metalloporphyrin administration. Induction of heme oxygenase by heavy metals is usually associated with destruction of cytochrome P-450; this can be prevented by simultaneous administration of SKF 525A. This protective effect was ascribed to formation of a complex of SKF 525A with cytochrome P-450[23]. A similar protective effect of metyrapone has recently been described in hepatocyte culture[24]. It is conceivable that cimetidine similarly protects cytochrome P-450 from increased heme oxygenase activity.

Our finding that cimetidine administered chronically induces heme oxygenase is in apparent contrast to the findings of Marcus and co-workers[20]. These authors found reduced activity of this enzyme 30 min after cimetidine administration. This apparent discrepancy is easily resolved when one considers the effect of cimetidine on heme oxygenase activity in vitro (fig. 6). Inhibitory concentrations of cimetidine are easily achieved in the liver (table 2). It is therefore likely that Marcus et al. were seeing a direct inhibitory effect of cimetidine and not depression of enzyme content.

The inhibitory effect of cimetidine on heme oxygenase may explain the lack of an effect of cimetidine on heme turnover in spite of an increase in heme oxygenase activity. The direct inhibitory action of cimetidine may lead to derepression of a feed-back loop and thereby to induction of the enzyme. Inhibitory concentrations of cimetidine are achieved for about 4 h after a single dose of cimetidine (table 2).

In conclusion, cimetidine does not alter hepatic heme catabolism. However, it increases heme oxygenase activity after chronic administration. The lack of an increase in heme breakdown in spite of the increased activity of this enzyme could be due to formation of a protective complex of cimetidine with cytochrome P-450, as postulated for other inhibitors of oxidative drug metabolism[23,24]. Alternatively, it could be due to the inhibitory activity of cimetidine on heme oxygenase activity demonstrated in vitro.

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Short Communications

Starvation-induced changes in the autoradiographic localisation of valine uptake by rat small intestine

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Summary. We report here the effects of a 72-h fast on the localisation of Na-dependent [^{3}H]-valine uptake by rat small intestine. Starvation results in the earlier appearance of valine transport during cell migration and an enhanced accumulation of the amino acid at the villus tip.
Key words. Intestine; adaptation; absorption.

During the course of a day, the intestinal epithelium is exposed to wide fluctuations in the nutrient composition of the intraluminal fluid. Enterocytes making up this cell layer must therefore be capable of a rapid adaptation of their transport properties if homeostasis is to be maintained. Starvation represents an attractive model for the study of the mechanisms involved in intestinal adaptation since the condition is easy to produce in experimental animals and the effects on nutrient uptake have a rapid onset[3]. While much is known concerning changes in villus morphology following the removal of food[4], no information is available concerning the effects of starvation on the development of transport activity during cell migration along the villus. In order to address this problem, we have performed autoradiography on intestine from either fed or fasted rats to determine the cellular location of amino acid uptake. Valine was chosen as a probe for the study since this is an essential amino acid in this animal.

