

THE EFFECT OF ACUTE YELLOW ATROPHY ON METABOLISM AND ON THE COMPOSITION OF THE LIVER *

WILLIAM C. STADIE AND DONALD D. VAN SLYKE
NEW YORK

In the present paper we report chemical analyses of the blood and urine made in a case of acute yellow atrophy for three days before death, together with analysis of the liver removed at necropsy three hours after death. The study was directed especially toward the nitrogen metabolism and the acid-base balance of the patient. Besides other determinations, including those of urea, quantitative estimations of the amino-acid nitrogen in the urine, blood and liver were performed for the first time in such a case, so far as we can ascertain.

The results are of interest in two connections: (1) the pathologic metabolism of acute yellow atrophy, and (2) the rôle of the liver in the normal handling of nitrogenous products. The condition is one in which the liver as a functioning organ suffers almost complete, if not complete, destruction, without similar apparent injury to other organs; and the failure in yellow atrophy of definite steps in nitrogenous metabolism may be taken as an indication of the possibility that such steps are normally achieved either by the liver or with its aid.

REVIEW OF LITERATURE

Since Frerichs¹ (1861) established the presence of leucin and tyrosin in both the liver and urine in acute yellow atrophy, there has been basis for the belief that in this disease the liver proteins are digested to amino-acids, which are excreted as such in the urine. Schultzen and Reiss² (1869) are quoted as declaring that the presence of leucin and tyrosin in the urine indicates acute yellow atrophy as definitely as the presence of albumin indicates nephritis. In nearly all cases reported, however, the amino-acids have been identified only by microscopic examination of the crystals, and Röhmann³ (1888) with the utmost care was unable definitely to identify either leucin or tyrosin in the antemortem urine in a case terminating fatally. Röhmann explained the failure of these substances to appear in some cases of yellow atrophy by the hypothesis that amino-acids are excreted only when formed at a rate faster than they can be destroyed in the body. In his case an aromatic acid, probably p-hydroxy-phenyl lactic acid, formed by replacing the NH₂ group of tyrosin by an OH (Ellinger and Kotake,⁴ 1910), was obtained from the urine. Its presence indicated that the body could still deaminate tyrosin, even though the product of deamination failed of further combustion.

* From the Hospital of The Rockefeller Institute for Medical Research.

1. Frerichs: *Klinik der Leberkrankheiten*, Braunschweig, 1861.

2. Schultzen and Riess: *Hammarsten's Lehrb. d. Physiol. Chem.*, Ed. 8, Wiesbaden, 1914, p. 705.

3. Röhmann: *Berlin. klin. Wchnschr.* 861, 882, 1888.

4. Ellinger and Kotake: *J. Biol. Chem.* 5:129, 1908.

Neuberg⁵ (1909) claimed to have obtained from the blood serum in a fatal case tyrosin, leucin and lysin, the three amino-acids together amounting to about 6 gm. per liter of blood. This indicated the presence of so great an amount of amino-acids in the blood, that the investigators believed they must be due to autolysis of the muscles as well as the liver. At the same time, the nitrogen distribution in the urine was normal, 76 per cent. of the total nitrogen being in the form of urea. The results of the blood and urine analyses are both so unusual and so completely at variance with each other (i. e., tremendous amounts of amino-acids in the blood with no apparent disturbance of the nitrogen distribution in the urine) that without confirmation their acceptance as accurate in a quantitative sense is difficult.

Wells⁶ (1909) has published the most complete examination of the liver in yellow atrophy. He submitted the noncoagulable, water-soluble extract to Fischer's ester process for isolation of the mono amino-acids, and to Kossel's procedure for the diamino acids, and identified histidin, lysin, tyrosin, leucin, glycin, alanin, prolin, glutaminic acid and aspartic acid, the total amount being 8.67 gm. from the entire liver. To judge from the usual losses by the ester method, the amounts present were probably twice as great as those actually obtained. Wells proved beyond doubt that the liver in acute yellow atrophy does contain considerable amounts of the various amino-acids yielded by proteins on hydrolysis. That previously only leucin and tyrosin were, as a rule, found was obviously due to the fact that these are the amino-acids which are most easily obtained by crystallization.

