THE CHEMISTRY OF THE LIVER IN CHLOROFORM NECROSIS (DELAYED CHLOROFORM POISONING).

BY H. GIDEON WELLS.

(From the Pathological Laboratory of the University of Chicago.)

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Recently I reported the results of a complete analysis of a liver exhibiting the typical conditions of acute yellow atrophy, and while engaged in this analysis had the good fortune, through the kindness of Dr. F. S. Tufts, to perform an autopsy upon the body of a young man who had died a few hours before from the condition of acute necrosis of the liver that occasionally follows the administration of chloroform. This liver has now been analyzed in the same manner as was the acute yellow atrophy liver, and certain steps have been duplicated with two normal livers for comparison, all four livers having been obtained from robust young men in the third decade of life. Some of the final figures of all four analyses were included in the paper upon acute yellow atrophy, but in the following report will be considered particularly the chemistry of the liver in chloroform necrosis.

Delayed chloroform poisoning is an extremely serious, often fatal sequence of the use of chloroform as an anesthetic, which has only recently become generally recognized. Attention was called to it in this country particularly by the articles of Ballin and of Bevan and Favill, and in England by Guthrie, while the chief recent article in German literature is that of Guleke. It is not my purpose here to go into the literature, or to discuss the clinical and anatomical features of the case, which has been done more extensively in another place. Briefly, the features of the effects...
of delayed chloroform poisoning are as follows: After an opera-
tion, which may involve any part of the body but which is most
frequently abdominal, and in which chloroform has been used
as the anesthetic,\(^1\) the patient begins as if upon the road to an
uneventful recovery; but after a period of from 12 to 72 hours
(generally about 24 to 36 hours), symptoms of restlessness, fear
and delirium appear, passing into coma which terminates after
two to four days, as a rule, in death. The persons are almost
always young persons, never past middle life, and the cases
reported in the literature seem to fall into two groups. In one,
which is seen chiefly in children, the manifestations are similar
to those of acid intoxication, with the so-called acetone odor of
the breath and the presence of acetone and diacetic acid in the
urine, more or less cyanosis, and sometimes distinct air hunger;
anatomically in these cases there is usually found a well marked
fatty degeneration of the kidneys, myocardium and liver, the
changes in the latter being described as chiefly in the periphery
of the lobules. The other type of poisoning has been observed
in young adults, and differs in that with the onset of delirium
jaundice appears, and usually becomes profound, cutaneous hem-
orrhages often develop, there is more or less tenderness in the
region of the liver, the area of liver dulness decreases, leucin
and tyrosin may be found in the urine, and the clinical picture is
similar to that of acute yellow atrophy; at autopsy the liver is
found decreased in size, friable, yellow, extremely degenerated,
with necrosis of by far the majority of the liver cells, and there
is more or less necrosis and fatty degeneration of the kidneys.

The case that I have studied is of the second type, in which the
hepatic changes are for the most part similar to those of acute
yellow atrophy, and may be described briefly as follows: A vigor-
ous young man, 28 years of age, was operated upon for gallstones,
chloroform being given in a rather liberal manner. The opera-
tion, which amounted merely to an exploratory laparotomy, was
of short duration, and for the first 48 hours after the patient was
in good condition; then restlessness began, rapidly passing into
delirium and coma; jaundice appeared and became pronounced,

\(^1\) There are, perhaps, a few instances in which a similar effect has
been produced by ether.
and death occurred 100 hours after the operation. At the autopsy, performed twelve hours after death, the chief changes were found in the liver, which had been reduced in size so that it weighed but 1050 grams, this being rather under than over two-thirds the normal weight for a man of this size and age. It had a wrinkled capsule, and was strikingly shrunken, flabby, soft and friable; it appeared as if badly decomposed, except that it had a sweetish odor, and it was of a yellow color on the cut surface. Microscopically the cells in the center of each lobule were found necrosed, greatly decreased in size and number, granular, containing many small droplets, and causing an extremely disorganized appearance of at least two-thirds to three-fourths of each lobule; only the cells at the very periphery of each lobule still retain the power of taking the nuclear stains, and even they are very granular and contain numerous large droplets of fat. These changes are quite similar to those seen in the early stages of acute yellow atrophy, differing chiefly in the considerable amount of fatty change that is present.

CHEMICAL ANALYSIS.

