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# THE DIFFERENTIAL EFFECTS OF TWO SALTS OF 5-FLUOROURACIL AND OF DIETARY RESTRICTION ON ABSORPTION BY RAT SMALL INTESTINE

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**Abstract**—Water absorption, measured *in vitro* in the perfused rat small intestine, following injection of the Tris or sodium salt of 5-fluorouracil (5FU) has been compared with that following a food restriction regime which mimicked the food intake pattern of rats after injection of 5FU. Water absorption rates were decreased both after administration of 5FU and after food restriction but the decrease following 5FU injection was much greater. Also, the Tris and sodium salts of 5FU had different effects on the food intake pattern after injection but had very similar effects on intestinal absorption of water. Hence the effects of 5FU on absorption cannot wholly be attributed to a reduced food intake. Four reference systems have been used to express water absorption rates: whole small intestine, unit length of intestine, intestinal dry weight and mucosal DNA content. The importance of the choice of reference system is discussed. Due to the heterogeneity of the mucosal cell population the use of DNA and dry weight as reference systems could not give an unequivocal indication of whether the changes in absorption rates resulted from a change in the number of absorbing cells or from a change in the absorptive capacity of each absorbing cell. Severe systemic toxicity, including death, was observed after injection of the Tris salt of 5FU, but not after injection of the sodium salt.

Chemotherapy with antimetabolites such as 5-fluorouracil is often restricted by toxic side effects, especially those affecting the rapidly proliferating tissues including the gastrointestinal tract [1]. Severe impairment of intestinal absorption after administration of 5FU has already been shown in the rat [2–4]; alteration of intestinal enzyme activities has also been shown both in rat and man [3–6]. Although these effects may be directly ascribed to the 5FU it must be noted that 5FU administration to rats is followed by a severe reduction in food intake [4, 5] and that food restriction *per se* is known to cause changes in the absorptive and enzymic activities of the small intestine [7–18]. However, the nature of these changes is unclear since the literature on the effects of starvation on the intestine contains much apparently conflicting information: this may be due, in part, to the use of different reference systems [8, 19].

Therefore we have now measured the absorption of water *in vitro* in the small intestine from rats, fed *ad lib*, whose food intake and body weights were monitored daily before and for 3 days after an injection of 5FU. We have also measured intestinal absorption and body weights of uninjected rats whose food intakes were restricted to the extent observed in the 5FU-injected animals. Since changes in absorption rates may result from (a) a change in the number of absorbing cells, (b) a change in the absorptive capacity of each absorbing cell, or (c) a combination of these, we have measured the mucosal DNA content as an index of changes in total cell numbers, although this of course is not specific for absorbing cells. In order to characterise the changes in absorption rates as a result of food restriction and to establish the effect of the reference system we have also used three other reference systems: whole small intestine, unit length of intestine and intestinal dry weight.

Since our preliminary observations indicated that the Tris and sodium salts of 5FU produce markedly different mortality and systemic toxicity we compared the effects of both salts on intestinal absorption. Although they have different effects on the animals' food intakes, they produce almost identical effects on intestinal absorption, and so can be used as a further means of dissociating the effects of the drug from those of food restriction.

## MATERIALS AND METHODS

*Animals.* Male and female rats of a local Wistar strain and weighing 160–200 g were used. They had been kept in conditions of controlled day-length with free access to water and Oxoid Diet 86 (Oxoid Ltd., Basingstoke, Hants., U.K.) for at least a week before use.

*5-fluorouracil treatment.* Rats were weighed and, under light ether anaesthesia, injected i.p. with a sterile solution of 5FU (Roche Products Ltd.) as either the Tris (384 μmoles/ml) or the sodium (192 μmoles/ml) salt. The mean dose was 1.44 mmoles/kg body weight. Some rats received an injection of Tris placebo (Roche Products Ltd.). All injections were given between 11:00 and 11:30 hr British Summer Time (BST). Body weights and food intakes were recorded at 11:00 BST each day.

