THE FORMATION OF GLYCOCYAMINE IN ANIMAL TISSUES*

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It was shown in preceding communications that glycocyamine is converted into creatine by surviving liver slices (1). Our findings indicated that the methylating agent is methionine or a derivative of methionine. Liver slices can methylate glycocyamine rapidly enough to permit assignment to the liver alone, if necessary, of the task of making good the loss of creatine and creatinine in the urine. This holds for the livers of all mammals studied. We found no evidence of this methylating mechanism in any other tissues, except possibly slight activity in the kidney. In the pigeon the kidney is as effective in this respect as the liver.

These experimental facts were corroborated (as far as the rat is concerned) by experiments on living animals with tracers. Bloch and Schoenheimer, using N^{15}, found that glycocyamine is readily converted to creatine (2). Du Vigneaud and his collaborators fed rats methionine with deuterium in the methyl group; after only 3 days a relatively large quantity of deuterium was found in the muscle creatine (3).

Glycocyamine has had a favored position among the possible precursors of creatine. It is nearer to creatine structurally than any other precursor which has been proposed; and its convertibility to creatine in the living organism has been proved. The case against glycocyamine rested on two arguments: (1) that only a small fraction, 5 to 15 per cent, of administered glycocyamine is methylated, and (2) that glycocyamine had not been found as a normal constituent of animal tissues. It has, however, been isolated by Weber from human and dog urine (4).

* A summary of this work has appeared (*Science*, **91**, 551 (1940)).

The findings with surviving liver slices and the tracer studies reinstated glycocyamine, more firmly than before, as a possible normal precursor of creatine. The quantity of creatine synthesized daily is so large that, if glycocyamine is its normal precursor, an active mechanism for the formation of glycocyamine must exist. We undertook a search for this mechanism.

The first necessity was an adequate analytical method. Such a method is described in the preceding communication (5).

With it we have found that kidney slices rapidly form glycocyamine from arginine and glycine. All other tissues tested are negative in this respect.

This interaction of arginine and glycine is also catalyzed by thoroughly macerated cell-free kidney tissue suspended in a phosphate buffer solution.

The formation of glycocyamine from arginine and glycine is a new biochemical reaction which may be called "transamidination." We propose that the enzyme be designated "glycine-transamidinase." The discovery of this reaction provides direct proof that arginine and glycine are precursors of creatine.

Bloch and Schoenheimer fed ammonia containing N^{15} to rats and later found the isotope in the amidine nitrogen of creatine. After glycine containing N^{15} was fed, the isotope was found in creatine in the sarcosine nitrogen. In a later communication the same authors presented more direct evidence (again obtained by the use of N^{15}) which confirmed the findings we had reported that glycocyamine is formed by the transfer of the amidine group from arginine to glycine. They fed $l(+)$-arginine with N^{15} in the amidine group to rats (6); afterwards the creatine in the muscles had a far higher isotopic content than after the administration of isotopic ammonia, urea, or any other amino acid except glycine. It was so high that they considered that the amidine group of creatine must have originated from arginine.

The present communication contains the details of our experimental procedure, our findings on the effects of a fairly large number of amino acids and derivatives of arginine and of glycine, surveys of the capacity for glycine transamidination of the organs of a number of common experimental animals, and the results of some studies of the effect of concentration of reactants, pH, and time on the rate of transamidination.

Technique and Results

The tissue slice technique used and the details of the reaction vessels are described in a previous communication (7).

At the end of an experiment the contents of the reaction vessels were transferred with the slices to test-tubes graduated at 20 ml.; the vessels were washed with three 2 ml. portions of 0.02 M phosphate buffer solution at pH 6.0 and the washings added to the main solution. The pH was adjusted finally to 6 with a drop of 0.5 N hydrochloric acid. The test-tubes containing the slices, the main solutions, and washings were kept in a boiling water bath for 10 minutes, after which they were cooled to room temperature, and made up to the 20 ml. mark with water and mixed by shaking. These solutions were then filtered. 5 ml. of the clear protein-free filtrate were analyzed for glycocyamine by the procedure described in the preceding communication (5).

The coagulated slices and protein in each test-tube were transferred to small glass weighing dishes, heated overnight at 105°, and, after cooling in a desiccator, were weighed.

