CLXXXII. TRANSAMINATION IN PIGEON BREAST MUSCLE

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D. M. NEEDHAM [1930] first observed that in pigeon breast muscle added glutamic acid disappears from the tissue while the concentration of amino groups, as measured by the Van Slyke method, remained unchanged. She concluded that the amino group of glutamic acid is transferred to "some reactive carbohydrate residue" to form a new amino-acid. Braunstein & Kritzmann [1937; 1938] succeeded in elucidating this reaction in detail. They showed that glutamic acid reacts as follows:

(1) Glutamic acid + α-ketonic acid ⇌ α-ketoglutaric acid + amino-acid. The "reactive carbohydrate residue" of Needham is thus an α-ketonic acid. The reaction is reversible and proceeds with great rapidity under suitable conditions. It is a general reaction, occurring in almost every animal tissue, and has been termed "transamination" [Schaeffer & Le Breton, 1938; Cohen, 1939; Braunstein, 1939]. The widespread occurrence and the rapid rate of transamination suggest that the reaction plays an important part in tissue metabolism, although its significance is as yet by no means clear.

The detailed study of transamination has been hampered by the lack of suitable analytical methods. The procedure used by Braunstein & Kritzmann [1937] does not appear to be very satisfactory for the authors themselves [1938] question their analytical data in several instances. Since the determination of glutamic acid synthesis in the presence of α-ketoglutaric and amino-acids is one of the ways of measuring transamination, a more reliable method for glutamic acid determination was recently worked out by the author [1939].

The preliminary experiments with this method showed that although most tissues are capable of bringing about transamination, the different tissues show great differences in detail. In muscle, for example, only three amino-acids, *l*(+) glutamic, *l*(−)aspartic and *l*(+)alanine react rapidly; two others, *dl*-α-aminobutyric and *l*(+)valine react slightly; while the other amino-acids do not react appreciably. In kidney, on the other hand, many more amino-acids react.

This paper is confined to a study of transamination in pigeon breast muscle. Experiments on the rates of transamination in this tissue and the reactivity of different amino-acids are reported.

Procedure and methods

Principle of the procedure. For the study of transamination it is desirable to separate this reaction from other reactions in which amino- and α-keto-acids take part. Many of the other reactions require molecular O_2 and they can therefore be eliminated by working under anaerobic conditions. Since it is simpler we have preferred this method to the use of inhibitors. Comparative experiments showed that the results obtained under anaerobic conditions are not essentially different from those obtained aerobically in the presence of bromoacetate [Braunstein & Kritzmann, 1937], an inhibitor which prevents certain

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side reactions. Neither method however eliminates the side reactions completely, for glutamic acid slowly disappears anaerobically, as well as aerobically in the presence of iodoacetate, without the addition of ketonic acids. The mechanism of this reaction is not clear; it may be due to transamination to ketonic acids provided by the muscle. The rate of the reaction however is relatively slow so that it generally does not interfere with experiments on transamination of added substrates.

Transamination was studied by the determination of glutamic acid formed on the addition of α-ketoglutaric acid and amino-acids. In all experiments a blank was carried out with α-ketoglutaric acid alone to determine the glutamic acid formation from preformed NH_2-donators (NH_3 or amino-acids). The values of the "blanks" in most experiments were considerable, showing that a relatively high concentration of NH_2-donators is present in pigeon breast muscle.

An increased glutamic acid formation from added α-ketoglutaric acid and amino acids may be considered as conclusive proof of transamination. Euler *et al.* [1939] studied the disappearance of oxaloacetic acid in the presence of amino-acids and they concluded that transamination occurs when the added amino-acids increased the rate of disappearance of oxaloacetic acid. This type of experimentation may suggest that transamination takes place, but cannot provide conclusive proof. The demonstration of aspartic acid formation in this reaction would in our view be the only conclusive evidence of transamination.

Experimental procedure. Pigeon breast muscle was chilled immediately after the death of the animal and finely divided in a Latapie mincer. The minced muscle was suspended in 0·10 *M* phosphate buffer, *p*H 7·4. Unless otherwise stated the muscle suspension used in the different experiments consisted of 1 part muscle plus 7 parts phosphate buffer. The suspension (3 ml.) was pipetted into the main compartment of conical Warburg manometer flasks provided with a side arm, which contained the substrate, and a centre well which contained a stick of yellow phosphorus. The substrates were used as neutral 0·2 *M* solutions (0·4 *M* for *dl*-amino-acids) and usually added in amounts to give a final concentration of 0·017 *M*. Substrates which were soluble with difficulty were weighed directly into the side arm of the cup.

