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THE EFFECT OF ORAL METHOTREXATE ON THE RAT INTESTINE*

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Abstract—The effects of the administration of methotrexate to rats at different oral doses has been studied. The administration of a single dose of 6 mg/kg provokes little response from the intestine; no morphological alterations could be detected, and only marginal changes in absorptive capacity were perceived. However, the ingestion of a sub-lethal dose (40 mg/kg) induces serious lesions of the intestinal mucosa by arresting cellular regeneration in the crypts. In spite of the morphological damage, the active absorption of amino-acid persists, albeit at a reduced level, until nearly all the mucosal surface has been desquamated. However, when a certain level of destruction has been sustained, the function falls suddenly to zero. The metabolic activity of the mucosa, as witnessed by respiration, glucose consumption, and lactic acid production, reflects the changes in absorptive capacity.

Successive daily administrations of the drug at the lower level eventually induce the same changes that occur after a single high dose.

The ingestion of this drug has provided an excellent model by which the functional activity of the intestinal mucosa can be correlated with the histological findings. Two cases are presented where the morphological pattern would not predict the state of functional viability of the tissue. After 48 and 96 hr, following the administration of a single high dose of methotrexate, there is extensive and severe mucosal derangement, but sometimes a considerable though depressed transport capacity is maintained. On the other hand, rats surviving the high dose of methotrexate have a normal histological pattern after 8 days, though their functional activity is still depressed.

These findings suggest that clinical studies on intestinal biopsy samples should include functional tests as well as histological examinations whenever possible.

THE GENERAL therapeutic use of folic acid antagonists[3] has necessitated a study of their side effects on other tissues. The action of the drugs on rapidly regenerating tissues, such as the intestinal mucosa, has been reviewed recently[4, 5] and the mitotic arrest in metaphase has been demonstrated. The histological damage engendered in the tissue after administration of these drugs resembles very closely that caused by X-irradiation[6, 7] which has been more extensively studied. Nevertheless, it has been suggested that the malabsorption caused by excessive irradiation is not secondary to the tissue damage, but is due to a direct effect on the absorption mechanism which is

* Preliminary reports of this work were given[1, 2] to the 1965 meetings of the Gesellschaft für Nuclearmedizin (in Lausanne) and the Société Suisse de Gastroentérologie in (Genève).

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conspicuous before any morphological alterations can be detected.[6, 8] It will be seen that this is not the case following the administration of anti-mitotic drugs.

Although a large number of studies on the histological changes in the intestine following the administration of folic acid antagonists have appeared,[9–18] only a few succinct investigations into the effect of the drugs on intestinal transport and metabolism have been carried out.[9–12] Following the discovery that morphological examination does not necessarily provide a precise indication of the functional state of the intestine,[19] it has become of utmost importance to carry out functional tests simultaneously with histological studies in order to obtain a complete characterization of the extent of the lesions caused by the administration of a drug. The use of methotrexate has provided us with a very satisfactory model to determine the correlation between functional, metabolic, and morphological findings. With this aim in mind, a routine test for intestinal function, based on the ability of the intestinal mucosa to absorb nutrients against a concentration gradient has been developed,[20] and justified.[1, 21] Changes in this parameter, which is the essential and specific property of the intestine, are found to correlate closely with the metabolic activity of the tissue, reflected in its respiration and its glycolytic capacity, but under certain conditions, profound divergences have been noted between the functional viability measured biochemically and that which would have been predicted from purely morphological considerations.

METHODS

Male hybrid rats weighing 200–300 g were used for all experiments. The animals were allowed free access to food and water before use.

The experimental animals received by stomach tube 1 ml of an aqueous solution of amethopterin (Methotrexate, Lederle) dissolved at the required dose level. Preliminary experiments showed that the LD_{50} was of the order of 60 mg/kg, so our experiments were carried out at dose levels either just below this, or considerably lower. 6 mg/kg and 40 mg/kg were chosen arbitrarily. Control rats received 1 ml of water by stomach tube.

At the appropriate time after the administration of the drug, the animal was anaesthetized with ether, a section of the ileum was removed, and dissected into small rings, as has been described in detail elsewhere.[22] Of the randomized samples, some were used for histological examination, others for the study of absorptive capacity, and a third group underwent metabolic analysis.

The determination of the transport component of the uptake of L-phenylalanine by intestinal rings has been described in detail previously.[20, 22] Briefly, the tissue slices are incubated in a solution of 5 mM ^{14}C-L-phenylalanine in oxygenated Krebs bicarbonate buffer, with or without the addition of 1 mM 2:4-dinitrophenol (DNP). After the incubation (1 hr at 37°), the fragments are washed, weighed, and dissolved in 30% aqueous potassium hydroxide solution. The ^{14}C content of these solutions is then determined in a liquid scintillation counter, as described previously,[22] and compared directly with the specific activity of the incubation medium, aliquots of which are counted simultaneously. The total accumulation of amino acid in the absence of DNP is accounted for by two mechanisms, active transport and passive diffusion,[20] while in the presence of DNP, the former is inhibited. Hence the difference between

the two values represents a measure of the active transport component of the amino-acid uptake. It is the value that is utilized in the statistical treatment of the results.

The metabolic measurements were carried out after an incubation of 1 hr at 37° in Krebs-Ringer phosphate buffer containing 2 mg/ml glucose. The incubations took place under an atmosphere of 95% oxygen and 5% carbon dioxide in the case of the glycolysis measurements, and in air in the case of the respiration. The tissue respiration was determined by noting the diminution of oxygen pressure in a Warburg manometer, and aerobic glycolysis was measured by gauging the disappearance of glucose and the liberation of lactate into the medium. These methods are described and discussed in detail in a previous work.[23] Glucose was measured by the *o*-toluidine method[24] and lactate by the method described by Kirk.[25]

The intestinal samples for histological examination were fixed in formol, and embedded in paraffin, from which sections of 6 μ were cut out. The specimens were stained for study with haemalum and eosin.

Various statistical analyses have been employed in this work. In general, the arithmetic means of different groups of animals are compared by means of Student's *t*-test. In the case of the animals receiving a single high dose (40 mg/kg) of methotrexate, an analysis of the type of distribution of the experimental values was also undertaken. This study was performed on single groups of animals or on composite populations resulting from the amalgamation of two groups whose means do not differ significantly (according to the *t*-test). In addition, the homogeneity of such composite populations was tested by means of Fisher's *F*-test.

