OBSERVATIONS ON TRANSIMINATION IN LIVER HOMOGENATES

by

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BORSOOK AND DUBNOFF[1] have shown that citrulline reacts in kidney cortex with glutamic acid or aspartic acid to form arginine; they proposed the term "transimination" for this reaction. COHEN AND HAYANO[2] found that the reaction also occurs in liver homogenates. The chief facts concerning the mechanism of transimination reported so far are as follows:

1. Molecular oxygen and the cytochrome system are required, irrespective of whether glutamic acid or aspartic acid is the reactant (BORSOOK AND DUBNOFF[1], COHEN AND HAYANO[2]).

2. Glutamic acid reacts 4 to 6 times more rapidly than aspartic acid or asparagine when liver homogenates are used (COHEN AND HAYANO[2]) whereas in kidney slices glutamic and aspartic acids react at about the same velocity (BORSOOK AND DUBNOFF[1]).

3. Adenosine triphosphate and Mg ions must be present (COHEN AND HAYANO[2]) when homogenates are used.

4. Malonate inhibits the formation of arginine from citrulline and glutamic acid (COHEN AND HAYANO[2]).

5. The inhibition by 0.0025 M malonate is abolished by 0.005 M fumarate or L-malate (FAHRLÄNDER, FAVARGER, NIELSEN AND LEUTHARDT[3]).

No theory accounting for these observations has as yet been put forward and the details of the mechanism of transimination must still be regarded as obscure. RATNER[4] has recently suggested, on the basis of experiments with extracts of acetone dried beef liver, that aspartic acid rather than glutamic acid is the immediate nitrogen donator, according to the scheme

$$\text{L-aspartate} + \text{citrulline} = \text{L-arginine} + \text{L-malate}.$$

This would account for the observations (4) and (5), but is difficult to reconcile with (1) and (2). The present work reveals further facts which indicate that the mechanism of transimination is more complex than RATNER's[4] scheme suggests.

EXPERIMENTAL

Procedure. Liver homogenates, prepared in general according to COHEN AND HAYANO[2] were used. The liver, mostly from rats, was homogenised in the apparatus of POTTER AND ELVEHJEM[5] or a similar stainless steel instrument. The medium was a phosphate saline (KREBS AND EGGLESTON[6]) with additional $MgCl_2$ (1 ml 0.1 M $MgCl_2$ per 80 ml saline). The homogenates were used without centrifugation. Usually 3 ml homogenate were measured into the main compartment of a conical Warburg vessel; substrates to be added, dissolved in a total volume of 1 ml were placed in the side-

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arm. The substrate concentrations stated in the Tables are final concentrations in the reaction mixture and the data given in the Tables all refer to 4 ml suspension. The amino acids used in this work were the optically active isomerides of the L-series. Substrates and homogenate were mixed just before the manometers were attached to the water bath. At the end of the incubation period the enzymic activity of the homogenate was stopped by the addition of 1 ml of 2 N HCl. The bath temperature was 40°. The analytical methods for the determination of glutamic acid, urea and NH_3 were those previously described from this laboratory[7, 8]. As the liver homogenate contained a powerful arginase any arginine formed during the experiments was assumed to be quantitatively converted into urea. The formation of urea on addition of citrulline was therefore taken to represent the formation of arginine.

TABLE I

EFFECT OF TISSUE CONCENTRATION ON THE FORMATION OF UREA FROM CITRULLINE AND GLUTAMATE IN RAT LIVER

(Incubation 40 min; 0.001 M adenosinetriphosphate; citrulline and glutamate 0.005 M (= 448 μl per flask))

Dilution of liver	μl urea found with		
	Citrulline	Glutamate	Citrulline plus glutamate
10-fold	232	0	440
20-fold	35	0	435
30-fold	5	20	270
40-fold	22	7	295