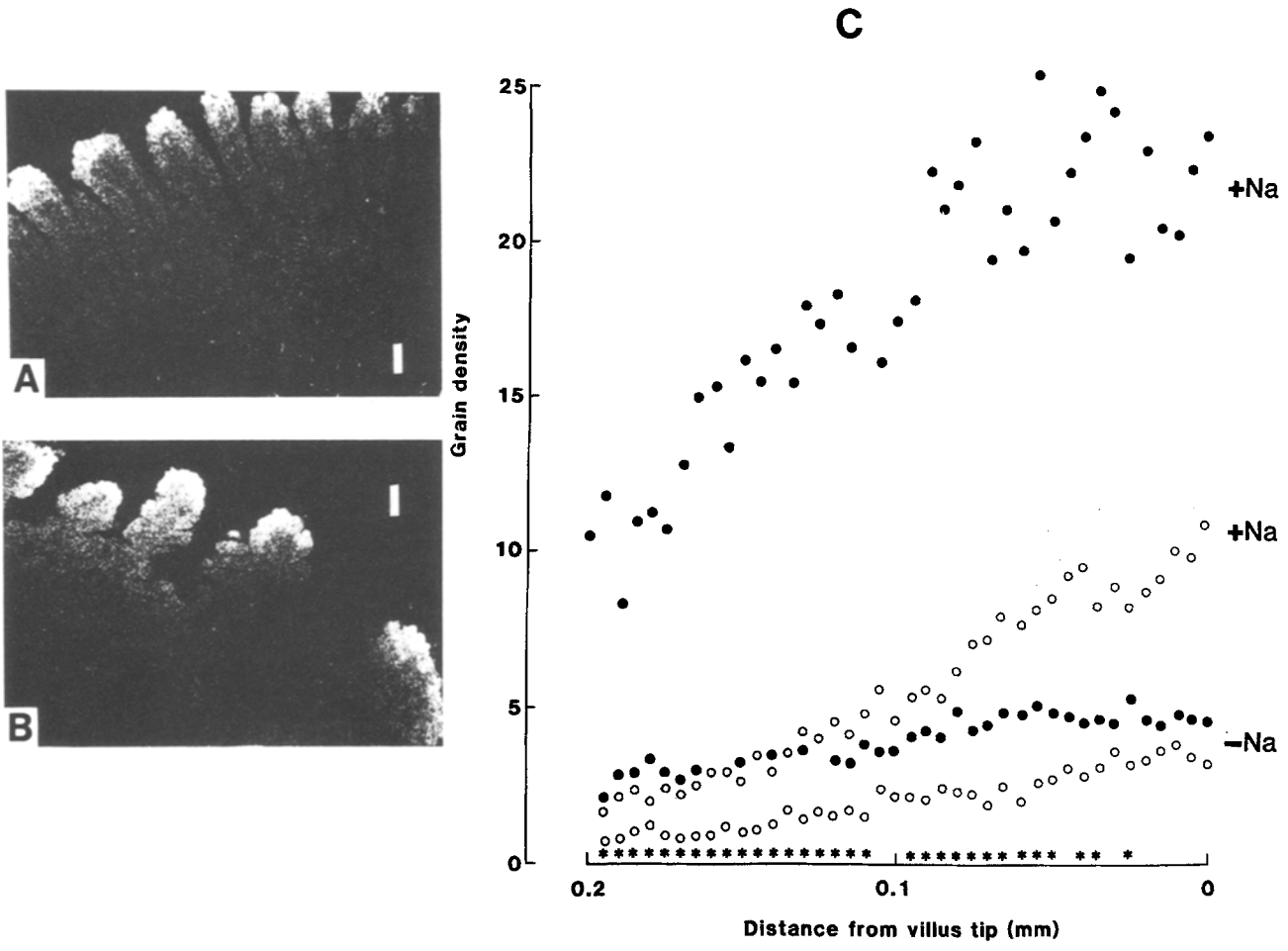

Figure 1. *A* and *B* Sections of jejunum from fed and 72-h fasted rats respectively showing silver grain deposits resulting from [^{3}H] valine uptake. Tissue was incubated in Na-loaded buffer. Vertical scale bar represents 100 µm. *C* Grain density as a function of distance from the villus tip under Na-free and Na-loaded (143 mM) conditions. Open circles represent the fed condition and solid circles the fasting state. The number of villi/animals used to calculate the mean values were as follows: Fed (+Na) 28 villi from 13 animals; Fed (−Na) 13 villi from 5 animals; Fasted (+Na) 12 villi from 7 animals; Fasted (−Na) 16 villi from 6 animals. *denotes significant difference between fed and fasted conditions under Na-free conditions.

Materials and methods. Male, Sprague-Dawley rats (230–250 g) were either maintained on Diet 41B (Grain Harvester, UK) up to the time of experimentation or fasted for 72 h. Both groups were allowed free access to water. All animals were housed individually in suspended wire bottom cages to minimize coprophagy. Animals were anaesthetised with pentobarbitone sodium (90 mg/kg; i.p.) and intestinal segments (3–4 cm in length) were taken at points 10–15 cm from the ligament of Trietz (designated jejunum) and 10–15 cm proximal to the ileocaecal junction (designated ileum). The incubation and autoradiographic methods used have been described previously[5]. Tissue sections were washed through with cold saline (154 mM), opened longitudinally and clamped, mucosal side uppermost, between two halves of a superfusion chamber. The mucosal surface was preincubated at 37 °C for 10 min with bicarbonate saline[6] made Na-free by the substitution of choline chloride, choline bicarbonate and KH_2PO_4 for NaCl, $NaHCO_3$ and NaH_2PO_4 respectively. Incubation solutions were equilibrated with 95% O_2:5% CO_2 and fed by gravity from a reservoir and removed by suction. At the end of the preincubation period, the chamber was drained and the mucosa exposed to either Na-free buffer containing 10 mM L-valine (Sigma UK Ltd) and 270 µCi/ml L-[3,4]^{3}H valine (Amersham International) or Na-loaded (143 mM) buffer containing 10 mM L-valine and 90 µCi/ml of the labelled amino acid. Stirring of the solutions at 700 rpm during the 45-s incubation period ensured immediate and continual contact of all parts of the villus with the medium. Phosphate buffered saline (pH 7.3) containing 4% v/v glutaraldehyde and 2% w/v sucrose was used to remove extracellular valine and link the transported amino acid to intracellular protein at the end of incubation. Tissue sections were transferred to fresh glutaraldehyde-containing medium for 2 h before being washed twice at 4 °C, over a period of 24 h in phosphate buffered saline to remove excess fixative. The tissue was then dehydrated by passing through ascending grades of ethanol and embedded in glycomethacrylate[7]. Histological sections (3 µm) were mounted on well-cleaned microscope slides, coated with Ilford K2 photographic emulsion containing 2% glycerol and stored for 12 days at 4 °C in the dark. Slides were developed in D-19 (Kodak Ltd) and mounted in Gurr's Xam medium.