For the analysis of the theoretical distribution, the experimental values within each group or combination of groups are first divided into classes of equal intervals.[2] The normality of the distribution obtained is then tested by probit analysis, and the theoretical distribution is calculated.[26] The probability of this adjustment is then tested by Pearson's chi-squared test. In all tests, the *P* value of 0·05 has been utilized as the limit of significance. In the case of the probit analysis, the linearity of the probits corresponding to the cumulative frequency of each distribution was estimated. All these methods have been described in detail by Lamotte.[26]

RESULTS

Toxicity

Preliminary experiments showed that the LD_{50} for a single oral dose of methotrexate was of the order of 60 mg/kg body wt. when a complete survival test was carried out. This is considerably lower than the semi-lethal dose published by Ferguson *et al.*,[13] who nevertheless used the same preparation of methotrexate, but employed pure-bred rats of smaller weight. But it is our experience that larger rats tolerated the drug better than smaller ones, even after a correction in the dosage for the increased body weight had been applied. So it is difficult to account for this discrepancy.

In our experiments on animals receiving a single dose of 40 mg/kg of the drug, the mortality was of the order of 30 per cent; an accurate assessment was not made, since the rats were killed on successive days for the biochemical tests, and several of those killed appeared moribund. Following the high acute doses, there was always a latent period of three days, before the rats began to succumb to the intoxication.

No rat died after a single dose of 6 mg/kg although there was one rat in one series that displayed considerable signs of intoxication when it was killed 48 hr after the

administration. Since its intestinal viability was nearly zero, there is a high possibility that it would have died eventually. After daily doses, the first animals perished after five successive administrations.

Macroscopic changes

After a single dose of 6 mg/kg, few outward signs of disturbance were visible, except in the one case noted above. Diarrhoea only occurred in rare cases, and then only after 48 or 96 hr. After daily doses, the results paralleled the findings with the higher acute dose.

After a large single dose (40 mg/kg), serious signs of intoxication were evident after 48 hr in nearly every animal. The symptoms, which have been listed elsewhere,[13] included severe watery diarrhoea, anorexia and loss of weight, a rough coat with general untidiness and lethargy, and lesions of all the mucous membranes of the body. On laparotomy, gross distension of the stomach and intestines was noted. The small intestine was extensively shortened; indeed in certain cases its entire length was reduced to about 20 cm, and it was maintained in rigour with a diameter of about twice normal. The mucosa appeared to have been entirely desquamated, and the lumen was filled with evil-smelling liquid, and occasionally contained a little blood.

Histological findings

No pronounced histological modifications were found in any of the samples from rats receiving one small dose (6 mg/kg).

The changes following the administration of 40 mg/kg were extensive. After 24 hr, although the functional activity was normal, considerable vasodilatation of the capillaries and oedema of the lamina propria was apparent. This was coupled with flattening of the villi and mitotic arrest in the crypts (Fig. 1). After 48 hr, the extent of the lesions greatly increased: there was severe cellular infiltration of the entire lamina propria, serious degeneration of the epithelial cells, with vacuolization of the cytoplasm and pycnosis of the nucleus, together with cystic degeneration of the crypts. The lesions remained limited to the mucosal part of the tissue, and did not affect the muscularis mucosae and the muscular layers. From the histological point of view, there was no sharp dividing line between the samples that have decreased functional activity (Fig. 2), and those whose function is abolished (Fig. 3).

After 96 hr, however, the picture changes (Figs. 4–6). The epithelial cells of the villi have largely disappeared (once again the functional activity is correlated only to a slight degree with the level of deterioration of the epithelial layer), but mitotic patterns may once again be seen in the crypts, since at this stage, the effects of the drug on the regenerative cells have diminished. The recovery has already set in, and after another 4 days (Fig. 7), the histological pattern has reverted to normal.

Biochemical results

After a single dose of 6 mg/kg methotrexate, the change in intestinal absorptive capacity is small (Table 1). Ninety-six hours after the ingestion, the absorption is significantly smaller than that of the control animals, although this difference is not large. A progressive decline in the active transport capacity of the intestines has been noted, following the administration of 6 mg/kg methotrexate, but the significance of this finding is questionable.

