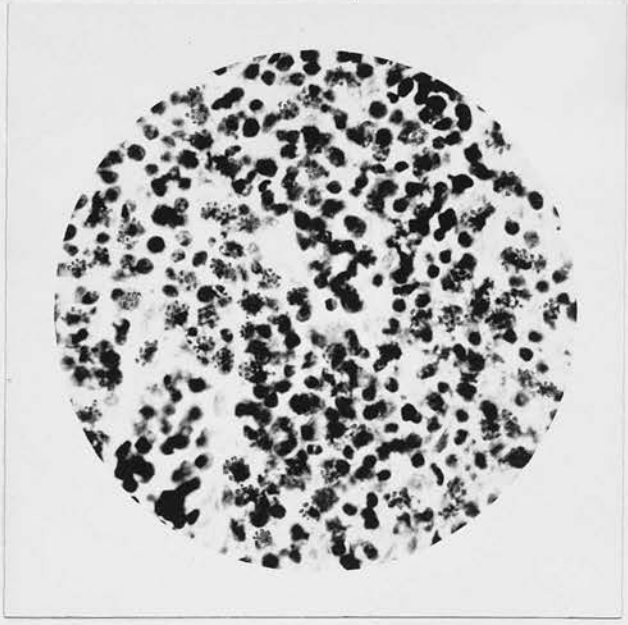


Fig. 62. x 600.

Figs. 61 and 62. Malpighian Bodies showing an extensive infiltration of polymorph leucocytes among their lymphoid cells. The polymorphs are the granular cells seen in the figures. (Haem. and Eos.)

(1) In some Malpighian bodies cells possessing a clear cytoplasm and a round or oval vesicular nucleus appear among the lymphocytes and pyknotic granules. These cells which would appear to be of the nature of endothelial cells or mononuclear leucocytes act as phagocytes toward the lymphocytic débris with the in-:gestion of which they become markedly swollen (Figs. 57 and 58). Such macrophages are sometimes scattered in fair numbers throughout the Malpighian bodies.

(2) In the immediate neighbourhood of several other Malpighian corpuscles showing disintegration of their lymphocytes numbers of polymorphonuclear leuco-:cytes may be seen to have accumulated in the pulp (Figs. 59 and 60). These cells are sometimes gathered there in great hosts surrounding the Malpighian bodies completely or being localised more or less at one side. Polymorphs, moreover, are to be found among the dis-:integrated lymphocytes of the adjacent Malpighian bodies. Such infiltration may be comparatively slight, but on the other hand may be so severe that the whole lymphoid structure is densely overrun with them (Figs. 61 and 62). A Malpighian body may thus come to be composed of about equal numbers of polymorphs and lymphocytes amongst which are scattered the granular remains/

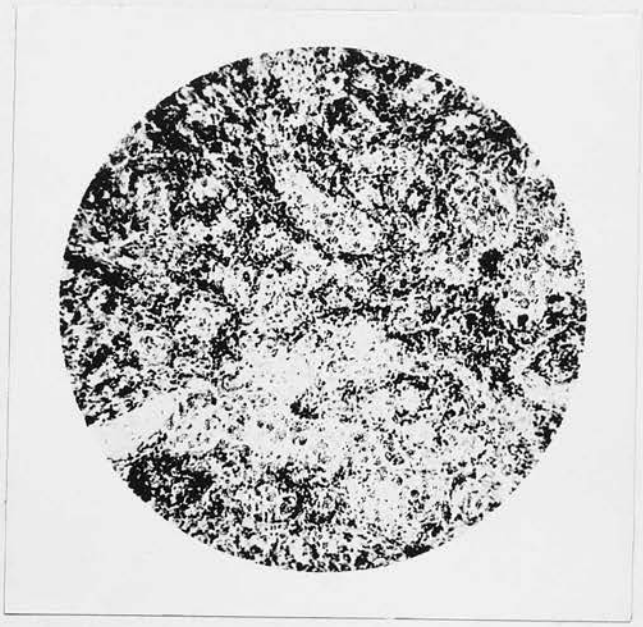


Fig. 63. x 85.

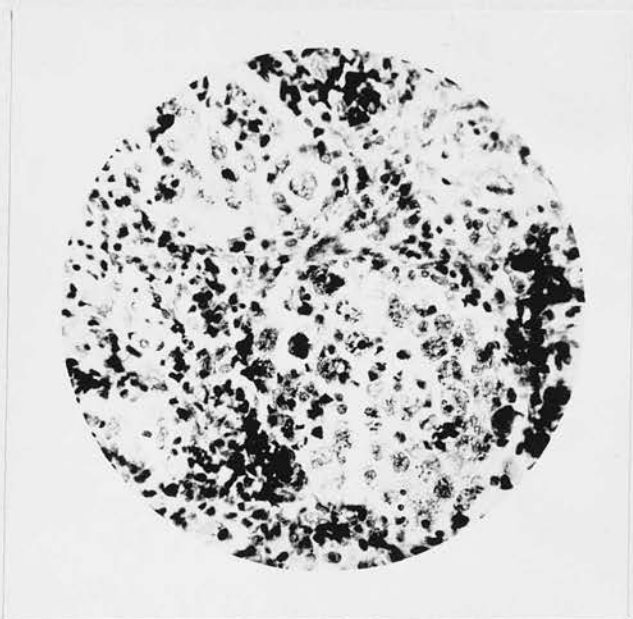


Fig. 64. x 380.

Figs. 63 and 64. Spleen from a case of chronic poisoning to show the dilated condition of the pulp sinuses and the marked increase therein of endothelial cells most of which exhibit a granular content of haemosiderin. (Haem. and Eos.)

remains of the necrosed lymphocytes. The pulp sur-:rounding such polymorphous zones is invariably the seat of extreme congestion.

It is to be noted that these changes - necrosis of lymphoid cells, phagocytosis by endothelial macrophages and infiltration of polymorphs - affect the Malpighian bodies only insevere cases of intoxication. Further, they are by no means constant in their occurrence; thus they occurred in only one of five experiments though the dosage was in all cases the same. In con-:ditions of subacute and chronic poisoning the Malpig-:hian bodies beyond slight congestion of their capill-:aries may present no abnormality.

The endothelial cells lining the sinuses of the pulp, especially in cases of chronic intoxication, become swollen and prominent. This is accompanied by a varying increase in the number of endothelial cells scattered throughout the pulp (Figs. 63 and 64). A marked increase in these cells has been observed within 12 hours. They may become extremely numerous particularly in the more chronic conditions of poison-:ing where the sinuses - in such conditions often markedly dilated - exhibit great numbers of them.

These endothelial cells act as phagocytes toward the/

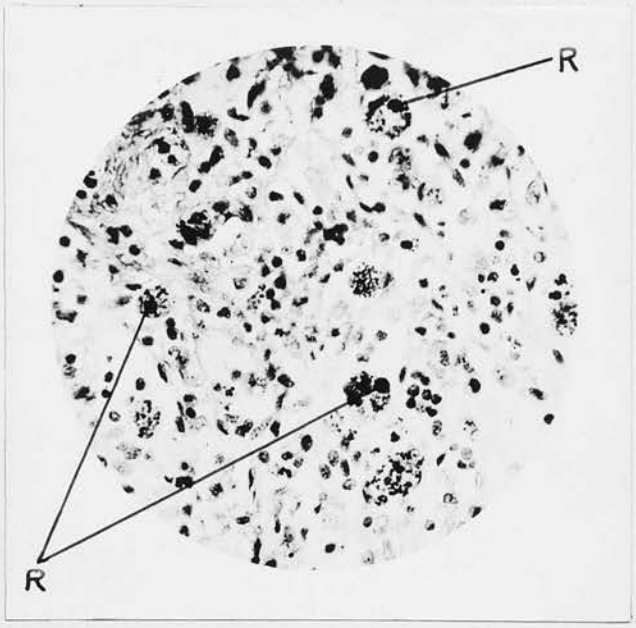


Fig. 65. x 500.

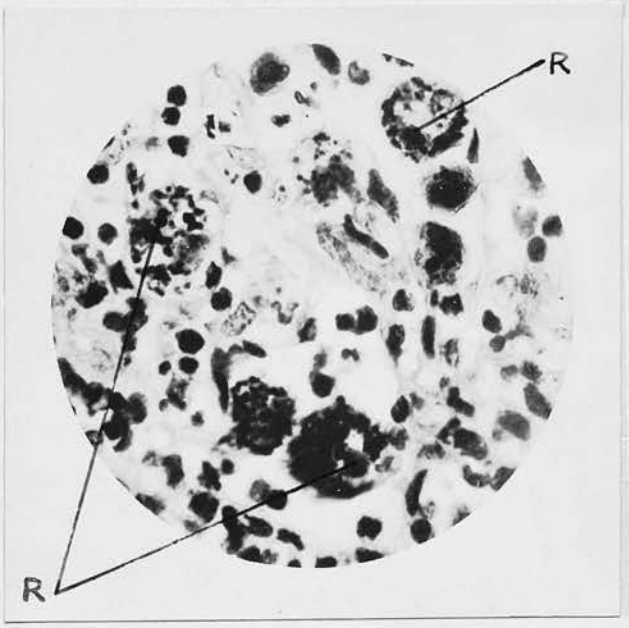


Fig. 66. x 700.

Figs. 65 and 66. Spleen showing proliferated endothelial cells which are acting as phagocytes toward effete red cells. R indicates red cells which have just been engulfed. Some of the phagocytes have become markedly swollen with iron-pigment derived from the red cells. (Haem. and Eos.)

the red blood corpuscles two or three of which may often be seen lying in the cytoplasm of the macro-:phages.(Figs. 65 and 66). In sections stained haem-:atoxylin and eosin it is seen that after ingestion the haemoglobin content of each corpuscle undergoes division into small granules; these granules lose their eosinophilic properties and become converted first into granules of reddish-brown or purple colour, and finally into spherules of a golden-yellow tint which give a positive Prussian Blue Reaction and are, therefore, of the nature of haemosiderin. All stages in red cell destruction with the ultimate formation of iron pigment may be visible in the same cell. As they indulge more and more in the destruction of red cells the phagocytes increase markedly in size and become loaded with pigment so that in conditions of chronic intoxication many of the phagocytes may be very large. The nucleus of cells so loaded with pigment is not in-:frequently seen to be pushed to one side and sometimes a large phagocyte may exhibit two or even three nuclei.

Transformation of haemoglobin into haemosiderin is well demonstrated in sections stained Prussian Blue and counterstained with neutral red. Then it is seen that the earliest granules derived from the red cells do not give the reaction, but stain a reddish colour with/

with the counterstain. Not infrequently the first granules to give the reaction appear in a narrow zone round the periphery of the cell, the granules in the centre still staining red. Gradually all the granules come to give the reaction so that ultimately a phago-:cyte may exhibit only a mass of blue granules.

After they become loaded with pigment many of the endothelial cells appear to undergo disintegration with liberation of their pigment content. The excessive accumulation of pigment in the cytoplasm of the cells, in other words, appears so to interfere with their metabolism that they undergo degeneration and ultim-:ately die. At least this seems to me to be the orig-:in of many of those small accumulations of haemosid-:erin pigment often to be observed lying free in the pulp-sinuses in cases of marked phagocytosis. It is possible, however, that some of these small amounts of haemosiderin and also isolated granules lying extra-:cellularly in the sinuses have been produced by lysis of red cells in the sluggish circulation of the pulp. But regarding this point I cannot pronounce definitely.

The end-result of this phenomenon of exaggerated phagocytosis is the deposit in the splenic pulp of an excessive amount of haemosiderin pigment. This de-:posit/

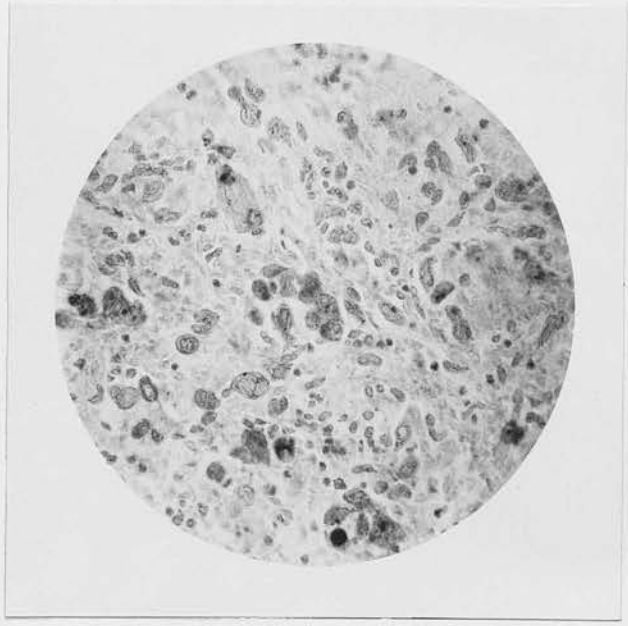


Fig. 67. Spleen stained by the Prussian Blue Method to show the deposit of iron-pigment in its pulp-sinuses. x 160.

deposit is particularly well demonstrated by the Prussian Blue Method (Fig. 67).