*Dietary restriction.* The daily food intake of unin-

jected rats was restricted to that observed on each of the first three days following 5FU injection (see Fig. 1). Some rats were fasted for 24 hr and then taken for immediate experiment. Others were fasted for 48 hr and then caged individually and allowed 5.4 g of food in the next 24 hr. The rats had free access to water and the bedding was changed daily to minimise coprophagy. All rats were weighed daily at about 11:00 BST.

*Intestinal perfusion* in vitro. The 'segmented-flow' technique for single-pass luminal perfusion, and the apparatus, were as detailed by Fisher and Gardner [20]. Jejunum plus ileum from the ligament of Treitz to the ileo-caecal valve was rinsed with oxygenated $NaCl/NaHCO_3$ solution at 38° and connected to the perfusion apparatus while the rat was maintained under light ether anaesthesia. The intestine was removed and the animal killed only after complete luminal perfusion had been established since neglect of this leads to decreased absorption rates [21] and increased release or leakage of cellular peptidases [22]. The perfusion medium was the modified Krebs–Henseleit bicarbonate solution (pH 7.4) equilibrated with $CO_2 + O_2$ (5:95) at 38° used by Fisher and Gardner [20]. It contained glucose (28 mmoles/l and phenol red (141 nmoles/ml; 50 μg/ml); the latter serves to check for the presence of 'leaks'. Water absorption rates were determined directly from the weight of fluid secreted on to the serosal surface of the intestine during three consecutive 5 min periods [see 20].

*DNA estimation.* Since there is a small loss (approx. 16% per hour) of mucosal DNA during luminal perfusion [22], DNA contents were measured on unperfused intestines. Hence estimates of both absorption rates and DNA content were not available for the same intestine. The intestine was rinsed with oxygenated $NaCl/NaHCO_3$ solution at 38° and removed under light ether anaesthesia.

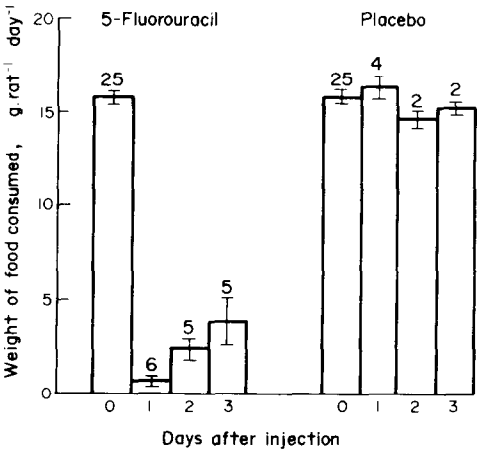


Fig. 1. Daily food intakes of female rats during the first 3 days following 5FU (Tris salt) or Tris placebo injection and of control rats. Values are mean ± S.E.M. and the number of observations on groups of 8 rats is shown above the bars.

Immediately, the mucosa was scraped on a dry glass sheet over ice in a cold room and homogenised with 7.5 ml of chilled NaCl solution (154 mmoles/l). DNA in the homogenate was determined by the ethidium bromide fluorescence method of LePecq and Paoletti [23] as modified by Karsten and Wollenberger [24] except that the RNAase was used at 20 mg/ml, the incubation time at 37° was extended to 1 hr, and all volumes were doubled. Samples were assayed in duplicate at each of three dilutions 1:80, 1:160 and 1:320. A standard curve was set up daily with freshly prepared calf thymus DNA (Sigma London Chemical Co.).

*Dry weight.* After perfusion the intestine was dried to constant weight at 75°.