Immediately after the autopsy the liver was cut into thin slices, and 850 grams (which constituted 81 per cent of the entire liver) was placed in a large volume of 95 per cent alcohol. After hardening was completed the tissue was ground up in a meat chopper and extracted repeatedly with fresh quantities of boiling alcohol under a reflux condenser, the alcoholic extracts being filtered off and united. Twice the residue was dried and ground to a powder in a mill during the alcohol extraction, and after this it was extracted in a Soxhlet apparatus with absolute ether until nothing more could be removed. The ether and alcohol extracts were united and evaporated to dryness under vacuum at a temperature not exceeding 40°, the residue obtained weighing 127.5 grams, or 15 per cent of the entire fresh weight of liver taken. A sample of the fresh liver tissue was also weighed and dried, and the water content was found to be 72.4, leaving 27.6 per cent of solids. The liver residue left after this extraction was then extracted in a shaking machine repeatedly with fresh quantities of water, then with water at 50° to 60°, these extracts
Chloroform Necrosis

being united and evaporated to dryness at 40° to 45° in vacuo; the weight of the dried extract was 5.03 grams. The residue of liver tissue was then extracted with boiling water, changed frequently, until the extracts were practically colorless. These three distinct extracts (alcohol and ether, cold and warm water, hot water) and the insoluble residue of coagulated liver tissue were now available for separate analysis.

The hot water extract was concentrated to 100 cc., and four volumes of absolute alcohol added; the precipitate was filtered off, washed in 95 per cent alcohol, redissolved in hot water and reprecipitated with alcohol. On dissolving this precipitate again it was found to give a good Millon's reaction, a bluish biuret reaction, but not the Hopkins-Cole reaction for tryptophan. On cooling it gelatinized, and when dialyzed for three days under toluol in a celloidin sac the diffusate contained no appreciable amount of solids and did not give the biuret reaction, indicating the absence of proteoses and peptones. Therefore the precipitate was pure, or nearly pure, gelatin. Determination of nitrogen in an aliquot part of the solution indicated a total of 0.302 gram nitrogen, which corresponds to 1.68 gram of gelatin (assuming that gelatin contains 18 per cent of nitrogen); this amounts to but 0.72 per cent of the total solids of the liver.

Evaporation of the alcoholic filtrate from the gelatin precipitation yielded 3.81 grams of solids, which was added to the 5.03 grams of residue from the cold and warm water extracts; determination of the nitrogen in an aliquot part of this mixture indicated the presence of 0.894 gram of nitrogen, or 16 per cent of the total solids in these watery extracts. This solution gave good biuret and Millon reactions, and a faint reaction for tryptophan. On addition of several volumes of alcohol to the watery solution a granular precipitate was obtained, which was found to consist chiefly of arsenic from the embalming fluid.1 The filtrate gave a strong biuret reaction, and upon concentration to 50 cc. and addition of five volumes of absolute alcohol there was obtained a typical hygroscopic proteose-peptide mixture, which weighed

1 An attempt at embalming had been made, but fortunately for my purposes this was very unsuccessful; the liver was not at all hardened except a small part of the surface where embalming fluid injected into the peritoneal cavity had come in contact with it.
three grams when dry. This proteose-peptone mixture was then dissolved in 50 cc. of water and precipitated fractionally with ammonium sulphate according to the method of Pick, with the following result:

First fraction (half saturation). A brown gummy mass, weighing about 0.5 gram. This gave a slightly purplish biuret reaction, and was probably a mixture of gelatin and proteoses.

Second fraction (two-thirds saturation). About 0.2 gram more of brownish gummy precipitate, which gave a good pink biuret reaction and a good tryptophan reaction.

Third fraction (saturation). Only a very slight precipitate, giving a good biuret reaction and fair tryptophan reaction.

Fourth fraction (filtrate). Gave only a faint biuret reaction, therefore probably only a small amount of peptone present.

