On Arginase and its Participation in Urea Synthesis in the Liver

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Since Bollman, Mann & Magath (1924) demonstrated that *in vivo* mammalian liver was the only site for the synthesis of urea, much effort has been directed to the investigation of the details of this synthesizing mechanism. The theory of the 'ornithine cycle' (Krebs & Henseleit, 1932) offered a plausible explanation, until Neber (1935), London & Alexandry (1937) and Leuthardt (1938), in similar experiments *in vivo* and *in vitro*, were unable to confirm the essential role attributed to ornithine in urea synthesis. Trowell more recently (1942), in perfusion experiments, failed to find more than a transitory effect on urea synthesis by the other members of the 'ornithine cycle', citrulline and arginine; he found, however, a catalytic action of ornithine under his experimental conditions. The experiments of Schoenheimer, Ratner & Rittenberg (1939) confirming the 'cycle' are not unambiguous. The workers after feeding with N^{15} found a high percentage of the isotope both in the urea and in the protein-linked arginine but not in the free arginine. They formulated the hypothesis that 'free arginine' and protein arginine are continuously exchanged in the liver, and that urea is formed from arginine while the latter is in the free state, for peptide-linked arginine is not hydrolyzed by arginase. Furthermore, the amide-N fraction of the liver protein of the carcass contained only slightly less N^{15} than the amidine fraction of the arginine, a finding which supports the idea that amide-N may possibly play a part as important as that of arginine in the synthetic mechanism (Leuthardt, 1938; Bach, 1939).

Bach (1939) suggested a new mechanism of urea synthesis in which glutamine was the principal ammonia carrier in place of ornithine and citrulline ('amide-N cycle') and explained the effect of citrulline on urea synthesis by its oxidation by keto-acids to glutamic acid and glutamine. He suggested a similar possibility for ornithine. Arginine and arginase were not considered to participate in this mechanism.

The abundant occurrence of arginase in mammalian liver, however, and the ease with which it can be demonstrated, may have influenced the general opinion for a long time in favour of arginine being the only source of urea. Clementi's empirical rule (Clementi, 1914) that arginase is present in the livers of ureotelic and absent from those of uricotelic

animals, although it cannot be applied without limitations, has contributed much to this view. Leuthardt & Glasson (1942) attempted to reconcile the importance attributed by Leuthardt (1938) and Bach (1939) to glutamine with the essential role earlier given to arginine and arginase in urea synthesis. They suggested, though without much experimental evidence, an extension of the 'ornithine cycle' to include glutamine, which acts as ammonia carrier and transfers its ammonia to ornithine, whence the cycle continues to arginine and urea.

In view of these conflicting views on the problem of urea synthesis and the part played by arginine and arginase, an attempt was made to decide if urea synthesis could take place in the liver without the participation of arginase. Experiments to this effect were described by Bach & Williamson (1942) who showed that urea synthesis was not impeded when arginase was inhibited by excess ornithine. Details of this preliminary work are given in this paper, which also includes experiments on the nature of 'soluble' and 'tissue' arginase. Arginase is a highly soluble enzyme and will in experiments *in vitro* partially diffuse out of the tissue into the surrounding medium. Any effect observed with arginase might therefore be interpreted as originating from the extracellular part of the enzyme, the portion within the intact tissue not having come into play (Krebs, 1943). The participation of this 'tissue arginase' in the experiments, the similarity of 'tissue' and 'extracellular' arginase with respect to their Michaelis constant, to the effect of O_2 on the enzymes, and to the inhibition by ornithine, are shown in the first part of this paper, together with experiments on the accessibility of the tissue to arginine and ornithine. The remaining experiments are devoted to the main problem of the participation of arginase in urea synthesis.

METHODS

Animals. The rats used were similar to those previously employed (Bach, 1944).

Washing of slices. The method of slicing and weighing was also described in the above paper but the washing of the slices after weighing needs special attention. Each portion of slices was placed into a shallow 5 ml. dish, through which a steady stream of Ringer's solution was passed from a capillary tube. The dishes stood on a glass platform inside a crystallizing dish so that the overflow could be collected. After 5 min. washing in running Ringer's solution the dishes

were emptied, refilled with fresh Ringer's solution and the washing continued. This procedure was repeated and finally the slices were placed into fresh Ringer's solution before being transferred to the Barcroft vessels.

