J. Physiol. (1983), **341**, *pp.* 441–452
With 4 text-figures
Printed in Great Britain

MODIFICATION BY DIET AND ENVIRONMENTAL TEMPERATURE OF ENTEROCYTE FUNCTION IN PIGLET INTESTINE

BY M. J. DAUNCEY, D. L. INGRAM, P. S. JAMES AND M. W. SMITH
From the Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

(*Received 9 December 1982*)

SUMMARY

1. Intestinal morphology, enterocyte life span and alanine transport have been studied in the small intestine of piglets fed different amounts of food at high and low environmental temperatures.

2. Villus height and crypt depth were both greater in pigs maintained on a high energy intake. Environmental temperature produced negligible effects on intestinal structure.

3. Enterocyte life span increased from 45 h in pigs kept at 35 °C to about 70 h in animals living at 10 °C. A low energy intake prolonged enterocyte life span at an environmental temperature of 10 °C.

4. The Na-dependent fraction of alanine uptake, judged by analysis of autoradiographs and by measurement of alanine-dependent short-circuit current, was greater in intestines taken from pigs maintained on a restricted diet. This effect, which appeared to be due to changes in the number of carriers (J_m) rather than the apparent affinity of the carrier for the amino acid (K_m), was most noticeable using intestines taken from pigs kept at 10 °C.

5. The Na-independent fraction of alanine uptake remained unchanged either by alterations in diet or in the environmental temperature at which pigs were kept.

6. Restricting the diet of pigs at low environmental temperature leads to a relative increase in the capacity of the intestine to absorb alanine through an Na-dependent process. This increase appears to be caused by an extension of enterocyte life span rather than by any change in the time of onset or rate of expression of carrier function in a single enterocyte.

INTRODUCTION

Animals kept at different environmental temperatures have completely different energy requirements because, in the cold, energy is needed specifically for thermoregulation. It follows that, for animals kept at different environmental temperatures on the same energy intake, the one in the cold will be relatively underfed. Semi-starvation of animals at constant environmental temperature has already been shown to cause changes in the capacity of intestines to absorb nutrients (see Neale & Wiseman, 1969; Debnam & Levin, 1976; Syme & Levin, 1977). These dietary-induced effects could be organized locally, as a direct response to changes in the amount of

nutrients ingested or more indirectly, as part of a general mechanism whereby animals increase the efficiency at which they utilize nutrients when maintained on a restricted diet. One aim of the present work was to test for similarities in intestinal adaptation to reduced energy intake and low environmental temperature to try to distinguish local from general effects on transport. Young pigs were used in this work because of their rapid rate of growth and their toleration of extreme changes in temperature and energy intake.

It has been common practice in the past to measure the transport of a chosen nutrient and relate these measurements to changes in intestinal structure. It is now possible, however, to study the transport of amino acids directly at the level of the enterocyte using a technique of quantitative autoradiography described previously (King, Sepúlveda & Smith, 1981). Results obtained using this technique have been combined with measurements of cell kinetics in the present work to distinguish some of the changes taking place in the intestinal transport of alanine. A preliminary report of part of the work has already been published (Smith, Dauncey & Ingram, 1982).

METHODS

Animals

Piglets bred at Babraham from a herd of Large Whites were removed from the sow on day 14 and housed in pairs at a constant temperature of 27 °C for a period of 3 days. During this time they were allowed access to unrestricted amounts of solid food (Supercreep, RHM Agriculture, Brooks Hasler Ltd, Essex; energy value 1·9 MJ 100 g^{-1}). The pigs were separated on day 17 and given a high or low energy intake, increasing in amount from 100 to 250 g and 50 to 125 g feed (H and L pigs respectively) over a period of 10 days. The final levels of food intake, attained between 49 and 56 days of age, at the time pigs were killed for experiment, were 600 and 300 g for H and L pigs respectively. Changes in environmental temperature were started on day 17, final temperatures of 35 °C or 10 °C being reached by day 28.

The result of these various manipulations was to produce four types of experimental animal (hot and cold pigs both maintained on high or low energy intakes). These have been referred to as 35H, 35L, 10H and 10L pigs throughout the text. The food which was given once a day was normally eaten within a period of 2 h. Before experiment, pigs were fasted for a period of 24 h, before being pre-medicated with Ketamine and killed by intracardiac injection of sodium barbiturate.