In only two of my experiments have I observed any phagocytosis of white cells. In one of these experiments numerous examples of phagocytosis of polymorphs can be made out. Some of the larger phagocytes in this experiment contain not only several red cells, but also a few polymorph leucocytes. After ingestion it is seen that both the cytoplasm and nucleus of a leucocyte lose their characteristic staining reactions and become broken up into a number of coarse granules which are more or less indistinguishable from those derived from disintegrated red cells.

DISCUSSION.

The most interesting phenomena are undoubtedly in relation to the Malpighian bodies. They include (1) necrosis and disintegration of their lymphoid cells: (2) phagocytosis of the lymphocytic débris by macrophages; and (3) an infiltration among the lymphocytic débris and in the pulp around the affected Malpighian bodies of numerous polymorphonuclear leucocytes.

In the first place it would appear that mercury in/

in large doses has a specific destructive effect on splenic lymphocytes and in this conclusion I am supported by Lyon's observations[17]. I have not observed mercury to have this effect on lymphoid cells elsewhere, e.g. lymph glands, Peyer's patches. But even in the spleen destruction of the lymphoid cells is by no means constant in its occurrence.

The accumulation of polymorphs in the pulp and in the affected Malpighian bodies with accompanying congestion resembles the phenomena characteristic of an acute inflammatory lesion with suppuration. But here it is to be noted that the polymorphs are everywhere healthy in appearance and show no signs of degeneration as inflammatory cells commonly do. Further, the infiltration of polymorphs into the Malpighian bodies does not appear to be with a view to phagocytosis of the lymphocytic fragments for microscopic evidence of inclusions in the polymorphs is altogether lacking. Again, it is a noteworthy feature that polymorph infiltration is never observed in relation to those Malpighian bodies which show the presence of endothelial phagocytes as described above. Now it is through the activity of these cells that the débris of the degenerated lymphocytes is removed. The natural

conclusion/

conclusion, therefore, is that in the absence of these phagocytes substances are derived from the cellular débris which diffusing outward from the Malpighian body exert a strongly positive chemiotactic action on the polymorph leucocytes of the blood. Hence the ac-:cumulation of polymorphs in the pulp and neighbouring Malpighian bodies.

This phenomenon is, indeed, one of importance. It goes to prove that the products of necrotic and dis-:integrated tissues sometimes possess the power of at-:tracting polymorphs to the seat of necrosis - an observation which in its turn has a bearing on the pro-:cess of 'suppuration' - that phase of inflammation characterised by much tissue destruction and accumul-:ation of polymorphs. Whatever the relation between bacteria or their toxins and polymorphs may be it would appear highly probable from my concluding ob-:servation above that in a localised lesion of infect-:ive origin the products of tissue-destruction play a part in attracting polymorphs to the seat of infection.

Phagocytosis of red cells is a constantly recur-:ring phenomenon and the degree to which it occurs in chronic cases may be striking. The amount of haemo-:siderin pigment deposited in the pulp may in consequ-:ence/

consequence be markedly increased. Destruction of red cells will be referred to again later in connect-:ion with the lymph glands and bone marrow.

Phagocytosis of polymorphs has been observed, but is by no means an outstanding feature of my experiments. This is rather remarkable considering the very extens-:ive destruction of red cells.

CONCLUSIONS.

1. In all conditions of intoxication with corrosive sublimate the splenic pulp is the seat of congestion - severe in acute cases and of moderate degree in sub-:acute and chronic cases.

2. In severe, acute conditions of poisoning corrosive sublimate may exert a specific destructive influence on the lymphoid cells of the Malpighian bodies. This influence has not been observed to affect the lymphoid cells of other tissues.

3. Destruction of the lymphoid cells of the Malpig-:hian bodies may be followed by either:-

(a) The appearance amongst the lymphocytic débris of mononuclear cells which act as phagocytes toward the nuclear fragments;

or (b) An infiltration of polymorphonuclear leuco-:cytes/

leucocytes throughout the affected Malpighian bodies and a marked accumulation of such cells in the neighbouring pulp.

These observations suggest that the products of cell-destruction sometimes exert a strongly positive chemiotactic action on polymorph leucocytes.

4. Corrosive sublimate increases the process of blood-destruction by endothelial macrophages in the pulp of the spleen and so leads to an excessive deposit of haemosiderin pigment in that organ.

5. Phagocytosis of polymorphs in the splenic pulp is not an outstanding feature.

BONE MARROW

LITERATURE.

The changes produced by corrosive sublimate in the marrow and blood have been investigated by several workers.

Keyes (1876)[51] found that in healthy men and syph-:ilitics small doses of mercury increased the number of red cells, while larger doses diminished the red count. These findings have been corroborated by Lindstroem (1898)[52] in the case of syphilitics.

Heilborn (1878)[53] administered corrosive sublimate by subcutaneous injection to rabbits in doses of 5 - 65 mgms. He found the marrow markedly congested with much atrophy of its fat-cells, but no increase of its nucleated red cells.

Raimondi (1880)[54] gave to three rabbits by the mouth repeated doses of 1 - 20 mgms. corrosive sublim-:ate. He describes the blood as hydraemic containing few red corpuscles and an increased number of white; and the marrow as containing much extravasated blood, less fat than usual and few nucleated cells.

Schlesinger (1881)[55] experimenting on rabbits and dogs/

dogs with small doses of corrosive sublimate found a substantial increase in the red cell count - as much as 2 million per cmm.

Stockman and Charteris (1903)[56] administered corrosive sublimate subcutaneously in 1 mgm. doses daily and also calomel by the mouth in daily doses of 6 cgms. All their animals died within 4 weeks. The first effect noted by them was a very striking increase in the number and size of the blood-vessels accompanied by complete atrophy of the fat-cells. Small extravasations of blood were common. Gelatinous degeneration occurred early and was extreme. They did not observe any increase in the erythrocyte count or haemoglobin content of the blood.

Allan Gray (1925) gave to a series of ten rabbits daily doses of mercuric chloride ranging from 5 - 50 mgms. Gelatinous degeneration of the marrow was produced in only one case which received 5 mgms. per day for 51 days. A slight reduction in the red cell count was also noted.

PERSONAL/

Fig. 68. Bone marrow showing a marked degree of vascular engorgement with some reduction in the amount of adipose tissue. (Haem. and Eos.) x 140.

Fig. 69. Bone marrow showing severe congestion and considerable extravasation of blood into the adipose tissue. (Haem. and Eos.) x 180.

PERSONAL OBSERVATIONS.

Eosine and Methylene Blue. In all of thirteen bone marrows examined congestion is present varying in degree between slight and very marked. The most extreme degree of hyperaemia is noted in the marrow of Experiment 9 where the animal succumbed 12 hours after receiving a large dose intra-orally (Fig. 68). In a marrow such as this, the seat of extreme congestion, the majority of the capillaries are greatly dilated and from some of them extravasation of blood is seen to have taken place into the tissue (Fig. 69). Extreme vascular engorgement is accompanied by a reduction in the amount of adipose tissue present, many of the fat-cells undergoing a diminution in size or disappearing entirely to make room for the dilated capillaries. In proportion as the congestion is less marked this disappearance of the fatty marrow is less in evidence.

In each of five cases, viz. Experiments 6, 10, 14, 15, and 16, the duration of which varied between 3 and 14 weeks the marrow exhibits a well-marked leucoblastic reaction (Fig. 70). The reaction is in one or two of these cases so pronounced that little fatty marrow remains/

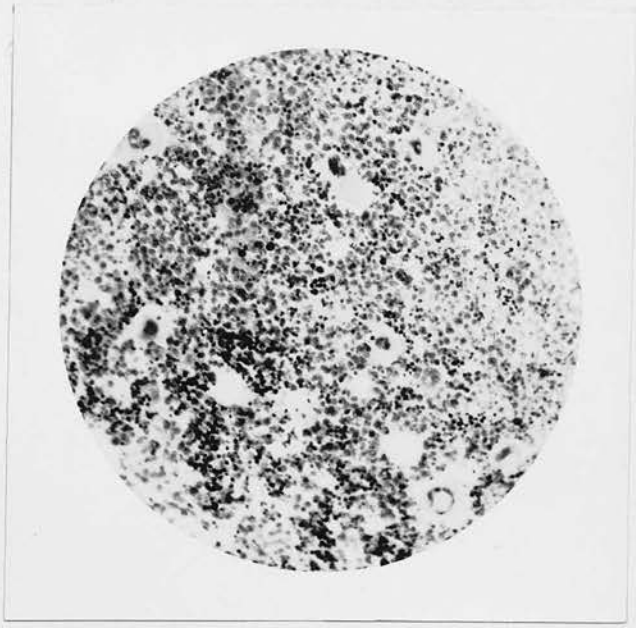


Fig. 70. Bone marrow from middle of shaft of femur showing well-marked leucoblastic reaction. (Haem. and Eos.) x 160.

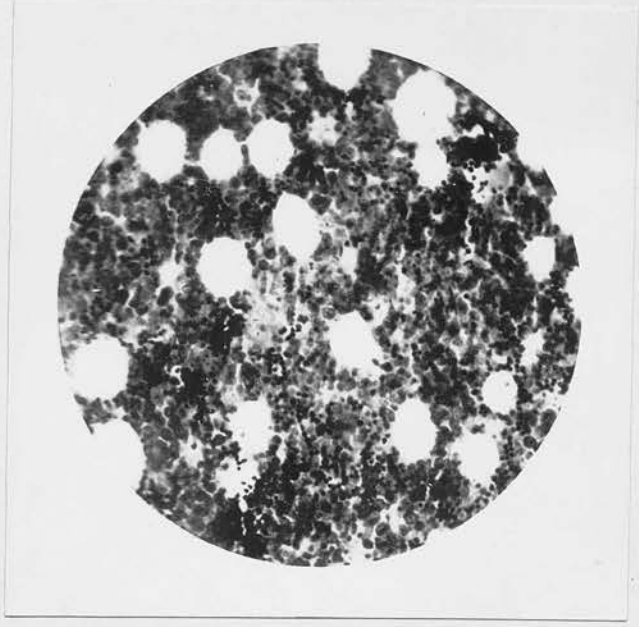


Fig. 71. Bone marrow from middle of femur showing a moderate leuco-:blastic reaction. The leuco-:blastic elements are beginning to lose their staining powers and are becoming merged into a homogeneous mass - early gelatinous degenerat-:ion. (Haem. and Eos.) x 200.

remains. The occurrence of this reaction in the mar-:row is occasionally accompanied by a visible increase of white cells in the vessels of the various organs, kidney, liver, heart, etc., but since I never carried out white-cell counts I cannot state that a leucocyt-:osis was actually present in these cases. The vas-:cular appearances are certainly suggestive.

In contrast with the above-described leucoblastic reaction I have never in any experiment remarked the occurrence of any reaction on the part of the erythro-:blastic elements.

In Experiment 7 which lasted 5 days the haemo-:poietic elements show early signs of undergoing de-:generation. They have gone far to losing their individual staining powers and are becoming merged into a more or less homogeneous mass. The intervening fat cells in this case still look healthy. This is the only instance in my series of experiments where gelat-:inous degeneration is observed to have attacked the marrow (Fig. 71).

In no experiment have I ever observed any destruct-:ion of red-cells progressing in the marrow. Partic-:ular attention has been paid to the marrow of cases in/

in which the endothelial phagocytes of the spleen and liver are seen to be actively engaged in destroying red blood cells. But even in cases showing this phen-:omenon in marked degree or in which the spleen shows a heavy deposit of iron-pigment no destruction of red corpuscles by endothelial macrophages is to be seen in the marrow. The absence of this phenomenon under such circumstances is, indeed, surprising.

CONCLUSIONS.

1. Congestion is a constant feature in the marrow. According to the intensity of the intoxication it varies in degree between slight and very marked. Ex-:travasation of blood may occur from the dilated cap-:illaries.

2. In proportion as the hyperaemia is marked there is a disappearance of the fatty marrow. In the presence of extreme vascular engorgement the reduction in fatty marrow may be considerable.