Composition of the insoluble coagulated residue. The residue of insoluble liver substance left after all the extractions had been completed, was dried to a constant weight and analyzed. The total nitrogen was determined by the Kjeldahl method in a sample, and found to amount to 14.48 per cent of the entire weight, indicating that the substance is nearly pure protein. The distribution of this nitrogen in the form of monamino, diamino and amid nitrogen was determined in two samples according to the Hausmann method as used by Osborne and Harris with the following results:

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
<th>Average</th>
<th>Per cent total nitrogen.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amid nitrogen</td>
<td>0.52</td>
<td>0.58</td>
<td>0.55</td>
<td>3.9</td>
</tr>
<tr>
<td>Humus nitrogen</td>
<td>0.82</td>
<td>0.80</td>
<td>0.81</td>
<td>5.7</td>
</tr>
<tr>
<td>Diamino nitrogen*</td>
<td>4.25</td>
<td>4.17</td>
<td>4.21</td>
<td>30.0</td>
</tr>
<tr>
<td>Monamino nitrogen†</td>
<td>8.45</td>
<td>8.45</td>
<td>8.45</td>
<td>60.3</td>
</tr>
<tr>
<td>Totals</td>
<td>14.04</td>
<td>14.00</td>
<td>14.02</td>
<td></td>
</tr>
</tbody>
</table>

* This fraction also contains part of the purins.
† The monoamino nitrogen was determined directly in an aliquot part of the filtrate from the phosphotungstic acid precipitate, and not by difference as is usually done. On this account the total nitrogen obtained, 14.02 per cent is a trifle less than all the nitrogen present, 14.48, but this statement of results is probably more nearly accurate than when the determination is made by difference.

Other portions of this liver residue were also analyzed for sulphur, iron and phosphorus, with the following percentage results:

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphur</td>
<td>0.80</td>
<td>0.78</td>
<td>0.79</td>
</tr>
<tr>
<td>Phosphorus*</td>
<td>0.85</td>
<td>0.83</td>
<td>0.90</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.98</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>0.42</td>
<td>0.57</td>
<td>0.50</td>
</tr>
<tr>
<td>Ash</td>
<td>0.93</td>
<td>0.98</td>
<td>0.95</td>
</tr>
</tbody>
</table>

* On account of the unexpectedly high figure for phosphorus in this analysis, in which the combustion was made by fusing with sodium carbonate and sodium nitrate, another pair of determinations was made by Neumann's method, which gave the slightly higher figures seen in the second row.

**FATS AND LIPIDS.** The extracts with alcohol and ether, which had been evaporated to dryness *in vacuo*, weighed 127.5 grams, and this material was in turn thoroughly extracted with ether, which dissolved out 75 grams, presumably fats and lipoids, this constituting 8.8 per cent of the total fresh weight or 32 per cent of the solids of the liver. Therefore 18.8 per cent of the entire weight of the fresh liver was proteins and extractives, 8.8 per cent fats and lipoids, and 72.4 per cent water. The ether extract was evaporated to dryness, dissolved in absolute alcohol and made up to 250 cc., of which two 20 cc. samples were taken for cholesterin determinations and two 10 cc. samples for lecithin.

**Cholesterin** was determined according to the method of Ritter,¹ in which the fats are saponified with sodium alcoholate, dried out in a large volume of salt, and extracted thoroughly with ether which dissolves out the cholesterin but not the soaps. Traces of sodium alcoholate, soaps, and glycerin escaping into the ether extract are removed by shaking out with water, and the cholesterin weighed directly after evaporating off the ether. The amount found in the samples taken (0.353 gram in 2/6 of the extract) corresponds to a total of 4.4 grams in the 850 grams of fresh liver substance analyzed, or 5.4 grams in the entire liver; this indicates that cholesterin constituted 0.52 per cent of the fresh weight; 1.9 per cent of the total dry weight, 2.9 per cent of the fat-free dry substance, or 5.9 per cent of the ether-soluble material.

Lecithin was determined by the method recommended by Koch and Woods in which the lecithin is precipitated from an aqueous emulsion with acid chloroform, combusted by Neumann's method, and the phosphorus determined as magnesium pyrophosphate. The amount of phosphorus present, 0.0212 gram, in the samples analyzed, corresponds to 0.558 gram of lecithin in the sample, or 12.95 grams in the extract from 850 grams of liver, representing a total of 16 grams of lecithin in the entire liver. This indicates that lecithin constituted 1.5 per cent of the fresh weight, 5.5 per cent of the total solids, 8.1 per cent of the fat-free solids, and 17.3 per cent of the ether-soluble material.