Even the results of Wells, however, did not entirely solve the problem from a quantitative standpoint. Van Slyke and Meyer⁷ (1912), and Abel, Rowntree and Turner⁸ (1914) showed that amino-acids in considerable amounts are normal constituents of the blood and tissues. The conceptions of the changes in liver atrophy, and their significance in regard to liver function, therefore, became dependent on the question whether or not the amounts of amino acids formed and excreted in acute yellow atrophy vary significantly from the normal. In the present paper we are able to answer this question in the affirmative, and thereby confirm the older conceptions of the metabolism in this disease and, we believe, place them on a more complete experimental basis.

REPORT OF CASE

The patient, female, aged 29 years, had been well to within one day of admission to the hospital. Her first symptoms were nausea and vomiting, at first mild, but persistent and gradually increasing in intensity. She had no chills, fever or pain. There was slight headache. Bowels were slightly constipated. The vomitus consisted of greenish, bile-tinged fluid.

On admission to the hospital, physical examination showed temperature was 100, pulse 75, respirations 20. Sclerae showed a very slight but definite icterus. Heart and lungs were negative. Abdomen negative. Liver and spleen not felt. Skin negative; no jaundice.

Second day.—Patient continued vomiting all day; no pain. Temperature 99.6. Definite jaundice had developed over the entire body.

Third day.—Vomiting persistent and intractable. She was unable to retain even water by mouth. Jaundice much more marked.

Fourth day.—She was very weak and restless. No pain; no headache. All measures to allay the continuous vomiting were unsuccessful. Jaundice deeper. The abdomen, heart and lungs continued negative. Pulse 90, respirations 20, temperature 100.0 F.

5. Neuberg and Richter: Deutsch. med. Wchnschr. **30**:499, 1904.

6. Wells: Arch. Int. Med. **9**:628, 1907.

7. Van Slyke and Meyer: J. Biol. Chem. **16**:197, 1913.

8. Abel, Rowntree and Turner: J. Pharmacol. Exper. Therap. **5**:275, 1914.

Fifth day.—Patient vomited continually. Later she gradually sank into coma. No convulsions. Sclerae and skin of a deep greenish-yellow color. Pupils were widely dilated but equal, and reacted slightly to light. Fundi oculi showed nothing. Blood pressure, 155/70. Abdomen soft. Liver and spleen not felt. Liver dullness extended from fourth rib to just below the costal margin. All extremities quite rigid, no Kernig. Exaggerated knee jerks on both sides. Marked double ankle clonus. Definite Babinski on the left side, none on the right. No Chvostek or main d'accoucheur.

Lumbar puncture: No increased pressure. Fluid clear, cells 5 per c.c.; spinal fluid urea nitrogen, 0.073 gm. per liter. No bile (Smith's test).

In the late afternoon rigidity of the extremities and Babinski disappeared, and the knee jerks became less marked. No convulsions at any time or further evidence of tetany. No subcutaneous hemorrhages; at point of pressure, however, by the bed clothes there were small areas of ecchymosis. No diminution of the liver dullness made out during the day.

Sixth day.—Patient continued comatose, temperature and pulse rapidly rising. At 8 a. m., temperature was 108; pulse, 145; respirations, 44. No decrease of liver dullness made out.

Patient died at 8:33 a. m.

Wassermann completely anticomplementary.

Necropsy findings.—Macroscopic: Skin was deeply jaundiced, of a yellowish green color. Lungs were normal, except for a few scattered, hemorrhagic patches. Heart was negative. Abdomen: Omentum and the mesentery showed everywhere numerous hemorrhagic areas from 3 to 5 cm. in diameter. Stomach and intestines, gallbladder, pancreas, kidneys, suprarenals and uterus were normal. Ovaries contained few cysts. Spleen not enlarged; extremely dark in color. On section it was quite soft. Cut surface was a very dark purplish red. Malpighian bodies were not well made out.

Liver was quite markedly diminished in size. It was not found fallen away from the anterior abdominal wall. Its upper border corresponded with the fourth rib; its lower border to the costal margin. Weight, 1,000 gm. Capsule was smooth, and the parenchyma shining through appeared quite yellowish. Liver substance was extremely friable and cut with great ease. On section, the liver surface showed at the center of each lobule a dark reddish area surrounded by a lighter yellow zone. This appearance was quite uniform throughout the entire liver, except that there were occasional patches from 5 to 10 mm. in diameter of a darker red, and these areas were sunken below the general surface. Other areas, larger in size, were yellowish and studded with small reddish points representing the centers of the lobules. In these yellowish patches the centers of the lobules were smaller and less numerous than in other parts of the liver. There were no areas which suggested adenomatous hyperplasia.