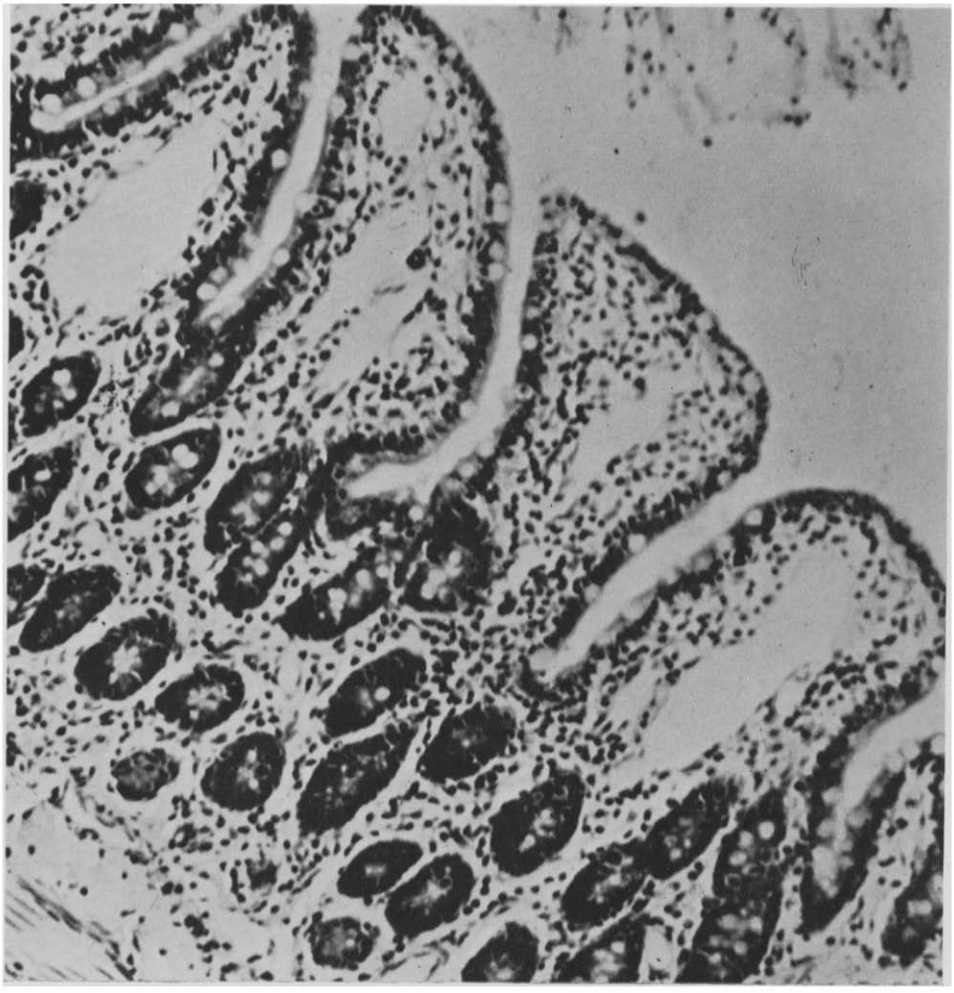

FIG. 1 shows an intestinal section (× 63) taken 24 hr after the administration of a single dose of methotrexate (40 mg/kg). Despite the flattening of the villi and the oedema of the lamina propria, the function was normal.

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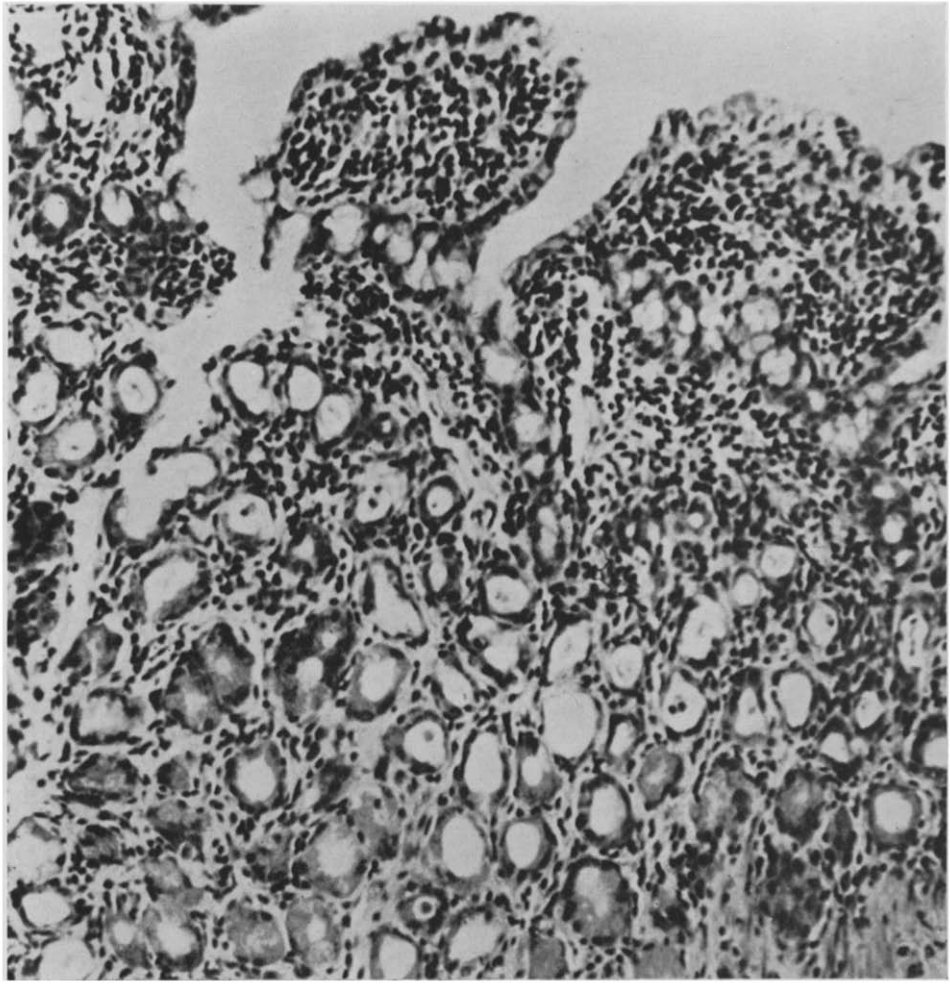

FIG. 2 shows an intestinal section (× 63) of an animal with reduced function, 48 hr after the administration of 40 mg/kg methotrexate. There is very heavy cellular infiltration, and severe degeneration of the villous architecture.