Autoradiographical analysis of alanine transport

Pieces of tissue taken from the middle of the small intestine were first superfused with Na-free bicarbonate medium for a period of 10 min. [^{3}H]alanine (1 mM), made up in the presence and absence of Na in bicarbonate medium equilibrated with 95 % O_2 + 5 % CO_2 (100 μCi ml^{-1}), was then presented to the mucosal surface of the intestine for a period of 45 s. These solutions were maintained at 37 °C and stirred at 750 rev min^{-1} throughout the period of incubation. Uptake was stopped by the addition of phosphate-buffered saline containing 4 % (v/v) glutaraldehyde and 2 % (w/v) sucrose and the tissue processed for autoradiography as described previously (King *et al.* 1981).

The density of Ag grains in Eosin-stained sections of intestinal mucosa was determined subsequently by microdensitometry (Vickers Instruments, York) using a 5 μm spot of light to obtain a series of individual readings from the tips of villi down to levels where readings of density became close to background. All values of optical density were converted to intracellular concentrations of alanine by comparison with gelatin sections containing known amounts of ^{3}H. Further details of this method of quantification are given in the paper of Paterson, Sepúlveda & Smith (1982).

Electrical analysis of alanine transport

Three pieces of tissue taken from the middle of the intestine of each pig were mounted in Ussing-type chambers for the recording of short-circuit current and allowed to equilibrate for

10 min in bicarbonate saline gassed with 95 % O_2 + 5 % CO_2 at a temperature of 37 °C. Small volumes of solutions containing alanine were then added to medium bathing the mucosa and the increases in short-circuit current recorded. The final concentration of alanine present in the mucosal solution varied from 0·5 to 40 mM. A standard concentration of alanine was added at regular intervals throughout the experiment to compensate for variations in tissue sensitivity. Calculation of dose-effect curves and further details concerning the method used to measure short-circuit current have both been published previously (Henrique de Jesus & Smith, 1974; Smith, James & Paterson, 1981).

Measurement of enterocyte life span

Best estimates of enterocyte life span involve making replicate measurements of [^{3}H]thymidine location within the intestinal epithelium at a number of different times following the injection of isotope. The size of 7–8-week-old pigs, however, makes it too expensive to carry out this procedure in detail. As a compromise it was decided to inject two pigs with [^{3}H]thymidine from each experimental group (1 mCi kg^{-1}). One animal was then killed 24 h and the other 48 h after injection. The leading front of labelled enterocytes was identified subsequently in autoradiographs and their life span calculated assuming enterocytes to be produced at the base of crypts and migration to take place at a constant rate. Some support for the first assumption was provided by the observation that radioactively labelled cells were still being produced near the base of crypts 24 h after isotope had been injected.

The mean crypt depth and villus height in each pig was determined from ten measurements using sections of intestine stained with Haematoxylin and Eosin.

Materials

The following radioactive compounds were obtained from Amersham International Ltd, Amersham, Bucks: L-[2,3-^{3}H]alanine (30–50 Ci $mmol^{-1}$); [*methyl*-^{3}H]thymidine (47 Ci $mmol^{-1}$). All other reagents used were of A.R. grade.

RESULTS

Effect of varying energy intake and environmental temperature on pig intestinal structure

Seven-week-old pigs kept at 35 °C or 10 °C on two levels of energy intake were killed and measurements of intestinal structure carried out on sectioned material of mid-intestine as described above. The results obtained are summarized in Table 1.

Increasing the energy intake by a factor of two was associated with an increase in both villus size and crypt depth at both environmental temperatures. Maintaining pigs at 10 °C rather than 35 °C was without effect on crypt depth or villus height provided the energy intake remained low. There was some temperature-dependent increase in villus size in cold compared with warm pigs maintained on a high energy intake (539 *vs.* 422 μm; $P < 0{\cdot}05$), but this was not associated with any significant change in crypt depth ($P > 0{\cdot}1$). It is concluded from these initial experiments that energy intake rather than environmental temperature is the main originator of change in intestinal structure in these animals. Subsequent experiments were designed to test how these changes in structure might be related to changes in the capacity of the intestine to absorb neutral amino acids.

Alanine-induced changes in intestinal short-circuit current

The uptake of neutral amino acids by mammalian intestine has recently been described as taking place on two carriers, one of low affinity which is Na-independent and another of high affinity which operates only in the presence of Na (Paterson, Sepúlveda & Smith, 1979, 1980*a*). Amino acid uptake through the Na-dependent

TABLE 1. Effect of changing energy intake and environmental temperature on pig intestinal structure

	Experimental conditions			
	35H	35L	10H	10L
Villus height	422±24 (n.s.)	371±19 (n.s.)	539±36**	339±36**
Crypt depth	302±11*	243±19*	276±15*	227±17*

Each value gives the mean villus height or crypt depth in μm±S.E. of the mean, based on determinations carried out on six pigs. 35 and 10 refer to the environmental temperatures of the pigs (°C); H and L refer to the high and low energy intakes defined in the text. Differences between values for H and L at 35 or 10 °C, assessed by paired *t* test analysis, have the following significance: *$P < 0.05$; **$P < 0.02$; n.s., not significant.

