NITROGEN METABOLISM OF THE ISOLATED TISSUES OF THE RAT

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(Received for publication, May 13, 1935)

Little study has been devoted to the anabolic aspects of nitrogen metabolism in animals. The reason, of course, has been the difficulty of obtaining experimental conditions in which these can be observed, measured, and analyzed. The experiments of Krebs and Henseleit (1) on the formation of urea from ammonia with Warburg's method of surviving slices of liver suggested that this method might be useful in a direct attack on a number of problems of nitrogen anabolism in animals; *i.e.*, it might be possible to observe reactions in which there is a gain in free energy. It appears that for these reactions the intact cell structure is necessary.

With this end in view suitable micromethods were developed for the determination of ammonia, urea, uric acid, creatine (and creatinine), and allantoin. These are described in a previous communication (2). In addition, in the experiments described below amino nitrogen was determined by formol titration with the colorimetric procedure of Northrop (3) and the microvolumetric technique of Linderstrøm-Lang and Holter (4). The details of their adaptation are described below.

With these methods a survey was made of the nitrogen metabolism of the liver, kidney, diaphragm, spleen, and small intestine of the rat. The following results of general significance in nitrogen metabolism were obtained. Deamination occurs at a significant rate only in the kidney and liver. With a mixture of amino acids resulting from the complete enzymatic hydrolysis of egg albumin the rate of urea formation in the liver per unit weight of tissue was the same as ammonia formation in the kidney. As the liver is approximately 4 times the size of the two kidneys, the bulk of the

deamination *in vivo* therefore probably occurs in the liver. The main site of urea formation, as Krebs and Henseleit have shown, is the liver. But it is not the only tissue in which urea can be formed from the catabolism of protein. Arginine is also hydrolyzed in the small intestine to urea (and probably ornithine, though the latter was not measured). No urea formation could be detected in any of the other tissues. Uric acid is formed in every tissue except the liver. Allantoin formation occurs only in the liver. Uric acid is converted in the liver quantitatively and very quickly to allantoin. The precursors of allantoin in the liver are substances other than uric acid. Per unit weight the most active tissue in uric acid or allantoin formation is the small intestine, of which the mucosa is by far the most active part. These results indicate that the uricolytic index in the rat is a measure of the fraction of uric acid which escapes the liver. New formation of creatine was observed in all the tissues except the spleen. With liver but with no other tissue this increase was greater when digest was added to the Ringer's solution. No active creatinine formation as distinguished from the spontaneous production of creatinine from creatine could be detected in the kidney, liver, or diaphragm, either from the creatine in these tissues, or from added creatine.

The animals used in these experiments were Wistar Institute adult white rats. They were fasted 24 to 48 hours, and killed by stunning. The kidney, liver, and spleen were sliced free-hand with a safety razor blade on a filter paper moistened with Ringer's solution. Before slicing the kidney and spleen their capsules were removed. The diaphragm was cut away as close to the ribs as possible, freed from connective tissue, and each half cut into two equal portions. The small intestine (jejunum and upper part of ileum) was freed from mesentery and fat, cut into lengths about 15 mm. long, slit open, and washed thoroughly with a stream of Ringer's solution from a pipette, after any visible intestinal contents had been picked off.

The slices and tissue sections were washed in Ringer's solution prepared according to the formula of Krebs and Henseleit, containing 0.1 per cent glucose, and equilibrated at 37° with a gas mixture containing 95 per cent oxygen and 5 per cent carbon dioxide. Each experiment consisted of a series containing the blanks, in which the tissues were extracted and analyzed immediately

after the washing, and a number of others in which the composition of the experimental Ringer's solutions was varied. In each member of the series relatively large amounts of tissue were used, 20 to 100 mg. of dry weight, requiring, for a large experiment, three or four animals. The slices or tissue sections were distributed in Petri dishes containing about 20 cc. of equilibrated glucose-Ringer's solution, one dish for each member of the series. When a sufficient amount of tissue had been obtained, the slices were transferred from the Petri dishes to the experimental vessels, containing as a rule 5 cc. of Ringer's solution with some other substance dissolved in it whose influence was being investigated. The slices were impaled on tungsten spikes carried on a glass ring into which they were fused. As a rule somewhat less than half an hour elapsed from the killing of the first animal until all the slices were immersed in the experimental solutions.

