Fatty Degeneration of the Blood.

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Erroneous as histological research has proved Hunter's view of the organisation of effused blood to be, yet Hunter was the first to discern the biological truth that the blood is alive—alive in as strict a sense as is any other component part of the living body.

To view the blood as a tissue is a natural extension of the same conception, a tissue, that is, of mobile elements, and one in which the plasma represents a fluid, in place of a solid, intercellular material. Yet even in regard to this quality we may discover gradations between the opposite extremes like those presented by blood and cartilage, in such intermediate forms as mucous tissue, where the intercellular substance may be equally well called semi-fluid or semi-solid.

If there is a paradox in such an application of words, it is not so much that the incongruity marks an error of conception as that terms used in one stage of science become inadequate to compass biological truth, which as it grows under observation defies the artificial limitations which language would impose upon it.

In a similar manner has the common conception of a crystal been dissipated by the sheer force of observation. For the researches of Lehmann and of Schenck* have made it clear that certain fluids may retain the characteristic crystalline feature of containing doubly refractive bodies. The conception of a fluid crystal is not less paradoxical than that of a fluid tissue.

One peculiarity in the constitution of the blood whereby it differs from other tissues is, of course, the fact that although its cells, as in the more stable forms, undergo division, both mitotic and amitotic, and have in this sense a life of their own, yet the primary sources of its elements are the blood-forming organs—the bone-marrow, the hæmolymph and lymphatic glands, or other lymphatic tissue, and the spleen.

In considering diseases of the blood, therefore, there is a double question involved: there are firstly the changes in the blood arising from disease of the blood-forming organs, and, secondly, the abnormal changes of the formed blood, arising from lesions proper to the blood itself.

* 'Flüssige Kristalle,' Leipzig, 1905; cited by J. C. Adami, F.R.S., and L. Aschoff, Roy. Soc. Proc.,' B, vol. 78, 1906.

Conceiving of the blood, then, as a complex tissue, and subject like any other to parasitic and bacterial infection, or partaking in the morbid changes of many other specific diseases, it might be imagined that it would likewise be subject to degenerations, and more particularly to the chief and most widespread of all of these, viz., fatty.

The blood-degeneration which has been as yet most studied (other than the so-called polychromatophilic of the red cells) is that usually regarded as glycogenic, and consists in the abnormal, *i.e.*, excessive, appearance of glycogen, chiefly in the finely granular polymorphonuclear leucocytes, although the granules occur also in the eosinophile or coarsely granular polymorphonuclear cells, and to a lesser degree in the lymphocytes.

To this form of degeneration (which is known also as iodinophilic or iodophilic, since the grains and granules take a brown colour after treatment with iodine solution) we will refer again in connection with certain ambiguous appearances met with by ourselves in blood-films treated with Scharlach solution.

If we except pathological effusions, such as those of pleurisy and peritonitis and purulent exudations, the process of fatty degeneration has hitherto been looked for and studied only in the solid tissues. Yet it will be apparent on theoretical grounds that in cases where fatty degeneration of the last-named tissues is due to a deficient oxygen-carrying power of the blood, or to a toxic condition of it, the state of the blood might react upon itself so as to induce similar changes in its own cells.

The chief causes of fatty degeneration in general, excluding those of a local character which produce only a local change and have no interest in connection with the present subject, are profound anæmias and certain forms of toxæmia, whether bacterial or of inorganic causation, as especially illustrated in phosphorus poisoning.

In the first the fatty degeneration of the various organs results from the reduction in the oxygen-carrying power of the blood. In the second, it results directly from the injurious action of the toxin upon the cells of the tissue.

Our inquiry, therefore, is: Does the blood itself suffer similarly in these two groups of morbid conditions?

(1) Do the leucocytes (leaving the red cells out of consideration) suffer from a defective oxygen-carrying power of the blood itself?

(2) Do the leucocytes suffer from the direct action of toxins circulating in the blood in profound toxæmia?

In the blood, as in other tissues, the distinction must be drawn between fatty infiltration and fatty degeneration.

Without entering into the chemical question as to the source of the fat in fatty degeneration, how far, *i.e.*, it is due to a transformation of the proteid of the cell (as once held), and how far to the accumulation of fat reaching the cell from without and appearing in it because not utilised, the cardinal difference between the two processes remains, even though the source of the fat should be the same in both.