Microscopic: Only the liver and kidney appeared abnormal under the microscope. The uterus, spleen, suprarenals, pancreas, lungs and bladder were examined, with negative results.

Kidney: There are scattered throughout the cortex small areas of hemorrhage between the tubules and in Bowman's capsule. Glomeruli appear quite normal, but the tubules show here and there a moderate degree of fatty infiltration and cloudy swelling. Otherwise the parenchyma was normal.

Liver: Universally, the histologic picture is characterized by an extensive destruction. Throughout most of the liver, cells are represented by only faint outlines with completely or partially disintegrated nuclei. In some areas the liver cells are represented only by a structureless debris, while in others the outlines of the cell bodies can be faintly made out still in lobular arrangement. There is an extensive fatty infiltration. These changes involve practically all of the lobule, but are most extensive at the periphery. At the center of each lobule the bile duct epithelium is practically intact, and here may be found quite generally very few liver cells in a better state of preservation. Some of the

bile ducts are surrounded by well preserved liver cells arranged in strands. Scattered throughout, mainly at the periphery of the lobules, are areas of blood extravasation.

Analytical Methods.—Urine Analyses: Chlorids were titrated by the Volhard method. Urea was determined by Van Slyke and Cullen's⁹ (1917) modification of Marshall's urease method, using Squibb's urease prepared from Jack beans, ammonia by the aeration technic described by Van Slyke and Cullen. The total amino-acid nitrogen was determined as described by Levene and Van Slyke¹⁰ (1912), the creatinin by Folin's¹¹ (1905) method, and the uric acid by Folin and Shaffer's¹² (1901) method. Total acetone bodies were estimated by Van Slyke's¹³ (1917) gravimetric method. The titratable acid was determined according to Folin¹⁴ (1903) by titration with phenolphthalein in the presence of potassium oxalate.

Blood Analyses: The urea was determined as described by Van Slyke and Cullen¹⁵ (1914), the amino-acid nitrogen by Van Slyke's nitrous acid method as described by Whipple and Van Slyke¹⁶ (1918). The plasma bicarbonate was estimated by the carbon dioxid capacity method of Van Slyke and Cullen⁹ (1917).

Liver Analysis.—Water content: Three samples of from 2 to 3 gm. weight each from different lobes of the liver were dried in glass dishes at 110 C. to constant weight. The results were 71.6, 71.6 and 71.9 per cent. water.

Total Nitrogen.—The dried samples used for water determination were Kjeldahlled. The results were 8.0, 8.36 and 9.0 per cent. of nitrogen calculated on the dried samples, and 2.28, 2.34 and 2.51 per cent. calculated on the fresh samples, an average of 2.38 per cent. of the fresh substance, or 8.45 per cent. of the dried. Using the approximate factor 6.25 for conversion of nitrogen figure into protein figure, this would indicate that 5.3 per cent. of the dry substance was protein.

Fat.—Three samples of from 6 to 12 gm. from different lobes were let stand over night with 100 c.c. of 95 per cent. alcohol each. The alcohol was poured off, the liver samples were minced, and were extracted with ether in a Soxhlet apparatus. The alcohol extracts were concentrated nearly to dryness, taken up with ether, and combined with the ether extracts. The latter were concentrated to dryness, and taken up with petroleum ether in weighed flasks. After the petroleum ether had been mostly removed on the water bath, the flasks were dried in an evacuated desiccator over sulfuric acid to constant weight. The results were 12.7, 13.8 and 14.0 per cent. of fat, an average of 13.5 per cent. of the fresh liver, or 47.7 per cent. of the dry substance.