Anaerobic conditions were maintained by filling the vessels with N_2 and by the yellow phosphorus in the centre well. The vessels were shaken at 40° and unless otherwise stated the substrate was added from the side arm after 5 min. shaking. At the end of the experimental period the flasks were detached and 1 ml. 10 % H_2SO_4 added to stop the reaction. The solutions were washed into graduated cylinders with 6–7 ml. distilled water, 1 ml. 10 % Na_2WO_4 was added and the solutions made up to a volume of 15–20 ml. The solutions were filtered and an aliquot of the clear filtrate employed for the glutamic acid determination.

Analytical procedure. Glutamic acid was determined by the method recently described by the author [1939]. Interfering succinic acid, and malonic acid when added, were removed by ether extraction before the glutamic acid determination. The aliquot of the clear filtrate was acidified with 2 ml. 10 % H_2SO_4 and extracted with ethyl ether for 2 hr. The ether was removed from the aqueous phase and the latter brought to a *p*H of approximately 5 by the addition of alkali in the presence of a drop of bromocresol purple. The glutamic acid in the aqueous phase was then determined.

The results are expressed as μl. glutamic acid (147 mg. glutamic acid being equivalent to 22,400 μl.). This procedure was used since the glutamic acid was determined as μl. O_2 (1 μl. O_2 is equivalent to 2 μl. glutamic acid).

Results

Glutamic acid formation from amino-acids and α-ketoglutaric acid

The question was first investigated as to which amino-acids react with α-ketoglutaric acid to form glutamic acid. In these experiments a relatively long incubation period of 40 min. was chosen to allow for the formation of measurable amounts of glutamic acid from slowly reacting amino-acids. The results of these experiments are listed in Table I.

Table I. *Glutamic acid formation from α-ketoglutaric acid and different amino-acids*

Substrate conc. 0·017 *M* (for *dl*-amino-acids, 0·034 *M*). N_2. Yellow phosphorus. 40°.

Amino-acid added	Period of incubation min.	μl. Glutamic acid formed: Without amino-acid	μl. Glutamic acid formed: With amino-acid	Increase in glutamic acid due to added amino-acid μl.	% of added α-keto-glutaric acid converted into glutamic acid
l(−)Aspartic acid	40	161	815	654	49
l(+)Alanine	40	96	636	543	40
l(+)Valine	40	96	220	124	9
l(−)Phenylalanine	40	96	170	74	—
l(−)Tyrosine	40	142	204	62	
l(−)Cysteine	40	94	152	56	—
d(−)Valine	40	96	149	53	—
l(−)Histidine	40	96	146	50	—
d(+)Histidine	40	96	140	44	—
l(−)Leucine	40	142	180	38	—
d(−)Alanine	40	130	160	30	—
l(+)Citrulline	40	142	168	26	—
l(+)*iso*Leucine	40	142	166	24	—
l(−)Methionine	30	138	156	18	—
l(+)Arginine	40	96	99	3	—
Glycine	40	96	95	—	—
l(+)Ornithine	40	142	104	—	—
l(−)Proline	40	142	142	—	—
d(+)Phenylalanine	40	96	92	—	—
l(−)Tryptophan	40	142	124	—	—
dl-Serine	40	142	140	—	—
dl-Lysine	30	138	113	—	—
		Homologous series			
dl-Alanine	40	130	564	434	32
dl-α-Aminobutyric acid	40	130	266	136	10
dl-α-Aminovaleric acid	40	130	145	15	—
dl-α-Aminohexoic acid	40	130	150	20	—

Of the 21 different amino-acids tested, *l*(−)aspartic acid and *l*(+)alanine are the only ones which show a large glutamic acid formation; *dl*-α-amino-butyric acid and *l*(+)valine show a slight activity. None of the remaining amino-acids is significantly active.

