THE CHEMISTRY OF THE LIVER IN ACUTE YELLOW ATROPHY.*

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Chemical studies of the liver in true acute yellow atrophy are extremely few in number; of these but one or two have been made since the studies of Kossel and Fischer furnished improved methods for the isolation of monamino and diamino acids, and in no instance has a thorough study of the entire composition of the liver been made. Idiopathic acute yellow atrophy offers us the most striking example of *intra-vitam* autolysis of tissues that occurs in human pathology, excelling even pneumonia in interest because in the latter it is merely an inflammatory exudate that is digested, whereas in acute yellow atrophy the liver tissue itself is destroyed, and often as much as three quarters of the entire parenchyma is removed by autolysis within a few days. Hence we should expect to find the liver showing many interesting chemical changes, especially in regard to the products of the autolytic process. But scanty information on this subject is as yet available. Many years ago Frerichs first detected leucin and tyrosin in the liver in such cases, by microscopic means, and later observers have isolated these substances from extracts of the liver. The only instance found in which modern methods have been used is in the report by A. E. Taylor.[1] This author was able to detect no free arginin, histidin or lysin by the method of Kossel and Kutscher; but by Fischer's ester method he obtained and identified 0.35 gram of leucin and 0.612 gram of aspartic acid. Tyrosin was not isolated. The same author studied an instance of the rather similar form of hepatic atrophy that is occasionally observed as a sequel of chloroform

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[1] *Zeit. f. physiol. Chem.*, 1902, xxxiv, 580; *Jour. of Med. Research*, 1902, viii, 424.

anesthesia, and found in the liver 4 grams of leucin, 2.2 grams of tyrosin, and 2.3 grams of arginin nitrate.

Sootbeer[2] has reported a case of supposed acute yellow atrophy in which a small piece of liver tissue was examined for amino acids with negative results. Beebe[3] investigated two livers, said to be examples of acute yellow atrophy, for their pentose content, but found no decided alteration; if anything the amount of pentose was somewhat larger than usual. Heffter[4] found the amount of lecithin decreased in two livers which he calls, without description, acute yellow atrophy; but it is evident from his analytical figures of the water and fat content that he was dealing with some other condition. Perls and v. Starck[5] found the proportion of water greatly increased in the liver of acute yellow atrophy, a finding corroborated by Taylor and others. They also found the proportion of fat quite similar to that of the normal liver, ranging in their cases from 4.2 to 8.7 per cent. of the weight of the fresh liver, in marked contrast to the 25 to 30 per cent. found in fatty livers. Beyond these few reports there seem to be no analyses recorded in the available literature.

Having the fortune to obtain an autopsy a few hours after the death of a patient with a typical case of this rare disease, thus obtaining the entire liver in a very fresh condition, it seemed desirable to study the material at my disposal in as thorough and systematic a manner as possible. The case from which the material came was a very typical one, of so-called idiopathic acute yellow atrophy, and the anatomical features have already been described elsewhere.[6] Briefly, the chief features were the following:

A young man, aged 20 years, was taken slightly sick without evident cause, the first symptom being jaundice. He gradually grew weaker, and after a week became bedridden. After about four weeks he became unconscious, the jaundice deepened, delirium set in, the area of liver dullness decreased, and there was tenderness in the region of the liver. Death occurred six weeks after the onset of the illness, and two weeks after the illness had become severe. Autopsy was performed three and one-half hours after death, and the chief

[2] *Arch. f. exp. Path. u. Pharm.*, 1903, l, 294.
[3] *Amer. Jour. of Physiol.*, 1905, xiv, 237.
[4] *Arch. f. exp. Path. u. Pharm.*, 1891, xxviii, 97.
[5] Quoted by Quincke, *Spec. Path. und Ther.*, 1899, xviii, 297.
[6] Wells and Bassoe, *Jour. of the Amer. Med. Assoc.*, 1905, xliv, 685.

changes were in the liver, which weighed but 970 grams. The description of the liver follows:

The surface of the left lobe and the margin of the right lobe are decidedly wrinkled, while the upper part of the right lobe is not shrunken and appears much firmer. The left lobe and the margin of the right may be doubled on themselves. On section, the lobules of the shrunken portion are seen to be much smaller than normal, while the lobules of the remainder of the liver tissue are enlarged. The left lobe is of uniform consistence, and is more pliable but not softer than normal liver. The margin of the right lobe is the same except that scattered through it can be felt pea-sized nodules, some of which can be seen protruding on the surface. These nodules are most abundant at the junction of the atrophied and large portions of the liver; in the upper part of the liver these nodules can not be felt. The cut surface of the atrophic portion is of a brownish-red color, becoming lighter yellow in the enlarged portions. The lobules of the large part are enlarged, yellow in color, the centers being darker that the periphery and the cut surface is irregular. The bile ducts are not distended. In the atrophied portion the lobules are very small and of normal color. The small nodules described above on section correspond to islands of yellow tissue lying in the red. The lymph glands at the hilum are enlarged, brownish at the center and pale at the periphery. The *gall bladder* is not enlarged and is of normal size. It contains a very thick green-black bile. No concretions are demonstrable. The mucous membrane appears normal. Bile ducts are all patent.