Extraction of Nonprotein Nitrogen.—Three samples, totalling 303 gm., were cut with scissors into pieces which were dropped into 1.5 liters of boiling water slightly acidified with acetic acid. After the tissues were coagulated, the water was decanted, the pieces were minced, and the extraction with hot water was repeated three times. The decanted extracts were filtered through glass wool, then mixed with 50 gm. kaolin, and filtered through paper with suction, the residue being washed with hot water. The clear amber filtrate was boiled down in an enamelled ware vessel to about 500 c.c., and poured into three volumes of absolute alcohol to complete the removal of proteins. The next day the pre-

9. Van Slyke and Cullen: J. Biol. Chem. **30**:289, 1917. Van Slyke, Stillman and Cullen: J. Biol. Chem. **30**:401, 405, 1917.

10. Levene and Van Slyke: J. Biol. Chem. **12**:301, 1912; **16**:125, 1913.

11. Folin: Am. J. Physiol. **13**:48, 1905.

12. Folin and Shaffer: Ztschr. physiol. Chem. **32**:552, 1901.

13. Van Slyke: J. Biol. Chem. **32**:455, 495, 499, 1917.

14. Folin: Am. J. Physiol. **9**:265, 1903.

15. Van Slyke and Cullen: J. Biol. Chem. **19**:211, 1914.

16. Whipple and Van Slyke: J. Exper. M. **28**:213, 1918.

cipitate which formed was filtered off, and washed with 80 per cent. alcohol. The filtrate was concentrated under reduced pressure and brought to a volume of 150 c.c.

Free Amino Nitrogen.—Two c.c. portions of extract were used for the determination of amino nitrogen by the nitrous acid method of Van Slyke¹⁷ (1912), the reaction being continued for 3.5 minutes at 22 C. The volume of gas yielded in three determinations was 9.55, 9.50 and 9.65 c.c. at 22 C., 761 mm., the average, 9.57 c.c., indicating 5.40 mg. of amino nitrogen, or 134 mg. per 100 gm. of fresh liver.

Peptid Nitrogen.—Five c.c. samples of the extract were mixed in test tubes with 5 c.c. portions of concentrated hydrochloric acid and heated for twenty-four hours at 100 C. The hydrochloric acid driven off on the water bath, and the residue diluted to 20 c.c., of which 2 c.c. portions were used for the amino nitrogen determination. The determinations yielded 3.72, 3.67 and 3.63, average 3.67 c.c. of nitrogen at 22 C., 759 mm., indicating 206 mg. of amino nitrogen per 100 gm. of fresh tissue. Subtracting the 134 mg. present as per amino nitrogen before hydrolysis, leaves 72 mg. as peptid nitrogen, freed by hydrolysis.

Urea and Ammonia Nitrogen.—These determinations were made as in urine (Van Slyke and Cullen,⁹ 1914), 5 c.c. of extract being used for urea and the same amount for ammonia. The results of triplicate determinations indicated 14.8 mg. of urea nitrogen and 34.5 mg. of ammonia nitrogen per 100 gm. of fresh tissue.

Creatinin.—Folin's method was used as for urine¹¹ (1905), except that the final dilution was less. Five c.c. of the extract were mixed with 15 c.c. of saturated picric acid solution, and 5 c.c. of 10 per cent. sodium hydroxid. At the end of five minutes the solution was diluted to 50 c.c. and read against a dichromate standard. The result, which may be somewhat high because of the relative concentration of sodium picrate, indicated 3.3 mg. of creatinin nitrogen per 100 gm. of fresh tissue.

Creatin Plus Creatinin.—Five c.c. of extract were heated at 100 C. with 5 c.c. of N hydrochloric acid for three hours. The hydrochloric acid was neutralized with concentrated sodium hydroxid, and 5 c.c. of 10 per cent. sodium hydroxid in excess were added with 15 c.c. of saturated picric acid. After five minutes the solution was diluted to 100 c.c. and read against a chromate standard. The reading indicated 17.6 mg. of creatin plus creatinin nitrogen, or 14.3 mg. of creatin nitrogen per 100 gm. of fresh liver tissue.

The technic used is similar to that employed by Janney and Blatherwick¹⁸ (1915). The difference is that the above authors used aluminum hydroxid to adsorb uncoagulated protein (presumably gelatin), while we used kaolin. Creatinin under proper conditions is adsorbed by kaolin (Greenwald,¹⁹ 1918), but we found by control tests that the kaolin preparation used by us in the amounts employed did not remove sufficient creatinin to significantly affect results such as were obtained.

