J. Physiol. (1981), **319**, *pp.* 355–368
With 1 *plate and* 6 *text-figures*
Printed in Great Britain

CELLULAR DISTRIBUTION OF NEUTRAL AND BASIC AMINO ACID TRANSPORT SYSTEMS IN RABBIT ILEAL MUCOSA

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(*Received 20 March 1981*)

SUMMARY

1. An autoradiographic technique is described whereby the cellular location of tritiated amino acids can be determined following uptake by rabbit ileal mucosa.

2. Stirring solutions in contact with the intestinal mucosa during measurement of rapid influx changes the quantity, but not the distribution, of alanine taken up by the tissue.

3. Conditions predicted to favour either a high affinity system (Ly_1) or a low affinity system (Ly_2) were used to measure lysine distribution following uptake. Maximal uptake for both transport systems occurred in fully differentiated enterocytes at the tips of villi. Initial maturation of the Ly_1 system, which was slow, was followed by a rapid phase of development. The Ly_2 system lacked this rapid phase of late development.

4. The cellular distribution of alanine entering on a low affinity Na-independent neutral amino acid carrier closely resembles that determined for the Ly_1 system for lysine entry.

5. Arginine is a potent inhibitor of lysine uptake through the Ly_2 system. Little or no diffusion of lysine appears to take place into rabbit ileal enterocytes.

6. The different distribution of the high and low affinity systems for lysine transport provides further support for their independent existence. It also suggests that more than one message exists for the switching on of amino acid transport function in differentiating enterocytes.

INTRODUCTION

It is now over 15 years since Kinter & Wilson first described how autoradiography could be used to study the cellular distribution of amino acids in intestinal tissue (Kinter & Wilson, 1965). At that time the authors were more concerned to establish which border of the enterocyte was responsible for the active transport of amino acids, but it was also mentioned that there was a gradient of intracellular accumulation along the villus which could not be explained by poor contact of the tissue with incubation medium. Surprisingly little has been published on this method until recently, when it was used in modified form to determine the villus distribution of alanine uptake in rabbit ileum (Paterson, Sepúlveda & Smith, 1980*a*). The results

of that work were encouraging, since they showed that quantitative autoradiography could be carried out on fixed instead of frozen tissue using tritiated instead of ^{14}C-labelled amino acids.

Other advances occurring during the past 15 years include a deeper understanding of how amino acids cross membranes. This has led, in the case of the rabbit ileum, to a rejection of the view that all neutral amino acids use a single mechanism to enter the mucosa (Curran, Schultz, Chez & Fuisz, 1967). There are now considered to be at least two mechanisms of entry, one with high affinity and low transport capacity and another with low affinity but high transport capacity (Sepúlveda & Smith, 1978; Paterson, Sepúlveda & Smith, 1979). Only the high affinity component is Na-dependent (Paterson *et al.* 1980*a*). Results presented in the previous paper suggest that neutral amino acids can also enter rabbit ileal mucosa on a third low capacity transport system showing high affinity for lysine (Paterson, Sepúlveda & Smith, 1981). This system is probably identical to the high affinity system described originally for lysine entry into rabbit ileal mucosa (Munck & Schultz, 1969). Independent evidence suggests that a similar situation exists in the small intestine of other mammals (Smith, James & Paterson, 1981).

Knowing the kinetic characteristics of these various transport systems allows one to predict which experimental situation will favour one system over another. The aim of the present work was to use an autoradiographic technique to measure the cellular distribution of these transport systems in some detail. The results obtained suggest that the low affinity system for lysine uptake has a distribution different from that of both the high affinity lysine system and the low affinity system for neutral amino acid uptake. These results are discussed in terms of possible selectivity in the process of enterocyte differentiation.

METHODS

Animals

White rabbits of the New Zealand strain weighing 2·5–3·5 kg were purchased from Morton Commercial Rabbits, Stansted, Essex. They were deprived of food but allowed free access to water for 24 hr before experiment.

Uptake measurements

Rapid influx of ^{14}C-labelled amino acids across the mucosal border of rabbit ileum, following a 10 min pre-incubation in Na-free medium, was determined, in the presence or absence of Na, over a period of 45 sec using tritiated polyethylene glycol (PEG-900) as a marker of extracellular space (Sepúlveda & Smith, 1978). The effect of stirring (0–700 rev/min) on alanine uptake was also measured using a variable speed stirring apparatus (Barker, Sepúlveda & Smith, 1980).

The tonicity of amino acid-containing solutions was maintained constant by varying both choline chloride and mannitol content as described previously (Paterson *et al.* 1981).

Autoradiography

Tritiated amino acids (6–400 μc ml.$^{-1}$) were presented to the luminal surface of rabbit distal ileum for a total period of 45 sec. The procedure for mounting the ileum in the uptake apparatus and the preliminary incubation of tissue in Na-free medium was as described above. Phosphate buffered saline, pH 7·3 (Dulbecco 'A', Oxoid Ltd., Basingstoke, Hampshire) containing 4% (v/v) glutaraldehyde and 2% (w/v) sucrose was used to remove extracellular amino acids and link intracellular amino acids to cellular protein at the end of incubation. The efficiency of this procedure was tested for by incubating paired pieces of tissue in 1 mM-tritiated amino acid. Both pieces of tissue were processed for counting in the normal way, but one was treated with glutaraldehyde before being placed in dilute HNO_3. Comparison of the counts obtained under these conditions

allowed one to calculate the percentage of absorbed amino acid becoming irreversibly fixed through treatment with glutaraldehyde. The percent fixation for Ala, Met, Gly, Ser, Arg and Lys was 59, 82, 89, 89, 106 and 112 respectively.

Pieces of ileum taken from the influx apparatus following initial contact with glutaraldehyde were transferred to fresh glutaraldehyde-containing medium for 2 h before being washed twice at 4 °C, over a period of 24 hr, in phosphate buffered saline to remove fixative. Tissues were embedded in glycol-methacrylate, using the method of Murgatroyd (1976), following dehydration by passage through ascending grades of EtOH. Sections cut at 3 μm on a Reichert–Jung Autocut rotary microtome (tungsten carbide knife) were coated with Ilford K2 photographic emulsion. Subsequent development was in Phen-X medium, sections being fixed in a solution consisting of 30% (w/v) $Na_2S_2O_3$ (2 parts) and 2% (w/v) $CrKS_2O_8$ plus 2% (w/v) $Na_2S_2O_5$ (1 part). Fixative was removed by washing in water, sections then being dehydrated and mounted in Gurr's Xam medium. The intensity of the Ag grains was enhanced, on some occasions, by incubating wet sections in Kodak silver intensifier solution (IN-5) for periods of 0·5–15 min. In this case sections were fixed in 30% (w/v) $Na_2S_2O_3$ for 2 min before being washed, dehydrated and mounted in Gurr's Xam medium for analysis by microdensitometry. This method has already been demonstrated to The Physiological Society (King, Sepúlveda & Smith, 1981).