system is coupled to that of Na with a stoichiometry of one (Paterson, Sepúlveda & Smith, 1980*b*). Work on other cells has shown that it is the Na-dependent uptake of amino acids which is subject to hormonal regulation (Guidotti, Borghetti & Gazzola, 1978). In the intestine it is possible to study the characteristics of this Na-dependent process indirectly by measuring the ability of amino acids to increase the short-circuit current (Smith *et al.* 1981). Results obtained using this method of analysis for intestines taken from pigs subjected to the four different conditions are given in Fig. 1.

Alanine applied to the mucosal surface of pieces of intestine clamped in Ussing-type chambers caused an increase in short-circuit current which was dependent on concentration. Increases produced by different concentrations of alanine were, however, significantly greater using tissue taken from pigs maintained on a low energy intake ($P < 0.01$; analysis of variance). This energy-dependent difference in current increase also appeared to be greater using tissue taken from pigs kept at low environmental temperature. Each set of data was fitted to a single hyperbola. Constants describing these curves are given in Table 2.

Values of K_m describing the alanine interaction with its Na-dependent carrier showed no significant variation between the four groups of animals. Calculating a common K_m for all experiments gave a final value of 5·4±0·4 mM. This is very similar to values reported previously using intestines taken from a variety of different species (Smith *et al.* 1981). The values for the number of carriers (J_m) for intestines taken from pigs maintained on a low energy intake were greater than those found using intestines taken from high intake animals ($P < 0.02$; paired *t* test). This difference was more pronounced using tissue taken from cold-adapted pigs.

Restricting energy intake appears, from these results, to increase the capacity of the pig intestine to take up alanine through an Na-dependent process without changing the affinity of the carrier for its substrate. The object of the following work was to test for confirmation of this result and establish the cellular basis for these energy-dependent changes in transport.

Quantitative autoradiography of alanine uptake by pig intestine

Pieces of intestine were exposed to [^{3}H]alanine for 45 s in the presence and absence of Na. Pieces of tissue were then removed and processed for autoradiography as

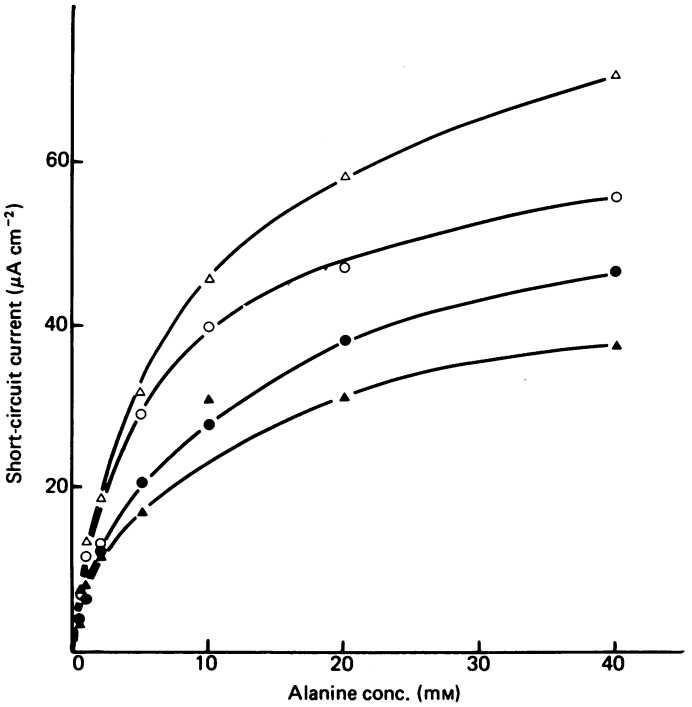

Fig. 1. Concentration dependence of alanine-induced changes in short-circuit current of pig small intestine. Pieces of mid-intestine taken from pigs maintained at environmental temperatures of 35 (○, ●) or 10 °C (△, ▲), fed a low or high energy intake (open and filled symbols respectively), were incubated in bicarbonate saline at 37 °C and the short-circuit currents recorded as described in the text. Values for current refer to the increases measured in the presence of different concentrations of alanine. Each value gives the mean of triplicate estimates carried out on tissue taken from six pigs. Curves fitted to these data have constants given in Table 2.