The vessels containing the tissue were then attached to Warburg manometers, partially immersed in the bath at 37°, and a gas mixture of 95 per cent oxygen and 5 per cent carbon dioxide blown through for 10 minutes. They were then sealed off, completely immersed in the water bath, and rocked at a rate of about 90 cycles per minute for the duration of the experiment, in most cases 4 hours. By means of the manometers the gas exchange was followed. Only those experiments were taken into account in which the tissues respired actively.

At the end of the experiment the tissues were extracted as follows: The Ringer's solution was pipetted into a 25 cc. volumetric flask. The slices were washed with two successive 5 cc. portions of 0.001 N HCl, and the washings added to the volumetric flask which was then immersed in a boiling water bath for 5 minutes. The slices were washed once more with 5 cc. of the acid, which was transferred to a 25 cc. test-tube, after which the slices were removed and added to the test-tube. The vessel itself was washed once again with 5 cc. of the acid which was then transferred to the test-tube. The test-tube containing the slices and washings was placed in a boiling water bath for 10 minutes, after which the liquid was poured into a small beaker. The slices, which were kept in the test-tube, were extracted in the boiling water bath twice more for 10 minute intervals with 5 cc. portions of acid, and finally for 5 minutes with 5 cc. of acid. All the washings

were collected in the beaker, evaporated down on a hot-plate, and added to the 25 cc. volumetric flask. After cooling to room temperature, the contents were diluted with 0.001 N HCl to the mark, and then filtered. The filtrate was used for the analyses. The precipitate of coagulated protein remaining on the filter paper was washed several times with distilled water and then added to the slices which were transferred to small glass cups (the rounded ends of small test-tubes) of about 10 mm. internal diameter, in which they were dried at 100–105° to constant weight.

The free amino nitrogen determinations were carried out as follows: 1 small drop of neutral red solution was added to 3 cc. of the filtrate in a 25 cc. Erlenmeyer flask, and dilute H_2SO_4 (0.135 N) was added until the solution was just acid, after which the solution was evacuated with a suction pump to remove the carbon dioxide. Dilute sodium hydroxide was now added to the solution until it was nearly neutral, and the solution again evacuated to remove any carbon dioxide added with the sodium hydroxide. Approximately 0.3 cc. was taken for titration. Another small drop of neutral red was added, and the solution then titrated to match a phosphate buffer at pH 7.0 containing the same concentration of neutral red. Then an equal volume of commercial formaldehyde, diluted 1:1 with water and neutralized to phenolphthalein, and 6 small drops of 0.2 per cent phenolphthalein were added. This mixture was titrated to match a standard consisting of Ringer's solution containing the same concentration of neutral red, and formaldehyde, one-sixth the concentration of phenolphthalein, and sufficient alkali to develop the maximum color of the phenolphthalein. The alkali used in the second stage minus the titration value of the same quantity of Ringer's solution (or water) similarly titrated in two stages and containing formaldehyde was taken as representing the amino nitrogen plus ammonia present.

