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# THE ACTIVE TRANSFER OF D-METHIONINE BY THE RAT INTESTINE *IN VITRO*

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Gibson & Wiseman (1951) showed that the L forms of a number of amino acids disappeared from the lumen of the intestine of the rat more rapidly than the corresponding D-enantiomorphs, and they regarded this as evidence for the existence of a stereochemically specific mechanism for active absorption of L-amino acids. Wiseman (1953) showed that from solutions of a number of racemic amino acids the L-enantiomorphs could be transferred against a concentration gradient by the intestine *in vitro*, but that this did not occur with the D-enantiomorphs, and these results were confirmed by Agar, Hird & Sidhu (1953). Matthews & Smyth (1954) showed that the L-enantiomorphs of some amino acids appeared in the blood stream in larger quantities than the D-enantiomorphs when the racemic amino acid was present in the lumen of the intestine. All these results were in keeping with the view that transport of L-amino acids involves an active process, and it was assumed that this did not apply to D-amino acids. Further evidence, however, suggested that this view on D-amino acid transfer must be revised, since it was shown by Jervis & Smyth (1959*a*) that competition between some D- and L-amino acids was possible, and also by Jervis & Smyth (1959*b*) that the rates of absorption of both the D- and L-enantiomorphs of histidine and methionine were not proportional to their concentration in the intestine. The following experiments were therefore undertaken to obtain more evidence for the possible existence of an active mechanism for transfer of D-amino acids. A preliminary account has been given by Jervis & Smyth (1958).

## METHODS

The D-amino acid chosen for study was D-methionine, partly because there is evidence that it appears to compete with L-histidine (Jervis & Smyth, 1959*a*) and also because it was desirable to use an amino acid for which a specific colorimetric method of estimation was available.

The general principle was to attempt to create experimental conditions where movement of D-methionine against a concentration gradient could be observed. It seemed possible that failures to achieve this in previous experiments were due to (1) the presence of L-methionine which competed for the mechanism and (2) the simultaneous movement of water. Fisher (1955) and Smyth & Taylor (1957) have shown that active movement of fluid

takes place in the intestine *in vitro*, and hence the relative rates of movement of solvent and solute will be an important factor in determining whether a substance can be concentrated. It was found that the necessary conditions could be achieved (*a*) by carrying out experiments where D-methionine was used without the L-form, (*b*) by using phosphate saline instead of bicarbonate saline, and (*c*) by using the ileum instead of the jejunum. It has been shown by Wilson & Wiseman (1954) and by Smyth & Taylor (1957) that these last two conditions reduce the rate of water transfer.

Male albino rats were used, and the intestinal preparation was the sac of rat everted small intestine as described by Wilson & Wiseman (1954). The details of the technique were as described by Parsons, Smyth & Taylor (1958), except that phosphate saline (Krebs, 1933) was used instead of bicarbonate saline. In making the sacs the small intestine below the duodenum was removed and divided into five approximately equal parts, and sacs were made from either the fourth or fifth segment counting from the jejunal end. The saline contained 500 mg glucose/100 ml. in all experiments and D-methionine was present initially in equal concentrations in the mucosal and serosal fluid. After shaking the sacs for 1 hr at 38° C the concentration of D-methionine was estimated in the mucosal and serosal fluids and the transfer of water and amino acid was calculated. The methods of calculating and expressing the results were those used by Parsons *et al.* (1958) with some small modifications. For the convenience of the reader the terms are defined as follows. The fluid in which the sacs are suspended is the mucosal fluid, the fluid inside the sac is the serosal fluid. The *mucosal fluid transfer* is the decrease in volume in mucosal fluid which occurs during the course of the experiment, the *serosal fluid transfer* is the increase in volume of serosal fluid. The *mucosal substrate transfer* is the amount of substrate (in this case D-methionine) which disappears from the mucosal fluid, the *serosal substrate transfer* is the increase in the amount of substrate in the serosal fluid; a decreased amount is described as negative serosal transfer. *Gut fluid uptake* and *gut substrate uptake* are the amounts of fluid or substrate not accounted for in the mucosal or serosal fluid. The difference between the final serosal and final mucosal concentrations is the *final concentration gradient*. The following additional terms are also used. The mucosal substrate transfer divided by the mucosal fluid transfer is the *mucosal concentration transferred*, and the serosal substrate transfer divided by the serosal fluid transfer is the *serosal concentration transferred*.

The transfer of methionine and fluid is expressed in amounts per unit weight of tissue. Parsons *et al.* (1958) used milligrams initial wet weight of tissue but this procedure gives figures which are inconveniently small, and therefore figures for transfer are given per gram initial wet wt.