3. In subacute and chronic cases of intoxication a leucoblastic reaction occurs in the marrow. This re-:action may be pronounced.

4. No erythroblastic reaction has ever been observed.

5. In one acute case widespread, early gelatinous de-:generation/

degeneration is a feature.

6. Phagocytosis of red cells by endothelial macro-:phage is conspicuous by its absence from the marrow throughout this series of experiments.

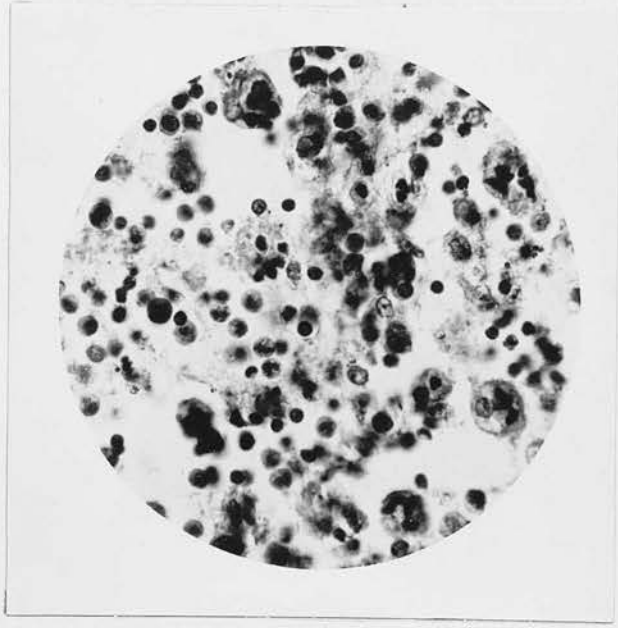


Fig. 72. x 500.

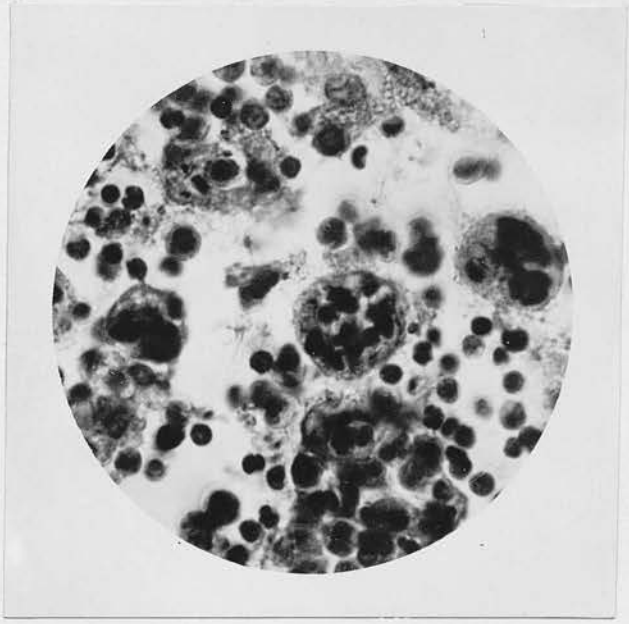


Fig. 73. x 800.

Figs. 72 and 73. Mesenteric lymph gland showing endothelial cells actively engaged in phagocyting erythrocytes lying free in the lymph-sinuses. Some of the phagocytes are greatly swollen with a content of numerous red cells. (Haem. and Eos.)

LYMPH GLANDS

PERSONAL OBSERVATIONS.

Haematoxylin and Eosin. My observations on the changes affecting the lymphoid cells of the Malpighian bodies led me to examine the mesenteric group of lymph glands. During these investigations I have in some cases remarked a definite increase, first, in the number of red cells, and, second, in the number of endothelial cells lying free in the sinuses. It can be seen, moreover, that the endothelial cells are engaged in actively phagocyting the red cells and occasionally the degree of phagocytosis to be seen in progress is remarkable (Figs. 72 and 73). This is the case, for example, in Experiment 11, where the endothelial phagocytes are numerous and large and not infrequently exhibit as many as eight or nine red cells in their interior. Intracellular disintegration of the red cells results as described in the case of the spleen in the production of granules which are at first still definitely eosinophilic, but which gradually assume the golden-yellow colour characteristic of haemosiderin. But in the lymph glands which I examined the majority of the phagocytes exhibit only red cells and intracellular/

intracellular destruction has resulted in the production of haemosiderin granules in the case of only a few macrophages. Such proliferation of endothelial cells and increased phagocytosis is observed both when the drug is administered intra-orally and intramuscularly.

Excessive red cell destruction in these glands is to be correlated with the same phenomenon exhibited by the Küpffer cells of the liver and by the endothelial cells of the spleen. These are, of course, all elements in the so-called reticulo-endothelial system which has of late years been brought into such prominence by Aschoff[57]. Such excessive phagocytosis is essentially an exaggeration of one of the normal processes of blood-destruction and is in all probability an indication that corrosive sublimate has an injurious influence on red cells. This conclusion is, indeed, highly probable considering the degree of anaemia which may develop in a person suffering from chronic mercurialism[58].

The endothelial cells of the bone marrow also form part of this system, but as has already been stated above they were never in this series of experiments observed to participate in red-cell destruction - even/

even when phagocytosis was proceeding vigorously in the spleen and liver. The reason for this inactivity on the part of the endothelial cells of the marrow is obscure.

CONCLUSIONS.

1. Corrosive sublimate increases the process of blood-destruction by endothelial macrophages in the sinuses of the mesenteric lymph glands.
2. The increased destruction of red blood cells observed in the spleen, liver and lymph glands indicates that corrosive sublimate has an injurious influence on red cells.

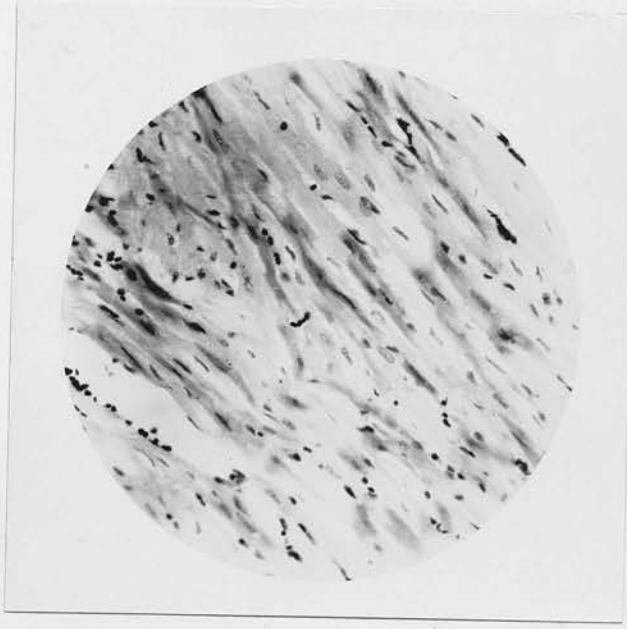


Fig. 74. x 200.

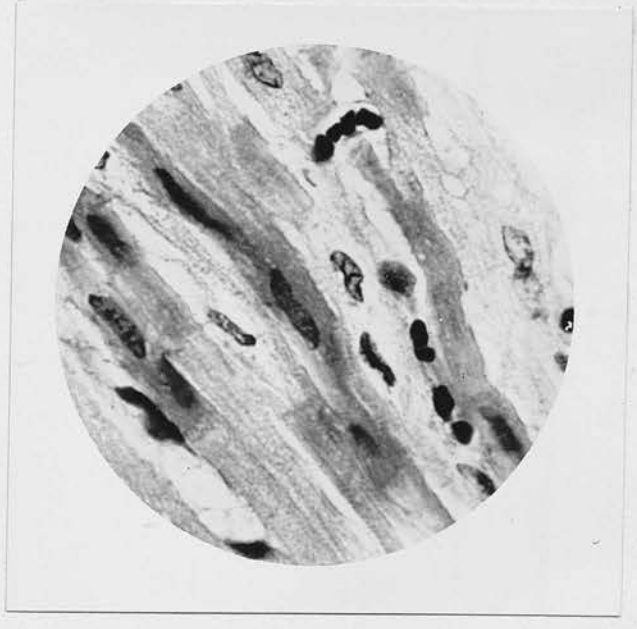


Fig. 75. x 800.

Figs. 74 and 75. Heart-muscle showing irregularity in staining with eosin. Individual or small groups of fibres have absorbed the eosin strongly so that they stand out bright red (dark in the figures) against the more faintly stained myocardium of the background. (Haem. and Eos.)

H E A R T

PERSONAL OBSERVATIONS.

Haematoxylin and Eosin. According to the severity of the intoxication the myocardium exhibits congestion of a severe, moderate or mild degree.

The muscle-fibres as a rule show an abnormal gran-:ularity of their cytoplasm. The minute cytoplasmic granules are distributed in parallel rows within the fibres and in consequence of their presence the normal striations of the muscle are rendered obscure. Most frequently the nuclei present no abnormality, but oc-:casionally a nucleus is swollen and vesicular in ap-:pearance. These observations substantiate a diagnos-:is of cloudy swelling.

In the majority of my experiments the myocardium presents a peculiar irregularity in staining (Figs. 74 and 75). This irregularity consists in little groups of fibres taking the eosin very strongly so that they stand out bright red against the more faintly-staining myocardium of the background. The affected groups sometimes comprise about a dozen fibres, sometimes three or four, occasionally only a single fibre or a segment of one. A feature of this irregularity is the/

Fig. 76. An arteriole surrounded by a zone of degenerated myo-:cardium (showing dark in the figure) which is in process of being infiltrated by small round wandering cells. (Haem. and Eos.) x 260.

the clearness with which these groups of eosinophilic fibres are demarcated from the surrounding myocardium. The nuclei of the affected fibres nowhere exhibit any distinctive changes. In the majority of cases the eosinophilic areas show no particular distribution, but in one experiment it is the muscle-fibres immediately around the larger capillaries that are most affected.

In connection with this irregularity in staining Experiment 8 demonstrates an additional feature of interest. A moderately large arteriole in cross-section is seen to be surrounded by a zone of eosino-:philic myocardium (Fig. 76). The latter is in a very degenerated condition and around it, moreover, there has accumulated a large number of small round wandering cells. These cells, besides, are in process of infiltrating the affected myocardium. In the lum-:en of the vessel there is at one side an accumulation of similar cells mixed with fibrin. The endothelial cells lining the vessel are swollen and in some cases almost desquamated.

Sudan III and Aldol. Of ten myocardia examined by these staining methods for the presence of fat eight gave positive results. Aldol has revealed the pre-:sence of fat in the myocardium after a period of 4 hours'/

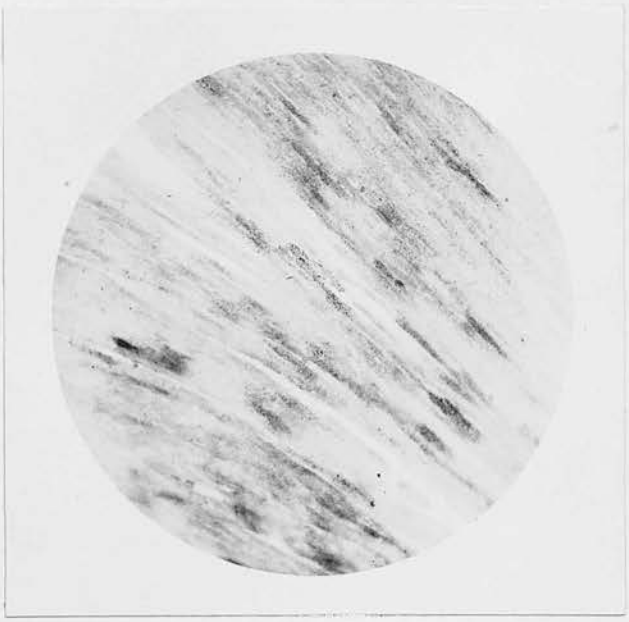


Fig. 77. (Aldol). x 45.

Fig. 78. (Sudan III and Haem.) x 45.

Figs. 77 and 78. Fatty degeneration of the myocardium. Note the small groups of muscle-fibres showing a granul-:ar content of fat. An excellent demonstration of the superiority of Aldol over Sudan III in defining the minutest fatty granules.

hours' intoxication.