In this way it was possible to obtain from the same liver comparable portions of slices which were practically free from urea and preformed urea-forming substrates and which under the same experimental conditions yielded similar results. Many of the experiments described in this paper depend on the comparability of the different portions of slices used. Table 1 gives a few examples of duplicate experiments. All weights of tissue given in the text refer to wet weight.

Table 1. *Duplicate experiments with liver slices*

Wet wt. of slices (mg.)	Time of incubation (min.)	Arginine concentration (g./100 ml.)	Urea-N produced (μ g.)
100	60	0.06	{490 460
100	50	0.05	{268 310
50	20	0.07	{181 175
50	15	0.007	{22 21
50	10	0.5	{493 466
50	20	0.5	{720 795
40	40	0.5	{1060 1030

Incubation of slices. See Bach (1944). Tissue slices and tissue extracts were incubated at 38°.

Estimation of urea-N. Urea estimation was carried out as before (Bach, 1939) by incubation of the experimental fluid with urease followed by ammonia distillation at a low temperature *in vacuo* (Parnas & Heller, 1924). In the later experiments a Kjeldahl apparatus of the Pregl type (Markham, 1942) was used. When large amounts of urea-N were present the manometric method of Krebs & Henseleit (1932) was applied. With the Markham apparatus a distillation could be carried out within 2 min., a time sufficiently short to avoid decomposition of urea and amino-acids (see Table 2). With the Parnas apparatus the ammonia was

distilled into 0.01 N-HCl, while with the Kjeldahl distillation into water was sufficient if the quantities distilled did not exceed 100 μ g. The distillate was titrated with 0.01 N-alkali or 0.01 N-HCl respectively by means of a horizontal Conway microburette, as in the earlier paper (Bach, 1939).

Krebs's (1942) criticism of the method of microtitration, implying that a reading of 0.2 ml. on a microburette cannot be taken accurately enough to yield satisfactory results, is contrary to all experience of microtitration. Most experimenters agree that 0.005 ml., i.e. a 40 times smaller quantity, can be accurately read on a 1 ml. microburette. 0.2 ml. 0.01 N-HCl corresponds approx. to 100 μ g. urea-N per sample (allowing for the fact that only 30–50% of the whole is used for the titration); an error of ± 0.005 ml. will therefore correspond to ± 2.5 μ g. urea-N. Conway (1939) calculated the standard deviation in titrating 1 ml. 0.01 N-acid with 0.01 N-alkali as 25×10^{-6} m.mol. = approx. 0.3 μ g. N or (allowing for the fractional portion used) = 1 μ g. per total sample. With quantities of 100 μ g. these deviations amount to approx. ± 1 –3%. This error is roughly the same as in the recovery experiments (Table 2), in which known quantities of urea-N were estimated in absence and presence of tissue slices. Thus Krebs's explanation of discrepancies between his own results and those of Bach by attributing erroneous titrations to the latter is without foundation. Furthermore, to support his argument Krebs chose a few unusually divergent values from among fifty or more reasonably consistent controls. Incidentally, it is these particular results criticized which have been independently confirmed both by Gornal & Hunter (1943) and by Borsook & Dubnoff (1943).

Reagents. *l*(+)-Arginine was prepared from gelatin according to Whitmore (1932) and *l*(+)-ornithine from arginine according to Hunter (1939).

Preparation of liver extracts containing arginase

(a) *Glycerol extracts.* Fresh rat liver (4 g.) was ground up with glycerol (16 ml.). The mixture was centrifuged and filtered through glass-wool. In the anaerobic experiments of Figs. 2b and 3 the filtrate was transferred to a Thunberg tube which was then evacuated. Immediately before the incubation a measured fraction of its content was quickly transferred to Barcroft vessels containing the other reagents and irrigated with a N_2/CO_2 mixture.

(b) *Aqueous extracts.* Liver acetone powder (1 g.) was ground up with water (10 ml.). The mixture was centrifuged and filtered through glass-wool.