Optimal tissue dilution

Table I shows the formation of urea in the presence of citrulline and glutamate at varying dilutions of the tissue. Glutamate alone did not produce appreciable amounts of urea at any tissue concentration. Citrulline alone yielded about 50% urea, on a molecule per molecule basis, when the dilution was 1 in 10, about 8% when the dilution was 1 in 20 and negligible amounts at higher dilutions. At all tissue concentrations tested the mixture of citrulline and glutamate yielded more urea than citrulline alone, but the effect of adding glutamate was greatest at the dilution 1 in 20, where 97% of the citrulline was converted into urea when glutamate was present against 8% in the absence of glutamate. The concentration of 1 in 20 therefore appears to be best suited for studying the reaction between citrulline and glutamate. COHEN AND HAYANO[2] reached a similar conclusion.

The mechanism of urea formation from citrulline alone at the higher tissue concentrations (1 : 10) is not clear. In some experiments citrulline alone yielded 0.80 equivalents of urea. The homogenates did not contain enough initial NH_3, or glutamate, or aspartate to account for the formation of urea by the known transimination reactions. Moreover the amounts of these 3 substrates did not appreciably change whilst urea was formed. The mechanism of urea formation under these conditions is under investigation.

Comparison of glutamate and aspartate

Under comparable conditions glutamate reacted with citrulline about 5 times faster than aspartate when the homogenate was diluted 20 fold. This applied to aspartate and glutamate concentrations between 0.00125 M and 0.005 M. These results confirm the findings of COHEN AND HAYANO[2]. It will later be shown that these differences can be abolished by the addition of various substances.

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Anaerobic transimination

Very small, though definitely measurable amounts of urea were formed when homogenates were incubated anaerobically with citrulline and glutamate and/or aspartate, but the rate was no more than 2 or 3% of that found aerobically under otherwise the same conditions. In these experiments O_2 was rigidly excluded by placing yellow phosphorus in the centre well of the manometer flask and adding the substrates from the side-arm after the complete absorption of any O_2 impurities of the N_2. Such formation of urea as occurred under these conditions can therefore not be explained by a reaction involving O_2.

Observations on the Reaction Between Citrulline and Glutamate

TABLE II

EFFECT OF FUMARATE, ASPARTATE AND α-KETOGLUTARATE ON THE MALONATE INHIBITION OF THE REACTION BETWEEN CITRULLINE AND GLUTAMATE

(One part of rat liver homogenised in 19 parts of saline medium. Citrulline 0.005 M; L-glutamate 0.005 M; Adenosinetriphosphate 0.001 M 40°; O_2. Incubation 40 min)

Exp. 1						
Additional substances . . .	none	Fumarate 0.002 M	Malonate 0.002 M	Malonate 0.002 M	Malonate 0.002 M	Malonate 0.002 M
				Fumarate 0.005 M	Fumarate 0.0025 M	Fumarate 0.00125 M
Total urea found (μl) . . .	338	343	39	353	359	367
Extra urea found as a result of addition of fumarate (μl)				314	320	328
Amount of fumarate added (μl)				448	224	112
Exp. 2						
Additional substances . . .	none		Malonate 0.002 M	Malonate 0.002 M	Malonate 0.002 M	Malonate 0.002 M
				Fumarate 0.000625 M	Fumarate 0.00031 M	Fumarate 0.000156 M
Total urea found (μl) . . .	391		144	301	292	215
Extra urea found as a result of addition of fumarate (μl)				157	148	71
Amount of fumarate added (μl)				56	28	14
Ratio $\frac{\text{extra urea found}}{\text{fumarate added}}$				2.81	5.28	5.06
Exp. 3						
Additional substances . . .	none		Malonate 0.005 M	Malonate 0.005 M	Malonate 0.005 M	Malonate 0.005 M
				Fumarate 0.000625 M	L-Aspartate 0.000625 M	α-Ketoglutarate 0.000625 M
Total urea found (μl) . . .	380		119	278	225	134
Extra urea found as a result of the addition of fumarate, aspartate or α-ketoglutarate (μl)				159	106	15
Amount of fumarate, aspartate or α-ketoglutarate added (μl)				56	56	56

