CXII. THE ACTION OF ARGINASE UPON CARBAMIDOARGININE

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THIS paper presents proof that extracts of liver contain an enzyme which hydrolyses *l* (+)-carbamidoarginine (α -uramido- δ -guanidovaleric acid) into urea and *l* (+)- α -carbamidoornithine (α -uramido- δ -aminovaleric acid). This enzyme must be either arginase or a new specific enzyme having similar action. The evidence supports the first, and more probable, of these alternatives. The point will be discussed later; for the present the identity of the enzyme will be taken as granted. Arginase has no demonstrable action upon arginine hydantoin.

Materials used

The carbamidoarginine and hydantoin used in our experiments were prepared, in part from arginine hydrochloride, in part from gelatin hydrolysates, according to Boon & Robson [1935]. The product in nearly saturated aqueous solution, had $[\alpha]_D^{25} + 17.4^\circ$ ($c = 0.776$; $l = 2.0$ dm.). As the observed rotation was rather small, the estimated specific rotatory power is subject to an error of perhaps 1° . There is also no certainty that the product examined was optically homogeneous.

Preliminary experiments

The first experiments attempted sufficed to show that incubation of carbamidoarginine with "crude arginase extracts" [Hunter & Dauphinee [1930] did actually lead to the production of urea, but much more slowly than the action of the same extracts upon equivalent quantities of arginine. In these preliminary experiments the digestion mixtures contained from the beginning urease as well as arginase, and the urea formed within a given interval of time was estimated from the CO_2 content of a conveniently large sample. The procedure was in fact identical with that followed by Hunter & Pettigrew [1937] in the manometric determination of arginine. Suitable controls, containing the substrate with (a) no enzymes, (b) urease alone, (c) liver extract only, showed that the CO_2 measured arose neither from the spontaneous decomposition of the substrate (a), nor from the direct action upon its uramido group of urease (b) or some unknown liver enzyme (c). The source of the CO_2 was therefore indubitably urea.

It could not be at once concluded that this urea arose from the direct action of arginase. The structure of carbamidoarginine suggested other possibilities. Liver enzymes might exist capable of (a) splitting off the uramido group as urea by a reaction analogous to deamination, or (b) splitting the $-\text{CONH}-$ group hydrolytically with liberation of arginine. In the first case arginase would have nothing to do with urea formation in our experiments or, if, as stated by Calvery & Block [1934], it can act upon argininic acid, would account for only a part of it; in the second it would be responsible only secondarily through its familiar action upon arginine.

A decision as to the actual mechanism of urea formation in our digests was first sought by determination of the amino groups simultaneously liberated. For various reasons this failed to yield an unambiguous result. A study, to be described later, of the influence of *pH* upon the process or processes involved proved no more than that arginase was indeed an agent in the production of the urea. The question whether it was acting upon carbamidoarginine or arginine itself could only, it seemed, be answered by isolating the second product of the reaction. Before this was attempted, it was obviously desirable to know whether, and in what conditions, carbamidoarginine could be completely decomposed by the liver extract. The following experiment is typical of those which were designed to elucidate this point.

Maximum effect of arginase upon carbamidoarginine

With the aid of heat 0.375 g. carbamidoarginine was dissolved in about 22 ml. 0.25 *M* phosphate-phenolsulphonate buffer [Hunter & Morrell, 1933] of *pH* about 10.6. The solution was cooled in a thermostat to 37°, and made up to 25 ml.; 15 ml. of this 1.5% solution (which, although supersaturated, did not crystallize during the course of the required manipulations) were mixed with 5 ml. of liver extract containing 400 units [Hunter & Dauphinee, 1930] of arginase. The mixture, which had *pH* about 9.1 was immediately returned to the thermostat.

At the end of 1, 2 and 3 days 5 ml. samples were withdrawn, acidified and boiled. The urea content of each sample was then determined by the urease-aeration method of Van Slyke & Cullen [1914]. The results, corrected by appropriate controls, were as follows:

Time of action hr.	Urea N found mg.	% of possible maximum
24	3.81	52.5
48	6.69	92.2
72	6.82	94.0

The "possible maximum" is taken as 7.256 mg. of urea-N, corresponding to the production of 1 mol. of urea from each mol. of substrate.