Table 2. *Ammonia and urea recovery experiments*

In presence of liver slices (mg.)	NH ₃ -N (μ g.)		Urea-N (μ g.)		Error (%)	Apparatus used (P = Parnas, K = Kjeldahl)
	Added	Found	Added	Found		
—	50	49.5	—	—	1	K
—	25.1	25.4	100	—	1	K
—	—	—	100	101.5	1.5	P
—	94.2	89.6	200	196	5 and 2	P
—	—	—	100	99.9	0	K
200	437.5	434	—	—	1	P
50	—	—	31	32.4	4.5	K
100	—	—	200	202	1	P
In presence of 4 mg. ornithine, 6 mg. glutamic acid, and 6 mg. lactic acid:						
100	92	94.2	200	191	2 and 4.5	P
In presence of 4 mg. ornithine and 2 mg. arginine:						
—	—	0	—	0	—	K

RESULTS

The participation of intracellular arginase in experiments with tissue slices

100 mg. liver slices were shaken in 5 ml. bicarbonate Ringer's solution in a Barcroft vessel, in absence of substrate, for 10 min. They were then transferred to another vessel containing fresh Ringer's solution and after 10 min. to a third and at 10 min. intervals to a fourth and fifth vessel. At the end of each 10 min. period arginine was added to the fluid from which the slices had been removed and the mixture was incubated for a further 10 min., after which the enzyme action was stopped by the addition of trichloroacetic acid and the urea formed was estimated. At the beginning of the fifth period of incubation arginine was added to the tissue suspension and the mixture was again incubated for 10 min. Any arginase found in solutions 1-4 must have diffused out of the tissue into the Ringer's solution during the first four incubations. Any arginase effect, however, found in the Ringer's solution of the fifth vessel must have been due both to intracellular and extracellular enzyme, since the substrate had been added in presence of the tissue.

Fig. 1. The participation of intra- and extracellular arginase of liver slices. Arginine 0.5% (final concentration) added for activity determination: total volume 6 ml. One sample (100 mg.) liver slices incubated for 10 min. periods in Barcroft vessels each containing 5 ml. fresh Ringer. At the end of each of four periods 1 ml. arginine HCl (3%) added to the fluid after removal of the slices and further incubated = urea-N production from extracellular arginase. After the fifth period arginine HCl added without removal of the slices = urea production from extracellular plus intracellular arginase.

The amount of urea produced in each of the solutions is a measure of the outflow of arginase from the tissue during the corresponding period and is plotted in Fig. 1. It will be seen that the outflow gradually decreased during the first three incubations, when the level of the outflow of arginase became almost constant, and a similar urea-N value would have been expected after the fifth incubation

had the arginine been added as in 1-4, namely after removal of the tissue. But it will be seen that, with tissue present, arginine hydrolysis in the fifth vessel rose to reach a new peak. This was obviously due to the participation of intracellular enzyme.

It can therefore be assumed that in such *in vitro* experiments 'tissue' arginase participates in the hydrolysis of arginine and also that the tissue is permeable to arginine.

Properties of arginase in liver slices and cell-free liver extracts

(a) *Michaelis constant (K_m)*. Figs. 2a and 2b demonstrate the determination of K_m for arginase in liver slices and in cell-free glycerol extract of liver tissue.

Fig. 2a. Determination of the Michaelis constant (K_m) for arginase in liver slices (50 mg.). Time of incubation 10 min. Total volume: 4 ml.

Fig. 2b. Determination of Michaelis constant (K_m) for arginase in glycerol extract (0.5 ml.) under anaerobic conditions. Time of incubation 10 min. Total volume: 4 ml.

The results, namely, K_m for 'tissue' arginase = $2.5 \times 10^{-3} M$ and K_m for extract = $2.75 \times 10^{-3} M$ -arginine, are as close as can be expected, considering the fact that each concentration of substrate had to be tested with a different portion of liver slices, and are consistent with the identity of the two forms.

(b) *The inhibitory effect of O_2 on arginase.* The effect of O_2 on arginase extracts, first observed by Edlbacher, Kraus & Leuthardt (1933), was studied with different O_2/N_2 mixtures in experiments with tissue slices and tissue extracts. In agreement with these workers, it was found that aqueous arginase extracts exposed to air were irreversibly damaged by O_2 , while glycerol extracts were less affected. Further treatment with O_2 would therefore have little or no effect on an aqueous arginase extract previously exposed, while it should be possible to demonstrate O_2 inhibition with a glycerol extract prepared under anaerobic conditions. From Fig. 3

Fig. 3. Effect of oxygen on arginase. Time of incubation 30 min. Note: urea production under anaerobic conditions is taken as 100%. Total volume: 4 ml.

it can be seen that arginase in glycerol extract was increasingly inhibited by O_2 treatment, while no effect was seen with aqueous extracts. The effect of O_2 treatment on arginase in liver slices during incubation was even more marked, because the enzyme within the intact tissue was better protected against atmospheric O_2 by the cellular structure during the preparatory stages of the experiment, than was the enzyme extracted from the cells. So there is no doubt that both in tissues and in glycerol extracts arginase shows a higher activity under anaerobic conditions.