DISCUSSION OF RESULTS

Excretion.—The comatose condition of the patient made collection of complete twenty-four hour specimens impossible, so that conclusions have to be drawn largely on the nitrogen distribution. The abnormalities in the latter during the last two days before death are the

17. Van Slyke: J. Biol. Chem., 1912.

18. Janney and Blatherwick: J. Biol. Chem. **21**:567, 1915.

19. Greenwald: J. Biol. Chem. **34**:103, 1918.

high ammonia ratio, and even more strikingly, the high amino acid nitrogen and the low proportion of urea nitrogen (Table 1). All three are explainable on the assumption that the liver had lost a part of its ability to transform amino-acid nitrogen into urea nitrogen, one portion being excreted in the form of unchanged amino-acids, and another in the form of ammonia.

TABLE 1.—SALT AND NITROGEN EXCRETION

Date, 1919	Output, C.c.†	Specific Gravity	Bile	NaCl, Gm. per L.	Total N, Gm. per L.		Urea N		Amino-Acid N		NH ₃ N		Creatinin N		Uric Acid N		Undetermined N	
							Gm. per L.	Per Cent. of Total N	Gm. per L.	Per Cent. of Total N	Gm. per L.	Per Cent. of Total N	Gm. per L.	Per Cent. of Total N	Gm. per L.	Per Cent. of Total N	Gm. per L.	Per Cent. of Total N
Feb. 28	1,070	1.013	+	3.11	0.392
Mar. 1	250+	1.022	++	1.04	13.90	7.04	47.2	0.61	4.1	0.862	16.2	0.65	4.4	0.185	1.2	4.65	32.7
Mar. 2	411+	1.032	++	0.28	10.94	5.68	51.9	1.75	16.0	1.880	17.2	0.54	2.0	0.128	1.7	0.96	8.1
Mar. 3	130+	1.031	+	1.38	16.64	7.75	46.6	2.22	13.3	1.925	11.6	0.49	2.9	0.417	2.5	3.82	23.0

* Tyrosin crystals were obtained from the combined specimens of urine not used for other analyses.

† Part of urine was lost on March 1, 2 and 3 because of patient's comatose condition.

The amount of nitrogen eliminated cannot be stated accurately because of the lack of twenty-four hour samples of urine. The probable minimum can, however, be approximately estimated from the creatinin. The creatinin nitrogen excretion of an individual of the patient's size (about 50 kg.) would normally be about 4.40 gm. The twenty-four hour excretions of nitrogen and of ammonia plus titratable acid, estimated in this manner, are given in Table 2. In an acute illness

TABLE 2.—APPROXIMATE TWENTY-FOUR HOUR NITROGEN AND ACID EXCRETIONS ESTIMATED FROM CREATININ OUTPUT

Date	A Creatinin N per L.	B Proportion of 24-Hr. Excretion in 1 Liter of Urine Sample Obtained, Estimated from Creatinin,	Estimated Excretion per 24 Hours = Excretion per Liter Urine	
			B	
	Mg.	A 400	Titratable Acid + NH ₃ , C.c. 0.1 N	Total N, Gm.
March 1	650	1.62	670	8.6
March 2	540	1.35	1,530	8.1
March 3	490	1.22	1,530	13.6

with pathologic tissue loss, the creatinin output might be increased. Even if it remained only normal at 0.40 gm. per day, however, the total nitrogen excretion on March 1, 2 and 3 would be calculated at 8.6, 8.1 and 13.6 gm., respectively, assuming that the ratio of twenty-four hour total nitrogen to the observed nitrogen per liter is as 0.40 to the observed creatinin nitrogen per liter. The first two figures

might be considered normal for a fasting individual (Lusk,²⁰ 1917) but the last is certainly high and corresponds with the incidence of fever.

Small amounts of acetone bodies were excreted, but in quantities not sufficient to be of significance for the acid-base balance of the body.

The low salt output was presumably due to lack of salt intake, rather than kidney retention.