The 0.3 cc. aliquots were delivered from a micropipette described by Linderstrøm-Lang and Holter, at a uniform rate with a constant pressure of nitrogen, into small vials 30 mm. high and 7 mm. in inside diameter. The titrating fluid, N/14 NaOH, was delivered from a microburette of the type described by Linderstrøm-Lang and Holter containing 100 c.mm. in a length of approximately 50 cm. The carbon dioxide of the air seriously interferes with the titration carried out on this micro scale. This difficulty was over-

come by carrying out the titration in a closed chamber kept free of air as follows: The vial was screwed up against a brass plate covering the top. Through small holes in this plate pass a small glass stirring rod, the tip of the burette reaching into the solution, and a small tube of about 1 mm. inside diameter extending for only a few mm. below the brass plate. Through this tube a rapid current of alkali-washed nitrogen was passed. This procedure kept the chamber sufficiently free of CO_2. The usual titration figures ranged from 12 to 40 c.mm. of N/14 NaOH for 0.3 cc. of solution. The blank was usually about 6 to 8 c.mm. The titrations were always carried out in triplicate. After some practise extremes differed by not more than 0.3 c.mm.

The present studies, to our knowledge, are the first in which an attempt has been made in experiments with surviving tissue slices to construct a balance sheet of the non-heat-coagulable nitrogen. In all previous studies only one or two constituents have been measured, and the possibility remained that the changes observed were in part or entirely in the initial constituents of the tissues induced by the addition of the substances whose effect was being investigated, and were not changes effected by the tissues in the added substances. By determining the total nitrogen and most of the known nitrogenous constituents at least the gross changes observed could be allocated to the initial constituents of the tissues or to the added digest.

Most of the results are summarized in Table I. They are averages of over 100 experiments. The individual deviations from these averages were small, and were in no case in the opposite direction from those given. The blank values are those obtained from the tissues boiled and extracted at the beginning of the experiment. The others are differences from these blank values when the tissues were maintained in Ringer's solution for 4 hours at 37°.

Considering first the changes in Ringer's solution alone, Table I shows that increases occurred in every category. Computed in terms of the whole animal for 24 hours, these changes are 6 to 10 times greater than the endogenous nitrogen metabolism of the rat (5). These might be interpreted therefore as autolytic changes; *i.e.*, they represent qualitatively abnormal disintegration of the cells. If this is the case, it may be expected that the rate

TABLE I

Changes in Nitrogenous Extractives Effected by Rat Tissues, in 4 Hours at 37.5°, per 100 Mg. of Dry Weight of Tissue

Tissue	Concentration of N added to Ringer's solution	Total soluble N	Amino N	Ammonia N	Urea N	Allantoin N	Uric acid N	Creatine N	Residual N
	mg. per 100 cc.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent total soluble N*
Liver	Blank	+0.74	0.15	0.004	0.048	+0.005	0	0.006	0.71
	0	+0.86	+0.32	+0.018	+0.22	+0.098	0	+0.002	44
	12	+0.67	+0.14	−0.019	+0.48	+0.120	0	+0.0056	
	56	+0.63	−0.11	−0.13	+1.05	+0.144	0	+0.0074	
Kidney	Blank	+1.05	0.25	0.016	0.05	0	0.003	0.042	66
	0	+0.87	+0.58	+0.21	0	0	+0.101	+0.024	29
	12	+0.85	+0.12	+0.50	0	0	+0.118	+0.026	
	56	+0.70	−0.16	+0.67	0	0	+0.178	+0.024	
Diaphragm	Blank	1.19	0.22	0.014	0.044	0	0	0.35	47
	0	+1.01	+0.49	+0.036	+0.014	0	+0.066	+0.061	41
	12	+1.26	+0.18	+0.04	+0.018	0	+0.058	+0.058	
	56	+0.89	+0.11	+0.04	−0.01	0	+0.052	+0.066	
Intestine	Blank	1.27	0.15	0.15	0.016	0	0.032	0.044	65
	0	+4.88	+2.53	+0.16	+0.09	0	+0.286	+0.018	35
	15	+4.28	+2.57	+0.14	+0.12	0	+0.300	+0.020	
	50	+4.56	+2.60	+0.20	+0.19	0	+0.350	+0.012	
Spleen	Blank	1.86	0.37	0.086	0.013	0	0.013	0.041	72
	0	+0.17	+0.42	+0.012	+0.04	0	+0.066	−0.017	52
	15	+0.20	+0.42	−0.053	−0.02	0	+0.081	−0.017	
	50	+0.32	+0.35	−0.065	−0.03	0	+0.090	−0.020	

of increase would not fall off quickly, and might even increase. Actually the maximum rate of increase was in the 1st hour, and became progressively less over a period of 6 hours. It seems therefore that the changes recorded in Table I may be related to the breakdown of labile nitrogenous constituents present in the cell in a limited quantity.

