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THE FATS OF PNEUMONIC EXUDATIONS

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(From the Sears Pathological Laboratory of the Harvard Medical School and the Pathological Laboratory of the Boston City Hospital)

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THE FATS OF PNEUMONIC EXUDATIONS.*

HENRY A. CHRISTIAN, A.M., M.D.

(From the Sears Pathological Laboratory of the Harvard Medical School and the Pathological Laboratory of the Boston City Hospital.)

In recent times much attention has been directed to the fats of the body, their distribution in the various tissues under normal and abnormal conditions, and the interpretation of their presence. No longer are Virchow's [1] criteria for the differentiation of fatty infiltration from fatty degeneration accepted. Rather, influenced by the work of Lebedeff,[2, 3] Rosenfeld,[4] Krehl and his pupils,[5] Taylor,[6, 7, 8] Lubarsch,[9] Fischler,[10] and others, there is a growing tendency to question even the existence of a fatty degeneration in the sense of a formation of fat in situ from the splitting up of the cell proteid and, instead, to consider that the condition is in reality a fatty infiltration with which is associated a varying degree of cell degeneration.

With this revival of interest in the tissue fats have come new technical methods and these have been applied to different tissues and processes, mainly to the liver, kidney, heart, and skeletal muscles. The purpose of this paper is to give the results of the application of some of these methods to the pneumonic lung.

The histology of pneumonia has been studied in its various aspects by Fox,[11] Finkler,[12] Kohn,[13] Hauser,[14] Bezzola,[15] Ribbert,[16] Aufrecht,[17] and Pratt.[18] In their papers very little or no consideration is given to the presence of fat in the various cells of the exudate. Fox [19] pictures and describes small cells with rounded nuclei, a few of which, he says, show fat drops in their interior. These occur in both red and gray hepatization. Pratt,[20] considering desquamated epithelial cells, says: "The protoplasm stained but lightly and was frequently vacuolated. Treatment with osmic acid showed the vacuolation was not due to fat."

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In many of the text-books of pathology fatty degeneration is considered as playing an important part in the stage of resolution (Ziegler,[21] Orth,[22, 23] Schmaus and Ewing[24]). Simon,[25] in studying the action of a proteolytic ferment in the process of resolution, notes the presence of fat in the expressed fluid after autodigestion in the incubator. However, with these exceptions, it seems that special attention has not been given to the fatty substances present in the cells of pneumonic exudations.

Technic. — For the study of the fats of the pneumonic lung several methods were employed: (*a*) the Marchi method for staining degenerated myelin sheaths of nerve fibres;[26] (*b*) the method of direct reduction of osmium tetraoxide ("osmic acid") by fat, the fett-osmium-reduction of Starke;[27] (*c*) the secondary reduction of fat thus treated by alcohol, the alcohol-osmium-reduction of Starke; (*d*) staining with Sudan III., a stain introduced by Daddi,[28] or with Scharlach R. (Fettponceau), recommended by Michaelis;[29] (*e*) the determination of solubility in alcohol and ether. In the first method celloidin embedding was employed, the sections cleared in chloroform and mounted in chloroform balsam; for the others frozen sections mounted in glycerin were used. All material was hardened and preserved in a ten per cent aqueous solution of commercial formaldehyde.

Sections treated with osmium tetraoxide were studied unstained, or counterstained with a saturated aqueous solution of saffranin. Those stained with Sudan III. or Scharlach R. were left unstained, or counterstained with hematein, methylene blue, toluidin blue, or methyl green in aqueous solutions. A one per cent solution of osmium tetraoxide in distilled water was made use of, and allowed to act on the sections for twelve to twenty-four hours. For staining with Sudan III. or Scharlach R., the sections were left for twenty-four to forty-eight hours in a solution in seventy per cent alcohol, supersaturated in the way suggested by Fischer[30] (saturate with boiling 70 per cent alcohol, and place solution

for twenty-four hours in incubator at 37° C.) or for ten to fifteen minutes in the alkaline solution of Herxheimer[31] (absolute alcohol 70 pts., water 10 pts., 10 per cent sodium hydroxide 20 pts., saturate with Sudan III. or Scharlach R.). The former method, with an alum hematein counterstain[32] gave the most satisfactory results. Of these two fat stains, Scharlach R. was found preferable, because it gives a rather brighter staining of the droplets of fatty substance, does not tint other tissue elements of the lung, and has less tendency to precipitate in the preparations.