TABLE 3.—ACID EXCRETION AND ALKALINE RESERVE

Date, 1919	Excretion per Liter of Urine			Total Acetone Bodies, C.c. 0.1 N	Blood Plasma Bicarbonate CO ₂ , Volume per Cent.
	NH ₃ , C.c. 0.1 N	Titrateable Acid, C.c. 0.1 N	Acid + NH ₃ , C.c. 0.1 N		
February 28.....	280	92.4	372		
March 1.....	616	460	1,076	64	
March 2.....	1,343	719	2,062	180	96.5
March 3.....	1,375	496	1,871	...	65.4
March 4.....	49.0

Blood Analyses.—The analyses of the blood, like those of the urine, indicate a loss of diaminizing function in the body (Table 4). The amino nitrogen contents on the last two days are respectively two and three times as great as the 7 to 8 mg. per 100 c.c. which represent the average normal (Bock,²¹ 1917). The apparent explanation is that the liver had lost the ability to transform amino-acids into urea, an ability which has been demonstrated in the normal liver by the perfusion experiments of Salaskin²² (1898) recently confirmed by Jansen (1915), and by the physiological experiments of Van Slyke, Meyer, Cullen and McLean (Van Slyke,²³ 1917).

TABLE 4.—UREA AND AMINO-ACID NITROGEN IN THE BLOOD

Date, 1919	Urea N, Gm. per L.	Amino-Acid N, Gm. per L.
February 28.....	—	—
March 1.....	—	—
March 2.....	0.123	0.140
March 3.....	0.088	0.173
March 4.....	0.159	0.263

The source of the amino-acids in the blood in our case was undoubtedly autolyzed tissue protein. The patient, because of the nausea, retained practically no food; the considerable amounts of nitrogen excreted (8 gm. or more per day) must have come from tissues autolyzed under the influence of the fasting and intoxication attending the disease.

20. Lusk: Science of Nutrition, Philadelphia, 1917.

21. Bock, J. C.: J. Biol. Chem. **29**:191, 1917.

22. Salaskin: Ztschr. f. physiol. Chem. **25**:128, 1898.

23. Van Slyke: Arch. Int. Med. **19**:56 (Jan.) 1917.

The blood urea remains within normal limits. Taken with the continued output of urea in the urine, it shows that the urea-forming function of the body, although diminished, was only partially lost, even on the day before death.

The blood plasma bicarbonate determinations (Table 3) gave a peculiar result on the first observation four days before death. The carbon dioxid capacity of 96.5 per cent. would indicate an abnormally high alkaline reserve. Unfortunately, the observation was not repeated on that day, and we are uncertain whether an increased blood bicarbonate actually existed, or whether there was an error in the determination. The carbon dioxid estimations were done in duplicate, but the carbon dioxid capacity might have been raised in vitro by contamination of the centrifuge tube or equilibrating funnel with alkali. That this may have occurred is made more probable by the fact that the excretion of titratable acid and ammonia on this day was above the usual normal limits.

On the last two days before death, there was a high excretion of titratable acid and ammonia, about double the normal, and on the day before death the plasma bicarbonate fell slightly below normal (49 per cent. of carbon dioxid). These facts indicate a definitely accelerated formation of acids in the organism, but neither the acid excretion nor the plasma bicarbonate indicates an acidosis sufficient to have in itself a definite effect on the patient's condition. (Compare Van Slyke,²⁴ 1917 [b]). In connection with the increased acid production it is of interest to note that Röhmann³ (1888) obtained unusual aromatic acids and considerable amounts of sarcolactic acid from the urine in a case of yellow atrophy.

Composition of the Liver.—The most striking change in the liver was the marked loss of substance, which is usually noted in acute atrophy (Wells,⁶ 1907). The liver weighed only 1,000 gm. instead of the 1,800 gm., which, for an individual of the patient's size, is normal, according to Frerichs¹ (1861).

The water content of 71.7 per cent. (Table 5) shows no increase over that of the normal liver analyzed by Wells, in fact is less than the latter (76.1 per cent.). In this respect this case differs from most of those in the literature, as the water content usually has been found high in acute yellow atrophy.

The fat content of 13.5 per cent. is decidedly high, the normal being quoted by Wells⁶ (1907) as about 3 per cent. The livers of acute atrophy of which analyses are reported in the literature (Wells, 1907, 1908) have been found to vary between from 2.0 and 8.7 per cent. in fat content, while those of phosphorus poisoning and fatty degenera-

24. Van Slyke: J. Biol. Chem. **32**:(b)455, 1917.

tion show from 25 to 30 per cent. In our case of acute atrophy the difference from the high fat values of phosphorus poisoning and fatty degeneration is much less striking than in any of the instances reported. It may be that the unusually rapid progress, with death a few days after the first symptom, prevented the complete combustion of the fat transported to or formed in the liver. The high fat content in our case is the cause of the normally high content of total solids noted in the preceding paragraph; fat increase makes up for the protein loss.

