EXPERIMENTAL LIVER NECROSIS; III. NITROGENOUS METABOLISM.[1]

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We present here the results of experiments carried out upon dogs in which the general state of the nitrogenous metabolism of the animals was studied before and after the injection of hæmotoxic immune sera. As has been stated elsewhere[2] the important organic lesion produced by such sera is a hepatic necrosis either focal or diffuse, according to the amount and strength of the serum employed. Such an experimental lesion seemed peculiarly adapted to the study of the function of the liver in general metabolism and of certain peculiar metabolic derangements which have an analogy to those noted in eclampsia, chloroform poisoning, vomiting of pregnancy and acute yellow atrophy. It must be clearly borne in mind, however, that this experimental method of producing hepatic necrosis allows of the infliction upon the liver of a single injury, in point of time, which if not followed by death is rapidly repaired and which is almost without other disturbing factors. Hence metabolic variations may be transient and last but one or two days, and for this reason the resulting disturbance is not comparable to that produced by a continually acting cause with progressive lesion.

The experiments were carried out upon dogs kept in the usual

[1] Conducted under grants from the Rockefeller Institute for Medical Research. Read before the American Association of Pathologists and Bacteriologists, Washington, May 7, 1907. Received for publication July 2, 1907.

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

well-ventilated metabolism cages and fed upon a purin-free diet of casein, cracker dust and lard. The amounts of the three dietary constituents varied for the different animals. They were calorifically sufficient and so regulated that the animal finally came into nitrogen equilibrium. The dogs were catheterized at the end of each twenty-four hour period and the urine thus obtained, added to that voided naturally. This was at all times carefully preserved from changes of any kind until the analytic work was completed. To the total twenty-four elimination was added distilled water to make the volume up to 800 cubic centimeters. When hæmoglobinuria or albuminuria caused the appearance of proteid in the urine this was removed by heat and acetic acid, the coagulum being thoroughly boiled out with water and the washings added to the urine.

Upon these twenty-four hour samples the following determinations were made: total nitrogen by the Kjeldahl-Gunning method; ammonia by the Shaffer method; urea by the Mörner-Sjoquist method; uric acid by the Hopkins-Folin method and creatinin by Folin's colorimetric adaptation of Weyl's qualitative test. The difference between the sum of these various factors and the total nitrogen is given as rest or undetermined nitrogen. Kynurenic acid was also looked for qualitatively in order to obtain evidence as to variations in the output of this substance.

The general procedure in these experiments was to place the animal upon nitrogen equilibrium and to conduct control determinations for a period of three days, after which the animal was injected with either normal or toxic sera and the experiment allowed to continue until death ensued or the metabolism had regained its normal level as shown by the control period. It is obvious that in experiments of this character it becomes extremely difficult, if not impossible, owing to variance in the toxicity of the sera and the susceptibility of the animals, to regulate the severity of the lesion, especially as the degree of the latter can be determined only by post-mortem examination. This fact prohibits the production in any two experiments, no matter how carefully planned, of absolutely comparable pathological conditions. This difficulty is brought out by the fact that although each new lot of serum was tested for lethal action on dogs of approximately the same weight and conditions as

those prepared for the experiment, only six out of twelve of the latter survived the first twenty-four period. These six, with a control dog receiving normal serum, constitute the experiments on which this communication is based.

These may be divided into four groups: one injected with normal serum in which no histological change took place; one with a weak toxic serum which caused no necrosis, but an extensive granular and vacuolar degeneration; four with toxic sera causing more or less extensive focal necroses and one with toxic serum producing diffuse necrosis.

TABLE I.

(Dog 18, normal serum.)

Date.	Total Nitrogen.	Urea.*	Ammonia.	Uric Acid.	Creatinin.	Undetermined Nitrogen.	Notes.
Nov. 10	6.51	4.99 / 76.8	0.556 / 8.5	0.018 / 0.28	0.677 / 10.4	0.269 / 4.0	
11	6.09	4.81 / 78.9	0.492 / 8.1	0.013 / 0.21	0.633 / 10.4	0.142 / 2.4	
12	6.52	5.25 / 80.5	0.493 / 7.6	0.020 / 0.30	0.607 / 9.3	0.150 / 2.3	Injected 12 M. Dose 1: 738. Vomited.
13	6.60	5.06 / 76.6	0.510 / 7.7	0.017 / 0.26	0.576 / 8.7	0.437 / 6.7	
14	6.06	4.72 / 77.8	0.482 / 8.0	0.021 / 0.34	0.539 / 8.9	0.298 / 5.0	