TABLE 2. Kinetic constants describing alanine uptake by pig small intestine

	Experimental conditions			
	35H	35L	10H	10L
K_m (mM)	6·3±1·3	4·6±0·7	5·6±1·0	5·3±0·8
J_m (μA cm^{-2})	48·8±4·8	57·5±8·7	42·6±8·3	74·3±12·8

Values of K_m and J_m were calculated from results shown in Fig. 1 assuming alanine effects on the short-circuit current to be mediated through a single carrier. The experimental conditions (35H, 35L, 10H and 10L) were as described for Table 1. All values give means±S.E. of the mean.

described previously (King *et al.* 1981). The concentrations of alanine in different parts of the intestinal mucosa, found subsequently using microdensitometry, are shown in Fig. 2.

The highest concentrations of alanine were always found at the tips of villi. This was true whether or not Na was present in the incubation medium. Including Na in the incubation medium caused a three- to four-fold increase in the ability of enterocytes to concentrate alanine. Intestines taken from pigs fed a low energy intake

appeared to concentrate alanine more effectively in the presence of Na than did those taken from high intake animals (Fig. 2*A*). No such difference was seen in the absence of Na (Fig. 2*B*). Lines of best fit to data obtained in the tip region of these villi were calculated. The slopes of these lines are given in Table 3. The intracellular concentration of alanine increased in the presence of Na by about 0·02 mM μm^{-1} travelled by the

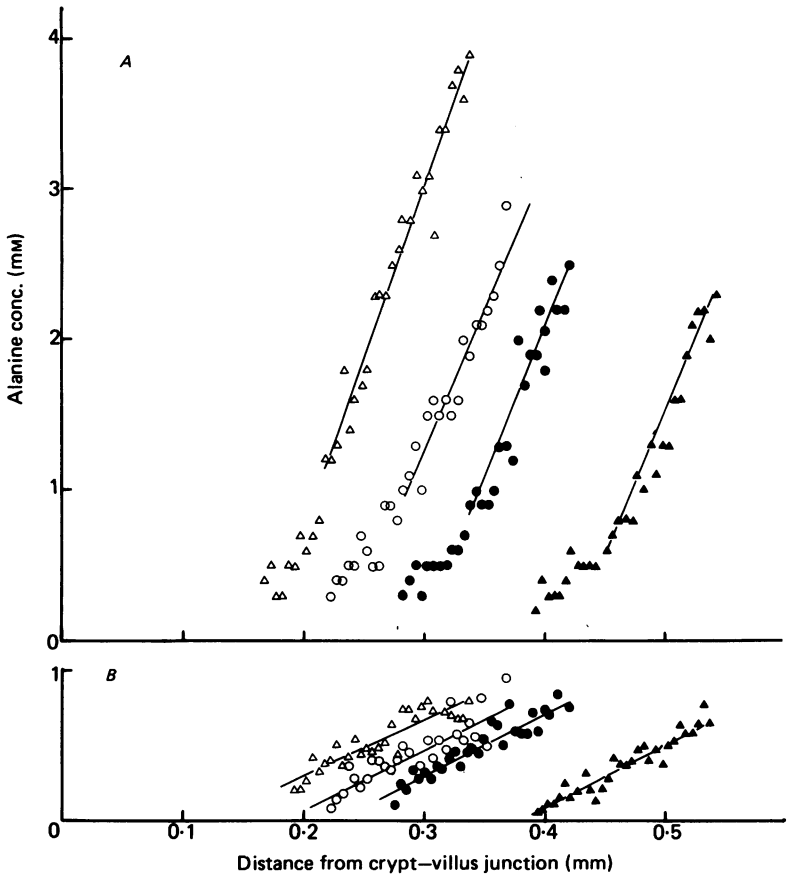

Fig. 2. Cellular distribution of alanine uptake by pig small intestine. Pieces of mid-intestine were incubated with 1 mM-[^{3}H]alanine (100 μCi ml^{-1}) for 45 s in the presence (*A*) and absence (*B*) of Na. Tissues were then fixed and processed for autoradiography as described in the text. Symbols describing the previous treatment of pigs (35H, ●; 35L, ○; 10H, ▲; 10L, △) are as described for Fig. 1. Each value of alanine concentration represents the mean of estimates carried out on twelve villi (four pigs). Lines fitting the data have slopes given in Table 3.

enterocyte during the final stages of enterocyte migration to the villus tip. The corresponding value determined in the absence of Na was 0·004 mM μm^{-1}. Neither of these values appeared to depend on the previous history of the animal.

In order to estimate the extent of Na-dependent alanine transport taking place in individual villi it was decided to calculate the mean slope for Na-independent uptake and to subtract the values obtained from this slope from the individual

estimates of alanine uptake carried out in the presence of Na. The resulting estimates of Na-dependent alanine uptake are shown in Fig. 3.