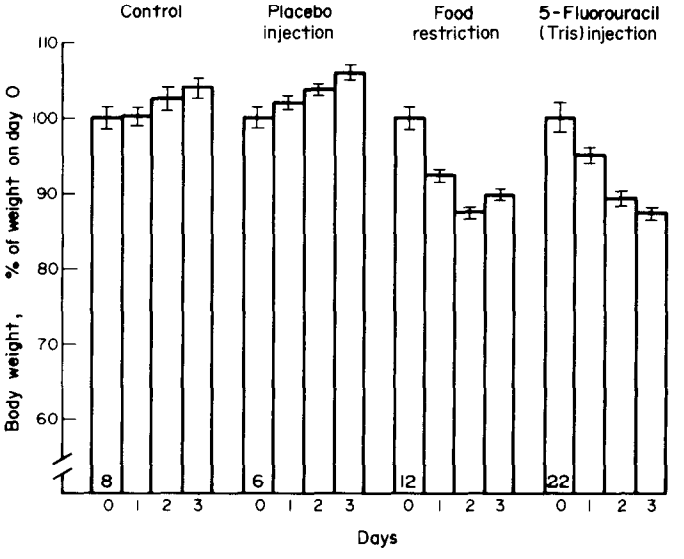


Fig. 2. Body weights of control female rats and on each of the first 3 days following Tris placebo injection, food restriction and 5FU (Tris salt) injection. Values are the means ± S.E.M. of the weights expressed as a percentage of the body weight on the day of treatment or the first day of food restriction (Day 0). Numbers of observations, each on a single animal, are shown within the bars for Day 0.

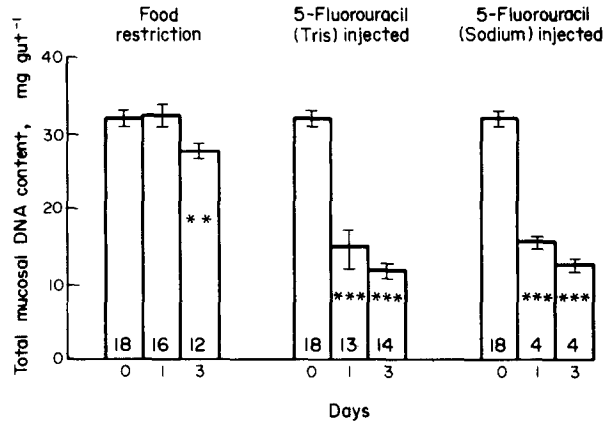


Fig. 3. DNA content of the mucosa from the whole jejunum plus ileum from control female rats, and on the first and third days of the food restriction regime and following 5FU (Tris or sodium salts) injection. Values are means ± S.E.M. and the numbers of intestines are shown within the bars. The significance levels of the differences from values in control rats are indicated thus: *** $P < 0.001$, ** $P < 0.02$, N.S. $P > 0.05$.

## RESULTS

### *Effects of 5-fluorouracil (Tris salt) on food intake*

Figure 1 shows the food intake of rats over each day after injection of the Tris salt of 5FU. During the first 24 hours the intake was only 5 per cent of the normal daily intake. By the third day it had risen to 25 per cent of that of control rats. However, injection of the Tris placebo had no effect on the daily food intake (Fig. 1).

### *Effects of 5-fluorouracil (Tris salt) and food restriction on body weight*

Figure 2 shows that the body weight decreased both after 5FU treatment and after the food restriction regime. The decrease following food restriction was slightly greater over the first two days than that after 5FU treatment ($P < 0.02$). The increased food intake between the second and third day resulted in an increase in the body weight of the food restricted rats; in contrast, the body weight of the 5FU-treated rats continued to decrease over this period.

### *Effects of 5-fluorouracil (Tris and sodium salts) and food restriction on mucosal DNA content*

One day after administration of either the Tris or sodium salt of 5FU the mucosal DNA content had decreased to 47 per cent of the normal content; in contrast, food deprivation for 1 day had no effect