Microdensitometry

The M85 scanning microdensitometer (Vickers Instruments, York, Yorks.) collects light passing through the specimen in a photomultiplier tube where it is integrated and displayed as a digital output of optical density. Machine settings for band width and spot aperture controls of 80 and 2 respectively and a wave-length setting of 650 nm were found to be suitable for recording Ag grain densities.

The eyepiece of the microdensitometer contained a grid so arranged as to lie parallel to the vertical axis of the villus to be analysed. The spot delineating the area of the section to be scanned was then placed in the grid covering the tip of the villus (corresponding to an area of 25 or 155 μm^2 depending on whether a $\times 40$ or $\times 100$ objective was being used). Optical density was recorded and the section was then moved so that the spot fell on tissue covered by the next square in the grid. In this way it was possible to move down the villus obtaining readings of density in discrete steps of 5 or 12·5 μm depending on the magnification used for scanning. Errors in density reading which could have arisen from the scanning of goblet cells instead of enterocytes were avoided by moving the scanning spot laterally into other areas of the mucosa. It was adopted practice to scan only the densest areas of Ag grain deposit found at each level on the villus.

Histology

Pieces of rabbit distal ileum were placed in Heidenhain's Susa fixative for general histology. Sections cut at 5 μm from paraffin-embedded tissue were stained with Alcian Blue/Curtis's Ponceau S (van Gieson's substitute) reagent, Weigert's iron haematoxylin being used to stain nuclei. Other sections were, on occasions, stained with haematoxylin and eosin for general purpose viewing.

Materials

The following radioactive compounds were purchased from The Radiochemical Centre, Amersham, Bucks.: L-[U-^{14}C]alanine (171 mc.m-mole^{-1}); L-[2,3-^{3}H]alanine (35 c.m-mole^{-1}); L-[U-^{14}C]lysine monohydrochloride (342 mc.m-mole^{-1}); L-[4,5-^{3}H]lysine monohydrochloride (90 c.m-mole^{-1}); L-[methyl-^{14}C]methionine (25 mc.m-mole^{-1}); L-[methyl-^{3}H]methionine (87 c.m-mole^{-1}); L-[U-^{14}C]serine (171 mc.m-mole^{-1}) and L-[3-^{3}H]serine (11 c.m-mole^{-1}). [1,2-^{3}H]Polyethylene glycol, mol. wt. 900 (6 mc.g^{-1}) came from NEN Chemicals Gmbh, D-6072 Dreieichenhain, West Germany.

Non-radioactive L-alanine, L-methionine, L-serine and L-arginine hydrochloride were purchased from BDH Chemicals Ltd., Poole, Dorset and L-lysine hydrochloride came from Cambrian Chemicals Ltd., Croydon, Surrey.

Materials used for histochemistry and autoradiography were usually of General Reagent grade. One exception was $AgNO_3$ purchased from Fisons Scientific Apparatus, Loughborough, Leics. as an Analytical Reagent. Phen-X medium was purchased from Ilford Ltd., Ilford, Essex. Gurr's Xam medium came from BDH Chemicals Ltd., Poole, Dorset. All other reagents, used for the measurement of amino acid uptake, were of A.R. grade.

RESULTS

Control experiments

The total number of transport systems responsible for alanine and lysine entry into rabbit ileal mucosa has been shown to be three in the absence of Na (Paterson *et al.* 1981). The aim of the present work was to identify sub-populations of enterocytes responsible for these three types of transport through the use of autoradiography. To do this it is first necessary to verify that the density integrated over the area of

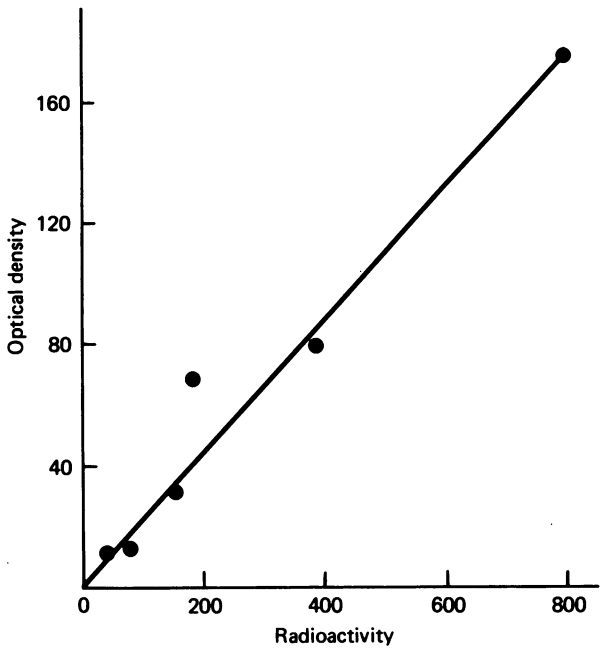


Fig. 1. A comparison of autoradiographic and spectrophotometric measurements of alanine uptake by rabbit ileal mucosa. Rabbit ileum was prepared for measurement of isotope uptake as described in the text. Six ports were used for the normal measurement of ^{14}C-labelled alanine uptake in the presence of 143 mM-Na using tritiated PEG-900 extracellular space marker. Alternate ports were incubated for 45 sec in tritiated alanine, these tissues then being processed for autoradiography as described in the text. Alanine was used at specific activities varying from 0·25 to 4·2 μc ml.$^{-1}$ (^{14}C-labelled) or 6–100 μc ml.$^{-1}$ (^{3}H-labelled) at a concentration of 1 mM. Results give mean integrated optical densities or radioactivity corrected for extracellular space (cpm $\times 10^{-3}$) obtained from experiments carried out on four rabbits.

tissue scanned is proportional to total uptake of amino acid. The scope of the problem is illustrated in Pl. 1.

