

EXPERIMENTAL LIVER NECROSIS. V. THE FATS
AND LIPOIDS.¹

BY HOLMES C. JACKSON, PH.D.,
Adjunct Professor of Physiological Chemistry,

AND

RICHARD M. PEARCE, M.D.,
*Professor of Pathology and Bacteriology,
Albany Medical College.*

(From the Bender Laboratory, Albany, N. Y.)

The results outlined in this communication constitute a partial report of a somewhat comprehensive investigation² now in progress of the chemical processes concerned in the variations occurring in the amounts of fats and lipoids in the hepatic cell under normal and various pathological conditions.

It has seemed advisable, in connection with the other investigations of liver necrosis here presented, to discuss at this time only that part of the general study which deals with the fatty changes in hepatic necrosis brought about by the injection of hæmotoxic immune serum.

The study of such lesions is of peculiar value in view of the attempts which have been made to bring into a relation of cause and effect the autolysis of the organ and the appearance of the fat. We have therefore with this point in view attempted to determine whether in the necrosis (autolysis) which follows the injection of the serum there occurs any alteration in the fat content correspond-

¹Conducted under grants from the Rockefeller Institute for Medical Research. Received for publication July 11, 1907.

²This investigation, including a study of the fatty changes occurring in various experimental lesions of the liver of the dog and of certain pathological conditions of the human liver, will be published later in full by H. C. Jackson and L. K. Baldauf. We wish to express here our indebtedness to Dr. Baldauf for the privilege of utilizing in this partial report that portion of his work which refers to necroses of the liver produced by hæmotoxic serum.

ing to changes observed in the nitrogenous constituents of the cell. Especial interest is attached to this question in view of the somewhat widely divergent opinions held as to the origin of the fat which appears in the so-called fatty transformation of various organs. That the fat does not arise from a peculiar decomposition of the proteid molecule in the cell, the fatty degeneration of Virchow, seems fairly well established. On the other hand, Rosenfeld³ and others hold that the fat makes its appearance as a simple infiltration from without when for any reason the cell has received an injury which seems to inhibit its oxidizing power. The appearance of fat in organs during phlorhizin poisoning is thus explained by Lusk.⁴ Waldvogel,⁵ however, who has investigated this question most thoroughly believes that the process is one closely allied to autolysis. His theory is that normally certain substances, which may be called combined fats, such as the ovovitellin of Hoppe-Seyler, or the lecithalbumin of Liebermann, hold the fatty radical in a combination which does not react to microchemical fat stains, such as Scharlach R or Sudan III, and which cannot be removed chemically by the ordinary fat solvents. These substances, however, during autolysis become split in such a manner that the fat radical is liberated in the form of protagon, jecorin, lecithin and even neutral fats. He supports this contention by experiments upon the livers of phosphorus-poisoned animals and upon normal livers undergoing autolysis, in which he has shown there is a marked augmentation during autolysis of such substances as protagon, jecorin, cholesterin, fatty acids and neutral fats. The lecithin, on the other hand, is diminished.

³ Rosenfeld, G., Fetbildung, *Ergebnisse der Physiologie*, 1902, i, 651; *ibid.*, 1903, ii, 50.

⁴ Ray, W. E., McDermott, T. S. and Lusk, G., On Metabolism During a Combination of Phosphorus Poisoning and Phlorhizin Diabetes, *Amer. Jour. of Physiol.*, 1900, iii, 139.

⁵ Waldvogel, Autolyse und fettige Degeneration, *Virchow's Arch.*, 1904, clxxvii, 1. Phosphorvergiftung und Autolyse, *Deut. Arch. f. klin. Med.*, 1905, lxxxii, 437. Waldvogel and Mette, Die Autolyse in Menschlichen fettige degenerierten Organen, *Munch. med. Woch.*, 1906, ix, 402. Waldvogel, Die durch Fermente bewirkten Umwandlungen bei der fettigen Degeneration, *Zeit. f. physiol. Chem.*, 1904, xlii, 200.

Siegert⁶ has also shown that, although in autolysis the ether extract of the liver does not increase, a marked rearrangement of the fatty compounds of the extract takes place, and that the jecorin rapidly suffers decomposition. This is not in accord with Waldvogel's results. In this connection it may be mentioned that Taylor⁷ has conducted experiments upon normal and phosphorus-poisoned frogs and finds that, although the absolute amount of free fat may not increase after the administration of phosphorus the combined fats estimated after digestion with pepsin-hydrochloric-acid suffer a marked diminution equivalent to two thirds of the amount originally present.