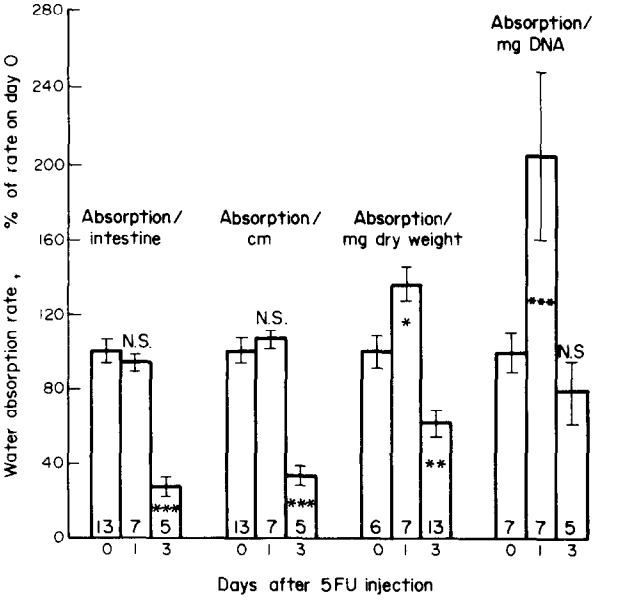


Fig. 4. Water absorption rates in jejunum plus ileum from female control rats and from female rats which had received 5FU (Tris salt) 1 and 3 days previously. Rates are expressed as a percentage of control (Day 0) rates using four different reference systems: whole small intestine, cm length of intestine, mg dry weight of intestine and mg mucosal DNA content. Values are means ± S.E.M. and the numbers of intestines are shown within the bars. The significance levels of the differences from the values in control rats are indicated thus: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.02$, N.S. $P > 0.05$.

on the mucosal DNA content (Fig. 3). By the third day after 5FU treatment the DNA content had decreased to 38 per cent of the control value; food restriction for 3 days resulted in a much smaller, but significant ($P < 0.02$), decrease in the mucosal DNA content to 86 per cent of the control value.

*Effects of 5-fluorouracil (Tris salt) and food restriction on water absorption*

The rates of water absorption after injection of 5FU or after food restriction were determined: these rates have been expressed relative to (a) total jejunum plus ileum, (b) unit length of intestine, (c) unit dry weight of whole small intestine and (d) mucosal DNA (Figs. 4 and 5). There was no significant change in water absorption rates expressed per whole small intestine or per unit length of intestine 1 day after 5FU treatment; by the third day after 5FU injection they had decreased to 30 per cent of control values. On the other hand when the absorption rates were expressed relative to total intestinal dry weight or to mucosal DNA a dramatic increase was observed after 1 day, and by the third day the absorption rates had decreased to 70–80 per cent of control values (Fig. 4). However, injection of a Tris placebo had no effect on the water absorption rates (Table 1).

After food deprivation for 1 or 3 days the water absorption rate expressed per whole small intestine was decreased to approximately 70 per cent of the control rate (Fig. 5). The decreased absorption rate was not quite significant when any of the other three reference systems were used.

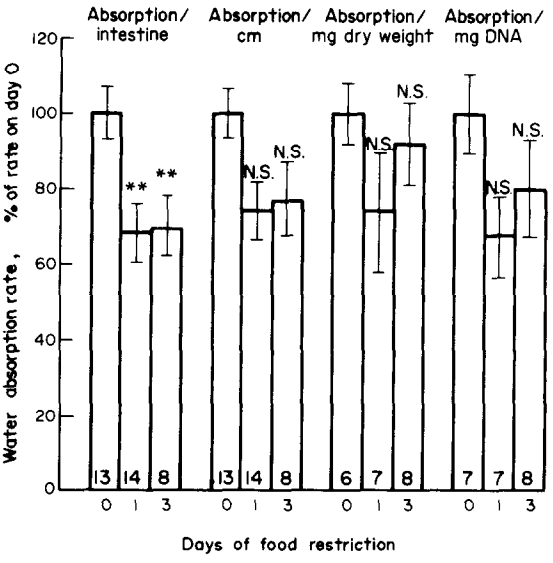


Fig. 5. Water absorption rates in jejunum plus ileum from control female rats and from female rats following food deprivation for 1 day or food restriction for 3 days. Rates are expressed as a percentage of control rates using each of four different reference systems: whole small intestine, cm length of intestine, mg dry weight of intestine and mg mucosal DNA content. Values are means ± S.E.M. and the numbers of intestines are shown within the bars. The significance levels of the differences from the values in control rats are indicated thus: ** $P < 0.02$, N.S. $P > 0.05$.