TABLE 5.—DRY MATTER, FAT AND TOTAL NITROGEN IN LIVER

	Per Cent.	Normal Values, per Cent.*	
Water.....	71.7	77.8	76.1
Dry matter.....	28.3	19.4	20.9
Dry matter as fat.....	13.5	5.0	3.0
Dry matter as protein calculated as $N \times 6.25$	14.9		

* From Wells (1908).

TABLE 6.—NITROGEN DISTRIBUTION IN LIVER

	Per 100 Grams Liver, Gm.	Proportion of Total N, per Cent.
A. Total N		
Total N.....	2.380	100.0
Nonprotein N.....	0.315	13.2
Protein N (by difference).....	2.065	86.8
B. Nonprotein N	Per 100 Grams Fresh Liver, Gm.	Proportion of Total Nonprotein N, per Cent.
Total nonprotein N.....	0.3130	100.0
Urea N.....	0.0148	4.76
Ammonia N.....	0.0345	10.95
Amino N.....	0.1340	42.50
Peptid bound N.....	0.0720	22.80
Creatin N.....	0.0143	4.54
Creatinin N.....	0.0033	1.05
Undetermined N.....	0.0421	13.40

The "dry matter not fat," which, as indicated by its nitrogen content, was nearly all protein, was much decreased, as it has been in all the reported cases of liver atrophy, whether due to poisoning or to acute disease. In our case the content of solids not fat, or approximately the protein, is 14.9 per cent. instead of the normal 20 per cent. of fresh liver. During the few days of the disease, the liver, therefore, in losing about 45 per cent. of its weight lost about 60 per cent. of the protein substance.

In the nitrogen distribution in the liver (Table 6) the main point of interest is the high content of amino-acid and peptid nitrogen. Compared with livers of normal dogs, the amino nitrogen, 0.134 per

cent. of the fresh tissue, is about three times as great (Van Slyke and Meyer,⁷ 1913). The observed amino nitrogen content indicates the presence of approximately 13 or 14 gm. of free amino-acids in the 1,000 gm. of liver tissue.

RÔLE OF THE LIVER IN NITROGENOUS METABOLISM

The apparent significance of these figures, along with those for the blood and urine, is that the liver protein was autolyzed at a rapid rate to amino-acids and peptids, chiefly the former, and that amino-acids produced by the abnormal autolysis of the liver and the normal autolysis of the rest of the body (other organs were not macroscopically or microscopically degenerated) were turned into urea to the extent of only about 60 per cent. instead of from 85 to 95 per cent. The excreted amino-acids formed as high as 16 per cent. of the total urinary nitrogen instead of the normal 2 per cent.

There was no indication at any time of a tendency for the urea nitrogen output to fall much below 50 per cent. of the total nitrogen. In view of the apparently complete degeneration of the liver cells the data consequently suggest the probability that, although deamination and urea synthesis without the liver are incomplete, nevertheless they can occur to such an extent that the greater part of the nitrogen normally excreted as urea is still in this form. Fiske and Sumner²⁵ (1914) found that dogs could form urea from injected glyocoll even after the abdominal viscera had been excluded from the circulation. It is uncertain whether a considerable part of the total urea synthesis normally occurs in parts other than the liver, or whether the process is taken up elsewhere only when the liver fails.

It appears that acute yellow atrophy, with the possible exception of fatal phosphorus poisoning, is the only clinical condition in which unusual amounts of amino-acids have been demonstrated to be formed and excreted as such, without change to urea or ammonia.

The most rapid autolysis unaccompanied by liver degeneration does not apparently result in the excretion of amino-acids. For example, we have not found the amino nitrogen of the blood or urine increased during resolution in pneumonia, although this process represents one of the most striking examples known of rapid autolysis *in vivo*. Nor was any abnormal increase in the blood amino nitrogen observed by Whipple and Van Slyke¹⁶ (1918) in dogs that were intoxicated by proteose or by intestinal obstruction in such a manner that the blood urea was raised by autolysis of body protein in a few hours to form two to five times the fasting level. Such urea changes indicate an amount of tissue digestion seldom met in disease, and not approximated in the apparently localized autolysis of yellow atrophy.