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Effects of fumarate, aspartate and α-ketoglutarate on the malonate inhibition

Data given in Table II (Exp. 1 and 2) show that 0.0025 M fumarate and even lower concentrations accelerated the formation of urea from citrulline and glutamate in the presence of malonate, partially abolishing the inhibition by this substance. Under the given conditions 1 molecule of fumarate caused a formation of more than 5 molecules of urea. Aspartate acted in the same manner (Table II, Exp. 3) whilst α-ketoglutarate had no appreciable effect on the malonate inhibition. Control experiments showed that fumarate (0.0006 M and 0.005 M) had no effect on urea formation from citrulline alone (in the absence of glutamate).

TABLE III

EFFECTS OF α-KETOGLUTARATE, CITRATE, SUCCINATE AND FUMARATE ON THE REACTION BETWEEN CITRULLINE AND GLUTAMATE

Conditions as in Table II; concentration of all added substances 0.005 M

Additional substances added	μl urea found				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
None	408	382	385	325	290
Malonate	199		122	102	41
α-Ketoglutarate	196	169	136		
Citrate	288	249		230	
Succinate		302		244	
Succinate; fumarate		291			
Malonate; citrate					163
Malonate; fumarate	390				
α-Ketoglutarate; fumarate		204			
Citrate; fumarate		262			

Inhibition by citrate, α-ketoglutarate and succinate

Citrate, α-ketoglutarate and succinate inhibited the reaction between citrulline and glutamate as shown in Table III. The inhibitory effect of α-ketoglutarate which has already been noted by COHEN AND HAYANO[2] was of the same order as that of malonate. The inhibitions by the 3 substances, unlike the malonate inhibition, were not removed by 0.005 M fumarate. In the presence of malonate citrate increased the rate of reaction. Experiments with lower citrate concentration (not recorded in Table III) showed that the citrate effect, in contrast to that of fumarate or aspartate, was not catalytic.

Observations on the Reaction Between Citrulline and Aspartate

Acceleration by α-ketoglutarate, fumarate and allied substances

The rate of urea formation in the presence of citrulline and aspartate showed considerable variations in different experiments (Table IV). Addition of fumarate (Exp. 4 and 5), α-ketoglutarate (Exp. 1), citrate (Exp. 6 and 7), succinate (Exp. 6 and 7) and small amounts of glutamate (Exp. 1) accelerated the reaction. The effects of α-ketoglutarate (Exp. 1), of glutamate (Exp. 1) and of fumarate (Exp. 5) were catalytic in that 1 molecule of the added substrate caused an additional formation of more than 1 molecule of urea. As liver homogenates can form glutamate from aspartate and α-ketoglutarate by transamination an acceleration by α-ketoglutarate might be expected on the assumption that glutamate rather than aspartate is the immediate nitrogen donator

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in transimination. It is however difficult to explain why α-ketoglutarate, citrate and succinate inhibit the formation of urea when glutamate and citrulline are added whilst they accelerate the reaction when glutamate is replaced by aspartate.

Inhibition by malonate

Malonate (0.005 M) inhibited urea formation on addition of aspartate and citrulline (Table IV, Exp. 3, 4 and 5) but the degree of inhibition varied and was small when the rate of urea formation is relatively low (Exp. 6). Small amounts of α-ketoglutarate (Exp. 2 and 3), glutamate (Exp. 2) or fumarate (Exp. 3 and 5) reduced the inhibition, α-ketoglutarate and fumarate being more effective than glutamate. Citrate likewise diminished the malonate inhibition while succinate has no appreciable effect.