In the one case the fat accumulates or is stored in a cell of which the cytoplasm is uninjured; in the other it appears for the reason that the latter is damaged and rendered incapable of utilising the fat which reaches it.

With the fat that is not free, but combined, in the cell, neither term deals; the presence of this is not recognisable, either by its microphysical or microchemical properties.

Technique.

At the outset it is obviously essential to exclude the use of fat solvents, like absolute alcohol and ether, as fixing reagents, and essential also that the blood should not be dried. One preliminary method we tried was the use of blood clot, but it proved less satisfactory than that of making films.

Microscopic sections are readily cut from cylinders of blood that has been received into, and allowed to clot in, narrow tubes, the clot being afterwards fixed in salt-formol followed by 80-per-cent. alcohol. Such sections can be stained for fat like those of other tissues. The method, again, of using washed corpuscles (*i.e.*, corpuscles washed in salt solution after having been separated by the centrifuge from blood in which clotting has been prevented by the addition of citrate of sodium) offers no special advantages, and after making a trial of it we did not adopt it.

The method finally chosen was that of making films on slides and cover glasses. The film from first to last must be kept moist, and treated, in short, throughout as a section of any tissue which is to be studied for the presence of fat. The reason for this will be obvious, for any fat in the leucocytes would, in the dried film, tend to transude, and become lost by diffusion in the surrounding area.

The film having been made on the slide in the usual manner, by drawing a second slide in front of the blood, the slide is immediately placed with the film downwards in a specially devised chamber of formol vapour.

The chamber we use is a deep, oblong glass with a ground edge, and is itself hardly larger in sectional area than an ordinary microscopic slide. This is packed nearly to the top with cotton wool thoroughly soaked with formol (40 per cent.), and on the top of the wet wool is laid a grating of perforated zinc, near either end of which is soldered a cross strip of zinc

wire; the latter prevents the film from touching the grating, through which the vapour freely passes to fix the film. The chamber is covered with a piece of overlapping plate glass, after vaseline has been painted over the edge.

Before the slide is prepared, cover-glass films can be made in the usual manner, and placed with the blood upwards upon the perforated zinc. The glass lid being immediately replaced, the slide film is prepared and introduced with the film downwards over the cover glasses, from off which it is kept by means of the cross pieces of zinc wire on which it rests.

After not less than 15 minutes (though the films may be left in the vapour for 24 hours or longer, if convenient) the slide is removed and at once placed in the vertical position in a glass of Scharlach for 24 or 48 hours. The cover glasses are placed separately in hollowed glass blocks of the dye, accurately covered to prevent evaporation.

After a certain amount of experience we adopted, as a check, the practice of treating one of the cover glasses after removal from the formol, and before its

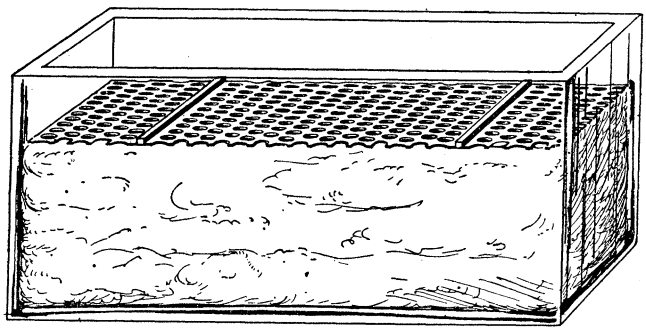

FIG. 1.—A diagram showing the glass formol chamber and zinc grating used to fix the blood films. The glass lid is not represented. Below the perforated zinc the space is filled with cotton wool soaked in formol solution, 40 per cent.

transference to the Scharlach, with absolute alcohol, followed by ether and again by absolute alcohol; the object of this was to remove any fat or fat-like bodies from the cells of the film. At first, in making the cover-glass films, we adopted the plan of adding salt solution to the blood on the cover, in order to prevent any possibility of the film drying; the film itself was made on each cover glass separately by means of a platinum loop. This precaution was found to be unnecessary, and we therefore discarded it.