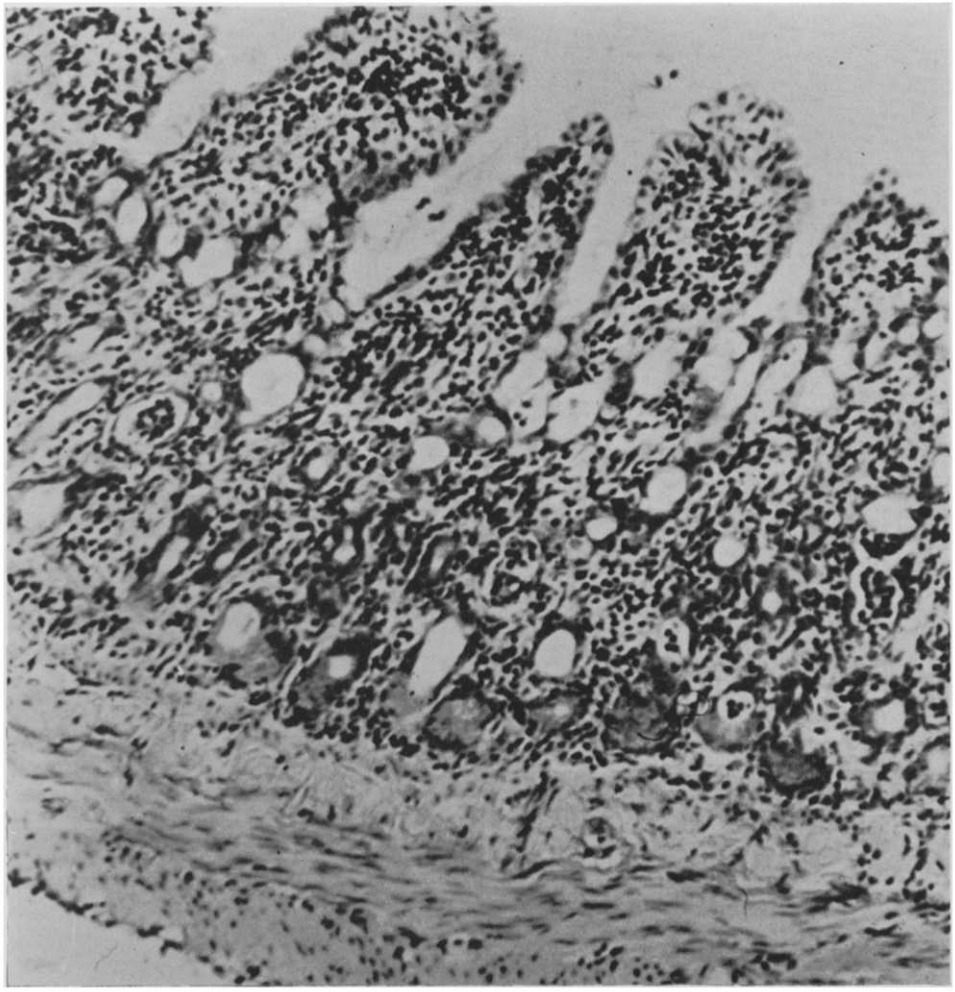

FIG. 3 shows another intestinal section (× 63) from an animal killed 48 hr after the administration of 40 mg/kg methotrexate. This intestine retained no active transport capacity, and its morphological pattern resembles that shown in Fig. 2.

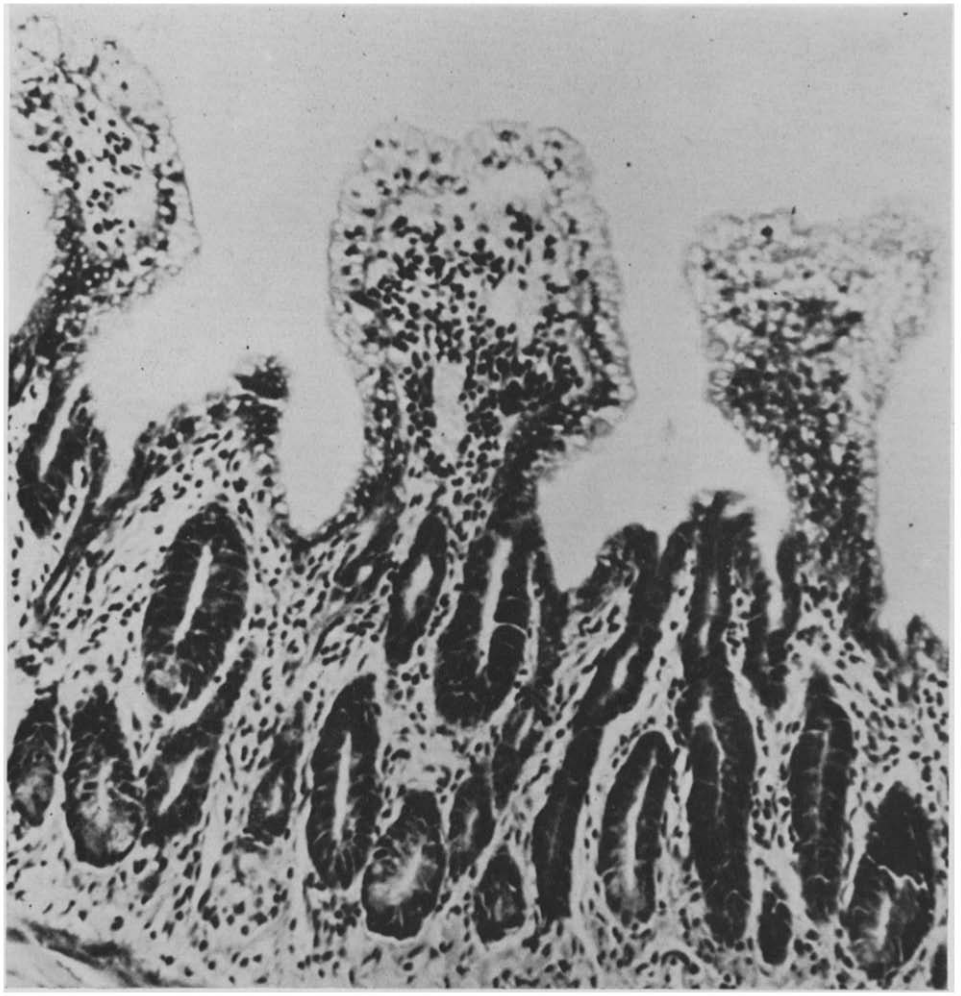

FIG. 4 shows the intestinal section ($\times$ 63) of an animal killed 96 hr after a single dose of 40 mg/kg methotrexate. Although the functional activity has been reduced to zero, mitotic patterns are now visible once more in the crypts.