These results differ in various points from those reported by Braunstein & Kritzmann [1938] whose experiments were carried out aerobically in the presence of bromoacetate. In order to ascertain whether the differences in the results are due to differences in experimental conditions, transamination was also studied under conditions similar to those of Braunstein & Kritzmann. It is seen from Table II that the results obtained aerobically in the presence of iodoacetate are essentially the same as those obtained anaerobically. If anything these experi-

Table II. *Glutamic acid formation from α-ketoglutaric acid and different amino-acids under aerobic conditions in presence of iodoacetate*

Substrate conc. 0·016 *M*. Iodoacetate conc. (final), 0·002 *M*. (Iodoacetate plus muscle suspension incubated 15 min. before substrate added). 1 part minced muscle plus 5 parts phosphate buffer. Reaction time, 60 min. Air.

Amino-acid added	Glutamic acid formed μl.	Increase in glutamic acid due to added amino-acid μl.	% of added α-ketoglutaric converted into glutamic acid
—	92	—	—
l(+)Valine	98	6	—
d(−)Valine	33	—	—
d(+)Histidine	98	6	—
l(−)Histidine	55	—	—
l(+)Arginine	127	35	—
l(+)Alanine	450	358	27
	No iodoacetate		
l(+)Alanine	214	122	9

ments show that the formation of glutamic acid is greater under anaerobic conditions. The inhibitory effect of iodoacetate on the aerobic disappearance of glutamic acid formed from *l*(+)alanine and α-ketoglutaric acid is seen from Table II. This is in agreement with the findings of Braunstein & Kritzmann [1937] who first reported this effect with bromoacetate.

Braunstein & Kritzmann [1938] found 14 different amino-acids to react with α-ketoglutaric acid in pigeon breast muscle, but the authors themselves question their results for 3 amino-acids. Of these amino-acids only the findings for alanine are confirmed by our experiments. From our data previously discussed it follows that the discrepancy between our results and those of Braunstein & Kritzmann cannot be attributed to a difference in experimental procedure. The different results may however be explained by the omission of a suitable control by Braunstein & Kritzmann. As can be seen from Table I α-ketoglutaric acid when added to a muscle suspension forms considerable amounts of glutamic acid in the absence of any added amino-acid. The failure of Braunstein & Kritzmann to take this into account is the chief reason for the high values obtained for all the amino-acids. An additional factor is to be found in the method used by Braunstein & Kritzmann for the determination of glutamic acid. The method employed by these workers measures the NH_2-N which is insoluble in alcohol after the addition of $Ba(OH)_2$ and thus would include aspartic acid and possibly other amino-acids. The high values for certain of the amino-acids, e.g. cystine, reported by these workers is probably due to the insolubility of these amino-acids under the conditions used for the analysis.

On the other hand, of all the amino-acids studied we find *l*(−)aspartic to be the most active. Braunstein & Kritzmann have reported no data for this amino-acid, presumably because their method does not permit the differentiation between glutamic and aspartic acids.

None of the amino-acids of the *d*-series tested was found to be active (Table I).

Effect of ketonic acids on the anaerobic disappearance of glutamic acid

Under anaerobic conditions glutamic acid slowly disappears when added to pigeon breast muscle [Needham, 1930; Braunstein & Kritzmann, 1937; Cohen, 1939]. Braunstein & Kritzmann [1937] showed that the disappearance of glutamic acid is markedly accelerated if pyruvic acid is added, and that the disappearance of glutamic acid is accompanied by the formation of equivalent

amounts of alanine. These authors thus demonstrated the reversibility of transamination.

In order to determine whether pyruvic acid can be replaced by other ketonic acids, the effects of a series of α- and other keto-acids on the anaerobic disappearance of glutamic acid were studied. If the activities of the members of the homologous series of α-ketomonocarboxylic acids are compared (Table III)

Table III. *Disappearance of added glutamic acid in the presence of ketonic acids*

Substrate conc. 0·017 *M*. N_2. Yellow phosphorus. 40°. Incubation period, 40 min.

Ketonic acid added	Glutamic acid added μl.	Glutamic acid found μl.	Glutamic acid disappeared μl.	% Glutamic acid disappeared
—	1344	1240	104	7·5
Oxaloacetic	1344	654	690	51·5
Pyruvic	1344	688	656	49
α-Ketobutyric	1344	1090	254	19
α-Ketovaleric	1344	1180	164	12
α-Ketocaproic	1344	1190	154	11
Mesoxalic	1344	1105	239	18
Acetopyruvic	1344	1162	182	13·5
Acetoacetic	1344	1192	152	11
Laevulic	1344	1360	—	—

it is seen that pyruvic acid is the most active, α-ketobutyric acid is slightly active, while α-ketovaleric and α-ketohexoic acids are not appreciably active. These results are in keeping with the findings of the reverse reactions (Table I). Of the α-ketonic dicarboxylic acids, oxaloacetic acid shows an activity of the same order as pyruvic whilst mesoxalic shows a small activity. Acetoacetic, acetopyruvic and laevulic acids are not appreciably active. Laevulic acid appears to inhibit the small anaerobic disappearance of glutamic acid.