It is seen that, given sufficient time and sufficient concentration of arginase, it is possible for a liver extract to decompose carbamidoarginine almost quantitatively. Under the conditions of the experiment an equivalent amount of arginine would have been completely hydrolysed by one-fifth of the enzyme in less than 12 hr.

Isolation and properties of α -carbamidoornithine

The product of the enzymic action expected (α -carbamidoornithine) had not hitherto been described, and its properties were therefore unknown. Attempts to isolate it by direct precipitation with phosphotungstic acid were unsuccessful. Alkaline mercuric acetate and other commonly employed precipitants were either ineffective or not sufficiently discriminating. The first success obtained was the recovery of a mixture of carbamidoornithine and its anhydride from the precipitate produced by phosphotungstic acid in a digest previously boiled with H_2SO_4 . This procedure was open to the objection that the products might have been artefacts produced by the condensation of urea with ornithine under the action of the hot acid; in any case the yield was very small. Finally, the simple, if far from quantitative, method was adopted of direct crystallization of the product from a concentrated digest. This was facilitated by the previous destruction of the accompanying urea by urease, a step which also obviated the possibility, just referred to, of the occurrence, during later manipulations, of a secondary

condensation. Sometimes the urease was allowed to act along with the arginase, at other times it was applied only after the latter had completed its action. Both procedures led to approximately the same yield of the material sought. The first of them is exemplified in the following detailed description of one experiment.

Carbamidoarginine (2 g.) was dissolved in water (200 ml.) at 37°, and the solution treated with 15 ml. of a very active aqueous solution of arginase together with 5 ml. of a 10 % extract of jack bean meal in 30 % alcohol. The arginase had been prepared from a watery extract of liver by double preprecipitation with acetone, the second precipitate being taken up in one-fourth of the original volume of water. The solution thus obtained contained 335 units of arginase per ml., so that the total amount used in the experiment was about 5000 units. By concentrating the enzyme in the manner indicated it was possible to use a large amount without introducing into the digest more than a minimum of extraneous material.

The mixture, protected by a layer of toluene, was placed in a thermostat at 37°, where it rapidly became alkaline through formation of $(\text{NH}_4)_2\text{CO}_3$. After 3 days it was neutralized with H_2SO_4 , heated to 100°, and treated with just enough more acid completely to coagulate the protein. It was then filtered into a Claisen flask, and, after addition of 100 ml. alcohol and 50 ml. saturated baryta water, was distilled *in vacuo* at 45° till completely free of ammonia. The residue was filtered from precipitated Ba salts, neutralized exactly to litmus with H_2SO_4 , filtered again from BaSO_4 and evaporated on the water bath.

During evaporation the solution darkened somewhat, and, when reduced to a small volume, still gave a precipitate with baryta. It was therefore treated again with excess of baryta, filtered, neutralized with H_2SO_4 , decoloured with a minimum of norite, filtered again and evaporated to dryness. The product was a practically colourless mass, in appearance almost entirely crystalline and weighing 1.6 g.

For purification the combined yields of crude material from several such experiments, amounting to 6.34 g. from 8 g. carbamidoarginine, were repeatedly recrystallized from water until the mother liquor gave no Sakaguchi reaction and the melting point of the crystals remained constant. This procedure removed some syrupy non-crystallizable material, but involved also a considerable loss of the fairly soluble main product. The final yield was only 1.35 g. of glistening snow-white crystals. (Found: C, 41.20; H, 7.32; N, 23.7 %. $\text{C}_8\text{H}_{13}\text{O}_3\text{N}_3$ requires: C, 41.12; H, 7.49; N, 24.00 %.)