Microscopically the usual changes of acute yellow atrophy are present. In the yellow areas the parenchyma cells are swollen, stain poorly, and in the center of the lobule have disappeared to a considerable extent; the bile vessels and periportal connective tissues have begun to proliferate. In the red areas the liver cells have entirely disappeared, leaving the very small lobules composed entirely of the original stroma and capillaries, and proliferating cells coming from the bile ducts about their periphery. When stained with Sudan III the absence of fatty changes is striking, only a few of the most atrophied liver cells containing a few minute fat droplets.

Description of Analysis.

Immediately after the autopsy, 700 grams of the liver, chosen in such a manner as to represent fairly all the different portions of the liver in proper proportions, was taken for analysis. It was cut up into thin slices and placed in strong alcohol, sealed, and kept in a dark place until the analysis could be undertaken. One sample was dried to constant weight, and the amount of water present was determined by difference; it was found to amount to 83.8 per cent., there being 16.2 per cent. of solids.

The alcohol-hardened tissue was cut into fine pieces, and extracted repeatedly with boiling 95 per cent. alcohol under a reflux condensor, all extracts being combined. It was then ground fine in a mill, and extracted several times more; this process of grinding and extracting being repeated four times. Finally the material was extracted in a Soxhlet apparatus, jacketed so that the temperature was kept near the boiling point of alcohol, until the extracts were colorless. The residue was then dried, and extracted repeatedly with ether and ligroin until

nothing more was dissolved out; all the extracts being united, and evaporated to dryness *in vacuo,* at a temperature under 45°.

The extracted liver tissue was now repeatedly extracted with water at room temperature, with much shaking; and then with water at 60°. These extracts were united and reduced to one liter, and analysis of a small fraction showed that they contained in all 1.94 grams of nitrogen. They gave a faint biuret reaction. Only traces of ammonia (less than 20 milligrams) could be detected by Folin's method.

For the next extraction of the liver tissue, boiling water was used, and the extraction continued until the extracts were nearly colorless. They were concentrated to 700 cubic centimeters, and, on cooling, the solution gelatinized. The nitrogen content was 1.835 grams and the solution gave Millon's reaction, but not the tryptophan reaction, being apparently nearly pure *gelatin.* As gelatin contains about 18 per cent. of nitrogen there were, therefore, in 700 grams of liver tissue approximately 10 grams of gelatigenous substance, and in the entire liver 13.8 grams; this constituted about 8.8 per cent. of the entire dry weight of the liver substance.

Composition of the Insoluble Residue.—After all extractions had been completed the residue was dried to constant weight, and analyzed. Total nitrogen was determined by the Kjeldahl method and found to be 15.6 per cent., indicating that the residue was very nearly pure protein. The distribution of this nitrogen in the form of monamino, diamino and amid nitrogen was determined according to the Hausmann method, as used by Osborne and Harris,[7] with the following results:

Sample.	*A*	*B*	Ave.
Amid nitrogen.	0.82	0.90	0.86
Melanoid nitrogen.	0.48	0.40	0.44
Diamino* "	4.15	4.11	4.13
Monamino** "	10.10	10.25	10.18
Total	15.55	15.66	15.61

* This fraction also contains part of the purin nitrogen.

** The monamino nitrogen was determined directly in an aliquot part of the filtrate, rather than by difference as is usually done. The large amount of phosphotungstic acid does not prevent successful combustion if sufficient sulphuric acid and time are used.

In view of Wakeman's[8] observation that in phosphorus poisoning of dogs the arginin of the liver seems to decrease more rapidly than the other diamino acids, it was attempted to determine the relative proportions of the diamino acids in the insoluble proteins of this specimen. The method of Kossel and Kutscher was followed. 35.9 grams of the extracted residue was hydrolyzed fourteen hours with sulphuric acid, and the determination of *histidin* made in the usual manner. There was found, in an aliquot part of the histidin precipi-

[7] *Jour. of Amer. Chem. Soc.,* 1903, xxv, 323.

[8] *Jour. of Exper. Med.,* 1905, vii, 292.

tate, nitrogen to the proportion of 0.540 gram, which corresponds to 2.05 grams of histidin. Unfortunately, the filtrate from the histidin, which contained the arginin and lysin, was lost through the breaking of a beaker, so the amount of these two substances cannot be determined. As the original material contained 4.13 per cent. of nitrogen precipitable by phosphotungstic acid, there was left as nitrogen in the solution not over 0.9 gram of nitrogen as lysin and arginin. If human liver protein has the same composition as dog liver protein the proportion of histidin found is relatively high in this specimen, indicating a low proportion of arginin or of lysin, or both.

