ON THE HEXON BASES OF LIVER TISSUE UNDER NORMAL AND CERTAIN PATHOLOGICAL CONDITIONS.

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INTRODUCTION.

The discovery by Drechsel that bases are among the decomposition products of proteid material was an important step for proteid chemistry. Drechsel found the bases lysin—a diaminocaproic acid—and lysatinin, and from the latter Hedin afterwards isolated the base arginin. Later A. Kossel added a third member to the group of hexon bases [1] by the discovery of histidin. The fact that these three bases, lysin, arginin, and histidin, are obtainable from nearly all proteid matter makes their investigation of much interest, and it is a matter of great importance that Kossel and his pupils have been able to devise methods for their satisfactory quantitative estimation.

The interest attaching to a knowledge of the actual yield of the hexon bases relates not merely to strictly physiological questions connected with the proteid molecule. It has long been a question with pathologists whether the various degenerations of parenchymatous cells were not attended by alterations in the constitution of the proteid molecule during life, but the methods heretofore at the disposal of investigators have not permitted any satisfactory answer to be given to the question.

In the hope of throwing some light on these fundamental problems, I have been led, at the suggestion of Professor A. Kossel, to study this condition of the proteid molecule with

[1] So called on account of each molecule containing six atoms of carbon.

regard to the yield of hexon bases in phosphorus poisoning.[2] The results now obtained through the study of the dog's liver in this condition and in chronic chloroform poisoning are sufficiently definite and uniform to justify their presentation, in the belief that they constitute a first step toward the correlation of histological and profound chemical alterations in the proteids of living cells.

The work on the normal and phosphorized dogs was performed in Professor Kossel's laboratory in Heidelberg; the remainder of the work was done in collaboration with Professor Herter, to whom I am indebted for the opportunity of making this contribution.

METHOD.

The final separation and isolation of the hexon bases, in this series of experiments, was carried out according to the method of Kossel and Kutscher.[3]

The principle of the individual separations depends upon the insolubility of the silver compounds of arginin and histidin in a fluid saturated with barium hydrate, lysin remaining in solution, and also upon the insolubility of the silver compound of histidin in a fluid rendered faintly alkaline with barium hydrate, the arginin, under these conditions, remaining in solution.

The early part of the procedure varied somewhat from the method above referred to.[4] The method adopted was as follows:

A nitrogen determination [5] was made upon portions of the fresh organ as well as the dried one, which had been used for water estimation[6]; 150 grams of the finely divided tissue were mixed with 900 c. c. water and 450 grams H_2SO_4,

[2] A preliminary report appeared in the *Berliner klinische Wochenschrift*, 1904, xli, 1065.

[3] *Zeitschr. f. physiol. Chemie*, 1900, xxi, 165.

[4] It may be well to state that an attempt was made to keep the mode of procedure in all the cases studied as much alike as possible, thus obtaining results which, if not absolute, were at least more nearly comparable.

[5] Duplicate nitrogen determinations, according to Kjeldahl, oxidizing by aid of $CuSO_4$ and K_2SO_4, were made throughout the analyses, and a control of the nitrogen content of precipitates and fluids was kept by making nitrogen estimations upon aliquot parts of filtrates, observing correction for fluid taken.

[6] It is interesting to note the slight loss of nitrogen through the process of drying to a constant weight at 105° C. Of fourteen instances of nitrogen determinations upon fresh as well as upon dried tissue of the organ, the average loss of total nitrogen was 0.047 %.

sp. gr. 1.80. The mixture was boiled in a flask with an upright condenser on a paraffin bath fourteen hours. After cooling and diluting, the small amount of fatty matter remaining undissolved was removed by filtration.[7] A hot solution of 1000 grams of crystallized $Ba(OH)_2$ was added to the hot filtrate, thus removing about 70 % of the H_2SO_4. The bulky precipitate was filtered off and thoroughly washed,[8] and the filtrate evaporated to a certain point (approximately one litre), until the H_2SO_4 content was brought to 5 % by volume, this proving to be the most favorable concentration for the subsequent precipitation. At this point, the hexon bases were thrown out of solution as phosphotungstates.[9] The precipitate was filtered off and washed with water containing 5 % by volume of H_2SO_4 and a few drops of phosphotungstic acid. After complete washing, the precipitate was rubbed up with a small amount of water, and decomposed on the water bath with $Ba(OH)_2$ in very slight excess.

