reported a 36-47% recovery of this material in feces after oral administration and only trace amounts in urine. It is possible that the methiodide is metabolized in a different manner than is the dihydrochloride and/or that the mouse metabolizes these materials differently than does the dog. However, it should be pointed out that the method of extraction in mouse studies (hot ethanol) is not specific for the unchanged methiodide and that when a similar extraction was performed on dog feces after 4M20 administration the presence of at least 4 similarly colored metabolites was demonstrated.

Kelsey *et al.*(5) and Mead and Koepfli(6) have followed detoxication of quinine by liver slices *in vitro* and found a product whose properties suggest an oxidation occurring at 2 position in the quinoline moiety. This metabolic change would appear to be analogous to that which occurs with 4M20.

Summary. 1. A specific sensitive method for detection in biological materials of 4M20 has been presented. 2. Plasma levels, urinary and fecal excretion of 4M20 in the dog after intravenous and oral administration were studied. These studies indicate almost complete destruction of the material after administration by both routes. Less than 0.5% of unchanged material was recovered in urine after intravenous administration and only traces were found in feces. After oral administration only traces of unchanged material were found in urine and feces. 3. The major metabolite of the drug was isolated by means of chromatography. It has been partially characterized as oxidation product with hydroxylation occurring at position 2. Other undetermined changes also occur in the molecule.

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Effects of Croton Oil on Sugar Absorption by Isolated Surviving Guinea Pig Intestine.* (24752)

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Croton oil has been known to act as a tumor induction promoter or cocarcinogen since 1944(1,2,3). It also has mild carcinogenic properties(4,5,6). In an attempt to ascertain what metabolic mechanisms may be influenced by the presence of croton oil, the effects of this substance on intestinal absorption of sugars, in the presence and absence of some amino acids, have been studied. The experiments were carried out using the perfusion technic of Darlington and Quastel(7). This technic has been fully described and will not be described further except to point out that it consists of the circulation at 37°C of an oxygenated Ringer-bicarbonate medium (the mucosal solution) through the lumen of a segment of surviving guinea pig intestine. The intestinal segment is also bathed by an oxy-

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TABLE I. Effects of Croton Oil on Glucose and Galactose Absorption by Isolated Surviving Guinea Pig Intestine.

Mucosal sol.: Ringer-bicarbonate (Ca-free) solution. Sorbose, 200 mg %; glucose or galactose, 250 mg %. Croton oil, as stated.

Serosal sol.: Ringer-bicarbonate (Ca-free) solution.

Gas: 93% O_2 + 7% CO_2, or 93% N_2 + 7% CO_2.

Time: 75 min. Temp.: 37°C.

Mucosal sol.	Serosal sol.	
	Rate of appearance, μM/hr	
% croton oil (v/v)	Glucose	Galactose
Aerobic		
Control* .0	31.3 ± 2.2	33.2 ± 2.3
.1	24.7 ± 3.1	
.2	20.2 ± 2.8	
.5	12.1 ± 2.2	24.6 ± 2.2
1.0	12.2 ± 1.7	24.3 ± 2.3
Anaerobic		
Control* .0	10.0 ± 3.0	
1.0	12.1 ± 2.2	

* No oil.

genated Ringer-bicarbonate medium circulating around it. This is called the serosal solution. The rates of transport of substances from the mucosal solution to the serosal solution are measured. The method has already been used for study of absorption of amino acids(8), of mixtures of sugars(9), and of the effects of cations on sugar absorption(10) by isolated surviving guinea pig intestine. The croton oil (and its fractions) are suspended in the Ringer-bicarbonate medium (mucosal solution) by homogenization in a Waring Blendor.

Results. Differential effects of croton oil on glucose and galactose absorption. The aerobic rate of intestinal absorption of glucose is greatly diminished by the presence of croton oil,† the rate being inversely proportional to the concentration of croton oil in the mucosal solution. This may be seen from the results given in Table I. Croton oil also affects galactose absorption but to a lesser degree than glucose (Table I). These results favor the view that separate mechanisms exist in the guinea pig intestine that are concerned with transport of glucose and galactose (see 9,12).

The presence of croton oil, however, has no effect on transport of glucose by isolated guinea pig intestine under anaerobic conditions *i.e.* on the passive diffusion of glucose through the intestinal wall (Table I). Nor is there any effect of croton oil on diffusion of sorbose into the intestine. It is evident, therefore, that one or more constituents of croton oil affect the mechanisms involved in active transport of glucose.