Maximal Rates of Transimination

To compare the rates of urea formation from aspartate with that from glutamate under optimal conditions further experiments were carried out in which different substrate combinations were added to the same liver homogenate. The periods of incubation in these experiments were short (20 min) because under optimal conditions the reaction may reach virtual completion before the end of a 40 minute period. The highest rates were obtained when aspartate, citrulline and small amounts of α-ketoglutarate (see Table V) were present. The combination glutamate and citrulline with a small amount of aspartate was only a little less efficient. Thus, apart from citrulline, both glutamate and aspartate, the former replaceable by α-ketoglutarate, are required for maximal rates.

Effect of adenosine triphosphate

It has been suggested by FAHRLANDER, FAVARGER, NIELSEN AND LEUTHARDT[3] that the effect of fumarate in the presence of malonate might be due to the generation of ATP or other energy-rich phosphate bonds. If this were the case addition of ATP should have an effect similar to that of fumarate. However, increasing the concentration of ATP from 0.001 M to 0.005 M ATP or addition of 0.0075 M phosphoglycerate had no major stimulating effects on the system citrulline-glutamate-malonate under aerobic conditions, or on the systems citrulline-glutamate, citrulline-aspartate and citrulline-aspartate-ketoglutarate under anaerobic conditions. Small effects of 0.005 M ATP, amounting to less than 10% of the aerobic effect of fumarate were observed anaerobically in some, but not in every experiment.

DISCUSSION

Complex nature of transimination

The experiments reported in this paper supplement the observations on transimination by previous authors listed in the introduction. The new findings are:

1. Catalytic amounts of fumarate or aspartate abolish the malonate inhibition of the urea formation from citrulline and glutamate.

2. Citrate, α-ketoglutarate, and succinate inhibit the urea formation from citrulline and glutamate. These inhibitions, unlike the malonate inhibition, are not reversed by fumarate.

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TABLE

EFFECTS OF VARIOUS SUBSTANCES ON THE REACTION

Conditions as in Table II. Citrulline and aspartate 0.005 M

Exp. 1. Additional substances:	none
Total urea found (μl)	135
Extra urea found as a result of addition of α-ketoglutarate or glutamate (μl)	
Amount of α-ketoglutarate or glutamate added (μl)	
Exp. 2 (same liver as Exp. 1). Additional substances:	Malonate 0.005 M
Total urea found (μl)	51
Extra urea found as a result of addition of α-ketoglutarate (μl)	
Amount of α-ketoglutarate added (μl)	
*Exp.*3. Additional substances:	none
Total urea found (μl)	207
Extra urea found as a result of addition of α-ketoglutarate (μl)	
Amount of α-ketoglutarate added (μl)	
Exp. 4. Additional substances:	none
Total urea found (μl)	225
Extra urea found as a result of addition of fumarate (μl)	
Amount of fumarate added (μl)	
Exp. 5. Additional substances:	none
Total urea found (μl)	246
Extra urea found as a result of additional fumarate (μl)	
Amount of fumarate added (μl)	
Exp. 6. Additional substances:	none
Total urea found (μl)	179
Extra urea found as a result of addition of citrate or succinate (μl)	
Amount of citrate or succinate added (μl)	
Exp. 7. Additional substances:	none
Total urea found (μl)	70
Extra urea found as a result of addition of citrate or succinate (μl)	
Amount of citrate or succinate added (μl)	

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IV

BETWEEN CITRULLINE AND ASPARTATE

α-Ketoglutarate 0.001 M 371 236 90	L-Glutamate 0.001 M 368 233 90	L-Glutamate 0.0002 M 240 105 18		
Malonate 0.005 M α-Ketoglutarate 0.001 M 200 149 90	Malonate 0.005 M L-Glutamate 0.001 M 99 48 90	Malonate 0.005 M L-Glutamate 0.0002 M 58 7 18		
Malonate 0.005 M 54	Malonate 0.005 M α-Ketoglutarate 0.00125 M 282 228 112	Malonate 0.005 M α-Ketoglutarate 0.000625 M 156 102 56	Malonate 0.005 M Fumarate 0.000625 M 138 84 56	
Fumarate 0.005 M 417 192 448	Malonate 0.005 M 82	Malonate 0.005 M Fumarate 0.005 M 389 307 448		
Fumarate 0.000625 M 401 155 56	Malonate 0.005 M 74	Malonate 0.005 M Fumarate 0.000625 M 188 114 56		
Citrate 0.005 M 370 191 448	Succinate 0.005 M 382 203 448			
Succinate 0.005 M 180 110 448	Citrate 0.005 M 418 348 448	Malonate 0.005 M 63	Malonate 0.005 M Succinate 0.005 M 72 9 448	Malonate 0.005 M Citrate 0.005 M 132 69 448