A portion of this residue was also analyzed for sulphur, phosphorus, iron and ash, with the following percentage results:

	I.	II.	Ave.
Sulphur.	0.80	0.84	0.82
Phosphorus.	0.51	0.49	0.50
Iron.	1.33	1.12	1.22
Ash.	2.0	1.8	1.9

Fats and Lipoids.—All the extracts with alcohol, ether, and ligroin were united, and evaporated to dryness *in vacuo* at a temperature below 45.° (In the distillate there was always a minute amount of a yellow, gummy substance, the nature of which was not determined.) The residue was thoroughly extracted with ether, and then with ligroin, and the extracted material, which constituted but a small part of the entire residue, was freed from ether and ligroin. The ether-soluble material weighed 17.2 grams, had a decidedly acid reaction, and the odor suggested the presence of a trace of acetic acid. It was dissolved in alcohol and the free acids neutralized with one-tenth normal sodium hydroxide solution, of which 35.2 cubic centimeters were required, indicating the presence of 0.92 gram of *free fatty acids* reckoned as oleic acid. The material was dried down with a large volume of sand, and extracted with ether, leaving the salts of the free fatty acids in the sand. The ether extract showed a yellowish green fluorescence, and examined spectroscopically showed the absorption bands of *urobilin*. The ether extract was reduced to 100 cubic centimeters and 20 cubic centimeters were taken for analysis for phosphorus, to determine the proportion of *lecithin*. The amount of phosphorus found in this fraction was 0.0236 gram, indicating the presence of 3.1 grams of lecithin in the entire material, assuming that lecithin contains 3.8 per cent. of phosphorus. This corresponds to a total of 4.4 grams in the entire liver, or 0.45 per cent. of its fresh weight, and 17.6 per cent. of all the ether-soluble material.

The lecithin was removed by precipitating with four volumes of acetone, and the process repeated. The total weight of the solid material left after removing the lecithin, allowing for the fraction taken for determination of phosphorus, was 14.0 grams, and consisted, presumably, of neutral fats and cholesterin. The *cholesterin* was determined by Ritter's method, after saponifying the fats with sodium alcoholate, drying out in a large volume of salt, and extracting with ether. A small amount of sodium alcoholate carried out by the ether, and also traces of glycerin, were removed by shaking out with water, and the cholesterin ob-

tained in a very pure condition by crystallizing out of hot alcohol. The quantity obtained was 1.904 grams, which corresponded to 2.38 grams in the entire material analyzed, or 2.05 per cent. of the dry weight. The free fats, estimated by difference after removing the cholesterin, weighed 11.62 grams, corresponding to 16.2 grams in the entire liver, or 10.3 per cent. of the entire dry weight.

Free Volatile Fatty Acids were sought in the soaps obtained on neutralizing the original ethereal extract, by distilling in acid solution, and collecting the distillate in tenth-normal sodium hydroxide. But 1.1 cubic centimeters of the alkaline solution was neutralized, indicating the presence of mere traces of volatile acids. The free higher fatty acids were isolated by cooling the hot acidified aqueous solution; in this way 0.9 grams of free fatty acids were obtained. *Lactic acid* was sought in the filtrate, but no crystallizable zinc salt could be obtained.

The amount of fat held in the tissue in a form not available to extraction by alcohol and ether was determined by digesting five grams of the extracted residue with pepsin-hydrochloric-acid mixture, and the material thus obtained, after desiccation, was extracted with ether. Only 11 milligrams of ether-soluble material was extracted, indicating that at the most not over 0.3 per cent. of the total dry weight of the liver tissue was combined fat not removable by prolonged alcohol extraction.

Soluble, Non-coagulable Proteins.—These were studied in the hot and cold water extracts. As before mentioned, the hot water extract seemed to consist solely of *gelatin,* and contained 1.835 grams of nitrogen, corresponding to about 10 grams of gelatin. The cold water extract and the extract at 60° C. contained but a minute quantity of gelatin, gave the biuret reaction, and contained 1.94 grams of nitrogen. These extracts were separately evaporated to a small bulk and let stand in the cold, but no crystallization occurred. They were therefore united, evaporated to a thick syrup, and slowly poured into a large volume of 95 per cent. alcohol, to precipitate the proteids. The alcoholic solution was separated by filtration, and the proteins redissolved and reprecipitated four times. The alcoholic filtrates were united, evaporated to dryness, dissolved in water, and slowly concentrated after decolorization with animal charcoal. A small quantity of typical *tyrosin* crystals appeared, which gave the usual tests for that substance and weighed 0.25 gram. From the tyrosin filtrate a small amount of material with a sour flavor, resembling meat extract, crystallized out and this was added to the original alcohol extracts for study later, as was also the mother liquor.

The proteins precipitated by alcohol were dissolved in hot water, cooled, and let dialyze in a collodion sac for six days in the cold (with thymol, toluol and chloroform) against distilled water, which was frequently changed. The diffusate was concentrated, and found to contain but a small quantity of material, which reacted positively to the biuret, Millon's and tryptophan tests. The dialysate solidified into a jelly-like cake inside the dialyzing bag, and gave the Millon's but not the tryptophan test. These two solutions, containing respectively diffusible and non-diffusible proteins, were examined for their contents in proteins, proteoses and peptones, and non-protein nitrogen, according to the method of Bigelow and Cook.[9]

[9] *Jour. of Amer. Chem. Soc.,* 1906, xxviii, 1485.