The bases, thus thrown back into solution, were removed from the insoluble barium phosphotungstate by filtration and complete washing with hot water, and the slight excess of $Ba(OH)_2$ in the filtrate was thrown out with CO_2.[10] After concentration, the $BaCO_3$ was filtered off and washed, and nitrogen estimated in the filtrate. After acidifying with HNO_3, silver nitrate solution was added in slight excess, as indicated by the yellow-colored precipitate formed when a drop of the clear fluid was brought in contact with a drop of solution of barium hydroxide on a watch glass.

The small quantities of purin bodies which were brought down with the phosphotungstate precipitate separated at this point and were removed by filtration.[11] The clear fluid was then saturated with $Ba(OH)_2$. The precipitate (A) contained arginin and histidin; the fluid (B), lysin.

(A) The precipitate, quickly filtered and washed with baryta water and suspended in weak H_2SO_4 solution, was decomposed with H_2S. After removing the H_2S by evaporation, and the precipitated $BaSO_4$ and Ag_2S by filtration and washing, nitrogen was estimated in a portion of the filtrate. The remainder of the filtrate was neutralized with $Ba(OH)_2$, and $Ba(NO_3)_2$ added to complete the precipitation. The $BaSO_4$ was filtered off and washed. The filtrate was acidified with HNO_3, and $AgNO_3$ added in slight excess. It sometimes occurred that traces of purin bodies separated at this point; if so, they were removed by

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[7] In nine determinations the average percentage of total nitrogen in this residue was 0.049.

[8] The $BaSO_4$ holds back nitrogen in spite of thorough washing. Although in each case the precipitate was washed with over 25 litres of hot water, the average percentage of total nitrogen which was held back was found, as a result of nine determinations, to be 0.111.

[9] In order to insure uniformity, the phosphotungstic acid reagent was always prepared by the method of Drechsel. *Berichte d. deutschen chem. Gesellschaft*, 1887, xx, 1454.

[10] The fluid at this point in every case was of a deep rose color, and this color appeared later, although in less degree with lysin. The nature of the color reaction was not investigated.

[11] Their amount, in terms of nitrogen, varied from 0.02 to 0.037 % of total nitrogen, the average of seven determinations being 0.0273.

filtration. The fluid was carefully neutralized with $Ba(OH)_2$, then more $Ba(OH)_2$ was added cautiously from a burette until a drop of the clear supernatant fluid failed to give any turbidity with a drop or two of a weak odorless solution of ammoniacal silver. The precipitate was quickly filtered and washed. The precipitate (a) contained histidin; the filtrate (b), arginin.

(a) The histidin silver was decomposed in the usual manner with H_2S in the presence of H_2SO_4. The H_2S was removed by evaporation, the Ag_2S filtered off and washed, and a portion of the filtrate subjected to a nitrogen estimation, upon which determinations the values for histidin in the various tables below are based.[12]

The remainder of the filtrate, made to contain $2\frac{1}{2}$ % by volume of H_2SO_4, was treated with $HgSO_4$ in slight excess according to the method of Kossel and Patten.[13] The histidin mercury was decomposed with H_2S, the H_2S driven off by heat, and the HgS removed by filtration. After removing the H_2SO_4 by $Ba(OH)_2$, the excess of $Ba(OH)_2$ by CO_2, and the last traces of $BaCO_3$, the fluid was rendered slightly acid with HCl, evaporated, and allowed to crystallize, the histidin being weighed as the dichloride.[14]

