

## OBSERVATIONS ON TRANSIMINATION IN LIVER HOMOGENATES

by

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BORSOOK AND DUBNOFF<sup>1</sup> 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<sup>2</sup> 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<sup>1</sup>, COHEN AND HAYANO<sup>2</sup>).

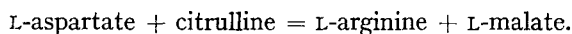
2. Glutamic acid reacts 4 to 6 times more rapidly than aspartic acid or asparagine when liver homogenates are used (COHEN AND HAYANO<sup>2</sup>) whereas in kidney slices glutamic and aspartic acids react at about the same velocity (BORSOOK AND DUBNOFF<sup>1</sup>).

3. Adenosine triphosphate and Mg ions must be present (COHEN AND HAYANO<sup>2</sup>) when homogenates are used.

4. Malonate inhibits the formation of arginine from citrulline and glutamic acid (COHEN AND HAYANO<sup>2</sup>).

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

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<sup>4</sup> 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 donor, according to the scheme



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<sup>4</sup> scheme suggests.

### EXPERIMENTAL

*Procedure.* Liver homogenates, prepared in general according to COHEN AND HAYANO<sup>2</sup> were used. The liver, mostly from rats, was homogenised in the apparatus of POTTER AND ELVEHJEM<sup>5</sup> or a similar stainless steel instrument. The medium was a phosphate saline (KREBS AND EGGLESTON<sup>6</sup>) with additional  $\text{MgCl}_2$  (1 ml 0.1 M  $\text{MgCl}_2$  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-

*References p. 328.*

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  $\text{NH}_3$  were those previously described from this laboratory<sup>7, 8</sup>. 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  $\mu\text{l}$  per flask))

| Dilution of liver | $\mu\text{l}$ urea found with |           |                              |
|-------------------|-------------------------------|-----------|------------------------------|
|                   | Citrulline                    | Glutamate | Citrulline<br>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<sup>2</sup> 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  $\text{NH}_3$ , or glutamate, or aspartate to account for the formation of urea by the known transamination 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<sup>2</sup>. It will later be shown that these differences can be abolished by the addition of various substances.

*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<sub>2</sub> 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<sub>2</sub> impurities of the N<sub>2</sub>. Such formation of urea as occurred under these conditions can therefore not be explained by a reaction involving O<sub>2</sub>.

*Observations on the Reaction Between Citrulline and Glutamate*

TABLE II

EFFECT OF FUMARATE, ASPARTATE AND  $\alpha$ -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<sub>2</sub>. Incubation 40 min)

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

References p. 328.

*Effects of fumarate, aspartate and  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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                 | $\mu$ l urea found |        |        |        |        |
|---|--------------------|--------|--------|--------|--------|
|   | Exp. 1             | Exp. 2 | Exp. 3 | Exp. 4 | Exp. 5 |
| None . . . . .                              | 408                | 382    | 385    | 325    | 290    |
| Malonate . . . . .                          | 199                |        | 122    | 102    | 41     |
| $\alpha$ -Ketoglutarate . . . . .           | 196                | 169    | 136    |        |        |
| Citrate . . . . .                           | 288                | 249    |        | 230    |        |
| Succinate . . . . .                         |                    | 302    |        | 244    |        |
| Succinate; fumarate . . . . .               |                    | 291    |        |        |        |
| Malonate; citrate . . . . .                 |                    |        |        |        | 163    |
| Malonate; fumarate . . . . .                | 390                |        |        |        |        |
| $\alpha$ -Ketoglutarate; fumarate . . . . . |                    | 204    |        |        |        |
| Citrate; fumarate . . . . .                 |                    | 262    |        |        |        |

*Inhibition by citrate,  $\alpha$ -ketoglutarate and succinate*

Citrate,  $\alpha$ -ketoglutarate and succinate inhibited the reaction between citrulline and glutamate as shown in Table III. The inhibitory effect of  $\alpha$ -ketoglutarate which has already been noted by COHEN AND HAYANO<sup>2</sup> 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  $\alpha$ -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),  $\alpha$ -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  $\alpha$ -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  $\alpha$ -ketoglutarate by transamination an acceleration by  $\alpha$ -ketoglutarate might be expected on the assumption that glutamate rather than aspartate is the immediate nitrogen donor

in transimination. It is however difficult to explain why  $\alpha$ -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  $\alpha$ -ketoglutarate (Exp. 2 and 3), glutamate (Exp. 2) or fumarate (Exp. 3 and 5) reduced the inhibition,  $\alpha$ -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  $\alpha$ -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  $\alpha$ -ketoglutarate, are required for maximal rates.