In experiments shown in Fig. 4 the irreversibility of O_2 inhibition was further illustrated. Two identical portions of slices, each divided into three equal parts, were incubated with arginine, one anaerobically and the other aerobically. For this purpose the Warburg vessels containing the tissue and the experimental mixture with arginine in the side-bulb were irrigated with the gas mixture for 10 min. both before and after being immersed in the water tank. 30–40 min. after arginine had been added to the experimental mixture, the first part of the aerobic and anaerobic portions were taken out and the urea produced by them was estimated. The second part of each portion was then incubated another 30–40 min. under the same conditions. The gaseous conditions of the third parts of each portion were reversed, i.e. the one which had been incubated under anaerobic conditions was irrigated with an O_2/CO_2 mixture for 10 min. and vice versa.

As a result it will be seen that in the initial period urea production was 2–3 times greater under anaerobic conditions than under aerobic; further that aeration of the anaerobic portion almost completely

Fig. 4. Hydrolysis of arginine by arginase in liver slices (50 mg.) under anaerobic and aerobic conditions. Arginine HCl, 0.3%. Total volume: 5 ml.

inhibited any further urea production, while the replacement of O_2 in the aerobic portion by N_2 had scarcely any effect, the tissue being irreversibly damaged by shaking in 95% O_2 .

Finally, in experiments shown in Fig. 5 the effect of O_2 on arginase is compared with that on the urea-synthesizing mechanism in liver slices. It will be

Fig. 5. The effect of O_2 on urea production by hydrolysis of arginine and by synthesis from ammonium lactate and ornithine in liver slices. Arginine HCl, 0.3%; ornithine HCl, 0.1%; lactate, 0.2%; NH_4Cl , 0.01%. Note: urea production by aerobic synthesis and by anaerobic hydrolysis is taken as 100%. Total volume: 4 ml.

seen that while arginase works most actively under anaerobic conditions and is gradually inhibited by O_2 , urea synthesis is completely inhibited in absence of O_2 and increases with rising O_2 content of the gas mixture.

The conclusions to be drawn from the above experiments are twofold: (1) There is a close similarity between the behaviour of extracted and 'tissue' arginase as shown by their Michaelis constants and by the effect of O_2 , so that cellular

environment apparently has little or no effect on these properties of the enzyme. (2) The enzyme within the intact cell structure is, however, better protected against atmospheric O_2 than the enzyme in aqueous solution.

(c) *Inhibition of arginase by ornithine.* In the search for a specific inhibitor of arginase the effect of ornithine was investigated. Its inhibitory action first observed by Gross (1920) on arginase in aqueous liver extracts can also be demonstrated on arginase in liver slices. The experiments shown in Fig. 6 give evidence for the similarity of the ornithine effect on tissue arginase and arginase extract of comparable strength.

Fig. 6. The inhibition of arginase by various concentrations of ornithine in liver slices and cell-free extract. Arginine HCl, 75 mg./100 ml. Time of incubation 20 min. Total volume: 4 ml.

Fig. 7. The inhibition of arginase by excess ornithine in liver slices (progress curve). 50 mg. slices; arginine HCl, 15 mg./100 ml.; ornithine, 1.6%. Total volume: 5 ml.

The lower concentration of ornithine needed to inhibit arginase in extracts than in tissue slices raises the problem whether intracellular arginase is accessible to ornithine added to the suspension of tissue slices. Experiments shown in Fig. 7 made it clear that there was an immediate response after either arginine or ornithine had been added to the experimental mixture. Since the inhibition of arginase by ornithine is complete, and since the above experiments have shown that intracellular arginase participates in these *in vitro* reactions, both substrates must have had immediate access to the

tissue cells and the fact that no time lag was observed is evidence against a low tissue permeability for either.