For the time being, until the factors influencing the rate of production of the various substances have been studied further, the data will be interpreted only as revealing the presence or absence of certain potentialities in the different tissues.

The changes in total soluble nitrogen show that protein hydrolysis, *i.e.* the production of non-heat-coagulable nitrogen, may occur in all the tissues. The very large changes observed with intestine are not surprising in view of the active secretory function of this tissue and the hydrolyzing enzymes elaborated there.

The changes in the amino nitrogen and residual nitrogen are additional evidence that some hydrolysis of polypeptides or protein occurred. The largest absolute changes were again in the intestine, but the greatest relative change indicated by the residual nitrogen figures was in the kidney.

In accord with the observations of Krebs (6), the most extensive ammonia formation was in the kidney. The amount of ammonia formed by the intestine is relatively small, considering the enormous increase in free amino nitrogen.

The most active urea formation was, of course, in the liver. But some urea was also formed in the intestine. The values given for the other tissues fall just within the limits of uncertainty of the method.

Allantoin is formed in the liver; uric acid in all other tissues. The very small quantities in the blanks indicate that both these substances are very diffusible, and hence are easily washed out. Experiments in which liver and kidney slices were incubated together proved (taken in conjunction with the specificity of the method) that the substance formed in the kidney is uric acid. Under these conditions no uric acid was found. The allantoin was the sum of the allantoin formed by the liver alone plus the uric acid formed by the kidney alone. Uric acid added to Ringer's solution in which liver slices were suspended was later quantitatively recovered as allantoin.

Increases in total creatine were found with every tissue except the spleen. The largest increases were with diaphragm, the least with liver. The large amounts of creatine in the blanks were to be expected, since most of the creatine is probably held in the tissues as phosphocreatine.

The proof is not as complete with the creatine as it is with uric acid that the chromogenic substance with alkaline picrate is creatinine, derived from creatine. There is no enzymatic method which could be used on the micro scale necessary here; and the quantities are too small to be extracted and identified in the form of a well defined derivative, such as the creatinine zinc chloride, for example. Nevertheless, there are good reasons for accepting the figures as indicative of creatine. First the chromogenic substance with alkaline picrate is adsorbed on Lloyd's reagent and washed. Secondly, the quantity obtained before autoclaving in 0.2 N HCl is very small. Thirdly, the blank values are lower than the creatine figures quoted for these tissues (7).

It has long been known that creatine (and creatinine) synthesis occurs normally in young animals from both exogenous and endogenous protein nitrogen (7, 8) and may apparently be increased by any of a large number of amino acids (9). The data in Table I constitute one of the first pieces of direct evidence that creatine synthesis may be continually in progress in the normal adult animal.

The figures in Table I in the experiments with nitrogen added to the Ringer's solution represent the difference between the amounts found at the end of the experiment minus the blank values and minus the quantities of the various substances added. Where the resulting figure is less than that obtained with Ringer's solution alone, it indicates either retardation of the "autolytic" process, or removal of some of the added material, or both. Where the resulting figure is greater than in plain Ringer's solution, in view of the changes in total soluble nitrogen, it may be taken that the difference represents action of the tissues on some constituent of the added digest.

In most experiments we preferred to use a mixture of amino acids resulting from the complete enzymatic hydrolysis of egg albumin, rather than a single amino acid. For the purposes of this survey it seemed preferable to present to the tissues a physiological mixture of amino acids in approximately physiological concentration.