The diffusate was concentrated, made up to 150 cubic centimeters, and divided into three fractions of 50 cubic centimeters each.

Fraction I analyzed for total nitrogen, gave 0.144 gram, or 0.432 gram for the entire material.

Fraction II was made slightly acid with sulphuric acid. Zinc sulphate was added to saturation; the solution was let stand over night, filtered; the filtrate was washed with saturated zinc sulphate solution, and the proteose nitrogen in the precipitate was determined. I found 0.015 gram, corresponding to about 0.27 gram of *proteoses* in the entire diffusate.

Fraction III 15 grams of salt and 30 cubic centimeters of a saturated tannic acid solution (containing 1 milligram of nitrogen in blank) were added at a temperature below 15°. The solution was made up to 100 cubic centimeters and let stand over night in the cold. 50 cubic centimeters were filtered off and the nitrogen in this fraction, which contained extractives, ammonia compounds, and possibly amino acids, determined. 0.0441 gram nitrogen, or 0.264 gram in the entire solution, was found as non-protein nitrogen. In the tannic acid precipitate there was, therefore, by difference, 0.122 gram of nitrogen in the form of *peptones*.

The dialysate was analyzed in the same way, but equal amounts of nitrogen were obtained in both the zinc sulphate precipitate and the total nitrogen fraction, indicating the total absence of peptones and non-protein nitrogenous substances.

Amino Acids.—These were contained chiefly in the portion of the alcohol extracts that was insoluble in ether. This material was dissolved in hot water, and nitrogen determined in a sample. The result indicated the presence of 4.69 grams of nitrogen in this fraction, which was then studied according to the method followed by Schumm[10] in his study of autolysis in leukemic spleens, certain modifications being introduced as indicated during the work. As Schumm has detailed his methods quite fully, it does not seem necessary to repeat the description in this place. Determination of the nitrogen in the filtrate of the phosphotungstic acid precipitate showed a total of 1.541 grams in forms precipitable by this reagent, which were presumably diamino acids and purins; while 3.148 grams nitrogen was presumably in the form of monamino acids, ammonia compounds, and simple extractives.

The following substances were isolated from the phosphotungstic precipitate:

I. *Free purins,* the total quantity containing 0.069 gram of nitrogen; both *xanthin* and *hypoxanthin* were isolated in characteristic forms (hypoxanthin silver nitrate, xanthin silver), but no guanin or adenin seemed to be present. Quantitative estimation of the purins was not attempted because of the minute quantity of each present, but the hypoxanthin was considerably the more abundant of the two.

II. *Diamino Acids.*—Histidin was present to the amount of 0.64 gram, and *lysin* amounting to 1.04 grams, as estimated from the amount of nitrogen contained in the fractions in which they were present. The lysin was isolated as the picrate, and identified by its crystalline form. *Arginin* could not be isolated, and a determination of nitrogen in the fraction in which it should have been precipitated showed only traces of nitrogen.

[10] *Hofmeister's Beiträge,* 1906, vii, 175.

Monamino Acids.—For the isolation of the monamino acids there was available the filtrate from the phosphotungstic acid precipitate of the original alcoholic extract, and also the non-protein portion of the aqueous extracts which had already yielded 0.25 gram of tyrosin. After removal of the phosphotungstic acid with barium hydroxide in the usual manner the two solutions were united, and the amino acids first sought by simple fractional crystallization. By this means 0.45 gram more of *tyrosin* was obtained. This was identified by means of its typical appearance when crystallizing out, its insolubility in glacial acetic acid, by which it was purified, and by the intense Millon's reaction.

Similarly by fractional crystallization 4.16 grams of approximately pure *leucin* was obtained from several fractions, which, when purified, had a decomposition point of 291°. The copper salt formed from this had the typical appearance of the copper salt of leucin, and contained 19.8 per cent. of copper, the theoretical content of this salt being 19.6 per cent. Nitrogen in the purified product was found to be 10.85 per cent., the theoretical amount for leucin being 10.69 per cent. It is possible that a certain amount of amino-valerianic acid may have been mixed with the leucin, but it was not attempted to make a separation because of the small amount of material.

About two grams of other material also came out on further concentration, which seemed to consist largely of *bile salts;* it also gave Jaffe's reaction for *creatinin,* but the amount was not quantitatively determined.

The solution was then saturated in the cold with hydrochloric acid gas, and a small amount of typical *glutaminic acid hydrochloride* was obtained; this was united with that obtained later by the ester method, and the combined products purified and studied together.