The rabbit ileal mucosa consists of enterocytes measuring approximately 5 × 30 μm (Pl. 1 *A*) and the scanning spots have a diameter of 5 or 12·5 μm (Pl. 1 *B*). The maximum proportion of mucosa scanned for Ag grains represents about 30 % of the total available area. In addition it was decided to scan only the top 250 μm of each villus, since it is only this area of the mucosa which shows pronounced uptake of tritiated amino acids under both stirred (Pl. 1 *C*) and unstirred (Pl. 1 *D*) conditions.

Individual measurements made down this length of villus using a 5 or 12·5 μm spot were summed to give a final reading called the integrated optical density. These values are compared in Fig. 1 with influx estimated directly in adjacent pieces of tissue. Uptake was from a solution of 1 mM-alanine presented to the mucosal surface of the ileum over a sixteen fold range of specific activity.

TABLE 1. Effect of stirring on amino acid uptake by rabbit ileal mucosa. The three amino acids were presented separately to individual pieces of rabbit distal ileum at a concentration of 0·1 mM. Some solutions were stirred at 600 rev/min. Uptake was determined using autoradiography (integrated optical density) or scintillation spectrophotometry (n-mole cm^{-2} min^{-1}). *R* gives the ratio of uptake measured under stirred and unstirred conditions. All results were obtained using a total of three rabbits; Na concentration 143 mM

	Amino acid uptake					
	Scintillation spectrophotometry			Autoradiography		
Amino acid	Stirred	Unstirred	*R*	Stirred	Unstirred	*R*
Ala	1·42	0·81	1·8	285	148	1·9
Met	2·94	1·30	2·3	610	261	2·3
Ser	4·62	1·87	2·5	479	312	1·5

Integrated optical density was directly proportional to total extractable radioactivity measured over the whole range of specific activity tested. Experiments mentioned in Methods show the retention of tritiated amino acid within the mucosa to be high following glutaraldehyde fixation. The technique used to determine integrated Ag grain density appears to reflect quantitatively the total uptake of amino acid measured using more conventional methods of analysis. Further evidence in support of this conclusion is given below in connection with results summarized in Table 1.

One remaining problem encountered when using autoradiography is the time needed to obtain a result following experiment. Control experiments show Ag grain density to be directly proportional to the time radioactive sections are exposed to photographic emulsion (Fig. 2*A*: uptake from 0·1 mM-methionine solution used at a specific activity of 62·5 μc . $ml.^{-1}$). Suppose that 30 days were needed to produce an optical density great enough to measure activity. This time could be shortened considerably by Ag intensification after 10 days exposure. Such treatment causes a four- to fivefold increase in Ag grain density within a period of 5 min (Fig. 2*B*) with negligible effect on background reading. Longer exposure to intensifier produces no further increase in density. It was decided, on the basis of these experiments, to use the technique of physical enhancement routinely in all subsequent experiments.

Effect of stirring on the cellular distribution of amino acid uptake by rabbit ileal mucosa

Previous experiments carried out using 1 mM-alanine in the presence and absence of Na show uptake to be confined to the upper third of individual villi (Paterson *et al.* 1980*a*). Present results, which confirm earlier findings, show stirring to increase the overall uptake of alanine (Pl. 1*C* and *D*). This effect seems to take place through

a reduction in the K_m of the Na-dependent system for alanine with no measurable effect on J_{max} (Paterson, Sepúlveda & Smith, 1980*b*). Similar effects have been reported previously when measuring sugar and amino acid influx into intestinal tissue (Wilson & Dietschy, 1974). The possibility that these results might reflect a change in cellular distribution of amino acid uptake has not, however, been investigated.

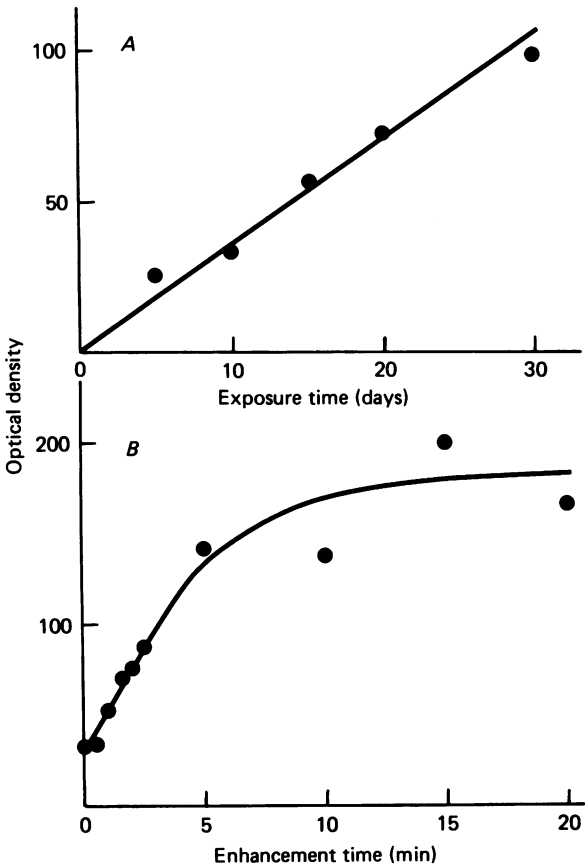


Fig. 2. Relation between Ag grain density and time of exposure of sections to photographic emulsion (*A*) or Ag intensifier solution (*B*). Pieces of rabbit distal ileum were prepared for measurement of rapid isotope uptake as described in the text. Methionine (0·1 mM) at a specific activity of 62·5 μc ml.$^{-1}$ was presented to the mucosa for a period of 45 sec in the presence of 143 mM-Na. The tissue was then processed for autoradiography using exposure times varying from 5 to 30 days (*A*). Some sections exposed to photographic emulsion for 10 days were then incubated in Ag intensifier solution for 0–20 min (*B*). Each point represents a mean integrated density obtained from measurements carried out on three villi.