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TABLE V

RATE OF UREA FORMATION FROM VARIOUS SUBSTRATE COMBINATIONS

1 part of rat liver homogenate in 19 parts of saline medium; ATP 0.001 M; O_2; 40°; 0.005 M citrulline; period of incubation 20 min. All substrate concentrations 0.005 M unless otherwise stated. The amount of urea present in the homogenate at the beginning of the incubation was 42 μl in Exp. 1, 62 μl in Exp. 2, and 44 μl in Exp. 3.

Exp. 1 Additional substrates:	none	Glutamate	Glutamate Fumarate 0.00125 M	Aspartate	Aspartate Fumarate 0.00125 M	Aspartate α-Ketoglutarate 0.00125 M	Aspartate Glutamate 0.00125 M
Urea found (μl) . . .	104	264	268	146	275	343	243
Exp. 2 Additional substrates:	none	Glutamate	Glutamate Aspartate 0.00125 M	Aspartate	Aspartate Fumarate 0.00125 M	Aspartate α-Ketoglutarate 0.00125 M	Aspartate Glutamate 0.00125 M
Urea found (μl) . . .	168	285	351	249	292	402	386
Exp. 3 Additional substrates:	Glutamate Fumarate	Glutamate Fumarate Malonate	Aspartate α-Ketoglutarate	Aspartate α-Ketoglutarate Malonate	Aspartate Glutamate	Aspartate Glutamate Malonate	
Urea found (μl) . . .	299	306	327	328	423	379	

3. The urea formation from citrulline and aspartate is catalytically accelerated by glutamate, α-ketoglutarate or fumarate.

4. The urea formation from citrulline and aspartate is inhibited by malonate. This inhibition is reduced or abolished by α-ketoglutarate, glutamate or fumarate.

5. The highest rate of urea formation is found when the combinations citrulline plus aspartate plus α-ketoglutarate or citrulline plus glutamate plus aspartate are present.

Thus the urea formation from citrulline and aspartate on the one hand, and from citrulline and glutamate on the other, have some features in common whilst others differ. Both reactions are inhibited by malonate and in both cases the inhibition is reversed by fumarate. Differences concern the effects of α-ketoglutarate and citrate which inhibit the glutamate system and accelerate the aspartate system.

It is obvious that transimination is a highly complex reaction. The fact that the highest rates are found when both a C_4- and C_5-dicarboxylic acid are added together suggests that both types of compound are required. The observations on the malonate inhibition and its reversal support this conclusion. It is premature at this stage of knowledge to propose a comprehensive hypothesis on the mechanism of transimination.

Malonate inhibition

An inhibition of a reaction by malonate may be taken as evidence that succinic dehydrogenase takes part in the reaction. The fact that the inhibition of transimination can be abolished by adding various substrates such as fumarate or aspartate shows that succinic dehydrogenase is not *directly* required for transimination, but takes part in the formation of a substance which is required for transimination. The nature of this substance is uncertain.

Variability of the rate of transimination

As already pointed out the rate of urea formation from citrulline and aspartate showed considerable variation. Since small quantities of fumarate or glutamate or related substances may greatly affect the rate of reaction, the variability may be due to differences from liver to liver in the quantities of these substances.

