EXPERIMENTAL LIVER NECROSIS; I. THE HEXON BASES.[1]

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This communication is the first of a series of five, which presents the results of a somewhat comprehensive investigation of the chemical changes associated with experimental liver necrosis and which includes, in addition to that here presented, studies of the nitrogenous metabolism, nuclein metabolism, the activity of the intracellular hepatic enzymes and the changes in the fats and lipoids of the liver.

The advantage of undertaking such a comprehensive study was suggested by the investigations[2] which one of us had previously made of the necrosis caused in the liver of the dog by the intravenous or intraperitoneal injection of hæmagglutinative and hæmolytic immune sera. The lesions so produced are frequently focal and resemble in a general way those of eclampsia while the diffuse lesions, with the associated repair, are more or less similar to certain stages of acute yellow atrophy. It seemed plausible therefore that a study of the chemistry of such lesions, readily produced experimentally, might throw light not only on certain functions of the liver but might offer new knowledge of value in explaining

[1] Conducted under grants from the Rockefeller Institute for Medical Research. Read by title before the Society of Experimental Biology and Medicine, New York, March 20, 1907. Received for publication July 2, 1907.

[2] Pearce, R. M., The Experimental Production of Liver Necroses by the Intravenous Injection of Hæmagglutinins, *Jour. of Med. Research,* 1904, xii, 329; 1906, xiv, 541. Experimental Cirrhosis of the Liver, *Jour. of Exper. Med.,* 1906, viii, 64.

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some of the problems of eclampsia, acute yellow atrophy and similar lesions in man.

The study of the hexon bases was undertaken in the hope of determining the character of the processes underlying the production of *intra vitam* autolysis. We have therefore attempted to determine the relative enzymotic power of the normal and the necrotic liver in regard to the synthesis or decomposition of the hexon bases. The importance of these bodies, as products of autolysis, has been emphasized by Wakeman[3] in his study of the livers of dogs poisoned with phosphorus or repeatedly anæsthetized with chloroform. In addition to the study of fresh tissues, we have investigated also the changes occurring in hexon nitrogen during antiseptic autolysis of liver substance.

The experimental lesions under consideration may be described, without going into detail, as hyaline necroses with little or no leucocytic reaction, the position and extent of which vary according to the amount of serum administered and the resistance of the animal. Small doses cause focal lesions more or less isolated and irregularly distributed; large doses produce a diffuse necrosis which spares only the tissue about the larger portal spaces. The lesions are found chiefly near the surface of the liver, but may occur in the deeper portions. In animals dying within a few hours after injection and before the appearance of necrosis an intense congestion of the entire portal system exists with, in the liver, innumerable thrombi composed of fused red cells.

In addition to such material from the dog, the livers of horses presenting degenerative and necrotic lesions, occurring in the course of immunization with various bacterial products, have also been utilized. These lesions, which have been described fully elsewhere by Pease and Pearce[4] and more recently by Lewis,[5] consist of widespread necrosis associated not infrequently with an extensive deposition of amyloid and usually accompanied by hæmorrhages.

[3] Wakeman, A. J., On the Hexon Bases of Liver Tissue under Normal and Certain Pathological Conditions, *Jour. of Exper. Med.*, 1905, vii, 292.

[4] Pease, H. D., and Pearce, R. M., Liver Necrosis and Venous Thrombosis in Horses Actively Immunized with Diphtheria and Tetanus Toxins and with Streptococci and their Products, *Journ. of Infect. Diseases,* 1906, iii, 619.

[5] Lewis, P. A., Hæmorrhagic Hepatitis in Antitoxin Horses, *Jour. of Med. Research,* 1906, xv, 449.

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A series of fifteen livers, eleven from the dog and four from the horse, has been examined. Of the former, five were normal, four showed focal necroses and two diffuse necrosis; of the latter two were normal and two were examples of necrosis associated with extensive amyloid transformation.