A microdensitometer (Model M85 Vickers Instruments, UK) was used to quantify grain density on the developed slides. Scanning was carried out at 1000 × magnification and a wavelength of 650 nm moving in discrete steps of 5 µm beginning at the villus tip. Optical density was recorded at 36–40 sequential points covering a total villus length of 180–200 µm. Readings were limited to a maximum of three villi in tissue sections from any one animal.

For measurements of villus height, 1-cm sections of jejunum and ileum were removed, washed through with NaCl (154 mM) and placed in 10% formol saline. The tissue was later blocked in paraffin wax, sectioned longitudinally (5 µm) and stained with

Haemotoxylin and Eosin. Villus height was determined using a microscope fitted with a projection attachment (final magnification, × 500). The use of a graticule allowed prior calibration of the projection area.

Results are expressed as mean ± SE of the mean. Figures in parentheses indicate the number of villi used to achieve the group mean. Differences between means were evaluated by a Students t-test for unpaired data and considered not significant at $p > 0.05$.

Results. Na-dependent valine transport by jejunum from fed rats could first be detected at a point 160 µm from the villus tip (figs 1A and 1C). Reference to the values for villus height given in the table implies that this distance represents the top 37% of the villus structure. Once expressed, uptake increased rapidly until the enterocyte reached the villus tip (fig. 1C). Interestingly, starvation for 72 h resulted in the earlier appearance of valine uptake during enterocyte transit along the villus. Na-dependent uptake could now be detected at a point in excess of 200 µm from the villus tip (figs 1B and 1C). Since starvation results in a decreased villus height in the jejunum (table) it can be calculated that, following starvation, the zone of active valine uptake is considerably expanded and is equivalent to a minimum of 56% of the upper villus length. Starvation also caused an increased Na-dependent valine uptake in the upper villus (fig. 1C). Values obtained at the villus tip, for example, were 10.75 ± 0.94(28) and 23.19 ± 3.22(12) for sections from fed and fasted animals respectively ($p < 0.001$). Incubation of jejunum from fed rats in a Na-free buffer resulted in a reduced uptake of valine at all levels of the villus, maximum grain density being 3.21 ± 0.59(13). Although starvation was without effect on maximum grain density at the villus tip under these Na-free conditions (4.47 ± 0.62(16); $p > 0.1$), consistent differences were recorded lower down the villus (fig. 1C).

Effect of 72 h starvation on villus height. Results are given as mean ± SE of the mean with number of observations in parentheses

	Villus height (µm) Jejunum	Ileum
Fed	432.0 ± 6.2 (100)	277.8 ± 5.5 (107)
72 h starvation	358.4 ± 4.2 (109)	269.4 ± 8.7 (78)
p	< 0.001	> 0.3 < 0.4

Analysis of data obtained from the ileum gave similar results to those from the jejunum. Thus, in the presence of Na, fasting was associated with an increase of some 84.7% in maximal grain density at the villus tip (fed: 19.17 ± 3.76(11); fasted: 35.40 ± 6.13(11), $p < 0.05$ (fig. 2C)). For tissue incubated under Na-free conditions, starvation was without effect on maximum grain density at all points along the villus. In contrast to the jejunum, villus height in the ileum was very similar in fed and 72 h starved conditions.