It happens that among the possible, if hardly probable, products of the experiment there are two already known substances of the formula $\text{C}_8\text{H}_{13}\text{O}_3\text{N}_3$: One is α -hydroxy- δ -guanidinovaleric (argininic) acid, the other δ -carbamidoornithine (citrulline). Our substance was neither of these, for it gave no Sakaguchi reaction and contained therefore no guanidine group; moreover it neither dissolved CuO nor gave the ninhydrin reaction and was therefore not an α -amino-acid). It did give reactions characteristic of the grouping $\text{NH}_2\cdot\text{CO}\cdot\text{NH}-$, such as the yellow-green colour with *p*-dimethylaminobenzaldehyde and HCl [Barrenscheen & Weltmann, 1922], or the purple colour with furfuraldehyde and HCl [Schiff, 1877]. Formaldehyde titration indicated the presence of one amino group.

Manometric determination of amino groups [Van Slyke, 1929] yielded rapidly rather more than one equivalent of nitrogen, and after prolonged action about two. (Found after 2.75 min. 11.5 % N and after 2.5 hr. 17 % N; calc. for one NH_2 group 8 % N.)

Similar results, both for the formaldehyde and the nitrous acid methods, have been reported by Wada for citrulline.

These properties, coupled with its origin, left little doubt that the substance was α -carbamidoornithine (I). The ease with which it could be converted into an anhydride (see below) afforded further evidence in favour of this conclusion. The constitution of the substance is therefore established with reasonable certainty. At the same time it is demonstrated that arginase can act directly upon carbamido-arginine.

α -Carbamidoornithine is only moderately soluble in water, although much more so than carbamidoarginine. Like the latter it readily forms supersaturated solutions. It crystallizes in large bright pointed prisms or in elongated hexagonal plates. Heated in an open capillary tube it melts at $205.5\text{--}206^\circ$. Its aqueous solutions are neutral to litmus and dextrorotatory ($[\alpha]_D^{20} = +22.3^\circ$; $c = 1.948$; $l = 2$ dm.; $\alpha = +0.87^\circ$); Presumably the substance has the same configuration as natural arginine, and its proper designation is $l(+)\text{-}\alpha$ -carbamidoornithine.

A 0.4% solution gives no precipitate with flavianic acid, picric acid, mercuric acetate or sulphate, Millon's or Nessler's reagents, silver nitrate (with or without ammonia), potassium bismuth iodide or potassium ferrocyanide. With phosphotungstic acid it gives no immediate precipitate, but yields after many hours a scanty crystalline deposit of thick rhombic plates. With mercuric acetate and sodium carbonate it gives at once a heavy white precipitate. With ferric chloride it gives in the cold a yellowish brown colour, similar to that given by glycine. This is a general, although far from specific, reaction of α -uramido-acids.

Carbamidoornithine anhydride

α -Carbamidoornithine ought, it was thought, on heating with dilute acid, to form a hydantoin. The following experiment was carried out in the hope of realizing this expectation. 0.5 g. of $l(+)\text{-}\alpha$ -carbamidoornithine was dissolved in 10 ml. of 6% (by vol.) H_2SO_4 , and heated for 3 hr. in a boiling water bath. The H_2SO_4 was then removed by baryta, and the filtered solution evaporated to dryness. The product (0.446 g.) was completely crystalline and only slightly coloured. One recrystallization from water yielded a perfectly white product. (Found: C, 45.7; H, 6.93; N (Kjeldahl), 26.2; N (Dumas), 26.7%. $\text{C}_6\text{H}_{11}\text{N}_3\text{O}_2$ (carbamidoarginine $-\text{H}_2\text{O}$) requires: C, 45.82; H, 7.06; N, 26.75%.)

The substance was evidently an anhydride of carbamidoornithine and was supposed at first to be the expected ornithinehydantoin (II). Several of its properties however excluded this supposition. In the first place, its aqueous solution was neutral to litmus; secondly, it gave, quite as strongly as its mother

substance, the Barrenscheen-Weltmann and Schiff reactions, pointing to the persistence of the uramido grouping; and, thirdly, even prolonged treatment with HNO_2 , in the Van Slyke method for determination of primary amino groups, failed to liberate as much as one atom of nitrogen. ($\text{NH}_2\text{-N}$ found; after 3 min. 1.79; after 30 min. 4.87; after 2.5 hr. 5.93%. One NH_2 requires 8.92% $\text{NH}_2\text{-N}$.)