Table I is the detailed protocol of a typical experiment. A significant amount of glycocyamine is formed when arginine alone is added to the Ringer's solution. Glycine without arginine also leads to a slight increase in glycocyamine. When both amino acids are added together, the increase in glycocyamine is more than 10 times the increase with arginine alone. The increases obtained with arginine alone and with glycine alone indicate either the presence of these amino acids in the free state (more glycine than arginine) or their formation in small amounts by autolysis within the slices.

In the experiment whose results are recorded in Table I, and in a number of others, creatine analyses were carried out with the specific bacterial (NC) enzyme of Dubos and Miller (8, 9). No evidence of creatine formation was found.

The steps in the proof that the substance we were measuring was glycocyamine were as follows: Autoclaving in acid solution produced a substance which was adsorbed by Lloyd's reagent and gave a positive test with the Jaffe reagent. This chromogenic material was not digested (before autoclaving) by the NC bacteria of Dubos and Miller under conditions in which creatine and creatinine were completely digested. A strongly positive Saka-

guchi test was obtained in the unautoclaved solution after all the arginine was removed by exhaustive adsorption on permutit. The depth of color which the unknown solution gave with the Jaffe reagent (after autoclaving) corresponded, assuming it to be glycocyamine, to the intensity of color it gave in the Sakaguchi reaction after removal of the arginine. A liter of solution was

TABLE I

Protocol of Typical Experiment Showing Formation of Glycocyamine from Arginine and Glycine by Rat Kidney Slices

Ringer's solution, 38°, 4 hours.

Dry weight of slices (1)	Ringer's solution (2)	0.02 M arginine in Ringer's solution (3)	0.04 M glycine in Ringer's solution (4)	Concentration of glycocyamine in aliquot taken for analysis (5)	Glycocyamine found per 100 gm. fresh tissue (6)
mg.	*ml.*	*ml.*	*ml.*	*mg. per cent*	*mg.*
26.4	4			0.02	6
30.4	4			0.04	11
22.5	3	1		0.10	36
21.0	3	1		0.11	42
20.0	3		1	0.04	16
22.0	3		1	0.05	18
23.6	2	1	1	1.03	349
21.4	2	1	1	0.97	363

The figures in Column 6 are obtained by multiplying those in Column 5 by 8000 and dividing them by the dry weight of the tissue in mg. (Column 1). The figure 8000 is obtained as follows: the solution is diluted 5-fold before analysis; in the course of analysis it undergoes a further 1:1 dilution; the results in Column 5 expressed as mg. per cent must be divided by 25, since there were only 4 ml. of the original reaction solution; to express the results on the basis of 100 gm. of fresh tissue, the factor $100{,}000/5 \times W$ is used, W being the dry weight in mg. given in Column 1. The factor therefore is $5 \times 2 \times 1/25 \times 100{,}000/(5 \times W) = 8000/W$.

now collected in which kidney slices had acted upon arginine and glycine, and which contained, according to analysis, about 50 mg. of glycocyamine. The glycocyamine was isolated by adsorption on Lloyd's reagent in acid solution, elution with baryta, removal of the arginine by repeated adsorption with permutit, and crystallization from glacial acetic acid as glycocyamine acetate. These crystals were the characteristic needles and thin prisms (10).

The free glycocyamine was regenerated from the acetate by boiling in dilute aqueous solution and crystallized by evaporation of the water. 25 mg. of crude glycocyamine were thus obtained. It was thrice recrystallized from water, with a final yield of 11 mg. of the pure dry material which was analyzed. It gave the following figures.[1]

Observed.	C 30.8, H 5.95, N 35.8
Theoretical for glycocyamine.	" 30.8, " 6.0, " 35.9

TABLE II

Formation of Glycocyamine by Rat Kidney Slices from Arginine and Glycine or Glycine Derivatives

Glucose-Ringer's solution, 3 hours, 38°. Concentration of arginine 0.005 M; glycine or derivatives 0.01 M.