(b) The filtrate containing the arginin was saturated with $Ba(OH)_2$, and the precipitate quickly filtered off and washed with baryta water. After decomposing the silver arginin with H_2S in the presence of H_2SO_4, removing the H_2S and filtering off the precipitated Ag_2S and $BaSO_4$, a nitrogen estimation was made upon a portion of the fluid. The arginin results recorded in the tables below are based upon these nitrogen estimations. From the remainder of the fluid the H_2SO_4 was removed with $Ba(OH)_2$ and the excess of $Ba(OH)_2$ with CO_2. After the last trace of $BaCO_3$ was filtered off, the fluid was neutralized with HNO_3, concentrated, and the crystals of arginin nitrate finally obtained and weighed.[15]

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[12] In view of the report by Kutscher and Steudel (*Zeitschr. f. physiolog. Chemie*, 1903, xxxix, 12) relating to the untrustworthiness of the Kjeldahl method for the estimation of nitrogen in certain definite bodies, among them histidin and lysin, an objection might be raised to this procedure. But of the two choices—basing the results upon the nitrogen estimation, or upon the weight of the final crystallized product—the former is probably the more accurate. I may say that the duplicate nitrogen determinations upon which the histidin and lysin values are based, were, more often than not, in absolute agreement. The results obtained by Sörensen and Pedersen (*Zeitschr. f. physiolog. Chemie*, 1903, xxxix, 513) in their nitrogen determinations of lysin compounds by the Kjeldahl method agree closely with the theoretical requirements.

[13] *Zeitschr. f. physiolog. Chemie*, 1903, xxxviii, 39.

[14] The histidin nitrogen calculated from the weighed histidin dichloride ($C_6H_9N_3C_2$. 2HCl) was, as a rule, somewhat lower than the value based on the Kjeldahl determination. Of nine specimens studied, the average nitrogen was 0.0519 grm. by Kjeldahl, and 0.048 grm. by weight.

[15] The nitrogen based on the Kjeldahl determination and that calculated from the weighed arginin nitrate ($C_6H_{14}N_4O_2$. $HNO_3+\frac{1}{2}H_2O$) agreed fairly closely. In some instances the latter showed a somewhat higher value, due probably to the unremoved traces of $BaCO_3$. The average of the nine specimens studied gave 0.1772 grm. by Kjeldahl and 0.1850 grm. by weight.

(B) The fluid containing the lysin was acidified with H_2SO_4 and the excess of silver used in the previous precipitation removed with H_2S. After evaporating off the H_2S, the rather bulky precipitate of $BaSO_4$, together with the Ag_2S, was filtered off and washed. After concentrating to a convenient bulk, the lysin was again thrown out of solution with phosphotungstic acid, filtered, washed, decomposed with $Ba(OH)_2$, and the excess of $Ba(OH)_2$ removed by CO_2 in the usual manner, after which a nitrogen determination was made upon a portion of the fluid. The results for lysin recorded in the tables are based upon these nitrogen estimations.[16] From the remainder of the fluid, after evaporating, the lysin was separated as a picrate. After washing, recrystallizing, and weighing the lysin picrate, the lysin left in the mother liquor and washings was again thrown out of solution by phosphotungstic acid. In carrying out this method, the picric acid was first removed with ether, after which the process was repeated as before. The weight of the lysin picrate recovered was added to the weight of the main portion.[17]

Hexon Bases in Normal Dog's Liver.—For the sake of comparison normal values were established by taking the livers of two healthy, well-nourished dogs, weighing 10 and 13 kilos respectively. The dogs were killed, about fourteen hours after feeding, by bleeding. The livers appeared normal. To ascertain if the small amount of blood left in the liver after death by bleeding produced any appreciable effect upon the results, a third normal and fairly well-nourished dog of about 9 kilos weight was taken. After a similar bleeding, the liver of the last dog was washed with five litres of Ringer's solution, thus removing practically all the blood from the tissue. The results obtained are recorded in Table I, Division A.