The general appearance of the curves produced was very similar to that seen in Fig. 2*A*. Alanine uptake decreased in linear fashion on moving down the villus to a depth of either 100 (10H, 35H and 35L pigs) or 150 μm (10L pigs). There was, in

TABLE 3. Regression analysis of alanine transport by pig intestinal villi

Experimental condition	Slope (mM μm^{-1})	
	Na present	Na absent
10H	0·020 ± 0·001	0·0043 ± 0·0003
10L	0·023 ± 0·001	0·0037 ± 0·0003
35H	0·020 ± 0·002	0·0042 ± 0·0003
35L	0·019 ± 0·001	0·0039 ± 0·0004

Estimates of slope ± S.E. of the mean were obtained from data plotted in Fig. 3. Experimental conditions (10H, 10L, 35H and 35L) were as described for Table 1.

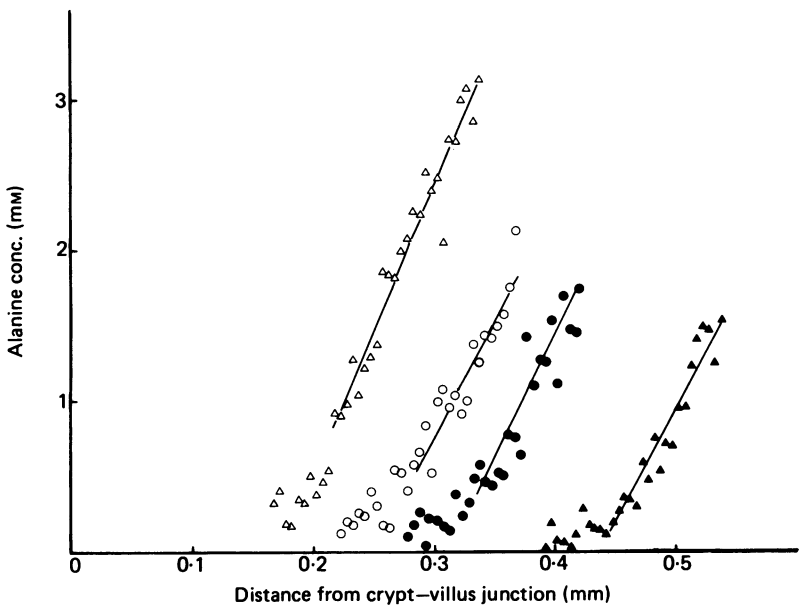

Fig. 3. Cellular distribution of Na-dependent alanine uptake by pig small intestine. The values for alanine concentration were calculated from results shown in Fig. 2 using methods described in the text. Symbols describing the previous treatment of pigs are as described for Fig. 1. Lines of best fit have been plotted for data obtained in the region of the villus tips.

addition, a second minor component to uptake extending a further 50 μm towards the crypt–villus junction. This second component appeared more noticeable using intestines taken from pigs maintained on a high energy intake. The ability of enterocytes at the villus tip to concentrate alanine, through an Na-dependent process during 45 s incubation, was calculated by regression analysis. Values obtained from this analysis (3·1, 1·8, 1·7 and 1·5 mM for intestines taken from 10L, 35L, 35H and

10H pigs) ranked in the same order as values of J_m calculated earlier from measurements of short-circuit current (Table 2).

Enterocyte turnover and alanine uptake

The increased ability of enterocytes from pigs on a low energy intake to take up alanine through an Na-dependent process could arise because they mature earlier, because the normally maturing enterocyte stays longer on the villus, or because the rate of carrier synthesis in individual enterocytes increases. Estimates of enterocyte life span were obtained for each of the four groups of experimental animals to try to distinguish between these various possibilities.

TABLE 4. Enterocyte life span in pig intestine

Time from thymidine injection (h)	Enterocyte life span (h)			
	35H	35L	10H	10L
24	44·2	42·0	60·4	84·9
48	48·0	48·0	70·5	70·7
Mean values	46·1	45·0	65·5	77·8

[^{3}H]thymidine (1 μCi g^{-1}) was injected intraperitoneally into pigs which were killed 24 or 48 h later. The highest point on the villus to which labelled enterocytes had migrated was measured and enterocyte life span calculated assuming mitosis to be confined to the base of crypts. The experimental conditions (35H, 35L, 10H and 10L) were as described for Table 1.

Pigs taken from two litters were injected intraperitoneally with [^{3}H]thymidine and the animals killed 24 or 48 h later. Labelled enterocytes migrating up the villi were identified subsequently by autoradiography and the enterocyte life span calculated as described in the Methods section. The results obtained are given in Table 4.