Arginine	Glycine or glycine derivative	Glycocyamine found per 100 gm. fresh tissue
		mg.
−		8
+		35
−	Glycine	19
+	Betaine	44
+	Glutathione	296
+	Glycine	382
+	" anhydride	8
+	Glycylglycine	436
+	Glycolic acid + ammonia	6
+	Hippuric acid	35
+	Leucylglycine	254
+	Sarcosine	109
+	" anhydride	6

Table II summarizes the relative effectiveness of glycine and some glycine derivatives as precursors of glycocyamine. The effect of the glycine peptides is accounted for on the hypothesis that these are first hydrolyzed and that it is the free glycine which reacts with arginine to form glycocyamine. The argument is as follows: The rate of glycocyamine formation is proportional to the concentration of free glycine (Table VIII). When glycine

[1] We are indebted to and wish to thank Dr. A. J. Haagen-Smit for these analyses.

peptides were the source of the glycine, the concentration of free glycine was initially 0 and only in the course of the 3 hour experimental period did it approximate 0.01 M, whereas when glycine itself was added the initial concentration was 0.01 M. Hence smaller amounts of glycocyamine were formed from glutathione, leucylglycine, and hippuric acid than from the same initial concentration of glycine. Glycylglycine gave a higher value than glycine, because on hydrolysis it yields 2 molecules of glycine and as a result the concentration of glycine rose well above 0.01 M before the end of the 3 hour period.

It follows, if the above is the correct explanation of the effect of glycine peptides, that rat kidney contains a dipeptidase for leucylglycine, an enzyme which liberates glycine from glutathione, and no enzymes capable of forming free glycine at a significant rate from betaine, glycine anhydride, or hippuric acid.

The results with sarcosine indicate that this substance is demethylated without deamination in rat kidney. Separate analyses showed that no creatine was formed; this proved that demethylation of the sarcosine had occurred prior to the transamidination. The kidney contains, therefore, an enzyme which demethylates sarcosine.

These findings with sarcosine are complemented by those of Bloch and Schoenheimer (2, 11) who, using N^{15} as a tracer, found that sarcosine is converted to glycine *in vivo* and that in the course of the demethylation the glycine nitrogen originally attached to the carbon chain is not replaced. Their experiments therefore excluded intermediate deamination of sarcosine in the course of its conversion to glycine.

Analogous to its inability to hydrolyze glycine anhydride the kidney is unable to hydrolyze sarcosine anhydride.

The negative result shown in Table II with glycolic acid and ammonia indicates that rat kidney is unable to form glycine from these two substances at a significant rate.

The experiments summarized in Table III revealed that rat kidney evidently is able to synthesize arginine from citrulline. The other possible amidine donators which were tested, guanidine, ornithine, and urea, were negative.

The positive result with citrulline was retested in a number of more adequately controlled experiments. A group of typical

TABLE III

Formation of Glycocyamine by Rat Kidney Slices from Glycine and Arginine or Other Possible Donators of Amidine Group

Glucose-Ringer's solution, 3 hours, 38°. Concentration of glycine 0.01 M; of arginine or other amidine donators 0.005 M.

Glycine	Amidine donator	Glycocyamine formed per 100 gm. fresh tissue
		mg.
−		35
−	*l*(+)-Arginine	68
+	"	498
+	*l*(+)-Citrulline + ammonia	330
+	Guanidine	16
+	*d*(−)-Ornithine + ammonia	12
+	Urea	33

TABLE IV

Formation of Glycocyamine from Glycine and Citrulline by Rat Kidney Slices and by Cell-Free Macerate of Rat Kidney

Slices in glucose-Ringer's solution, macerate in 0.1 M phosphate buffer, pH 7.0, 3 hours, 38°. Glycine 0.01 M; amidine donator (ammonia, arginine, citrulline, or ornithine) 0.005 M.

	Glycine	Amidine donator	Glycocyamine formed per 100 gm. fresh tissue
			mg.
Kidney slices	−		22
	+		31
	−	Ammonia	18
	−	Arginine	68
	−	Citrulline	58
	+	Arginine	357
	+	Citrulline	200
	+	" + ammonia	227
	+	Ornithine + "	8
Cell-free macerate	−		30
	+	Arginine	120
	+	Citrulline + ammonia	59

results is shown in Table IV. Citrulline was only slightly less[8] effective than citrulline plus ammonia.

Included in Table IV are some typical results obtained with a

cell-free macerate of kidney. This suspension was less active than an equivalent amount of kidney tissue in the form of slices but qualitatively the results were in every respect the same.

The suspension was made by the homogenizing procedure of Potter and Elvehjem (12). The kidney was stripped of its capsule, split down the longitudinal axis, the pelvic fat and the medulla cut away, the remainder homogenized with 4 times its weight of 0.01 M phosphate buffer at pH 7.0, and the resulting suspension passed through gauze. Such a suspension retains all its transamidinase activity for at least 2 months if kept in an ice box.

