XXIX. PROBLEMS OF NITROGEN CATABOLISM IN INVERTEBRATES.

III. ARGINASE IN THE INVERTEBRATES, WITH A NEW METHOD FOR ITS DETERMINATION.

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ARGINASE was discovered in mammalian liver by Kossel and Dakin [1904, 1, 2] who showed that the products of its action are ornithine and urea. Since that time the enzyme has attracted a great deal of interest from many points of view, but for present purposes it is sufficient, without going into the history of the subject, to point out that until very recently it was generally supposed that a small proportion of the urea excreted by mammals arises by the action of the liver arginase upon ingested arginine. Krebs and Henseleit, however, brought forward convincing evidence [1932] for supposing that the whole of the urea so excreted is elaborated by means of a cyclical mechanism involving arginase. In the meantime a generalisation of the greatest comparative importance was made by Clementi [1914; 1915], who pointed out that arginase could be detected in the livers of animals having a ureotelic metabolism, but not in cases where the metabolism is uricotelic in character. Manderscheid [1933] has extended the work of Krebs and Henseleit [1932] to other vertebrates and found the same cyclical system operating in those having the ureotelic type of metabolism, thus providing an explanation for the empirical rule enunciated by Clementi.

The distribution of arginase among vertebrate tissues has been carefully studied by a number of workers [Edlbacher, 1915; Hunter and Dauphinee, 1924, 1, 2; Edlbacher and Bonem, 1925] but the invertebrates have received scant attention. Table I presents the results obtained in the few cases hitherto

Table I.

Animal and species	Part examined	Arginase	Author
Termite larvae	Whole body	—	Clementi [1918]
Snail (<i>Helix pomatia</i>)	Hepatopancreas	+	"
Crayfish (<i>Astacus fluviatilis</i>)	"	—	"
Starfish (<i>Pisaster ochracea</i>)	Caecae	—	Hunter and Dau-
Crab (<i>Cancer productus</i>)	Hepatopancreas	—	phinee [1924, 2]
Clam (<i>Saxidomus giganteus</i>)	Digestive glands	—	"

examined. From these results Hunter and Dauphinee [1924, 2] concluded that "arginase is an enzyme almost, if not entirely, peculiar to the vertebrates." But it is known that many and possibly all invertebrates excrete urea to some extent [Delaunay, 1927; 1931]. The facts that *Helix* excretes some 20 % of its waste nitrogen in the form of urea and that it was the only invertebrate then known to contain arginase led Baldwin and Needham [1934] to investigate the possibility of a cyclical synthesis of urea in *Helix* itself. The presence of arginase in

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the hepatopancreas was confirmed, and the enzyme was also detected in the nephridium, but we were forced to the conclusion that the urea excreted by this snail arises, not synthetically, but from ingested arginine. This brought the snail into the same category as the bird [Clementi, 1932; 1933] and raised the question as to how far the excretion of urea by invertebrates in general can be attributed to the action of a tissue arginase upon ingested arginine. The present paper offers a partial answer to this question in the form of the results obtained for a variety of invertebrates by means of the new and very sensitive method which has been devised for the detection and estimation of arginase.

METHOD.

The essential basis of all the methods which have been employed for the detection of arginase consists in allowing the enzyme to act upon arginine and determining the amount decomposed in one or another of several ways. Of these the most suitable for quantitative purposes is that of estimating the urea which is liberated. The earliest quantitative studies of the distribution of arginase were those of Hunter and Dauphinee [1924, 2] who used a colorimetric method of their own for the estimation of urea [1924, 1]. Their method however was open to objections on theoretical grounds, as was pointed out by Edlbacher and Röthler [1925, 1], who introduced a second quantitative method and defined a new unit of arginase activity; in this case the urea determinations were carried out by a distillation method. But the most delicate method yet introduced for the estimation of urea is that of Krebs and Henseleit [1932]. Here the urea is decomposed by a urease preparation acting at p_H 5; ammonia is effectively bound at this p_H but the CO_2 is liberated and measured manometrically. The sensitivity of this method is at least 100 times that of the distillation method employed by Edlbacher and Röthler [1925, 1], and the manometric technique was therefore adopted for present purposes. It must be mentioned, however, that Weil and Russell [1934] have used the same principle in their studies of blood arginase, their method being published shortly before the present work was completed.