The use of alcoholic solution of Scharlach as a fat stain requires much care, for the dye readily precipitates. The solution we employed was made by saturating 75 per cent. absolute alcohol in the cold, and subsequent filtration through paper first wetted with alcohol. In the case of a slide, the latter was

placed straight from the formol chamber into the Scharlach solution, and after 24 or 48 hours withdrawn, washed for a few seconds in 75-per-cent. alcohol, then in distilled water, and after this stained in a bath of hæmalum for three minutes; this was followed by washing in distilled water, and then in tap water, the film being finally protected with a long cover glass after the application of Farrant's medium. The cover glasses were prepared in a similar manner.

Our observations were commenced in April, 1906, and on May 29, 1906, at the meeting of the Pathological Society of London, held on that date,* we made a preliminary statement to the effect that we had discovered the existence of fatty degeneration in the blood in certain morbid conditions. We are not aware that any similar observation has been published before this announcement. The only work of a similar kind of which we know, indeed, is that carried out by Professor Antonio Cesaris-Demel, at the Institute of Pathological Anatomy of the University of Pisa. Of this a preliminary note was published very shortly after our own, viz., in June, 1906, having been communicated to the Royal Academy of Medicine of Turin at its sitting of June 8, 1906.† The title of the note is, "On Degenerative Changes of the Leucocytes of the Blood, studied by the Method of Coloration in the Fresh State." The author gives no detailed account and no figures, although he states that the stain used by him was Sudan III, one closely allied to that used by ourselves.

Our examination of blood films extends altogether to 79 cases and comprises various morbid conditions. Nevertheless, it is in comparatively few that we have satisfied ourselves of the presence of fat in the leucocytes, *i.e.*, of points or droplets presenting the same physical characters and coloration with Scharlach as those seen, *e.g.*, in the typical form of fatty degeneration of the cardiac muscle or renal epithelium.

List of Diseases in which the Blood was examined for Fatty Degeneration.

Pernicious anæmia.	Myelæmia.
Toxæmia of pregnancy.	Influenza.
Subphrenic abscess.	Exophthalmic goitre.
Chlorosis.	Acute pneumonia.
Diphtheria.	Chronic Bright's disease.
Appendicitis.	Purpura.
Lymphadenoma.	Pericarditis (rheumatic).
Acute meningitis.	Anæmia (secondary).

* 'Brit. Med. Journ.,' June 2, 1906.

† 'Giornale della R. Accademia di Medecina di Torino,' Giugno-Luglio, 1906.

Post nephric abscess.	Septic nephritis.
Anæmia of undeterminal origin.	Myxœdema.
Pleurisy and pericarditis.	Hepatic suppuration.
Septic bronchopneumonia.	Ulcerative endocarditis.
Carcinoma of pylorus.	Rickets.
Sub-acute rheumatism, probably gonorrhœal.	Acute cerebro-spinal meningitis.
	Diabetes and lipæmia.

In the case of some of these diseases more than one example was studied.

List of Diseases in which Fat was found in the Leucocytes.

Chlorosis.	Toxæmia of pregnancy.
Chronic Bright's disease and anæmia of chlorotic type.	Acute pneumonia.
	Purpura.
Carcinoma of pylorus and anæmia of chlorotic type.	Diabetes and lipæmia.
	Myelæmia.
Influenza.	Lymphadenoma.
Pleurisy and pericarditis.	Acute cerebro-spinal meningitis.

If these cases are analysed it will be found that most of them come in the category of toxic diseases, some of an acute kind, others of a chronic. Of the three cases of Myelæmia in which fatty degeneration of the finely granular leucocytes was found, one was acute, two were chronic.

The degree of fever present in these toxæmic diseases varied according as the condition was one of intoxication or infection. In one of the most pronounced examples of fatty change, viz., the toxæmia of pregnancy, the pyrexia was very slight.

In the toxic series may be placed:—

Toxæmia of pregnancy.	Influenza.
Acute pneumonia.	Pleurisy and pericarditis.
Myelæmia.	Lymphadenoma.
Purpura.	Chronic Bright's disease (associated with some degree of chlorotic anæmia).
Acute cerebro-spinal meningitis.	

In regard to this group of cases we believe that the fatty change present in the finely granular polymorphonuclear leucocytes results from the direct action of the toxic substances present in the blood itself; that is to say, that the change is one of proper fatty degeneration as distinguished from the ingestion or storage of fat by a healthy cell.