The solution left after these various crystallizations was then esterified three times after the method of Emil Fischer, and the esters were set free and fractionated in the usual manner. The following fractions were obtained:

	Temperature.	Pressure.	Weight of Ester.
1	to 65°	10 mm.	1 gram.
2	65–100	10	2.5
3*	100–120	1–6	1
4	120–190	4–6	1
5	Residue.	—	3.5

* Fractions 3 and 4 were obtained by the use of the mercury pump. As no liquid air condensation was used the gases formed by decomposition at the higher temperatures prevented a successful evacuation, so that pressures less than 4 mm. could not be obtained.

The esters were hydrolyzed in the usual way, and the amino acids separated by fractional crystallization.

From Fraction 1 was obtained 0.2 gram of a white, crystalline material, sweet-tasting, with a melting point of 240°, and containing 18.48 per cent. of nitrogen—calculated for *glycocoll,* 18.67 per cent.

From Fraction 2 was obtained, by precipitation from hot aqueous solution

with alcohol, 0.3 gram of white, rhombic crystals, with a melting point of 274°, a sweetish taste, and containing 15.60 per cent. of nitrogen—calculated for *alanin,* 15.73 per cent. The alcoholic filtrate from the alanin contained, when evaporated, a yellowish, non-crystalline residue, with the odor characteristic of *pyrollidin carbonic acid.* A copper salt was readily formed, which was entirely soluble in absolute alcohol; this salt weighed 0.475 gram, corresponding to 0.354 gram of prolin. The dehydrated salt (dried at 115° until of constant weight) yielded 22.03 per cent. of copper; calculation for the copper salt of prolin being 21.81 per cent. A small amount of crystalline substance, resembling leucin in its properties, was also obtained from this fraction, but not identified.

Fraction 3 yielded but a minute quantity of a sour-tasting, non-crystalline substance, which was too small in amount to identify.

Fraction 4 was examined in the usual way for phenylalanin, but if present, it was in too small quantity to be isolated. Aspartic acid was also sought for, but could not be found.

Fraction 5, the residue left after the fractional distillations, did not yield any phenylalanin, but there was abundant evidence of the presence of some sulphur-containing compound. On hydrolyzing with barium hydrate, and saturating the concentrated, barium-free filtrate with hydrochloric acid gas, there was obtained a precipitate of typical crystals of the hydrochloride of *glutaminic acid.* This was united to the glutaminic acid previously obtained, decolorized with animal charcoal, and recrystallized, pure glutaminic acid hydrochloride to the amount of 1 gram being obtained. This was analyzed, and gave the following figures:

C, 33.15 per cent.; H, 5.61 per cent.; N, 7.70 per cent.

Calculated for glutaminic acid hydrochloride:

C, 32.70 per cent.; H, 5.45 per cent.; N, 7.63 per cent.

The chlorine was removed from the filtrate with lead oxide, and from the filtrate was obtained by condensation and crystallization 0.28 gram of beautiful needles, with an extremely acid taste. A copper salt was formed, which contained 33.2 per cent. of copper, the theory for the copper salt of *aspartic acid* being 32.7 per cent.

DISCUSSION OF RESULTS.

The most interesting feature of the results obtained in this analysis is the quantity and the number of different amino acids that were found free in the liver tissue. The actual quantity of each amino acid that was isolated from 700 grams of liver tissue (which constituted but 72 per cent. of the entire liver) was as follows:

	Gram.
Histidin	0.64
Lysin	1.04
Tyrosin	0.70
Leucin	4.16
Glycocoll	0.20
Alanin	0.30
Prolin	0.35
Glutaminic acid	1.00
Aspartic acid	0.28
Total	8.67

The quantities given above indicate nothing as to the actual amounts of the free amino acids that were present in the liver, as will be appreciated by anyone who has worked with these substances, for our analytic methods are so imperfect that the quantities obtained represent merely minimal figures, and account for only such quantities of each amino acid as I could obtain in sufficient purity for positive identification. How small a part of the total quantity of amino acids that was really present in the liver is represented by the isolated and indentified amino acids, is shown by the fact that the 8.67 grams of amino acids obtained accounts for but about 1.5 grams of nitrogen, out of a total of about 5 grams of non-protein nitrogen that was present in the liver extracts from which the amino acids were obtained. In the entire material used there was but about 64 grams of protein, which would contain about 10 grams of nitrogen; therefore it is noteworthy that nearly one-third of the nitrogen of the liver was in a water-soluble, non-protein form. If we take into consideration the fact that the patient in this case had been sick some six weeks, the question arises: Could so large an amount of readily soluble substances, such as free amino acids, accumulate in the liver and remain in it for any considerable part of this time? If we estimate the protein lost from this liver during the course of the disease, which resulted in a decrease of its fresh weight to 970 grams (the normal being about 1650 grams), and make allowance for the relatively high proportion of water in the diseased liver, we find that it amounts to approximately 210 grams. This would contain approximately 30 grams of nitrogen, so that the soluble, non-protein nitrogen