*Comparison of the Tris and sodium salts of 5-fluorouracil*

Within 3–4 hours of injection of the Tris salt of 5FU there were signs of severe systemic toxicity: the animals were cold and lethargic, and many showed obvious respiratory distress. Some died (16% out of a total of 260 rats injected) within 24 hr. In contrast, no such signs of toxicity were apparent after injection with the sodium salt of 5FU at the same dose and, after injection of 350 rats, we have not recorded a single death.

A marked difference was observed between the effects of the Tris and sodium salts of 5FU on both food intakes and body weights (Fig. 6). After injection of the Tris salt the food intake decreased to only 7 per cent of the normal intake in the first 24 hr (Fig. 6A). A gradual daily increase in the food intakes followed so that by the third day the total food intake, since treatment, was only 11 per cent of the normal intake over the same period. This contrasts with a value of 34 per cent when the sodium salt of 5FU was injected. After injection of the sodium salt the food intake in the first 24 hr decreased to 50 per cent of the normal intake; it continued to decrease and had fallen to 7 per cent of the normal daily intake only by the third day.

These differences in food intake were also reflected in significant differences in the effects of the two salts of 5FU on the rats' body weights (Fig. 6B): at each day studied the rats which had received

Table 1. Water absorption rates in control intestines from female rats and 1 and 3 days after injection of a matched Tris placebo*

| | Absorption per intestine (%) | Absorption per cm (%) | Absorption per mg dry weight (%) |
|---|---|---|---|
| Control | 100 ± 6.87 (13) | 100 ± 6.50 (13) | 100 ± 7.87 (6) |
| Day 1 | 99.67 ± 7.20 (7) | 113.55 ± 12.18 (7) | 101.37 ± 8.19 (7) |
| Day 3 | 99.20 ± 5.56 (13) | 107.28 ± 12.57 (13) | 109.40 ± 7.47 (13) |

* Absorption rates are expressed as a mean percentage (±S.E.M.) of control rates in uninjected animals, and the numbers of intestines are shown in parentheses. No values are significantly different from 100%.