The fineness of the fatty points, their varying number in different cells in the same film, and the fact that they occur in abundance in cases where the condition of the patient excludes the possibility of so large an amount of fat being merely ingested from the blood by the circulating leucocytes, all point to this conclusion. Thus in the three cases of acute pneumonia the blood was taken, in one, on the sixth day, two hours after the crisis; in another, on the fifth or sixth day, before the crisis; and in the third, five hours after the crisis. Furthermore, the fact that in such an acute disease fatty degeneration actually does occur in the renal epithelium and cardiac muscle proves the ability of the toxins to effect such damage also upon the circulating leucocytes.

That the degeneration of the leucocytes has occurred in the blood and not in the bone-marrow may be held as almost certain, since had so marked a change taken place whilst the cells were still in the marrow, it is unlikely that they would have entered the vessels; they would have remained *in situ*. Upon the actual condition of the bone-marrow we have, with one exception, no observations.

When, as in myelæmia, the fat occurs likewise in myelocytes in the blood, we should think, similarly, that the change occurred in the cells after they had reached the blood-stream.

The leucocytes most degenerated probably die, and might by their disintegration furnish a certain amount of free fat in the plasma.

Not that this is the sole source of the free fat in the blood which was present in some of the cases, for its amount is too great for such an explanation. Most of the free fat must be regarded as derived from the intestine, or as transferred from the common connective tissue to the blood plasma for the use of the more important organs. The fat in some of the leucocytes under these circumstances is doubtless ingested, but the fact that in some of the cases where the fat in the leucocytes was well pronounced no free fat was present in the plasma shows that this cannot be advanced as the sole explanation.

The presence of fat in some of the myelocytes of the blood film in myelæmia, again, cannot be due to ingestion, since these cells have no phagocytic powers. It might have been thought, *a priori*, that the presence of fat in the finely granular polymorphonuclear leucocytes would be a sign of grave significance as indicating not only a high degree of toxæmia but an interference with the function of cells, the phagocytic integrity of which is of such importance in the cure of a disease like pneumonia. For the leucocytes so altered must have lost for the time their physiological capacities. They would be much in the same condition as the pus cells in an acute abscess,

rendered fatty by the toxic action of pyogenic micro-organisms. Indeed, a study of cover-glass films made from the pus of an acute abscess and treated precisely as those of blood reveals the presence of scarlet points and droplets within a large number of the finely granular polymorphonuclear leucocytes, which are exactly matched by those in the leucocytes of certain of the blood films described. In some of the pus cells (as in the blood) the red points are quite minute (as they were in the case of influenzal pleurisy); in others larger (as in one of the cases of acute pneumonia); or still more so, as they were in the case of pyloric carcinoma associated with chlorotic anæmia.

It is noteworthy, too, that in the pus cells so affected the nuclei may exhibit no fragmentation. Nor have we ever seen nuclear fragmentation in the fatty leucocytes in blood films.

This prognostic conjecture, nevertheless, is not borne out by observation. Thus in the three cases of acute pneumonia in which a certain number of the polymorphonuclear leucocytes contained fat, recovery ensued. In one case the blood was examined on the sixth day, two hours after the crisis; in another on the fifth or sixth day, before the crisis; and in another five hours after the crisis.

The actual number of leucocytes showing the fatty change in these three cases, however, was not excessive, and although the phagocytic power of such cells would, doubtless, be diminished or destroyed, the great excess of leucocytes present would compensate for such a loss. Within two or three days after the crisis the leucocytosis has disappeared; the leucocyte count is practically normal.

This rapid disappearance of the leucocytic excess from the blood is largely due to the vast immigration of leucocytes into the infected organs, the lung more especially; the surplus yet remaining would disappear, as having no further purpose to serve. For all observation goes to show that the rapid amelioration following the crisis is due to the activity of the finely granular leucocytes.

MacDonald* has shown that the opsonic index attains its maximum at the crisis. And Rosenow† concludes from his work that pneumonia serum has no pneumococcidal effect; that the opsonin during and shortly after crisis (in cases that recover) seems to rise above the normal, and that the pneumococcidal action of pneumonic blood is the result of the combined action of serum and leucocytes—phagocytosis and intraphagocytic digestion. It must not be forgotten, moreover, as a general consideration, that the

* 'Path. Soc. Trans.,' London, vol. 57, p. 45, 1906.