Discussion. The positional dependence of active valine uptake in the fed condition is clearly evident from the data shown in figures 1C and 2C. Other amino acids have been shown to be similarly handled by the small intestine of the rat[8] and other mammalian species[5,9]. Interestingly, active uptake by the entire enterocyte population has been noted using intestine from the newborn pig[9]. Our study is the first to report the effect of starva-

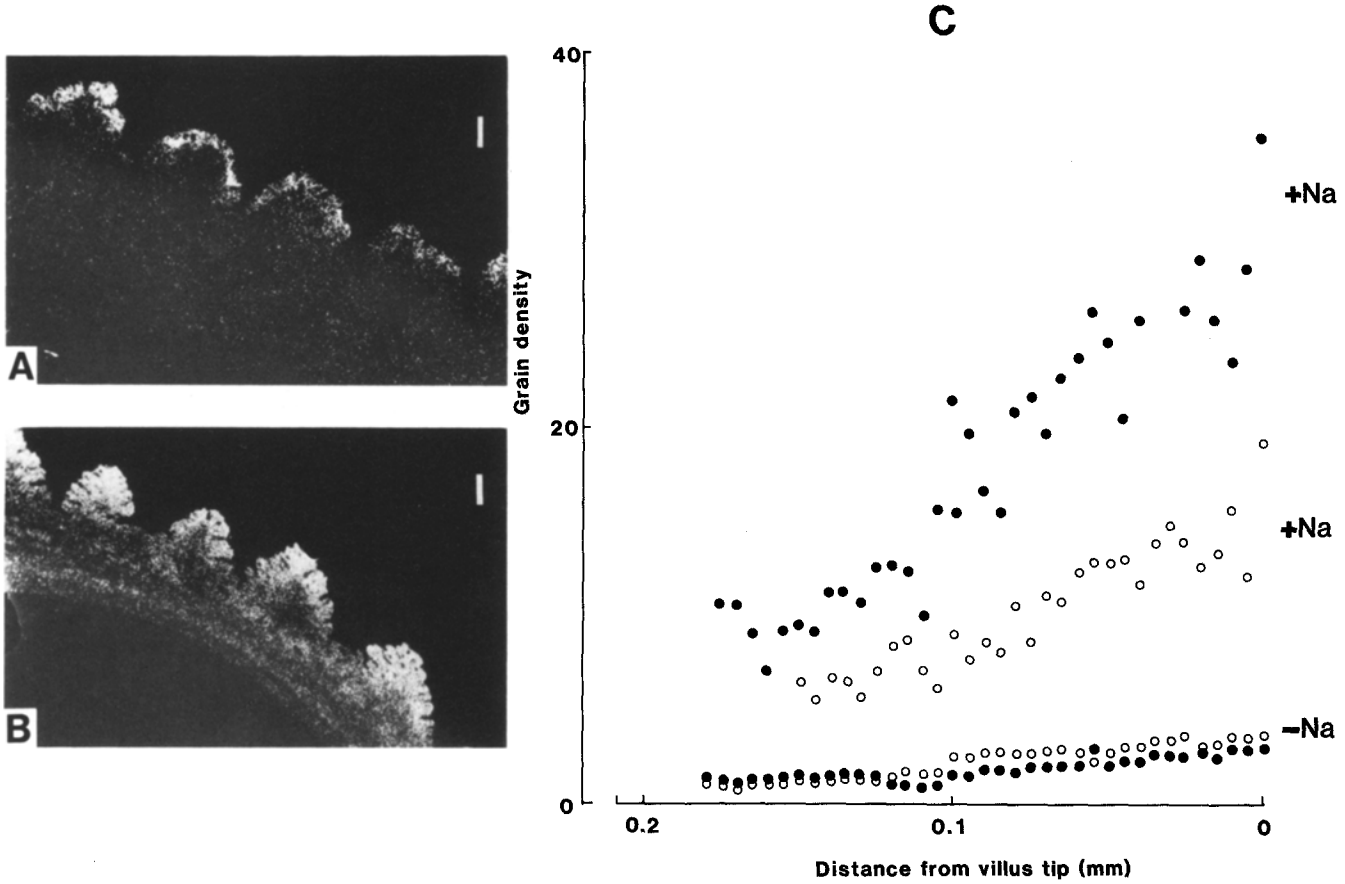

Figure 2. *A* and *B* Sections of ileum from fed and 72-h fasted rats respectively showing silver grain deposits resulting from [^{3}H] valine uptake. Tissue was incubated in Na-loaded buffer. Vertical scale bar represents 100 µm. *C* Grain density as a function of distance from the villus tip under Na-free and Na-loaded (143 mM) conditions. Open circles show the fed condition and solid circles the fasting state. The number of villi/animals used to calculate the mean values were as follows: Fed (+Na) 11 villi from 4 animals; Fed (−Na) 12 villi from 4 animals; Fasted (+Na) 11 villi from 5 animals; Fasted (−Na) 15 villi from 5 animals.