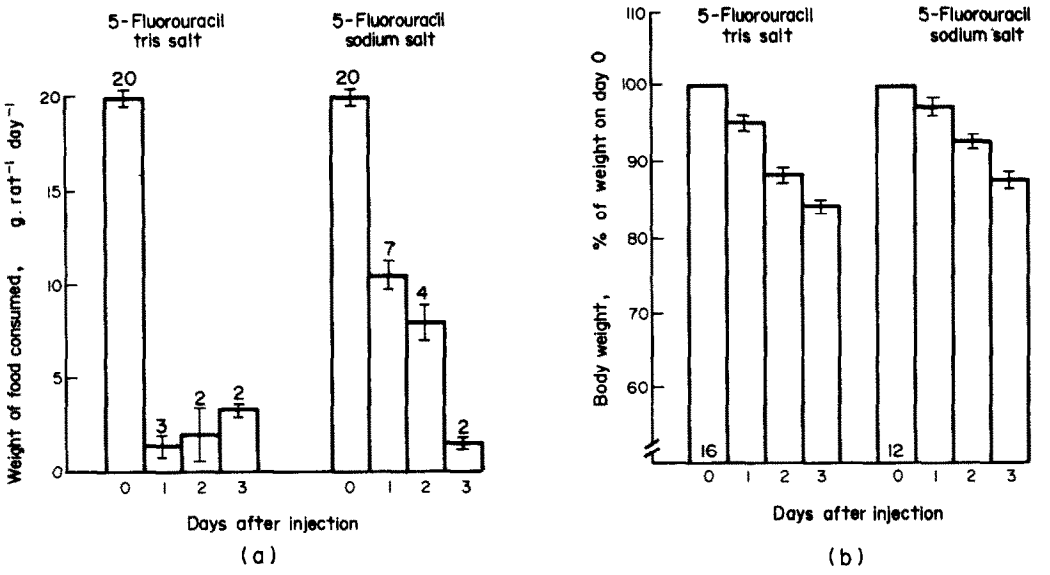


Fig. 6. (a) Daily food intakes of male rats during each of the first 3 days following injection of the Tris and sodium salts of 5FU and of control rats. Values are means ± S.E.M. and the number of observations on groups of 8 rats is shown within the bars. (b) Body weights of male rats on each of the first 3 days following injection of the Tris and sodium salts of 5FU and of control rats. Values are means ± S.E.M. of the weights expressed as a percentage of the body weight on the day of injection (Day 0). Numbers of observations, each on a single animal, are shown within the bars for Day 0.

the sodium salt had lost less weight than those injected with the Tris salt ($P < 0.02$).

In contrast, however, the intestinal effects of the two salts were indistinguishable. Water absorption rates on each of the first 5 days after injection were affected to the same extent with both salts (Fig. 7). Also we noted that the incidence of diarrhoea was independent of the salt of 5FU used. The two salts had identical effects on the mucosal DNA content (Fig. 3).

It should be noted that the results in Figs 6 and 7 were obtained from male rats: all the preceding experiments were performed on female animals. Since the control (uninjected) animals had different food intakes and growth rates between the sexes (Figs 1 and 6a; and Figs 2 and 6b, respectively) all comparisons should be made between like sexes.

## DISCUSSION

Our results extend those of earlier studies which showed that 5FU administration was followed by biochemical and physiological impairment of intes-

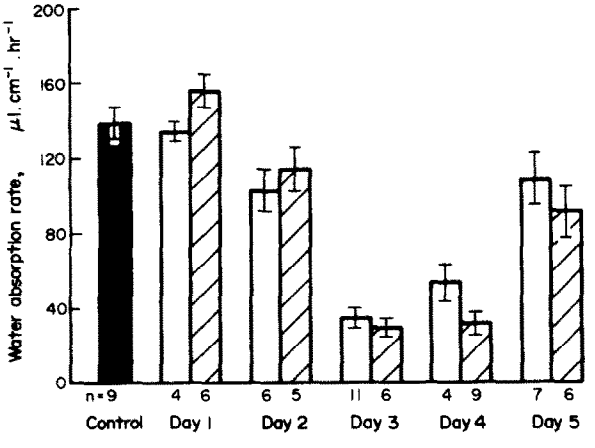


Fig. 7. Water absorption rates in jejunum plus ileum from male rats on each of the first 5 days following injection of Tris-salt (open bars) and sodium-salt (hatched bars) of 5FU and in control rats (solid bar). Values are means ± S.E.M. and the number of intestines is shown below each bar.

tinal function; in particular they show clearly several fundamental differences between the changes in absorption rates following 5FU injection and those after a food restriction regime designed to mimic the animals' food intake after 5FU injection.

Water absorption by the whole small intestine is greatly decreased after 5FU injection (Fig. 4). However the reduced food intake by itself results in a far smaller, although significant ($P < 0.02$), decrease in water absorption (Fig. 5). Hence the effects of 5FU on absorption cannot wholly be attributed to food restriction. This result agrees with Levin's [2] observation of decreased absorption of glucose, galactose and fructose in fasted rats after 5FU injection when compared with fasted controls. Also, by using both Tris and sodium salts of 5FU, which have different effects on the food intake pattern of rats after injection (Fig. 6) we have demonstrated further the difference between the effects of 5FU on absorption and the effects of the consequent reduced food intake on absorption. The intestinal toxicity of the two salts is very similar (Fig. 7) and they have identical effects on the mucosal DNA content (Fig. 3) although the overall food intake during the 3 days after injection of the sodium salt of 5FU is about 3 times that after injection of the Tris salt.