Rôle of O_2

It is noteworthy that O_2 is necessary even when a combination of aspartate and α-ketoglutarate (which by transamination rapidly yields glutamate and oxaloacetate) or aspartate and glutamate are added; also that ATP and sodium phosphoglycerate replace no more than a small fraction of the activity of O_2. Hence the role of O_2 cannot be fully explained by the part it may play in the interconversion of the C_4- and C_5-dicarboxylic acids and the generation of energy rich phosphate bonds.

SUMMARY

The formation of urea through the two "transimination" reactions

L-citrulline + L-glutamate ⟶ L-arginine ⟶ urea (1)

L-citrulline + L-aspartate ⟶ L-arginine ⟶ urea (2)

was studied in liver homogenate.

Catalytic amounts of fumarate or aspartate abolish the malonate inhibition of reaction (1).

Citrate, α-ketoglutarate and succinate inhibit reaction (1) but these inhibitions, unlike the malonate inhibition, are not reversed by fumarate.

Reaction (2) is catalytically accelerated by glutamate, α-ketoglutarate or fumarate.

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Reaction (2) is inhibited by malonate and this inhibition is reduced or abolished by α-ketoglutarate, glutamate or fumarate.

The highest rates of urea formation occur when the combinations citrulline plus aspartate plus α-ketoglutarate or citrulline plus glutamate plus aspartate are present.

The findings are discussed with reference to the mechanism of transimination. It is concluded that transimination is a highly complex reaction. The details of its mechanism are obscure.

RÉSUMÉ

La formation d'urée par les deux réactions de "transimination":

L-Citrulline + L-glutamate ⟶ L-arginine + urée (1)

L-Citrulline + L-aspartate ⟶ L-arginine + urée (2)

a été étudiée dans des homogénates de foie.

Des quantités catalytiques de fumarate ou d'aspartate suppriment l'inhibition de la réaction (1) par le malonate.

Le citrate, l'α-cétoglutarate et le succinate, inhibent la réaction (1) mais ces inhibitions, contrairement à celle provoquée par le malonate, ne sont pas supprimées par le fumarate.

La réaction (2) est accélérée catalytiquement par le glutamate, l'α-cétoglutarate ou le fumarate.

La réaction (2) est inhibée par le malonate, et cette inhibition est réduite ou supprimée par l'α-cétoglutarate, le glutamate ou le fumarate. La vitesse de formation de l'urée la plus élevée apparaît lorsque sont en présence la citrulline + l'aspartate + l'α-cétoglutarate, ou la citrulline + le glutamate + l'aspartate.

L'importance de ces observations pour l'explication du mécanisme de la transmination est discutée. La transimination est une réaction très compliquée dont les détails restent encore obscurs.

ZUSAMMENFASSUNG

Die Harnstoffbildung durch "Transiminierung"

L-Citrullin + L-Glutamat ⟶ L-Arginin ⟶ Harnstoff (1)

L-Citrullin + L-Aspartat ⟶ L-Arginin ⟶ Harnstoff (2)

wurde in homogenisierter Leber untersucht.

Fumarat oder Aspartat heben in katalytischen Mengen die Malonate-Hemmung der Reaktion (1) auf.

Citrat, α-Ketoglutarat und Sukzinat hemmen Reaktion (1). Diese Hemmungen werden durch Fumarat nicht aufgehoben.

Reaktion (2) wird durch Glutamat, α-Ketoglutarat oder Fumarat katalytisch beschleunigt.

Reaktion (2) wird durch Malonat gehemmt. Diese Hemmung wird durch α-Ketoglutarat, Glutamat oder Fumarat teilweise oder vollständig aufgehoben.

Die Geschwindigkeit der Harnstoffbildung durch Transiminierung ist am grössten, wenn die Substratkombination Citrullin plus Aspartat plus α-Ketoglutarat oder Citrullin plus Glutamat plus Aspartat anwesend sind.

Die Befunde werden im Hinblick auf den Mechanismus der Transiminierung erörtert und es wird gefolgert, dass Transiminierung eine komplizierte Reaktion ist, deren Mechanismus unklar ist.

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