In all these cases the myocardium is the seat of diffuse, widespread fatty degeneration every fibre being affected. In addition, however, areas here and there exhibit a much more abundant fatty deposit (Figs. 77 and 78). These areas comprise individual fibres or groups of fibres and in shape, size and distribution are very similar to the strongly eosinophilic zones described above. Though perfectly diffuse in its distribution the fatty degeneration has thus in every case a patchy character in addition. Over large areas of myocardium the deposit of fat may be very marked, yet even here the patchy manner in which certain groups of muscle-fibres are more heavily affected is still obvious.

The deposit of fat is always in the form of a great number of small granules or globules. These are distributed in closely arranged longitudinal rows (Figs. 79 and 80). In areas where the myocardium is the seat of very early fatty degeneration the muscle-fibres exhibit only a few granules of minute size placed at irregular distances from each other, the intervals between them being occasionally fairly great. As the degree of fatty degeneration increases in sev-

:erity/

Fig. 79. x 240.

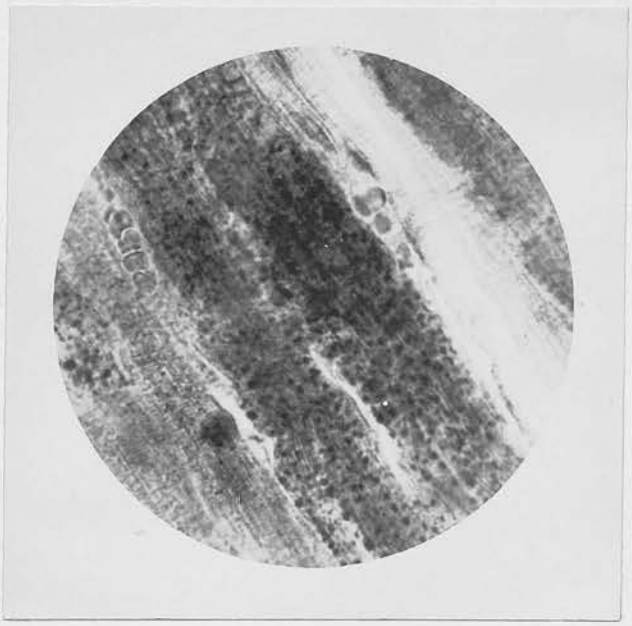


Fig. 80. x 900.

Figs. 79 and 80. Fatty degeneration of the myocardium. The fat is deposited in the muscle-fibres in the form of small granules or globules disposed in closely arranged longitudinal rows. Note the precision of the Aldol Method in defining the minutest fatty granules.

severity the granules become more numerous and at the same time larger. The consequence is that in heavily affected zones the globules attain to fairly large di-:mensions and are placed in close apposition to one an-:other. Sometimes the globules are so large that co-:alescence of neighbouring granules would appear to have occurred.

It has already been stated that in one experiment, viz. 9, the muscle-fibres immediately around the larger capillaries are more strongly stained with eosin than the myocardium elsewhere. In the interpretation of this change it is interesting to note that in frozen sections stained aldol the fibres around the larger capillaries are the seat of more advanced fatty degen-:eration than the remainder of the myocardium. These perivascular fibres contain a somewhat greater number of granules and the latter are all distinctly larger than the granules elsewhere.

Bichromate Method. This staining method reveals that each heart-muscle fibril normally contains within its substance a continuous row of short, thick, bacillary rods placed end to end. Between the ends of adjacent rods there is a brief space. The rods in adjacent fibrils are so placed that definite transverse striat-:ions/

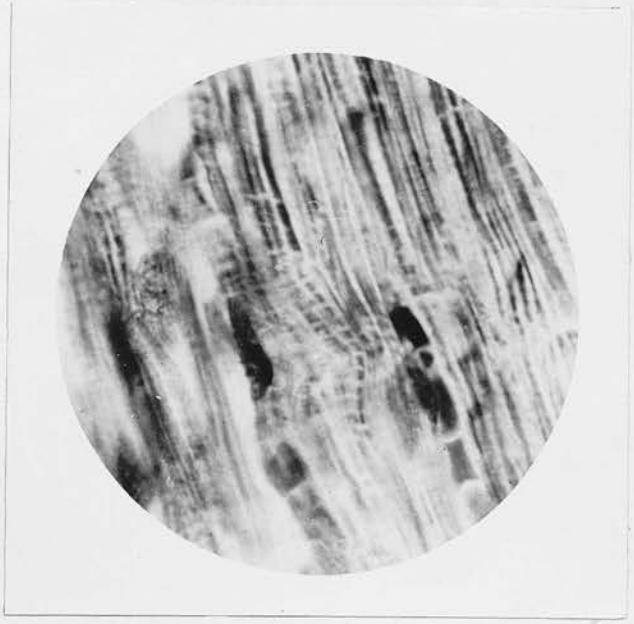


Fig. 81. Heart-muscle stained by the Bichromatin Method. Each fibril contains a continuous row of short, bacillary rods placed end to end and so situated in re-:lation to those of adjacent fibrils that transverse striat-:ions are produced. x 1500.

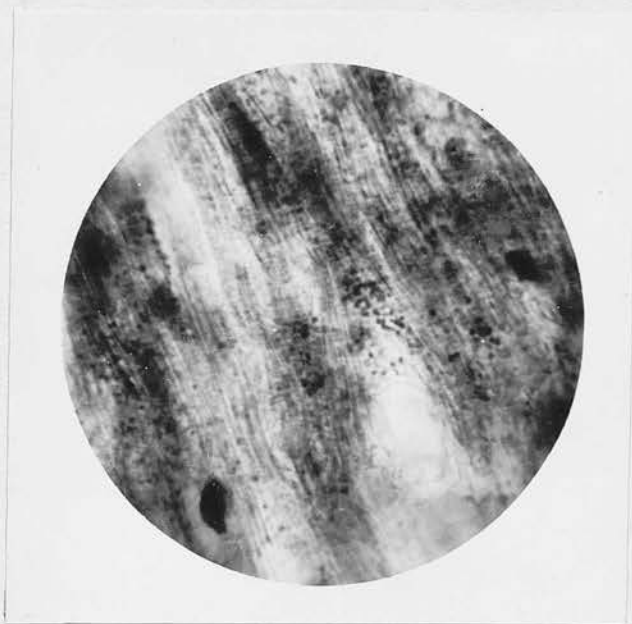


Fig. 82. Heart-muscle showing transformation of the rods into granules. (Bichromate). x 1500.

striations are produced (Fig. 81). Although well de-:monstrated by this method these short rods cannot with certainty be proved to represent mitochondria. The most that can be said is that they are specialised rods of cytoplasm which have been well demonstrated by haema-:toxylin after mordanting with bichromate.

In the myocardium of several animals subjected to corrosive sublimate poisoning I remarked certain chang-:es in these cytoplasmic rods. Half of the myocardia proved by aldol and sudan III to be the seat of fatty degeneration reveal no change in these rods. In the remainder the rods are seen to have been converted in-:to spherical granules of varying sizes, some compar-:atively small, others much larger (Fig. 82). They continue like the rods to lie in parallel, longitudin-:al rows, but owing to their irregularity in spacing they show no attempt at forming transverse striations. These alterations in the rods occur sometimes uniform-:ly over the myocardium, sometimes only in patches dis-:tributed irregularly here and there.

DISCUSSION.

The irregular way in which the heart-muscle stains with eosin after the animal has been subjected to cor-:rosive/

corrosive sublimate poisoning is a phenomenon which attracted my attention in my first experiment. To be-:gin with I was inclined to look upon it as an artifact, but when I found it recurring in the majority of my subsequent experiments I was unable to regard it long-:er in that light. In seeking for an explanation I naturally turned to the results revealed by frozen-sections stained aldol. Here I found that not only was every muscle-fibre the seat of fatty degeneration, but groups of fibres here and there always exhibited a much more abundant deposit of fat than the myocardium intervening. In shape, size and distribution these groups of fatty fibres are very similar to the small areas of muscle which stain abnormally strongly with eosin. Further, every myocardium revealed by aldol to be the seat of patchy fatty degeneration exhibits this irregularity in staining with eosin. The relat-:ion between the groups of fatty fibres and the eosino-:philic areas is also supported by an observation made in Experiment 9. In this experiment it is the muscle-fibres immediately around the larger capillaries that are most strongly stained with eosin and in frozen sections stained aldol the fibres in the same situation are/

are the seat of more advanced fatty degeneration than the myocardium elsewhere. It seems to me, therefore, that the scattered areas of myocardium staining strong-:ly with eosin are identical with those showing the most advanced degree of fatty degeneration. If this be true it means that as it is undergoing fatty degen-:eration the cytoplasm of the heart-muscle fibres be-:comes more eosinophilic in character: the cytoplasm, in other words, undergoes some kind of chemical change whereby it is rendered abnormally basic in its react-:ion.

The patchiness which is superadded to the diffuse distribution of the fatty degeneration is commonly at-:tributed to the fact that the affected groups of fib-:res are pararterial in position. Thus situated these fibres are thought to be less well supplied with oxygen and nutriment than fibres placed nearer the or-:igin of the coronary artery, and are, therefore, like-:ly to suffer most under the influence of any toxin circulating in the blood. Whether or not this is actually so is difficult to decide.

The findings as revealed by the Bichromate Method are not easy to interpret. The most that can be said is that the cytoplasmic rods which are revealed so clearly/

clearly by this method may as a result of the intox-:ication become granular in shape and irregular in dis-:tribution. I would like to draw a correlation be-:tween these granules and those of fatty degeneration stainable by aldol and sudan III. But in several cases myocardia proved definitely to be the seat of widespread fatty degeneration have been found to ex-:hibit a uniformly normal state of their cytoplasmic rods or the rods have only been granular in small areas. I find it, therefore, impossible to draw any parallel between the findings of the bichromate method and those revealed by aldol or sudan III.

CONCLUSIONS.

1. The myocardium exhibits congestion of severe, mod-:erate or mild degree according to the intensity of the poisoning.
2. The myocardium is the seat of cloudy swelling and fatty degeneration. The latter is diffuse in dis-:tribution, but has always a superadded patchy charact-:er, certain groups of muscle fibres being more severe-:ly affected than the remainder of the myocardium.
3. In the majority of my experiments the myocardium presents a peculiar irregularity in staining with eos-:in/

eosin. This consists in certain groups of fibres absorbing the acid dye more strongly than the intervening myocardium. These groups of fibres are in all probability identical with those patches of myocardium which exhibit the most advanced degree of fatty degeneration. If this be so it indicates that fatty degeneration in heart-muscle is accompanied by a chemical change in the cytoplasm whereby the latter becomes more basic in its reaction.

4. The bichromate method reveals that each myocardial fibre contains several rows of short, bacillary rods - "cytoplasmic rods" I have termed them - which are so arranged as to form definite transverse striations.

Half of the myocardia proved by aldol and sudan III to be the seat of fatty degeneration reveal no change in these rods. In the remainder the rods have been converted into spherical granules of varying size. I cannot prove that there is any relation between these granules and the granules of fatty degeneration stainable by aldol and sudan III, though there would appear to be some such relation.

L U N G

PERSONAL OBSERVATIONS.

Haematoxylin and Eosin. In eight of nine experiments the lungs present no abnormality. In one instance the pulmonary tissue is severely congested, but otherwise shows no departure from the normal.

It is clear, therefore, that corrosive sublimate produces no constant change in the pulmonary tissue.

LARGE INTESTINE

LITERATURE

It is well known (Kaufmann[57], McCallum[58], Muir[59]) that corrosive sublimate may produce severe membranous inflammation of the wall of the large bowel.

Kaufmann states that the inflammatory lesions are neither constant nor always confined to the large intestine. The lower portion of the ileum may also be involved or exceptionally it alone. Kaufmann (1889)[16] endeavoured to show that the intestinal lesions are not due to the direct corrosive action of mercury excreted by the bowel, but due to the fact that mercury poisoning produces stasis and results in the formation of hyaline thrombi in the capillaries with sufficient circulatory disturbances to render the intestinal mucosa non-resistant to the invading intestinal bacteria. This interpretation is supported by Heinz[60], but disputed by others (Falkenberg[61], Lyon[17]). Ricker[62] is of the opinion that mercury irritates the vasomotor system of the intestines and causes vascular dilatation, slowing of the current, stasis and subsequent necrosis.

Harmon (1928)[32] reports four cases of corrosive sublimate/

sublimate poisoning by intravenous injection in humans. Distributed through the entire colon of Cases I and III were numerous circumscribed reddish-grey ulcers. The colonic mucosa in Case IV was hyperaemic, oedematous and showed petechial haemorrhages, but no necrosis or ulceration was present. The colon in Case II present-:ed no abnormality.

PERSONAL OBSERVATIONS.