The quantitative dependence of alanine uptake on speed of stirring is shown in results summarized in the inset to Fig. 3. Experiments were carried out using 0·1 mM-alanine in the presence of Na. Alanine uptake increased markedly as the speed of stirring rose from 0 to 300 rev/min. Stirring at higher speeds produced no further increase in uptake. A stirring speed of 600 rev/min was chosen, on the basis of these

results, to study the effect of stirring on the cellular distribution of alanine uptake. The results produced are shown in Fig. 3. Alanine uptake was confined mainly to enterocytes occupying the top 50 μm of individual villi. This applied whether or not the solution was stirred at 600 rev/min. There was virtually no alanine uptake by enterocytes found 150 μm or more below the tips of villi. A more sensitive test for

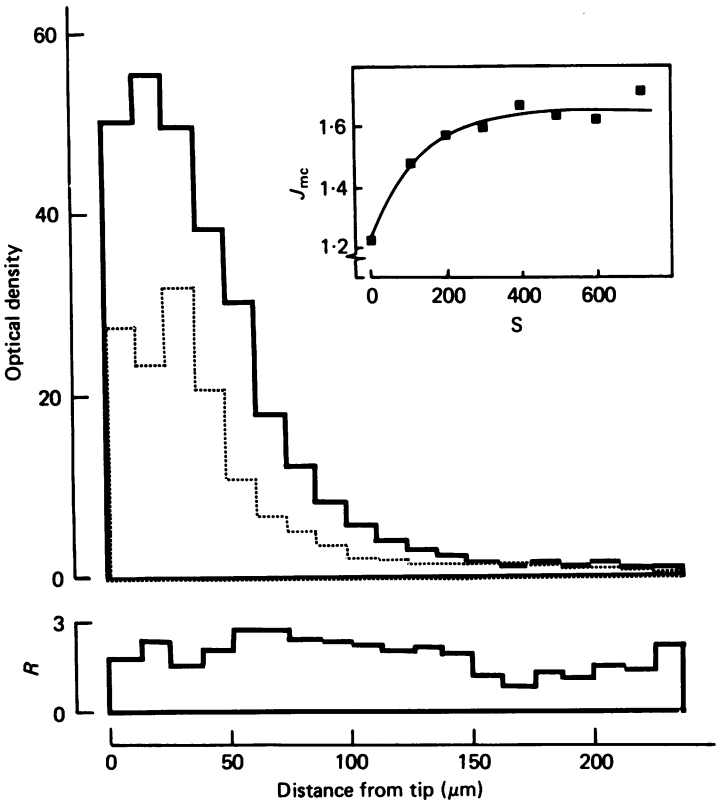


Fig. 3. Effect of stirring on the villus distribution of alanine uptake by rabbit ileal mucosa. Alanine (100 μc ml.$^{-1}$: 0·1 mM) was presented to the mucosal surface of rabbit distal ileum for a period of 45 sec in the presence of 143 mM-Na under both stirred (600 rev/min) and unstirred conditions. All tissues were processed for autoradiography as described in the text. Values of optical density (stirred, solid line; unstirred, broken line) represent the means of estimates carried out on six villi. *R* gives the ratio o.d. stirred: o.d. unstirred for different parts of the villus. The inset shows the effect of stirring (S = rev/min) on total alanine uptake in n-mole cm^{-2} min^{-1} (J_{mc}) measured using scintillation spectrophotometry. Alanine concentration, 0·1 mM; mean values for uptake based on eight determinations.

cellular heterogeneity is provided by dividing one uptake by another for each region of the villus analysed. The resulting ratio, which has been plotted in Fig. 3, shows no consistent pattern of variation. Similar results, not shown, were found when using serine and methionine. It is concluded from these results that functional differences rather than local limitation of substrate availability determine the pattern of cellular distribution observed.

Pieces of tissue adjacent to those taken for microdensitometry were used to determine alanine uptake by scintillation spectrophotometry. Comparisons were made between these results and those obtained by integrating individual optical densities plotted in Fig. 3. Results, including those obtained with serine and methionine, are summarized in Table 1. The net effect of stirring at this low substrate concentration was to increase uptake by a factor of 2. This applied irrespective of

TABLE 2. Predicted amino acid uptake by different transport systems in rabbit ileal mucosa. Rabbit ileal mucosa was incubated for 45 sec in the absence of Na with tritiated lysine (240 μc ml.$^{-1}$) at a concentration of 1 or 50 mM, or with tritiated alanine (400 μc ml.$^{-1}$) at a concentration of 1 mM in the absence and presence of 50 mM-non-radioactive lysine. The predicted percentage uptakes by four systems of transport, calculated using constants derived previously (Paterson *et al.* 1981), is compared with integrated optical densities obtained from autoradiographs of sectioned tissue. S_1 and S_2, Na-dependent and Na-independent transport systems for neutral amino acids. Ly_1 and Ly_2, high and low affinity Na-independent transport systems for lysine. Values of integrated optical densities give means ± S.E. of estimates carried out on six villi

Amino acid (mM)	Predicted uptake				Integrated density
	S_1	S_2	Ly_1	Ly_2	
Lys (1)	0	0	78	22	392 ± 58
Lys (50)	0	0	17	83	18 ± 2
Ala (1)	0	51	49	0	85 ± 12
Ala:Lys (1:50)	0	98	2	0	15 ± 3

the amino acid used or the method chosen for analysis. It was decided to use stirring in all subsequent uptake experiments.

Cellular distribution of amino acid transport systems in rabbit ileal mucosa

Rabbit ileum was incubated in the presence of tritiated lysine and alanine under conditions predicted to favour one or other of the various systems available for transport. The rationale for these experiments and the initial results obtained are shown in Table 2. Four transport systems are listed. Two of them (S_1 and S_2) carry neutral amino acids. One of them (S_1) is included only for completeness sake, since its Na dependence renders it inactive under the experimental conditions used (Na-free). The high affinity system for lysine (Ly_1) is shared by alanine but the low affinity system (Ly_2) is not. Using tritiated lysine at 1 mM should produce autoradiographs in which 78% of the total radioactivity corresponds to material take up through the Ly_1 system. Repeating the experiment at 50 mM-lysine should give results 83% specific for the Ly_2 system. Alanine used at a concentration of 1 mM will marginally favour uptake through S_2. This can be increased to a 98% specificity by inhibiting alanine uptake through the Ly_1 system with 50 mM non-radioactive lysine. All these predictions are based on kinetic constants derived in the previous paper (Paterson *et al.* 1981).

The actual integrated densities obtained under these different experimental conditions are also shown in Table 2. Decreasing the specific activity of lysine fiftyfold causes a 95% inhibition of isotope uptake. This compares with a predicted fall of 82%. Inclusion of non-radioactive lysine in medium containing 1 mM-alanine reduces alanine uptake by 82%. This compares with a predicted inhibition of 49%. The

agreement between these two sets of Figures is reasonable considering the errors involved in determining J_{max} for low affinity systems.