Methods.—As the necrotic lesion in the dog's liver reaches its greatest extent in twenty-four to forty-eight hours after injection, all livers used in the study of the hexon bases were taken from animals killed at the end of the latter period. In the case of the horses the livers were removed within one hour after death.

After cutting away the larger vessels and the coarser tissue about the hilum the organ was subdivided and passed through the hashing machine. Small portions were weighed and set aside for drying and upon these total nitrogen estimations were made later. At the same time two portions of 100 grams each of moist liver were placed in flasks. To one, 600 cubic centimeters of water and 300 grams of sulphuric acid were added and hydrolysis carried on for fourteen hours in a paraffin bath. The other sample was suspended in 600 cubic centimeters of water and with it were thoroughly mixed about fifty cubic centimeters of toluol. This flask plugged with cotton was allowed to stand at room temperature for periods of one or two months. At the end of this time it was examined bacteriologically, and if found free of bacteria, 300 grams of sulphuric acid were added and the material hydrolyzed in the same manner as the control. Subsequent treatment was carried out according to the method outlined by Wakeman except that the process was continued only to the point of making nitrogen determinations of fractions "A" (arginin and histidin) and "B" (lysin) after removal of the barium and silver by means of sulphuric acid and hydrogen sulphide. For our purpose no advantage was to be gained by separating arginin and histidin.

Dry Solid Content.—An examination of the figures in the table indicates that the dry solid content of the dog livers with scattered focal necroses did not vary from the normal average of 24 per cent. In the case of the diffuse lesions, where the autolysis of the protoplasm was obviously more marked, the dry solids were reduced to an average of 20.8 per cent. The figures referring to normal livers

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TABLE—*Hexon Bases.*

Nature of Experiment.		Determination on Fresh Tissue.						On Autolysed Tissue.			
		Per Cent.			* Per Cent. Nitrogen as			Per Cent. of N_2 ppt. by Phospho-tung. Acid.	* Per Cent. Nitrogen as		
Dog.	Condition of Liver.	Dry Substance.	Nitrogen in Dry Substance.	N_2 ppt. by Phospho-tung. Acid.	Arginin and Histidin.	Lysin.	Purin Bases.		Arginin and Histidin.	Lysin.	Purin Bases.
3	Normal	24.8	12.1	13.2	4.3 / 32.5	3.6 / 27.1	5.3 / 40.4	Not bacteria-free			
4	Normal	18.4	10.9	15.0	6.0 / 40.0	4.9 / 32.7	4.1 / 27.3	19.8	8.4 / 42.4	9.8 / 49.5	1.6 / 8.1
13	Normal	26.8	11.8	Used only for autolysis				18.3	8.0 / 43.7	7.3 / 39.9	3.0 / 16.4
14	Normal	24.5	9.2	Used only for autolysis				17.6	7.5 / 42.6	7.6 / 48.2	2.5 / 14.2
17	Normal	25.0	11.2	19.6	4.3 / 36.2	7.4 / 37.7	5.1 / 26.1	20.4	8.0 / 39.2	7.9 / 38.7	4.5 / 22.1
Av.		23.9	11.3	15.9	6.0 / 36.2	5.3 / 32.5	4.8 / 31.3	19.0	8.0 / 42.0	8.2 / 42.8	2.9 / 15.2
5	Few focal necroses	24.4	21.4	12.2	5.6 / 45.8	4.2 / 34.4	2.4 / 19.8	9.0	4.1 / 45.5	2.7 / 30.0	2.0 / 24.5
7	Few focal necroses	16.6	19.1	14.5	7.3 / 50.2	5.1 / 35.1	2.1 / 14.7	10.2	4.9 / 48.0	3.5 / 34.3	1.9 / 17.7
9	Numerous focal necroses	26.9	22.8	10.2	4.8 / 47.2	3.2 / 31.5	2.1 / 21.3	6.7	2.3 / 34.3	2.6 / 38.8	1.8 / 26.9
10	Numerous focal necroses	25.6	23.7	8.5	3.6 / 42.3	2.5 / 29.4	2.4 / 28.3	6.8	2.7 / 39.8	1.9 / 27.9	2.1 / 32.3
Av.		23.4	21.8	11.4	5.3 / 46.4	3.8 / 32.6	2.2 / 21.0	8.2	3.5 / 41.9	2.7 / 32.8	2.0 / 25.3
29	Extensive diffuse necrosis	21.9	12.7	30.0	15.6 / 52.0	11.6 / 38.6	2.8 / 9.4	23.9	11.5 / 48.1	10.2 / 42.7	2.2 / 9.2
60	Extensive diffuse necrosis	19.8	13.2	25.6	12.7 / 49.6	11.3 / 44.1	1.6 / 5.3	20.7	10.1 / 48.8	9.5 / 45.9	1.1 / 5.3
Av.		20.8	12.95	27.8	14.2 / 50.8	11.5 / 41.4	2.2 / 7.4	22.3	10.8 / 48.5	9.9 / 44.3	1.7 / 7.3