#### *Effect of adenosine triphosphate*

It has been suggested by FAHRLANDER, FAVARGER, NIELSEN AND LEUTHARDT<sup>3</sup> 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,  $\alpha$ -ketoglutarate, and succinate inhibit the urea formation from citrulline and glutamate. These inhibitions, unlike the malonate inhibition, are not reversed by fumarate.

*References p. 328.*

TABLE  
EFFECTS OF VARIOUS SUBSTANCES ON THE REACTION  
Conditions as in Table II. Citrulline and aspartate 0.005 M

|  |                               |
|--|-------------------------------|
| Exp. 1. Additional substances:<br><br>Total urea found ( $\mu$ l) . . . . .<br>Extra urea found as a result of addition of $\alpha$ -ketoglutarate or glutamate ( $\mu$ l) .<br>Amount of $\alpha$ -ketoglutarate or glutamate added ( $\mu$ l) . . . . .      | none<br><br>135               |
| Exp. 2 (same liver as Exp. 1). Additional substances:<br><br>Total urea found ( $\mu$ l) . . . . .<br>Extra urea found as a result of addition of $\alpha$ -ketoglutarate ( $\mu$ l) . . . . .<br>Amount of $\alpha$ -ketoglutarate added ( $\mu$ l) . . . . . | Malonate<br>0.005 M<br><br>51 |
| Exp. 3. Additional substances:<br><br>Total urea found ( $\mu$ l) . . . . .<br>Extra urea found as a result of addition of $\alpha$ -ketoglutarate ( $\mu$ l) . . . . .<br>Amount of $\alpha$ -ketoglutarate added ( $\mu$ l) . . . . .                        | none<br><br>207               |
| Exp. 4. Additional substances:<br><br>Total urea found ( $\mu$ l) . . . . .<br>Extra urea found as a result of addition of fumarate ( $\mu$ l) . . . . .<br>Amount of fumarate added ( $\mu$ l) . . . . .  | none<br><br>225               |
| Exp. 5. Additional substances:<br><br>Total urea found ( $\mu$ l) . . . . .<br>Extra urea found as a result of additional fumarate ( $\mu$ l) . . . . .<br>Amount of fumarate added ( $\mu$ l) . . . . .   | none<br><br>246               |
| Exp. 6. Additional substances:<br><br>Total urea found ( $\mu$ l) . . . . .<br>Extra urea found as a result of addition of citrate or succinate ( $\mu$ l) . . . . .<br>Amount of citrate or succinate added ( $\mu$ l) . . . . .                              | none<br><br>179               |
| Exp. 7. Additional substances:<br><br>Total urea found ( $\mu$ l) . . . . .<br>Extra urea found as a result of addition of citrate or succinate ( $\mu$ l) . . . . .<br>Amount of citrate or succinate added ( $\mu$ l) . . . . .                              | none<br><br>70                |

References p. 328.

## IV

## BETWEEN CITRULLINE AND ASPARTATE

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

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<sub>2</sub>; 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  $\mu$ l in Exp. 1, 62  $\mu$ l in Exp. 2, and 44  $\mu$ l in Exp. 3.