In the method of Edlbacher and Röthler [1925, 1] the arginase was prepared in the form of a glycerol extract of the tissue under examination. Increasing quantities of this extract were incubated for 60 minutes with 10 ml. 1 % arginine solution and 5 ml. of a glycine buffer, the p_H being 9.5 and the temperature 37° . A few drops of toluene were added as an antiseptic. At the end of the period of incubation the enzyme was destroyed by heat, the urea formed being decomposed by urease and the ammonia so set free estimated by titration after being distilled off. A standard curve was now plotted relating urea production to enzyme concentration, the latter being expressed in convenient arbitrary units. The ratio of activity to enzyme concentration was not constant, but fell off with increasing concentration of the enzyme, but the form of the curve was found to be the same for all the tissues examined, with a single exception in the case of the kidney of the bird. This curve could therefore be used as a standard for interpreting the urea production directly in terms of arginase concentration. The number of "Arginase Units" per g. of tissue could then be calculated, and hence the "Arginase Number" of the particular tissue.

This simple procedure however could not be applied at once to invertebrate materials. Different tissues did not, it was found, give superposable curves for the activity-concentration relation, and it was thought that better agreement might be obtained if the enzyme were allowed to act upon an excess of substrate

and if the period of its action were reduced. Arginine was therefore used at a concentration of 2 % and an incubation period of 30 minutes only was allowed, and this, since the temperature was 28° instead of 37°, was equivalent to only one-fourth of the period used by Edlbacher and Röthler [1925, 1]. It will be remembered that those authors themselves stressed the importance of measuring the initial rate of the arginase-arginine reaction.

With these modifications it was found that the amount of urea produced was directly proportional to the enzyme concentration, provided that the total urea formed was not more than 4 mg. under the conditions adopted as standard. A number of curves are plotted in Fig. 1 and show this relation clearly. The slope

Fig. 1. See text for explanation. * Guinea-pig; • *Limnaea*; o *Viviparus*; x *H. pomatia*; Δ *H. aspersa*; □ *Carcinus*. The data for *Carcinus* are plotted on the lower scale of weights.

of the straight part of the curve thus gave the urea production per unit weight of tissue, *i.e.* the relative richness of the tissue in arginase. All the results are here expressed in the Q_H notation of Krebs and Henseleit [1932], *i.e.* as $\mu\text{l. urea-CO}_2$ per mg. dry weight of tissue per hour, the temperature being 28°, the activities so expressed being denoted by the symbol Q_H^{28} . It is possible to express the data given by Edlbacher and Röthler [1925, 2] in the same terms, and it seemed better to use Krebs and Henseleit's mode of expression than to add yet another arbitrary unit to the list already defined.

The conditions of temperature and p_H were 28° and 9.5 respectively. Hunter and Dauphinee [1933], Hunter and Morrell [1933] and Hunter [1934] have made a thorough study of the effects of these and other factors upon the reaction and point out that although the enzyme has an optimum p_H of 9.5–9.8 under certain conditions [Hunter and Morrell, 1924; Edlbacher and Bonem, 1925] it is very labile at such a p_H , the lability being much increased by rise of temperature. Consequently there is in practice an optimum neither of temperature

nor of p_H but "at p_H 9.8 the optimum for most conditions will be lower than 40° and for many lower than 30° " [Hunter, 1934]. The temperature chosen was 28° ; it seemed possible that the enzyme, coming from invertebrate sources, might be more labile than that from vertebrate materials, and this actually appeared to be the case. Fig. 2 shows two comparable curves obtained at p_H 7.4 and 9.5

Fig. 2. Time curve of arginase-arginine reaction at 28° (*Limnaea* enzyme).
○ at p_H 9.5; ● at p_H 7.4.

respectively. That at 7.4 shows that the arginase of *Limnaea stagnalis* is rapidly inactivated even at 28° , whereas mammalian preparations undergo only very slow inactivation under such conditions [Hunter and Dauphinee, 1933]. At 9.5 the *Limnaea* enzyme was practically destroyed in 3 hours, a circumstance which again indicates the desirability of dealing only with the earliest stages of the reaction.

The p_H was controlled by means of a glycine buffer; Weil and Russell [1934] report that such a buffer causes slight inhibition of the arginase of rat blood but no such effect was noticed here. The use of a p_H of 9.5 seems especially suitable since urease, which appears to be of frequent occurrence in invertebrate tissues [Albrecht, 1920; Przyłeki, 1922; Baldwin and Needham, 1934], is here practically inactive and cannot interfere appreciably with the urea produced by the action of arginase. Indeed, no evidence of such interference was observed during this work.