Amino-acids and purins. These were sought in the part of the original alcohol extract that was left after ether extraction, and in the non-protein portions of the watery extracts. These were united, dissolved in water, and made up to 1000 cc., and the nitrogen in a 10 cc. sample determined by Kjeldahl's method, 46.41 mg. of nitrogen was found in the sample, corresponding to 4.641 grams of nitrogen in the entire extract, the total weight of which was 45 grams. This material gave a strong Millon reaction, but only a very faint biuret reaction. It was examined for amino acids and purins, following with slight modifications the method used by Schumm in his study of autolysis in leukemic spleens. As Schumm has given his procedure in detail it is unnecessary to give here the details of the analysis. Briefly, the diamino acids and purins were separated from the monamino acids by precipitating in 5 per cent sulphuric acid solution with phosphotungstic acid. From this precipitate, which contained 1.386 gram of nitrogen, were isolated the following substances:

1. Free purins. The total quantity obtained contained 0.0974 gram nitrogen, and from it was isolated 0.16 gram of hypoxanthin silver nitrate, and a smaller quantity of xanthin silver. Adenin and guanin could not be found.

2. Diamino acids. After removal of the purins from the phosphotungstic acid precipitate, the diamino acids were sought according to the method of Kossel and Kutscher. In the histidin fraction was found 91 mg. of nitrogen, which corresponds to

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1 This Journal, i, p. 203, 1906.
0.34 gram of histidin. The arginin fraction contained but 32.2 mg. of nitrogen, and yielded a small amount of long rhombic crystals, but the amount was so small that it is impossible to tell whether this was arginin or not.

The fraction that should have contained the lysin contained 0.404 gram of nitrogen, but from it no lysin picrate could be obtained; instead there was a gummy mass, which gave a strong biuret and good Millon and tryptophan reactions. This was soluble in strong alcohol, and only a small part was precipitated by saturating the solution with ammonium sulphate: the filtrate from this precipitate gave strong biuret and Millon reactions, and a faint tryptophan reaction, and therefore was either peptone or polypeptid, most probably chiefly the latter.

III. Monamino acids. After removal of the phosphotungstic and sulphuric acids from the filtrate from the diamino acid precipitate, the filtrate was concentrated and by fractional crystallization was obtained 2.70 grams of what seemed to be chiefly a mixture of leucin and tyrosin. By extraction with glacial acetic acid 0.26 gram of pure tyrosin was obtained from the insoluble residue, which was identified by its typical cottony appearance when crystallizing out after purification, its insolubility in glacial acetic acid, and its intense Millon reaction.

By careful recrystallization of the filtrate from the tyrosin after removal of the acetic acid and decolorization with animal charcoal, leucin was obtained to the amount of 1.5 gram. This was converted into a copper salt by boiling with fresh copper oxide, and the typical bluish-white copper salt of leucin was readily obtained in pure condition. After drying this at 112° a nitrogen determination was made, and 8.77 per cent of nitrogen was obtained, the theory for the copper salt of leucin being 8.67 per cent of nitrogen. Copper was determined, and found to be 19.9 per cent, the theory for the copper salt of leucin being 19.6 per cent.

Upon saturating with hydrochloric acid gas the concentrated filtrate from the leucin-tyrosin crystallization, a considerable amount of crystalline material came out, but on further examination this was found to be entirely inorganic, and not glutamic acid hydrochloride. The filtrate from this was then esterified three times according to the method of Emil Fischer, and about
10.5 grams of raw esters was obtained. This was fractionated in the usual manner except that only the water pump was used, the pressure throughout the distillation being 9 to 12 mm., and the following fractions were obtained.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Temp. up to 60°; weight of esters</th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>&quot; 60°-100°; &quot; &quot; 1.9</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>&quot; 100°-188°; &quot; &quot; 1.3</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Residue &quot; &quot; 2.5</td>
<td></td>
</tr>
</tbody>
</table>

The first three fractions were hydrolyzed by boiling in water eight hours with inverted condenser, and then evaporated to dryness in vacuo over sulphuric acid at 45°. Fraction I yielded 0.58 gram of a crystalline white substance having a sweet taste and melting at 240°; probably chiefly glycocoll. Fraction II yielded 0.99 gram of a substance similar to fraction I, except that it had a mixed sweet and bitter taste and contained a few waxy crystals resembling leucin; it melted at 250° and was presumably a mixture of glycocoll with small amounts of higher amino acids. Fraction III yielded 0.81 gram of a brownish, semi-solid substance, smelling like prolin and slowly forming a small amount of crystalline substance.