Croton oil inhibition of active glucose absorption by isolated intestine and the state of health of the animal. It has been frequently observed that when guinea pigs are not in a healthy condition (for example, when they have symptoms of diarrhoea) that croton oil inhibition of glucose absorption is diminished. With animals that are obviously healthy consistent inhibitions of glucose absorption by croton oil are observed.

Effects of oils on glucose transport by isolated surviving guinea pig intestine. Investigations were made as to whether the depressing effect of croton oil on glucose absorption is nonspecific, and due perhaps to the presence of fats. Glucose absorption was studied in the presence of various oils, namely: castor oil, olive oil and cod liver oil. Castor oil inhibits glucose absorption but the inhibition is much less than that due to croton oil. Olive and cod liver oils have very little or no influence on this process (Table II) at comparable

TABLE II. Effects of Oils on Glucose Absorption by Isolated Surviving Guinea Pig Intestine.

Mucosal sol.: Ringer-bicarbonate (Ca-free) solution. Sorbose, 200 mg %; glucose, 250 mg %. Oil: as stated.

Serosal sol.: Ringer-bicarbonate (Ca-free) solution.

Gas: 93% O_2 + 7% CO_2.

Time: 75 min. Temp.: 37°C.

	Serosal solution
% oil (v/v) in mucosal sol.	Rate of appearance of glucose, μM/hr
Control (no oil)	31.3 ± 2.2
.5 castor oil	22.1 ± 2.5
" olive oil	27.4 ± 2.4
" cod-liver oil	27.4 ± 1.9
" castor oil + .02 M DL-phenylalanine	26.6 ± 2.5
1.0 castor oil	21.8 ± 3.0
" olive oil	26.2 ± 3.3
" cod-liver oil	30.0 ± 2.8
" castor oil + .02 M DL-phenylalanine	21.1 ± 4.0

† Commercial preparation (Messrs. W. J. Bush & Co., Canada).

concentrations. It follows that inhibition by croton oil is due to a specific factor present in this oil that is not present in olive oil or cod liver oil.

Effects of amino acids on croton oil inhibition of active glucose absorption. It is of interest to note that croton oil inhibition of glucose is prevented to some extent by the presence of certain amino acids. DL-phenylalanine (.02*M*), on adding to the mucosal solution, greatly diminishes inhibition of active glucose absorption due to croton oil. Glycine and DL-methionine are also effective but less so than phenylalanine. Typical results are shown in Table III. Experiments were carried out to ascertain whether the action of the amino acids can be simply explained by complex formation with substances in croton oil responsible for suppression of glucose absorption. Radioactive glycine was dialyzed in presence and absence of croton oil. Rates of dialysis of the amino acid were found to be equal in both cases. It is evident, therefore, that a non-dialyzable complex of glycine and a croton oil constituent is not formed. The effects of the enantiomorphs of phenylalanine on glucose absorption by isolated intestine in the presence of croton oil were then studied. It was found that whereas DL or L-phenylalanine diminishes croton oil inhibition of glucose absorption, D-phenylalanine has relatively little effect (Table III). These results indicate that the ability of phenylalanine to prevent croton oil from interfering with glucose absorption depends on the presence of the L-enantiomorph and point to the possibility of enzymatic involvement. It is important to note that, whereas DL-phenylalanine when present in the *mucosal* solution can prevent croton oil from interfering with glucose absorption, it cannot do so when present in the serosal solution (Table III).

It is probable that croton oil influences glucose absorption of isolated surviving guinea pig intestine by damaging the glucose intestinal transport mechanism. The fact that croton oil depression of glucose transport can be prevented by some amino acids suggests that its action is mediated by a phase of amino acid metabolism.

TABLE III. Effects of Croton Oil and Amino Acids on Glucose Absorption by Isolated Surviving Guinea Pig Intestine.

Mucosal sol.: Ringer-bicarbonate (Ca-free) solution. Sorbose, 200 mg %; glucose, 250 mg %. Croton oil as stated. All amino acids = 0.02 M.

Serosal sol.: Ringer-bicarbonate (Ca-free) solution.

Gas: 93% O_2 + 7% CO_2.

Time: 75 min. Temp.: 37°C.