It has already been stated that in this series of experiments two methods of administering the drug were employed - intra-oral and intramuscular. It is, therefore, interesting to contrast the lesions produc-:ed by each of these methods of administration.

In the case of seven animals to which the drug was administered by the intra-oral route six exhibited lesions of the colon. Of five animals treated intra-:muscularly two presented a pathological state of the large bowel. In no case of either series was any pathological change ever noted in the small intestine.

Further, I found that the lesions produced in the large bowel when the drug was administered intra-orally were very similar to those produced when the drug was given intramuscularly. Indeed, a comparison of the caecum/

Fig. 83. Caecum of a rabbit to which corrosive sublimate was administered intra-orally (Exper. II). The margins of the rugae are capped with a beaded, ir-:regularly swollen ridge of necrotic tissue. The morbid appearances are identical with those produced when the drug is administered intramuscularly - see Fig. 84. x 1½.

Fig. 84. Caecum of a rabbit to which corrosive sublimate was administered intramuscularly (Exper. 8). The margins of the rugae are capped with a beaded, irregularly swollen ridge of necrotic tissue. The morbid appearances are identical with those produced when the drug is administered intra-orally - see Fig. 83. x 1½.

caecum from Experiment 11 (Fig. 83) where the intra-oral method was employed with that from Experiment 8 (Fig. 84) where the drug was given intramuscularly proves the lesions to be absolutely identical in distribution and naked-eye appearances. In the intra-oral series the lesions were certainly more widespread and as will be shown reveal microscopically severer degenerative changes than those of the intramuscular series, but this difference is explicable on the grounds of larger dosages having been employed in the intra-oral series. In distribution and in naked-eye and microscopic appearances the lesions produced by the two methods of administration were in essence the same.

In the intra-oral series the lesions were restricted to the caecum and ascending colon. I found the most constant change to be an affection of the mucous folds of these parts. The margins of the rugae instead of being thin, smooth and transparent as they are normally in the caecum were capped with a beaded, irregularly swollen ridge of tissue of dark, reddish brown colour and necrotic appearance. The various folds exhibited this change in different degrees. The caecum from Experiment 7 not only presented the changes already described, but exhibited also two or three

necrotic/

necrotic patches between its rugae. These patches were of dark brown colour, raised above the surface, irregular in shape, and rough and ragged superficially. A patch of necrosis was also present in this case in the commencement of the ascending colon. As a rule the mucosa of the caecum and ascending colon between the rugae was congested though in one instance it was healthy-looking. In all cases where it was present the congestion diminished rapidly on proceeding along the ascending colon, the distal end of the latter be-:ing invariably normal.

The caecum from Experiment 8 where the animal had been treated by the intramuscular method showed its mucous folds to be affected as has already been de-:scribed above. The margins were in various degrees swollen, beaded, reddish-brown in colour, and very hard, almost gritty to the touch. One or two of the less affected folds exhibited only slight congestion along their margins, no swelling or beading of the tissue be-:ing present. In Experiment 14 (also intramuscular) there was present in the caecum about its middle a small round ulcer the size of a threepenny piece with greyish necrotic base and ill-defined margins. A necrosed patch was also present on an adjacent fold of mucous membrane.

Mucous/

Fig. 85. Mucous fold of Caecum from a rabbit treated intra-orally (Exper.11). The tissue throughout a considerable depth of the fold has undergone necros-:is with disappearance of villous structure. (Haem. and Eos.) x 60.

Fig. 86. Same as Fig. 85 (Exper. 2) showing extensive haemorrhage into the necrosed tissue of the fold. (Haem. and Eos.) x 40.

Mucous Fold of Caecum from Animal Treated Intra-Orally (Haematoxylin and Eosin). The tissue throughout a considerable depth of the fold has undergone necrosis (Fig. 85). The structural outlines of the villi are still recognisable in areas, but the cells have acquired a peculiar, hyaline appearance and are devoid of nuclei. In some parts cell-outlines have disappeared and the tissue consists merely of coarsely granular débris. The necrotic cells and granular débris have stained deeply with eosin. The tissue planes deep to the necrosed area of the fold are infiltrated with numerous mononuclear wandering cells of various sizes; most of them are small, but some reach large dimensions. They contain fragments of disintegrated cellular material and are accumulated at some points in very large numbers. Cellular débris is also to be seen lying free in the tissue planes. In this case (Experiment 11) no congestion or haemorrhage is visible. But in Experiments 1 and 2 very marked congestion of the capillaries is present and haemorrhage into the necrotic tissue is an outstanding feature (Fig. 86). In both these cases the necrotic zones and the tissue planes deep to them are infiltrated with numerous polymorphs and some mononuclears.

Caecal/

Fig. 87. Caecal fold from rabbit treated intramuscularly (Exper. 8). The tip of the fold has undergone necrosis. The necrot-:ic zone which is structureless and strongly eosinophilic is well-demarcated at its margin by a dense accumulation of mononuclear cells and polymorphs. (Haem. and Eos.) x 40.

Caecal Fold from Animal Treated Intramuscularly (Haem-:atoxylin and Eosin). Microscopic examination of the fold (Experiment 8) reveals that its tip has undergone definite necrotic changes (Fig. 87). The tissue in this region is structureless, almost homogeneous and strongly eosinophilic. Only here and there can the outlines of cells still be discerned. The necrotic zone is well demarcated at its margin by a dense ac-:cumulation of wandering, mononuclear cells and some polymorphs in the cytoplasm of which can be seen frag-:ments of disintegrated cellular material. Passing more deeply these phagocytic cells become less numer-:ous and finally one passes into healthy tissue. The mucosa of the remainder of the fold and of the caecum between the folds shows no departure from the normal. The necrosis affecting the extremity of the fold is not accompanied by any congestion or haemorrhage.

DISCUSSION.

It is interesting to note that both the intra-oral and intramuscular methods of administration pro-:duce similar lesions in the large intestine. No doubt the lesions in the intra-oral series occur more constantly, are wider in their distribution and involve more/

more intense degenerative changes than those of the intramuscular series, but these differences as already stated are explicable on the grounds of larger dosages being given intra-orally.

The fact that the intramuscular administration of the drug results in the production of lesions in the colon is highly suggestive of the idea that the large bowel shares in the endeavour to eliminate the poison from the circulation and that the poison passing through the capillaries of the intestinal mucosa exerts a destructive action on the latter with resultant ne-:crosis. I never observed the presence of hyaline thrombi in the capillaries such as has been described by Kaufmann and I, therefore, cannot agree with his statement that the intestinal necrosis is due to in-:farction. Kaufmann, it will be remembered, put for-:ward the same idea as an explanation of the damage sustained by the kidney: here again I have found it impossible as has already been stated to corroborate his findings and conclusions. To me it seems that the lesions are really due to the direct, destructive action of the poison during its elimination through the intestinal capillaries.

In the case of the intra-oral series of experi-:ments/

experiments it might be argued that the lesions in the colon were produced by the drug acting upon the mucosa superficially from the lumen of the gut. But against this supposition is the fact that it is scarcely poss-:ible for the drug to be transmitted from the stomach along the entire length of the small gut to the colon without leaving behind some traces of its passage and yet the small bowel in this series of experiments nev-:er presented any pathological abnormality. Once the drug has been absorbed from the stomach into the cir-:culation the case to all intents and purposes resembl-:es one in which the drug has been administered intra-:muscularly and the colon soon begins to eliminate the poison from the circulation.

It is interesting to note that the first parts of the intestinal mucosa to show evidence of injury are the tips of the caecal folds. No doubt the explan-:ation of this is the fact that the tips of these folds are transparent and very delicate so that the capill-:aries therein must come very close to the surface. The rugae of the ascending colon on the other hand are much thicker and, therefore, likely to eliminate the poison less readily.

CONCLUSIONS/

CONCLUSIONS.

1. Whether it be administered intra-orally or intra-:muscularly mercuric chloride produces lesions of a necrotic and membranous character in the large intest-:ine, particularly in the caecum and ascending colon.
2. These lesions are due to the direct action of the drug on the mucosa during its elimination from the blood. No thrombosis of vessels has ever been ob-:served.

SUPRARENAL GLAND

PERSONAL OBSERVATIONS.

Haematoxylin and Eosin. The suprarenal glands from 13 animals subjected to corrosive sublimate intoxicat-:ion have been examined microscopically. Of these five show no departure from the normal. The remaind-:er exhibit pathological changes of varying nature and severity.

Of the eight specimens showing some departure from the normal seven exhibit congestion of their paren-:chyma. Both cortical and medullary zones share in this hyperaemia which in the majority of cases is of mild degree. In two cases the hyperaemia is fairly marked. Most often the congestion is uniformly dis-:tributed throughout both cortex and medulla, but oc-:casionally it is most marked in the cortical tissue adjacent to the medulla.

The cytoplasm of the cortical cells, moreover, exhibits an abnormal granularity which may sometimes be very marked. This phenomenon is accompanied by de-:generative changes affecting the nuclei. Most fre-:quently the nuclei are swollen and vesicular; some-:times these vesicular nuclei have almost faded out of existence and occasionally nuclei are altogether in-:discernible/

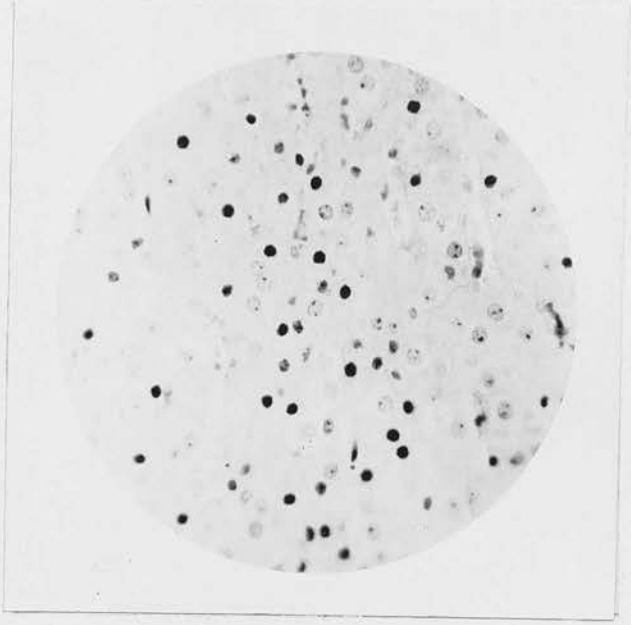


Fig. 88. Cortex of suprarenal gland showing cloudy swelling. The nuclei of many of the cells are intensely pyknotic. (Haem. and Eos.) x 340.

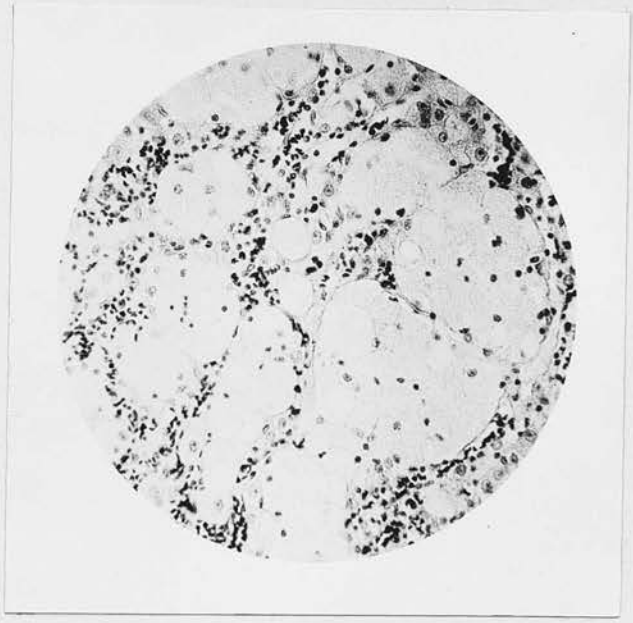


Fig. 89. Suprarenal gland showing areas of necrosis in the cortex. Note the disappearance of cell-outlines and nuclei, and the presence of moderately severe congestion in relation to the necrosed areas. (Haem. and Eos.) x 180.

indiscernible. Less commonly the nuclei are pyknotic and sometimes such pyknotic nuclei are very shrunken and misshapen (Fig. 88). In areas of the cortex where it is a marked feature the excessive granularity is not infrequently accompanied by disappearance of the cell-membranes. The tissue at such points can then be said to consist merely of a mass of granular material devoid of cell-membranes and through which are scatter-:ed the degenerated remains of some of the nuclei, the remainder having disappeared altogether (Fig. 89). As a rule vacuoles of various sizes, some of them fair-:ly large, are distributed through such granular mater-:ial; these vacuoles no doubt originally contained fat and glycogen. The above-described cytoplasmic and nuclear changes substantiate a diagnosis of cloudy swelling and necrosis.