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TABLE—*Hexon Bases (Continued).*

Nature of Experiment.		Determination on Fresh Tissue.					
		Per Cent.			* Per Cent. Nitrogen as		
Horse.	Condition of Liver.	Dry Substance.	Nitrogen in Dry Substance.	N_2 ppt. by Phosphotungstic. Acid.	Arginin and Histidin.	Lysin.	Purin Bases.
60	Normal	23.3	12.0	8.1	3.0 / 37.0	4.6 / 56.7	0.5 / 6.3
65	Normal	21.8	11.2	8.5	3.9 / 45.9	3.1 / 36.5	1.0 / 17.6
Av.		22.6	11.6	8.3	3.5 / 41.5	3.9 / 46.6	0.75 / 12.0
42	Necrosis and amyloid	20.0	21.7	13.5	2.9 / 21.4	8.2 / 60.7	2.4 / 17.9
69	Necrosis and amyloid	21.4	13.7	13.3	1.2 / 9.0	10.2 / 76.7	1.9 / 14.3
Av.		20.7	17.7	13.4	2.1 / 15.2	9.2 / 68.7	2.2 / 16.1

* Figures in upper left hand corner are calculated on the total nitrogen; those in the lower right hand corner on the total hexon nitrogen.

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agree somewhat closely with those of Wakeman, who, however, noticed a slight decrease in the percentage of dry substance in the livers of dogs poisoned by phosphorus. This is somewhat surprising since he describes these livers as markedly fatty. If the protoplasm of which seventy-five per cent. is water is replaced by fat, which contains none, the dry solids should increase instead of decrease. If Wakeman's figures are correct they show a marked increase in the water content of the organs during phosphorus poisoning. That such an increase may occur did not appear in our investigation[6] of fatty changes in the liver.

Total Nitrogen Content.—Several interesting facts developed from the analysis of the nitrogen of the dry solids. The average of the five determinations for normal tissue was 11.3 per cent. in agreement with Wakeman; while that of the livers with lesions of a scattered focal character was 21.8 per cent.

This, of course, indicates a deposition or heaping-up of nitrogenous material in the hepatic cell. That such a process may take place even under physiological conditions is evident from the results of experiments carried out by Seitz.[7] This investigator found that by feeding hens and geese excessive amounts of meat a true deposition of nitrogenous substances occurred in the cells of the liver. This increase amounted in some instances to 300 per cent.

In our experiments the quantity of nitrogen in the hepatic cell was almost doubled in the organs with scattered necrotic lesions. This condition allows of an explanation similar to that offered for the infiltration of fat in tissues during phosphorus or phloridzin poisoning. In such lesions the cells have lost in part their power to oxidize properly the sugar or other materials placed at their disposal by the circulating blood and hence the starving cell, in its endeavor to spare its own protoplasm from destruction, stores up fat for purposes of oxidization.