All the preceding observations on transamination in pigeon breast muscle can be explained on the assumption that three different enzyme systems are concerned with transamination in this tissue, one each for the three reactions:

Glutamic acid $\leftrightharpoons$ α-ketoglutaric acid
Aspartic acid $\leftrightharpoons$ oxaloacetic acid
Alanine $\leftrightharpoons$ pyruvic acid

The slight activities of *l*(+)valine and *dl*-α-aminobutyric acid, on the one hand, and α-ketobutyric acid and mesoxalic acid on the other, may be explained by assuming slight affinities of *l*(+)valine, *dl*-α-aminobutyric and α-ketobutyric acids for the alanine $\rightleftharpoons$ pyruvic acid system, and of mesoxalic for the aspartic $\rightleftharpoons$ oxaloacetic acid system. It is well known that the affinities of other enzyme systems, for example, lactic dehydrogenase, are not strictly limited to one substrate. Homologous compounds may also react but the rate of reaction rapidly falls off as the carbon chain is lengthened. If this holds for the transaminating enzymes, then the failure of the higher homologues of alanine and pyruvic acid to react is easily understood. The fact that α-aminobutyric acid is physiologically a rare compound makes the explanation of its activity difficult on any other basis.

Glutamic acid formation from amino compounds other than α-amino-acids

A number of different amino compounds—β-, δ-, and ε-amino-acids, amines and aminopurines—were tested for glutamic acid formation in the presence of

α-ketoglutaric acid, but no significant increase was observed with any of these compounds (Table IV). These findings confirm those of Braunstein & Kritzmann [1938].

Table IV. *Glutamic acid formation from α-ketoglutaric acid and amino compounds other than α-amino acids*

Substrate conc. 0·017 *M*. N_2. Yellow phosphorus. 40°.

Amino-acid added	Period of incubation min.	Glutamic acid formed: Without amino-acid μl.	Glutamic acid formed: With amino-acid μl.
β-Alanine	30	85	114
δ-Aminovaleric	30	85	114
ε-Aminohexoic	30	85	92
dl-β-Aminobutyric	40	130	127
Adenine	40	138	95
Histamine	40	138	118
Putrescine	20	122	125
Spermine	40	122	116
Heptylamine	40	122	92

Rates of formation and disappearance of glutamic acid by transamination

The data in the previous sections are not accurate measurements of the rate of transamination, since in cases where the rate is very rapid, as with *l*(−)aspartic acid and *l*(+)alanine, the reaction comes to an equilibrium before the end of the incubation period. To determine the rate of transamination shorter periods of incubation were therefore chosen. The data from such experiments are shown in Figs. 1, 2 and 3.

From Fig. 1, it is seen that the initial rates are very rapid for *l*(−)aspartic acid and *l*(+)alanine. In the case of *dl*-α-aminobutyric acid and *l*(+)valine, the initial rates are also appreciable but the curves show an early plateau, for some unexplained reason. This is not due to a true equilibrium since the plateaux reached from both sides of the reaction lie at different levels (Fig. 2).

Under the experimental conditions employed the reaction:

(2) glutamic acid + pyruvic acid ⇌ ketoglutaric acid + alanine

has an equilibrium constant

$$\frac{[\text{glutamic}] \times [\text{pyruvic}]}{[\text{ketoglutaric}] \times [\text{alanine}]}$$

of approximately 1 (see Fig. 2). This is in agreement with the previous finding reported by Braunstein & Kritzmann [1937]. None of the remaining systems shown in Fig. 2 shows any evidence of reaching an equilibrium.

The reaction:

(3) glutamic acid + oxaloacetic acid ⇌ ketoglutaric acid + aspartic acid

reaches an equilibrium even faster than reaction (2) (Fig. 3). The equilibrium constant of this reaction is of an order similar to that of reaction (3). Owing to some side reactions in which oxaloacetic acid disappears, reaction (3) does not come to a standstill and this complicates the accurate determination of the equilibrium constant.