found in this liver would represent about one-sixth of all the nitrogen that could have been released by the destruction of the quantity of liver tissue that was lost during the six weeks illness. Although it is possible that so large a proportion of the liberated amino acids as this might have been retained in the liver, it hardly seems probable in view of their relatively easy solubility. It may be recalled in this connection that Neuberg and Richter[11] found a total of 2.127 grams of free amino acids in 350 cubic centimeters of blood from a patient with acute yellow atrophy, and estimated that the entire blood must have contained as much tyrosin at this time as could have been formed by hydrolysis of the entire liver. Hence they were obliged to conclude that there must be some other source for the free amino acids than the autolysis of the liver, and they suggested the possibility that they come through the intestinal walls without undergoing the normal synthesis, because of some pathological alteration in this structure. Whatever the source of the amino acids may be, the large quantity of free, soluble, non-protein nitrogen present in the liver in my case would seem to be in favor of the contention of Neuberg and Richter that there must be some source for these substances other than the autolyzing liver itself.

The large number of different amino acids that were found is not more than might be expected in the light of the studies of Fischer and his colleagues. Already leucin, tyrosin, lysin, arginin, and aspartic acid have been found free in the liver or blood of persons dying with acute yellow atrophy or similar conditions. Glycocoll has been found in the urine of phosphorus-poisoned dogs,[12] and Wohlgemuth[13] has also found alanin, glycocoll and arginin in human urine in phosphorus poisoning. So far as I have been able to find in the literature, the finding of prolin (pyrrolidin carbonic acid) and glutaminic acid free in the tissues or fluids of either human or animal material has never been before recorded. There is no evident reason why all the amino acids that have been isolated from proteins by Fischer and others might not be found in dis-

[11] *Deut. med. Woch.*, 1904, xxx, 499.
[12] Aberhalden and Barker, *Zeit. f. physiol. Chem.*, 1904, xlii, 524.
[13] *Zeit. f. physiol. Chem.*, 1905, xliv, 74.

eased tissue in which autolysis has occurred; but the rapid absorption of these substances and their early destruction by desamidizing enzymes cause the amounts present at any one time to be so small as to make their successful isolation by the available methods extremely difficult. In my material, arginin and phenylalanin were sought in vain, and no tryptophan reaction was given by the protein-free extracts. Serin, iso-serin, and cystin were not sought, but a sulphur-containing compound was present among the esters obtained by the Fischer method.

Small quantities of proteoses and peptones seem to have been present, as shown by the biuret reaction of the original aqueous extracts and of the diffusate from the protein solution obtained by cold and hot water extraction of the liver. The quantity of the nitrogen found in the different fractions obtained by the Bigelow and Cook methods, indicated the presence of about one-third gram of proteoses, and one-fourth gram of peptones.

Free xanthin and hypoxanthin were also found in minimal quantities, the latter predominating; the total quantity isolated contained but about 0.1 gram of nitrogen. The failure to obtain guanin and adenin is readily explained, since these substances are known to be soon changed by the guanase and adenase of the liver into xanthin and hypoxanthin.[14]

Wakeman's interesting studies on the changes in composition of the liver of dogs during phosphorus poisoning[15] render a study of the composition of the liver proteins in the human liver during acute yellow atrophy of value. The result of the analysis by Hausmann's method of the insoluble proteins of my specimen of acute yellow atrophy, of two normal livers, and of one liver in delayed chloroform poisoning with extreme necrosis of the liver cells, is shown in the following table:

	Acute atrophy.	Normal (anemic).	Normal (congested)	Chloroform necrosis.
Amid nitrogen.	5.5	3.7	4.8	3.9
Humus "	3.6	3.4	4.9	5.7
Diamino "	26.2	32.8	30.0	30.0
Monamino "	64.8	60.3	60.2	60.3

[14] Jones and Austrian, *Zeit. f. physiol. Chem.*, 1906, xlviii, 110.

[15] *Jour. of Exper. Med.*, 1905, vii, 292.

There seems to be present here, as Wakeman found in his dogs' livers, a decrease in the diamino nitrogen, although this is not so striking as in Wakeman's material. Possibly the slighter decrease observed in the acute yellow atrophy liver depends in part upon an increase in the purins present on account of regenerative cell multiplication, for in the Hausmann method of determining nitrogen distribution the purins are partly precipitated with the diamino compounds. It was impossible to determine the relative proportion of each of the three diamino acids, because of accidental loss after the histidin had been separated. However, the proportion of the nitrogen present in the form of histidin (0.54 gram) to that present as arginin and lysin (0.94 gram) is larger than the normal proportion, and suggests that either the arginin or the lysin, or both, were decreased much below the normal.

Interesting figures as regards sulphur, phosphorus and iron in the insoluble residue of extracted liver tissue were obtained, as follows:

	Acute atrophy.	Normal (anemic).	Normal (congested).	Chloroform necrosis.
Sulphur.	0.82	0.75	0.77	0.79
Phosphorus.*	0.90	0.27	0.21	0.50
Iron.	1.22	0.2	0.4	0.5

* Average of four analyses of each specimen.