Fraction IV was shaken out with ether and water until the ether-soluble portion was removed. This ether extract was hydrolyzed by evaporating with concentrated hydrochloric acid, but only a very small amount of crystalline material, mixed with a brownish amorphous substance, was obtained, insufficient for purification and identification as phenylalanin which would be found in this fraction if present. The watery extract was hydrolyzed by boiling with barium hydroxide, and the barium salts allowed to crystallize out, but after removing the barium from the crystalline material no aspartic acid or other crystalline organic substance could be found. The filtrate from the crystalline barium salts was freed from barium, concentrated to a few cubic centimeters, and saturated with hydrochloric acid gas; after standing on ice over night without crystallization the solution was inoculated with a crystal of glutamic acid hydrochloride and a considerable amount of typical crystals of this salt came out. This salt was separated by filtration through asbestos in a Gooch crucible, and weighed 0.58 gram. It was recrystallized,
and a nitrogen determination made, 7.74 per cent of nitrogen being found, agreeing well with the theory for the hydrochloride of glutamic acid, which calls for 7.63 per cent of nitrogen.

The chlorine was removed from the filtrate from the glutamic acid with lead oxide, and after removal of the lead with hydrogen sulphide a very small amount of slightly crystalline greenish material was obtained. This was redissolved and a copper salt was made, which was of a greenish yellow color and manifestly impure; therefore it was impossible to determine whether aspartic or any other amino acid was present in this fraction.

Fractions I and II were united, dissolved in 10 cc. of hot water, and on addition of three volumes of hot absolute alcohol there was obtained no precipitate, and none appeared on cooling, indicating the absence of any appreciable amount of leucin or alanin. Therefore the solution was evaporated to dryness, dissolved in a minimum amount of hot absolute alcohol, saturated with dry hydrochloric acid gas and cooled. After inoculating with crystals of the hydrochloride of glycocoll ethyl ester a small amount of crystals of this type appeared. The yield being unsatisfactory the chlorine was removed with lead oxide, and a copper salt was made of the amino acid in the filtrate. On analysis of the first fraction of this salt obtained on crystallization 13.58 per cent of nitrogen and 30.2 per cent of copper were obtained, the theory for the copper salt of glycocoll being nitrogen, 13.40 per cent, copper 30.8 per cent. Therefore, most if not all of the first two samples, amounting to 1.57 gram, is glycocoll.

The third fraction, which resembled prolin, was made into a copper salt, and the salt treated with absolute alcohol which dissolved out a greenish, noncrystalline material, leaving the crystals of the copper salt. This was analyzed for nitrogen, and 8.0 per cent found. The theory for the copper salt of prolin is 9.62 per cent, for aspartic acid 7.22 per cent, and for leucin 8.67 per cent. Possibly the salt obtained was an impure mixture, but the amount available for analysis was too small to permit of duplicate analyses or for copper determinations; therefore the presence or absence of prolin cannot be determined, but from the appearance and odor of the material it is probable that prolin was present.
DISCUSSION OF RESULTS

This analysis has most interest when compared with the results obtained by the analysis of two normal human livers and a liver from a case of typical "idiopathic" acute yellow atrophy, which has been previously published, all four livers being from young men of about the same age. In the acute yellow atrophy liver the most interesting result was the isolation of a comparatively large number of amino acids in sufficient purity for their identification; in the chloroform necrosis a somewhat smaller number and smaller amounts were isolated, as shown by the following table:

<table>
<thead>
<tr>
<th></th>
<th>Acute atrophy</th>
<th>Chloroform necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidin</td>
<td>0.64</td>
<td>0.34</td>
</tr>
<tr>
<td>Arginin</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Lysin</td>
<td>1.04</td>
<td>?</td>
</tr>
<tr>
<td>Tyrosin</td>
<td>0.70</td>
<td>0.26</td>
</tr>
<tr>
<td>Leucin</td>
<td>4.16</td>
<td>1.50</td>
</tr>
<tr>
<td>Glycocollel</td>
<td>0.20</td>
<td>1.57</td>
</tr>
<tr>
<td>Alanin</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Prolin</td>
<td>0.35</td>
<td>present ?</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1.00</td>
<td>0.58</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Phenylalanin</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

Total: 8.67 4.25

With these results may be incorporated the results obtained by A. E. Taylor, who has also examined for amino acids the extracts from a case of acute yellow atrophy and one of chloroform necrosis. From the acute yellow atrophy liver he isolated 0.35 gram of leucin and 0.612 gram of aspartic acid; from the chloroform necrosis 2.3 gram of arginin nitrate, 2.2 grams of tyrosin, and 4.0 grams of leucin.