*Chemical.* The D-methionine was obtained commercially, and freedom from contamination with L-methionine was tested by using L-amino acid oxidase from *Neurospora*. The D-methionine was estimated either colorimetrically by the method of McCarthy & Sullivan (1941) or enzymically with D-amino acid oxidase prepared from kidney according to the method of Bender & Krebs (1950).

## RESULTS

### *Transfer of* D-*methionine against a concentration gradient*

The results of a typical experiment with D-methionine are included in Table 1. In referring to this experiment it shall be understood that the figures given for D-methionine and fluid transfer refer to the amounts/g wet wt. of intestine. Initially D-methionine was present in equal concentrations (19·5 mM) in both mucosal and serosal fluids. During the course of the experiment 53·0 $\mu$moles of D-methionine and 1·09 ml. fluid left the mucosal fluid, the D-methionine concentration in the fluid leaving the mucosal side

being 48·6 mM, i.e. greater than the concentration of 19·5 mM initially present in the mucosal fluid. Of the D-methionine and fluid leaving the mucosal side 33·0 $\mu$moles of methionine and 0·50 ml. fluid were retained in the gut and the rest was transferred to the serosal fluid. The gut uptake of methionine was relatively greater than the gut uptake of water, and hence the fluid transferred to the serosal side contained a smaller concentration (33·8 mM) of methionine than that leaving the mucosal side. In spite of this it was still greater than the concentration initially present in the serosal side, and hence the final concentration (24·2 mM) in the serosal fluid was greater than the initial concentration. The total process resulted in D-methionine being moved against a concentration gradient.

TABLE 1. Transfer of D-methionine by sacs of rat everted intestine *in vitro*. In the experiments with inhibitors the initial concentration of KCN was $10^{-2}$ M and of 2:4-dinitrophenol $2 \times 10^{-4}$ M. Duration of experiment 1 hr. Negative values of the final concentration gradient mean that the final serosal concentration is less than the final mucosal, and negative values for serosal transfer mean a loss from the serosal side

| | | In presence of KCN | In presence of 2:4-dinitrophenol |
|---|---|---|---|
| Initial wet wt. of sac (g) | 0·84 | 0·90 | 1·04 |
| D-methionine | | | |
| Initial mucosal and serosal concn. (mM) | 19·5 | 19·2 | 20·4 |
| Final mucosal concn. (mM) | 17·6 | 18·8 | 20·0 |
| Final serosal concn. (mM) | 24·2 | 17·1 | 18·4 |
| Final concentration gradient (mM) | 6·6 | −1·7 | −1·6 |
| Mucosal transfer ($\mu$moles/g) | 53·0 | 7·4 | 9·5 |
| Serosal transfer ($\mu$moles/g) | 20·0 | −4·6 | −4·4 |
| Gut uptake ($\mu$moles/g) | 33·0 | 12·0 | 13·9 |
| Fluid | | | |
| Mucosal transfer (ml./g) | 1·09 | 0·04 | 0·19 |
| Serosal transfer (ml./g) | 0·59 | −0·07 | −0·14 |
| Gut uptake (ml./g) | 0·50 | 0·11 | 0·33 |
| Concentration of D-methionine transferred | | | |
| Mucosal (mM) | 48·6 | 187·3 | 50·8 |
| Serosal (mM) | 33·8 | 67·6* | 32·2* |

* This is the concentration of fluid moved from the serosal side into the intestine.

In eight other experiments very similar results were obtained. In two further experiments the pattern was similar except that the serosal transfer of fluid was smaller, a relatively larger amount of the mucosal transfer being retained in the tissue. In these cases the concentration of D-methionine transferred to the serosal side was even greater than in the experiment in Table 1.

### *Effect of inhibitors*

One method of demonstrating an active transport is by using inhibitors, and in the present experiments the inhibitors were 2:4-dinitrophenol and potassium cyanide. Two experiments were done with each inhibitor and one of each of these experiments is included in Table 1. It will be observed

that with the concentration used there was a marked inhibition of movement of both fluid and D-methionine. No serosal transfer of either took place and in fact solutions of D-methionine moved from the serosal fluid into the intestine.