The changes in total soluble nitrogen afford no evidence of any increased breakdown of tissue consequent upon the addition of amino acids; *i.e.*, of any stimulation of endogenous nitrogen metabolism by the added amino acids.

The amino acid and ammonia figures with liver and kidney are distinctly less than those with plain Ringer's solution. In the case of the liver they represent the conversion of amino nitrogen and ammonia to urea; in the kidney, deamination with the formation of ammonia. The negative figures indicate that the quantity of amino nitrogen or ammonia removed from the mixture of added amino acids was greater than that formed in Ringer's solution alone.

The amount of urea formed in the liver compared with the ammonia formation in the kidney shows that the liver is as active in the deamination of a mixture of amino acids, under comparable conditions, as the kidney. In this respect our results differ from those of Krebs who found in experiments with individual amino acids suspended in a phosphate buffer solution relatively much less urea formation in the liver than ammonia in the kidney. It is possible, in fact there is definite indication in Krebs' data, that the rate of deamination of amino acids in the liver, similar to the rate of urea formation from ammonia (1), is faster in a CO_2-bicarbonate-Ringer's solution than in the less physiological phosphate buffer solution at the same pH.

More urea was formed in the liver than could be accounted for by the amino nitrogen and ammonia which had disappeared. This excess was greater than the urea which could have arisen from the hydrolysis of all the added arginine. One of the sources of non-amino nitrogen convertible to urea is probably histidine. Edlbacher and Neber (10) have demonstrated the existence in the liver of an enzyme capable of converting at least one of the imidazole nitrogen atoms of histidine to ammonia. We found that histidine added to liver slices led to a marked increase in free ammonia and urea. In the experiments with the mixture of amino acids the urea formed could be accounted for by the amino nitrogen and ammonia which had disappeared, and by assuming that all the arginine was hydrolyzed and that the imidazole nitrogen of all the added histidine was converted to urea. It seems improbable from our experiments with histidine alone that all the histidine added with the digest was decomposed. It is more probable that there

are still other forms of non-amino nitrogen, proline for example, which the liver can convert to urea.

In the kidney only part of the amino nitrogen which had disappeared could be accounted for as ammonia, uric acid, or creatine. Krebs (6) similarly observed a much greater disappearance of amino groups when individual amino acids were added to kidney slices than could be accounted for by the increase in ammonia.

Table II shows the formation of urea from arginine in the small intestine. The urea formed in the Ringer's solution alone could have arisen from arginine liberated by the "autolysis" of the intestine. Table I shows there is a great increase in amino nitrogen when the small intestine is incubated in Ringer's solution for

TABLE II

Urea Formation by Rat Small Intestine, in 4 Hours at 37.5°, per 100 Mg. of Dry Weight of Tissue

Solution No.	Additional contents of Ringer's solution (5 cc.)	Urea N
		mg. × 10^3
1	Blank; tissue analyzed immediately	7
2	None	120
3	Complete enzymatic hydrolysate of egg albumin; N = 32 mg. %	112
4	(3) + 15 mg. % NH_3-N	100
5	(3) + 10 " % ornithine	105
6	(3) + 15 " % NH_3-N + 10 mg. % ornithine	82
7	20 mg. % arginine	365

4 hours at 37.5°. Table II also shows that no other amino acid, with or without added ammonia, nor ammonia itself can serve as a precursor of urea in the intestine.