These figures of themselves indicate nothing as to the actual quantity of free amino acids present, on account of the inadequacy of the analytic methods that are available for their isolation; in each case the amino acids recovered account for only a
small fraction of the nitrogen present in the solutions containing
them, and less than one-fourth of the total weight of the esters
obtained from the chloroform necrosis liver could be recovered
as amino acids. Nevertheless it is of interest to find that so
many of these constituents of the protein molecule can be found
free in degenerated livers, even if only in small amounts. These
two analyses furnish the only instances that I can find in the
literature of the isolation of free glutamic acid and free prolin
from either human or animal tissues or excretions; the identity
of the glutamic acid was completely established in both cases,
but the prolin, although almost certainly present in the extracts
from the chloroform necrosis liver, could not be isolated in suffi-
cient amount for analysis and identification.

It might be expected that more free amino acids would be
found in the chloroform necrosis liver, in which the reduction in
size of the autolyzing liver took place in a few days, than in
the acute yellow atrophy liver in which the process was of some
six weeks' duration. The fact that a smaller quantity of amino
acids was isolated from the chloroform necrosis liver may only
depend upon less success with the analysis, but it may mean that
there actually was a larger amount of free amino acids in the
acute yellow atrophy liver. If the latter explanation is correct
then we should be obliged to consider it as added evidence that
in acute yellow atrophy the free amino acids found in the liver and
secretions are not derived solely, or even chiefly, from the auto-
lyzing liver cells. Neuberg and Richter\textsuperscript{1} found larger amounts
of free amino acids in the blood in acute yellow atrophy than
could be accounted for by the destruction of liver tissue going on
at the time, and concluded that there must be some other source
for them, possibly the intestine. The rather large amount of
amino acids isolated from the liver in the case of acute yellow
atrophy mentioned above is in favor of the same idea, and the
smaller amount present in the more rapidly digesting liver with
chloroform necrosis might be looked upon as of similar signifi-
cance, were not the value of quantitative results obtained with
such materials and methods so very questionable.

Proteoses and peptones were also present, and apparently
much more abundantly in the chloroform necrosis liver, in the

\textsuperscript{1} Deutsch. med. Woch., xxx, p. 499, 1904.
extract from which were probably also considerable amounts of substances related to the polypeptids. The relative abundance of these substances intermediate between proteins and amino acids in the chloroform necrosis liver is presumably dependent upon the fact that here the autolysis was less advanced than in the acute yellow atrophy liver.

In both livers the presence of free xanthin and free hypoxanthin was established, and in about the same amount in each. Free guanin and adenin were absent from both livers, presumably because they are so readily converted into xanthin and hypoxanthin by the hepatic enzymes.

The composition of the coagulated and insoluble proteins of the liver left after thorough extraction with alcohol, ether, cold and hot water, is found to be quite the same in chloroform necrosis as in normal livers, as shown by the following table giving the results of analysis by Hausmann's method:

<table>
<thead>
<tr>
<th></th>
<th>Acute atrophy</th>
<th>Normal (anemic)</th>
<th>Normal (congested)</th>
<th>Chloroform necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amid nitrogen</td>
<td>5.5</td>
<td>3.7</td>
<td>4.8</td>
<td>3.9</td>
</tr>
<tr>
<td>Humus nitrogen</td>
<td>3.6</td>
<td>3.4</td>
<td>4.9</td>
<td>5.7</td>
</tr>
<tr>
<td>Diamino nitrogen</td>
<td>26.2</td>
<td>32.8</td>
<td>30.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Monamino nitrogen</td>
<td>64.8</td>
<td>60.3</td>
<td>60.2</td>
<td>60.3</td>
</tr>
</tbody>
</table>

In considering these figures it must be borne in mind that we are dealing with liver tissue from which not only the extractives, fats and lipoids have been removed, but also the greater part if not all the gelatigenous substances. Analysis of liver tissue by other observers in which these extractions have not been made are, it seems to me, of uncertain value, especially in pathological livers in which the great variation in fat as well as extractives and connective tissue can by themselves produce great alterations in the percentage figures, which are then incorrectly ascribed to the essential constituents of the liver cells themselves. Furthermore, it must be considered that the degree of regenerative proliferation and leucocytic invasion that is taking place in the liver will modify greatly the amount of purins and nucleoproteins. Taking these figures at their face value, however, they may be interpreted as meaning that the decrease in diamino nitrogen
which Wakeman\textsuperscript{1} found to be so striking in the livers of dogs poisoned with phosphorus, and which was found to a less extent in my case of acute yellow atrophy, was not exhibited by the liver showing extensive necrosis from chloroform. More recently Wakeman\textsuperscript{2} has analyzed a liver said to show acute yellow atrophy, in which there was found no decrease in the nitrogen of the bases. To draw any conclusions from these isolated observations, however, would not be warranted.

