tivity of NCP; (b) A 100% increase in total counts per minute recovered in collagen and a 50-60% increase in those of NCP per incubation; (c) a 700% increase in counts per minute in collagen and 200-300% increase in NCP per uterus; (d) A much higher ratio of specific activity of hydroxlysine from the treated rat to that from the untreated rat as compared with the ratio of specific activities of lysine from the same 2 groups of animals; (e) An effective dose of estradiol for stimulation of collagen synthesis is 1 μg with a dose-response time of 16 hours.

4. It is suggested that one of the important, if not the most important, action of estrogen in uterine connective tissue is concerned with the hydroxylation of lysine.

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Absorption of Short Chain Fatty Acids in Man.* (29505)

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Recently Barry and Smyth(1) investigated the absorption of short chain fatty acids from the gut of the rat and presented evidence to suggest that this was accelerated by an active transport system. Another explanation seemed possible(2) for diffusion of a compound across the gut wall besides being controlled by the compound's size and charge, is also modified by its lipid solubility(3,4).

We have, therefore, studied the relative rates of absorption of 7 short chain fatty acids from the human intestine and compared these with their partition into chloroform from an aqueous solution.

Patients studied and methods. The technique was similar to that used for studying sugar(5) and cortisol(6) absorption in man. The evening before the study the patients swallowed a double lumen polyvinyl tube ('Portex' M.L.T./B. external diameter 4.2 mm) to which had been attached a mercury bag. Next morning its position was checked fluoroscopically and the tube withdrawn slightly if necessary so that the mercury bag was approximately 40 cm past the duodenojejunal flexure. Fasting jejunal contents were collected from 7 patients. A solution contain-

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ing the mixture of acids was then pumped at 20 ml/min (using a Bowman constant infusion pump—Process and Instruments, Brooklyn, N. Y.) through the lumen which has an opening 35 cm from the end, *i.e.* the fluid entered the jejunum 5 cm past the duodeno-jejunal flexure. After 30 minutes equilibration samples of jejunal contents were siphoned off through holes cut into the other lumen of the tube 5 cm from the end, *i.e.* 30 cm below the point at which the solution entered the jejunum. The solution of acids also contained polyethylene glycol (PEG) molecular weight 4000 at a concentration of 0.5 g/100 ml. This substance is neither absorbed nor metabolized by the gut and may be used as a reference compound indicating dilution or concentration of the sample as it passes through the bowel(7): thus by knowing the concentration of acid in the aspirated and infused sample net absorption of acids/min/30 cm gut may be calculated.

The absorption of volatile acids from the colon was studied in a 55 year old woman who had had a colonic exclusion operation for chronic portal systemic encephalopathy (8). The operation leaves the patient with a fistula between the abdominal wall and the excluded colon. The colon was washed out by instilling 500 ml isotonic saline on 3 occasions and emptying after 10 minutes: on 2 further occasions 100 ml of a solution of short chain fatty acids and PEG were instilled and recovered after 10 minutes.

The fluid perfusing the gut contained the following short chain fatty acids, acetic, propionic, butyric, iso-butyric, valeric, iso-valeric and caproic in equimolar amounts, the final concentration being between 110 and 145 mM, and was made isotonic with plasma by addition of appropriate amounts of sodium chloride after the pH was adjusted to 7.0 with sodium hydroxide. Only a mixture of acetic, propionic, butyric, valeric and caproic acids were instilled into the colon: this solution was also isotonic at pH 7.0.

Analyses of samples. The pH of the aspirated sample was measured with a glass electrode. 1.5 ml $FeCl_3$ solution (1.0 g $FeCl_3 \cdot 6H_2O$ made up to 100 ml with water with addition of 2 drops of concentrated HCl) was added to 10 ml of intestinal sample, made up to 50 ml, shaken with 1.5 g $Ca(OH)_3$ and filtered. This precipitates protein and long chain fatty acids(9). Fifteen ml of the filtrate was acidified with sulphuric acid and the short chain fatty acids extracted with four 25 ml portions of ethyl-ether. The ether extract was dried with sodium sulphate and the acid content titrated with N/100 alcoholic KOH using bromthymol blue as the indicator.

The relative amounts of the individual acids were assessed by first adding enough dilute aqueous KOH to the ether extract to produce 2 phases—usually 3-4 ml. The aqueous extract of the sodium salts was then dried, and the liberated acids separated by gas chromatography(10). Polythylene glycol was estimated turbidometrically(11).

Partition of fatty acids into chloroform. On 3 occasions 10 ml of the equimolar solution of the short chain fatty acids (pH 7) such as was used in the perfusion experiments, was equilibrated against 400 ml chloroform. After standing overnight, the chloroform was run off into a flask, washed with 20 ml N NaOH and the alkaline aqueous solution of salts dried and analyzed in a similar way as were the intestinal samples.

Results. The mean fasting total short chain fatty acid concentration of the upper jejunal contents in the 7 patients was 7.7 mM (range 2-11.3) of which over 80% was acetic acid.