The optimum pH of glycine-transamidinase is in the neighborhood of pH 7.0 (Table V).

TABLE V

Effect of pH on Activity of Glycine-Transamidinase

38°, 4 hours. Glycine 0.01 M; arginine 0.005 M.

pH	Glycocyamine formed per 100 gm. fresh tissue
	mg.
6.0	214
6.5	259
7.0	320
7.5	278
8.0	246

The activity of glycine-transamidinase in cell-free solution is unaffected by 0.001 M potassium cyanide or by carrying out the reaction *in vacuo*. For example, one extract under the same conditions as those described above formed 67 mg. of glycocyamine per 100 gm. of fresh tissue; in the presence of 0.001 M potassium cyanide it formed 80 mg. and anaerobically, 72 mg.

The following amino acids and amides were tested with rat kidney slices and arginine as possible precursors of the glycine radical in glycocyamine. The initial concentration in every case was 0.005 M. They were all negative: *d*-alanine, *dl*-alanine, *l*-asparagine, *l*-aspartic acid, *l*-cysteine, *l*-cystine, *d*-glutamic acid, *d*-glutamine, *l*-hydroxyproline, *l*-histidine, *dl*-isoleucine, *l*-leucine, *d*-lysine, *dl*-methionine, *dl*-norleucine, *l*-proline, *dl*-phenylalanine, *dl*-serine, *d*-threonine, *l*-tryptophane, *l*-tyrosine,

and *d*-valine. These negative results indicate that under the conditions of these experiments none of these amino acids is a precursor of glycine.

Glycine-transamidinase activity was found in the kidney of every animal tested except the frog (Table VI). Whenever activity was found in kidney slices, it was also found in the cell-free extract. The beef and sheep kidneys were used at least 24 hours after the animals were slaughtered; they were obtained in a butcher shop. In the cases of all the other animals the kid-

TABLE VI

Formation of Glycocyamine from Glycine and Arginine by Kidney Slices and by Cell-Free Macerate of Kidney of Various Animals

Slices in glucose-Ringer's solution, macerate in 0.1 M phosphate buffer, pH 7.0. Glycine 0.01 M; arginine 0.005 M. 38°, 3 hours.

Animal	Glycocyamine formed per 100 gm. fresh tissue by	
	Kidney slices	Cell-free macerate
	mg.	*mg.*
Beef		190
Cat	93	32
Dog	281	480
Frog		0
Guinea pig	38	14
Pigeon	27	16
Rabbit	187	160
Rat	357	120
Sheep		160

neys were removed immediately after the animals were killed and the extracts made soon afterwards. The negative results with extracts of frog kidney call for further study.

Liver slices and cell-free extracts of heart and of muscle of all the animals listed in Table VI were tested for glycine-transamidinase activity. Except in the case of the pigeon (see below) they were all negative. The blood, brain, intestine, and spleen of the rat were also examined; they were negative. Rat liver slices also gave negative results with glycine plus arginine, urea, or guanidine.

Before the advent of Weber's method no glycocyamine could be demonstrated in animal tissues. With this method glyco-

cyamine was detected in urine (4), in intestine, testes, and kidney (13).

Using our more sensitive method, we found glycocyamine to be widely distributed in the tissues of the rat. The concentrations (mg. per 100 gm. of fresh tissue) were blood 0.5 to 1; brain, heart, liver, skeletal muscle, and spleen 3 to 6; small intestine 10; kidney (cortex) 15 to 30.

The question arose whether transamidination in the kidney can be sufficiently rapid under physiological conditions to account for the total production of creatine in the body as indicated by the

TABLE VII

Rate of Glycocyamine Formation by Rat Kidney Slices at 38° from Arginine Initially 0.005 M and Glycine 0.01 M

Time	Glycocyamine per 100 gm. fresh tissue		
	Found	Formed	Average rate of formation per hr.
hrs.	*mg.*	*mg.*	*mg.*
0	32		
0.5	95	63	126
1.0	164	132	132
2.0	315	283	142
4.0	505	473	118
6.0	741	709	118

daily excretion in the urine of creatine and creatinine. A number of experiments were carried out to obtain what information we could on this point.