The results obtained by these methods were controlled by paraffin sections stained with hematein and eosin. Unless this is done, error may arise with the osmium preparations from mistaking for fat, pigment already present in the lung either hematogenous or extraneous in origin. Further, in material preserved in formaldehyde a dark, granular precipitate sometimes appears. All of these substances may present much the same appearance as osmium reduced by fat and lead to confusion. Another source of error as pointed out by Poll[33] may arise from the reduction of the stain subsequently used by remains of osmium tetraoxide in sections not thoroughly washed in water.

Material.—Typical examples of the various forms of exudation in pneumonia have been studied, representing the earlier and later stages of the disease. Tissue from different portions of ten lungs has been examined. The classification of the cellular exudate given by Pratt[18] has been followed.

In one lung the exudate consists of non-granular mononuclear cells, with a smaller number of polymorphonuclear leucocytes, a varying admixture of fibrin, and a few scattered, desquamated, epithelial cells. The cells, with very few exceptions, are well preserved, and show no evidence of degenerative changes. In the polymorphonuclear leucocytes of both alveoli and bronchi are numerous droplets of a substance which stains bright orange-red with Sudan III. and Scharlach R. These droplets are small. As a rule, they measure less than one micron in diameter, though occasionally

they are larger. The number in a single cell varies from one to twenty or more; generally there are six to ten. They are irregularly distributed in the cytoplasm, sometimes grouped together, but do not occur in the nucleus. In many alveoli they are found in almost every polymorphonuclear leucocyte. They are sometimes found in the mononuclear cells, but oftener these are free from them. They appear also in some of the desquamated epithelial cells. Besides these small droplets, a cell is occasionally seen which contains droplets two or three times their size, and staining similarly. All of these cells lie within the alveolus or bronchus. Sometimes an intravascular leucocyte contains similar small droplets.

Sections from this same lung treated by any of the osmium tetraoxide methods give very different pictures from the above. In them the mononuclear and polymorphonuclear cells very infrequently contain droplets of a brown or black color. The very few droplets present are larger in size than the ones common in the Sudan III. or Scharlach R. preparations. As a rule they are not very dark in color, though they may become darker after secondary reduction in alcohol. In the desquamated epithelial cells are numerous masses, rather coarse, often irregular in contour, and black or brownish-black in color. Examination of paraffin sections, untreated with osmium tetraoxide, shows that most of the latter and some of the former are pigmented masses already present and not reduction products of the osmium treatment. That the droplets staining with Sudan III. or Scharlach R. are present and unaffected by the osmium tetraoxide is shown by their appearance in large numbers when osmium preparations, not previously treated with ether or strong alcohol, are stained with one of these fat stains.

All of these droplets before osmium treatment are soluble in ether and less readily soluble in strong alcohol, as shown by their not appearing in section stained with Sudan III. or Scharlach R. after being extracted with ether or strong alcohol. Eighty per cent alcohol does not dissolve them, or dissolves them extremely slowly.

In another lung the exudate contains a larger number of polymorphonuclear leucocytes and numerous phagocytic cells with leucocyte inclusions. In this are found many more droplets which reduce osmium tetraoxide than in the first. The number of these, however, is greatly exceeded by the droplets staining with Sudan III. or Scharlach R., and this increment appears to come largely in the form of small droplets.