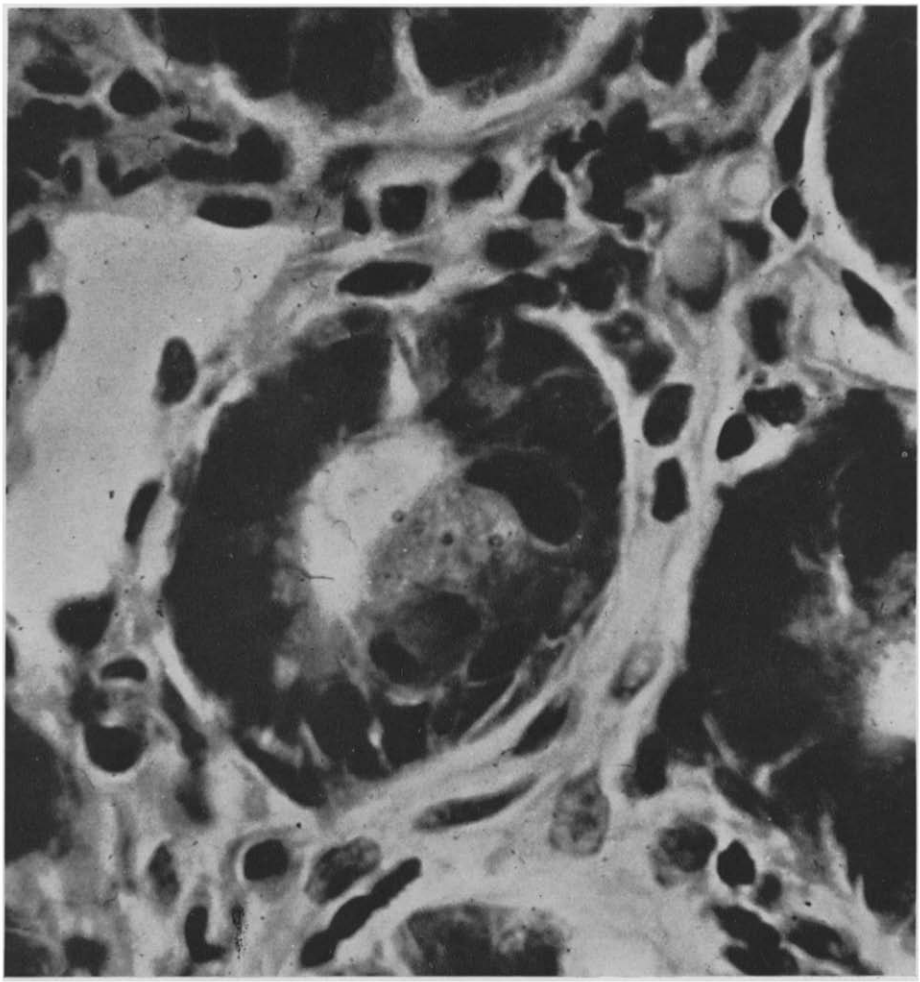

FIG. 5 shows a detail of Fig. 4 (× 350) demonstrating the presence of mitotic patterns in the crypts.

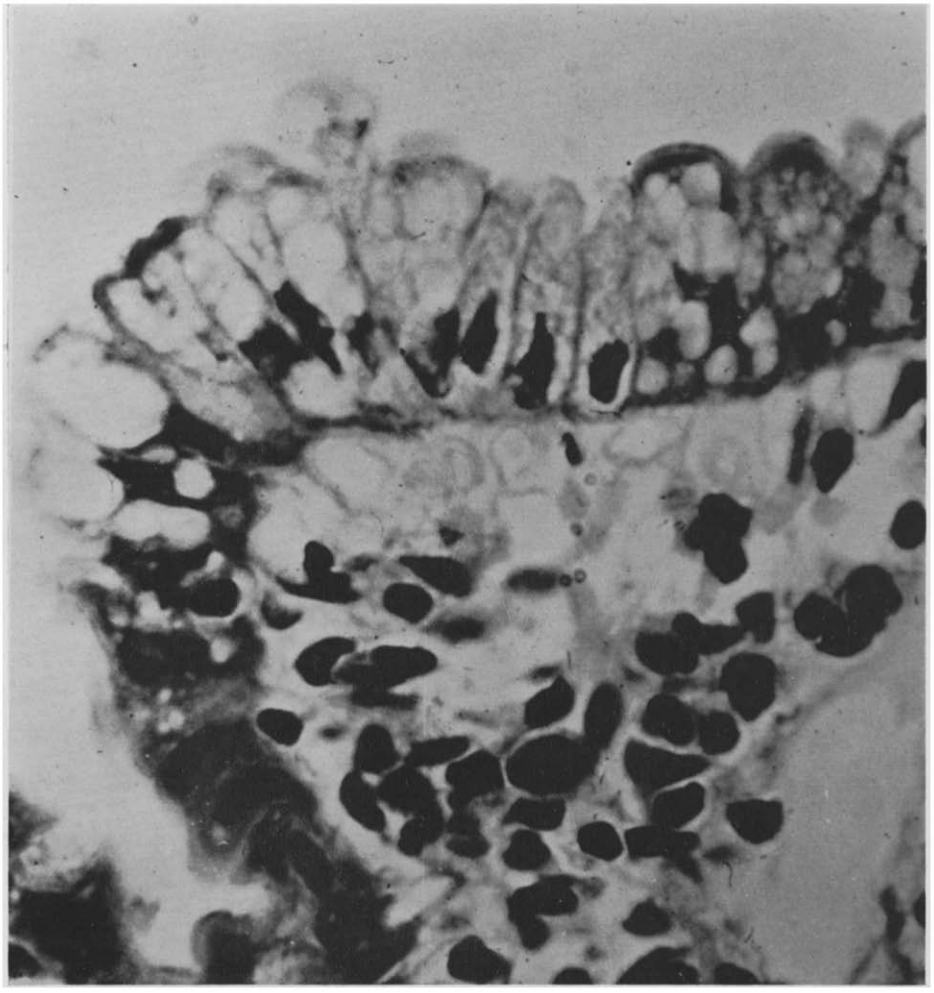

FIG. 6 is a further detail of Fig. 4 (× 350) showing the degenerate epithelial cells of the villi, with pycnosis of the nuclei and vacuolization of the cytoplasm.

