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THE EFFECT OF CONCENTRATIONS OF AMINO ACIDS ON THEIR RATE OF ABSORPTION FROM THE INTESTINE

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The relation between the rate of absorption of a substance from the intestine and its concentration has frequently been used as a criterion of the process involved in absorption. Hober & Hober (1937), Lathe (1943) and others have concluded that the rate of absorption of amino acids is not proportional to the concentration in the intestine, and regarded this as evidence of the presence of an active process concerned with absorption. Hetenyi & Winter (1952) found that the rate of absorption of glycine, histidine and β -alanine was not proportional to concentration, while in the case of proline there was a straight-line relationship. The following experiments were undertaken to study more carefully the relationship between the rate of absorption and concentration of amino acids, and furthermore the D- and L-enantiomorphs of different amino acids were compared. A preliminary account has been given by Jervis & Smyth (1959*b*).

METHODS

Albino rats were anaesthetized with pentobarbitone sodium and the procedure for study of absorption was that described by Jervis, Johnson, Sheff & Smyth (1956). By this technique it is possible to measure both the rate of absorption and also the concentration of the substance in the lumen of the intestine. The amino acids, neutralized by addition of NaOH, were dissolved in NaCl solution 0.9% (w/v) and circulated through the lumen of the intestine for 20 min. At the end of this period the amount remaining in the intestine was washed out and estimated, and from this and that initially added the amount absorbed was calculated.

Chemicals. The amino acids used were D- and L-methionine and histidine and were obtained commercially. Histidine was estimated by the method of Macpherson (1946) and methionine by the method of McCarthy & Sullivan (1941). Urea was estimated by the microdiffusion method of Conway, as described by Hawk, Oser & Summerson (1947).

RESULTS

Relation between concentration and rate of absorption

The results of these experiments are summarized in Figs. 1 and 2, which show the relation between the initial concentration of various enantiomorphs and the amounts absorbed. From these results it is clear that in the case of all four amino acids the rate of absorption is not proportional to the concentration, and at higher concentrations increase in rate fails to keep pace with the increase in the concentration. In the case of L-histidine, D-histidine and D-methionine the rate has not reached a maximum value at the highest concentration used. In the case of L-methionine it would appear that the mechanism is already saturated at 50 mM, and in fact the average rate of absorption is somewhat smaller at 100 mM than at 50 mM, although not significantly so.

In order to investigate further the significance of these results, similar experiments were done with urea, which might be supposed to move by diffusion only. The results of these experiments are seen in Fig. 3. This presents a very different type of picture, there being a direct relationship between the average values for concentrations and the rate of absorption. The regression line cuts the abscissa not at zero but at a concentration of about 9.86 mM (59 mg urea/100 ml.), and the probable reason for this is the presence of urea in the blood. Spector (1956) gives a value of 19–35 mg/100 ml. for the concentration of urea in rats' blood, but when the animal is absorbing urea the concentration is probably higher than this. Too much attention need not be given to the exact point where the regression line cuts the abscissa, as we have measured the initial concentration of urea instead of the average concentration during the experimental period. Similar curves for urea have been obtained by Lathe (1943) in experiments on Thiry-Vella loops in dogs and by Hetenyi & Winter (1952) for rats. These results strongly suggest that both L- and D-enantiomorphs of histidine and methionine are absorbed by a process which is not explained by simple diffusion.

Michaelis-Menten kinetics

The usual concept of active transport involves combination of the substance transported with some other substance which may be a carrier or possibly an enzyme involved in the formation of the carrier. If this process is the rate-limiting factor then the relation between the rate of absorption and concentration might be expected to conform with the scheme of kinetics discussed by Michaelis & Menten (1913). Without defining the physical significance of the results obtained, it seemed profitable to inquire in the first place whether the relation between the rate of absorption and concentration of the amino acids studied fits in with the Michaelis-Menten scheme. Investigations of this sort

have been made on intestinal absorption of glucose by Fisher & Parsons (1953) and Ricklis & Quastel (1958), and for phosphate by McHardy & Parsons (1956). In attempts to apply these kinetics a number of approximations must be made. The rate of absorption must be measured over a reasonable time, in