It took approximately 45 h for enterocytes to reach the tips of villi in pigs kept at 35 °C. This time was extended considerably when animals were kept at 10 °C. Energy intake appeared to have only a small effect on enterocyte life span and then only when animals were kept at an environmental temperature of 10 °C. The inability of energy intake to affect enterocyte life span at an environmental temperature of 35 °C reflects the maintenance of an equilibrium existing between cell production rate and crypt–villus size. A similar effect has already been reported for rats fed different diets (Syme & Smith, 1982). The general extension of enterocyte life span at an environmental temperature of 10 °C and the possible additional dependence of enterocyte life span on energy intake at low environmental temperature, were both unexpected findings. The effects these changes had on the time of onset and rate of expression of Na-dependent carrier function are shown in Fig. 4.

Enterocytes produced in the crypts of pigs kept at 35 °C were about 35 h old before they began to absorb alanine through an Na-dependent process. Ten hours later these cells were extruded into the intestinal lumen. Reducing the environmental temperature to 10 °C led to a near doubling of the time needed for enterocytes to reach the stage where they first began to absorb alanine. Alanine-transporting enterocytes were also retained longer on villi obtained from pigs living at 10 °C maintained on a restricted diet (10 and 15 h longer respectively for pigs fed a high and low energy intake). The rate at which individual enterocytes increased their capacity to concentrate alanine

during their final period of migration was not markedly affected by environmental temperature or energy intake ($0{\cdot}24 \pm 0{\cdot}04$, $0{\cdot}18 \pm 0{\cdot}01$, $0{\cdot}20 \pm 0{\cdot}01$ and $0{\cdot}19 \pm 0{\cdot}01$ mM h^{-1} for enterocytes studied in 35H, 35L, 10H and 10L pigs).

DISCUSSION

Energy intake and intestinal function

The finding in the present work that eating smaller amounts of food leads to a decrease in villus height and crypt depth in pig small intestine confirms earlier work on feeding and fasting carried out by a number of investigators in different species.

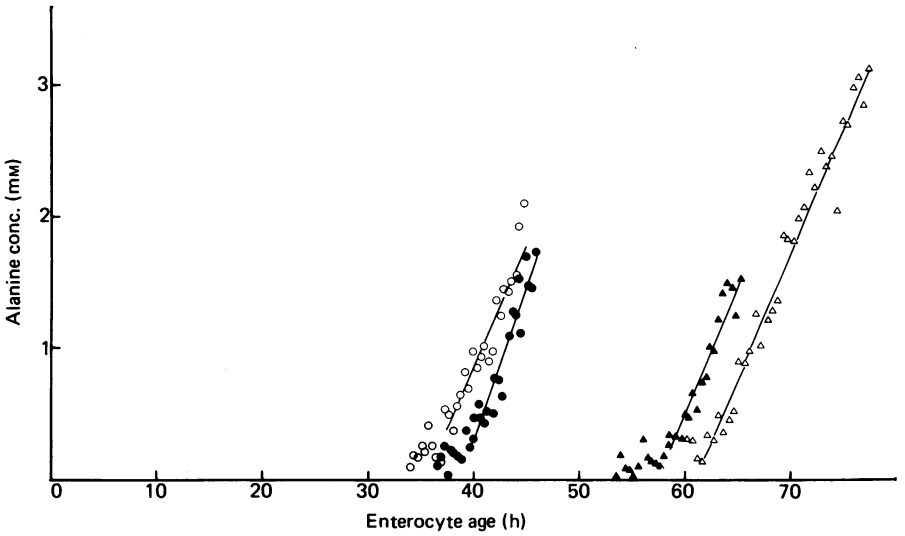

Fig. 4. Relation between enterocyte age and its ability to take up [^{3}H]alanine through an Na-dependent process. The intra-enterocyte concentrations of alanine are those plotted in Fig. 3. The time base was obtained from independent estimates of enterocyte life span given in Table 4. Symbols describing the previous treatment of pigs are as described for Fig. 1. Lines of best fit have been plotted for data obtained in the region of the villus tips.

These effects of fasting on intestinal structure occur rapidly in the rat (McManus & Isselbacher, 1970). Similar effects can be produced, over a longer period of time, by feeding rats different amounts of protein (Syme, 1982; Syme & Smith, 1982). Substitution of parenteral for enteral feeding in rats also leads to a diminution of both villus height and crypt depth (Levine, Deren, Steiger & Zinno, 1974). It is suggested from this type of work that it is the local presence of nutrients within the intestinal tract which is largely responsible for the maintenance of intestinal structure. The present finding that villi from 10H pigs are significantly longer than those from 35H animals (Table 1), even though both receive the same amount of food, raises the possibility that other factors besides luminal nutrition are involved in maintaining villus height. A similar inference has been reached recently from work showing relative differences in jejunal and ileal structure to be maintained over long periods of time in fetal tissue transplanted to the kidney capsule (MacDonald & Ferguson, 1982).