If the anhydride is not a hydantoin, it seems that it must have been formed by ring closure between the carboxyl group of the carbamidoornithine and the δ -amino group in which case it must be 3-uramido-2-piperidone (III). Ornithine and related substances are already known to be easily convertible into piperidones, e.g. by attempted esterification [Fischer & Zemplan, 1909]. Again, δ -methylaminovaleric acid is converted into 1-methyl-2-piperidone by heating to 160° [Fischer & Bergmann, 1913]. Other instances of the same transformation have been reported by Thomas *et al.* [1923] and by Bergmann & Köster [1926, 1927]. Among these the partial conversion of δ -methylornithine into 1-methyl-3-amino-2-piperidone by boiling with 20 % HCl [Thomas *et al.*, 1923] is especially apposite, because the ring closure took place in dilute aqueous acid, a medium which in other cases (such as that of the ornithine-aminopiperidone transformation) has been found to favour rather the reverse reaction [Fischer & Zemplan, 1909; Bergmann & Köster, 1926]. The factors, which, in a given case, determine the direction actually taken by the reaction, remain obscure. Although there can be little doubt concerning the piperidone structure of the anhydride here encountered, complete certainty must await its synthesis; for 3-uramido-2-piperidone has not hitherto been described.

The anhydride crystallizes in rather thick elongated prisms, sometimes straight, but usually lancet- or whetstone-shaped, having multifaceted square-cut or obtusely pointed ends. It melts sharply at $228-228.5^\circ$. It is only moderately soluble in water. The specimen in hand had probably been racemized during preparation, for a 2 % solution showed no optical activity. Like α -carbamidoornithine it does not dissolve CuO; unlike it, it gives no colour with ferric chloride. It gives a scanty greenish grey precipitate with Nessler's solution, and a heavy white one with mercuric acetate and sodium carbonate. It is more readily precipitated by phosphotungstic acid than α -carbamidoornithine; from a 0.4 % solution precipitation begins in an hour, and yields in time a quite considerable deposit of elongated narrow prisms, arranged either singly or in clumps. No precipitate is given by any of the other reagents tried with carbamidoornithine. A 0.1 % solution is not precipitated even by phosphotungstic acid.

It may be added that the mother liquor from the above-described recrystallized anhydride had a definitely alkaline reaction, and that upon concentration it yielded not only a second crop of crystals indistinguishable in appearance from the first, but also (after removal of this and further concentration) a small amount of material of an entirely different crystalline form. The quantity obtained was too small for satisfactory study; but it is possible that this material was ornithinehydantoin, and that the reaction had actually followed in part the direction at first expected.

Influence of pH upon the arginase hydrolysis of carbamidoarginine

The following experiments were carried out before the isolation of carbamidoornithine had demonstrated that we were dealing with the direct action of arginase upon carbamidoarginine. They may now be presented as showing the effect of pH upon a defined reaction. Incidentally they exemplify the kind of result which first proved that liver extracts do in some manner produce urea from carbamidoarginine.

Into a series of 20 ml. volumetric flasks there were introduced mixtures, each of which contained 5 ml. of a nearly saturated (about 0.8 %) solution of carbamidoarginine, 10 ml. of a selected phosphate-phenolsulphonate buffer [Hunter & Morrell, 1933], and a convenient amount (1 or 2 ml.) of an arginase-containing

liver extract. All mixtures were made up in duplicate, one of each pair being used immediately for the determination of pH , while the others, accompanied by appropriate blank controls, were kept at room temperature for a period varying in different experiments from 18 to 24 hr. Each was then treated with dil. H_2SO_4 till just yellow to phenol red, whereupon its urea content was determined by the manometric urease method of Van Slyke [1927].

Fig. 1. Curve I. Carbamidoarginine, 0.834%. Arginase, crude, 40 units. Time, 18 hr. Curve II. Carbamidoarginine, 0.80%. Arginase, crude, 70 units. Time, 24 hr. Curve III. Carbamidoarginine, 0.80%. Arginase, purified, 60 units. Time, 24 hr. Curve IV. Carbamidoarginine, 0.80%. Arginase, purified, 60 units. Time, 22 hr. Curve V. Arginine hydantoin, 0.80%. Arginase, 60–70 units. Time, 24 hr.