The medullary parenchyma shows pathological changes less often than the cortical. When they oc-:cur they consist of slightly excessive granularity in the cytoplasm of the medullary cells together with some vesicularity of their nuclei; pyknosis is a less common nuclear change than in the case of the cortical cells. The occurrence of congestion in the medulla has already been mentioned.

CONCLUSIONS/

CONCLUSIONS.

1. The suprarenal glands may exhibit no departure from the normal in sections stained haematoxylin and eosin. Of 13 suprarenal glands examined five present no pathological abnormality.

2. Of the remainder all but one exhibit congestion of their parenchyma. This congestion is as a rule slight and uniform in distribution; occasionally it is fairly marked and most in evidence in the cortical tissue ad-:jacent to the medulla.

3. In affected cases the cortical cells are the seat of cloudy swelling moderate in severity as a rule, but occasionally proceeding to actual necrosis. The med-:ullary cells show cloudy swelling less often, and this is always less marked than in the case of the cortex.

B L O O D F A T

PERSONAL OBSERVATIONS.

The sequence of events which led me to suspect that the total amount of fat in the blood underwent a change in corrosive sublimate intoxication is interest-:ing. The earliest observation - one which I made in frozen-sections in my first experiment - was the fact that the plasma of the blood within the lumina of all the vessels stained diffusely with sudan III. This phenomenon has been noticed in nearly all my experi-:ments, but its exact significance was to begin with not properly understood. Another early discovery in this connection consisted in the observation that the lymphatics of the portal tracts in the liver were more heavily laden with fat than usual (Fig. 51). As I have already stated under the section on Liver - "the extent to which the periportal lymphatics are swollen with fat is sometimes remarkable". But again the significance of this more or less isolated observation remained at first undetermined. Thirdly, I have al-:ready drawn attention to the constancy with which fat-granules have been observed in the Küpffer cells of the liver (Figs. 48 and 49). This phenomenon, too, I observed in my earliest experiments and the origin of/

Fig. 90. Interlobular arteriole of kidney showing the presence of numerous globules of fat in the blood-stream. (Sudan III and Haem.) x 700.

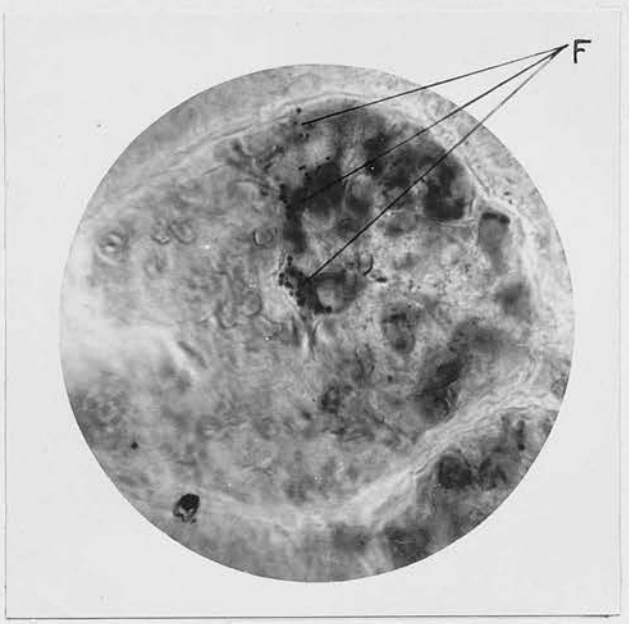


Fig. 91. Glomerulus showing small globules of fat (F) in some of the capillaries of the tuft. (Sudan III and Haem.) x 540.

of the fat-granules puzzled me. Finally in Experi-:ment 7 frozen-sections of kidney stained sudan III revealed the presence in the renal vessels of actual globules of fat (Fig. 90). To quote from my notes on this experiment:- "A considerable quantity of fat is to be seen in the vessels of both cortex and medulla. The plasma in long segments of the interlobular arter-:ioles stains a diffuse, homogeneous yellow and fre-:quently amongst this are scattered numerous well-defined globules of fat showing considerable variation in size. The extent to which the plasma is laden with such granules is frequently a noteworthy feature. The capillaries of the glomeruli likewise show a pale-yellow homogeneous content amongst which is scattered a great number of minute fat-globules (Fig. 91). Ev-:ery glomerulus without exception is so affected. The inter-lobular capillary plexus and the vessels of the medulla are similarly affected". In this experiment actual globules of fat are to be noted also in the cap-:illaries of the lungs. Examination of the liver and heart fails to reveal the presence of globules in their capillaries though the plasma is as usual stained dif-:fusely yellow with sudan III.

The discovery of actual globules of fat in the blood-stream opened up new vistas of thought. Two possibilities/

possibilities suggested themselves - the presence of this fat in the blood-stream indicated either (1) an increase in the total amount of fat in the blood: or (2) a precipitation by the mercury of fat normally present in the blood-stream. The first suggestion entailed a true increase in the amount of blood-fat: the second merely an apparent increase.

In order to detect which of these probabilities was the correct one it became necessary to make actual estimations of the total blood-fat before and after the administration of the drug. The results of four experiments are given below. All the estimations were made on oxalated blood drawn off in the morning after the animal had fasted overnight for a period of 12-14 hours. In each case the animal's fasting blood-fat was estimated prior to the administration of any mer-:cury, and then following the administration estimat-:ions were made at varying intervals of hours or days according to the acuteness or chronicity of the ex-:periment. The animals were not given special diet, but were allowed to eat as they wished of ordinary food (bran and green meat). Control experiments were car-:ried out with animals living under the same conditions and feeding on the same kind of food; venesection was performed on these animals also after a 12 hour fast.

In/

In two of the four experiments the CO_2-Combining Power of the blood was determined at the same time as the blood-fat. As will be shown later some interesting results also accrue from this investigation. Both blood-fat and CO_2-Combining Power estimations were very kindly performed by Dr Robert Gaddie of the Medical Chemistry Department. In estimating the blood-fat Dr Gaddie employed a modification of a method originally devised by Stewart and White in 1925[3]; and the CO_2 -Combining Power of the blood he estimated by means of van Slyke's Method[69].

Experiment/

Experiment 10.

Dosage: 1 mgm. Corrosive Sublimate ($\frac{1}{2}$ cc. 1 in 500 solution) daily intramuscularly.

Animal given $HgCl_2$		Control Animal	
Day of Experiment	Total Blood-Fat in Mgms. per cent.	Day of Experiment	Total Blood-Fat in Mgms. per cent.
1st (normal)	443	1st	394
6th	554	6th	393
9th	667	11th	429
12th	753	13th	467
16th	767	17th	488
20th	810	21st	480
23rd	460	24th	488
27th	597	28th	480

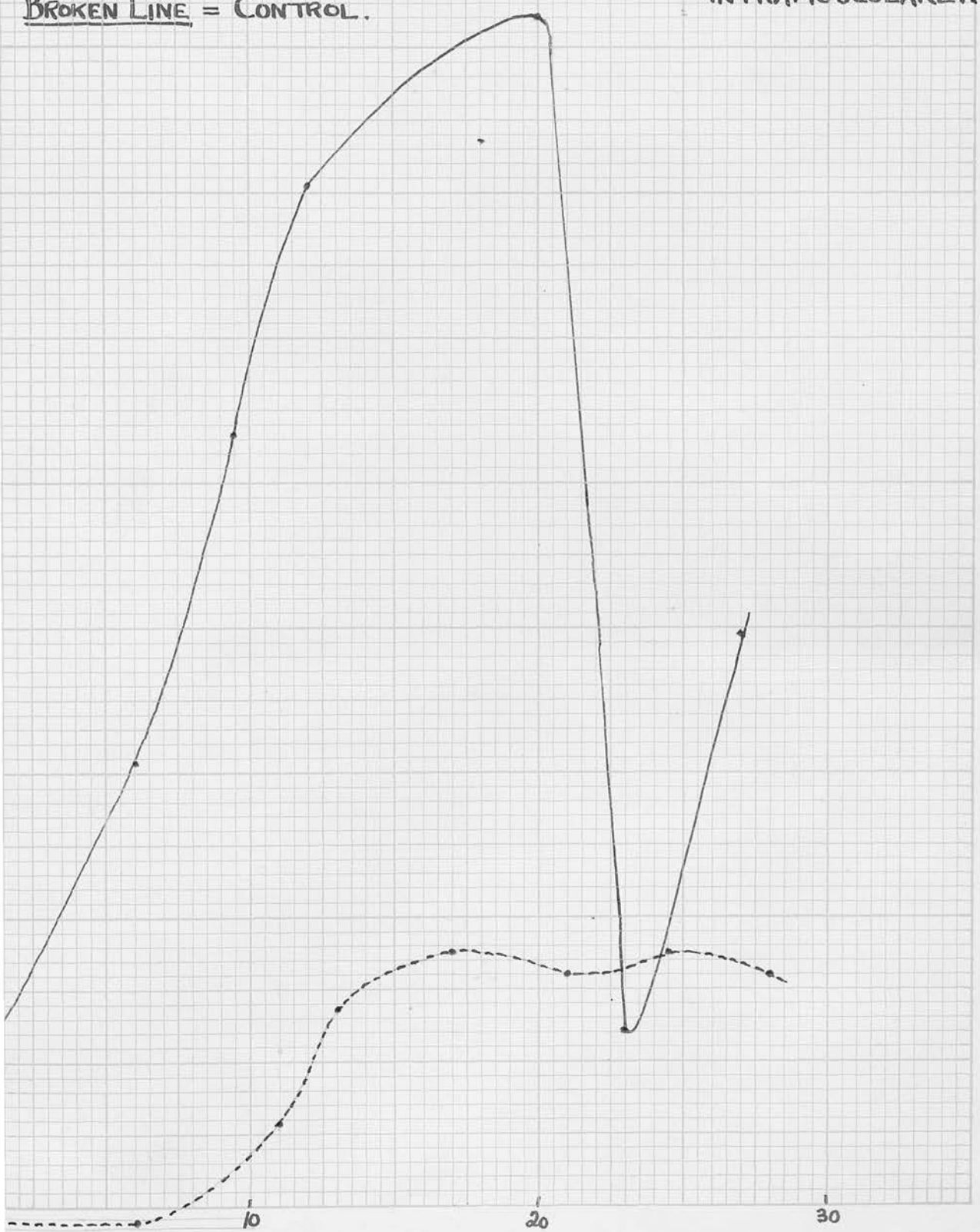
Throughout this experiment the animal receiving the mercury continued to eat well. By the 27th day the animal had lost a little weight but showed no other ill-effects of the intoxication. For a graphic representation of the above figures see Graph 1.

Experiment/

EXPERIMENT 10. GRAPH I.

CONTINUOUS LINE = BLOOD FAT OF ANIMAL TREATED WITH CORROSIVE SUBLIMATE : DOSAGE = 1MGM. ($\frac{1}{2}$ CC. IN 500 SOLN.) DAILY INTRAMUSCULARLY.

BROKEN LINE = CONTROL.



DURATION OF EXPERIMENT IN DAYS.

Experiment 11.

Dosage: 60 mgms. per kilo.(1 in 500 solution) intra-orally.

Duration of Experiment.	Total Blood-Fat in Mgms. per cent.	
	Animal given $HgCl_2$	Control Animal
-	410	375
4½ hours	621	480
24 "	1548	375
48 "	848	400
72 "	1186	415

The poisoned animal ate nothing during the entire period of the experiment. The control animal was, therefore, starved for a similar period while the blood-fat estimations were being made. Poisoned ani-:mal died on 4th day. See Graph 2.

