temperature, the solution was heated to 65° for a few minutes, cooled, and then titrated twice with 0.1 molar NaOH. A sharp end point was obtained in both cases, and the results checked closely, *viz.*, 1.0716 and 1.0715.

**Summary.**

A dinitrobenzoylene urea has been discovered whose monosodium salt is a very sensitive indicator for hydrogen-ion concentrations between the limits of 6 and 8 on the Sörensen scale, changing from colorless to greenish yellow.

Structurally, and in its behavior as an indicator, it resembles *p*-nitrophenol more closely than any of the other well-known indicators. Like the latter, its chief disadvantage is its yellow color, which renders it unsuitable for work in artificial light.

It is but slightly affected by neutral salts, not at all by chloroform or toluene, proteins (egg albumen) have no more influence upon it than upon *p*-nitrophenol; its color fades very slightly in a week, and is unchanged by nitrous acid. It can be used in cold or in boiling (100°) solutions. It gives a sharp end point with $NH_4OH$ and HCl, but cannot be used to titrate carbonates.

For the preparation of neutral ammonium citrate solutions, for fertilizer or soil analysis, it should prove superior to rosolic acid (commercial Coralline).

It can be prepared easily from anthranilic acid by the method described.

NEW YORK, N. Y.

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[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY OF THE UNIVERSITY OF ILLINOIS.]

# A COMPARATIVE STUDY OF THE DISTRIBUTION OF UREA IN THE BLOOD AND TISSUES OF CERTAIN VERTEBRATES WITH ESPECIAL REFERENCE TO THE HEN.

BY WALTER G. KARR AND HOWARD B. LEWIS.

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The problems of protein metabolism and protein requirements of the organism are today among the most absorbing and fundamental in the whole realm of physiological chemistry. Closely associated with these problems is the problem of the formation and distribution of urea, since this has long been recognized as the chief end-product of protein metabolism in the higher vertebrates, with the exception of birds and reptiles, in which its place as the chief end-product of nitrogenous metabolism is taken by the more complex uric acid. Although much work has been done on the relation of urea to the intermediary metabolism of protein and amino acids, many problems remain to be solved, the solution of which is now a possibility with the more suitable and accurate

methods of analysis developed in the last few years. The site of the formation of urea has not been determined, whether its synthesis be a function of the liver only, or one of all the tissues, nor is the significance of the variations of the urea content of the blood and tissues clearly defined. In view of this it has seemed of value to make a comparative study of the urea in the blood and tissues of several species of vertebrates, including also those animals whose main end-product of nitrogen metabolism is not urea.

Many of our recent developments in the knowledge of the rôle of urea in intermediary metabolism are due to the perfection of the methods for the determinations of small amounts of urea. Older methods were disadvantageous in that they required rather large amounts of blood, which necessitated experiments on large animals or the death of the animal to obtain sufficient blood for analysis. They were also inaccurate, as our present methods show us, in that either some of the urea was lost in the removal from the solution of interfering substances, or other substances were broken down and their decomposition products determined as urea. Of our present methods, those of Folin[1] and Benedict[2] have generally given satisfactory results in urine and other macro-analyses. But since they are known to break down the allantoin and a small part of the uric acid, creatinine, etc., quantities which are negligible in a macro-analysis, errors in the determination of small amounts are thereby introduced which render the methods of little value in tissue analysis. The most satisfactory method at present seems to be the urease method of Marshall[3] as modified by Van Slyke and Cullen.[4] The urease method is entirely specific for urea, also short and easy of operation, and with Folin's[5] aeration method for ammonia makes a very suitable and accurate determination of urea. Moreover, no removal of protein or other substance is necessary, thus avoiding loss in that respect.

On the distribution of urea in the animal organism many figures are available, but due to the inaccuracy of the older methods of determination, the figures of the last few years only would seem to be trustworthy. Schöndorff[6] reported a large number of figures on the urea content of tissues, but, due to the methods used, these are all apparently too high. He showed, however, that the distribution of urea was general in the body and the quantities approximately equal in different tissues, although his figures are not very consistent on this point. Marshall and Davis[7]

[1] *Z. physiol. Chem.*, **32**, 504 (1901); *J. Biol. Chem.*, **11**, 507 (1912).
[2] *J. Biol. Chem.*, **8**, 405.
[3] *Ibid.*, **14**, 283 (1913).
[4] *Ibid.*, **19**, 211 (1914).
[5] *Z. physiol. Chem.*, **37**, 161 (1902).
[6] *Pflüger's Arch. Physiol.*, **74**, 307 (1899).
[7] *J. Biol. Chem.*, **18**, 53 (1914).