Determination of insoluble sulphur, phosphorus and iron in the extracted residues of these livers, diseased and normal, gave results that are difficult of interpretation. The following table gives the percentage amounts of these inorganic elements in the residues:

\begin{table}
\centering
\begin{tabular}{lcccc}
\hline
\hline
Sulphur & 0.82 & 0.75 & 0.77 & 0.79 \\
Phosphorus* & 0.90 & 0.27 & 0.21 & 0.90 \\
Iron & 1.22 & 0.2 & 0.4 & 0.5 \\
\hline
\end{tabular}
\begin{flushright}
* Average of four analyses of each specimen.
\end{flushright}
\end{table}

While the sulphur is practically constant in amount in all four specimens, in spite of the great structural changes in the two diseased livers, the insoluble phosphorus in each of the latter is increased to about four times the amount present in the normal livers. The increase in the phosphorus in the acute yellow atrophy may be readily explained as the result of the great proliferative activity exhibited by the cells of the stroma and bile ducts in areas where regeneration is taking place, which causes the presence of large numbers of new cells rich in nucleic acid. No such explanation is available for the increase of phosphorus in the chlofoform necrosis liver, however, for in this specimen there is not only no proliferation, but also by far the majority of hepatic nuclei have disappeared, making it doubly hard to account for this decided increase of insoluble phosphorus. It is barely possible that some of the lecithin phosphorus has been so fixed

\textsuperscript{1} Journ. of Exper. Med., vii, p. 292, 1905.
\textsuperscript{2} This Journal, iv, p. 119, 1908.
that it cannot be extracted from the cells, in view of the fact that
there has been some loss of lecithin in both livers, but it is not
probable that such a change could account for more than a small
fraction of the increase in phosphorus that was observed. In any
case these figures show that necrosis of the liver cells, with dis-
appearance of the majority of stainable nuclei, is not necessarily
associated with a decrease in the amount of insoluble phos-
phorus as would be expected.

In agreement with the histological picture, the strikingly large
amount of gelatin obtained from the acute yellow atrophy liver
as a result of the connective tissue proliferation present in this
condition was not found in the liver of chloroform necrosis.
While the acute yellow atrophy liver yielded 13.8 grams of gelai-
tin or 10.1 per cent of the dry, fat-free tissue, a normal liver
yielded but 3.2 per cent of the dry fat-free tissue as gelatin, and
in the chloroform necrosis but 1.5 per cent was gelatin.

There was no such increase of the proportion of water in the
liver as is found constantly in acute yellow atrophy, as shown in
the following table:

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>Fat</th>
<th>Fat-free dried substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal liver (Quincke)</td>
<td>76.1</td>
<td>3.0</td>
<td>20.9</td>
</tr>
<tr>
<td>Normal liver (Wells)</td>
<td>77.6</td>
<td>5.0</td>
<td>17.4</td>
</tr>
<tr>
<td>Acute atrophy (Perls)</td>
<td>81.6</td>
<td>8.7</td>
<td>9.7</td>
</tr>
<tr>
<td>&quot; (Perls)</td>
<td>76.0</td>
<td>7.6</td>
<td>15.5</td>
</tr>
<tr>
<td>&quot; (v. Starck)</td>
<td>80.5</td>
<td>4.2</td>
<td>15.5</td>
</tr>
<tr>
<td>&quot; (Taylor)</td>
<td>85.8</td>
<td>2.0</td>
<td>12.2</td>
</tr>
<tr>
<td>&quot; (Wakeman*)</td>
<td>79.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; (Wells)</td>
<td>83.8</td>
<td>2.5</td>
<td>13.7</td>
</tr>
<tr>
<td>&quot; (Voegtlin†)</td>
<td>78.0</td>
<td>6.6</td>
<td>15.4</td>
</tr>
<tr>
<td>Phosphorus poisoning (v. Starck)</td>
<td>60.0</td>
<td>29.8</td>
<td>10.0</td>
</tr>
<tr>
<td>Fatty degeneration (v. Starck)</td>
<td>64.0</td>
<td>25.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Chloroform necrosis (Wells)</td>
<td>72.4</td>
<td>8.8</td>
<td>18.8</td>
</tr>
</tbody>
</table>

* This Journal, iv, p. 119, 1908.
† Johns Hopkins Hospital Bull., xix, p. 50, 1908.