It is likely that these values are within the range of normal variation. Probably the influence of the small percentage of blood left in the liver tissue after death by bleeding may be disregarded, and, with one exception, in the subsequent examples studied, the last traces of blood were not removed from the liver preparatory to analysis.

Hexon Bases in the Liver of Dogs Poisoned with Phosphorus.—Seeking a condition showing marked degeneration of the liver

[16] See footnote 12, page 295.

[17] The average of the nitrogen values in the specimens studied was 0.1261 grm. by the Kjeldahl method, and 0.1147 grm. by crystallizing and weighing as lysin picrate ($C_6H_{14}N_2O_2 \cdot C_6H_2(NO_2)_3OH$). The amount obtained by the latter method was, as a rule, somewhat less than was indicated on the basis of the Kjeldahl estimations.

cells and one in which we might expect changes influencing the structure of the proteid molecule itself, observations were undertaken upon the liver in phosphorus poisoning.

Two dogs were placed under the phosphorus treatment. The first (No. IV, Table I), weighing 10 kilos, received during a period of 32 days, thirteen subcutaneous injections of a moderately strong solution of phosphorus in olive oil. The first nine injections were of 0.5 c. c. each, and they were followed by one injection of 0.6 c. c., 0.7 c. c., 0.7 c. c., and 0.8 c. c. each. It was not until after the ninth injection, on the twenty-sixth day, that the animal began to show symptoms. One day after the last injection the dog was killed by bleeding. He had lost 1.7 kilos in weight, and the liver showed the appearance of phosphorus poisoning, although in less marked degree than the next animal employed.

This dog (No. V, Table I), weighing 10.3 kilos, received during nine days six subcutaneous injections of 0.5 c. c. each of a saturated solution of phosphorus in olive oil. The third day after the last injection the animal died. A few minutes after death the blood was removed from the liver by washing with Ringer's solution, as in the case of normal dog No. 3. The dog had shown marked symptoms of depression and complete loss of appetite, and diarrhœa; he had lost 1.4 kilos in weight. The liver was extremely fatty, showing the typical appearance of phosphorus poisoning.

The results obtained with these two animals are recorded in Table I, Division B. A comparison of averages may be seen in Table II, and the percentages of diminution of the bases, compared with the normal values, are given in Table III.

In comparing the values obtained from the two phosphorus-poisoned dogs with the values regarded as normal, one is struck with the marked diminution of the hexon bases as a whole in the former. Thus the average nitrogen content of the bases in the normal liver tissue is 17.04 % of the total nitrogen (Table II), while the content of the liver of the phosphorus dog (IV) is only 10.72 %, a falling off of 37.1 % (Table III). In the case of the more advanced phosphorus poisoning (V), the nitrogen of the

hexon bases is only 8.11 % of the total nitrogen (Table II), or a loss of 46.01 % (Table III) from the corresponding normal value of 15.02 % of Exp. III. Of the bases, arginin suffers the greatest relative diminution, and its loss is a trifle more marked in Case V than in the others. Thus the average arginin nitrogen in the liver of the normal dog is 9.62 % of the total nitrogen, while in that of the phosphorus dog (IV) it is only 5.05 % (Table II), a diminution of 47.52 % (Table III). In the washed dogs' livers the normal arginin nitrogen value is 8.72 %, while in the phosphorus dog (V) it is only 4.14 %, thus making a loss of 52.49 %.