That the cells at the margin of the necroses under consideration do accumulate fat we have observed in our study of the histological changes occurring in these livers.[6] It seems distinctly possible that

[6] See fifth paper of this series, "The Fats and Lipoids" in this number of the *Journal.*

[7] Seitz, W., Die Leber als Vorrathskammer für Eiweissstoffe, *Arch. f. ges. Physiol.,* 1906, cxi, 309.

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during the initial stage of congestion and thrombosis and in the early stages of necrosis, the imperfectly nourished and slightly injured cells may heap up nitrogenous material also. When, however, the lesion is more extensive, the storing up of nitrogen is not so evident as shown by the fact that the nitrogen of the dry solids remains more nearly normal. This is to be explained by assuming that the nitrogen stored up in the persisting liver cells is sufficient to more than balance the loss by autolysis in the necrotic areas.

Wakeman's figures for the nitrogen content of livers after phosphorus poisoning show a diminution equivalent to 35.6 per cent. and a corresponding decrease in the hexon base nitrogen. This indicates, according to his view, that that part of the proteid molecule involving the hexon bases has not undergone a relatively greater decomposition than the other nitrogenous substances. It would seem to us that the low nitrogen content of the phosphorus livers is wholly, or in greatest part, due to the large amount of fat present.

The Hexon Bases.—Wakeman's results indicate that in the dog the average nitrogen content of the bases in the normal liver tissue is 17.04 per cent. of the total nitrogen, while in the liver of dogs poisoned with phosphorus it is only 10.72, a falling off of 37.1 per cent. The livers of dogs receiving chloroform showed 13.6 per cent., a decrease of 20 per cent. In these figures Wakeman sees evidence of increased autolysis in hepatic cells affected by phosphorus or chloroform. Although he mentions definite necrosis in but one of his livers, the cell destruction in phosphorus poisoning, gradual as it is, is such that our results ought to fall, as they do, somewhat into line with his.

Thus as an average of three normal livers we find the figures concerning the total content of hexon bases, based on nitrogen content attributable to them, to be 15.9 per cent. of the total nitrogen. In the case of the scattered focal necrosis, the percentage is slightly decreased to 11.4 as an average of four determinations, but the absolute amount is increased. That is gram for gram of dry substance there occurs an increase in the absolute amount of hexon bases which however appears as a decrease in percentage on account of the high nitrogen content of the dry substance.

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This absolute increase in hexon content of dry substance is greater and more clearly accentuated in the livers of those dogs in which the necrosis is more diffuse. Here the nitrogen of the dry substance is almost the same as that of the normal, 12.95 per cent. as average of two determinations; but the hexon base nitrogen content rises to 27.8 per cent. of the nitrogen of the dry substance.

This observation is extremely interesting in that it points most strongly to the preponderance of the autolytic process over the synthetic in the more widespread forms of necrosis with early repair. The figures show a definite increase in the hexon base content of the necrotic cell, although the accumulation of nitrogen in this lesion, 12.95 per cent., could not occur to such a marked extent as it did in the focal lesion, 21.8 per cent., because of the lessened number of persisting living cells capable of storing up nitrogen. A rearrangement of nitrogen, the result of autolysis in the larger areas of necrosis, therefore took place as shown by the hexon nitrogen content of 27.8 per cent., as compared with that of 11.44 per cent. in the focal lesion and 15.9 per cent. in the normal. This great increase of hexon bases may be due in part also to disturbances of the circulation accompanying the necrosis which prevent the diffusion and removal of the bases from the liver. In this connection attention may be called to Jacoby's[8] observation that leucin and tyrosin are not found in the liver of phosphorus poisoning when no disturbance of the hepatic circulation exists.