As pointed out above the method of surviving tissue slices holds out the hope of observing and possibly of analyzing anabolic processes. In the physicochemical sense the synthesis of urea from ammonia and carbon dioxide is such a process because in this "half reaction" there is a gain in free energy which must be obtained from an energy-yielding reaction with which it must be coupled.[1] From the physiological point of view urea formation

[1] The formation of urea from ammonia and carbon dioxide may be written as

$$2NH_3 + H_2CO_3 \text{ (aqueous)} = CO(NH_2)_2 + 2H_2O \quad (1)$$

is, on the contrary, a catabolic phenomenon. This complete antithesis between physicochemical and physiological classification raises the question of whether it will be profitable to retain the classical concepts of anabolism and catabolism. Of anabolic processes in both the physiological and physicochemical sense we have obtained clear indications only of the formation of amino groups from ammonia, in the kidney and in the spleen.[2] The data

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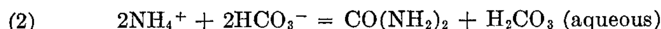
or as



(2) $$2NH_4^+ + 2HCO_3^- = CO(NH_2)_2 + H_2CO_3 \text{ (aqueous)}$$

According to Equation 1 ΔH for the conditions in the plasma is -6600 calories; for Equation 2 $+11{,}700$ calories. The difference is the energy of ionization. The reaction in Equation 1 is exothermic, in Equation 2 endothermic. *A priori* either reaction is possible. From consideration of the heat changes alone it is obviously not safe even to guess whether the synthesis of urea from ammonia and CO_2 could proceed spontaneously or not; *i.e.*, whether it is dependent on another energy-liberating reaction or not. This uncertainty is removed when the free energy data are taken into account. Since the ions and the undissociated molecules are in equilibrium, the free energy change ΔF is the same whether calculated from Equation 1 or 2, and under the conditions existing in the plasma amounts to $+14{,}300$ calories. The synthesis of urea under these conditions therefore cannot proceed spontaneously.

[2] The formation of an amino acid from ammonia and a non-nitrogenous acid may involve a positive or a negative free energy change. It would be unsafe therefore without definition of the actual chemical reaction which has occurred to conclude that an increase in free amino groups at the expense of ammonia necessarily implied the existence of another reaction coupled with it yielding the energy for this synthesis. For example, the free energy change, ΔF, in the oxidative deamination of alanine, $CH_3CH(NH_3)^+COO^- + \frac{1}{2}O_2 = CH_3C(O)COO^- + NH_4^+$, is approximately $-39{,}000$ calories, when all the reactants are taken at 1 M concentration. From this value the equilibrium relationship

$$((\text{Pyruvate}^-)\ (NH_4^+))/((\text{alanine}^\pm)\ (O_2)^{0.5}) = K = 10^{29.3}$$

is enormously in favor of deamination. The synthesis of alanine under physiological conditions therefore can proceed only if coupled with another energy-yielding reaction. On the other hand, in the reaction *l*-aspartate$^\pm$ = fumarate$^=$ + NH_4^+ the equilibrium expression is

$$((\text{Fumarate}^=)\ (NH_4^+))/(\text{aspartate}^\pm) = 0.011$$

In our experiments where the concentration of ammonia was approximately 0.003 M, an initial concentration of fumarate of 0.116 per cent (0.01 M) would spontaneously in the presence of the suitable enzyme lead to the conversion of approximately half the ammonia to aspartate, without the intervention of any other reaction (11, 12).

will be published later as part of a detailed and extensive study of this phenomenon alone.

From the data in Tables I and II we may infer that, while protein hydrolysis may occur in all tissues, deamination of amino acids takes place only in the liver and kidney. But since the rate of determination of a mixture of amino acids under comparable conditions is approximately the same in both tissues, and as the liver is 4 to 5 times as large as both kidneys combined, the bulk of the deamination *in vivo* probably takes place in the liver. The following approximate computation points to the same conclusion. A fair estimate of the blood flow through the kidneys of a dog weighing 10 kilos is 200 liters in 24 hours (13). The average increase in ammonia in the blood of the renal vein over that in the artery 2 minutes after the injection of alanine is 2 to 3.3 mg. per liter (14, 15). The normal difference is of the order of magnitude of 1 to 2 mg. per liter. If the high figure is taken, the total nitrogen deaminized in the kidney in 24 hours would be less than 1.0 gm. The urinary urea nitrogen in a dog in 24 hours may be over 30 gm. This figure seems even beyond the possibilities of blood flow through the liver. The preferential catabolism of nitrogen in the liver is facilitated by the storage of amino acids in the liver in the first hours after the passage of amino acids into the blood stream (16), and by the presence there of large quantities of labile nitrogen (17).