No antiseptics were employed since the period of incubation was only 30 minutes. Quite apart from any effect it may have had upon arginase itself, the introduction of toluene resulted in vapour pressure effects in the manometers, and stationary readings could not be obtained in spite of prolonged equilibration in the bath. Chloroform was successfully tried in a few of the earlier experiments since, on account of its lower boiling-point, it could be boiled off before samples were taken into the manometric flasks.

Finally, the method of extracting tissue arginase with glycerol has not been employed. At best this is a long and somewhat cumbrous process, and Hunter and Dauphinee [1930] have obtained maximum yields of the enzyme from fresh mammalian liver by extraction with water only for 2 minutes at 60° .

The details of the method may now be described, and it should be pointed out that these details must be closely observed in order to secure comparable results with different tissues.

SOLUTIONS AND EXPERIMENTAL TECHNIQUE.

(1) *Arginine solution.* This contained 5 % of arginine in the form of its carbonate. A little of the Universal Indicator of B.D.H., Ltd. was added and the p_H brought to 9.5 by passing in CO_2 .

(2) *Glycine buffer.* A stock solution containing M glycine plus M NaCl was prepared. For use, 7 parts of this received 3 parts of M NaOH; such a mixture, diluted 10 times, has a p_H of 9.5 at 28° (interpolated from the data given by Britton [1929]).

(3) *Urease solution and acetate buffer.* These solutions were prepared according to the directions of Krebs and Henseleit [1932], whose method was followed exactly except that for the sake of convenience the estimations were carried out at 28° instead of 37° .

(4) *Preparation of tissue "brei."* The organs selected for analysis were dissected from animals in good physiological condition. Adherent tissues were removed as far as possible, surplus water was absorbed by means of filter-paper, and the material dropped into a chilled dish. When enough had been collected, representative samples were removed and weighed on the torsion balance for the determination of the dry weight, the rest being weighed and transferred to a mortar. Enough sand was added to give a thin paste and the tissue then thoroughly ground. After the addition of a little water the p_H was brought to 9.5 by the addition of N NaOH and the mixture filtered through a loose plug of glass wool into a measuring cylinder and washed through. The volume was now made up to the desired value and the p_H finally adjusted if necessary, the Universal Indicator being employed externally.

It is essential that the preparation and neutralisation of the "brei" be carried out as rapidly as possible on account of the lability of arginase.

(5) *Experimental procedure.* A series of pyrex boiling-tubes (6×1 in.) were prepared and numbered. Each received 0.5 ml. of the buffer and sufficient water to bring the final volume to 5 ml. A quantity of the arginine solution having been set to warm to 28° in the bath, the "brei" was prepared, and samples were at once measured into the tubes. The latter were then promptly lowered into the bath and left for exactly 5 minutes; 2 ml. of the arginine solution were then added to the contents of each tube (except the control), the additions being made serially and at 10-second intervals. Table II shows the quantities taken in

Table II.

Tube	1	2	3	4	5	6 (control)
Buffer ml.	0.5	0.5	0.5	0.5	0.5	0.5
Water "	0.0	0.5	1.0	1.5	2.0	2.0
"Brei" "	2.5	2.0	1.5	1.0	0.5	2.5
Arginine "	2.0	2.0	2.0	2.0	2.0	0.0

typical experiments. After 30 minutes, the interval being measured with a stop watch, each tube received 1 drop concentrated HCl to neutralise the arginine and break down the buffer, followed by sufficient glacial acetic acid (usually 3–5 drops) to bring about precipitation of the proteins. This brought the p_H to a value at which urease is still fairly active, and the tubes were therefore plunged at once into a briskly boiling water-bath, where they were allowed to remain for

2 minutes. This inactivated the enzymes and usually sufficed to coagulate all the proteins present, which were then filtered off. Crystal clear filtrates, slightly yellow in colour, were usually obtained, and of these samples were taken into the manometric flasks for the determination of their urea contents. In addition to the control on the tissue itself (tube 6 in Table II) additional controls were run on each new lot of the arginine solution, since the arginine used was found to contain a little urea.