The result was that the final concentration gradient was negative, i.e. the final serosal concentration was smaller than the final mucosal concentration. The high concentration of D-methionine transferred from the mucosal side in the presence of cyanide might occasion some surprise, suggesting that the D-methionine transfer mechanism was to some extent cyanide-resistant. This conclusion is not warranted, as is shown by the following consideration. Initially there is no D-methionine in the intestine. Even if the tissue is dead it is conceivable that a small amount of D-methionine may diffuse in from both sides. If the fluid movement is very small this may result in movement of a very high concentration of D-methionine. Examination of Table 1 shows in fact that a high concentration (67 mM) of D-methionine also diffuses into the intestine from the serosal side. There is thus no need to postulate any active transport of D-methionine in the presence of cyanide.

The effects of 2:4-dinitrophenol are also shown in Table 1 and are very similar to those obtained with potassium cyanide. The absolute amount of mucosal transfer was greatly reduced, methionine actually disappeared from the serosal side, and no movement against a concentration gradient took place.

*Competition between* D- *and* L-*enantiomorphs of methionine*

The previous sections offer evidence for an active movement of D-methionine when L-methionine is not present. If the views of Jervis & Smyth (1959*a*) on competition are correct it might be expected that even if the other conditions remained the same movement of D-methionine against a concentration gradient could no longer be shown in the presence of L-methionine. Experiments were carried out to test this point, in which the conditions were similar to those described in earlier sections, except that DL-methionine was used instead of D-methionine. In these experiments D-methionine could not be estimated colorimetrically and D-amino acid oxidase was used.

The results of an experiment of this type are shown in Table 2. This table includes results from both the fourth and fifth segments of intestine. In the fourth segment DL-methionine was present, in the fifth segment D-methionine only. It is apparent from these results that the presence of L-methionine had an inhibitory action on the transfer of D-methionine. No movement against a concentration gradient took place, and the absolute transfer was greatly reduced. In contrast to the reduction in methionine

transfer there was no obvious inhibition of fluid transfer, and the inhibition caused by L-methionine is thus more specific than that due to cyanide or dinitrophenol. Somewhat similar results were obtained in two further experiments, the only difference being that in both of these the inhibition of D-methionine transfer in the presence of L-methionine was even more striking, and the serosal D-methionine transfer was negative, i.e. some D-methionine disappeared from the serosal fluid. It will be observed that this also happened in the case of inhibition with 2:4-dinitrophenol and potassium cyanide.

TABLE 2. Competition between D- and L-methionine for intestinal transport by sacs of rat everted intestine. The sacs were made from the fourth and fifth segments of intestine. Duration of experiment 1 hr. Negative values of the final concentration gradient mean that the final serosal concentration is less than the final mucosal, and negative values for serosal transfer mean a loss from the serosal side. It should be noted that DL-methionine was present in sac 4, whereas only the D-enantiomorph was present in sac 5

| Segment | Sac 4 | Sac 5 |
|---|---|---|
| Initial wet wt. (g) | 0·78 | 1·05 |
| Initial concn. of D-methionine (mM) | 18·3 | 18·3 |
| Initial concn. of L-methionine (mM) | 20·0 | — |
| D-methionine | | |
| Final mucosal concn. (mM) | 18·8 | 16·1 |
| Final serosal concn. (mM) | 11·6 | 25·6 |
| Final concentration gradient (mM) | −7·2 | 9·5 |
| Mucosal transfer (μmole/g) | 19·1 | 48·5 |
| Serosal transfer (μmole/g) | 3·3 | 20·4 |
| Gut uptake (μmole/g) | 15·8 | 28·1 |
| Fluid | | |
| Mucosal transfer (ml./g) | 1·52 | 1·07 |
| Serosal transfer (ml./g) | 1·02 | 0·53 |
| Gut uptake (ml./g) | 0·50 | 0·54 |
| Concentration of amino acid moved | | |
| Mucosal (mM) | 12·5 | 45·4 |
| Serosal (mM) | 3·2 | 38·5 |

## DISCUSSION

The results show that in the experimental conditions used D-methionine was transferred against a concentration gradient, and hence it would seem probable that there is an active mechanism involved in its transfer. This is supported by the fact that the transfer was abolished by 2:4-dinitrophenol and potassium cyanide, and by competition with L-methionine. It is also in agreement with the findings of Jervis & Smyth (1959*c*) that the rate of absorption of D-methionine, like that of L-methionine, is not proportional to the concentration present in the intestine. The competition between D- and L-forms of the same amino acid is of considerable interest in relation to the work of Jervis & Smyth (1959*a*) who found competition between L-methionine and D-histidine for intestinal absorption and to a lesser extent between L-histidine and D-methionine. The concept of