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

Table I shows the result of the injection of normal serum. With this may be compared also Tables VI and VII, in which it is seen that the toxic serum was preceded by an injection of normal serum. In two of these the dose[3] of the normal serum was greater than that of any injection of toxic serum. It will be seen that it was practically without effect although in Dog 48 (Table VI) a slight increase in the output of total nitrogen was evident. A consideration of the nitrogen partition indicates, however, that this increase is mainly at the expense of the rest-nitrogen and is to be explained by traces of foreign proteid of the rabbit's serum injected, too small to be removed by the ordinary methods. We possess, there-

[3] The figures representing dosage, for example 1:600, indicate that the dose was in the proportion of one cubic centimeter of serum to 600 grams of body weight.

fore, a series of controls upon which to base our conclusions concerning the effect of the toxic sera.

Table II gives the figures obtained as the result of injecting a weak serum,[4] which caused extensive hepatic degeneration of the granular and vacuolar type but no necrosis.

TABLE II.

(Dog 12, Degeneration, No Necrosis.)

Date.	Total Nitrogen.	Urea.*	Ammonia.	Uric Acid.	Creatinin.	Undetermined Nitrogen.	Notes.
Oct. 15	4.33	3.67 84.5	0.157 3.6	0.006 0.14	0.302 7.0	0.195 4.76	
16	3.77	3.24 85.7	0.131 3.5	0.005 0.13	0.285 7.6	0.109 3.07	
17	3.76	3.29 87.3	0.120 3.1	0.004 0.13	0.284 7.6	0.062 1.87	
18	3.16	2.72 87.1	0.104 3.3	0.004 0.13	0.241 7.6	0.060 1.87	
19	4.23	3.56 84.1	0.090 2.1	0.004 0.09	0.315 75.	0.261 6.21	Injected 4.30 P.M; weak toxic serum; dose 1:715.
20	3.37	2.66 78.9	0.165 4.9	0.003 0.09	0.288 8.5	0.254 7.66	
21	3.19	2.69 84.3	0.161 5.1	0.006 0.19	0.268 8.4	0.065 2.01	
22	3.02	2.60 86.1	0.122 4.0	0.002 0.06	0 260 8.7	0.036 1.14	Killed.

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

It is seen that a slight but transient rise in the total nitrogen occurred. A small part of this increase is attributable to the foreign proteid injected, with a corresponding rise in the undetermined nitrogen, both in absolute and percentage amounts. The absolute amount of urea nitrogen increased during the first twenty-four hours after injection but not in sufficient quantities to keep pace with the increase in total nitrogen, hence a percentage decrease occurred. The following day both the percentage and absolute amounts diminished markedly to be followed on the succeeding days by return to the normal percentage of urea output. The absolute quantity remained low since the total nitrogen did not return to nor-

[4] The serum of a rabbit which was not bled until six weeks after immunization against dog's blood. Sera obtained so long after injection frequently show diminished hæmagglutinative and hæmolytic power.

mal. The ammonia output during the twenty-four hours succeeding the injection suffered a decided diminution both in percentage and absolute figures. This decrease was exceedingly transient, since on the succeeding days the reverse occurred and both the absolute and percentage figures went much above the normal. The uric acid and creatinin showed no change but the rest-nitrogen increased considerably on the first and second days following the injection.

The reaction[6] for kynurenic acid was positive on the day subsequent to the injection and passed off gradually. This would indicate an increase in proteid destruction.[7]

Of most importance is the diminution in the percentage of the total nitrogen eliminated as urea associated with a somewhat corre-

TABLE III.

(Dog 25, Focal Necroses.)