In this lung the phagocytic cells are of interest. The included cells are almost always polymorphonuclear leucocytes or their remains. Some of these included leucocytes contain numerous orange-red droplets, while the cytoplasm of the phagocyte is free from them. In others both phagocyte and inclusion contain these droplets or they may be present only in the cytoplasm of the phagocyte, while the ingested leucocyte shows more or less evidence of digestion, or they are absent from both. Those inclusions in which the digestive process is advanced practically never contain fat droplets. Also there are present large cells, containing no inclusions, but otherwise similar to the phagocytic cells, which are either free from or contain orange-red droplets. Many though not all of these droplets also reduce osmium tetraoxide.

These appearances suggest that the leucocyte is ingested with its fatty content, and that in the process of intracellular digestion the fat droplets are removed from the leucocyte and reappear in the phagocyte. The leucocyte generally lies in a distinct vacuole. There is no evidence of a direct passage of the droplets through this, and it seems probable that here we have to do with a process similar to that in absorption of fat from the intestine, where, from the work of Kischensky,[34] it appears that most of the fat passes through the epithelial cells in some soluble form, during which condition it is not stainable by any of the fat staining methods, though, after its passage, it reappears in the form of stainable droplets. This presupposes a fat splitting with subsequent fat synthesis due probably to an enzyme action. The present view of the reversibility of the action of enzymes

as advanced by Hill[35] and by Kastle and Loevenhart[36] and the regular occurrence of the fat splitting enzyme, lipase, in the blood serum and many organs, as shown by Hanriot[37] and Loevenhart,[38] give further support to the view that the fatty substance present in the leucocyte is split up, passes into the cytoplasm of the phagocyte, and is there built up again into its original form.

In lungs representing the latter stages of gray hepatization or beginning resolution, in which the cut surface of the lung has lost its granular appearance, exudes much purulent fluid, and begins to have a yellowish tinge, microscopic examination shows the remaining fibrin to be granular or coalesced into irregular masses, and the cells of the exudate, mainly polymorphonuclear leucocytes, to be very granular and vacuolated. The cells are evidently undergoing rapid degeneration, and are being absorbed into the lymphatics. These cells are filled with droplets, which reduce osmium tetraoxide and which stain with Sudan III. or Scharlach R. The droplets are generally rather large, and the only difference between the two kinds of preparations is a possible greater richness of droplets in those stained with Sudan III. or Scharlach R. The lymphatics are crowded with the same cells containing the same droplets.

In one lung with a hemorrhagic exudation into the alveoli there are numerous vacuolated, desquamated, epithelial cells, but few other nucleated cells. This is from an autopsy in which the consolidation occupied the central portion of several lobes in a patient who had chronic diffuse nephritis, dilatation and hypertrophy of the heart, and general anasarca. Here the epithelial cells, both desquamated and attached to the alveolar wall, are crowded with rather large droplets staining intensely with Sudan III. and Scharlach R. and reducing osmium tetraoxide actively.

Similar cells are very numerous in a case of unresolved pneumonia in which organization of the exudate is advanced and which is complicated by a disseminated tuberculosis.

Discussion. — In the earlier stages of pneumonic exudation

there is present in the cells, particularly in the polymorphonuclear leucocytes, a substance in the form of small droplets, which is soluble in strong alcohol and in ether, insoluble in water and in 80 per cent alcohol, which stains intensely with Sudan III. and Scharlach R., and which does not reduce osmium tetraoxide. In the later stages, in addition, there is present likewise in the cells, generally in the form of larger droplets, a substance which has the same solubility properties, which also stains intensely with Sudan III. and Scharlach R., but which does not reduce osmium tetraoxide. In other words, the exudation cells of pneumonia contain two substances of a fatty nature, differing in some of their microchemical reactions, the one appearing earlier than the other.

Two questions naturally arise: What are these substances? What is their source?