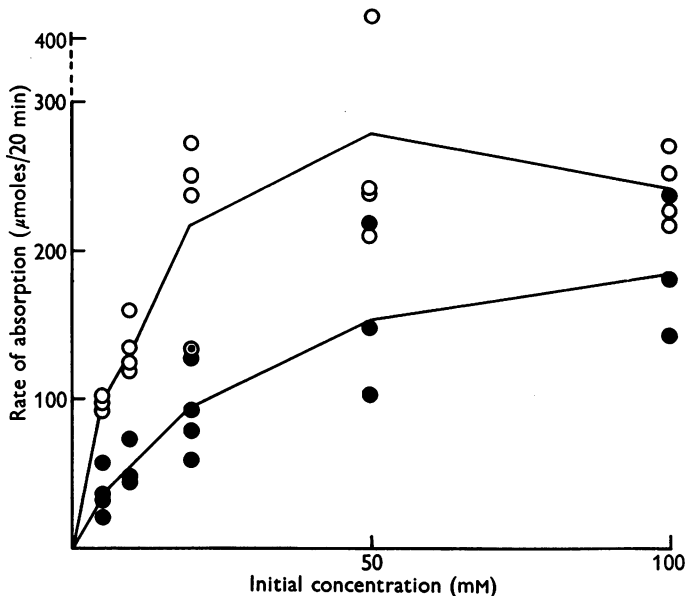


Fig. 1. Rate of absorption of L-methionine (○) and D-methionine (●) from the intestine of the rat at various initial concentrations. The lines have been drawn to join the average rates at each concentration.

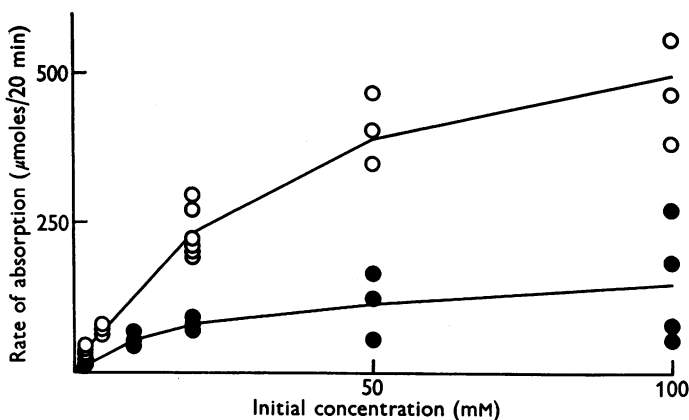


Fig. 2. Rate of absorption of L-histidine (○) and D-histidine (●) from the intestine of the rat at various initial concentrations. The lines have been drawn to join the average rates at each concentration.

the present case 20 min, so that in fact figures for an average rate must be taken. The concentration used may either be the average concentration or the initial concentration. While the present method of investigation would permit a final sample to be taken and hence an average of initial and final concentration to be obtained, this was not done in the present series because the taking of an extra sample would reduce the accuracy of estimating the total amount of substance absorbed. The initial concentration is therefore used. Fisher & Parsons (1953) have considered the implications of using the initial concentration and the average rate in the Michaelis–Menten equation, and have

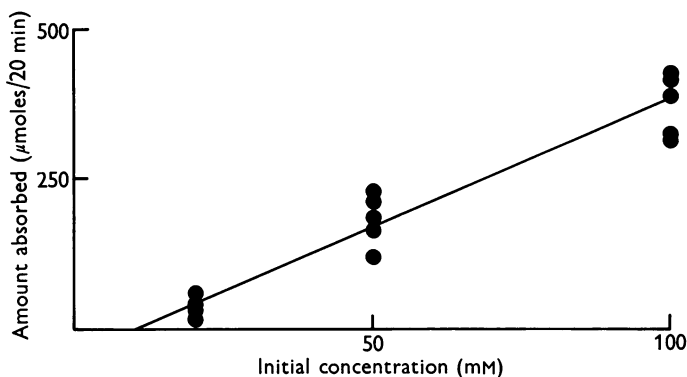


Fig. 3. Rate of absorption of urea from the intestine of the rat at various initial concentrations. The straight line is the regression line ($y = 4.36x - 42.99$) calculated from the experimental data.

concluded that a similar relationship will exist between these as exists between the average concentration and average rate, although the constants will have different values. The most convenient method of applying Michaelis–Menten kinetics is to write the equation in the form used by Lineweaver & Burk (1934).

$$\frac{1}{v} = \frac{\kappa}{V S} + \frac{1}{V}, \quad (1)$$

where v = rate of absorption, V = the maximum rate of absorption at infinite concentration, S = concentration and κ = Michaelis constant.