† 'Journal of Infectious Diseases,' vol. 3, June 30, 1906, Chicago.

degenerating cells in the blood may recover, like those, *e.g.*, of the kidney in toxic diseases, in which the damage done to the renal epithelium is shown by the temporary presence of albumen and casts in the urine.

In all the cases we have examined the fatty changes were confined to the finely granular polymorphonuclear leucocytes, that is, to the cells which take the active part in phagocytosis; the most active cells, as being the more highly differentiated, are those most affected by the toxin.

We have never seen any such change in the lymphocytes—a class of cell devoid of phagocytic power.

The inactivity of the lymphocytes is a striking comment upon the fanciful views once held, that the lymphatic tissue, whether of the tonsils, lymphatic glands, or intestinal track, constituted a highly important line of defence against microbial invasion. As a matter of observation it is in the solitary and Peyerian glands of the intestine that tubercular infection takes place; and once a tubercular process has commenced in a member of a lymphatic chain it spreads from gland to gland until the whole are involved.

Anæmia.

In this group of diseases the following of the cases in which fat was found in the leucocytes may be placed:—

(1) Chlorosis.

(2) Carcinoma of the pylorus, associated with anæmia of the chlorotic type.

In chlorosis the fatty changes occurring in the finely granular polymorphonuclear leucocytes are attributable to the deficient oxygen-carrying power of the blood. No direct observations upon the state of the organs appear to have been made in this disease, although it is commonly assumed that they are affected with fatty degeneration; the dilatation of the heart observed clinically being especially so explained. Observations upon the blood-forming organs are equally wanting.

In chlorosis the most marked change in the blood is the excess of water—hydræmic plethora. The number of red cells in a given volume is diminished; the absolute number is increased; and similarly, whilst the percentage of hæmoglobin may be reduced to 40 or even to 30, the total hæmoglobin content remains normal, and the oxygen-capacity undiminished.

As the reduction in the oxygen-carrying power affects the tissues supplied by the blood, so it may affect the nutrition of the leucocytes in the blood itself.

In the particular case of chlorosis in which fat occurred in the leucocytes the blood was taken at 2 o'clock p.m., the patient having had no food since

breakfast. The plasma, as studied in the blood films, contained free fat, the presence of the latter, under the circumstances mentioned, being possibly due to its less ready manipulation in consequence of the excessive bulk of water in the blood.

The amount of fat in the finely granular leucocytes in this case is very striking; every cell held a certain number of fine scarlet points or droplets, and many are loaded. How much of the fat in the leucocytes is to be ascribed to ingestion, and how much to degeneration, it is impossible to say.

As in the case where œdema follows venous obstruction the transudation occurs only after the stasis has led to damage of the endothelium, so in a somewhat similar way, in the case of anæmia, we should regard the deficient oxygenation as involving injury to the leucocytes; the intracellular fat appears in consequence.

In the case of pyloric carcinoma there was an associated chlorotic anæmia arising from the ill-nourished condition of the body in general, including the blood-forming organs.

The fat droplets in the leucocytes, of which only a certain number were affected, are of conspicuous size, the change being a far advanced one. No free fat was present in the plasma.

And finally, in the case of diabetes and lipæmia, in which typhoid infection terminated life, the amount of free fat in the plasma makes it clear that much of the fat in the leucocytes must be ascribed to phagocytic ingestion, but how much to this, and how much to the toxic condition of the blood, cannot be stated. The peritoneal fluid removed after death was full of fat droplets. The bone-marrow showed a highly abnormal number of large endothelial cells holding an abundance of multiple fat droplets. The gall-bladder held no bile, and its contents were so white that they were at first thought to be purulent; microscopic examination revealed the presence of free fat and shed columnar cells.

It is interesting to note, in passing, that the bases of these cells were loaded with fat, which had evidently been ingested by them whilst *in situ*, as it is by those of the intestine. The absorptive capacity of the epithelium of the gall-bladder has been shown, also, by Aschoff.