Mucosal solution	Serosal solution	
	Rate of appearance (μM/hr)	
	Glucose	Phenylalanine
Glu	31.3 ± 2.2	
" + .5% co	12.1 ± 2.2	
" + " + gly	26.1 ± 2.9	
" + gly	27.4 ± 4.2	
Glu + .5% co + DL-ph	24 ± 2.4	27.9 ± 3.9
Idem + D -ph	14.3 ± 2.4	15.9 ± 2.1
" + L -ph	23.6 ± 1.5	30.3 ± 2.1
Glu + DL-ph	37.9 ± 3.3	39.8 ± 2.4
Glu + 1% co + DL-ph	21.8 ± 1.4	32.6 ± 2.7
Idem + D -ph	14.3 ± 2.4	18.5 ± 2.1
" + L -ph	21.5 ± 1.5	34.2 ± 2.2
" + DL-ph*	12.9 ± 1.4	
Glu + 1% co	12.2 ± 1.7	
Idem + gly	18.3 ± 2.9	
" + DL-me	15.0 ± 1.7	
Glu + .5% co + DL-me	18.1 ± 2.1	
" + DL-me	32.3 ± 2.2	

Glu, glucose; co, croton oil; gly, glycine; ph, phenylalanine; me, methionine.

* Glucose and croton oil were present in mucosal solution, but DL-phenylalanine was present only in serosal solution.

Croton oil inhibition of glucose transport and tumor promoting activity. It is conceivable that the tumor promoting property of croton oil is linked with an inhibitory effect on glucose transport, or metabolism, and thereby on the cell energetics. While this work was in progress, Lijinsky(11) reported on isolation of active tumor-promoting fractions of croton oil. Dr. Lijinsky has kindly given us samples of his fractions and preliminary experiments have been carried out to ascertain whether there is any relationship between tumor-promoting activity (as tested by Lijinsky) and inhibition of glucose transport in isolated intestine. Results of preliminary experiments, carried out in collaboration with Mr. W. Paranchych of this Institute, suggest that there may be a correlation between tumor-promoting activity of the croton oil fraction and its

ability to inhibit active glucose absorption in the isolated surviving guinea pig intestine. For example, one fraction (at a concentration of 0.1%), stated to be active as a tumor promoter, gave an inhibition of glucose transport of 68% whereas another fraction, stated to be inactive, gave no inhibition of glucose transport at concentrations up to 0.5%. It is intended to proceed further with this work when more of the croton oil fractions become available.

Summary. 1. Croton oil (0.5%), when present in an oxygenated Ringer medium circulating through lumen of isolated surviving guinea pig intestine, inhibits active glucose absorption. It has relatively little effect, under identical experimental conditions, on active galactose absorption. 2. Castor oil has much smaller activity than croton oil. Cod liver oil and olive oil, under same experimental conditions, have little or no effect on glucose absorption by the isolated intestine. 3. Presence of L-phenylalanine in the mucosal solution diminishes inhibition of active glucose absorption by croton oil. D-phenylalanine, under same conditions, has little or no effect. L-phenylalanine when present only in the serosal solution has little or no effect on croton oil inhibition. Glycine and DL-methionine resemble, but are less effective than, L-phenylalanine in diminishing croton oil inhibition. Results indicate that croton oil inhibition may be mediated through some phase of intestinal amino acid metabolism.

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Oxidation of L-glutamic Acid in Relation to Antigenic Composition of *Salmonella typhi.** (24753)

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Enzymatic activity of various strains (Vi, O,H) of *Salmonella typhi* in relation to their antigenicity has been undertaken by Shrivastava *et al.*(1), who observed a marked difference in oxidative metabolism of glutamic acid and tyrosine of various strains containing Vi antigen in comparison with those devoid of it, maximum oxygen consumption with L-glutamic acid being higher with Vi rich strains. They concluded, therefore, that the Vi antigen is responsible for higher metabolic activity of strains containing this antigen. On the other hand, Baron, using Vi strains in V forms and W variants, demonstrated that each strain behaved identically in oxidative metabolism of amino acids, and that the difference observed cannot be correlated with presence or absence of the Vi antigen. Consequently, the observed variations in oxidative metabolism may be due to enzymatic differences inherent to each strain(2). In the present study we investigated whether different strains of *S. typhi* with optimally developed Vi antigen, or devoid of it, in comparison with H-91 and O-901 strains showed any difference in metabolic patterns to oxidize

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