|   |                       |                                   |                                      |  |                                    |   |                                     |
|---|-----------------------|-----------------------------------|--------------------------------------|--|------------------------------------|---|-------------------------------------|
| <i>Exp. 1</i><br>Additional substrates: | none                  | Glutamate                         | Glutamate<br>Fumarate<br>0.00125 M   | Aspartate  | Aspartate<br>Fumarate<br>0.00125 M | Aspartate<br>$\alpha$ -Ketoglutarate<br>0.00125 M | Aspartate<br>Glutamate<br>0.00125 M |
| Urea found ( $\mu$ l) . . .             | 104                   | 264                               | 268                                  | 146  | 275                                | 343   | 243                                 |
| <i>Exp. 2</i><br>Additional substrates: | none                  | Glutamate                         | Glutamate<br>Aspartate<br>0.00125 M  | Aspartate  | Aspartate<br>Fumarate<br>0.00125 M | Aspartate<br>$\alpha$ -Ketoglutarate<br>0.00125 M | Aspartate<br>Glutamate<br>0.00125 M |
| Urea found ( $\mu$ l) . . .             | 168                   | 285                               | 351                                  | 249  | 292                                | 402   | 386                                 |
| <i>Exp. 3</i><br>Additional substrates: | Glutamate<br>Fumarate | Glutamate<br>Fumarate<br>Malonate | Aspartate<br>$\alpha$ -Ketoglutarate | Aspartate<br>$\alpha$ -Ketoglutarate<br>Malonate | Aspartate<br>Glutamate             | Aspartate<br>Glutamate<br>Malonate                |                                     |
| Urea found ( $\mu$ l) . . .             | 299                   | 306                               | 327                                  | 328  | 423                                | 379   |                                     |



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

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

5. The highest rate of urea formation is found when the combinations citrulline plus aspartate plus  $\alpha$ -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  $\alpha$ -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*

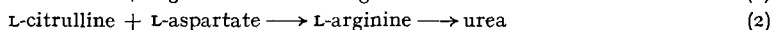
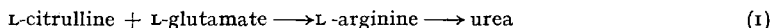
As already pointed out the rate of urea formation from citrulline and aspartate showed considerable variation. Since small quantities of fumarate or glutamate or related substances may greatly affect the rate of reaction, the variability may be due to differences from liver to liver in the quantities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

#### *Rôle of $O_2$*

It is noteworthy that  $O_2$  is necessary even when a combination of aspartate and  $\alpha$ -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



was studied in liver homogenate.

Catalytic amounts of fumarate or aspartate abolish the malonate inhibition of reaction (1). Citrate,  $\alpha$ -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,  $\alpha$ -ketoglutarate or fumarate.

*References p. 328.*

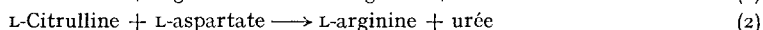
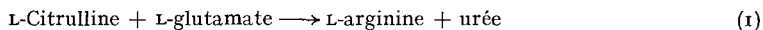
Reaction (2) is inhibited by malonate and this inhibition is reduced or abolished by  $\alpha$ -ketoglutarate, glutamate or fumarate.

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

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

### RÉSUMÉ

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



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' $\alpha$ -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.

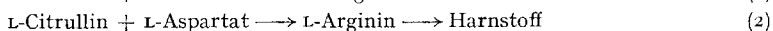
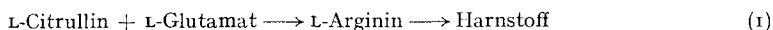
La réaction (2) est accélérée catalytiquement par le glutamate, l' $\alpha$ -cétoglutarate ou le fumarate.

La réaction (2) est inhibée par le malonate, et cette inhibition est réduite ou supprimée par l' $\alpha$ -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' $\alpha$ -cétoglutarate, ou la citrulline + le glutamate + l'aspartate.

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

### ZUSAMMENFASSUNG

Die Harnstoffbildung durch "Transaminierung"



wurde in homogenisierter Leber untersucht.

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

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

Reaktion (2) wird durch Glutamat,  $\alpha$ -Ketoglutarat oder Fumarat katalytisch beschleunigt.

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

Die Geschwindigkeit der Harnstoffbildung durch Transaminierung ist am grössten, wenn die Substratkombination Citrullin plus Aspartat plus  $\alpha$ -Ketoglutarat oder Citrullin plus Glutamat plus Aspartat anwesend sind.

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

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