(6) *Calculation.* The yields of urea, expressed in terms of $\mu\text{l.}$ of CO_2 and duly corrected for the tissue and solution blanks, were plotted against the amounts of tissue in the corresponding tubes. The yield per mg. of tissue was deduced from the slope of the curve in the manner already indicated, converted into $\mu\text{l. CO}_2$ per mg. dry weight of tissue and finally expressed as a Q_{H}^{28} value. It is proposed to refer to each such value as the "arginase index" of the particular tissue.

(7) *Preliminary tests.* Before following out the foregoing somewhat elaborate procedure it was desirable to discover approximately how much arginase, if any at all, was present in each tissue or organ examined. This was done by preparing a rather concentrated "brei," containing 100–300 mg. of tissue per ml. Of this 2.5 ml. were treated in the usual way, the two necessary controls being set up at the same time. Urea estimations were then carried out on the final filtrates, and the arginase index was approximately evaluated; fresh experiments were then set up in all cases to confirm negative results or to secure accurate values for the index where arginase was present. The data in Table II may be regarded as typical of those obtained in such preliminary experiments, but it should be pointed out that arginase was only regarded as present if the total amount of urea CO_2 exceeded $10\mu\text{l.}$ On the other hand, the disappearance of more than $10\mu\text{l. CO}_2$ might conceivably be taken as indicating the presence of urease in the tissue, but such indications would need further confirmation.

Table III.

Animal	<i>Littorina littorea</i>	<i>Mytilus edulis</i>	<i>Carcinus maenas</i> ♂
Part examined	HP	HP	HP
mg. tissue/ml. of "brei"	107	110	94
$\mu\text{l CO}_2$ found in			
(i) whole	69	79	432
(ii) tissue	0	5	5
(iii) solutions	66	81	66
$\mu\text{l CO}_2$ formed from arginine	3	-7	361
Arginase	absent	absent	present
Q_{H}^{28} (approx.)	0.05	0	11

HP, hepatopancreas.

RESULTS AND DISCUSSION.

The results of the experiments performed are presented in Table IV. The liver of the guinea-pig was examined in two cases with a view to comparing the values obtainable for Q_{H} by the new method and by that of Edlbacher and Rthler [1925, 1, 2]. From the data given by the latter authors it was possible to calculate an index of 930 for female guinea-pigs at 28° , the necessary temperature coefficient being obtained by interpolation from the data given by Hunter [1934]¹. The mean value found by the new method was 750 and it may be taken

¹ Krebs and Henseleit [1932] give an index of 4000 for the guinea-pig, but this figure refers to 37° and appears to have been calculated from Edlbacher and Rthler's data for the male animal; furthermore the dry weight was assumed to be 20 % instead of 28 % as was found here.

that the agreement between the two figures is satisfactory in view of the fact that the arginase content of the liver varies considerably from animal to animal as well as with conditions such as age and sex [Edlbacher and Röthler, 1925, 2].

A glance at the table of results suffices to show that the distribution of arginase is considerably wider than has hitherto been supposed. While two marine lamellibranchs (*Mytilus* and *Pecten*) gave no evidence of containing arginase, thus confirming Hunter and Dauphinee's finding in the case of *Saxidomus*, a fresh water species, *Anodonta*, contained the enzyme. This suggests that the distribution of the enzyme may be somewhat erratic although it is possible that

Table IV.

Phylum and class	Species	Part analysed	No. of specimens	Q_H^{28}
CRANIATA	<i>Cavia</i> ♀	Liver	1	772
MAMMALIA	(guinea-pig)		1	728
ECHINODERMATA	<i>Asterias rubens</i>	Hepatic caecae	1	1.6
ASTEROIDEA	(starfish)		1	1.4
			1(a)	0.9
ARTHROPODA	<i>Carcinus maenas</i> ♂	Hepatopancreas	1	ca. 11
CRUSTACEA	(shore crab)		1	15.5
			1	19.1
MOLLUSCA	<i>Mytilus edulis</i>	"	3	0.0
LAMELLIBRACHIATA	(mussel)		5	0.0
	<i>Pecten opercularis</i>	"	3	0.0
	(queen scallop)		5	0.0
	<i>Anodonta cygnaea</i>	"	4	2.8
	(river mussel)		4	2.9
GASTROPODA	<i>Buccinum undatum</i>	"	1	0.0
	(whelk)		1	0.0
	<i>Littorina littorea</i>	"	6	0.0
	(periwinkle)		10	0.0
	<i>Patella vulgata</i>	"	3	0.0
	(limpet)		6	0.0
	<i>Viviparus fasciatus</i>	"	6	719
	(river snail)			
	<i>Planorbis corneus</i>	"	12	22.5
	(ram's horn)			
	<i>Limnaea stagnalis</i>	"	10	682
	(pond snail)		14	587
	<i>Helix aspersa</i>	"	3	1230
	(common snail)		4	1240
	<i>Helix pomatia</i>	"	1(b)	1200
	(Roman snail)		2(c)	6500
			2(d)	933
	<i>Arion ater</i>	"	2	ca. 10
	(black slug)		4	11.6
			4	11.6