It is interesting to note the falling off of the total nitrogen in the cases under consideration. The average total nitrogen in 100 parts of the dry normal tissue is 11.41 (Table II); in the phosphorus dog of Exp. IV, the value is 7.34, a diminution of 35.67 % (Table III). The falling off of the nitrogen of the hexon bases in the same animal is 37.1 % (Table III). This would seem to indicate that that part of the proteid molecule, involving the hexon bases, has not in the pathological tissue undergone a greater relative decomposition than the other nitrogenous matter of the organ. In the case of the dogs whose livers were washed, the total nitrogen in 100 parts of the dry liver tissue fell from 12.48 (III) to 7.9 (V), a loss of 36.7 %. In this instance, however, the proteid matter involving the hexon bases of the phosphorus-poisoned tissue seems to have suffered a slightly greater relative breaking down, amounting to 46.01 % (Table III).

Hexon Bases in Livers of Dogs Repeatedly Anæsthetized with Chloroform.—In the course of some experiments upon dogs which had been repeatedly subjected to chloroform narcosis for longer or shorter periods, the opportunity to study possible changes in the hexon bases of the liver tissue was presented. Such a study seemed not without interest, especially in the light of the facts reported by Taylor to which reference will be made later.

In considering the results of the four instances of chloroform poisoning presented in Division C of Table I, it should be borne in mind that the first two dogs (VI and VII) had been under the

chloroform treatment for a somewhat shorter period than the other two, and showed therefore less advanced histological changes of the liver. Hence the four specimens, roughly speaking, fall into two groups.

The first dog (VI) was a moderately well-nourished animal of 9 kilos weight. It was placed under chloroform one hour daily for five days, after which it was killed by bleeding. Upon histological examination, for which, here as in the subsequent instances, I am indebted to Dr. Wm. R. Williams, the liver showed a moderate degree of fatty degeneration, which was rather more marked toward the centre of the lobules.

The next dog (VII) weighed 7.5 kilos and was well nourished. It was anæsthetized on nine occasions, each lasting about one hour, during a period of fifteen days. The dog ate very sparingly and became much emaciated. At the last administration of chloroform the dog died; but the arteries were quickly severed, allowing most of the blood to escape from the liver. Histological examination showed fairly marked fatty degeneration of the liver cells.

The third dog (VIII), weighing 12 kilos, was subjected to chloroform inhalation three times a week for 58 days, making in all twenty-five occasions of anæsthesia. The first nineteen periods of inhalation lasted one hour each, and the last six, one and a half hours each. Toward the close the animal became much emaciated and suffered a complete loss of appetite. He was finally killed by bleeding.

The histological examination showed marked hyalin degeneration of the liver cells, with considerable formation of new bile ducts, and an abundant production of richly cellular connective tissue.

The last dog (IX), weighing 5 kilos, and only moderately well-nourished, was under the chloroform treatment four weeks, receiving inhalations three times weekly, at first of one hour and later of one and a half hours' duration. The animal rapidly lost weight and became much emaciated. He was found dead in the cage, and hence it was impossible to free his liver from blood. In spite of this drawback, however, it seemed well to examine

the organ for possible changes in the hexon bases. The histological examination revealed very widespread and marked fatty degeneration of the liver cells, so that very few cells of normal appearance could be found.

In looking over the results in Table I, obtained from the chloroformed dogs, it is seen, as in the previous cases, that arginin has become much diminished in amount. This fact is especially true of the more advanced cases (VIII and IX), where the nitrogen of arginin falls off from 55 to 60 per cent. The arginin nitrogen of the other instances (VI and VII) shows far less diminution. In the latter cases, the histidin and lysin nitrogen content seems to be somewhat in excess of the values assumed as normal, the ratios of the bases being, however, in very close agreement, as is shown by the figures 3.18 and 3.25, as compared with the normal figures of 3.33 and 3.22. The histidin and lysin values of the more advanced lesions appear within the range of normal values or slightly under them.