Figure 4 shows the Lineweaver–Burk plot for L- and D-methionine. For simplicity of presentation only the average values are shown, but a summary of statistical data is given in Table 1. The regression lines drawn in Fig. 4 are calculated from the weighted regression analysis described in the appendix. Similar plots were made for the histidine enantiomorphs. These are not reproduced here, but the statistical data are included in Table 1. Tests for curvilinearity were applied to the Lineweaver–Burk plots for all four enantiomorphs and in all cases there was no evidence that a straight line was not the best fit for the experimental points. There is thus reason to believe

that the kinetics of absorption in the case of all four amino acids approximate to the Michaelis-Menten relationship. It is also seen from Table I that the Michaelis constant for L-methionine was lower than the constant for any other amino acid and differed significantly from the constant for L-histidine and D-methionine. It did not differ significantly from the values for D-histidine,

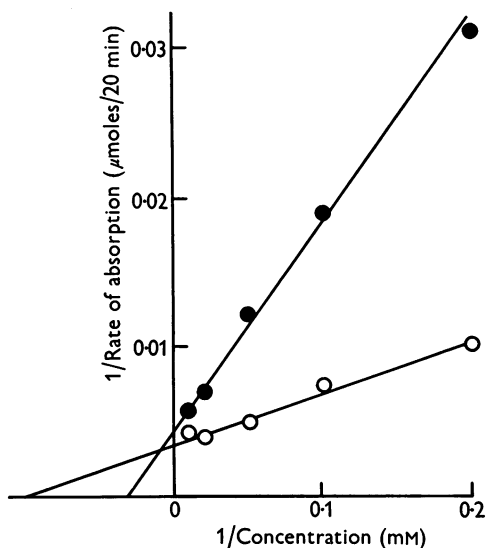


Fig. 4. Lineweaver-Burk plots for L-methionine (○) and D-methionine (●). The ordinate is the reciprocal of the rate of absorption expressed in $\mu\text{moles}/20\text{ min}$, and the abscissa the reciprocal of the concentration expressed as mM .

TABLE I. Statistical data on the Lineweaver-Burk plots for the four amino acids studied. For meaning of the symbols used in the table see appendix. In calculating the values of a and b the concentrations were expressed as mM and the rates of absorption as $\mu\text{moles}/20\text{ min}$

	L-methionine	D-methionine	L-histidine	D-histidine
$a \pm \sigma_a$	0.00616 ± 0.00023	0.00787 ± 0.00061	0.00338 ± 0.00124	0.01326 ± 0.00162
$b \pm \sigma_b$	0.0341 ± 0.0033	0.140 ± 0.023	0.0588 ± 0.0041	0.147 ± 0.026
$\left(\frac{a}{b} - x_0\right) \pm \sigma_{(a/b) - x_0}$	0.1048 ± 0.0189	0.03023 ± 0.01032	0.02481 ± 0.00457	0.05229 ± 0.01929
Estimated value of k (Michaelis constant; mM)	9.55	33.1	40.3	19.1
95% confidence limits of k (mM)	7.0-14.9	19.7-104.3	29.5-63.8	11.0-72.9

probably because of the lack of precision in determining this last value. The Michaelis constant for D-methionine and D- and L-histidine did not differ significantly from each other. It will be noted that the variability in k values was much greater in the experiments with the D-enantiomorphs as is shown by the wide range of 95% confidence limits.

DISCUSSION

The results indicate fairly clearly that the rate of disappearance of D- and L-methionine and histidine from the intestine is not proportional to the initial concentration, and they are thus in agreement with the views of Hober & Hober (1937), Lathe (1943) and Hetenyi & Winter (1952). Hetenyi & Winter (1952) did not state whether the histidine used was a racemic mixture, or the L-enantiomorph, but in either case the results would agree with our own. As regards L-histidine Gibson & Wiseman (1951) thought that the amount absorbed was roughly proportional to the amount administered. In this case, however, the experimental conditions were so different from our own that comparisons are very difficult. The higher concentrations used by Gibson & Wiseman were strongly hypertonic and might affect the water absorption with subsequent alteration in the distension of the intestine which could well alter the absorbing surface. In the present experiments the conditions of the intestine were probably more constant for the different concentrations applied. Furthermore, the results with urea suggest that our technique was such as to demonstrate a straight-line relationship between the rate of absorption and concentration, if such existed. The conclusion can therefore be drawn with some confidence that the rate of absorption of urea probably depends on its rate of diffusion through some part of the intestinal barrier, whereas this is not the case for amino acids.

The rate-limiting process in the case of amino acids is one which has kinetics approximating to the Michaelis-Menten scheme. This, however, as Fisher & Parsons (1953) have pointed out, does not prove that either a carrier mechanism or even an enzyme system is involved, although it would be consistent with these hypotheses. While the application of Michaelis-Menten kinetics would appear to be a logical stage in investigating the rate of any process such as intestinal absorption, we agree with Fisher & Parsons that unjustifiable conclusions can too easily be drawn from the finding that the absorption rate fits in with a scheme of this kind.