competition is now extended to competition between the enantiomorphs of the same amino acid. The effectiveness of L-methionine in competing with D-methionine is in keeping with the greater affinity of L-methionine for the transport mechanism, suggested by Jervis & Smyth (1959*c*) on the basis of investigations on the Michaelis constants in intestinal transport. It is also of interest to compare the results with those of Wiseman (1953), who first produced clear evidence of active transport of L-amino acids. In Wiseman's experiments racemic mixtures of amino acids were used and it was found that L-methionine but not D-methionine could be transferred against a concentration gradient. Our results confirm Wiseman's experimental observations, but suggest an interpretation of these which was not suspected at the time, i.e. that the failure to find active transport of D-methionine was in fact due to the presence of L-methionine. Wiseman (1955) also showed that there is a mechanism in the intestine for active transfer of amino acids for which a number of L-amino acids compete, and that L-methionine appears to have a greater affinity for the mechanism than the other L-amino acids tested. It appears from the present results and from those of Jervis & Smyth (1959*a*) that the same mechanism is capable of dealing with at least some D-amino acids, and the mechanism postulated by Wiseman (1955) is therefore not absolutely stereochemically specific. It should be stressed that the active transfer of D-methionine does not necessarily mean that all D-amino acids can be moved actively, and in fact preliminary experiments with D-histidine have not so far shown that this amino acid can be moved against a concentration gradient. The specificity of the mechanism could be such that it permits the transfer of many different L-amino acids, but being less favourable to the D configuration only permits the transfer of a smaller number of D-amino acids. Schemes for such a possibility have been suggested by Finch (1959).

The details of the mechanism of transfer of D-methionine still remain unknown. The inhibition by cyanide and by 2:4-dinitrophenol seemed at first to suggest an enzymic mechanism dependent on metabolic energy; but this does not necessarily follow, as the cyanide- and dinitrophenol-sensitive mechanisms may be involved only indirectly in transport, and may be more concerned with maintenance of the integrity of the cell, without which the transport mechanism cannot work. The preference of the mechanism for the L-enantiomorph, although also consistent with an enzymic mechanism, does not prove enzyme participation, as the stereochemical preference may be for sites on a non-enzymic carrier. On the other hand, competition between the D- and L-forms does not exclude direct participation of an enzymic mechanism. Although competition between two enantiomorphs of one substance for the same enzyme is unusual, there are other examples known of the action of an enzyme on one

enantiomorph being inhibited by the presence of the other enantiomorph, (Stahmann, Fruton & Bergmann, 1946; Massey, 1953), and there are also enzymes known which can act on both enantiomorphs of one substance. There is in fact an enzyme which will act on both L- and D-methionine (Kallio & Larson, 1955), but this particular enzyme is not likely to be involved in the present mechanism as it produces racemization, and the experiment quoted in Table 2, where the amino acid was estimated by D-amino acid oxidase, would seem to exclude this possibility. It must be concluded that the intestine possesses a mechanism for active transfer of both D- and L-methionine. The mechanism has a preference for the L-form, but it is not possible at present to suggest further details.

The results suggest that considerable care is necessary in deciding about the presence or absence of an active process in intestinal transfer, on the basis of movement or absence of movement against a concentration gradient. It is now well established that an active movement of water takes place in the intestine (Fisher, 1955; Smyth & Taylor, 1957) and the relative rates of water movement and solute movement must obviously be taken into consideration. The possibility of solutes being 'entrained' in an active water stream has been discussed by Fisher (1955), and the effects of 'solvent drag' have been discussed by Ussing and his co-workers (for references see Ussing, 1957). These considerations did not, however, include the possible case where solute and solvent are actively moved through a barrier by independent processes. It would seem possible in such cases that an active process concerned with solute movement might well be masked by one concerned with solvent movement. Thus, while achievement of concentration of a solute by the intestinal preparation can be regarded as evidence of the presence of a mechanism other than diffusion, failure to achieve a concentration is not necessarily evidence for the absence of such a mechanism. The relative rates of solvent and solute flow must be considered and conclusions should be made only in the light of such considerations.

SUMMARY

1. A study has been made of the transfer of D-methionine by everted sacs of ileum in conditions designed to reduce water transport.

2. Under these conditions D-methionine can be moved against a concentration gradient.

3. The movement against a concentration gradient is prevented by $2 \times 10^{-4}$M 2-4:dinitrophenol, $10^{-2}$M cyanide and by the presence of L-methionine.

4. It is suggested that the transfer mechanism for L-methionine is not absolutely stereochemically specific, and that D-methionine is able to utilize this mechanism, although it has a lower affinity than L-methionine.

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