As stated in previous papers in this series phosphorus poisoning does not seem to set up in the liver processes which are strictly analogous to autolysis following necrosis, hence the results to be presented must not be considered as strictly comparable to those obtained in other lesions.

Few attempts have been made to study the effect of a true necrosis upon the fat constituents of the cell. Dietrich,⁸ as the result of histological studies, claims that autolysis is not an important factor, since tissues introduced into the peritoneal cavity in collodion sacs do not show fatty change. When the tissue, however, is not enclosed in sacs, fat droplets appear which are present not in the cell substance but in the interstices of the tissue. He also tied off the renal arteries and found a "deposition" of fat around the necrotic areas. He believes, therefore, that fat will not appear if the cell is completely dead as in necrosis; but only when it continues to functionate incompletely, as, for example, must be the case with the cells around necrotic areas.

This opinion we can confirm as the result of the histological study of focal necroses caused by hæmotoxic serum. Frozen sections of formalin-hardened material stained with Scharlach R never show more fat, and usually less, than the surrounding adjacent

⁶Siegert, F., Das Verhalten des Fettes bei der Autolyse der Leber, *Beiträge zur chem. Physiol. u. Path.*, 1902, i, 114.

⁷Taylor, A. E., On Fatty Degeneration, *Jour. of Med. Research*, 1903, ix, 59.

⁸Dietrich, A., Experimente zur Frage der fettigen Degeneration *Münch. med. Woch.*, 1904, li, 1510.

normal liver tissue. There is always present, however, in lesions twenty-four to forty-eight hours old a very definite and striking accumulation of fat in the ring of more or less degenerated cells lying between the necrotic and normal liver. These cells correspond to those which in hæmatoxylin and eosin preparations present a vacuolated, granular protoplasm and pycnotic, poorly staining, nuclei.⁹

Di Cristina,¹⁰ who conducted experiments somewhat after the nature of those of Dietrich, and made chemical analyses by Rosenfeld's method, states that no increase of fat occurs in the necrosis caused by shutting off completely the renal circulation. Of considerable interest in connection with Dietrich's view are the experiments reported by Bainbridge and Leathes.¹¹ These investigators ligated the hepatic artery alone and thereby obtained an increase in fat but no necrosis. The ligation of the portal vein on the other hand resulted in atrophy of the cells and some necrosis, but no augmentation of fat. These experiments appear to confirm the idea that the cells must retain in part their normal function and be normally bathed with the circulating fluids in order to give rise to the appearance of fat within them.

In concluding this brief discussion which merely suffices to indicate the trend of opinion in regard to the subject under investigation it may be said that much of the discrepancy in the results reported can be safely ascribed to the varying methods employed especially in connection with the extraction of the fatty material from the tissue. Siegert has also emphasized the ease with which the extracted products undergo laboratory changes.

Methods.—The organs were removed from the body quickly, put through a hashing machine, weighed, and dried under absolute alcohol at about 70–80° C. At this stage the partially dry material was weighed and then ground in a machine to an impalpable powder. Part of this was further dried in a desiccator to constant

Pearce, R. M., Regenerative Changes in the Liver: A Study of Experimental Lesions in the Dog, *Jour. of Med. Research*, 1906, xv, 99.

¹⁰Di Cristina, Die chemischen Veränderungen bei fer fettigen Degeneration in Beziehung zur den anatomischen, *Virchow's Arch.*, 1905, clxxxii, 509.

¹¹Bainbridge, F. A. and Leathes, J. B., The Effect of Arterial or Venous Obstruction upon the Nutrition of the Liver Cells, *Biochem. Jour.*, 1906, ii, 25.

weight and upon this was calculated the dry substance and nitrogen content of the original tissue. Another weighed part was extracted in a Soxhlet with alcohol and chloroform successively according to the method of Rosenfeld. Each total extraction with alcohol and with chloroform lasted on an average thirty hours. In some cases the original partly-dried material was so fatty that a rough extraction with chloroform at room temperature preceded the grinding. This extract was added to the subsequent one obtained from the Soxhlet. The total fat was taken up in a definite volume of chloroform. An aliquot portion of this was evaporated to complete dryness at about 70° C. and from this was calculated the fat per cent. of the tissue.¹²