Nevertheless, the values obtained for the Michaelis constant are of interest and particularly the low value for L-methionine, suggesting a relatively high affinity of L-methionine for the transport mechanism. Wiseman (1955) found that L-methionine could compete successfully with L-histidine *in vitro* for the transport mechanism. Agar, Hird & Sidhu (1956) demonstrated the same effect *in vivo*, and Jervis & Smyth (1959*a*) found that L-methionine could compete successfully *in vivo* with L-histidine and D-histidine for intestinal absorption. All these results are consistent with the low constant for L-methionine obtained in the present experiments. The values of k for L- and D-histidine and D-methionine do not differ significantly from each other, and no conclusions could be drawn about their relative affinities for the transport

mechanism. The wide range of confidence limits in these cases draws attention to the need for more rigorous statistical investigation of the significance of k values in intestinal-absorption studies, where in general there is bound to be considerable lack of precision in the methods used. In their work on phosphate absorption McHardy & Parsons (1956) did state that the value of $1/k$ did not differ significantly from zero, but in the other studies referred to, k values were given without indication of the range of error. We would therefore stress that some caution is necessary in accepting k values without information on the statistical significance of the results.

When this work was started it was thought likely that the absorption of D-amino acids might be explained by a diffusion process only, while this would probably not be the case for L-amino acids, and from this point of view the results with D-amino acids were unexpected. More recently, however, other evidence on competition between D- and L-enantiomorphs (Jervis & Smyth, 1959*a*) has made us think that probably absorption of D-amino acids is more complex than we had previously thought and the present results confirm this view. The fact that there is preference for absorption of L-amino acids certainly suggests that an enzymic process is concerned at least in the absorption of L-amino acids. It seems likely that there is also a stage common to both L- and D-amino acids which shows kinetics approximating to the Michaelis-Menten scheme and probably it is at this stage that competition between the D- and L- forms takes place.

Jacobs & Hillman (1958) have recently studied absorption of D- and L-methionine in the rat by a procedure somewhat similar to our own, and have found that the two enantiomorphs were absorbed at about the same rate. The results presented here do not agree with this, but are in general agreement with those of Gibson & Wiseman (1951) and Wiseman (1953). Jacobs & Hillman (1958) used only a small part of the jejunum, and whether this is the reason for the discrepancy in the results is not certain. They did, however, find that the absorption of both D- and L-methionine was inhibited by deoxyypyridoxine and this is in keeping with our conclusions that the movement of both isomers involves processes other than diffusion.

APPENDIX

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Defining $x \equiv 1/S$, $y \equiv 1/v$, (2)

the regression line of y on x was identified with the relation (1) (p. 436), it being assumed that errors due to experimental technique and variation of biological material affected absorption only. It was clear that the variation in y tended to increase with x , to a degree which made the use of weighted

regression analysis necessary in the cases of D- and L-histidine and D-methionine, weighting coefficients being proportional to the inverse square of the smoothed standard deviation of values of y for different values of x in accordance with the usual statistical practice.

The underlying regression relationship may be expressed in the form

$$\text{average } y = \alpha + \beta(x - x_0). \quad (3)$$

where β is the slope of the line, and α its height at the arbitrary value $x = x_0$, which is usually chosen independently for each set of data to which (3) is fitted in such a way as to secure that the estimates a of α and b of β provided by the data are statistically independent. Comparing (3) with (1), we obtain

$$\kappa = ([\alpha/\beta] - x_0)^{-1} \quad (4)$$

so that from any particular set of data the estimate k of κ can be taken as

$$k = ([a/b] - x_0)^{-1}. \quad (5)$$

Since the proportionate error of the quantity in the bracket in (5) is not always small in the four cases examined, confidence limits for κ are best deduced as $[(a/b) - x_0] \pm 2\sigma_{(a/b) - x_0}]^{-1}$ where

$$\sigma_{(a/b) - x_0}^2 \sim \frac{b^2\sigma_a^2 + a^2\sigma_b^2}{b^4}.$$

The test of curvilinearity mentioned in the Results section above consisted of testing the significance of the regression coefficient of inverse absorption y on a second-degree polynomial orthogonal to the straight regression line (cf. R. A. Fisher, 1954). In none of the four cases was the 5% level of significance attained, showing that the data were all compatible with the assumption of a straight-line regression law.

SUMMARY

1. A study has been made of the relation between the rate of intestinal absorption of the D- and L-enantiomorphs of methionine and histidine, and their concentration in the lumen of the intestine.
2. It was found that in all cases the rate of absorption was not proportional to initial concentration, these showing a relationship which corresponded approximately to Michaelis-Menten kinetics.
3. It is suggested that L-methionine has a greater affinity for the transport mechanism than the other amino acids studied.
4. A statistical appendix gives a method for estimating the reliability of the Michaelis constant as determined in these conditions.

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