The cellular distribution of lysine at 1 and 50 mM concentrations is shown in Fig. 4. Individual optical densities are represented as a percentage of the total optical density determined over the top 250 μm of villus mucosa. This compensates for the

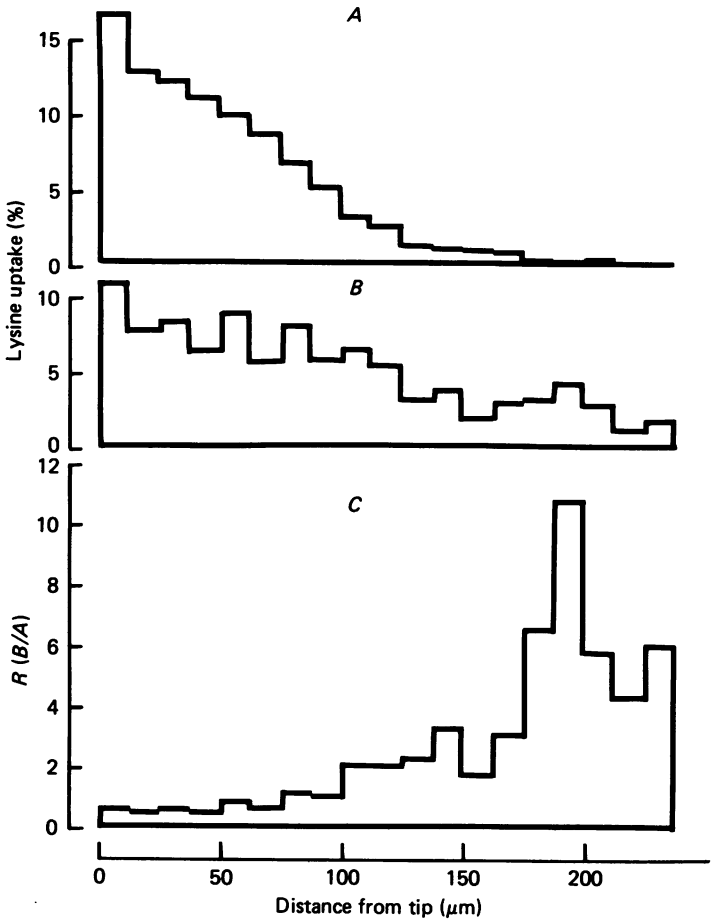


Fig. 4. Cellular distribution of lysine uptake. Rabbit ileum was incubated with tritiated lysine (240 μc ml.$^{-1}$) for 45 sec in the absence of Na at a concentration of 1 (*A*) or 50 (*B*) mM. Tissues were processed for autoradiography as described in the text. Values of optical density, taken to a villus depth of 250 μm, are represented, for each level on the villus as a percentage of the total uptake. Histograms give the means of estimates carried out on six villi. *R* is the density obtained using 50 mM-lysine divided by that obtained using 1 mM-lysine.

great difference in Ag grain density, produced by dilution of specific activity, without affecting the recorded pattern of isotope distribution. Maximal uptake of lysine through the Ly_1 system took place at the tips of villi. Uptake then fell steadily to reach negligible levels at a villus depth of 150 μm (Fig. 4*A*). Lysine uptake through the Ly_2 system was also high at the tips of villi, but the fall on moving down the villus was much more gradual than for the Ly_1 system (Fig. 4*B*). A more detailed

comparison of the two systems of uptake shows there to be relatively much more Ly_2 than Ly_1 system in enterocytes present in the lower regions of the villi. This discrepancy has more or less disappeared by the time enterocytes reach the villus tip (Fig. 4*C*). The distinction between Ly_1 and Ly_2 uptake is, in this instance, incomplete. The 20% overlap that occurs between these two systems (Table 2) will lessen the

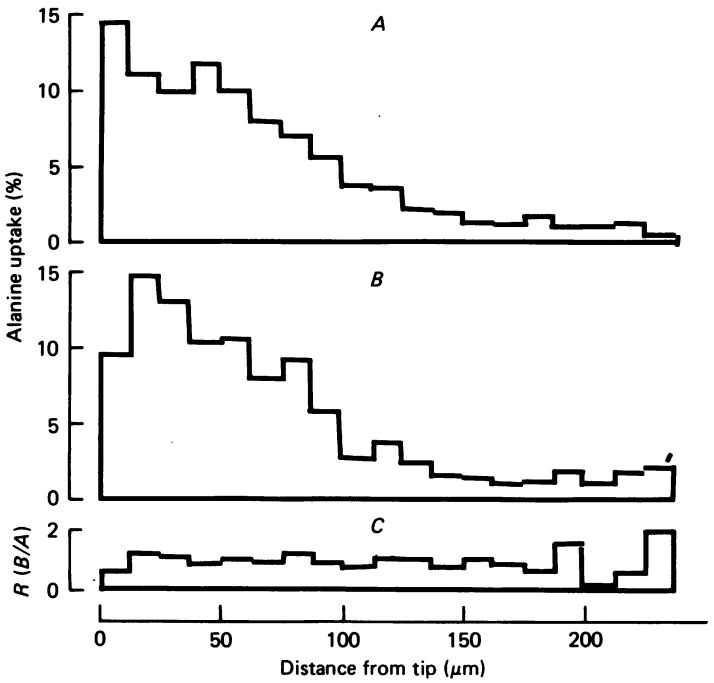


Fig. 5. Cellular distribution of alanine uptake. Rabbit ileum was incubated with tritiated 1 mM-alanine (400 μc ml.$^{-1}$) for 45 sec in the absence of Na with or without 50 mM non-radioactive lysine (*B* and *A* respectively). Tissues were processed for autoradiography as described in the text. Presentation of results is as described in the legend to Fig. 4. Histograms give the means of estimates carried out on six villi.

differences recorded above. There seems little doubt that the low affinity uptake mechanism for lysine transport has a distribution different from that seen for the high affinity system. The possibility that this distribution results from diffusion of lysine rather than mediated transport seems unlikely in view of the results summarized in Fig. 6.