Table VII shows that the glycine-transamidinase in rat kidney slices remained practically unimpaired for 6 hours. This is in accord with the stability of the enzyme in cell-free extracts.

In Table VIII are some figures on the effect of the concentration of the reactants, arginine and glycine, on the rate of transamidination. With equal arginine and glycine concentrations from 0.001 to 0.0001 M the rate was approximately linear with respect to concentration.

We can estimate what the rate of glycocyamine formation in the kidney must be to make good the loss of tissue creatine which appears in the urine as creatinine. Two human kidneys

weigh approximately 300 gm. An average figure for the creatinine excreted in the urine in 24 hours is 1.7 gm. To make good this loss the average hourly production of glycocyamine which is necessary must be approximately 25 mg. per 100 gm. of tissue per hour.

This rate of glycocyamine formation would have been obtained in the experiments of Table VIII with concentrations of glycine and arginine between 0.0005 and 0.001 M. This is probably the concentration range of these amino acids in kidney. The basis of this estimate is as follows: The arginine concentration in dog

TABLE VIII

Variation in Rate of Glycocyamine Formation by Rat Kidney Slices with Different Concentrations of Arginine and Glycine

Ringer's solution, 4 hours, 38°.

Initial concentration		Glycocyamine formed per 100 gm. fresh tissue	Initial concentration		Glycocyamine formed per 100 gm. fresh tissue	Initial concentration		Glycocyamine formed per 100 gm. fresh tissue
Arginine	Glycine		Arginine	Glycine		Arginine	Glycine	
mole per l.	*mole per l.*	*mg.*	*mole per l.*	*mole per l.*	*mg.*	*mole per l.*	*mole per l.*	*mg.*
0.02	0.01	190	0.005	0.02	617	0.005	0.005	360
0.01	0.01	590	0.005	0.01	533	0.0025	0.0025	310
0.005	0.01	533	0.005	0.005	361	0.001	0.001	134
0.0025	0.01	535	0.005	0.0025	252	0.0005	0.0005	78
0.00125	0.01	303	0.005	0.00125	201	0.0001	0.0001	20
0.000625	0.01	201	0.005	0.000625	103			

blood is between 2.6 and 3.9 mg. per cent (14); we have found 2.0 to 5.0 mg. per cent in human blood. This range corresponds to 0.0001 to 0.00025 M. The concentrations of these amino acids in the kidney are certainly much higher than in blood, since the total free amino nitrogen in kidney and other tissues is 10 times or more that in blood (15, 16). The rates of glycocyamine formation recorded in Table VIII are sufficient therefore to make good the total loss of creatine from the tissues. This estimate is, of course, based on the assumption that the rate of glycocyamine formation in human kidney *in vivo* is of the same order as in rat kidney slices *in vitro*, which seems not unreasonable.

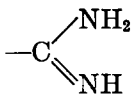
DISCUSSION

The experimental results given above, confirmed and complemented as they now are by the findings in studies with tracers, make a strong case for the following mechanism of creatine formation in animals: (*a*) arginine and glycine in the kidney form glycocyamine; (*b*) in the liver glycocyamine and methionine form creatine. The mechanisms for these reactions are widely distributed in mammals. The quantitative aspects of the data show that the transamidination mechanism for the formation of glycocyamine is fast enough to replace all the creatine lost from the body. Other mechanisms of creatine formation are not excluded; but the tracer studies indicate that the arginine-glycine-methionine mechanism is quantitatively the most important one *in vivo*.

The argument *contra* glycocyamine, that it had never been found as a constituent of animal tissues, antedated the development of suitable analytical methods. This argument is now completely disposed of by the detection of glycocyamine in practically all tissues.

The controversy whether arginine is a precursor of creatine (17, 18) may be considered as settled in favor of the affirmative. The path of its conversion to creatine, however, is different from what was generally believed. It was proposed that arginine was converted to glycocyamine by way of deamination and β oxidation; thus all of the nitrogen was derived directly from arginine. This made it necessary to explain away the evidence which suggested that glycine played some part in creatine formation, unless it be in the methylation of glycocyamine.