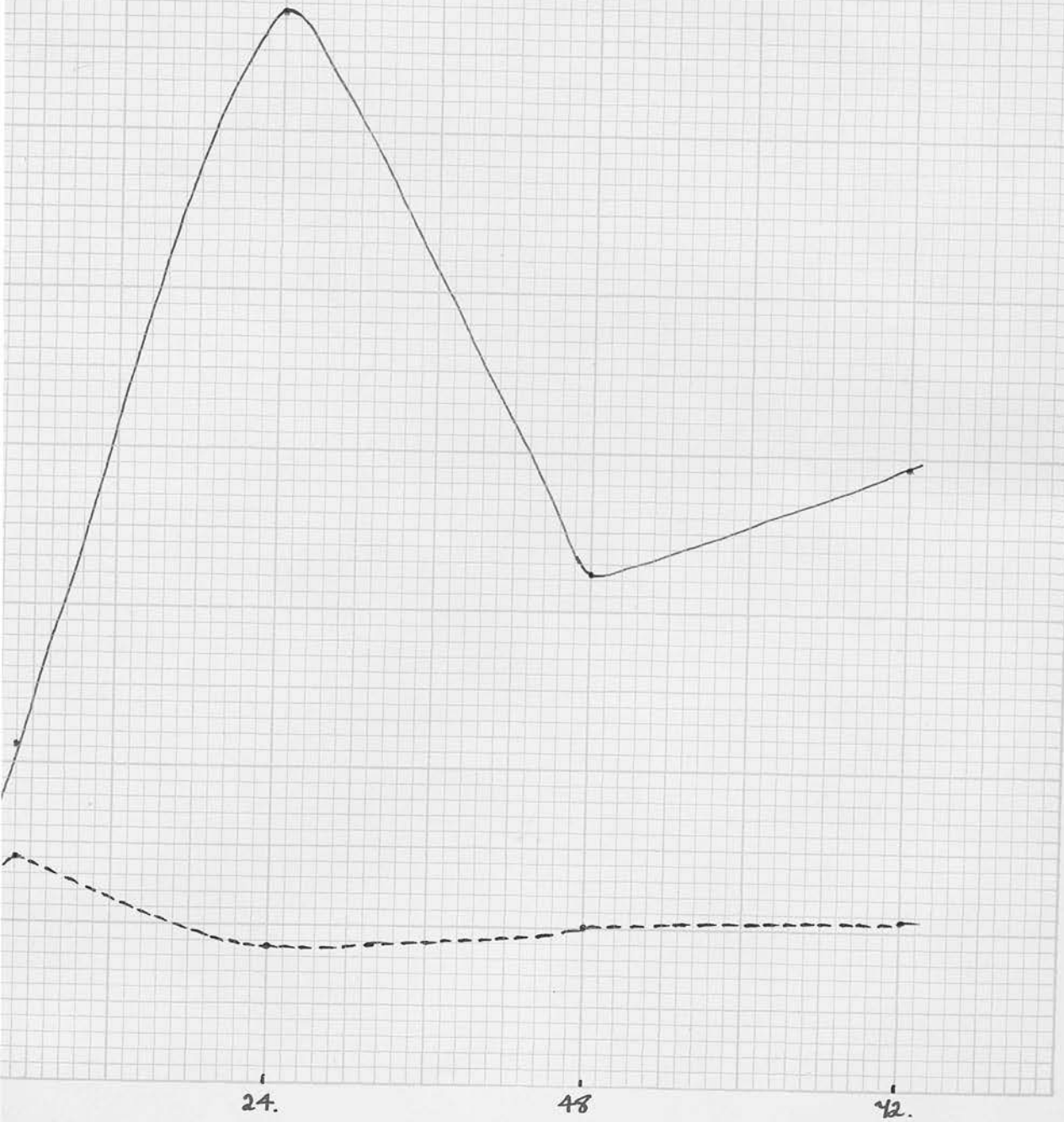
Experiment/

EXPERIMENT 11.

GRAPH 2.

CONTINUOUS LINE = BLOOD FAT OF ANIMAL TREATED WITH CORROSIVE SUBLIMATE : DOSAGE = 60 MGMS. per KILO (1 IN 500 SOLN.) INTRAORALLY.

BROKEN LINE = CONTROL.



DURATION OF EXPERIMENT IN HOURS.

Experiment 14.

Dosage: 14 mgms. per kilo. ($\frac{1}{2}$ per cent. solution) intra-muscularly.

Duration of Experiment	Total Blood-Fat in Mgms. per cent.		CO_2 - C.P. of Animal given $HgCl_2$ in volumes CO_2 per cent.
	Animal given $HgCl_2$	Control Animal.	
-	440	343	56.5
$3\frac{1}{2}$ hours	780	375	-
1 day	786	318	39
2 days	589	420	-
3 "	573	430	31
6 "	603	469	52
8 "	634	443	41

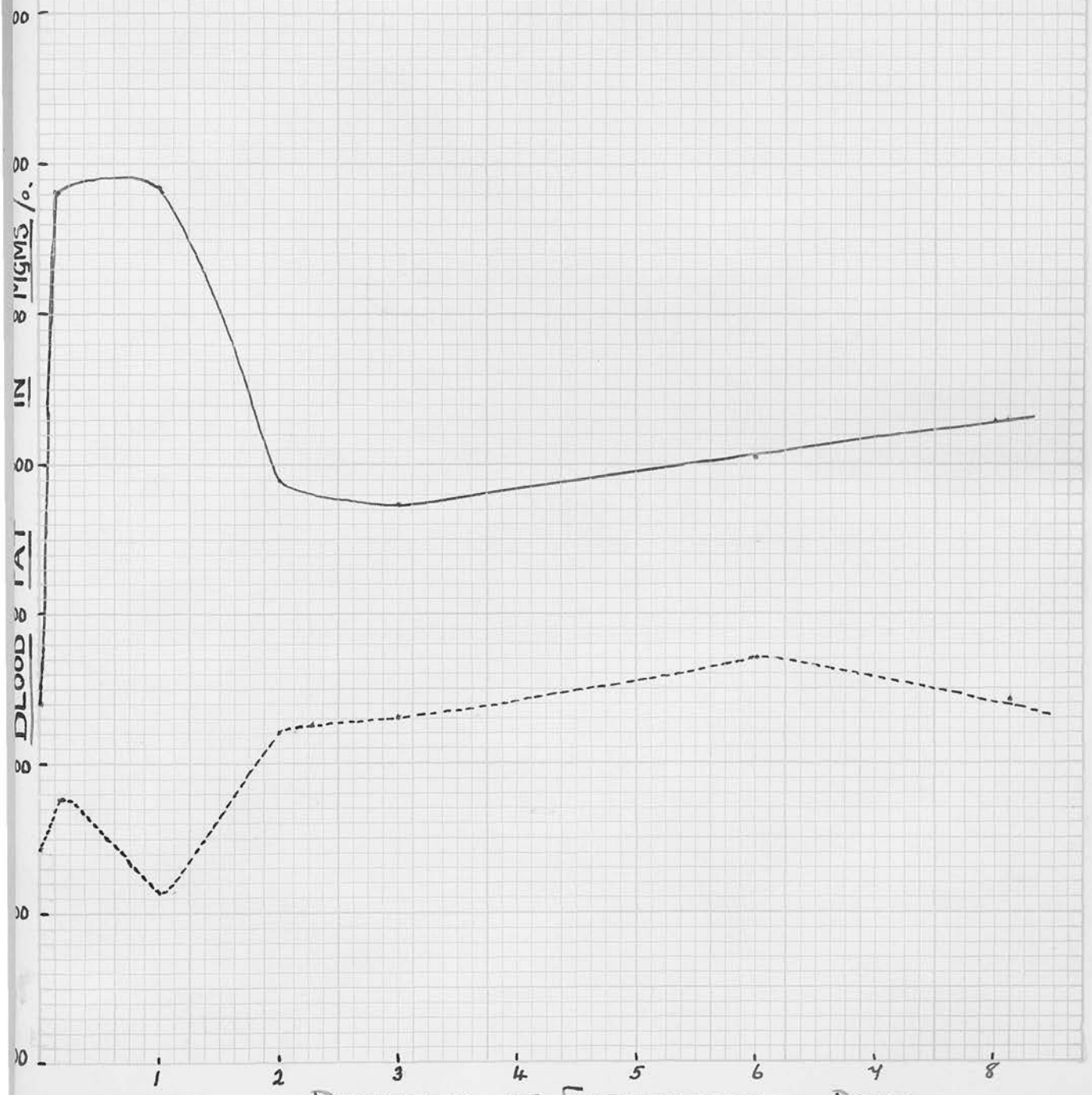
Animal ate well throughout this experiment, but by the 8th day its weight had fallen by 0.25 kilos. Otherwise the animal appeared healthy. See Graph 3.

Experiment/

EXPERIMENT 14. GRAPH 3.

CONTINUOUS LINE = BLOOD FAT OF ANIMAL TREATED WITH CORROSIVE SUBLIMATE : DOSAGE = 14 MGMS PER KILO (½% SOLN.) INTRAMUSCULARLY.

BROKEN LINE = CONTROL.



Experiment 16.

Dosage: 2½ mgms. (½ cc. ½ per cent. solution) daily intramuscularly. On 25th day was increased to 5 mgms. daily.

Animal given $HgCl_2$

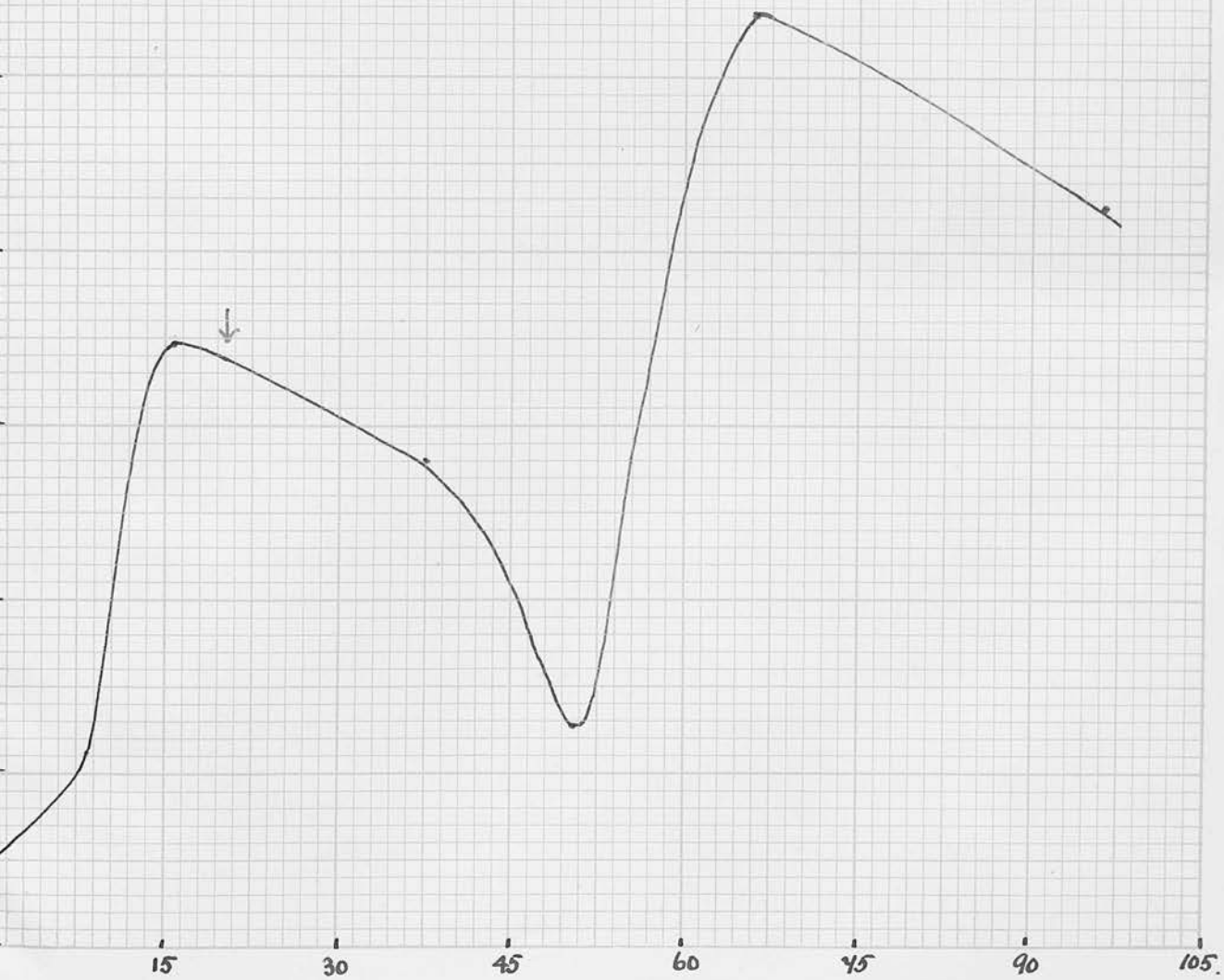
Duration of Experiment	Total Blood-Fat in Mgms. per cent.	CO_2 - C.P. in volumes CO_2 per cent.
Normal	450	61
8 days	510	69
16 "	748	49
38 "	680	61.5
50 "	529	60
66 "	934	50
86 "	867	-
96 "	822	64

During this experiment the animal ate well, but nevertheless its weight fell from 2.05 to 1.7 kilos. See Graph 4.