(d) *The fate of ornithine in liver metabolism.* It will be seen, however, that the inhibitory effect of ornithine is greatly reduced after prolonged incubation with tissue slices (Figs. 7, 9), while it remains almost constant in arginase extracts as shown in the experiments of Fig. 8. Table 3, section A, further

Fig. 8. The inhibition of arginase by excess ornithine in cell-free aqueous extract (progress curve). Arginine HCl, 75 mg./100 ml.; ornithine HCl, 0.48%; aqueous extract, 0.5 ml. Total volume: 5 ml.

illustrates this phenomenon. The results of section B of Table 3 are particularly interesting: when ornithine is pre-incubated with the tissue before arginine

Table 3. *Inhibition of arginase by excess ornithine*

(A) Effect of the period of incubation

Exp. no.	Arginase in	Ornithine added (g./100 ml.)	Period of incubation (min.)	Inhibition (%)
1	Liver slices	1.2	45	77
2	Liver slices	1.2	120	28
3	Cell-free extract	0.14	20	64
4	Cell-free extract	0.14	90	63

(B) Effect of time and temperature of pre-incubation of ornithine with liver slices

Exp. no.	Ornithine (g./100 ml.)	Pre-incubation		Inhibition (%)
		min.	temp.	
5	1	Not pre-incubated		76
6	1	30	18°	57
7	0.7	30	18°	51
8	0.7	30	37°	24

Note. In Exps. 1-5 ornithine and arginine were added simultaneously. In Exps. 6-8 arginine was added after pre-incubation of ornithine with the tissue.

is added, its inhibitory effect on arginase in the following period of incubation is reduced and this reduction is enhanced if pre-incubation is carried out at a higher temperature. From these results it is possible to conclude that ornithine is being

metabolized by enzymic systems present in the intact liver tissue but absent in liver extracts. If, therefore, ornithine were exposed to such a metabolic attack before or after arginase comes into play it would be partly used up and, with the decreasing concentration of the inhibitor, arginase becomes less and less impeded. A higher temperature would intensify any such metabolic action.

Urea synthesis with inhibited arginase

The strong inhibitory action of excess ornithine on the arginase of liver slices can be used to throw light on the problem of the participation of arginase in the synthesis of urea in the liver. For if arginase were considered indispensable for the synthesis of urea an inhibition of the enzyme should also stop the formation of urea. In the experiments recorded in Fig. 9 four portions of liver slices, each weighing 200 mg., were incubated in bicarbonate Ringer's solution (curves 1-4).

Fig. 9. Urea synthesis with and without inhibition of arginase. 200 mg. liver slices; arginine, 7.5 mg./100 ml.; ornithine, 0.4 and 1.6% respectively; Na lactate, 0.2%; NH_4Cl , 0.01%. Total volume: 5 ml.

For curve 1 arginine was added to demonstrate the presence of arginase; for curve 2 arginine and excess ornithine were added, as a result of which urea production was strongly inhibited in the initial period. In the experiments for curve 3 the Ringer contained ammonium lactate and small quantities of ornithine in concentrations identical with those used by Krebs & Henseleit (1932) for urea synthesis in liver slices. It will be seen that 60 $\mu\text{g. urea-N}$ were formed in 60 min. In the experiments for curve 4 the composition of the experimental mixture was identical with that of the experiments for curve 3, except that excess ornithine in quantities equal to those used for the inhibition of arginase were added.

Curve 4 shows that in spite of strong inhibition of arginase in the first 30-40 min. (see curve 2) the synthesis of urea was entirely unimpeded or even slightly accelerated in comparison with that shown in curve 3, where arginase was not inhibited.

Arginine content of liver slices

Urea production from arginine with both uninhibited and inhibited arginase as shown in curves 1 and 2 of Fig. 9 depends, amongst other things, on the quantities of arginine added. With too small a quantity of arginine used in the experiments for curve 2 inhibition would appear to be unduly enhanced and may not have corresponded to physiological conditions. It was therefore decided to choose a concentration of arginine not below but rather above physiological level. The actual concentration of arginine in the tissue during urea synthesis was therefore estimated.