Relation of Precipitate "A" (Arginin and Histidin) and Precipitate "B" (Lysin) to the Total Hexons.—Wakeman, from a consideration of his results on these fractions, concludes that in the autolysis which occurs in the cell in phosphorus poisoning the arginin suffers a greater destruction than do the other bases, probably through the action of arginase, which splits arginin into ornithin and urea.[9] His tables show that of the 17.0 per cent. of the nitrogen of the total bases in the normal tissues, 11.8 per cent. is to be attributed to arginin and histidin and 5.2 to lysin. In phosphorus poisoning, on the other hand, the nitrogen of the bases amounts to

[8] Jacoby, M., Ueber die Beziehungen der Leber und Blutveränderungen bei Phosphorvergiftung zur Autolyse, *Zeit. f. physiol. Chem.*, 1900, xxx, 174.

[9] Kossel, A., and Dakin, H. D., Ueber die Arginase, *Zeit. f. physiol. Chem.*, 1904, xli, 321.

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10.7 per cent., of which only 6.8 per cent. belongs to the arginin and histidin and 3.8 per cent. to the lysin. This indicates a decrease during autolysis of 42.3 per cent. for the arginin and histidin, but only 26.8 per cent. for the lysin.

If, however, one considers these figures from the standpoint of the relationship which the precipitates " A " and " B " bear to the total hexon bases of the normal and phosphorus dogs, an entirely different view is obtained. In the normal tissue precipitate " A " (arginin and histidin) forms 69.6 per cent. and precipitate " B " (lysin) 30.4 per cent. of the total bases; whereas in the phosphorus livers the former is 63.9 per cent. and the latter 36.1 per cent. Hence the decrease in the fraction " A " is only 5.7 per cent. and this is offset by the corresponding increase in fraction " B." This diminution in the arginin and histidin content of the hexon base fraction of the livers of phosphorus-poisoned animals compared with the normal is so slight that it hardly seems warrantable to attribute it to the action of arginase.

Our figures for the total hexon bases (15.9 per cent.) in the normal agree well with those found by Wakeman, while those for the focal necroses have suffered a percentage decrease which is somewhat comparable to that noticed by him in phosphorus poisoning. Wakeman's percentage decrease, however, was also an absolute one while ours in reality was an absolute increase, as has been explained above. The proportion which precipitates " A " and " B " bear to the total is markedly different, however, from that which he notes. Our average in the normal tissues for the arginin and histidin fraction is 36.2 per cent. as against 69.4; the lysin fraction 32.5 per cent. more nearly agrees with his 30.3 per cent. If we exclude the purin bases our results become more comparable and agree better in percentage.

We have also found a much greater percentage of the total nitrogen due to purin bases. Wakeman's figures show an average of 0.0273 per cent. for normal and pathological, while ours showed 4.8 per cent. for the normal tissue against 2.2 per cent. for the focal necrotic lesions and the same for the diffuse necrosis.

As to variations which the individual bases undergo in their relation to the total bases, our results point to an increase from the

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normal of 36.2 per cent. to 46.4 during the focal necrosis and to 50.8 per cent. during diffuse necrosis for arginin and histidin. The lysin fraction shows no change in the focal necroses as compared with the normal, but in the diffuse necrosis it increased 24.3 over the normal in agreement with Wakeman.

In the autolysis *in vitro,* which is discussed in the next paragraph, the normal fraction " A " represented 42.0 per cent. of the whole bases and fraction " B " 42.8 per cent.—differences from the normal which are well within the limit of error. Hence the absolute increase from 6.0 and 5.3 in the unautolyzed to 8.0 and 8.2 per cent. of the total nitrogen in the tissue after autolysis was in exact relation to the increase in total hexon nitrogen. The same is true for the autolyzed tissue with necrosis of all types.

This would emphasize more markedly the point made above that small evidence can be adduced to show that an enzyme, arginase, is acting on the arginin, decreasing its amount during autolysis. Such action is not shown by our figures and moreover in our investigation of intracellular hepatic enzymes[10] we could not obtain an active arginase from the necrotic dog's liver, though it was found in the normal.