It is interesting to observe that in the chloroformed animals, and hence in striking contrast to the phosphorized dogs, there seems to be no abnormal falling off of the total nitrogen of the tissue examined. Thus the average total nitrogen in 100 parts of the dry liver tissue of the four chloroformed dogs is 11.32 (Table II); the corresponding average in the normal dogs is 11.41. In view of this fact, it would seem that in the chloroformed animals, particularly in the two in which there is quite a marked diminution of the nitrogen of the arginin, a transformation, rather than an absolute loss, of the nitrogen has taken place in the tissue.

In glancing over the ratios of the nine cases studied (Table I), the relation of the lysin to the histidin, with one or two exceptions, remains remarkably constant. The average of the nine ratios is 3.19.

CONCLUSION.

The results above reported lead to the conclusion that while in the degenerating cells chemical changes are taking place tending toward a diminution of the hexon bases as a whole,

they affect the arginin especially. One may picture the process either as a partial or as a complete breaking down of certain proteid material more or less rich in hexon bases, leaving behind proteid matter poorer in bases.

The meaning of these changes is, however, obscure, and with the limited number of known facts bearing upon the subject, it would seem idle even to attempt to formulate an hypothesis to explain them. Certain work of other investigators is, however, suggestive in this connection.

Kossel and Dakin,[18] for instance, as illustrated by their work upon the simple proteid body clupein, found that a partial destruction of the proteid molecule, involving the arginin group, is brought about by a ferment furnished by the animal organism. When subjected to the hydrolytic action of a mineral acid, clupein yields arginin in considerable abundance. But if the clupein is first acted upon by the ferment arginase found in the liver, and then subjected to acid hydrolysis, the yield of arginin is appreciably diminished. Among the cleavage products in the latter instance are the components of arginin, namely, ornithin and urea. It would seem, therefore, as if the ferment had loosened the union between the ornithin and urea in the arginin group, so that upon subsequent hydrolysis a diminution of arginin resulted. In the cases studied by me, it may be that the conditions were favorable for some such ferment action as that above described, and hence the relatively low yield of arginin. No attempt, however, was made to ascertain if ornithin were present in the urine. Its presence there would seem not wholly unlikely when one considers the diminished power of oxidation of the phosphorus-poisoned cell, although Thompson [19] has shown that arginin or ornithin when administered to a healthy dog as food or by hypodermic injection is eliminated for the most part as urea, no ornithin being found.

There might seem to be a conflict between this view and the results recently published by Wohlgemuth,[20] but it must be

[18] *Zeitschr. f. physiolog. Chemie*, 1904, xlii, 181.
[19] *The Journal of Physiology*, 1905, xxxii, 137.
[20] *Zeitschr. f. physiolog. Chemie*, 1905, xliv, 74.

borne in mind that the influences at work causing the breaking down of the proteid molecule are probably quite diverse in character. Wohlgemuth has recently shown for the first time that a diamino acid may actually find its way into the urine in phosphorus poisoning. He found arginin in the urine not only in rabbits poisoned with phosphorus but also in a patient suffering from phosphorus poisoning. On the other hand, he was unable to find lysin in the urine. This fact is of especial interest in view of the evidence set forth in this paper that the arginin base is lost to the proteid molecule more rapidly than the lysin base. The correspondence between the findings in the liver and in the urine is thus a close one.

How much of the arginin liberated from the proteid molecule may find its way into the urine is of course uncertain. It seems reasonable to suppose that a portion of the base is acted upon by the arginase ferment in the manner already described.

Of the seventeen to eighteen cleavage products of the proteid molecule thus far isolated, the hexon bases are among the most stable. One or more of these bases have been found in practically all proteid matter thus far investigated[21]; in fact arginin is so uniformly present that Kossel[22] has made the suggestion that it is the kernel of the proteid molecule. At all events, the question may be asked, whether, if the influences at work in the altered liver tissue were of a general character causing a diminution of the hexon bases, the monoamino acid groups would not suffer even a greater diminution; and since the pathological condition is undoubtedly associated with impaired oxidation, their presence should not be expected in the urine. As a matter of fact, Ignatowski[23] found considerable quantities of monoamino acids in the urine of patients suffering from gout, pneumonia, and leukæmia, though under normal conditions no monoamino acids were found in the urine, indeed, not even after the subcutaneous injection of glycokoll.