In spite of the great loss of parenchymatous tissue in the acute yellow atrophy liver the proportion of sulphur is quite the same as for the normal livers. This is in agreement with the findings of Wohlgemuth[16] in phosphorus poisoning of dogs, the liver tissue of which showed practically no decrease in the proportion of sulphur. The proportion of phosphorus is, however, much increased over the figures for the normal livers, being increased by about four times. This is partly explained by the great proliferative activity of the cells of the stroma and bile ducts in the areas in which regeneration is occurring, resulting in the presence or large numbers of new nuclei rich in nucleic acid; but it does not seem that this proliferation is sufficient to explain a four-fold increase in phosphorus. Probably part of the phosphorus of the cells that

[16] *Biochem. Zeit.*, 1906, i, 161.

have undergone degeneration is present in some insoluble form, for the high figure obtained with the liver in chloroform necrosis shows that necrosis of the cells with disappearance of the majority of stainable nuclei is not associated with a decrease in the insoluble phosphorus. The very considerable amount of iron found in the acute atrophy liver is in agreement with the large amount of blood present in the areas of "red atrophy," where the space formerly occupied by liver cells is filled by dilated capillaries; furthermore, iron-containing pigment is usually found deposited in considerable amounts in the liver in this disease, presumably because of the extensive hemolysis produced by the cholemia.

Gelatigenous substances seem to have been excessively abundant, not only relatively, but to a less extent absolutely. The relative increase is largely the result of the fact that in this disease the parenchyma cells are destroyed while the stroma is not injured, and the absolute increase depends upon the regenerative proliferation of the connective tissue. From 700 grams of liver substance analyzed, 10 grams of gelatin were obtained, which corresponds to 13.8 grams of gelatin in the entire liver, 1.4 per cent. of the entire weight of the fresh substance, and 10.1 per cent. of the weight of the dry, fat-free tissue. A normal human liver was analyzed in the same way, and a total of 9.2 grams of gelatin was found in the entire liver, corresponding to 0.57 per cent. by weight of the entire liver, and 3.2 per cent. of the dry, fat-free substance. Another liver, from an acute case of chloroform necrosis, showed but 1.5 per cent. of the fat-free substance in the form of gelatin.

In common with all other recorded analyses, the proportion of water in the liver was found to be very excessive. This is due chiefly to the filling of the spaces left by the destroyed liver cells with blood, and partly, perhaps, to a considerable degree of intracellular edema (cloudy swelling or hydropic degeneration). But 16.2 per cent. of the entire liver was solids, there being 83.8 per cent. of water. This is seen to agree well with other analyses given in the accompanying table.[17]

The great loss of parenchyma is best shown if we consider that the dry substance of the entire liver weighed but 157 grams,

[17] Modified from Quincke, *Spec. Path. und Ther.*, 1899, xviii, 297.

	Water.	Fat.	Fat-free Dried Substance.
Normal liver.	76.1	3.0	20.9
Acute atrophy (Perls).	87.6	8.7	9.7
" " (Perls).	76.9	7.6	15.5
" " (v. Starck).	80.5	4.2	15.5
" " (Taylor).	85.8	2.0	12.2
" " (Wells).	83.8	2.5	13.7
Phosphorus poisoning (v. Starck).	60.0	29.8	10 0
Fatty degeneration (v. Starck).	64.0	25.0	11 0

whereas a normal liver contains 375 to 425 grams of solids; and furthermore, as the amount of connective tissue was rather increased and the fat not much below normal, the loss of parenchyma must represent at least two-thirds that of the entire liver.

As shown by the above table, the acute yellow atrophy liver does not show an accumulation of fat, having not far from the same amount of fat as the normal liver. The yellow color of the organ, which has commonly been assumed to represent fatty changes, is due to the large amount of bilirubin present; this can be shown by placing the tissue in some oxidizing solution, such as potassium dichromate, when the color of the liver at once becomes green. Even the apparent fat increase observed in many pathological conditions, in which the microscope shows a great amount of intracellular fat in the form of fine granules, while chemical analysis shows no fat increase, is entirely wanting in acute yellow atrophy; for in the two typical specimens that I have had the opportunity to examine with special stains, the amount of fat was strikingly small, only occasional cells being found containing a few granules. Therefore, whatever the cause of acute yellow atrophy may be, it cannot be classed among the steatogenetic poisons. As nearly every other degenerative change in the liver is accompanied by more or less fatty metamorphosis, it is remarkable that in this disease, which causes so great an alteration of the liver, no fatty changes occur. Certainly the cause of acute yellow atrophy must differ in some essential respect from the bacterial toxins, phytotoxins, zootoxins and most of the organic and inorganic hepatic poisons with which we are familiar, since all these cause greater or less fatty changes in the liver. In view of the prominence in this disease of autolytic changes, which are supposed to liberate invisible intra-

cellular fat and to make it microscopically visible, one might expect an abundance of this apparent fatty degeneration, but such is not the case.