Measurement of alanine uptake through the S_2 system will always be contaminated by uptake taking place through the Ly_1 system (Table 2). This can be reduced to negligible proportions, however, by the inclusion of 50 mM-lysine in the alanine incubation medium. Cellular distribution of alanine measured under these conditions is shown in Fig. 5*B*. Uptake of alanine on the S_2 system, which is most noticeable near the villus tip, falls away steadily to negligible levels at a villus depth of 150 μm. It is interesting to compare this pattern of distribution with that found using 1 mM-alanine in the absence of lysine, a situation which splits alanine entry equally between the S_2 and Ly_1 systems (Table 2). The distribution of alanine measured under

these conditions is remarkably similar to that determined in the presence of 50 mM-lysine (Fig. 5*A* and *B*). The ratio for uptake determined under these two conditions was approximately 1 for all parts of the villus analysed (Fig. 5*C*). The inability of a large Ly_1 component to influence the over-all pattern of alanine uptake suggests that both the S_2 and Ly_1 systems mature with the same time course during the later stages of enterocyte differentiation.

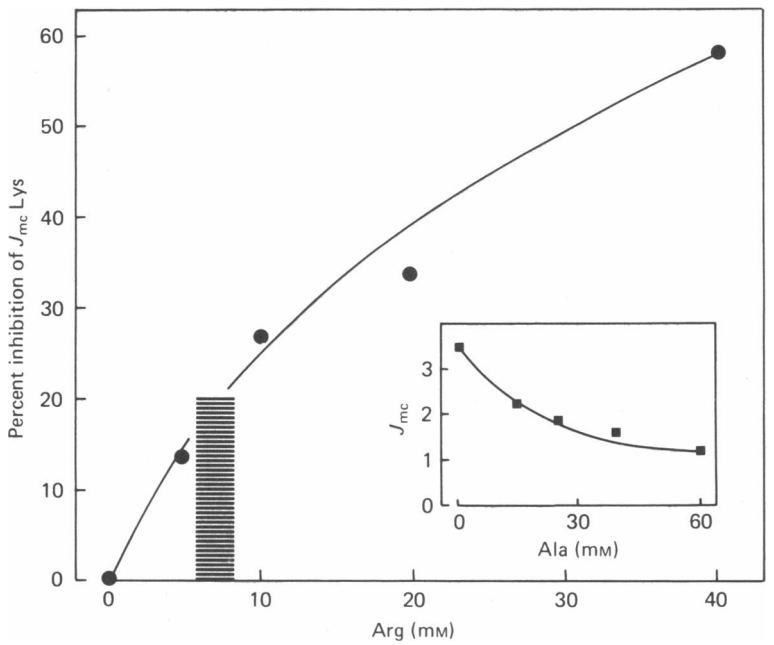


Fig. 6. Alanine and arginine inhibition of lysine uptake. Rapid uptake of lysine by rabbit ileal mucosa was measured at a concentration of 30 mM and 0·25 mM using arginine and alanine respectively as inhibitors of transport in the absence of Na. Results show the percent inhibition of lysine uptake (J_{mc} Lys) by arginine (main Figure) and the inhibition of lysine uptake in n-mole cm^{-2} min^{-1} (J_{mc}) by alanine (Fig. inset). The maximal percent Ly_1, component to total lysine uptake at 30 mM is shown by the column. All estimates represent means based on determinations carried out on eight rabbits.

Arginine inhibition of lysine uptake by rabbit ileal mucosa

The Ly_2 system for lysine transport, shown previously to have a villus distribution different from that of the S_2 or Ly_1 systems, could arise from diffusion. Experiments to test this hypothesis use arginine as an inhibitor of lysine uptake under conditions where the Ly_2 system predominates (30 mM-lysine). Arginine might also affect the Ly_1 system, however, and it is necessary to know the maximal contribution this system makes to total lysine uptake. This was determined by measuring the ability of alanine to inhibit lysine uptake under conditions where the Ly_1 system predominates (0·25 mM-lysine). The results obtained are shown in the inset to Fig. 6.

Alanine is a potent inhibitor of lysine uptake under these conditions. An Inui & Christensen plot of these results gives a straight line with intercept showing 74% inhibition of lysine uptake at an infinitely high concentration of alanine. This is taken

to be the fraction of lysine uptake entering the mucosa on the Ly_1 system at a concentration of 0·25 mM. The calculated J_{max} for this system, assuming a K_m for lysine of 1 mM, is 13 n-mole cm^{-2} min^{-1}.

The second part of the experiment, carried out on the same piece of ileum, measures the uptake of lysine from a concentration of 30 mM in the presence of increasing concentrations of arginine. Lysine uptake in the absence of arginine is $65·2 \pm 8·2$ n-mole cm^{-2} min^{-1}. The maximal contribution of system Ly_1 to this uptake (13 n-mole cm^{-2} min^{-1}) represents 20% of the total. This is shown by the column in Fig. 6: 10 mM-arginine causes a bigger inhibition of lysine uptake than could be accounted for by the Ly_1 system. Increasing the concentration of arginine caused a progressive increase in inhibition of lysine uptake (58% inhibition using an arginine concentration of 40 mM). Clearly most of the lysine uptake on the so-called Ly_2 system is taking place through a mediated pathway.

DISCUSSION

Part of the work described in the present paper serves to confirm previously published findings. It was already known, for instance, that amino acids taken up by the hamster jejunum would be concentrated preferentially in the tips of villi (Kinter & Wilson, 1965) and there has already been a crude attempt to quantitate this effect in rabbit ileum (Paterson *et al.* 1980*a*). Present results show that this distribution does not arise from the presence of unstirred layers or from insufficient contact of the lower villus regions with isotope. One would presume that the crypt cells are also unable to take up amino acids from the intestinal lumen, since this is true of similar cells shortly after they appear upon the villus, but this is difficult to test directly. Plugs of mucus tend to block the entrance to crypts and this could restrict penetration of isotope (Kinter & Wilson, 1965).

The villus distribution of amino acids following uptake through the Ly_1 and S_2 systems is similar. It consists of four phases spread over a total villus length of $450 \pm 9·6$ μm (mean ± S.E. of twenty-eight estimates). The first phase extends from about 450–250 μm from the villus tip. Enterocytes in this region are unable to take up measurable amounts of alanine or lysine. The second phase, which is one of limited amino acid uptake, stretches from 250 to 100 μm from the villus tip. Transport increases steadily as cells migrate through this region, uptake doubling over a distance of 100 μm. The third phase is one of rapid increase in transport capacity stretching from 100 to 50 μm from the villus tip. The rate of increase in amino acid uptake seen to take place during this stage of cell differentiation is approximately 25 times that found previously. Finally there is a phase where no further change is observed. This occurs as enterocytes travel the last 50 μm of villus surface before being extruded into the intestinal lumen.