Furthermore, the loosening of the amino acids from the

[21] Cohnheim, *Chemie der Eiweisskörper*, pp. 42–47.
[22] *Ber. d. deutsch. chem. Gesellschaft*, 1901, xxxiv, 3214.
[23] *Zeitschr. f. physiolog. Chemie*, 1904, xlii, 371.

proteid molecule is suggested by the fact that Taylor [24] found such acids in the liver of a patient who died from a hepatic disease of obscure etiology, but which he was inclined to attribute to chloroform poisoning. Taylor found not only leucin and tyrosin in the liver, but also arginin, a fact not without interest in view of the diminished arginin content found in the livers of the chloroformed dogs after acid hydrolysis.

Moreover, the falling off of the hexon bases under the conditions studied seems quite in accordance with some results recently reported by Levene.[25] He has shown that certain cleavage products obtained by the action of mineral acids upon self-digested pancreas, spleen, and liver, are much diminished when compared with the products obtained from the fresh glands. The lysin and arginin of the digested liver, for example, showed a diminution of over 50 per cent.

It is now well established that in course of the process of aseptic autolysis, the proteids of the liver cell undergo decomposition into simpler substances, and Jacoby [26] showed that during life autolysis may go on in portions of the liver in which the circulation has been hindered. But of greater significance still in this connection, is the observation made by Jacoby on the autolytic changes in the liver during phosphorus poisoning. He found that when the normal liver substance is permitted to autolyse the solution of the liver substance is a slow one. On the other hand, under similar conditions the liver of a phosphorus-poisoned animal undergoes rapid and almost complete solution. The difference in the behavior of the normal and damaged liver points to an increase of normal ferment action in the case of the poisoned organ. It thus seems reasonable to suppose that in phosphorus poisoning we have during life an exaggerated breaking down of the proteid molecule associated with an over-action of certain ferments, and among them probably arginase.[27] The pathological

[24] University of California Publications, *Pathology*, 1904, i, 43.

[25] *The American Journal of Physiology*, 1904, xii, 276.

[26] *Zeitschr. f. physiolog. Chemie*, 1900, xxx, 174.

[27] It would appear as if the rôle of arginase in phosphorus poisoning might be advantageously subjected to experimental study. By means of the method

process in the liver during life may, therefore, be thought of as proceeding in the same general direction as the process of postmorten autolytic decomposition.

By means of further studies along lines indicated in this paper, it should be possible to gain a deeper insight into numerous pathological processes. The changes in amyloid degeneration are among those which promise to be better understood through the application of the new methods of chemical analysis. Moreover, it cannot be doubted that pharmacology as well as toxicology has much to gain from a study of what happens to the proteid molecule under the influence of poisons.

of Kossel and Dakin (*Zeitschr. f. physiolog. Chemie*, 1904, xlii, 181), it is possible to separate arginase from the liver and to measure its activity upon arginin. If the view above expressed be correct, it should be possible to determine the presence of arginase in increased amount in the liver of animals poisoned with phosphorus.