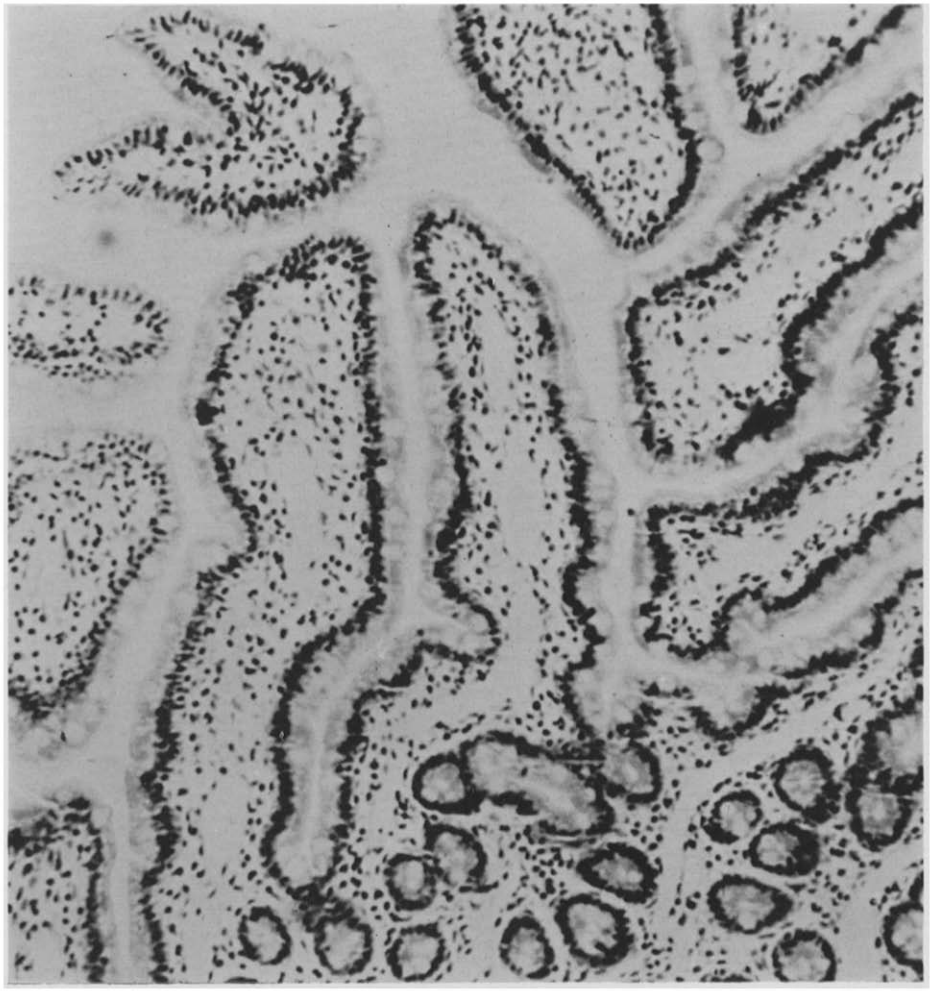

FIG. 7 shows the intestinal section (× 63) of an animal killed 8 days after the administration of 40 mg/kg methotrexate. Although the functional activity was still somewhat reduced, the histological pattern was normal.

TABLE 1. INTESTINAL ABSORPTIVE CAPACITY FOLLOWING SINGLE DOSE OF 6 mg/kg OF METHOTREXATE

Time after administration		Uptake of L-phenylalanine (μmoles/100 mg tissue, wet wt.) Alone	In presence of DNP	Active transport component
Controls	(11)	1·943 ± 0·0924	0·665 ± 0·0242	1·278 ± 0·0808
6 hr	(8)	1·918 ± 0·165	0·649 ± 0·0466	1·269 ± 0·125
24 hr	(8)	1·884 ± 0·157	0·718 ± 0·0606	1·166 ± 0·118
48 hr	(8)	1·625 ± 0·193	0·594 ± 0·0346	1·031 ± 0·165
96 hr	(11)	1·630 ± 0·104	0·624 ± 0·0342	1·006 ± 0·0781

Difference between control transport and that of 96-hr group is significant at the 2·5 per cent level (by Student's *t*-test). Numbers in parentheses represent the number of animals in each group. Values expressed ± S.E.M.

The administration of a sub-lethal dose of methotrexate (40 mg/kg) has a considerable effect on the transport capacity of the intestine; these modifications depend on the time between the administration of the drug and the measurement of the functional capacity of the tissue (Table 2). On comparing the means of each group with that of the control population, it is seen that the 6-hr group is significantly more active, the 24-hr group is not significantly different, but the 48-hr and 96-hr groups are considerably less active than the controls. The 6-day group has a mean that is higher than that of the 48-hr or 96-hr groups, but it is still significantly lower than the control. Finally, the 8-, 11- and 15-day populations are not significantly different from the normal animals.

TABLE 2. ABSORPTIVE CAPACITY OF INTESTINE FOLLOWING SINGLE DOSE OF 40 mg/kg OF METHOTREXATE

Time after administration		Uptake of L-phenylalanine (μmoles/100 mg tissue, wet wt.) Alone	In presence of DNP	Active transport component
Controls	(24)	1·535 ± 0·0500	0·624 ± 0·0135	0·912 ± 0·0475
6 hr	(8)	1·904 ± 0·0721	0·607 ± 0·0243	1·297 ± 0·0785
24 hr	(8)	1·675 ± 0·0752	0·640 ± 0·0140	1·035 ± 0·0702
48 hr	(22)	0·813 ± 0·0739	0·500 ± 0·0131	0·314 ± 0·0625
96 hr	(18)	0·778 ± 0·1049	0·471 ± 0·0193	0·307 ± 0·0887
6 days	(10)	1·190 ± 0·1015	0·553 ± 0·0200	0·637 ± 0·0876
8 days	(11)	1·433 ± 0·0578	0·631 ± 0·0244	0·803 ± 0·0418
11 days	(7)	1·494 ± 0·0705	0·628 ± 0·0316	0·866 ± 0·0636
15 days	(5)	1·559 ± 0·0713	0·630 ± 0·0449	0·928 ± 0·0486

Active transport component calculated by subtraction; this value is treated in the statistical analysis shown in Fig. 8. Numbers in parentheses represent the number of animals in each population.

In order to carry out a distribution analysis of these results, the values of the 6- and 24-hr groups were amalgamated to form a composite population, as were the values of the 48- and 96-hr groups, those of the 6- and 8-day groups, and those of the 11- and 15-day groups. Application of the *F*-test confirmed that homogeneous populations were obtained by this treatment, with the exception of the 6-hr/24-hr population.

Nevertheless, a probit analysis affirmed that each of these populations could be described by a normal distribution, with the exception of the 48-hr/96-hr population. On examining a histogram of the observed frequencies of the 48-hr/96-hr population, two maxima are revealed, one in the region of zero, and the other in the region of 600 (mμmoles/100 mg tissue). Hence this population consists of two sub-groups, one of which is asymmetrical with a maximum at zero, the other being symmetrical with a maximum near 600. A probit analysis confirmed that this latter is a normal distribution. The frequency distributions calculated from the data discussed above are shown in Fig. 8: the X^2-test did not reveal any significant differences between observed