Date.	Total Nitrogen.	Urea.*		Ammonia.		Uric Acid.[5]	Creatinin.		Undetermined Nitrogen.		Notes.
Dec. 2	4.77	3.55	76.5	0.443	9.1		0.405	8.4	0.372	6.0	
3	5.42	4.39	81.0	0.507	9.4		0.362	6.7	0.161	3.3	
4	5.24	4.11	78.4	0.440	8.4		0.298	5.7	0.392	7.5	
5	4.97	3.86	77.6	0.421	8.5		0.335	6.7	0.354	7.2	
6	6.02	4.68	77.4	0.496	8.2		0.362	6.0	0.482	8.4	Injection 2:30 P.M. toxic serum dose 1 : 1000. Vomited.
7	5.85	4.77	81.5	0.443	7.6		0.351	6.0	0.286	4.9	Hburia.
8	5.92	4.95	83.6	0.313	5.3		0.323	5.3	0.334	5.8	Hburia.
9	9.33	7.66	82.1	0.587	6.3		0.430	4.6	0.653	7.0	Hburia.
10	6.46	5.40	83.5	0.376	5.8		0.360	5.6	0.324	5.1	
11	8.15	7.16	87.8	0.404	4.9		0.368	4.5	0.218	2.8	
12	8.22	7.07	86.0	0.461	5.6		0.366	4.5	0.323	3.9	No food taken; killed.

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

[5] Amounts so small that they were not calculated.

[6] Treatment of the urine with bromine water.

[7] Mendel, L. B. and Jackson, H. C., On the Excretion of Kynurenic Acid, *Amer. Jour. of Physiol.*, 1898, ii, 1.

TABLE IV.

(*Dog 43, Focal Necroses.*)

Date.	Total Nitrogen.	Urea.*		Ammonia.		Uric Acid.		Creatinin.		Undetermined Nitrogen.		Notes.
Feb. 2	7.74	6.31	81.5	0.444	5.7	0.018	0.23	0.417	5.4	0.551	7.2	
3												Fæces mixed with urine.
4	6.95	5.50	79.1	0.406	5.8	0.013	0.17	0.338	4.9	0.693	10.0	
5	6.45	5.37	83.2	0.335	5.2	0.015	0.23	0.280	4.3	0.450	7.1	Injected 10:30 A.M. toxic serum dose 1 : 1738. Vomited.
6	9.91	8.48	85.5	0.478	4.8	0.073	0.73	0.343	3.5	0.836	8.5	Injected 3 P.M. toxic serum; dose 1 : 1200.
7	10.56	8.88	84.1	0.578	5.5	0.045	0.42	0.405	3.8	0.652	6.2	
8	7.65	6.31	82.5	0.479	6.2	0.009	0.12	0.386	5.0	0.466	6.2	
9												Urine lost.
10	4.85	3.84	79.2	0.288	5.9	0.007	0.14	0.341	7.2	0.374	7.6	Killed.

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

sponding increase in the percentage output of ammonia and rest-nitrogen. This is the urinary picture which recently has been described as associated with the hepatic disorder supposed to underly the symptoms of chloroform poisoning, toxæmia of pregnancy and like conditions and which will be discussed more in detail after our results have been completely given.

Tables III, IV, V, VI and VII present the results of the experiments in which a true necrosis, either focal or diffuse, was obtained.

In these experiments the injection of the toxic sera[8] was always quickly followed by a more or less marked increase in the elimination of total nitrogen which *persisted* for several days. During the first or second twenty-four hour period, after injection, occurred a slight increase in the percentage of urea nitrogen (three to five per cent.) which was followed on the succeeding days by a drop to normal, and in one experiment below normal. The ammonia nitro-

[8] In three of these experiments the first injection was followed by a second after a varying interval. This fact renders the figures after the time of the second injection less comparable.

gen percentage of the total nitrogen diminished gradually after the injection and reached its lowest point about the second or third day, after which, in some cases, as in Dogs 49 and 43, it returned to normal; in others, as in Dogs 25, 45 and 48, it remained low. The more advanced the repair at the time of death, the nearer the percentage of ammonia nitrogen had returned to the normal.

The uric acid nitrogen suffered a marked, though transient, increase.[9] In the three experiments where successive injections were given, the second injection in each instance caused an increase of uric acid on the following day after which it returned to normal.

The absolute creatinin nitrogen output was noticeably augmented after injection; this increase, however, was not quite in the same proportion as the total nitrogen, hence the creatinin nitrogen per cent. of the total tended at times to show a slight diminution.

TABLE V.