TABLE I

Division.	No. of experiment.	Weight of liver in grams.	Dry substance in 100 parts of moist liver tissue.	Nitrogen in 100 parts of dry liver tissue.	Nitrogen of bases in 100 parts of dry liver tissue:			Nitrogen of bases in percentage of total Nitrogen:			Bases in 100 parts of dry liver tissue:			Ratio of bases in liver tissue:		Remarks.
					arginin.	histidin.	lysin.	arginin.	histidin.	lysin.	arginin ($C_6H_{14}N_4O_2$) in grm.	histidin ($C_6H_9N_3O_2$)	lysin ($C_6H_{14}N_2O_2$)	*arginin.* histidin.	*lysin.* histidin.	
A. Normal.	I.	370	30.13	11.54	1.036	0.240	0.563	8.982	2.077	4.876	3.22	0.88	2.93	3.66	3.33	Normal dog. Killed by bleeding.
	II.	435	28.50	11.28	1.156	0.271	0.618	10.25	2.407	5.483	3.59	1.00	3.22	3.59	3.22	Normal dog. Killed by bleeding.
	III.	180	22.59	12.48	1.088	0.298	0.488	8.720	2.388	3.912	3.38	1.10	2.55	3.07	2.32	Normal dog. Liver washed.
B. Phosphorus.	IV.	194	26.74	7.34	0.370	0.133	0.284	5.046	1.809	3.868	1.15	0.490	1.48	2.35	3.02	Phosphorus dog. Killed by bleeding.
	V.	260	21.90	7.90	0.327	0.099	0.214	4.143	1.256	2.710	1.02	0.366	1.12	2.79	3.06	Phosphorus dog. Liver washed.
C. Chloroform.	VI.	340	24.12	11.96	0.762	0.371	0.832	6.369	3.103	6.961	2.37	1.37	4.34	1.73	3.18	Chloroform dog. Killed by bleeding.
	VII.	275	24.66	11.32	0.785	0.324	0.746	6.935	2.862	6.592	2.44	1.20	3.89	2.04	3.25	Chloroform dog. Killed by bleeding.
	VIII.	—	22.47	11.49	0.436	0.193	0.531	3.798	1.678	4.624	1.35	0.712	2.77	1.90	3.89	Chloroform dog. Prolonged treatment. Killed by bleeding.
	IX.	—	31.33	10.51	0.458	0.224	0.540	4.360	2.133	5.139	1.42	0.827	2.82	1.72	3.41	Chloroform dog. Prolonged treatment. Found dead.

TABLE II

(1) Division.		(2) No. of experiment.	(3) Dry substance in 100 parts of moist liver tissue.	(4) Nitrogen in 100 parts of dry liver tissue.	(5) Nitrogen in percentage of total Nitrogen:				(6) Bases in 100 parts of dry liver tissue:			
					arginin.	histidin.	lysin.	the three bases.	arginin.	histidin.	lysin.	the three bases.
A. Nor.		Average I. II.	29.32	11.41	9.616	2.242	5.180	17.04	3.41	0.94	3.08	7.44
B. Phos.		IV.	26.74	7.34	5.046	1.809	3.868	10.72	1.15	0.49	1.48	3.12
C. Chlor.		Average VI. VII. VIII. IX.	25.65	11.32	5.366	2.444	5.829	13.64	1.90	1.027	3.45	6.38
Washed liver.	Nor.	III.	22.59	12.48	8.720	2.388	3.912	15.02	3.38	1.10	2.55	7.03
	Phos.	V.	21.90	7.90	4.143	1.256	2.710	8.11	1.02	0.366	1.12	2.51

TABLE III

Division.	No. of experiment.	Diminution of Nitrogen in percentage of corresponding normal nitrogen values.[1]					Diminution of bases in percentage of corresponding normal values in dry liver tissue [2]:			
		Nitrogen in 100 parts of dry liver tissue.	Nitrogen of arginin.	Nitrogen of histidin.	Nitrogen of lysin	Nitrogen of the three bases.	arginin.	histidin.	lysin.	the three bases.
B. Phos.	IV.	35.67	47.52	19.32	25.33	37.10	66.27	47.87	51.94	58.06
	V. Liver washed.	36.70	52.49	47.41	30.73	46.01	69.82	66.73	56.08	64.28
C. Chlor.	Average VI. VII. VIII. IX.	0.79	44.20	9.01[3]	12.53[3]	2.00	44.28	9.26[3]	12.01[3]	0.81

[1] Calculated from columns 4 and 5, Table II.
[2] " " column 6, Table II.
[3] Increase.