tion on the expression of Na-dependent amino acid accumulation by mammalian small intestine. Valine uptake displayed a high Na-dependency in intestine from both fed and 72 h fasted rats. Uptake under Na-free conditions was, however, consistently higher in the jejunum but not the ileum following starvation. At present, we have no explanation for this regional difference.

The membrane events responsible for these dietary-induced changes in valine accumulation are unresolved. One possible explanation comes from studies of the potential difference across the brush border membrane (V_m) which is an important component of the total driving force for Na-linked amino acid uptake into enterocytes[10].

The process of cell movement along the villus is associated with marked changes in V_m, the value being highest at the villus tip[11]. This hyperpolarisation may be related to the structural and functional differentiation which occurs during enterocyte migration since both an elongation of individual microvilli[12] and a raised intracellular K concentration[13] have been observed during the initial phase of enterocyte development. Recently we reported that starvation results in a hyperpolarisation of the microvillus membrane of both the jejunum and ileum[14]. The enhanced valine accumulation shown in figures 1 and 2 powered by the increased membrane potential difference may, in turn, be due to a slower cell turnover time and reduced transit along the villus[15]. Lengthening the residence time would result presumably in a population of more mature cells at the villus tip.

The systemic and/or luminal factors responsible for the changes in active valine accumulation are unknown. It is of interest, however, that several studies have implicated pancreatic glucagon in the adaptation to transport following starvation[16–18]. In addition, recent work has shown that the chronic administration of this hormone to rats induces a hyperpolarisation of the microvillus membrane[19]. Preliminary observations on the effect of glucagon on the localisation of [^{3}H] valine uptake during enterocyte migration reveal striking similarities to the changes reported in this present study, i.e. valine uptake occurs earlier during enterocyte development resulting in a significantly higher maximum grain density at the villus tip[19]. It is therefore possible that the increased secretion rate of the hormone during fasting[20] is an important factor in the adaptation of valine transport.

In conclusion, it appears that starvation results in an enlarged functional absorptive surface area and an increased ability of individual enterocytes to accumulate valine. This adaptation may compensate, at least in part, for the reduced anatomical surface area which has been observed during fasting[4].

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Effects of storage and processing on the nutritive value of certain Nigerian foods

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Summary. The effects of storage and processing on the nutritive value of two Nigerian foods – raw cowpea *(Vigna unguiculata)* flour and palm oil, have been investigated. Increased retention of both thiamine and riboflavin as a result of increased water activity (A_w) were recorded for cowpea flour stored for 6 months. A storage temperature of 5 °C led to minimal losses of both vitamins in the stored flour. Differences in physico-chemical characteristics were observed between palm oil produced by the traditional method and that produced by a modern commercial method. Increasing A_w led to decreasing loss of unsaturation in the traditionally-produced palm oil during 4-week storage at ambient temperature (25 °C).

Key words. Palm oil; raw cowpea flour; storage; nutritive value.

The problem of inadequate food supply, especially in the developing countries, is related not only to the total output of raw agricultural production but also to post-harvest factors such as processing and storage. These two post-harvest factors must be taken into consideration in evaluating the adequacy (in terms of total quantity and nutritional wholesomeness) of food supplies in developing countries like Nigeria.

Although cowpea *(Vigna unguiculata)* is consumed, primarily, for its high protein content[9], it could also act as a good source of some vitamins. Accordingly, the influence of water activity[6] and of temperature on the thiamine and riboflavin contents of the stored raw flour, prepared from the seeds, was investigated.

The traditionally-produced palm oil marketed in the Nigerian traditional open markets is used by a large segment of the population, probably for reasons of cost. However, it would appear that no comparative studies have yet been undertaken to compare the physico-chemical quality attributes of the traditionally-produced palm oil with those of palm oil produced by modern commercial methods. The present studies address this problem. The differences in atmospheric humidity under which palm oil is