The Ly_2 system of amino acid entry can also be detected in enterocytes located 250 μm from the villus tip. What distinguishes this system from the rest is the apparent inability of enterocytes to initiate a second rapid phase of carrier synthesis (or activation). The ability to take up lysine on the Ly_2 system continues to increase slowly right up to the point of enterocyte extrusion, uptake doubling for every 100 μm of villus surface covered. The possibility that this pattern of distribution could result

from diffusion rather than from mediated transport has been excluded on the basis of arginine inhibition experiments. The similar distribution of the Ly_1, Ly_2 and S_2 systems during the initial phase of enterocyte maturation raises the possibility that all systems might be initiated through a common mechanism. The fact that the Ly_1 and S_2 systems later enter into a much more rapid phase of development could imply that the control mechanism for these systems changes or that this later stage of functional differentiation is initiated by an entirely different set of circumstances.

The presence of tight junctions linking the apical poles of enterocytes has been reported for both crypt and villus (Tice, Carter & Cahill, 1979; Madara, Trier & Neutra, 1980). EDTA disruption of similar tight junctions in another epithelium, the frog urinary bladder, results in a time dependent mixing of membrane components previously restricted to the apical surface (Pisam & Ripoche, 1976). One can assume, by analogy, that it is the presence of tight junctions in the crypt epithelium which allows the cells to express anatomical and biochemical asymmetry at an early stage in development. The time course with which other components of the microvillar membrane appear is variable. Aminopeptidase is already detected in crypt cell membrane at high activity while alkaline phosphatase and disaccharidase activities become important only in microvillar membranes of the villus region (Gratecos, Knibiehler, Benoit & Séméria, 1978). Some turnover of *m*RNA continues throughout the lifetime of a villus enterocyte providing ample opportunity for the initiation of protein synthesis associated with changed microvillar membrane function (Morrison & Porteous, 1980). The basolateral membrane of the enterocyte appears much less susceptible to change during development, Na^+/K^+-dependent, ouabain-sensitive ATPase showing constant specific activity throughout the life of the enterocyte (Gratecos *et al.* 1978).

The problems associated with understanding how differentiation takes place in this tissue are concerned both with the nature of the signal initiating synthesis of new proteins and the way in which these molecules are directed to one membrane rather than another. Insertion or activation of amino acid carriers into the microvillar membrane involves an additional feat of cellular organization, for the basolateral membrane has been shown to be able to take up amino acids throughout the cell's existence (Kinter & Wilson, 1965) and this is shown, in upper villus enterocytes at least, to take place through multi-carrier systems (Mircheff, van Os & Wright, 1980). In this case it is not the synthetic machinery which has to be activated during differentiation but the means to redirect carriers, possibly in varying proportions, to the microvillar membrane. Much experimental work is needed to unravel mechanisms underlying the differentiation of transport. The approach used here to examine the contribution of individual cells to amino acid transport provides one way of approach to this type of problem.

We should like to thank Mr A. L. Gallup for photography of intestinal villi and Mr L. G. Jarvis for preparation of histological specimens.

REFERENCES

BARKER, D. V., SEPÚLVEDA, F. V. & SMITH, M. W. (1980). A versatile multi-stirring apparatus used for rapid flux studies in the intestine. *J. Physiol.* **303**, 4–5*P*.

CURRAN, P. F., SCHULTZ, S. G., CHEZ, R. A. & FUISZ, R. E. (1967). Kinetic relations of the Na-amino acid interaction at the mucosal border of intestine. *J. gen. Physiol.* **50**, 1261–1286.

GRATECOS, D., KNIBIEHLER, M., BENOIT, V. & SÉMÉRIVA, M. (1978). Plasma membranes from rat intestinal epithelial cells at different stages of maturation. I. Preparation and characterization of plasma membrane subfractions originating from crypt cells and from villous cells. *Biochim. biophys. Acta* **512**, 508–524.

INUI, Y. & CHRISTENSEN, H. N. (1966). Discrimination of single transport systems. The Na-sensitive transport of neutral amino acids in the Erhlich cell. *J. gen. Physiol.* **50**, 203–224.

KING, I. S., SEPÚLVEDA, F. V. & SMITH, M. W. (1981). Autoradiographic analysis of amino acid uptake by rabbit ileal mucosa. *J. Physiol.* **316**, 1–2*P*.

KINTER, W. B. & WILSON, T. H. (1965). Autoradiographic study of sugar and amino acid absorption by everted sacs of hamster intestine. *J. cell Biol.* **25**, 19–39.

MADARA, J. L., TRIER, J. S. & NEUTRA, M. R. (1980). Structural changes in the plasma membrane accompanying differentiation of epithelial cells in human and monkey small intestine. *Gastroenterology* **78**, 963–975.

MIRCHEFF, A. K., VAN OS, C. H. & WRIGHT, E. M. (1980). Pathways for alanine transport in intestinal basal lateral membrane vesicles. *J. Membrane Biol.* **52**, 83–92.

MORRISON, A. & PORTEOUS, J. W. (1980). Changes in the synthesis of ribosomal ribonucleic acid and of poly(A)-containing ribonucleic acid during the differentiation of intestinal epithelial cells in the rat and in the chick. *Biochem, J.* **188**, 609–618.

MUNCK, B. G. & SCHULTZ, S. G. (1969). Lysine transport across isolated rabbit ileum. *J. gen. Physiol.* **53**, 157–182.

MURGATROYD, L. B. (1976). The preparation of thin sections from glycol methacrylate embedded tissue using a standard rotary microtome. *Med. Lab. Sci.* **33**, 67–71.

PATERSON, J. Y. F., SEPÚLVEDA, F. V. & SMITH, M. W. (1979). Two-carrier influx of neutral amino acids into rabbit ileal mucosa. *J. Physiol.* **292**, 339–350.

PATERSON, J. Y. F., SEPÚLVEDA, F. V. & SMITH, M. W. (1980*a*). A sodium-independent low affinity transport system for neutral amino acids in rabbit ileal mucosa. *J. Physiol.* **298**, 333–346.