25. Fiske and Sumner: *J. Biol. Chem.* **18**:285, 1914.

In order that amino-acids in exceptional amounts shall escape deamination and appear in abnormal amounts in the urine, it appears necessary not only that the liver shall be injured, but that its loss of function shall be profound, and, as stated above, such loss of function apparently has been observed in man with certainty only in acute atrophy. We have been unable to confirm the statement of Labbé and Bith²⁶ (1911) that it occurs in diabetes (Van Slyke and Stillman, unpublished results). It does not occur in the toxemias of pregnancy, despite the marked degenerative changes that occur in the liver (Losee and Van Slyke,²⁷ 1917). Levene and Van Slyke¹⁰ (1912) failed to observe it in two cases of cirrhosis. Chesney, Marshall, and Rowntree²⁸ (1914) report somewhat increased amino-acid nitrogen in both blood and urine in more than 50 per cent. of a series of cases with apparent liver insufficiency from various causes. However, the upper limits for amino nitrogen which they assumed were so low (1.5 per cent. of the urine nitrogen, and 3 mg. per 100 c.c. of blood) that they are readily exceeded in normal individuals (Levene and Van Slyke,¹⁰ 1912, 1913; Bock,²¹ 1917; Cullen, Ellis and Van Slyke,²⁹ 1915). The only case in their series with definitely high blood amino nitrogen was one of arsphenamin poisoning, with 12.4 mg. per 100 c.c.

In pathologic liver conditions caused experimentally, it appears to be likewise unusual, unless liver degeneration is almost complete, to find increased amino acid content in blood or urine. Whipple and Van Slyke¹⁶ (1918) failed to observe it in the blood of dogs with Eck fistulas (unpublished results). Levene and Van Slyke¹⁰ (1912) did not find it in the urines of dogs in which Opie and Dochez had caused liver degeneration by phosphorus and chloroform poisoning. Only Marshall and Rowntree³⁰ (1915) observed an increase in the blood amino nitrogen (up to 21 mg. per 100 c.c.) shortly before death in dogs poisoned with phosphorus.

In pathologic conditions not involving the liver, Bock²¹ (1917) found very markedly increased blood amino nitrogen only in some cases of nephritis, in which amino acids are retained along with other urinary constituents.

SUMMARY

An increased excretion of ammonia and titratable acids was observed in the last days of illness, and a fall of plasma bicarbonate to slightly below normal on the day before death. Even at this time, however, the deviations from the normal were too small to indicate that acid intoxication was a significant factor in the condition.

26. Labbé and Bith: *Progrés méd.* **27**:581, 1911.

27. Losee and Van Slyke: *Am. J. Med. Sc.* **153**:94, 1917.

28. Chesney, Marshall and Rowntree: *J. A. M. A.* **63**:1533, 1914.

29. Cullen, Ellis and Van Slyke: *J. A. M. A.* **64**:126, 1915.

30. Marshall and Rowntree: *J. Exper. M.* **22**:333, 1915.

The results afford confirmation of a quantitative character for the belief that amino-acids are formed by autolysis in the atrophying liver, and circulate and are excreted as such in unusual amounts.

The excretion of amino-acids did not appear to be due to increase in their rate of formation, for the total protein katabolism was not abnormally or even unusually rapid. The excretion appeared due rather to loss of power to deaminate amino-acids at even an ordinary rate.

A review of the known instances of rapid intra vitam autolyses not involving the liver indicates that tissue waste alone does not cause increase of amino acids in the blood and urine. In conditions of less profound liver injury (eclampsia) a marked decrease may occur in the proportion of urinary nitrogen present as urea and ammonia, which are partly replaced by as yet unidentified nitrogenous substances (undetermined nitrogen) ; but excretion of definitely abnormal amounts of amino acids appears to result only when the destruction of the liver cells is almost complete.

These observations support the view that in the deamination of amino acids and the synthesis of urea the liver bears a part which cannot be entirely assumed by the rest of the body.