DISCUSSION/

EXPERIMENT 16. GRAPH 4.

CONTINUOUS LINE = BLOOD FAT OF ANIMAL TREATED WITH CORROSIVE SUBLIMATE : DOSAGE = $2\frac{1}{2}$ MGMS. ($\frac{1}{2}$ CC $\frac{1}{2}$% SOLN.) DAILY INTRAMUSCULARLY. AT ARROW DOSE WAS INCREASED TO 5MGMS..



DURATION OF EXPERIMENT IN DAYS.

DISCUSSION.

From a study of the figures given in the above tables and of the preceding graphs it is clear that corrosive sublimate causes an increase in the amount of fat in the blood. The increase, moreover, is both immediate and marked. Thus in Experiment 11 after the lapse of 4½ hours the blood-fat had risen by over 200 mgms. per cent. and in Experiment 14 the blood-fat rose 340 mgms. per cent. in 3½ hours. In Experiment 14 the level at which the blood-fat stands at the end of 24 hours, viz. 1548 mgms. per cent., is remarkable. In the case of the other three experiments (10, 11 and 16), one of them acute, the other two more or less chronic in character, it is noticeable that the highest level of the blood-fat (taking the crest of the first wave in Experiment 16) is in each case in the neighbourhood of 800 mgms. per cent. Whether that occurrence is accidental or whether it is associated with some definite underlying physiological action is difficult to say. The similarity of the three experiments in this respect and also the fact that doubling the dosage at the crest of the first wave in Experiment 16 produced no further immediate rise seems to indicate that this phenomenon is not accidental.

The/

The graphs indicate that in each case the rise in blood-fat is succeeded by a fall - abrupt in the acute cases of intoxication, slower in the more chronic ex-:periments. Thus in Experiment 11 the blood-fat fell from 1548 to 846 mgms. per cent. in 24 hours, and in Experiment 14 from 786 to 589 mgms. per cent. in a like period of time. The graphs of Experiments 10 and 16 which were more chronic in character show a slower fall in the height of the blood-fat. The latter, however, does not in any of these curves fall to its original, fasting level. And finally in every instance this fall is succeeded by a second rise of varying degree.

The discovery that mercury causes a definite in-:crease in the amount of fat circulating in the blood opens up many new channels of thought and enquiry. We naturally ask - Is this rise in blood-fat produced by mercury alone or is this action shared by other drugs? Whence comes this fat that appears in the blood? What is the mechanism whereby the fat is called forth into the circulation? Does the discovery that the admin-:istration of mercury produces a lipaemia shed any light on the mechanism whereby the physiological mig-:ration of fat is effected? What is the value of this lipaemia? What is the explanation of the fall in blood/

blood-fat after the initial rise and why is this fall succeeded, as it apparently is, by a second rise?

To begin with it may be said that mercury is not alone in this action. Lattes (1911)[63] has shown that phlorrhizin and phosphorus are also capable of producing a definite rise in the blood-fat. Both these drugs he administered to fasting animals. In the case of those treated with phlorrhizin the blood-fat in all but one of 13 dogs rose higher than the normal average; in 10 of them the blood-fat rose to higher figures than the largest amount found in any of his 13 normal animals; and in 2 of them to twice as much. Terroine (1914)[64] also administered phlorrhizin to 4 fasting dogs and found a notable increase in the blood-fat of 3 of them. Lattes treated 7 dogs with phosphorus and in 5 of these obtained higher blood-fat figures than in any of his normal animals; but the maximum figure observed was not nearly so great as that obtained with phlorrhizin. Mansfeld (1910)[65], on the other hand, poisoning 4 dogs with phosphorus obtained a rise in the blood-fat in only one case.

Mercury then shares with phlorrhizin and phosphorus the ability to produce a definite increase in blood-fat. Comparing my results with those obtained by/

by Lattes in his phosphorus experiments it is interest-:ing to note that while in two animals he found the blood-fat increased to twice the largest amount found in any of his normal animals, I have myself secured an increase in Experiment 11 to a figure 3½ times the largest amount given by a normal animal. It is scarcely possible, however, to arrive at any conclusion from the above results regarding the individual capac-:ity of these drugs (phosphorus, phlorrhizin and mer-:cury) to produce a lipaemia. Such would require carefully graded and controlled experiments.

Some further points of interest arise here. Thus it is well known that phosphorus and phlorrhizin act in-:tensely upon the liver and produce in that organ a very marked degree of fatty degeneration and infiltration. Moreover, Fiegl (1913)[66] has found that in the disease known as acute yellow atrophy of the liver which bears some resemblance to phosphorus poisoning the blood-fat concentration may be increased to 5 or 6 times the nor-:mal and as is well known the liver in this condition is found post-mortem to be the seat of widespread nec-:rosis and fatty deposit. In all these conditions of intoxication (phosphorus, phlorrhizin and acute yellow atrophy) the marked accumulation of fat in the liver is principally due to an upset in the process of fatty in-:filtration/

infiltration which proceeds normally in the liver. It is here that mercurial poisoning presents a contrast. I have already indicated that the liver in my experiments never showed any fatty deposit. Mercury does not damage the liver-cells to the extent of producing fatty degeneration and does not, as is to be expected under such circumstances, cause any accumulation of infiltrated fat in them - in spite of the marked increase of fat in the circulating blood. It might be argued that no infiltrated fat appears in the liver-cells for the reason that the mercurial poison renders the cells incapable of absorbing fat from the blood-stream. But that idea is hardly tenable in the absence of any signs of fatty degeneration in the liver-cells.

Whence comes this fat? Since these drugs effect a rise in the blood-fat of fasting animals it is clear that the excess of fat is not derived from the food. Two sources of fat still remain - (1) the animal's organs (liver, kidney, heart, etc.) and (2) the adipose tissues (subcutaneous, intermuscular, etc.) where fat is stored normally. In determining which of these is the actual source of the fat reference might with advantage be made to Rosenfeld's well-known experiments with/

with phosphorus. Rosenfeld (1902)[67] starved a dog until its depôt fat was exhausted and then fed it on mutton-fat so that the latter accumulated in the animal's adipose tissues. The dog was next subjected to phosphorus poisoning and the fat which accumulated in the animal's liver was shown to have the iodine index of mutton-fat. A similar result is also obtained when the experiment is carried out with phlorrhizin which likewise causes marked accumulation of fat in the liver. This fat could be derived only from the fat stores and must have been transported thence by the blood. In all probability, therefore, the fat which appears in the blood consequent upon the administration of mercury is derived from the fat-depôts just as it is in phosphorus or phlorrhizin poisoning. To be certain of this fact it would, of course, be necessary to determine which of the fatty constituents of the blood it is that undergoes increase; if it is the lipoid fraction then the organs would be the source of the fat; if the glyceride fraction then the fat would be derived, as I strongly suspect it is, from the adipose tissues.

What is the mechanism whereby these drugs produce a rise in blood-fat? Does their action in this respect throw any light on the normal arrangement whereby fat migration/

migration is effected? The physiological migration of fat is generally so balanced that the cells remove it from the blood just as fast as it is discharged into the blood-stream from the fat-depôts. In view of our knowledge regarding endocrine and hormone action it is possible that this physiological discharge of fat into the circulation is brought about under the influence of some agent liberated by those cells which are in need of fat. In the light of such a hypothesis mercury or phosphorus would appear to take the place of this spec-:ific hormone and because the drug is administered in excess the amount of fat called forth is likewise in great excess. It may be, of course, that these drugs do not act directly on the fat-cells. They may act on the cells of the various organs themselves and so cause them to liberate the fat-discharging hormone. In the absence of any direct action on the fat-depôts themselves this last statement must in its widest sense be true - i.e. wherever it is located the action of the drug is such as to produce agents or factors that dis-:tributed by the blood bring about the discharge of fat.

The graphs at any rate show that fat accumulates in the blood in amounts greater than can at first be handled by the organs. What is the explanation of the/

the fall in each of the curves? In the case of Ex-:periments 11 and 14 where single doses of corrosive sublimate were given it is conceivable that the de-:crease in blood-fat is due to the action of the drug passing off. This would allow the organs absorbing fat at ordinary speed to reduce the blood-fat to the levels shown in the curves. But the decrease is also marked in Experiments 10 and 16 where daily doses of corrosive sublimate continued to be administered even while the blood-fat was undergoing a diminution in a-:mount. Is it possible that the adipose tissues be-:gin at a certain stage to react less intensely to the drug so that their output diminishes? Or do the cells of the peripheral organs acquire the power of absorb-:ing fat more rapidly from the circulation? Either possibility or both acting together would result in a fall in blood-fat. At present it is difficult to de-:cide in favour of any one of these possibilities.

Neither is the significance of the second rise clear. Its occurrence might be explained on the grounds that the cells of the various organs having done their utmost to cope with the excess of fat sup-:plied them ultimately become incapable of dealing with more and consequently fat accumulates again in the blood/

blood.

Stewart, Gaddie and Dunlop (1931)[3] have shown that when fasting patients are subjected to fairly vigorous muscular exercise there is a definite increase in the amount of blood-fat. They also showed that this rise is preceded by a fall in the CO_2-Combining Power of the blood - indicating a drop in the latter's alkali reserve. Whether or no there is any association between the fall in alkali reserve and the discharge of fat into the circulation it is hard to say. But a point of interest arises here for McNider (1924)[29] has shown that in mercurial poisoning there is a definite diminution in the alkali reserve of the blood - a finding I was able to confirm in two of my experiments (14 and 16) by determination of the CO_2-Combining Power of the blood. Thus in Experiment 14 the CO_2-Combining Power of the blood fell from a normal figure of 56.5 to 31 volumes CO_2 per cent. in 3 days and in Experiment 16 from 61 to 49 volumes CO_2 per cent. in 16 days (see Tables on pages 148 and 149). McNider endeavoured to prove that this tendency to acidosis was the cause of the renal lesions, but in my opinion the reverse is the case, the fall in alkali reserve being due to the renal lesion inhibiting the excretion of acid bodies from/

from the blood - as is known to occur in nephritis gen-:erally. In both my experiments in which the CO_2-Combining Power was determined there was a definite rise in blood-fat, but the CO_2 - Combining Power fig-:ures were so variable as to make it impossible to cor-:relate the changes in blood-fat and alkali reserve. Whether there is any association at all between the fall in alkali reserve as a result of the renal lesion and the rise in blood-fat is as in the case of Stew-:art's experiments a question difficult to answer and one requiring further investigation. But in my opin-:ion the lipaemia is so instantaneous and so marked as to make it almost impossible for me to see any such re-:lation. Certainly I have shown that at the end of $3\frac{1}{2}$ hours there may be a very marked increase in blood-fat and there seems to me to be within that period scarcely time for the kidney lesion to effect any very marked change in alkali reserve.

Graphs 2 and 3 each show in the case of the con-:trol experiments a small initial rise 4 hours after the first blood-fat estimation. This may be explain-:ed on the grounds of Stewart's findings that muscular exercise produces an increase of blood-fat. The re-:sistance of the animal during the performance of the first/

first venesection involving as it did some struggling made itself manifest in an increase of blood-fat at the time of the second estimation a few hours later. But this rise as the curves indicate is small and is alto-:gether overshadowed in the case of the mercurialised animals by the marked increase effected by the admin-:istration of the drug.

In the foregoing pages I have endeavoured to dis-:cuss in so far as possible the pros and cons of my experimental findings regarding the changes in blood-fat produced by mercury. It is clear that this is a vitally important and interesting subject, but one, nevertheless, fraught with great difficulties and much obscurity. I have sought to explain the various phen-:omena observed in ways that seem to me sound and reas-:onable, but the fact that I have frequently found it impossible to proceed far without beginning to postul-:ate and theorise makes it clear that our knowledge of fat-transportation and metabolism is still sadly lack-:ing and in need of much further research.

CONCLUSIONS/

CONCLUSIONS.

1. Corrosive sublimate produces an increase in blood-fat which is both immediate and marked.

2. This initial increase in blood-fat is succeeded by a decrease - abrupt in acute cases of intoxication, slower in more chronic cases - but the falling blood-fat does not return to its original, fasting level.

3. This fall in blood-fat is followed by a secondary rise of varying intensity.

4. The initial increase in blood-fat is associated with a decrease in the reserve alkali of the blood.

5. The source of the fat that appears in the blood, and the relation between the increase of blood-fat and decrease of reserve alkali are discussed.

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