500 mg. thoroughly washed slices were incubated for 30 min. with ammonium-lactate and ornithine in optimal concentration (Krebs & Henseleit, 1932) for urea synthesis. At the end of this period of incubation the urea formed was estimated and a measured portion of the experimental fluid, free from slices, was transferred to another vessel for a second period of incubation (45 min.) with a cell-free liver extract containing arginase. The activity of this enzyme preparation had previously been tested and its urea content determined. The experimental mixture used in the second incubation thus contained initially a known fraction of (a) urea synthesized during the first period of incubation, plus (b) urea initially present in the tissue extract. Any quantity in excess of (a) + (b) found at the end of the second period of incubation must have been formed in this period by the action of arginase on any arginine present. Since the arginase extract was arginine-free any arginine found must have originated from the first experimental fluid used for the incubation of the tissue slices.

Table 4 gives examples of the experiments described above: the example showing the higher arginine content corresponds with the presence of 220 $\mu\text{g. arginine HCl}/500 \text{ mg. tissue}$ or 88 $\mu\text{g.}/200 \text{ mg.}$

Table 4. *Determination of the arginine content of liver slices (500 mg.) during urea synthesis*

Initial incubation period (min.)	$\mu\text{g. urea-N}$ found			Urea-N formed by hydrolysis of arginine: (2) minus (1 + 3)	Urea-N ex-pressed as arginine HCl ($\mu\text{g.}$)
	(1) After initial period of incubation	(2) After second period of incubation	(3) In arginase extract used for second incubation		
30	65	111.5	17	29.5	220
90	168	191	17	6.0	45

tissue. In the experiments shown in Fig. 9, 375 $\mu\text{g. arginine HCl}$ were added to 200 mg. tissue (curves 1-2), i.e. more than four times the quantity found according to Table 4.

The main conclusion drawn from the experiments in Fig. 9 must be that arginase does not necessarily participate in the urea synthesis. This conclusion is based on the fact that, with the arginase activity inhibited, urea production from arginine occurs at a low rate, whilst on the other hand that from ammonium lactate is unimpeded. This discrepancy would be even more marked if the arginine concentration for curve 2 were to be still further reduced.

DISCUSSION

Participation of 'tissue' arginase in the experiments. The above experiments were designed to prove that urea synthesis from ammonium lactate remains unimpeded when the arginase in the tissue is strongly inhibited by excess ornithine. However, evidence had to be given that ornithine inhibited not only the arginase which diffused from the cells into the surrounding medium, but also the enzyme within the intact tissue. For this purpose it was first shown that the 'tissue arginase' participates in the hydrolysis of arginine in liver slice experiments (Fig. 1) and hence that the tissue is permeable to arginine, and further that the activity of arginase in liver slice experiments, like that in tissue extracts, can be fully inhibited by ornithine. This proves that the tissue is also permeable to ornithine. The evidence is supplemented by experiments showing that the enzyme had similar properties whether outside or inside the intact cellular structure. Thus a similar Michaelis constant and a similar behaviour towards oxygen and ornithine was found for 'tissue' arginase and cell-free arginase.

Inhibitory effect of ornithine on arginase. The effect of ornithine on arginase is specific and is caused by mass action in shifting towards the left the equilibrium in the reaction: $\text{arginine} \rightleftharpoons \text{ornithine} + \text{urea}$. Urea has a similar inhibitory effect on arginase (Vorochenko, 1936).

Metabolism of ornithine. Independently of this inhibitory action on arginase, ornithine is probably metabolized by the intact tissue but not by aqueous tissue extracts. For this assumption only indirect evidence could be secured by the findings that the inhibiting power of ornithine decreases with longer periods and higher temperature of incubation. The fate of ornithine in liver metabolism is a problem apart from its possible connexion with urea synthesis. Work in this direction is in progress.

Effect of O_2 on arginase. The irreversible inhibitory effect of O_2 on arginase in tissue extracts previously described by Edlbacher *et al.* (1933) was also observed with arginase in liver slices. Whereas tissue arginase appears to be extremely sensitive towards O_2 —the activity of arginase in yeast can even be fully suppressed by aeration (Edlbacher, Becker & v. Segesser, 1938)—urea synthesis greatly depends

on an ample supply of O_2 . This is clearly illustrated in experiments shown in Fig. 5 from which it can be seen that the oxygenation conditions under which urea synthesis takes place are unfavourable for arginase, while urea synthesis is entirely suppressed at low oxygen pressures almost optimal for the activity of arginase. While it is improbable that there is no intermediate condition under which both reactions may take place simultaneously, the experiments reported are not very favourable for the postulate that arginase is an obligatory intermediate enzyme in the synthesis of urea.