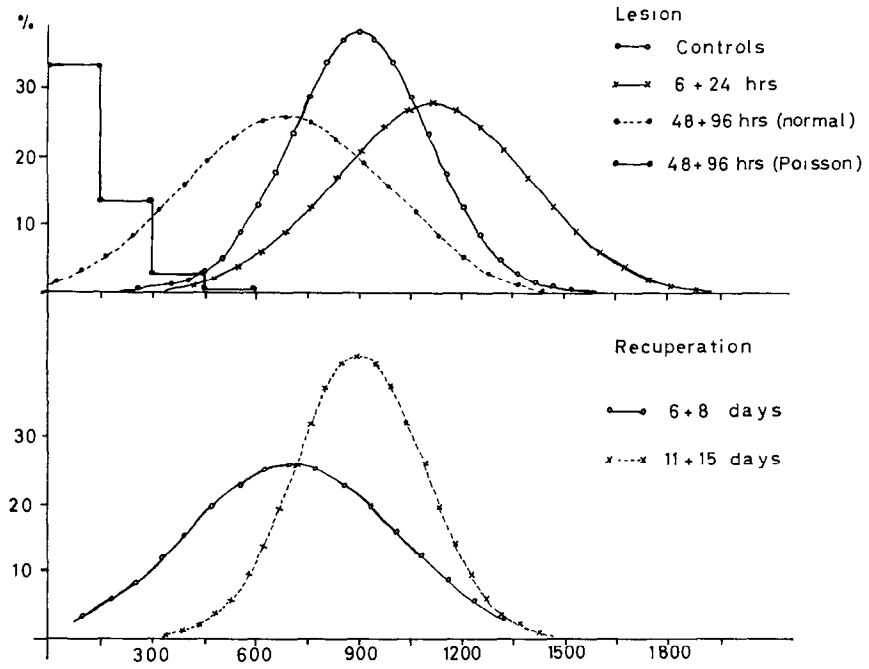

FIG. 8. Distribution curves of the results given in Table 2. Each curve represents the theoretical distribution of the intestinal absorptive capacity of the different populations of animals, corresponding to the various stages in the evolution of the injury provoked by the oral administration of 40 mg/kg of methotrexate. The composite populations are obtained by amalgamating different groups of rats, wherever statistically permissible (see text). The 48-hr/96-hr population is comprised of two different groups: one is represented by a normal curve with diminished activity, and the other by a Poisson distribution with its maximum at zero.

The statistical methods used to determine the theoretical distributions from the observed frequencies are described by Lamotte.[26] The abscissa represents the active transport component of amino-acid uptake (see Table 2), and is expressed in mμmoles of L-phenylalanine absorbed per 100 mg of tissue, wet wt. The number of rats in each population is given in Table 2.

and calculated distributions. Each population is represented as a normal distribution, with the exception of the 48-hr/96-hr group, which consists of a combination of a normal distribution about a reduced mean, and a Poisson distribution with a maximum at zero.

The replacement of the individual results by theoretical distribution curves is not contradicted by the statistical analyses employed: the F-test, X^2-test, and probit

analysis all agree insofar as no significant difference is revealed which would exclude our hypotheses. However, in the 6-hr/24-hr population, the probit analysis indicates the presence of a normal distribution, and the X^2-test demonstrates that the theoretical distribution satisfactorily explains the observed frequencies, but nevertheless the *F*-test demonstrates a significant difference between the two sub-groups of which the population consists. It certainly seems that immediately following the administration of the drug, an increase in transport capacity is observed, but this may be an experimental artefact for the following reasons: the results in Table 2 are compiled from five experiments on five different series of rats (of the same strain, but perhaps of slightly different age or nutritional status), but experiments lasting for 6 and 24 hr were only carried out on the first two groups of rats. Comparison with the control animals of those two series of rats shows no significant difference between Control, 6-, and 24-hr groups. For this reason, in our earlier analysis of the results of these experiments,[1, 2] a total composite population combining control/6-hr/24-hr animals was used as the base-line activity. However, probit analysis and the X^2 test show that this composite population cannot be replaced by a unique normally-distributed one; therefore it has now been divided into two sub-groups. For these reasons, the results for the 6-hr/24-hr population should be treated with circumspection.

On the other hand, the distribution analysis gives a good indication of what happens in the following stages of the intoxication. In the 48-hr/96-hr population, there are two groups of animals: one group have intestines that have lost their ability to absorb amino acids actively, the intestines being dead from the functional point of view, and the transport capacity being close to zero. The probability of a finite activity is small and the values are distributed according to a Poisson function. On the other hand, a second section of the population may be represented by a normal distribution about a reduced mean, indicating the existence of a finite though reduced functional activity in these animals. In physiological terms, this indicates that the intestinal mucosa is able to withstand the damage during the evolution of the injury up to a certain point, after which there is a sudden sharp fall to zero.

However, histological recovery starts on the fourth day, so that if the terminal stage does not occur too early, recovery of function may also take place. The transport capacity of the intestine however recuperates slowly: the *F*-test indicates that the activity of rats 6 and 8 days after the administration of the drug is a homogeneous function once more, and the population may be represented by a normal distribution with its mean very close to that of the viable section of the 48-hr/96-hr population. The 11-day/15-day population may be represented by a normal distribution with its mean very close to that of the control animals.

The results of the experiments where daily doses were administered to the rats are given in Table 3. In this experiment, there is a significant rise in the transport capacity after three doses (6 mg/kg) of the drug, before the heterogeneity of the population sets in once more. This rise can be equated to anorexia and wasting of the animal. It has been shown[27] that hunger induces a reflex adaptation of the intestinal tissue to enable it to concentrate non-electrolytes to a greater extent. We have described a similar effect in an earlier work[23] where daily doses of neomycin provoked a similar intestinal response. After the fourth successive dose of 6 mg/kg methotrexate, the dual response was once more observed: some rats lost their intestinal viability completely, whereas others continued to absorb at a reduced level. Since the response appeared very

similar to that occurring after a single high dose, the experiments were not further pursued.

In Table 4 are summarized the results of the metabolic studies on intestinal slices from rats receiving a single dose of methotrexate of either 6 mg/kg or 40 mg/kg. The lower dose did not elicit any changes in the metabolic activity of the intestine, but 48 hr after the higher dose, respiration and glycolysis were both depressed

TABLE 3. ABSORPTIVE CAPACITY FOLLOWING MULTIPLE DOSES (6 mg/kg) OF METHOTREXATE

No. of doses	No. of animals	Uptake of L-phenylalanine (μmoles/100 mg tissue, wet wt.) Alone	In presence of DNP	Active transport component
Controls	4	1·750 ± 0·0785	0·678 ± 0·0388	1·072 ± 0·0605
2	4	1·813 ± 0·116	0·572 ± 0·0458	1·241 ± 0·0885
3	4	2·079 ± 0·0997	0·654 ± 0·0200	1·425 ± 0·0834
4	4	0·977 ± 0·308	0·525 ± 0·0484	0·452 ± 0·259
5	4	0·717 ± 0·234	0·476 ± 0·0218	0·241 ± 0·213

6-mg/kg doses of methotrexate administered daily *per os*, the animals being sacrificed 24 hr after their final dose. After four and five doses, the groups became heterogeneous; in the former case, two of the four rats were moribund, the active transport component having fallen practically to zero, and in the latter group, three of the four rats were moribund. Difference between three-dose population and controls is significant at the 2 per cent level, and between five-dose population and controls at 1 per cent level (by Student's *t*-test).