If the rates for reactions (2) and (3) are expressed as $Q_{\text{glutamic acid}}$,

$$\frac{\mu\text{l. glutamic acid formed}}{\text{mg. dry wt. of tissue} \times \text{hr.}},$$

values of 39 and 44 respectively are obtained. Q_{O_2} values for respiration of pigeon breast muscle under optimum conditions are of the same order.

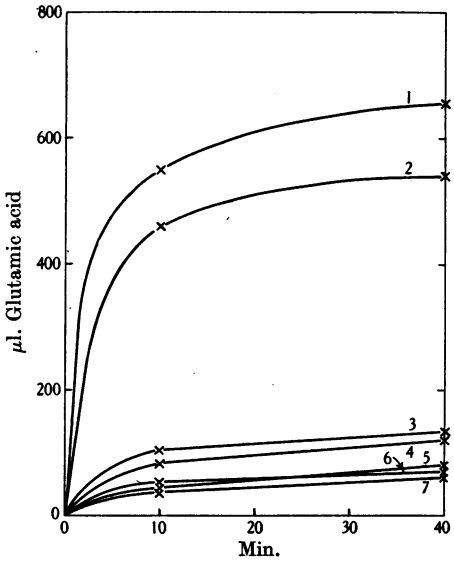

Fig. 1.

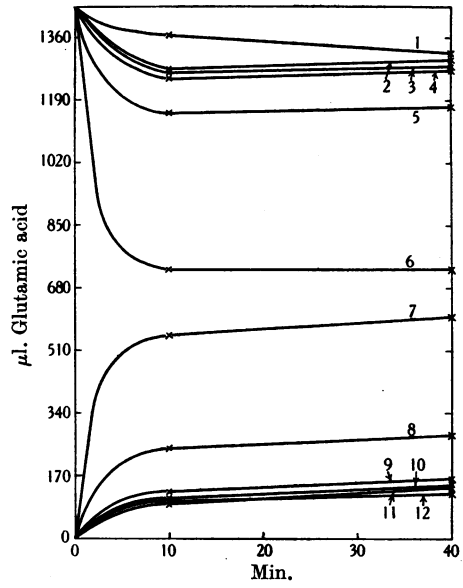

Fig. 2.

Fig. 1. Rate of glutamic acid formation from α-ketoglutaric acid and amino-acids. Substrate conc. 0·017 *M* (for *dl*-amino-acids, 0·034 *M*). N_2. Yellow phosphorus. 40°. (Corrected for blank.) Curves: 1, *l*(−)Aspartic acid + α-ketoglutaric acid; 2, *l*(+)alanine + α-ketoglutaric acid; 3, *dl*-α-aminobutyric acid + α-ketoglutaric acid; 4, *l*(+)valine + α-ketoglutaric acid; 5, *l*(+)phenylalanine + α-ketoglutaric acid; 6, *l*(−)cysteine + α-ketoglutaric acid; 7, *l*(−)tyrosine + α-ketoglutaric acid.

Fig. 2. Rate of glutamic acid formation in the presence of α-ketoglutaric acid plus amino-acids, and rate of glutamic acid disappearance in the presence of ketonic acids. Substrate conc. 0·017 *M* (*dl*-amino-acids, 0·034 *M*). N_2. Yellow phosphorus. 40°. Curves: 1, Glutamic acid; 2, glutamic acid + acetoacetic acid; 3, glutamic acid + α-ketohexoic acid; 4, glutamic acid + α-ketovaleric acid; 5, glutamic acid + α-ketobutyric acid; 6, glutamic acid + pyruvic acid; 7, α-ketoglutaric acid + *dl*-alanine; 8, α-ketoglutaric acid + *dl*-α-aminobutyric acid; 9, α-ketoglutaric acid + *dl*-α-aminovaleric acid; 10, α-ketoglutaric acid + *dl*-α-aminohexoic acid; 11, α-ketobutyric acid + *dl*-β-aminobutyric acid; 12, α-ketoglutaric acid.