The Presence of Granules in the Leucocytes, which Stain of a Deep Brown Colour.—Scharlach Granulation.

From the foregoing list of diseases in which fat was found in the finely granular polymorphonuclear leucocytes, some, in which a fatty degeneration might have been particularly anticipated, are conspicuous by their absence.

Two such would certainly be diphtheria and pernicious anæmia. In both these diseases, however, we have found the leucocytes laden with fine granules, which, though deeply stained, do not exhibit the transparency and proper red colour of ordinary fat.

We may first give a list of the cases in which these deep brown granules occurred; the phenomenon itself we may call, for convenience, Scharlach granulation :—

Subphrenic abscess.	Diphtheria (6 cases).
Exophthalmic goitre.	Appendicitis.
Pneumonia and empyema.	Pericarditis (rheumatic).
Pernicious anæmia.	Acute meningitis.

In discussing the nature of these granules, various possibilities require consideration. That the granulation is not a mere precipitate of Scharlach within the cytoplasm of cells that have been imperfectly fixed is shown by the fact that the diphtheria blood films (in which, amongst others, it occurred) were fixed in formol vapour for over 24 hours.

It is noteworthy that the normal granules of the eosinophile leucocytes exhibit a somewhat similar coloration, though of a less intense degree. Against the identity of the granulation with the substance composing the eosinophile grains, however, must be placed the circumstance that the latter are equally well coloured in both pathological and normal blood films after the films have been treated with absolute alcohol; the fine granules under discussion, on the contrary, are not demonstrable by means of Scharlach if the films are first treated with absolute alcohol.

The degree of Scharlach granulation, moreover, present in some cases (every finely granular leucocyte being laden) excludes, we think, the explanation that the granules in question are eosinophile, which have been discharged from coarsely granular cells, disintegrated, and subsequently ingested by the finely granular leucocytes.

In this connection it may be further mentioned that no eosinophilia has been present in any of the cases as a possible source of such an excessive amount of Scharlach-staining substance.

The Scharlach granulation is not due to the coloration of the proper granules of the cell; for in some of the leucocytes comparatively few granules occur, and in these cells the proper granules are recognisable, though unstained.

The granulation is not unlike the glycogenic, as displayed in the leucocytes after treatment of blood films with iodine solution. In most cases the iodine reaction takes the form of a diffuse brown coloration of the cytoplasm of the

finely granular polymorphonuclear leucocytes, but it may take that of a fine stippling. A still further likeness is to be found in the occasional presence of similar iodophilic granules in the plasma; for fine extra-cellular granules may occur also in the Scharlach preparations.

Although the iodophilic reaction may be seen in normal blood, in pronounced degree it occurs only under pathological conditions, and observers are agreed in viewing it as evidence of cell degeneration, brought about in most cases by a toxic cause.

We exclude this explanation, however, for the reason that the Scharlach granulation is not to be observed in control cover-glass films if treated with absolute alcohol before being transferred to the dye. The glycogenic reaction, on the contrary, is as readily obtained after this treatment as without it.*

We have, moreover, found that glycogen is uncoloured by Scharlach. If fat-free glycogen (from the rabbit's liver) is dissolved in distilled water and precipitated by alcohol, the wet sediment, when treated with Scharlach for 48 hours and examined microscopically, is seen to consist of fine spherical granules which are quite untouched by the dye. The same sediment stains of a mahogany brown when submitted (after drying on an albuminised cover glass) to the action of iodine vapour for 20 minutes, and afterwards mounted in Ehrlich's iodine medium.

The absence of the Scharlach granulation when the blood films have first been treated with absolute alcohol excludes the possibility of the granules being blood pigment which has been ingested by the leucocytes.

We have found the Scharlach granulation in some of the finely granular polymorphonuclear leucocytes in cover-glass films made from the splenic pulp in a case of acute peritonitis, the films having been fixed in formol and treated precisely like those of the blood. In this observation, also, the exclusion of blood pigment is brought out by the fact that the deep brown granules are not to be seen in the films stained by Leishman's method.

Does the Scharlach granulation represent a substance in which fat is not free, but combined? Is it allied to lecithin or myelin, both of which are known to occur in many kinds of cell? Lecithin stained from egg-yolk is not coloured with Scharlach.†

In sections of an enlarged prostate which we have examined, although the presence of fat was demonstrated by Scharlach treatment in some of the epithelial cells, the free granules in the acini (generally held to be lecithin) were quite uncoloured.