(*Dog 45, Focal Necroses.*)

Date.	Total Nitrogen.	Urea.*		Ammonia.		Uric Acid.		Creatinin.		Undetermined Nitrogen.		Notes.
Feb. 3	6.70	5.50	82.1	0.342	5.1	0.014	0.21	0.274	4.1	0.570	7.5	
4	6.57	5.37	81.7	0.491	7.5	0.013	0.20	0.296	4.5	0.400	6.1	
5	6.43	5.05	78.5	0.452	7.0	0.012	0.18	0.274	4.1	0.642	10.2	
6	6.52	5.33	81.7	0.455	7.0	0.013	0.20	0.265	4.1	0.457	7.0	Injected 10 A. M. toxic serum; dose 1 : 1500
7	8.99	7.51	83.5	0.560	6.2	0.105	1.17	0.359	4.0	0.456	5.1	
8	7.97	6.25	78.4	0.524	6.6	0.010	0.13	0.300	3.9	0.886	11.0	Injected 10 A. M. toxic serum; dose 1 : 1000
9	5.30	4.01	75.6	0.441	8.3	0.055	1.04	0.298	5.7	0.928	9.4	
10	10.30	8.20	79.6	0.646	6.3	0.030	0.28	0.349	3.4	1.075	10.4	
11	8.37	6.71	80.2	0.598	7.1	0.021	0.25	0.313	3.7	0.728	8.8	
12	7.45	5.99	80.0	0.447	6.0	0.009	0.12	0.326	4.5	0.678	9.4	Killed

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

[9] For a detailed discussion of this subject see fourth paper of this series, "Nuclein Metabolism," in this number of the *Journal.*

TABLE VI.

(Dog 48, Diffuse Necrosis.)

Date.	Total Nitrogen.	Urea.*	Ammonia.	Uric Acid.	Creatinin.	Undetermined Nitrogen.	Notes.
Feb. 19	6.80	5.90 86.7	0.374 5.5	0.018 0.26	0.373 5.5	0.135 2.0	
20	6.55	5.53 84.4	0.360 5.5	0.018 0.27	0.364 5.6	0.278 4.2	
21	6.38	5.41 84.8	0.358 5.6	0.024 0.37	0.326 5.2	0.228 4.0	Injected 5 P. M. normal serum; dose 1:600.
22	7.00	5.76 82.3	0.406 5.8	0.024 0.34	0.338 4.8	0.472 6.8	
23	6.53	5.50 84.2	0.376 5.8	0.021 0.32	0.326 5.0	0.307 4.7	
24	6.10	5.00 82.0	0.400 6.6	0.018 0.30	0.314 5.1	0.368 6.0	Injected 10 A. M. toxic serum; dose 1:1155.
25				0.036			Vomitus mixed with urine.
26	8.78	7.58 86.3	0.365 4.1	0.018 0.20	0.345 4.0	0.472 5.4	
27	9.11	6.80 (?) 76.4	0.551 6.0	0.018 0.20	0.349 3.8	1.392 (?) 13.6	Injected 10 A. M. toxic serum; dose 1:600. Vomited. Hburia.
28	11.12	9.24 83.1	0.418 3.8	0.130 1.17			Vomiting; refused food.
Mar. 1				0.083			Died.

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

The undetermined or rest-nitrogen which in a general way may be said to indicate the output of amino-acids, polypeptids or proteose-like bodies[10] also underwent a decided increase after injection.

An increase in kynurenic acid elimination after injection was noticed at times, but this was slight at the best and in no way corresponds to the increase which occurs after the administration of phosphorus, phlorhizin and large quantities of meat. In these latter instances Mendel and Jackson[11] showed that the increased kynurenic acid output was associated with augmented endogenous

[10] Salkowski, E., Zur Kenntnis der Alkohlolunlöslichen bzw. colloidalen Stickstoffsubstanzen im Harn, *Berl. klin. Woch.*, 1905, xlii, 1581, 1618.

[11] Mendel, L. B. and Jackson, H. C., On the Excretion of Kynurenic Acid, *Amer. Jour. of Physiol.*, 1898, ii, 1.

or exogenous destruction of proteid material containing the tyrosin nucleus.

As has already been stated, we believe that the effect of the blood changes produced by the serum is directed almost entirely upon the liver and represents a single attack upon this organ. Two coincident conditions, which however do not affect the results in any manner, require perhaps a brief notice. In the first place, vomiting usually occurs and persists for a short time, five to ten minutes, after the injection. The feeding and injection were so arranged that nothing was lost in this manner and as the vomiting also occurred when normal serum was used, with no apparent effect on the metabolism, we believe this factor may be disregarded. It has occasionally, however, caused the loss, on account of admixture of vomitus, of a day's urine.