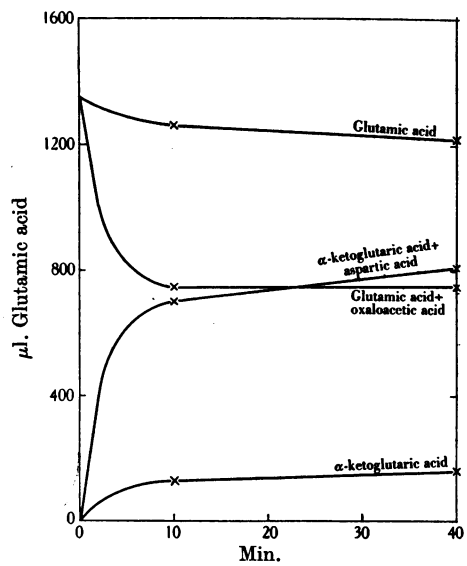

Fig. 3. Rate of glutamic acid formation in the presence of α-ketoglutaric acid plus aspartic acid, and rate of glutamic acid disappearance in the presence of oxaloacetic acid. Substrate conc. 0·017 *M*. N_2. Yellow phosphorus. 40°.

Effect of small amounts of aspartic acid on transamination

The lack of a suitable method for the determination of aspartic acid has so far prevented an investigation of the reaction:

(4) oxaloacetic acid + amino-acid ⇌ aspartic acid + ketonic acid

(except when the amino-acid is glutamic acid). However, reaction (4) can be studied in an indirect way by coupling it with reaction (3). Experimentally this means the addition of α-ketoglutaric acid to the system represented by reaction (4). The balance sheet of reactions (3) and (4) is:

(5) α-ketoglutaric acid + amino-acid ⇌ glutamic acid + α-ketonic acid.

Thus it should be possible to detect transamination according to reaction (4) by the addition of α-ketoglutaric acid, aspartic acid and another amino-acid to a muscle suspension. When these three substances are added, reaction (3) takes place and oxaloacetic acid is formed. Oxaloacetic acid would then be expected to react according to reaction (4), and aspartic acid would be regenerated. Since aspartic acid is expected to act in the manner of a catalyst in this reaction, small concentrations of aspartic acid should suffice to bring about an effect. With this in mind previously studied amino-acids were tested with and without the addition of aspartic acid. From the data in Table V it is seen that there is no significant

Table V. *Effect of small concentrations of aspartic acid on transamination from α-ketoglutaric acid and different amino-acids*

Final conc. of α-ketoglutaric acid and amino-acids, 0·016 *M*. Final conc. of aspartic acid, 0·0016 *M*. Substrate added after 5 min. incubation. N_2. Yellow phosphorus. 40°.

Amino-acid added	Period of incubation with substrate min.	μl. Glutamic acid formed: Without aspartic acid	μl. Glutamic acid formed: With aspartic acid	Increase in glutamic acid due to aspartic acid μl.
—	40	110	194	84
l(+)Alanine	40	520	560	40
l(+)Valine	40	164	218	54
l(−)Phenylalanine	40	140	210	70
l(+)Arginine	40	129	222	93
l(−)Leucine	40	138	241	103

increase in glutamic acid formation from the various amino-acids in the presence of aspartic acid, beyond that of aspartic acid itself. The conclusion may be drawn from these experiments that oxaloacetic acid reacts with no amino-acids other than glutamic acid and alanine. However direct proof for the reaction with alanine has not been produced.

The effect of inhibitors on transamination

The effects of different inhibitors on transamination are listed in Table VI. In these experiments the inhibitors were incubated with the muscle suspension for 15 min. before the substrate was added from the side arm.

Transamination is markedly inhibited by high concentrations of cyanide. As seen from the data the percentage inhibition is roughly proportional to the concentration of cyanide. The effect of high concentrations of cyanide suggests that the inhibition may be due to cyanohydrin formation [Green & Williamson, 1937]: however, the 30 % inhibition by 10^{-3} *M* cyanide indicates that some other basis for the inhibition may exist.

Table VI. *Effect of inhibitors on glutamic acid formation from α-ketoglutaric acid and l(+)alanine and l(−)aspartic acid*

Final substrate conc. 0·015 *M*. Inhibitor incubated with muscle suspension for 15 min. before substrates added. N_2. Yellow phosphorus. 40°.