In Fig. 1 are shown graphically the results of four such experiments. In Exp. I and II the enzyme preparation applied was a "crude arginase extract", containing in the first instance 40, in the second 70, units of arginase in the 1 ml. which was used. For Exp. III and IV the crude extract employed in Exp. II was freed from protease (and possibly from other contaminating enzymes) [Dauphinee & Hunter, 1930] and increased to 2 ml., which supplied 60 units of arginase. Exp. III was carried out simultaneously with Exp. II, and is directly comparable with it. Exp. IV was performed a few days later in the endeavour to approach more closely to the actual optimum than the other experiments had done.

The results of these experiments are expressed on the graph as percentages of the maximum amount of urea derivable from the guanidine group alone. Few in number as they are, they leave no doubt concerning either the general shape of the curve or the approximate position of the optimum at pH 9.6–9.8. In both respects the agreement with the pH -activity curve of arginase acting upon arginine [Hunter & Morrell, 1933] is sufficiently evident. There is even, in Exp. I, a suggestion of the peculiar inflexion which was observed to distort that curve between pH 7.5 and 8; although, in the absence of confirmatory observations, the present curve has of course been represented as perfectly smooth. It appears

therefore, that the replacement of one hydrogen of the α -amino group of arginine by the radicle $\text{NH}_2\text{—CO—}$ has not notably affected the $p\text{H}$ -activity relations of the arginase. The optimum $p\text{H}$, in particular, remains essentially the same.

Arginine hydantoin and arginase

There are included in Fig. 1 (Curve V) the results of two experiments with a 0.8% solution of arginine hydantoin hydrochloride, carried out exactly as with carbamidoarginine, the enzyme used in one being that of Exp. II, in the other that of Exp. III. The CO_2 -pressures measured did not in any instance exceed 9 mm. Hg, and the results are for all practical purposes negative.

Relative activities of arginase towards arginine and carbamidoarginine

Under the conditions prevailing in Exps. II–IV an equivalent quantity of arginine would have been completely hydrolysed in about 3 hr. It is obvious, then, that arginase acts much more slowly upon carbamidoarginine than upon its usual substrate and it was attempted to express this difference quantitatively as follows.

Exp. 1. A liver extract was treated with several volumes of acetone; the precipitate was dissolved in water, precipitated a second time by acetone and finally taken up in one quarter of the original volume of 50% glycerol. The arginase content of this very active solution was then determined by the method of Hunter & Dauphinee [1930], the substrate being a 0.75% solution of arginine hydrochloride, and the amount of enzyme solution used being 0.1 ml. (1 ml. of a tenfold dilution). At the same time a parallel determination was run in which the arginine solution was replaced by a carbamidoarginine solution of equivalent concentration (0.766%). In this case, in order to get a result of satisfactory magnitude, it was necessary to use 1 ml. of undiluted enzyme solution. The actual or apparent arginase concentration indicated by each determination was estimated by use of the graph given by Hunter & Dauphinee. It should be noted that in this experiment the arginase used was not fully activated; for by the addition of cobalt its activity could be increased about 60%.

Exp. 2. This experiment was similar to the first, but was carried out with another enzyme concentrate which had been treated with an appropriate amount of cobalt nitrate [Hellerman & Perkins, 1935]. The activity of this concentrate was so great that with the arginine substrate the amount used had to be reduced to 1 ml. of a twentyfold dilution (0.05 ml. of the original solution). For the carbamidoarginine 1 ml. of undiluted enzyme remained, as before, a convenient amount.

The results of these experiments are shown below:

Exp. no.	Substrate	Enzyme solution ml.	Urea-N produced mg.	Apparent arginase units per ml.	Activity ratio
1	Arginine	0.1	3.24	168	1:168
	Carbamidoarginine	1.0	0.48	1	
2	Arginine	0.05	3.17	324	1:162
	Carbamidoarginine	1.0	0.91	2	

Within the limits of the method the "activity ratios" calculated from the two experiments are identical. The value found (taken as 1 : 165) may be subject to some correction, for, although each substrate solution was buffered, as the method prescribes, in exactly the same way, it is possible that the $p\text{H}$ of the two digests was not exactly the same. It is assumed that the velocity curves of arginine and carbamidoarginine hydrolysis are of identical form.