TABLE VII.

(*Dog 49, Focal Necroses.*)

Date.	Total Nitrogen.	Urea.*	Ammonia.	Uric Acid.	Creatinin.	Undetermined Nitrogen.	Notes.
Feb. 22	5.24	4.33 82.6	0.366 7.0	0.021 0.40	0.215 4.1	0.308 5.9	
23	5.48	lost	0.320 5.9	0.018 0.33	0.218 4.0		
24	5.46	4.41 80.8	0.345 6.3	0.021 0.38	0.208 3.8	0.476 8.7	Injected 10 A. M. normal serum; dose 1 : 600
25				0.018			Vomitus mixed with urine
26	5.25	4.34 82.7	0.297 5.7	0.021 0.40	0.194 3.7	0.398 7.5	Trace albumin
27	5.63	4.77 84.8	0.320 5.7	0.022 0.39	0.204 3.6	0.314 5.5	Injected 10 A. M. toxic serum; dose 1 : 628. Vomited
28	7.07	6.07 85.6	0.245 3.5	0.014 0.20	0.189 2.7	0.452 8.0	Hburia
Mar. 1	9.14	7.56 82.7	0.270 2.8	0.029 0.32	0.225 2.5	1.056 11.7	Hburia
2	8.45	6.98 82.6	0.428 5.1	0.017 0.20	0.221 2.6	0.804 9.5	Hburia
3	6.97	6.04 86.6	0.370 5.3	0.022 0.31	0.214 3.1	0.324 4.7	Hburia
4	5.70	4.92 86.3	0.366 6.4	0.020 0.35	0.196 3.4	0.198 3.6	Hburia. Killed

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

In the second place hæmoglobinuria, sometimes but not always, makes its appearance after twenty-four hours. This condition, however, could not have produced the changes in the general metabolism which we have described since in two of the experiments (43 and 45), where no hæmoglobin or bile appeared in the urine, the results were the same as in those showing a well-marked hæmoglobinuria. This agrees with the observations of Samuely[12] who found in experimental anæmia, produced by means of pyrodin, that the appearance of hæmoglobin or bile in the urine stood in no direct relationship to the changes which took place during the anæmia. On the other hand, Andrea[13] in a series of experiments, in which various hæmolytic substances (phenylhydrazin, pyrogallol, p-phenylendiamin, glycerin) were administered to rabbits, has found an increase of urea after the initial injection, but a decrease of one third after subsequent injections. The increase he explains by destruction of hæmoglobin and the decrease as due to impaired hepatic function.

Another possibility, however, must also be considered. This is that the temporary anæmia which is simultaneously produced as the result of the primary action of the serum on the red cells may originate changes in oxidization capable of accounting for some of the results. If the anæmia were general in character and of the type which occasions a greatly diminished oxidative power throughout the body, such as is noticed after carbon monoxide poisoning, then we should expect to find, among other disturbances, the elimination of incompletely oxidized products of catabolism, such as lactic acid. We have searched for the appearance of this substance in the urines of five of the animals showing necrosis, but have failed to find it. This, with other facts, appear to justify the exclusion of the factor of diminished oxidation.

In this connection it is of considerable interest to note also that in the type of anæmia produced by Samuely the power of the body to oxidize aromatic compounds, such as phenylalanin and cystein,

[12] Samuely, F., Stoffwechseluntersuchungen bei experimenteller Anämie, *Deut. Arch. f. klin. Med.*, 1907, lxxxix, 220.

[13] Andrea, P., Influenca della sostanze emolitiche sulle funzioni ureogenetica ed antitossica del fegato, *Arch. Int. de Pharmacodyn. et de Therapie*, 1905, xiv, 389.

was somewhat decreased, but that the metabolism in regard to the fatty amino-acids was absolutely unchanged.

Upon the whole, therefore, it seems justifiable to designate, as the main causative factor in the production of the results obtained, the necrotic lesions more or less diffusely distributed throughout the liver.

Several attempts have been made to study the influence of hepatic necrosis upon the metabolism. Jacoby[14] was the first to study the effect of tying off the vessels supplying certain lobes of the liver. Unfortunately the animals did not survive the operation a sufficient length of time to allow observations upon the urine and he was compelled to content himself with demonstrating that products of autolysis were present in the lobes shut off from the circulation. Doyon and Dufourt[15] report that upon tying off the hepatic artery they obtained a diminished formation of urea and increase in ammonia. Their results were somewhat unsatisfactory, however.