It has also been suggested that the rate of cell production in the crypt might be one of the most important parameters controlling villus height (Clarke, 1974). Again this seems unlikely to provide a full explanation for our present findings. Feeding a high energy intake at 35 °C or 10 °C always caused an increase in crypt depth (an indication that cell production rate has increased) and this was always associated with an increase in villus height. Separate determinations of enterocyte life span, however, show the over-all rate of cell production to be much reduced at low environmental temperature (Table 4). The balance 'set' between the rate of cell production and the height of villi appears, in this case, to be controlled by the environmental temperature at which the animal is kept.

Present work shows the Na-dependent transport of alanine to increase when the intake of energy is reduced. This can be seen indirectly by measuring the effect of alanine on short-circuit current (Fig. 1; Table 2) and directly by analysis of autoradiographs (Fig. 3). Similar effects in other types of cells can be induced by the presence of a wide variety of hormones (Guidotti *et al.* 1978). Of the hormones able to induce this effect glucagon, catecholamines and glucocorticoids are also known to be acting, at times of semi-starvation, to mobilize energy resources. These hormones could be acting, separately or together, to stimulate amino acid absorption across the pig intestine. This action of hormones, to stimulate Na-dependent amino acid transport in other cell types is generally detected as an increase in the number of carriers present (a J_m rather than a K_m effect). This control mechanism appears to be similar to that recorded for the 10L pigs. It is true that the ability of villus tip enterocytes to concentrate alanine through an Na-dependent process *is* increased in intestines taken from 10L pigs, but this difference seems to be due mainly to a prolongation of the time enterocytes stay on the villus. Increase in enterocyte life span enables the cell to extend its capacity to transport alanine through the Na-dependent process (J_m effect), but this takes place without any noticeable change in the rate at which carriers appear in the microvillar membrane (the slopes of the lines shown in Fig. 4 are similar).

The interest of the present work derives from the finding that the Na-dependent transport system is being stimulated selectively, that stimulation can be induced through changes in diet, and that this effect can be enhanced considerably by lowering the environmental temperature of the pig.

Environmental temperature and intestinal function

Placing pigs in the cold causes transient rises in the circulating levels of catecholamines and glucocorticoids (Barrand, Dauncey & Ingram, 1981; Blatchford, Holzbauer, Ingram & Sharman, 1978) and in the over-all rate at which thyroid hormones are utilized (M. Macari, M. J. Dauncey, D. B. Ramsden & D. L. Ingram, unpublished observations), but this does not, by itself, lead to any major change of intestinal structure or function. What *has* been described in the present work is an interaction between temperature and diet. Effects of reduced energy intake are likely to be enhanced at low environmental temperature, since a large part of the available energy is devoted to thermoregulation (Macari, Ingram & Dauncey, 1982). Such an interaction might be explained by the action of hormones which are secreted

in response to the high demand for energy in the cold and which also mobilize reserves when food intake is restricted.

None of the postulated changes in hormone release are likely to explain the marked increase in cell production rate seen to occur at high environmental temperature (Table 4). Neither can one suppose that a difference in rectal temperature of about 1 °C will be sufficient to directly affect the rate of cell production. Gastrointestinal hormones *do* have specific trophic effects on the intestine, but their involvement on this occasion seems unlikely, because their release is normally thought to be associated with feeding and because their effects on cell production are accompanied by changes in intestinal structure. Some appreciation of what actually might be happening to pigs kept at different environmental temperatures comes from comparison of their physical shape. Animals in the warm have extended body lengths and long limbs compared with their litter-mates kept in the cold (Smith *et al.* 1982). This appearance has some similarity to the clinical signs of exaggerated bone growth seen in children suffering from acidophilic adenomas (Daughaday, 1974). It is tempting to suggest that keeping young pigs at a high environmental temperature leads to an increase in the secretion and utilization of growth hormones and that this is responsible for the changes seen to take place in the rate of cell production in the intestine. Such an effect has, in fact, already been reported following injection of growth hormone into hypophysectomized rats (Leblond & Carriere, 1955).

Parts of the present Discussion have been rather speculative. Some excuse for this is provided by the belief that the effects of diet and temperature on transport are likely to be complex and that it is only the end result of several changes taking place which are being recorded. Implicating hormones in the changes produced seems to be warranted, however, and it is this area of research which we intend to study in the future.

We would like to thank Mr I. S. King who was responsible for preparing all the autoradiographs and Mr K. F. Legge for his technical support.