As to their nature two hypotheses may be advanced: (*a*) the two substances are the same in chemical composition, but differing in physical condition; (*b*) they are chemically different substances. According to the first, it may be supposed that one is in a more finely divided state than the other, or possesses some form of envelope, possibly albuminous in nature, to keep it in a state of emulsion, and that under these conditions it is not capable of being penetrated by osmium tetraoxide, and so there is no reduction. Against this hypothesis is the following: Elsewhere, as in the heart and kidney under conditions regarded as fatty degeneration, equally fine droplets appear which do reduce osmium tetraoxide. In milk where there is fat in a state of emulsion with a supposed albuminous envelope there are a few very fine droplets. The staining reactions of these can be studied in this way. Agar-agar such as is used in nutrient media for the growth of bacteria can be freed from fat by means of ether, melted, and before it cools mixed with a small quantity of milk. After solidifying, frozen sections of this mixture can be cut and treated in the same way as the frozen sections of lung. Doing this no evident difference in the richness of small droplets is found whether treated with osmium tetraoxide or stained with Sudan III. or Scharlach R.

The second hypothesis, that we have to do with two substances chemically different, appears more plausible. That substances with differing staining affinities are chemically different is generally accepted, though the converse is not true. Altmann[39] has shown that osmium tetraoxide is reduced by olein and oleic acid. As he puts it: "Das Osmium ist mithin nicht ein Reagens auf Fette in Allgemeinen sondern auf freie Oelsäure und Olein;" and on the presence of these depends the reaction of body fat with osmium tetraoxide. The work of Starke[27] and Handwerck[40] go to show that the palmitin and stearin components of body fat do not reduce osmium tetraoxide directly. Michaelis,[41] Fischer,[30] and others state that Sudan III. and Scharlach R. stain all fats and these alone. Kaiserling and Orgler[42] have studied in the adrenal, thymus, and elsewhere a substance which resembles ordinary fat, but which differs from it in being doubly refractile to polarized light and in staining only light gray with osmium, though staining red with Sudan III. and Scharlach R. These fatty substances evidently differ in staining reactions, as do those found in pneumonic exudations.

Of the two fatty substances in the lungs, the one occurring later in the disease agrees in its staining properties with body fat and is probably true fat. The one occurring early differs from true fat. It may be some form of palmitin or stearin; it may be the same as the substance described by Kaiserling and Orgler[42] and spoken of by them as myelin; or it may be some other fatty substance. The exact nature of it cannot be determined by its staining reactions. That it is not true myelin, such as occurs around nerve fibres, is shown by the fact that it does not reduce osmium tetraoxide. That true myelin is not present in our preparations appears from the fact that there is no difference between sections treated directly with osmium tetraoxide and those treated by the Marchi method. The chemical analysis of Orgler[43] also show differences between the substance found by him in the thymus and the myelin of the central nervous system.

What is the source of the two substances? The one is present early and is sometimes noted in the leucocytes while yet intra-vascular. This suggests that the substance is present in the leucocytes before they leave the blood vessel, and so forms a passive part of the exudation process. This is supported by the above observation and by finding the same droplets in leucocytes obtained from the circulating blood of pneumonia patients. Smears were made in the usual way of blood obtained by ear puncture.* These were placed in 10 per cent formaldehyde and then treated in the same way as the frozen sections of lung. The blood from nine patients was thus examined. The leucocytes of all showed the presence of droplets in every way identical with those found in the lung alveoli. Though it may be true that not all of this substance reaches the lung within leucocytes, a considerable part certainly has this source.

Does the other substance reach the lung alveoli in the same way? From the observations of Arnold[44] and others, we know that fat is often transported by leucocytes, and undoubtedly some in the lung may come in this way. However, as the true fat appears mainly late in the disease, when the migration of leucocytes is no longer actively taking place, it seems probable that the greater part is taken up by the cells in the exudate from the surrounding fluids in which it is present, and owing to injurious conditions acting on the cells is not utilized, but stored as fat — a fatty degeneration in the sense at present accepted.

Conclusions. 1. In pneumonic exudations two kinds of intracellular fatty substances are present. 2. The one differs in some reactions from ordinary body fat, appears early in the disease, and is in the main brought by the leucocyte from the circulating blood. 3. The other is identical in reactions with ordinary body fat, appears late in the disease, and has the same origin as the fat of so-called fatty degeneration elsewhere.

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