It must be noted that it is not practicable to mimic exactly the food intake pattern of the 5FU treated rats. While we can control the overall food intake we cannot easily control the hour-to-hour food intake of the rats. For example, when the food intake of rats is restricted all the food presented on the second day is eaten immediately, thus leaving a fast of at least 12 hours before perfusion on the third day. However this reservation is irrelevant with regard to the first day after injection of the Tris salt of 5FU since the food intake is negligible. A 24 hour fast has considerable effects on absorption; water absorption by the whole small intestine is decreased by 32 per cent of control values (Fig. 5). This indicates a potential hazard in the practice of routinely preparing animals for absorption experiments by an overnight fast [13, 15, 25], especially since this, for a nocturnal feeder, is equivalent to a 24 hour fast.

A change in absorption rate could be the result of a change in the number of absorbing cells or of a change in the absorbing capacity of each absorbing cell. Batt and Peters have therefore suggested that absorption rates should be expressed per enterocyte, estimated by mucosal DNA measurement, in order to differentiate between these two possibilities [26]. We find that, although there is no effect on the absorption rate by the whole small intestine on the first day after 5FU injection, the absorption rate expressed per mg DNA is increased by 100 per cent (Fig. 4A). Roche *et al.* [27] also reported a change in cell numbers with no change in the absorption rate, expressed per unit length of small intestine, 1 day after 5FU injection. Since 5FU inhibits DNA synthesis and therefore prevents crypt cell division in the intestinal epithelium, the 54 per cent decrease in the mucosal DNA content 1 day after injection probably represents a reduced number of crypt and immature villous cells (i.e. non-absorbing cells) with, at this stage, little or no change in the number of mature absorbing cells at the tip of the villi. Since DNA is not a specific index of the number of absorbing cells it is not possible to use it in a study of the effects of an antimetabolite on a heterogeneous population of cells to determine whether the absorptive capacity of individual absorbing cells has been altered. The DNA content is, of course, a good index of the *total* cell population.

We find that the absorption rate relative to dry weight is significantly less on the third day after 5FU injection than the control rate and less than that on the third day of food restriction. This result contrasts with that of Levin [2] on starved rats but we stress that this does not unequivocally indicate that the decreased absorption rate on the third day after 5FU injection is a result of a decrease in the absorptive capacity of individual cells as well as a decrease in the number of absorbing cells. The reservations already discussed with respect to DNA as a reference basis also apply to the use of dry weight, and prevent the solution of this problem.

Throughout this work we have used four different reference systems for the expression of absorption rates; our results illustrate clearly how this choice affects both the quantitative and qualitative pattern of results. Levin *et al.* [12] also showed that the direction of change of enzyme activities as a result of starvation can even be reversed by use of a different reference system. Food restriction or starvation can cause a decrease in absorption rates when referred to the whole or a specific length of the small intestine or, in contrast, an increase when expressed on a weight basis [7, 10, 14, 16]. The possibility of obtaining misleading results when absorption rates are expressed on a weight basis has been discussed elsewhere [14, 28]; we agree since food restriction for 3 days reduces the water absorption rate by the whole small intestine or per unit length of intestine, although no significant change is seen when the rates are referred to the dry weight of the intestine (Fig. 5).

Although we have used water absorption rates as an index of absorptive capacity it is probable that identical results would have been obtained if we had measured glucose absorption since a very strong positive correlation was previously observed between glucose and water absorption in control and 5FU treated rats (Ref. [3], p. 415).

The differences in food intake shown in Fig. 6a and signs of systemic toxicity (including death) following injection of the two different salts of 5FU, sodium and Tris, are dramatic although we cannot explain this phenomenon; nor do we know whether it is relevant to clinical chemotherapy. A number of reports of cardiotoxicity following 5FU administration (see review in ref. [29]) were published during the period when the Tris salt was available on the U.K. market and it would be interesting to know if the incidence of adverse effects has decreased since the introduction (November 1978) of the sodium salt). Authors seldom state the salt-form of drugs in their publications and clinical records, and our observations indicate that this may be important and should not be neglected.

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