REFERENCES

BARRAND, M. A., DAUNCEY, M. J. & INGRAM, D. L. (1981). Changes in plasma noradrenaline and adrenaline associated with central and peripheral thermal stimuli in the pig. *J. Physiol.* **316**, 139–152.

BLATCHFORD, D., HOLZBAUER, M., INGRAM, D. L. & SHARMAN, D. F. (1978). Responses of the pituitary-adrenal system of the pig to environmental changes and drugs. *Br. J. Pharmac.* **62**, 241–254.

CLARKE, R. M. (1974). Control of intestinal epithelial cell replacement: lack of evidence for a tissue-specific blood-borne factor. *Cell Tissue Kinet.* **7**, 241–250.

DAUGHADAY, W. H. (1974). The adenohypophysis. In *Textbook of Endocrinology*, ed. WILLIAMS, R. H., pp. 31–79. Philadelphia: W. B. Saunders and Company.

DEBNAM, E. S. & LEVIN, R. J. (1976). Influence of specific dietary sugars on the jejunal mechanisms for glucose, galactose, and alpha-methyl glucoside absorption: evidence for multiple sugar carriers. *Gut* **17**, 92–99.

GUIDOTTI, G. G., BORGHETTI, A. F. & GAZZOLA, G. C. (1978). The regulation of amino acid transport in animal cells. *Biochim. biophys. Acta* **515**, 329–366.

HENRIQUES DE JESUS, C. & SMITH, M. W. (1974). Sodium transport by the small intestine of new-born and suckling pigs. *J. Physiol.* **243**, 211–224.

KING, I. S., SEPÚLVEDA, F. V. & SMITH, M. W. (1981). Cellular distribution of neutral and basic amino acid transport systems in rabbit ileal mucosa. *J. Physiol.* **319**, 355–368.

LEBLOND, C. P. & CARRIERE, R. (1955). The effect of growth hormone and thyroxine on the mitotic rate of the intestinal mucosa of the rat. *Endocrinology*, **56**, 261–266.

LEVINE, G. M., DEREN, J. J., STEIGER, E. & ZINNO, R. (1974). Role of oral intake in maintenance of gut mass and disaccharide activity. *Gastroenterology* **67**, 975–982.

MACARI, M., INGRAM, D. L. & DAUNCEY, M. J. (1983). Influence of thermal and nutritional acclimatisation on body temperature and metabolic rate. *Comp. Biochem. Physiol.* **74A**, 549–553.

MACDONALD, T. T. & FERGUSON, A. (1982). Regulation of villus height: the role of luminal factors in determining the villus height gradient of mouse small intestine. In *Mechanisms of Intestinal Adaptation*, ed. ROBINSON, J. W. L., DOWLING, R. H. & RIECKEN, E. O., pp. 47–53. Lancaster: MTP Press.

MCMANUS, J. P. A. & ISSELBACHER, K. J. (1970). Effects of fasting versus feeding on the rat small intestine. Morphological, biochemical and functional differences. *Gastroenterology* **59**, 214–221.

NEALE, R. J. & WISEMAN, G. (1969). The use of dietary-restricted rat intestine for active transport studies. *J. Physiol.* **205**, 159–178.

PATERSON, J. Y. F., SEPÚLVEDA, F. V. & SMITH, M. W. (1979). Two-carrier influx of neutral amino acids into rabbit ileal mucosa. *J. Physiol.* **282**, 73–90.

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*). Stoichiometry versus coupling ratio in the cotransport of Na and different neutral amino acids. *Biochim. biophys. Acta* **603**, 288–297.

PATERSON, J. Y. F., SEPÚLVEDA, F. V. & SMITH, M. W. (1982). Distribution of transported amino acid within rabbit ileal mucosa. *J. Physiol.* **331**, 523–535.

SMITH, M. W., DAUNCEY, M. J. & INGRAM, D. L. (1982). Effect of diet and environmental temperature on amino acid absorption by piglet enterocytes. In *Digestive Physiology in the Pig*, ed. LAPLACE, J. P., CORRING, T. & RERAT, A., pp. 87–97. Les Colloques de l'INRA No. 12.

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.

SYME, G. (1982). The effect of protein-deficient isoenergetic diets on the growth of rat jejunal mucosa. *Br. J. Nutr.* **48**, 25–36.

SYME, G. & LEVIN, R. J. (1977). The effects of hypothyroidism and fasting on electrogenic amino acid transfer: possible evidence for multiple neutral amino acid carrier systems in rat jejunum. *Biochim. biophys. Acta* **464**, 620–628.

SYME, G. & SMITH, M. W. (1982). Intestinal adaptation to protein deficiency. *Cell Biol. Int. Rep.* **6**, 573–578.