Urea formation is also a predominantly hepatic function. The only urea originating from the catabolism of protein nitrogen which can be formed in the intestine can be derived only from arginine, absorbed from the intestinal canal during digestion, or from the systemic blood.

The data in Table I show that the digest employed contained some material which augmented the uric acid production by the kidney and spleen and of allantoin by the liver. The same digest inhibited somewhat the uric acid production of the diaphragm. On account of the difficulty of obtaining uniform samples of small intestine it has not been possible as yet to determine with certainty the effect of the digest on its uric acid production.

The precursor of uric acid and allantoin present in the digest has not yet been identified. Glycine, alanine, histidine, lysine,

arginine, tryptophane, glutamic acid, proline, alloxan, uracil, and urea all gave negative results. Experiments with the purines indicated that guanine, xanthine, and possibly hypoxanthine, but not adenine, may be the precursors here. It is possible that these purines were added with the enzyme preparations in the preparation of the hydrolysate. This is being studied further.

The relatively high concentration of creatine in the liver, kidney, and diaphragm blanks contrasted with the negligible quantity of uric acid or allantoin indicates the great difference in the diffusibility from the tissues of these substances. This difference is all the more striking because, as Table I shows, all the tissues form uric acid or allantoin at a much greater rate than they do creatine.

The data in Table I show some new formation of creatine from exogenous nitrogen in the liver. This result was obtained in every one of twenty-four experiments with animals of both sexes, varying in age from 4 to 12 months. Small as they are, the values with liver, we believe, are the most reliable in the whole series. The sampling was the most uniform with this tissue, large amounts could be used, and the amount of creatine in the blank was so little that absolutely small but relatively large differences could be determined easily. On the other hand, it is uncertain whether there was any increased formation of creatine with intestine and diaphragm when digest was added. In the case of the intestine the sampling was not sufficiently uniform; with the diaphragm the blank value was too large for the detection of small increases.

SUMMARY

1. Several aspects of the nitrogen metabolism of the isolated liver, kidney, diaphragm, and small intestine of the rat have been studied by use of surviving slices or sections of these tissues.

2. When these tissues were incubated in Ringer's solution, increases were observed in non-heat-coagulable soluble nitrogen, free amino nitrogen, and ammonia in all the tissues. In every tissue except the liver uric acid, without any allantoin, was formed. Allantoin was formed only in the liver, and from precursors other than uric acid. Creatine was formed in all the tissues except the spleen.

3. The addition of a complete enzymatic hydrolysate of egg

albumin led to a slight decrease in the amount of non-heat-coagulable soluble nitrogen, an increase in ammonia in the kidney, of urea in the liver and intestine, an increase in uric acid in the kidney and spleen, and allantoin and creatine in the liver.

4. Deamination of amino acids occurred only in the kidney and liver, and at approximately the same rate in each tissue. From these results and certain general physiological considerations it follows that the bulk of the deamination *in vivo* probably occurs in the liver.

5. Though most of the urea derived from the catabolism of protein nitrogen is formed in the liver, some urea formation from arginine can occur in the small intestine.

6. Urea is formed in the liver from sources other than arginine, free amino nitrogen, or ammonia. One of these sources is probably histidine.

7. Uric acid is converted quantitatively to allantoin in the liver.

8. The significance of the observations on uric acid and allantoin formation is discussed in regard to the interpretation of the uricolytic index.

9. No acceleration of creatinine formation from creatine could be observed with slices of surviving liver, kidney, or diaphragm.

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