Hexons Resulting from Autolysis in Vitro.—It seemed worth while in view of the investigation, carried on synchronously, of intracellular hepatic enzymes[10] to determine the relation of the hexon bases to autolysis of the liver *in vitro.* For such observations the figures given above for autolysis during life serve as controls. Autolysis was allowed to proceed for varying lengths of time in the endeavor to determine whether the different organs showed varied degrees of autolysis. The periods selected, one and two months, were inadequate to bring out this point, since the autolysis was completed or the reaction reached its equilibrium before one month. This was unfortunate as we thereby disregarded the important element, that of time, in this connection. The time element, however, is fully considered elsewhere[10] from another point of view.

A glance at the figures in the table shows that after autolysis of the normal organs the percentage of total nitrogen as hexon

[10] See second paper of this series, " Enzymes," in this number of the Journal.

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nitrogen was 19.0 per cent. as an average of four determinations. This increase of 18.8 per cent. over the normal hexon content is not marked, and if one examines the figures referring to the two dogs (4 and 17) upon which alone we have absolute controls, it will be seen that this increase is variable.

We are not inclined to attempt to explain this result in detail in this place, since the data which we will present in our study of the enzymes bear more decisively upon this matter. Suffice it to say that these figures, taken in connection with those of the diffusely necrotic organs where an increase also was evident, indicate that in the autolysis a transformation or rearrangement occurs by which nitrogenous atomic complexes, not normally yielding hexon bases, become altered into hexon bases or their combinations.

In all degrees of necrosis, the autolyzed material contained a smaller amount of hexon bases than the unautolyzed. In the liver with scattered focal necroses the decrease of the hexon nitrogen in per cent. of the total nitrogen amounted to 28.0 per cent.; in the diffuse necrosis 19.8 per cent. This would seem to imply that in the living tissue the hexon splitting enzyme is to some degree inhibited, probably through the action of the blood serum.

Hexon Bases in the Liver of the Horse.—The results obtained with the normal livers of horses agree in regard to the dry solids (22.6 per cent.) and the nitrogen of the dry tissue (11.6 per cent.) with those obtained for the dog. The nitrogen precipitable with phosphotungstic acid, however, is surprisingly low, amounting to only 8.3 per cent. It would seem inadvisable to attempt an explanation of this difference, since these animals were not absolutely normal, in that they had been utilized for the purpose of preparing antitoxin and had died during such treatment, though no lesions were found in the liver. The injection of bacterial products may set up processes in the cell which tend to reduce its hexon content without changes evident histologically. The percentage which fractions "A" and "B" of such livers bears to the total hexon content is not far removed from that found for each in the case of the normal dog liver.

The two lesions which served as examples of necrosis presented the complicating feature of amyloid. This fact renders the series

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not exactly comparable to the previous one. The increase in nitrogen of the dry substance over that of the normal is present here also, and the total hexon base nitrogen has increased to 13.4 per cent. This latter result may be caused by the heaping up of the bases as products of autolysis in the large necrotic areas. More probably, however, it is to be attributed to the amyloid degeneration, in which the normal cellular proteids with relatively small percentage (10–30 per cent.) of diamino-nitrogen are replaced by amyloid, the proteid constituent of which, in the liver and spleen as shown by Neuberg,[11] contains twice as much (50–60 per cent.) diamino-acid nitrogen. This rearrangement in the hexon content of the proteids of the organ has also resulted in a marked change in the relation which the arginin and histidin as well as the lysin bears to the total amount of hexon base precipitate. "A" forms only 15.2 per cent. and precipitate "B" 68.7 per cent. of the whole diamino-acid nitrogen.

SUMMARY.

1. The liver of the dog in which necrosis has been produced by injection of hæmatoxic immune sera is characterized in the less marked forms by a storing up of nitrogen in the persisting living cells, while in the diffuse forms the total nitrogen content is but slightly above the normal. This last is to be explained by the great diminution in persisting liver substance which limits the power of nitrogen accumulation.