Inhibitor	Final conc. *M*	Incubation period min.	Glutamic formed μl.	Inhibition %
	α-Ketoglutaric acid + *l*(+)alanine			
—	—	30	576	—
Cyanide	0·05	30	122	79
,,	0·01	30	288	50
,,	0·001	30	400	30
,,	0·0001	30	505	12
Malonate	0·10	30	484	10
,,	0·01	30	542	6
Pyrophosphate	0·01	30	534	7
NaF	0·02	30	490	15
Iodoacetate	0·002	30	550	5
Bromoacetate	0·002	30	526	9
As_2O_3	0·01	30	488	15
Octyl alcohol	Sat.	30	498	14
	α-Ketoglutaric acid + *l*(−)aspartic acid			
—	—	40	592	—
Malonate	0·10	40	436	26
,,	0·01	40	580	2

Malonate in concentrations as high as 0·10 *M* has only a slight effect on transamination in the presence of *l*(+)alanine and α-ketoglutaric acid. Transamination in the presence of *l*(−)aspartic acid and α-ketoglutaric acid appears to be somewhat more sensitive to 0·10 *M* malonate. However the inhibition at this high concentration is probably non-specific, since at lower concentrations (0·01 *M*) no inhibitory effect is observed with either *l*(+)alanine or *l*(−)aspartic acid.

NaF, As_2O_3 and octyl alcohol have small inhibitory effects, while pyrophosphate, iodoacetate and bromoacetate have no appreciable effect on transamination in the presence of *l*(+)alanine and α-ketoglutaric acid.

The results with As_2O_3 and octyl alcohol are in agreement with those reported by Braunstein & Kritzmann [1937] and Kritzmann [1938]. The results with malonate do not agree with the statement made by Braunstein [1939].

Discussion

It has been pointed out in a previous section of this paper that α-ketoglutaric acid forms glutamic acid when added alone to pigeon breast muscle. Preliminary experiments show that the anaerobic glutamic acid synthesis from ammonium α-ketoglutarate is too small to account for the total glutamic acid formed. This must mean that there are NH_2-donators other than NH_3 preformed in the muscle. Among the amino-acids, *l*(+)alanine and *l*(−)aspartic acid are the only ones which could act in this manner. This suggests that these two amino-acids, like glutamic acid [Cohen, 1939], occur free in pigeon breast muscle.

The fact that the three amino-acids which alone are active in transamination are also the only ones which have specific dehydrogenases in muscle [Thunberg, 1920–1; Ahlgren, 1924; Needham, 1930; Euler *et al.* 1938; Dewan, 1938] suggests that transamination depends on the activity of a dehydrogenase during some phase of the reaction. On this basis the limitation of transamination in pigeon breast muscle to those amino-acids which possess dehydrogenases may be understood.

SUMMARY

1. Transamination (Braunstein & Kritzmann) has been studied in pigeon breast muscle. Of 21 different α-amino-acids studied in the presence of α-ketoglutaric acid, *l*(−)aspartic acid and *l*(+)alanine are the most active in forming glutamic acid; *dl*-α-aminobutyric acid and *l*(+)valine are slightly active. None of the remaining amino-acids is appreciably active.

2. Of a series of ketonic acids tested, oxaloacetic and pyruvic acids show the greatest activity in causing the anaerobic disappearance of glutamic acid; α-ketobutyric and mesoxalic acids are slightly active; α-ketovaleric, α-ketohexoic, acetoacetic and laevulic acids are not appreciably active.

3. The data from these experiments can be explained by assuming the existence of three enzyme systems which are concerned with the following reactions:

Glutamic acid ⇌ α-ketoglutaric acid
Aspartic acid ⇌ oxaloacetic acid
Alanine ⇌ pyruvic acid

By combinations of these three systems the following reactions may be brought about:

(1) α-Ketoglutaric acid + alanine ⇌ glutamic acid + pyruvic acid.
(2) α-Ketoglutaric acid + aspartic acid ⇌ glutamic acid + oxaloacetic acid.
(3) Oxaloacetic acid + alanine ⇌ aspartic acid + pyruvic acid.

The occurrence of reaction (3) in pigeon breast muscle has not yet been conclusively demonstrated. The slight activity of certain homologues can be explained by a slight affinity for one of these enzyme systems.

4. Transamination in pigeon breast muscle is inhibited by high concentrations of cyanide. NaF, As_2O_3 and octyl alcohol have small inhibitory effects. Pyrophosphate, malonate, iodoacetate and bromoacetate have no appreciable effect.

5. Of a number of different amino compounds tested other than α-amino-acids, none has been found to be active in transamination.

6. Certain discrepancies between the results of Braunstein & Kritzmann [1938] and those reported here are discussed. The failure of these authors to use a suitable control in their experiments explains their statement that all α-amino-acids of the *l*-series are active in transamination in pigeon breast muscle.

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