From the clinical side of the question quite recently a considerable amount of data which bears upon the question at hand has accumulated. Many investigators have studied the urinary changes occurring in certain metabolic disorders associated with hepatic diseases. Thus Schittenhelm[16] reports that in chronic diseases of the liver the ammonia output in relation to the total nitrogen elimination is increased. Axisa[17] states that in liver abscesses the same change is associated with a marked decrease in the urea percentage. Ingelrans and Dehons[18] corroborate these findings in hepatic insufficiency and claim that cirrhosis gives the same picture as acute yellow

[14] Jacoby, M., Ueber die fermentative Eiweissspaltung und Ammoniakbildung in der Leber, *Zeit. f. physiol. Chem.*, 1900, xxx, 149.

[15] Doyon, M. and Dufourt, L., Contribution à l'étude de la fonction ureopoietique du foie; Effets de la ligature de l'artère hepatique et de alle de la veine porto (*Arch. de physiol. normal et path.*, 1898, S. 5, x, 522), Ref. in *Maly's Jahresbericht f. Thierchemie*, 1898, xxviii, 382.

[16] Schittenhelm, A., Zur Frage der Ammoniakausscheidung im menschlichen Urin, *Deut. Arch. f. klin. Med.*, 1903, lxxvii, 517.

[17] Axisa, E., Ueber Harnstoff und Ammoniakausscheidung im Harn bei Leberabszess, *Zent. f. innere Med.*, 1905, xxvi, 929.

[18] Ingelrans, L., and Dehons, M., La valeur clinique de quelques signes urinaires considérés comme révélateurs de l'insuffisance hepatique, *Arch. de* med. exper. et d'anat. path., 1903, xv, 188.

atrophy. De Rossi,[19] on the other hand, offers evidence that not all diseases in which lesions of the liver are present show this altered relation of urea to ammonia elimination and concludes that the liver is not the only seat of the formation of urea.

In regard to acute yellow atrophy, vomiting of pregnancy, eclampsia, delayed chloroform poisoning and phophorus poisoning, recent investigations seem to show that the hepatic lesions, which are found to be present at autopsy in these conditions, are an important causative factor in the disturbance of metabolism. This disturbance shows itself in the urine by a marked diminution in the output of urea and an increase of the ammonia in relation to the total nitrogen elimination. Williams[20] assumes that the urinary picture of pernicious vomiting of pregnancy with its high percentage ammonia output is sufficiently definite to render it a valuable aid in determining the question of inducing labor. On the other hand, he contends that in the condition known as eclampsia there is a diminution in the total nitrogen and percentage urea with no very pronounced ammonia variation. Stone[21] believes that the vomiting of pregnancy is the result of a toxæmia, the lesions of which are primarily an acute degeneration of the liver amounting sometimes to necrosis and resembling in the fatal cases those of acute yellow atrophy. Zweifel's[22] researches confirm the opinion that the causative factor in eclampsia is a diminished oxidation which shows itself in the production and elimination of considerable quantities of p-lactic acid. The increased ammonia output is the result of the neutralization of the excess of acids produced and the diminished urea is due to the removal of quantities of ammonia which normally would be synthesized into urea.

[19] De Rossi, S., Sul valore semeiologico dell'urea et dell'ammonica nelle lesioni epatiche, *Riforma Medica*, 1904, xx, 1177.

[20] Williams, J. W., Pernicious Vomiting of Pregnancy, *Surgery, Gynecology and Obstetrics*, 1905, i, 41; *Johns Hopkins Hospital Bul.*, 1906, xvii, 71; *Amer. Jour. Med. Sciences*, 1906, cxxxii, 132.

[21] Stone, W. S., The Toxæmia of Pregnancy, *Amer. Gynecology*, 1903, iii, 518; Some Further Notes on the Toxæmia of Pregnancy, *Med. Record*, 1905, lxviii, 295.

[22] Zweifel, Zur Aufklärung der Eklampsie, *Arch. f. Gyn.*, 1905, lxxvi, 537.

Ewing and Wolf[23] report observations made upon pregnant women from the results of which they conclude that the various conditions of eclampsia, vomiting of pregnancy and yellow atrophy are but different degrees or manifestations of the same disordered process which probably centers itself in the hepatic cells and leads to the deranged elimination of urea and ammonia. A similar disturbance of metabolism associated with necrosis of the liver, has been found by Bevan and Favill[24] in fatal chloroform poisoning.