PATERSON, J. Y. F., SEPÚLVEDA, F. V. & SMITH, M. W. (1980*b*). Effect of stirring on kinetic parameters of amino acid uptake by rabbit ileal mucosa *in vitro*. *J. Physiol.* **303**, 78*P*.

PATERSON, J. Y. F., SEPÚLVEDA, F. V. & SMITH, M. W. (1981). Distinguishing transport systems having overlapping specificities for neutral and basic amino acids in rabbit ileal mucosa. *J. Physiol.* **319**, 345–354.

PISAM, N. & RIPOCHE, P. (1976). Redistribution of surface macromolecules in dissociated epithelial cells. *J. cell Biol.* **71**, 907–920.

SEPÚLVEDA, F. V. & SMITH, M. W. (1978). Discrimination between different entry mechanisms for neutral amino acids in rabbit ileal mucosa. *J. Physiol.* **282**, 73–90.

SMITH, M. W., JAMES, P. S. & PATERSON, J. Y. F. (1981). Electrical properties of a Na^+-dependent amino acid transport system common to different mammalian intestines. *Comp. Biochem. Physiol.* **69A**, 231–236.

TICE, L. W., CARTER, R. L. & CAHILL, M. B. (1979). Changes in tight junctions of rat intestinal crypt cells associated with changes in their mitotic activity. *Tissue & Cell* **11**, 293–316.

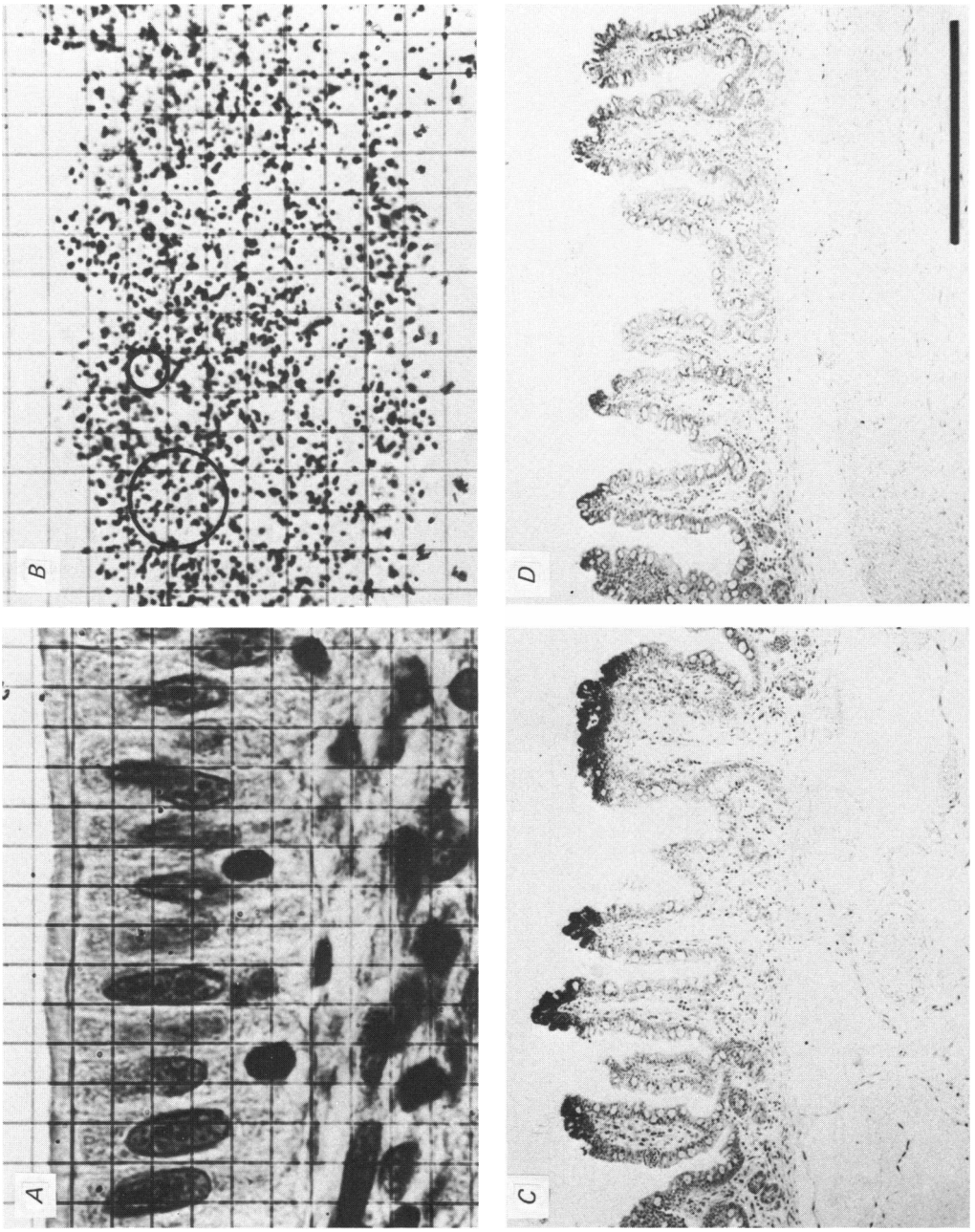
WILSON, F. A. & DIETSCHY, J. M. (1974). The intestinal unstirred layer: its surface area and effect on active transport kinetics. *Biochim. biophys. Acta* **363**, 112–126.

EXPLANATION OF PLATE

A, section of rabbit distal ileum stained as described in the text. The superimposed grid consists of squares measuring 5×5 μm. Section thickness 4 μm.

B, unstained section of rabbit distal ileum following exposure to photographic emulsion. The tissue had been exposed previously to 1 mM-alanine solution (100 μc ml.$^{-1}$), for a period of 45 sec in the presence of 143 mM-Na. The two circles represent scanning spots of diameter 5 and 12·5 μm. Section thickness 3 μm.

C and *D*, sections of rabbit ileum showing Ag grain deposits formed from tritiated alanine taken up during a 45 sec incubation in the presence of 143 mM-Na, while stirring at 700 rev/min. (*C*) or under unstirred conditions (*D*). The concentration of alanine was 1 mM; the specific activity was 100 μc ml.$^{-1}$; the time of exposure was 20 days and the enhancement time was 1 min. Sections cut at 3 μm were stained lightly with haematoxylin and eosin. Unstained sections were used for microdensitometry. Scale bar 1 mm.



(*Facing p.* 368)