The chloroform necrosis liver stands between the typical acute
yellow atrophy liver and the ordinary fatty liver, according to its
analytic figures as well as according to its histology. That
Chloroform Necrosis

is, there has been some replacement of water by fats, but not so much replacement of protein by water as in acute atrophy. These figures emphasize the fact that in acute yellow atrophy there is no increase of fat in the liver, and that in chloroform necrosis the amount of fat is distinctly increased, although not so much is found chemically as might be expected from the microscopic findings.

The amount of lecithin and choleseterol in this liver is by no means so greatly altered from normal as was found to be the case in acute yellow atrophy, as shown by the following table:

<table>
<thead>
<tr>
<th></th>
<th>LECITHIN</th>
<th>CHOLESTEROL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal average</td>
<td>Normal congested</td>
</tr>
<tr>
<td>Per cent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>of fresh weight</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>of total dry weight</td>
<td>6.3</td>
<td>6.95</td>
</tr>
<tr>
<td>of dry fat-free material</td>
<td>7.7</td>
<td>8.0</td>
</tr>
<tr>
<td>of ether-soluble substances</td>
<td>35.3</td>
<td>28.0</td>
</tr>
<tr>
<td>entire liver</td>
<td>23.7</td>
<td>22.4</td>
</tr>
</tbody>
</table>

While the total amount of lecithin has decreased, this is only in proportion to the decrease in the total size and weight of the liver; this proportional decrease has gone on in spite of a relative increase in the amount of simple fat, showing the same lack of correlation between the lecithin and the neutral fat which has been observed by others who have determined the lecithin content of organs showing fatty degeneration. Evidently, therefore, the increase in the fat content of the liver in chloroform necrosis is due entirely to simple fats. The choleseterol, on the other hand, has apparently remained in about the normal amount, and has not decreased with the lecithin and proteins; this is quite what might be expected from what we know of the tendency of choleseterol that is liberated by degenerating cells to remain at the place where it is formed.
The only other analysis of a liver showing chloroform necrosis that has been recorded in the literature is the one published by A. E. Taylor. This case was very similar to the one described above, both clinically and anatomically. The liver weighed 1200 grams, was very soft, friable, and "putty-like," showing microscopically widespread degeneration of the liver cells. It contained over 200 grams of fat, and from the extracts were obtained 4 grams of leucin, 2.2 grams of tyrosin, and 2.3 grams of arginin nitrate. The other constituents of the liver were not determined.

These two analyses corroborate one another in showing the presence of free amino acids in amounts large enough for identification in the liver of chloroform necrosis. The amino acids are presumably derived from autolysis of the liver cells, although it is by no means certain that part of the free amino acids found in the liver may not have come from some other source.

**SUMMARY.**

In the necrosis of the liver which occasionally follows chloroform anesthesia there is a rapid autolysis of the liver cells, resulting in a loss of as much as one-third or more of the solids in three or four days, and indicated chemically by the presence of free amino-acids, purins, proteoses, peptones and polypeptids in the liver. Several of the amino-acids were present in quantities large enough to permit of their isolation and identification. Despite the loss of nearly all the nuclear structures of the liver the amount of insoluble phosphorus was found in the specimen examined to be increased, without alteration in the amount of insoluble sulphur. The distribution of the nitrogen as mono- and diamino acids in the insoluble coagulated liver proteins is not different from that of the proteins of the normal liver. There is a moderate degree of fatty metamorphosis, the microscopic and chemical findings corresponding in this respect; this increase in ether-extractive material being due to infiltration of simple fats, while there is a slight decrease in the lecithin and no alteration in the amount of cholesterin. There is less replacement of proteins by water and more fatty infiltration than in acute yellow atrophy.

1 Univ. of Calif. Publ. (Pathol.), i, 43, 1904.
THE CHEMISTRY OF THE LIVER IN CHLOROFORM NECROSIS (DELAYED CHLOROFORM POISONING)
H. Gideon Wells


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