Participation of arginase in urea synthesis. Fig. 9 gives the crucial experiments bearing on the question of the participation of arginase in the synthesis. Even with an arginine concentration four times as strong as can be expected to exist in the tissue (Table 4), urea production from arginine with inhibited arginase (curve 3) cannot account to any significant extent for the synthesis of urea from ammonium lactate with arginase inhibited to the same degree (curve 2). These experiments were repeated with the same result: in both cases urea synthesis with inhibited arginase was slightly faster than with uninhibited arginase. The results clearly indicate the existence of a synthetic mechanism for urea other than, or in addition to, that of the 'ornithine cycle'.

Current ideas on the mechanism for the synthesis of urea. The results described in this paper may encourage the search for a new mechanism for the synthesis of urea. Whether there is any truth in the synthetic schemes suggested hitherto by Krebs & Henseleit (1932) or by Bach (1939) or others can only be finally decided by the chemical isolation of the reaction products. So far this has not been achieved for any scheme and indirect methods are

* Krebs (1943) postulated that under the conditions of Bach & Williamson (1942) only the small, extracellular part of the 'potential' arginase of the tissue participates in arginine hydrolysis and can be inhibited by ornithine. However, the participation of 'tissue arginase' has been demonstrated here.

He further argues that the remainder was uninhibited and participated in urea synthesis. Apart from the fact that it is difficult to know what importance can be attached to a calculation of the 'potential' activity of an enzyme (part of which had been removed by extensive washing) within the cellular structure from measurements on unwashed disintegrated cells, any such deduction cannot invalidate these experiments. For there is no ground for assuming that under the same experimental conditions the arginase hydrolyzing arginine supplied from outside the cell is different in nature from that hydrolyzing arginine synthesized within it from added ornithine. Therefore, if the observable proportion of extracellular plus intracellular arginase, however great or small, is inhibited by ornithine, it must be assumed that the arginase available for the synthesis observed under exactly the same experimental conditions is inhibited to the same degree.

rarely safe enough to decide the issue. The results of Gornal & Hunter (1943), who found accumulation of citrulline with added ornithine in tissue slices, suffer from the unspecificity of the colorimetric estimations of citrulline (the Fearon (1939) reaction works with all mono-substituted ureas) and from the high control values amounting in some cases to 60–80 % of the total changes (see Gornal & Hunter, 1943, Table IV). However, these workers and also Borsook & Dubnoff (1943) confirm Bach's findings (1939) that the ratio of urea-N found to ammonia-N disappeared was 1, instead of 2 as expected from Krebs's theory.

The present stage of knowledge with regard to the 'ornithine cycle' is well summarized by Borsook & Dubnoff (1943). Although, according to the authors, 'there is increasing expression of doubt regarding the ornithine cycle', there is in our mind always the possibility that the mechanism involving arginase exists side by side with others which may be called into action according to the conditions prevailing in the synthesizing tissue at the time.

Alternative role of arginase. Finally, a role of arginase, alternative to that as the principal catalyst in the synthesis of urea, may be considered. Cases are known where no parallelism is observed between the arginase content of a tissue and its urea production. Takahara (1938), for example, found a marked decrease in the arginase content of liver and a simultaneous rise of the blood-urea level after phosphorus poisoning, and Edlbacher & Koller (1934) report very high arginase contents of liver

sarcoma coupled with incapacity to synthesize urea. According to Edlbacher & Merz (1927), arginase activity in malignant tumours does not represent a partial reaction in the synthesis of urea but may be considered as a specific growth factor, or may even participate in protein synthesis by the transfer of N to non-nitrogenous substances (Edlbacher & Baur, 1938).

SUMMARY

1. A specific inhibition of arginase in tissue slices was obtained by adding large quantities of ornithine.
2. Urea synthesis in liver slices from ammonium lactate takes place unimpeded when arginase is thus inhibited.
3. While the arginase activity in liver slices is irreversibly inhibited by O_2 and is optimal under anaerobic conditions, urea synthesis from ammonium lactate depends on ample oxygenation.
4. These findings support the idea of a synthesizing mechanism other than, but not necessarily excluding, the 'ornithine cycle'. This new mechanism is independent of arginase.
5. Evidence was obtained that the tissue arginase, as well as that which passed into the solution, participated in the experiments described.
6. The different theories of the mechanism of urea synthesis are discussed and an alternative role of arginase is indicated.

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