For our purposes it was not thought necessary to keep separate for analysis the alcohol and chloroform extracts as Waldvogel does with alcohol and ether. This procedure is exceedingly time consuming and we have employed another and simpler method which we believe has given results equally definite. Instead of attempting to decide whether the fat compounds present in the extract underwent any change or rearrangement, such as is described by Waldvogel during autolysis of normal tissues and in phosphorus poisoning, it seemed sufficient to determine the nitrogen and phosphorus pentoxide content of the extract, and from these figures to calculate the relationship of the nitrogen to the phosphorus. Since the molecule of the lecithins contains one nitrogen and one phosphorus atom the relationship P:N = 1:1; in jecorin, however, this ratio is 1:4 and in protagon about the same, varying from 1:3.4 to 4.8. The latter figure is calculated from the analyses of Dunham.¹³ It is seen, therefore, that the greater the preponderance of substances of the jecorin and protagon type in the extract the higher would be the P:N ratio. On the other hand, if these substances should undergo an autolytic change whereby lecithin and fatty acids were

¹² We have employed chloroform in this connection, since, in the first place, it is a much readier solvent for the fatty compounds than either sulphuric or petroleum ether, and secondly, because all of the ether we could obtain reacted distinctly acid to phenolphthalein, a fact already alluded to by Baldauf, Chemistry of Atheroma and Calcification (Aorta), *Jour. of Med. Research*, 1906, xv, 355.

¹³ Dunham, E. K., Further Observations on the Phosphorized Fats in Extracts of the Kidney, *Proc. of Soc. for Exper. Biol. and Med.*, 1905, ii, 63.

produced, this ratio should fall to the neighborhood of 1 : 1. Hence from variations in this ratio, changes in the fatty constituents of the extract should be readily determined.

Measured portions of the chloroform extract were analyzed in duplicate as follows:¹⁴

Total nitrogen by the Kjeldahl-Gunning method; phosphorus pentoxide by the usual fusion procedure and weighing as magnesium pyrophosphate and the iodine equivalent as outlined by the Association of Official Agricultural Chemists.¹⁵ With some exceptions portions of the tissues analyzed were stained with hæmatoxylin and with Scharlach R for the purpose of determining roughly in a comparative way the extent of the necrotic lesion and the fat content.

Results.—The table presents the results obtained in the analysis of four dog livers with normal, four to five, per cent. fat content, one apparently normal but very fatty liver with 21.9 per cent., five with focal necrosis and three with diffuse necrosis of varying degree. As can be seen no relation exists between the degree of necrosis and the amount of fat present. The high amount of fatty material which is present in the normal liver, extractable by the newer method of Rosenfeld, is at first glance surprising, but is in accord with the results of recent investigators. The fat per cent. of all the necrotic livers falls between the normal limits with the exception of 43 and 21 which are above the normal, but these do not represent the most extensive necrosis. Experiments 28 and 29, with the most diffuse necrosis, show normal amounts of fat.

The point which was emphasized in one of the previous papers¹⁶ concerning the percentage of dry substance in the fatty livers is well shown in the table. Whenever the fat per cent. rose above normal the per cent. of dry substance rose almost proportionately. This relation is readily seen by referring to that column in the table in which is given the per cent. of fat-free dry substance. With two exceptions, the dry substance without fat falls between 18.8 to

¹⁴In most instances the chloroform had to be *completely* removed before the commencement of the analysis.

¹⁵U. S. Dept. of Agriculture, 1899, Bulletin 46, 50.

¹⁶See first paper of this series, "The Hexon Bases" in this number of the *Journal*.

26.7 per cent. and this surprisingly small variation is in no definite relation to the amount of fat present. The two exceptions are one with normal and one with high fat content. Waldvogel has claimed that as the fatty autolysis increases the water content of the tissue also rises. We would be inclined to ascribe this rather to the nitrogenous autolysis *in vivo* which as we have pointed out elsewhere¹⁶ tends to diminish the amount of dry substance if the circulation is not too greatly impaired. This is shown clearly in Experiments 27, 21, 28 and 29, with the most pronounced necrosis, in which it is seen that the figures for the dry fat-free substance lie on the lower edge of the variation limit for this factor.