By the same want of colourability, after treatment with formol, myelin

* J. Barnicot, 'Journal of Pathology,' vol. 11, p. 304.

† T. R. Elliott and I. Tuckett, 'Journal of Physiology,' vol. 34, p. 350.

may be excluded. In medullated nerve fixed in formol and afterwards teased and immersed in Scharlach solution for 24 hours, the myelin of the individual fibres exhibits practically no coloration; it is equally unaffected by osmic acid, if the nerve is first fixed in formol.

As regards the coloration of human fats, that is, of their fatty acids which are the proper colourable substances, pure oleic acid is stained of the typical red colour with 75-per-cent. alcoholic solution of Scharlach.

From human liver Leathes has extracted another fatty acid, the colourability of which we have been able to test upon cover-glass smears. The smear, after being treated with formol vapour, stained with Scharlach, and finally washed in distilled water and mounted in Farrant's medium, is transparent, and of an orange or orange-brown colour; it presents nothing suggestive of the Scharlach granulation.

From human heart-muscle Leathes has also extracted a fatty acid other than oleic, and not identical with that referred to from the liver.

The fact, however, that in fatty degeneration of this muscle, as studied in diphtheria, the fat that appears is from the first stained of a brilliant red by Scharlach shows that, whatever fat besides olein may be present, it is not the substance constituting the Scharlach granulation.

There is, to conclude, the possibility that the granulation is due to a soap, the colloidal nature of which prevents its diffusion into the substance of the cell.

Various fermentative capabilities have been ascribed to the leucocytes. They are certainly proteolytic; and both amylolytic and lipolytic powers have been attributed to them.

It is conceivable that the fat droplets, on their first appearance in the leucocytes, might be split, with the resulting formation of an intracellular soap arising from the combination of fatty acid with calcium, sodium, or potassium.

Using pure oleate of calcium (the only soap of the three not soluble in water), we find that cover-glass smears, after being treated as the blood films, fail to exhibit the dark brown colour and opacity of the Scharlach granulation; the particles take a pale brown or orange tint.

For the present, therefore, we are forced to leave the nature of the Scharlach granulation undetermined. Nevertheless, its appearance in diseases in which a fatty degeneration of the leucocytes might have been anticipated, and the fact that in some cases of acute pneumonia the proper Scharlach reaction of the points in the cells is obtained, whilst in others it is the brown opaque Scharlach granulation, makes us conclude that the granulation is indicative of a degeneration; and seeing that it is not

obtainable after the treatment of the blood films with absolute alcohol, we conclude that it is allied to that known as fatty.

In conclusion, we desire to express our thanks to Dr. G. Buckmaster, and to Dr. J. B. Leathes, for criticism and help in regard to certain of the points raised by the investigation.

Some Points in the Development of Ophiothrix fragilis.

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The subject of which the present communication is a brief preliminary account has occupied my attention during the last five years, and a full account will be published shortly. Meanwhile, a brief outline of the main results is given here.

Ophiothrix fragilis is one of the commonest species of British Ophiuroidea. Especially in the neighbourhood of Plymouth, where the material for this research was obtained, this species is found in immense swarms, so that, on certain kinds of bottom, hundreds of individuals are brought up with each haul of the dredge. During the months of June and July it spawns, and on some occasions the Plankton captured above the beds of gravelly sand on which it lives is full of its larvæ in all stages of development.

A large quantity of this Plankton, captured during July, 1899, formed the principal part of the material, but this was supplemented by artificial fertilisation carried out in the laboratory of the Marine Biological Association in the years 1898, 1899, and 1905.

The eggs are small (about 0·1 mm. in diameter) and opaque owing to the possession of a reddish-brown yolk. The development was observed to be completed in 28 days, but the rate of development depends on the amount of food supply in the water, and it is probable that in the sea the eggs develop more quickly than under the most favourable circumstances in the laboratory.

Only on one occasion (June, 1898) was it found possible to rear the larvæ through their development until metamorphosis was completed; this was due to the presence of an unusual amount of Phytoplankton in the water