The nomenclature is that of Ellis [1926].

(a) Animal moribund.

(b) Starved and aestivating.

(c) Just wakened from hibernation and well fed.

(d) Just wakened from hibernation but no food eaten.

further work would reveal a strict correlation between the presence of arginase and a terrestrial or fresh-water habitat. It will be seen that of all the molluscs examined only the terrestrial and fresh-water species contained the enzyme. The finding of arginase in a crustacean and in an asteroid suggests that it was on account of lack of sensitivity that the enzyme was not detected in *Astacus* and

Pisaster by the methods of Clementi [1918] and Hunter and Dauphinee [1924, 2] respectively. On the other hand the technique of Edlbacher and Röthler [1925, 1] would probably have been sufficiently delicate to allow of the detection even of the small arginase concentrations which were found in the animals so far mentioned, although the indices did not in any case exceed a value of 20, which is less than 3 % of the mean for the guinea-pig livers.

The examination of a moribund specimen of *Asterias* brought to light the fact that arginase content is dependent upon physiological condition, and this was further demonstrated in the case of *Helix pomatia* (see Table IV). Well-fed individuals of this species gave an index of 6500 as compared with 1200 and 933 for starved specimens, while only traces of arginase could be found in the slug *Milax sowerbii* and *Helicella itala* after these had been kept for about a fortnight without food. The results listed in Table IV refer to fresh healthy specimens except in the cases indicated; the marine forms, which were obtained from Plymouth, were in all cases used within a day or two of capture.

Turning now to consider the results obtained for gastropod molluscs, it will be seen that arginase could not be detected in any of the three marine forms which were tested, viz. *Buccinum*, *Littorina* and *Patella*. Yet the hepatopancreases of the fresh-water forms, *Limnaea* and *Viviparus*, contained the enzyme in a concentration quite comparable with that found in mammalian livers. Two terrestrial species, *H. pomatia* and *H. aspersa*, even surpass the mammalia in arginase content, the former having an index of about 6500 and the latter 1200 in well-fed specimens. In a previous paper [Baldwin and Needham, 1934] a value of 20.2 for the hepatopancreas "brei" of *H. pomatia* was published. The experiments in which this value was determined involved incubation of the "brei" with arginine for several hours at p_H 8.4 and 28°. The conclusions of our previous work are not much affected by the newer values here reported, but the fact that such low values were obtained emphasises the importance of measuring only the initial velocity of the arginase-arginine reaction when the arginase index of a given material is to be determined with anything approaching accuracy.

The present finding of indices of from 1000 to 6000 for the hepatopancreases of animals which are primarily uricotelic makes it still more certain that, if there be any homology whatever between the gastropod hepatopancreas and the vertebrate liver, Clementi's rule [1914; 1915] fails in its application to the invertebrata, and all the more so when it is remembered that the nephridium, and perhaps other organs too, contains much arginase [Baldwin and Needham, 1934]. At the same time, in view of the work of Krebs and Henseleit [1932], it seemed somewhat curious that the snail, possessing as it does an arginase content of the same order as that found in mammals, should excrete only 20 % of its waste nitrogen in the form of urea [Delaunay, 1927; 1931], while the mammalia excrete about 80 % in that form.