As regards the nitrogen content of the tissues a somewhat similar condition of affairs is evident, and although this point does not come directly into this part of the general subject it is of considerable interest. It will be seen that the per cent. nitrogen of the fat-containing dry substance varies within rather wide limits (6.7 to 12.7 per cent.). In those instances, however, where the fat content is high the nitrogen per cent. is low, as would be expected. If, however, the nitrogen of the fat-free dry substance is considered, it is found that the figures for this factor are surprisingly constant throughout (10.5 to 16.7 per cent.). Some of the lowest and highest values occur where, as regards the fat content, the liver tissue was perfectly normal. This indicates apparently that in conditions such as those under discussion, when an increase in fat-content occurs, the material which is represented by the nitrogen of the tissues suffers no decrease or increase in amount. If then the fat originates from some compound proteid antecedent, the proteid component remains apparently unchanged.

A consideration of the character of the fatty extract obtained from normal and necrotic tissues presents some interesting facts. In regard to the iodine equivalent, which indicates roughly the content of oleic acid in the fat mixture, it is evident that as the fat per cent. increases the iodine absorption factor falls below normal (Experiments 18, 43, 27 and 21). This low iodine factor associated with increased fat content would of course point to a diminishing content of oleic acid radicals as the fat is heaped up in the cell. This again is directly opposed to the finding of Waldvogel in phosphorus poisoning.

An examination of the nitrogen and phosphorus pentoxide percentage of the fatty extracts indicates that somewhat wide variations are present, the nitrogen varying from 0.3 to 3.1 and the phosphorus pentoxide from 1.1 to 7.3. It is evident, however, that the lower percentages are in those experiments in which the greatest amount of fat is present (Experiments 18, 43, 27 and 21). With these exceptions the percentage figures are quite regular. Only one explanation can be made of this difference. That is, that as fatty compounds make their appearance in the cell, those substances predominate which do not contain nitrogen or phosphorus, namely the neutral fats, fatty acids and cholesterol.¹⁷

Of particular interest is the ratio of phosphorus to nitrogen discussed above. It is seen from an examination of the figures in the table that this ratio remains remarkably constant regardless of the absolute variation in the figures. The ratio, with the exception of Experiment 5 which is high, varies only from 1 : 2.4 to 4.9. If we also exclude Experiments 21 and 27 the variation is still further reduced to 1 : 3.5 to 4.9, which is well within the limits of error or better perhaps within the calculation error as made from the published figures for the nitrogen and phosphorus percentages of such poorly defined substances as protagon and jecorin. This high ratio indicates that the nitrogen- and phosphorus-containing fats present in the extracts are of the protagon and jecorin type and remain so irrespective of the amount of fat appearing in the cell.

It must be admitted that some slight evidence does exist to indicate that perhaps under certain conditions these bodies may undergo autolysis. In the two experiments, for example 21 and 27 with a low ratio (1 : 2.4 to 3.0), considerable autolysis must have been going on during the extensive necrosis and the low ratio as a concomitant factor would point to the appearance in the extract of bodies such as the lecithins, which as previously stated possess a ratio of 1 : 1. In these experiments roughly one third of the nitrogen- and phosphorus-containing fats has been replaced by lecithin, according to this reasoning. We are somewhat sceptical, however, of the validity of this line of argument since it does not

¹⁷We possess confirmatory evidence upon this point in the results which have been obtained with the saponification equivalent. These will be published in a later paper.

agree with Waldvogel's observations; nor is the low ratio present in the two experiments (28 and 29) where one would expect it to be most markedly diminished since the necrosis was most pronounced. In these two instances, however, the ratio is normal. As the low ratio occurs in only two out of the eight experiments in which necrosis was present, we are more inclined to believe that some other factor is the true cause for the change.

SUMMARY.

1. Changes which occur in the fat content of the liver of dogs receiving hæmotoxic serum bear no relation to the degree of necrosis produced by this serum.

2. An increase in water content of the tissue seldom occurs, but where present is due to the nitrogenous autolysis rather than to the deposition of fat.

3. The appearance of fat in the cell is not associated with a decomposition of the proteid component of the compound fats, but rather to a simple splitting off of the fatty radical. This is shown by the slight variations occurring in the percentage nitrogen of the fat-free substance.

4. The iodine equivalent diminishes as the fat content increases. This would indicate that in the fatty changes which occur, fats other than those containing oleate radicals make their appearance.

5. The ratio of phosphorus to nitrogen in the alcohol-chloroform extract remains practically constant in all degrees of necrosis. Hence the substances of the protagon and jecorin type hold the same relation to the lecithins during the autolysis as they do normally.

6. In a general way it may be said that the results obtained in the microchemical staining of the fats with Scharlach R agree with those found by chemical extraction methods.