DISCUSSION

Under the action of a liver extract carbamidoarginine has been shown to yield urea equivalent to 94% of its guanidine group; yet in the most successful experiment only 22% of the corresponding quantity of carbamidoornithine has been recovered in a state of purity. The amount actually produced was undoubtedly much greater, for the method of isolation involved very considerable losses. Nevertheless it cannot be held as proved that the decomposition of carbamidoarginine was accomplished solely through the direct cleavage of its guanidine group. The possible intervention of other reactions, such as have been suggested earlier, cannot be excluded. The fact remains that the occurrence of the direct action itself was fully demonstrated.

That the enzyme responsible for this action is really arginase, will hardly be seriously doubted. The observation, that a given liver extract catalyses the hydrolysis of carbamidoarginine much less powerfully than that of arginine, is the only one which might, even remotely, suggest the contrary. If positive evidence of identity be required, it will be found (1) in the fact that the *pH*-activity curve of the effective enzyme is apparently indistinguishable from that of arginase and (2) in the observation that two different partly purified enzyme preparations, one of which had, and the other had not, been activated by cobalt, showed exactly the same ratio in their activities toward the two substrates. It would indeed be a coincidence if two separate enzymes were present in two different livers in the same proportion, maintained that proportion through a procedure of purification generally useful in the separation of enzymes, and were activated to equal extents by cobalt; and it would be just as remarkable if the identity of the ratios were the chance outcome of compensatory differences in the behaviours of the two enzymes at successive steps of the preparation.

Carbamidoarginine is the first substance, other than arginine itself, of which the susceptibility to arginase has been proved not merely by the detection of one product of the reaction, but also by the isolation and identification of the other. For a few other substrates susceptibility has been more or less definitely established by the demonstration that they yield urea under the action of liver extracts. Among these there are six, of which the chemical constitution is definitely known: α -monobenzoylarginine [Felix *et al.*, 1928], arginylarginine [Edlbacher & Bonem, 1925; Edlbacher & Burchard, 1931], δ -guanidino- α -hydroxyvaleric (argininic) acid [Calvery & Block, 1934], the iminodicarboxylic acid octopin (*l* (+)-arginine-*N-l*- α -propionic acid), its stereoisomeride *isooctopin* (*l* (+)-arginine-*N-d*- α -propionic acid) and its position isomeride *l* (+)-arginine-*N- β* -propionic acid [Akasi, 1937]. Klein & Tauböck [1932] would extend this list by the inclusion of canavanine (γ -guanidinooxy- α -aminobutyric acid). In addition it has been shown that under certain conditions, differing somewhat from those optimal for arginine itself, liver extracts can produce urea from protones [Kossel & Dakin, 1904; Dakin, 1907; Felix, *et al.*, 1933] and even from protamine [Felix *et al.*, 1932].

Negative results with a long list of guanidine derivatives [Dakin, 1907; Bierry & Ranc, 1909; Thomas, 1913; Clementi, 1915; 1916; Edlbacher, 1917; Kiesel, 1922; Thomas *et al.*, 1923; Edlbacher & Bonem, 1925; Poller, 1927; Abderhalden & Sickel, 1929; Baldwin, 1934; Hellerman & Perkins, 1935; Hellerman & Stock, 1936] have shown that arginase is not simply a general reagent for the guanidine group. Similarly negative results with arginine derivatives containing substituent radicles in either the guanidine group or the carboxyl group [Thomas *et al.* 1923; Edlbacher & Bonem, 1925; Meyerhof &

Lohmann, 1928; Felix *et al.* 1928; Klein & Tauböck, 1932; Calvery & Block, 1934; Dirr & Späth, 1935] indicate that susceptibility to arginase requires the presence of each of these in unmodified form. Presumably the enzyme must enter into some definite relation with both of these groups. The two groups must moreover be separated by a chain of not more than four carbon atoms, for the next higher homologue of arginine is not attacked [Steib, 1926, 2]. Whether lower homologues are acted upon by arginase has not been ascertained.