In view of all this it can readily be seen that the concensus of opinion favors the idea that the changes in the percentage elimination of urea and ammonia which are found to occur in these various conditions are but indications of the same functional lesion which centers itself in the hepatic cell. When necrosis of the liver occurs the cells which ordinarily synthesize ammonia into urea are out of function and the ammonia elimination is increased and the urea correspondingly falls.

With a full appreciation of the necessity of caution in transcribing deductions from the results of animal experiments to the explanation of pathological variations in the human organism, we feel that our results render somewhat doubtful the relationship between the hepatic necrosis of the vomiting of pregnancy, for example, and the urinary finding of a high percentage ammonia output. Wolf[25] has already justly criticized the conclusions of Williams in this regard and emphasizes the well-known fact that equally high percentages of ammonia are to be found when for any reason, as in inanition, the nitrogen or the calorific value of the diet becomes insufficient for the replacement of the wear and tear of the cell.[26] Schittenhelm has shown the influence of diet in this connection by experiments in chronic hepatic diseases where a high ammonia output is present. He noticed that upon increasing the fat of the diet

[23] Ewing, J. and Wolf, C. G. L., The Clinical Significance of the Urinary Nitrogen, II. The Metabolism in the Toxæmia of Pregnancy, *Amer. Jour. of Obstetrics*, 1907, lv, 289.

[24] Bevan, A. D., and Favill, H. B., Acid Intoxication and Late Poisonous Effects of Anæsthetics, *Jour. Amer. Med. Assoc.*, 1905, xlv, 691.

[25] Wolf, C. G. L., The Chemistry of Toxæmias in Pregnancy, *New York Med. Jour.*, 1906, lxxxiii, 813.

[26] Folin, O., Laws Governing the Chemical Composition of Urine, *Amer. Jour. of Physiol.*, 1905, xiii, 66.

a still further increase in the elimination of ammonia occurred and believes that the ammonia offers simply an indication of the lack of normal oxidation or catabolism of the ingested fatty acids. An examination of the results presented by Williams makes it seem very plausible that the diet in his cases is not an unimportant factor in the results. The figures for the total nitrogen indicate that the patients were practically in a state of diminished nutrition even approaching inanition since the amounts fall anywhere between four to eight grams per day, and more important still, the higher the total nitrogen the lower the ammonia and *vice versa* regardless of the severity of the condition. This same criticism can also be applied to the results of Ewing and Wolf. The daily total nitrogen elimination in their experiments is quite as low as that found by Williams, hence the high ammonia can be equally well attributed, in part at least, to similar causes.

The factor of low and insufficient diet was excluded in our experiments since the animals were upon exact equilibrium. We did obtain, however, severe hepatic lesions consisting of localized or diffuse necrotic areas and the ammonia output of our animals never showed more than the merest increase which was exceedingly transient. At this place emphasis must be laid upon the one experiment in which results comparable to, if not as pronounced as those of Williams and of Ewing and Wolf, were obtained. In this instance (see Table II.), however, the histological findings indicated that we were dealing not with a lesion of necrotic character but with an extensive and diffuse degeneration.

On account of the loose use of terms in pathology, this would seem to emphasize that a clear-cut differentiation between degeneration and necrosis[27] must be made histologically if we are to correlate the results of chemical studies and histological findings. The pathological condition "degeneration" does not imply autolysis which occurs only in necrosis. It is evident that one may occur without the other and therefore that the chemistry of the cell depends on its functional activity as determined by its physical state. As a reasonable explanation of why a difference in metabo-

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

lism must be expected in the conditions of degeneration as opposed to necrosis we would present the following: In a generalized hepatic degeneration the lesion affects the protoplasm of each and every cell of the whole organ without destruction of the nucleus. This degeneration may set up enzymotic disturbances, secondary in character, which are not connected directly with the actual life processes of the cell and which may readily again return to normal when the abnormal conditions of the cell are removed. Such a differentiation between the actual life processes of the cell and those of a secondary functional character finds best expression in the German words " Baustoffwechsel " and " Betriebstoffwechsel." When the disturbance of enzymotic equilibrium occurs, if we grant for the sake of argument the unproven hypothesis that the urea formation is the result of enzymotic relations, there would take place an interference in the production of urea from ammonium compounds without an increase in the output of total nitrogen. A simple rearrangement in the partition factors would evidence itself according to which the ammonia would increase as the urea correspondingly diminished. This is exactly the condition found in our experiment with diffuse degeneration.