The amount of lecithin seems not to have been out of proportion to the amount of fat present in the liver, the amount found corresponding to a total of 4.4 grams in the entire liver, or 0.45 per cent. of the fresh weight. In the liver of a man dying from accident, Balthazard[18] found 1.28 per cent. of lecithin. Orlow[19] found in the liver of infants from 0.29 to 1.34 per cent. of the fresh weight of lecithin. Heffter[20] found about 0.65 per cent. of lecithin in the liver of an executed criminal, and states that he found the proportion of lecithin in the liver of acute yellow atrophy to be decreased, but the cases he describes in support of this statement are probably of some other condition, for the dry weight is given as from 32.1 to 37.8 per cent. of the fresh weight, and of this from 55 to 68 per cent. is ether-soluble material; these figures agree with those of ordinary fatty metamorphosis.

Cholesterin was present to the amount of 3.23 grams in the entire liver, constituting 0.3 per cent. of the entire fresh weight, or 2 per cent. of the dry substance. The normal proportion of cholesterin in the liver is given by Orlow as 0.14 to 0.35 per cent. (fresh weight) in children. I have not been able to find figures on the proportion in the liver of adults, but it would presumably be at least as high, or higher (see note, p. 643).

The finding of small quantities of urobilin, bile salts and creatinin is what would be expected of such a liver, in a person dying with severe icterus. That free lactic acid and volatile fatty acids could not be found does not indicate that they may not have been formed in the living tissue, for they would have been rapidly absorbed and removed.

SUMMARY.

From the liver of a young man who died of typical, "idiopathic" acute yellow atrophy of the liver, after an illness of six weeks, there were isolated and identified the following amino acids: Histidin,

[18] *Compt. rend. Soc. biol.*, 1901, liii, 922.
[19] Abstract in *Biochem. Cent.*, 1907, v, 937.
[20] *Arch. f. exp. Path. u. Pharm.*, 1891, xxviii, 97.

lysin, tyrosin, leucin, glycocoll, alanin, prolin, glutaminic acid, aspartic acid. These were found free in extracts of the liver, and presumably represent products of the autolysis of liver cells, although the amount of soluble non-protein nitrogen present in the extracts was so large as to suggest that there must be some other source for these substances.

Small quantities of free proteoses and peptones, and of xanthin and hypoxanthin, were also found in the extracts.

In the insoluble proteins of the liver the proportion of diamino acids was decreased slightly as compared with normal livers. The proportion of protein phosphorus was increased, probably because of active regenerative proliferation, while the sulphur was normal in amount. Iron was increased because of the large quantity of blood in the liver and the hematogenous pigmentation of the liver cells.

Gelatigenous material was increased both absolutely and relatively, because of the loss of parenchyma and the proliferation of the stroma.

The proportion of water to solids was much increased, there having been a loss of over two-thirds of the entire parenchymatous elements of the liver. The amount of fat, lecithin and cholesterin was not far from that normal for the liver.

In conclusion, it gives me pleasure to express my indebtedness for advice and assistance to Professor Lafayette B. Mendel, in whose laboratory this study was begun. I am also under obligations to Professor A. P. Matthews of the University of Chicago for the use of his laboratory during part of the work.

Note.—Since this article was sent to the publishers I have completed the analysis of the fats and lipoids of three other livers, and the results are shown for comparison with the acute yellow atrophy liver in the following table:

It is apparent that the total amount of lecithin in this acute yellow atrophy liver is very greatly reduced, not only as to the actual amount present, but also in its relation to the other constituents of the liver. It would seem that although the liver has lost even as great a proportion of its fatty constituents as of its proteins, it has suffered an even greater loss in its lecithin. The

Specimen Number.*	Lecithin.				Cholesterin.			
	199	202	203	Ac. Atr.	199	202	203	Ac. Atr.
Per cent. of fresh weight.	1.6	1.4	1.5	0.45	0.26	0.37	0.52	0.3
" " " total dry weight.	6.3	6.25	6.2	2.9	1.0	1.7	1.9	1.8
" " " dry, fat free material.	7.7	8.0	8.1	3.2	1.25	2.1	2.9	2.1
" " " ether-soluble substances.	35.3	28.0	17.3	17.6	5.7	7.4	5.9	11.1
Grams in entire liver.	23.7	22.4	16.0	4.4	3.8	5.95	5.4	3.38

* 199 and 202 are normal human livers, from persons dying suddenly. 203 is the liver of a young man dying from delayed chloroform poisoning, with hepatic necrosis of an extreme type; this case will be the subject of a study which will be reported later.

significance of this observation cannot be estimated until we have more figures on the variations in the lecithins of the liver and other organs in health and disease. It is interesting to note, however, that in the liver showing severe chloroform necrosis, with considerable fatty change, there has also been a decrease in lecithin, although not so marked as in the acute yellow atrophy liver.

The cholesterin has not been so greatly reduced, for this liver shows about the same proportions, and nearly as large a total amount of lecithin, as the controls. The reduction in amount of neutral fats and lecithin causes it to form an unusually large proportion of the ether extract.