Bergmann and Zervas were the exception. They observed that "triacetyl anhydro arginine" and glycine ethyl ester reacted in the absence of water to give a fairly good yield of diacetyl glycocyamine ethyl ester (19); and the same arginine derivative with sarcosine ethyl ester gave diacetyl creatine ethyl ester (20). Their view was that, "der wesentliche Punkt der biologischen Kreatinbildung in einer direkten Umsetzung eines reaktionslustigen Argininabkömmlings mit einem Aminoäthanderivat beruht." This clear statement that the guanidine group of creatine arises *in vivo* by transamidination needs now to be modified in two respects: that an enzyme, glycine-transamidinase, and not a

reactive derivative or split-product renders the  group in arginine labile as it is in "triacetyl anhydro arginine." The enzyme arginase exerts a similar influence. The other modification is that glycine itself and not a derivative is the acceptor of the amidine group in the biological transamidination reaction.

We may now infer that one of the reasons for the essential character of arginine and methionine in the rat is that they participate in the formation of creatine. These amino acids, of course, also serve other functions, for example the rôle of arginine in urea formation and of methionine in other methylation reactions (3), in addition to their participation in the constitution of tissue protein.

The recent findings on essential amino acids for the chick indicate that arginine and glycine are required for creatine formation in this animal. Arnold *et al.* (21) reported that arginine is essential for rapid growth. This was confirmed by Klose *et al.* (22) who showed further that arginine is necessary for maintenance as well as growth. Recently Klose and Almquist reported that citrulline is as effective as arginine, whereas ornithine alone or with urea is ineffective (23). We have found (Tables III and IV) that glycocyamine is formed from citrulline and glycine, the citrulline presumably being first converted to arginine, while ornithine, with or without added ammonia, is completely negative.

The parallel between the amino acid requirements for creatine formation and for growth was extended further when Almquist *et al.* found that glycine is essential for the growth of the chick (24) and that creatine as a substitute for glycine is even more effective than glycine itself. Glycolic acid and betaine could not replace creatine (25).

We have examined the organs of the pigeon for transamidinase activity. Activity was found not only in the kidney but also in heart, liver, and skeletal muscle. The limiting amino acid appeared to be glycine; *i.e.*, nearly as much glycocyamine was formed when glycine alone was added as from glycine and arginine together. The differences between experimental and control were, however, small in absolute terms compared with those found in the kidneys of other animals. Although these

differences were greater than could be ascribed to analytical or sampling variations, we cannot consider our findings in the pigeon as established until more determinations have been made. For this reason the detailed figures are not presented here. We hope that other workers with facilities for a study such as this on birds may undertake the investigation. Our laboratory does not at present possess such facilities.

SUMMARY

1. Beef, cat, dog, guinea pig, pigeon, rabbit, rat, and sheep kidney form glycocyamine from arginine and glycine. This reaction is catalyzed by cell-free extracts of kidney as well as by surviving kidney slices.

2. It is proposed that this reaction be designated "transamidination," and the enzyme "glycine-transamidinase." The optimum pH of this enzyme is about 7.0. It is not affected by potassium cyanide nor by anaerobiosis.

3. Transamidination does not occur in the liver, heart, or skeletal muscle of the animals mentioned above; the blood, brain, and spleen of the rat were tested also and found inactive. It is possible that in the pigeon a low glycine-transamidinase activity resides in liver, heart, and skeletal muscle as well as in kidney.

4. Glycocyamine is also formed in the kidney from glycine and citrulline. Glycine plus ornithine (with or without ammonia), urea, or guanidine is negative in this respect.

5. A large number of amino acids, several amides, and anhydrides were tested as possible precursors of the glycine radical of glycocyamine. They were all negative, as was also glycolic acid plus ammonia.

6. Glycocyamine is formed from arginine and sarcosine. Evidence is presented that the sarcosine is first demethylated, thus being converted to glycine, indicating the presence of a demethylating enzyme in kidney. Sarcosine anhydride is negative.

7. The above findings, complemented by the tracer studies in the laboratories of Schoenheimer and of du Vigneaud, and in conjunction with our previous findings, prove the existence of the following mechanism of creatine formation in animals: arginine and glycine form glycocyamine in the kidney; the glycocyamine is methylated in the liver by methionine (or a derivative of methionine) to form creatine.

8. Quantitative aspects of the data indicate that all of the creatine formed in animals may normally be formed by this mechanism.

9. Evidence of the generality of transamidination is seen in the close parallel between the above findings and those on amino acids essential for the growth of the chick.

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