2. In all forms of necrosis there occurs an absolute increase of nitrogen precipitable by phosphotungstic acid (hexon bases) but the percentage increase, in relation to total nitrogen, diminishes in those forms (focal) in which the products of autolysis may be readily carried off by the blood stream and greatly increases in the diffuse form with large areas in which the circulation is seriously impaired.

3. Although the absolute amount of nitrogen representing arginin and histidin varies, a relative increase is evident when this fraction is compared with the total diamino-nitrogen. This increase corresponds to the degree of necrosis and attendant circu-

[11] Neuberg, Ueber Amyloid, *Verhand. d. Deut. path. Gesellsch.*, 1904, vii, 19.

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latory disturbance and indicates that in necrosis as opposed to degeneration (Wakeman) arginin is not split up by arginase. The lysin also bears a definite relation to the total hexon nitrogen.

4. The diamino-nitrogen of the normal liver after autolysis *in vitro* shows a slight variable increase over that of the unautolyzed, while the necrotic livers showed a decided decrease.

5. The diamino-acid nitrogen of normal horse liver is only about one half of that of the dog; the relative proportion of the bases is about the same. In necrotic livers with amyloid the diamino-nitrogen is markedly increased which is in accord with Neuberg's observations on the high hexon base content of amyloid.

Conclusions.—Upon the whole then the chemical processes occurring in the hepatic cell undergoing rapid or immediate necrosis and those accompanying a slow "degeneration," as for example in phosphorus poisoning, must be different and distinct as would be expected from the histological findings. In necrosis we find the cell in a complete state of disorganization and decomposition and hence autolysis begins immediately, but in the changes occurring in the cell in the so-called degenerations, as phosphorus poisoning, the nucleus remains intact, thereby insuring to a certain extent the life or at least partial function of the cell. That under the latter circumstance a disturbed condition does exist is evidenced by the heaping up of fat in the cell, and although the results of the various investigations upon the altered processes in the liver of animals poisoned with phosphorus tend to show that this change is an autolysis, in which certain amino-acids appear as the result of the splitting of the proteid molecule, it is not of the same type as that appearing in the necrotic cell. This is shown by a comparison of Wakeman's findings, which indicate definitely a diminution of hexons in the liver, with ours which show a great increase.

Examinations of the human liver, by a direct method without hydrolysis, but few in number, it is true, tend to the same conclusion. Taylor,[12] for example, found arginin in a liver with wide-

[12] Taylor, A. E., Ueber das Vorkommen von Spaltungsprodukten der Eiweisskörper in der degenerirten Leber, *Zeit. f. physiol. Chem.*, 1902, xxxiv, 580. On the Occurrence of Amino-acids in Degenerated Tissue, *Univ. of California Publications*, 1904, i (Path.), 43.

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spread necrosis, the result apparently of chloroform poisoning; from seven other livers, representing various lesions (dysenteric abscess, fatty degeneration, pyæmia and acute yellow atrophy), he was unable to isolate this substance. Soetbeer[13] also was unable to find hexon bases in a peculiar type of cirrhosis with acute degeneration.

This difference in the hexon content of the liver of "degeneration" and that of necrosis is so striking that it would appear to be due to a difference in the nature or rapidity of the cell destruction, though it may to some extent be explained by the disturbances of circulation which occur in the necrosis and which, presumably, are absent in degeneration.

Our observations show also that care must be exercised in drawing conclusions from results obtained by autolysis *in vitro*. It seems distinctly doubtful whether the autolysis of the cell which occurs under such circumstances has any relation to autolytic changes during life.

[13] Soetbeer, F., Ueber einen Fall von akuten Degeneration des Leberparenchyms, *Arch. f. exper. Path. u. Pharm.*, 1903, l, 294.

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