have given an accurate method for urea analysis in tissues by means of the enzyme urease. They have shown that in the dog urea is distributed approximately equally in all the tissues of the organism with the exception of the fatty tissues, kidneys, and urinary tract, that the figures for normal dogs vary from 18 to 31 mg. urea per 100 cc. or g., and that on injection of urea into the blood stream it is absorbed very rapidly, only about 10% being left in the circulation at the end of the injection. They consider that the low content of urea in fatty tissues is due to the small percentage of water in these tissues and the subsequent decreased power of absorbing the urea, and that the high content of the kidneys and urinary tract is due to saturation with urea which is in the process of excretion from the body. Folin and Denis[1] published analyses of the urea content of the blood of different species, showing that it varies with the species. The same observers found the urea content of human blood quite constant at 11–13 mg. urea N per 100 cc. Schwartz and McGill[2] reported figures from fifteen different authors on the urea content of human blood showing a range from 11 to 25 mg. urea N per 100 cc. Cullen and Ellis[3] have shown that the urea concentrations of human blood and cerebrospinal fluid are practically the same under normal conditions. Denis[4] analyzed the blood of the elasmobranch fish (dog fish, sand shark, and skate) and found the urea content to be much higher than that of mammals (800–1000 mg. urea N per 100 cc. blood). The urea content of the blood of the teleosts (goosefish) on the contrary was lower than that of mammals, being about the same as that found by Folin and Denis[5] for chickens (8–9 mg. urea N per 100 cc. of blood). The urea content of the blood of other fish was about the same as that of mammals. Von Schröder[6] found 2.46–2.71% urea in the blood of sharks and somewhat less in the muscles and liver. Baglioni[7] suggests that this is very important, as the presence of urea in these animals is necessary for the life-processes of the heart and probably all the organs and tissues. No results of the comparative analysis of tissues by the new and accurate methods have been reported other than those of Marshall and Davis[5] on the dog.

In the present series of experiments we have analyzed the urea content of the blood and tissues of various species of vertebrates. The animals were placed under ether anesthesia, bled, and the blood collected in a vessel to which a small amount of potassium oxalate was added to prevent clotting. The tissues were removed, immediately placed in

[1] *J. Biol. Chem.*, **14**, 291 (1913).
[2] *Arch. Intern. Med.*, **17**, 42 (1916).
[3] *J. Biol. Chem.*, **20**, 511 (1915).
[4] *Ibid.*, **16**, 389 (1913).
[5] *Loc. cit.*
[6] *Z. Physiol. Chem.*, 14, 576 (1890).
[7] *Centr. f. Physiol.*, **19**, 385.

weighed flasks containing ethyl alcohol and again weighed. The urea content was then determined by the method of Marshall and Davis.[1] The results are reported in Table I.

TABLE I.—THE UREA CONTENT OF THE BLOOD TISSUES OF VARIOUS SPECIES.
Results Expressed in Mg. Urea per 100 g. of Tissue.

| Species. | | Blood whole | Blood serum. | Liver. | Heart. | Lungs. | Thigh. | Abdomen. | Breast. | Kidney. |
|---|---|---|---|---|---|---|---|---|---|---|
| Guinea pig | 14 | 26 | .. | 25 | 22 | 23 | 17 | 16 | .. | 104 |
| Guinea pig | 7 | 26 | .. | 26 | 23 | 27 | 19 | 31 | .. | 50 |
| Guinea pig | 21 | 45 | .. | .. | .. | .. | .. | .. | .. | 152 |
| Guinea pig | 16 | 52 | .. | .. | .. | .. | .. | .. | .. | ... |
| Guinea pig | A | 43 | 42 | .. | .. | .. | .. | .. | .. | ... |
| Rabbit | | 60 | 60 | 65 | 38 | 40 | 32 | .. | .. | 155 |
| Turtle | 1 | .. | 38 | 17 | .. | .. | 38 | .. | .. | 52 |
| Turtle | 2 | 28 | .. | .. | .. | .. | .. | .. | .. | ... |
| Hen | A | 6 | .. | 15 | 11 | .. | 6 | .. | 10 | 10 |
| Hen | 2F | 9 | .. | 16 | 8 | .. | 9 | .. | 10 | ... |
| Hen | 2C | 12 | .. | .. | 10 | .. | 11 | .. | 11 | ... |
| Hen | 2D | 6 | .. | .. | 5 | .. | 11 | .. | 11 | ... |

The results of the analyses given in Table I show the variations of different species in the urea content of their blood and tissues. In agreement with the work of Bang[2] and Folin and Denis[1] there is considerable variation in the urea content between different species while individual variations of normal animals of the same species tend to fall within rather definite limits. The figures for the content of the blood and tissues of the guinea pig may vary from 25 to 50 mg. and the animal remain in an apparently normal condition. The data agree with those of Marshall and Davis[1] who showed that in the dog the urea was approximately equally distributed in all the tissues with the exception of the fatty tissues and the urinary tract. Thus in normal guinea pigs 7 and 14, the variations between tissues, except those of the urinary tract, were 16–31 mg. and 19–31 mg., respectively. On the other hand, the urea content of the kidneys, due presumably to contamination of the kidney tissue with urine was 104 and 50 mg., respectively. In these analyses and in others made in connection with a study of the influence of certain dietary factors on the urea concentration of the blood and tissues[3] we have observed wide variations in the urea content of the kidneys of guinea pigs maintained under similar experimental conditions (from 50 to 277 mg. per 100 g. tissue). We believe that these variations are to be attributed to differences in the concentration of the urine in the process of secretion at the time the animal was killed. The occurrence of considerable amounts of urea in turtle blood as a representative of the lower vertebrates is also

[1] *Loc. cit.*

[2] *Biochem. Z.*, **72**, 104 (1915).