Meanwhile Needham [1935] was examining the distribution of uric acid in the nephridia of a large number of molluscs, and on comparing our respective results it was found that there appeared to be a correlation between the arginase and uric acid contents of those forms which had been examined in common. Table V shows the average values found for Q_H and for uric acid content, the latter expressed as mg. HU per g. dry weight of nephridium, for those gastropods for which both sets of data are available. A test case of the greatest interest was found in the large black slug, *Arion ater*. As *Arion* appears at first sight to possess all the attributes of a truly terrestrial gastropod, it might have been expected to ally itself with other terrestrial forms such as *Helix* by having a high concentration of nephridial uric acid. This, however, was found not to be

Table V. *Relationship between hepatic arginase and nephridial uric acid in gastropods.*

Species	Habitat	Q_H^{28}	HU content
<i>Buccinum undatum</i>	Marine	0	4.1
<i>Littorina littorea</i>		0	1.5
<i>Patella vulgata</i>		0	0.5
<i>Planorbis corneus</i>	Fresh-water	22.5	40
<i>Limnaea stagnalis</i>		635	114
<i>Viviparus fasciatus</i>		719	35
<i>Helix aspersa</i>	Terrestrial	1235	128
<i>Helix pomatia</i>		6500	744
<i>Arion ater</i>		11	36

Data for Q_H^{28} are the average values from Table IV.

Data for uric acid are mean values calculated from the data of Needham [1935].

Fig. 3. Relation between arginase and uric acid in gastropods. (See text.)

the case; the amount of uric acid which could be detected was only a fraction of that to be found in the nephridia of the terrestrial snails as a group, and correlated with this low uric acid content was an arginase index of only 11. This is of the same order as those found for *Carcinus* and *Asterias*, neither of which would be regarded on any grounds as being primarily uricotelic. The relationship between the two sets of data is more clearly shown in Fig. 3, where they have been plotted on a double logarithmic scale. The points for the various forms lie well on the curve, with the exception of that for *Viviparus*, which differs from the others represented in being operculate and not pulmonate and perhaps might therefore be expected to behave in a rather different manner.

We have already seen how Krebs and Henseleit [1932] were able to demonstrate the functional importance of arginase in the elaboration of urea by the mammalian organism, and the discovery of this new and altogether unexpected correlation between arginase content and uric acid formation suggests that the enzyme is also of importance in the elaboration of uric acid by the Gastropoda. This group, together with the Insecta, appears to have specialised more than any

other invertebrate group in the production of uric acid as a nitrogenous end-product. To extend this notion to cover the birds, which are the chief producers of uric acid among the vertebrates, would plainly be difficult or impossible on account of the very small amount of arginase which is present in the avian organism [Edlbacher and Röthler, 1925, 2], but this does not detract from the possible value of the new hypothesis, since it has already been shown [Baldwin and Needham, 1934] that the bird and the snail probably make use of quite different chemical mechanisms in the formation of uric acid. Further work is therefore in progress with a view to testing the hypothesis.

A few interesting points remain to be discussed. In the first place, what is the source of the urea which plays such a large part in the nitrogenous excretion of the slug *Arion*? In our examination of the case of *Helix* we were unable to demonstrate any synthetic production of urea and came to the conclusion that the urea excreted by that animal arose almost entirely from ingested arginine under the influence of the arginase of the hepatopancreas and nephridium [Baldwin and Needham, 1934]. It was quite clear that the arginase cycle did not operate in *Helix* in spite of the high arginase index of its hepatopancreas. This must probably be true also in the case of *Arion* whose arginase index is relatively small, but in spite of this *Arion* is believed to excrete a greater proportion of urea than any other invertebrate so far examined [Delaunay, 1927; 1931]. It would be desirable to confirm Delaunay's analyses of the excreta of *Arion*, since, if they are reliable, they must point to the existence of some synthetic mechanism for the production of urea which is markedly different from that found in the vertebrates.

SUMMARY.

1. A new and sensitive method for the detection and estimation of arginase is described, together with a suitable mode of expression of the results.
2. A series of results has been obtained with the aid of this new method, and there is reasonable agreement between the values obtained by the new method and by its predecessor.
3. The examination of a variety of invertebrates has shown that arginase is much more widely distributed than has hitherto been supposed.
4. The arginase content of a given tissue is dependent upon its physiological condition, the enzyme tending to disappear in starvation.
5. On the grounds of a correlation between the arginase and the uric acid content of the hepatopancreases and nephridia respectively in a number of gastropods, it is suggested that arginase is concerned in the production of uric acid by members of this class. Thus in the elaboration both of urea and of uric acid, which represent the principal nitrogenous end-products of the non-sauropsid vertebrates and the gastropods respectively, the same enzyme, arginase, may be involved.

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