TABLE 4. METABOLIC ACTIVITY OF THE INTESTINE FOLLOWING METHOTREXATE ADMINISTRATION

Treatment	Oxygen consumption	Glucose consumption	Lactate production
Administration of 6 mg/kg			
Controls	2·08 ± 0·380 (8)	8·185 ± 0·920 (8)	1·115 ± 0·173 (8)
After 6 hr	2·14 ± 0·628 (8)	5·572 ± 0·508 (8)	0·742 ± 0·090 (8)
After 48 hr	1·85 ± 0·153 (8)	6·504 ± 1·495 (8)	1·075 ± 0·167 (8)
After 96 hr	1·56 ± 0·099 (7)	5·435 ± 1·192 (7)	0·865 ± 0·150 (7)
Administration of 40 mg/kg			
Controls	2·25 ± 0·178 (8)	6·151 ± 0·995 (8)	1·628 ± 0·242 (4)
After 6 hr	2·34 ± 0·206 (8)	5·979 ± 0·763 (8)	1·975 ± 0·379 (4)
After 48 hr	1·41 ± 0·119 (8)	3·626 ± 0·577 (8)	0·852 ± 0·594 (4)

Numbers in parentheses indicate the number of rats in each group studied. All values are expressed in μmoles/100 mg tissue, wet wt. No significant difference is observed in the low-dose group, but all parameters are significantly decreased (according to Student's *t*-test) 48 hr after administration of the higher dose: respiration is decreased at the 0·5 per cent level of significance, and glucose and lactate at the 5 per cent level.

(whereas only 6 hr after the ingestion of the drug, there was no change). This result is closely parallel to the findings on the absorptive capacity of the intestinal samples.

DISCUSSION

The most important fact that emerges from this study is the necessity to distinguish between morphological characteristics and functional activity in the case of the intestinal mucosa. We are not the first to point out that recovery of functional activity at the cellular level is retarded with respect to the apparent histological recuperation,

for Prasad and Osborne[19] supplied evidence that the intestine did not regain its full absorptive capacity for iron for several days after ostensible histological recovery following overdoses of X-rays. Their findings are confirmed by the similar results in the present investigation, since histological revival was complete after 8 days, whereas re-establishment of functional capacity was not achieved until the eleventh day. On the other hand, we have demonstrated in this work that the intestinal mucosa has an enormous reserve capacity, and is able to continue to absorb nutrients (even though often at a reduced level) in spite of very extensive histological derangement. These findings indicate that extreme care should be taken in forming a clinical diagnosis with the aid of histological evidence alone from intestinal biopsies: it is to be very strongly recommended that functional tests of the type used in this work are also carried out on human biopsy samples, as has been executed with conspicuous success in certain laboratories.[28–30]

It has been claimed that the malabsorption syndrome following massive X-irradiation of the intestine is not secondary to the cellular damage, but is due to a direct effect of the irradiation on the absorptive processes of the mucosa.[8] Indeed, Prasad and Osborne[19] maintained that the absorptive capacity for iron reached a minimum before maximum histological injury is noted. It seems probable that herein lies a difference between the effects of anti-mitotic drugs and overdoses of irradiation on the intestinal mucosa (although it must be recognised that different functional tests could give different results, and so direct comparison between the findings of Prasad and Osborne[19] and our own should be treated with a certain reserve). However, the administration of a high dose of methotrexate caused first morphological derangement, and then a drop in functional activity. No decrease in active transport was detected until extensive morphological damage had occurred, i.e. after 48 hr. Since the drug causes mitotic arrest, this delay is to be expected, since the renewal of the epithelial lining of the villi of the rat intestine occurs once every 2 days,[31] the new cells being formed in the crypts and migrating up the villi during their life cycle. Since the absorptive capacity is largely located on the villous tips,[32] this delay in onset of malabsorption is readily explained in terms of the histological changes. Therefore it seems that excessive X-irradiation induces inhibition of intestinal transport as well as mitotic arrest, whereas methotrexate provokes mitotic arrest, which then induces extensive histological damage and therefore absorptive dysfunction.

Although perhaps different lesions occur at the cellular level at the onset of intoxication following X-irradiation and ingestion of anti-mitotic drugs, the cause of the death of the animal is likely to be the same, namely the breakdown of the intestinal barrier which normally prevents leakage from the blood to the lumen.[33] The mechanism of this barrier in the normal animal is poorly understood, though it has recently been ascribed[34] to the differential between sodium and potassium ion concentrations in the intracellular and extracellular compartments. However, when this barrier ceases to exist due to the loss of intestinal viability (as a consequence of irradiation or ingestion of anti-mitotic drugs), sodium and water (in particular) may then leak freely into the lumen, and the animal is likely to die of dehydration. No attempt at electrolyte replacement was made in this study; but from the therapeutic viewpoint, it is encouraging to know that if the subject is maintained alive by electrolyte replacement during the critical period (2–6 days after the ingestion of the drug), he will probably recover after a latent period of malabsorption.

Finally, a comparison should be made between the findings presented in this paper and the work of other authors. Although many workers have studied the histological aspects of anti-mitotic administration, using methotrexate,[12, 17] aminopterin,[9–11, 14, 16] or 5-fluorouracil,[15, 18] and have reported findings similar to our own, very few authors have studied the effect of the drugs on intestinal absorption and metabolism. Wynn Williams[14] found a correlation between depressed fat absorption and mucosal damage following aminopterin administration, and Small *et al.*[10] reported decreased xylose uptake *in vivo* occurring simultaneously with characteristic morphological changes. Bognel[12] administered methotrexate to mice *per os*, and carried out *in vivo* sugar absorption tests. He found that a gastric retention factor, greatly increased after administration of the drug, prevented him from obtaining a constant malabsorption syndrome. Finally, Vitale *et al.*[9] and Zamcheck[11] have reported a severe fall in duodenal respiration 4 hr subsequent to aminopterin administration. We were quite unable to repeat their findings, and indeed our respiration studies paralleled the functional tests, as we had expected. On the other hand, Vitale *et al.*[9] found that the respiration of the duodenal tissue of a rat in the terminal state, where the mucosa had been largely desquamated, was within the normal range. Since it has been shown[35] that the muscular moiety of the intestinal tissue respires only to a third of the extent of the mucosa (when compared on a weight-to-weight basis), it is difficult to reconcile these findings with the state of the tissue. Maybe, as they themselves suggested,[9] their tissue preparations were heavily contaminated with free cells and bacteria.

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