On the other hand, in necrosis, the individual cell is destroyed and all its functions cease. Autolysis begins in the same way as it does when death supervenes as the result of the removal of the cell from the body. Under such circumstances there occurs a true protoplasmic decomposition from which the cell can never recuperate. Here the nucleus becomes involved as is shown by the histological picture and in the urine by the occurrence of a marked increase in the elimination of uric acid, purin bases[28] and phosphorus.

In necrosis, moreover, although many individual cells are dead and have ceased to functionate, there always remain, unless the whole organ becomes necrotic, in contradistinction to the condition of degeneration, many normal cells, ready and capable of assuming in a vicarious manner the function of those already dead. This " factor of safety " in the liver is well demonstrated by the partial extirpation experiments of Ponfick, while the power of other organs

[28] For a detailed discussion of this subject see fourth paper of this series, " Nuclein Metabolism," in this number of the *Journal.*

to assume the urea-forming function is shown by the numerous Eck fistula experiments.

In necrosis, therefore, all that is expected as a urinary finding is the appearance of an increase in the total nitrogen output and of the abnormal products of autolysis such as proteoses, polypeptids and amino-acids; and even these latter, in scattered focal necrosis, need not necessarily appear since as very little liver tissue is destroyed the remaining normal cells still possess the power of splitting these substances, formed by cellular digestion, just as they do similar products of intestinal digestion brought to them by the portal[29] vein.

Our results substantiate this theoretical expectation. In the experiments with diffuse necrosis a marked and continued augmentation in the total nitrogen and urea elimination occurred as the result of the removal of the products of autolysis. The diminution in the ammonia output may be ascribed naturally to the increase in the proteid catabolism. Finally the increase in undetermined nitrogen is not definitely to be ascribed to the amino-acids ordinarily considered in this connection since we have not found leucin and tyrosin in the urine in amounts which would compare with those found in acute yellow atrophy.[30] It is to be considered rather as in the " colloidal " form as described by Salkowski and Mancini.[31]

SUMMARY.

1. In focal and diffuse necroses of the liver due to hæmotoxic sera there occurs an increased elimination of total nitrogen with a corresponding augmented output of urea. The ammonia excretion becomes slightly diminished at first, but later rises somewhat above normal. The undetermined nitrogen is markedly increased.

2. In diffuse degeneration with no necrosis on the other hand only a slightly increased output of total nitrogen is evident. A

[29] Freund, E. and Tœpfer, G., Ueber den Abbau des Nahrungseiweisses in der Leber, *Zeit. f. exp. Path.*, 1906, iii, 632.

[30] Riess, L., Phosphorvergiftung und Leberatrophie, *Berl. klin. Woch.*, 1905, xlii (Ewald Festnummer 44 a, 54).

[31] Mancini, S., Studi un nuovo segno per la diagnosi di insufficienza epatica; Contributo allo studio dell'azeta colloidale nelle urine normali e pathologiche, (*Arch. di farmocol. speriment,* 1906) Ref. in *Biochem. Cent.*, 1906, v, 549.

rearrangement of the urea-ammonia proportion occurs in that the ammonia excretion is augmented while the urea elimination is correspondingly diminished. The undetermined nitrogen rises but little.

3. In control experiments with normal serum no effect is produced.

4. These results would appear to indicate that in lesions characterized by uniform degeneration of the liver parenchyma, in contradistinction to necrosis, there occurs no increased nitrogen elimination but merely a disturbance of the urea-forming function of the cell without the appearance in the urine of products of autolysis. On the other hand in necrosis, of even considerable extent, the total-nitrogen is greatly augmented, as is also the rest-nitrogen; while the production of urea, on account of the persistence of normally functioning liver cells, remains relatively unchanged.

This " factor of safety "[32] possessed by the liver is, we think, one of the most important results brought out in this investigation and must be given great weight in any consideration of the chemistry of hepatic disturbances.

[32] Meltzer, S. J., The Factors of Safety in Animal Structure and Animal Economy, *Jour. of Amer. Med. Assoc.*, 1907, xlviii, 655.