[3] Unpublished data from this laboratory.

of interest. In this connection it is worthy of note that urea has been found uniformly distributed in amounts varying from 20 to 30 mg. per 100 g. of tissue in the crayfish (*Cambarus virilis*).[1]

The results of the analysis of the blood of the hen are somewhat lower than those obtained by Folin[2] as he obtained 8 mg. urea N per 100 cc. These higher results may be due to the fact that he used a different method of analysis. In contrast to that of other animals the urea content of the tissues of the hen is low and that of the kidneys uniform with that of other tissues, which is in agreement with the fact that urea is not the end-product of nitrogenous metabolism in the hen. (Compare also Table II.) Analyses of the kidneys of hens for uric acid by the method of Benedict and Hitchcock[3] made in this laboratory have shown that the uric acid content of these organs is much greater than that of the other tissues as was to be anticipated from the composition of the hen's urine.

In order to determine the relation of urea to the intermediary nitrogenous metabolism in the avian organism, *e. g.*, hen, alanine was injected intramuscularly, the hens allowed to remain 2–5 hrs. and, after being placed under ether anesthesia, bled to death and the blood and tissues analyzed for urea. In one experiment urea was also similarly injected to show the distribution of urea following injection in the hen. The results are given in Table II.

TABLE II.—ANALYSES OF TISSUES OF HENS INJECTED WITH UREA AND ALANINE.
Results Expressed in Mg. Urea per 100 g. Tissue.

| No. | Amount injected. | Hrs. after injection. | Blood whole | Blood serum. | Liver. | Breast. | Thigh. | Heart. | Lungs. | Kidneys. |
|---|---|---|---|---|---|---|---|---|---|---|
| C | 2.5 g. alanine | 2 hrs. | 4 | .. | 10 | 10 | 7 | 7 | 4 | .. |
| D | 2.5 g. alanine | 3 hrs. | 4 | .. | 13 | 9 | 8 | .. | .. | 7 |
| E | 2.5 g. alanine | 5 hrs. | 7 | .. | .. | .. | .. | .. | .. | .. |
| 2E | 2.5 g. alanine | 3 hrs. | 5½ | 4 | .. | .. | .. | .. | .. | .. |
| B | 2 g. urea | 2 hrs. | 77 | 83 | 31 | 45<br>194[4] | 49 | .. | .. | 72 |

The results show that when urea is injected into the animal it is transferred to all tissues of the body although apparently more slowly than in mammals.[5] When alanine is injected there is no increase in the urea content of the tissues. This would seem to indicate that urea is not an important intermediary product in the transformation of the amino acids to the final excretory product, uric acid, in the metabolic processes of the hen.

In Table I analyses of the blood of rabbit A, and in Table II of hens

[1] Jewell, unpublished results from this laboratory.

[2] *Loc. cit.*

[3] *J. Biol. Chem.*, 20, 629 (1915).

[4] Sample taken from the site of injection.

[5] Marshall and Davis, *Loc. cit.*

B and 2E, show that the distribution of urea is approximately equal between serum and corpuscles of the blood, which confirms the results found by Bang[1] and older investigators.

**Summary.**

Values are given for the normal urea content of the blood and tissues of a number of species of vertebrates.

In vertebrates whose end-product of nitrogenous metabolism is urea, the kidneys are much higher in urea content than are the other tissues in which the urea content is about the same as that of the blood.

Hens injected with alanine do not show any increased amount of urea in the blood and tissues, indicating that urea is not one of the stages in the intermediary metabolism of amino acids in the hen. The kidneys of the hen have the same urea content as the other tissues, showing that urea is not present to any considerable extent in the kidney excretion of the hen.

URBANA, ILL.

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[CONTRIBUTION FROM THE LABORATORY OF THE NORTHWESTERN UNIVERSITY MEDICAL SCHOOL.]

# ON THE ASSUMED DESTRUCTION OF TRYPSIN BY PEPSIN AND ACID.[2]

BY J. H. LONG AND MARY HULL.

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This question is approached from two general standpoints. In one case it is a purely physiological one without reference to any therapeutic application whatever; in the second case the importance of the question comes from its bearing on the administration of ferments as remedies in disorders of digestion. Both points of view are interesting and important. In this laboratory the question comes up now as part of a problem dealing with the second point of view, and mainly through queries suggested by certain investigations prosecuted by the Council on Pharmacy and Chemistry of the American Medical Association, and particularly this question: What is the fate of the so-called pancreatins, administered by the mouth, in passing through the stomach? As digestive remedies have these preparations any real value? Do they, in any way, inhibit or diminish the activity of the pepsin in the stomach, or, on the other hand, are the components of the pancreas preparations themselves weakened or destroyed by the pepsin?

In part the queries suggested here are comparatively simple and easily

[1] *Biochem. Z.*, **72**, 104 (1915).

[2] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.