The integrity of the two terminal groups as a necessary condition for the action of arginase is generally agreed upon. The role of the α -amino group is not so clear. It is certainly not an indifferent feature of the substrate's structure. Arginase has no action at all upon δ -guanidinovaleric acid [Hellerman & Perkins, 1935; Hellerman & Stock, 1936]; and no arginine derivative is so readily hydrolyzed by it as arginine itself. Only in the case of carbamidoarginine has it been attempted to evaluate precisely the effect of a substituent radicle; but the records show clearly enough that any modification whatsoever of the amino group obstructs in greater or smaller degree the action of the enzyme. Replacement of one H by a methyl group is sufficient to inhibit that action completely [Steib, 1926, 1]; but the list of known substrates already given shows that certain other single substituents—the benzoyl radicle, the arginyl radicle, the carboxyethyl and, as now shown, the uramido group—may be introduced without rendering the substrate entirely resistant. Even a change in the spatial relations of the amino group does not present an absolute barrier to the enzyme; Edlbacher & Zeller [1936] have shown that, given a sufficient concentration of arginase, *d* (–)-arginine may be hydrolyzed almost as completely as its natural antipode. According to Calvery & Block [1934] the amino group may actually be entirely replaced by hydroxyl; but the positive results of these authors are not regarded by Hellerman & Perkins [1935] or Hellerman & Stock [1936] as due to the action of arginase.

In 1928 Felix *et al.* summed up the known facts, in so far as they affected the question of the amino group, by saying that that group must be present, but that one of its hydrogen atoms may be replaced by an acid (presumably even an amino-acid) residue. This summary some later writers have regarded rather rashly as an established general rule. Even if it prove to be true that the amino-group cannot be wholly replaced by another, the number of α -*N*-substituted arginines so far tested is much too small to permit of any generalization. It is true that the case of carbamidoarginine, as far as it goes, appears to confirm the rule; but the acid group here introduced is of a special type, and the product still presents a free amino group linked, although indirectly, to the α -carbon atom. It would be idle to speculate at this stage upon the possible influence of this characteristic. The whole question of the part played by the amino group in the mechanism of arginase action must in fact await the collection of an adequate body of experimental facts. A study of other arginine derivatives from this point of view has already been undertaken.

Felix *et al.* [1928] observed that in the action of arginase upon α -benzoyl-arginine, urea was produced only between *pH* 6.5 and 7.5, and not, as with arginine, in increasing amounts up to *pH* 9.8. This result was attributed to the low solubility of the substrate at alkaline reactions; but it has since been taken [Felix *et al.* 1932] as the basis of another rule—that, when the amino group of arginine carries an acid substituent, the *pH* optimum for arginase action is shifted. This rule was held to be exemplified in the action of arginase upon clupein and upon products of its partial hydrolysis [Felix *et al.* 1933], and was extended to include all proteins or peptides having a terminal arginine with its

carboxyl free. Whatever validity it may possess in such cases, monobenzoyl-arginine remains the only instance (and that admittedly a doubtful one) of its realization with a substance of known constitution. Data for argininic acid are lacking; but arginylarginine is freely split at pH 9.2 [Edlbacher & Burchard, 1931], for octopin and its isomerides the optimum is about 9.5 and for carbamido-arginine it has been shown to be about 9.7. To judge from these examples the nature of the substituting group has little effect upon the optimum; for in the first that group is of predominantly basic character, in the second it is an acid, and in the third it is neutral.

SUMMARY

1. It has been shown that liver arginase hydrolyses carbamidoarginine with the production of urea and α -carbamidoornithine.
2. The latter substance, not hitherto known, has been described, and from it there has been prepared an anhydride, also new, which has been shown to be probably 3-uramido-2-piperidone.
3. The pH optimum for the action of arginase upon carbamidoarginine is identical with that for its action upon arginine.
4. The activity of arginase toward carbamidoarginine is 165 times less than its activity toward arginine.
5. The bearing of these observations upon the specificity of arginase is discussed.

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