By the end of the perfusion there was some diarrhea on 4 occasions. This might have been due to direct irritation by the acids and their soaps, but in view of the fact that the solution was isotonic and its pH did not vary from 7 by more than 0.15, it is more likely that the solution acted as a bulk purge for under these conditions it was relatively poorly absorbed—only 0.89 (range 0.2-1.1) μMoles of short chain fatty acids were absorbed/min/30 cm jejunum.

In view of the fact that the small bowel had a measurable amount of acetate present in its lumen before the perfusion started, it was decided to express the rates of absorption in relation to propionic acid. Taking propionic acid as 1, it is seen from Table I

TABLE I. Relative Amounts of Short Chain Fatty Acids Recovered from the Jejunum and Colon, Compared with Their Partition into Chloroform.

	C_2	C_3	$IsoC_4$	nC_4	$IsoC_5$	nC_5	nC_6
Mean relative absorption							
Jejunum (7)*	1.6	1	.78	.72	.64	.54	.39
p†	.001	.001	.05	.02	.001	.001	
Colon (2)	1.41	1		.73		.59	.46
Partition into chloroform (3)		1	2.1	2.1	8.9	12.0	45

* No. of observations in parentheses. † Calculated by the paired t test.

that with increasing chain length there is relatively less of the acid recovered in the aspirated sample, thus the longer the fatty acid chain, the more quickly it was absorbed. The 2 isomers of butyric acid and valeric acid were also absorbed at different rates.

The 3 saline washings in the isolated colon contained 4 mM acetic acid before any acids were instilled, while after retention of the solution of the short chain fatty acids there was an overall absorption of 4 mMoles in 10 minutes, and the relative rates of absorption of the different acids were remarkably similar to those observed in the small bowel (Table I).

Partition of acids and salts into chloroform. The relative partition of the acids into chloroform from an equimolar solution at pH 7.0 is shown in Table I. With increasing chain length, there is increasing partition into chloroform.

Discussion. The relatively high concentration of acetate in the upper jejunal contents under fasting conditions may possibly be due to bacterial metabolism. This seems unlikely for the upper small bowel is said to be relatively sterile(12) and we have also found during absorption studies with sugars that after perfusing up to 6 l in 5 hours, the aspirated sample still contains comparable amounts of acetate. Under these conditions one would have expected bacteria and their products to have been swept down the gut. Another explanation is that the intestinal cells leak acetate into the bowel lumen.

The short chain fatty acids which we studied are weak electrolytes and their pK values are very similar, ranging from 4.76-4.85. This means that under the condition of perfusion at pH 7 the ratio of ionized/non-ionized acid is approximately 100/1. It is a general rule that ionized water soluble compounds do not diffuse across a membrane, but that their non-ionized components do(2,3,4). This is due to the greater lipid solubility of the non-ionized compound. Thus, relative absorption rate of weak electrolytes depend upon two factors, (1) their pK, which determines how much is ionized at a given pH, and (2) the relative lipid solubility of the non-ionized component. This latter has been tested for various drugs by equilibration against organic solvents such as heptane or chloroform (4). It must be realized that each solvent has slightly different properties which give only an overall indication of the properties of a lipid membrane surrounding cells. In our experiments, we equilibrated against chloroform at pH 7 to obviate even the slight difference in the pK of the acid. In fact the agreement between their partition into chloroform and their relative absorption rate is impressive, for although butyric and isobutyric acid partitioned equally into chloroform and yet had different rates of absorption, these were only significant at the 1 in 20 level. The very high ratio of acetate to propionate can probably be accounted for by the dilution of the perfusing fluid with the endogenous acetate.

The evidence cited by Barry and Smyth (1) that the rat small gut contains an active transport system for short chain fatty acids was: (1) *in vitro* preparations of rat small gut created a concentration gradient of the acids from the mucosal to serosal grade, and (2) the acids were absorbed at different rates. If in fact the main factor of importance in controlling the absorption of these acids were the relative lipid solubility of their

non-ionized form, then if *in vitro* the pH of the solution bathing the mucosa were lower than that bathing the serosa, a concentration gradient of the acids would be observed, for the serosal acids would be more ionized(2). Although Barry and Smyth(1) did not test this hypothesis, such a pH gradient has in fact been observed(13).

The fact that the absorption of these compounds from the large bowel followed the same pattern as the small bowel and that both could be predicted from their physical properties further suggests that it is not necessary to invoke an active transport system in man especially as the ability of the small bowel to absorb these acids is low, *e.g.* compared with glucose which, in spite of a higher molecular weight, is absorbed over 4 times as quickly. But it is possible that at such high concentrations as we used some inhibition of a transport system occurs(1).

Normally, these short chain acids are not an important feature of man's diet, although in ruminants they are a major product of cellulose fermentation in the rumen from where they are absorbed, metabolized and supply an important source of the animal's energy. It would seem that the greater the chain length the quicker they are absorbed by the squamous epithelium of the rumen (14).

In man, the main site of short chain acid formation is the large bowel, presumably the caecum, where they are most likely formed from bacterial action on cellular debris and unabsorbed carbohydrate. We have confirmed that faeces normally contain 10-20 mMoles/100 g wet weight(9,15) of which half is acetate, the rest being a mixture of the other short chain fatty acids and their isomers(9). Although this pattern may in fact reflect the metabolic pathways of colonic bacteria it may also reflect the more rapid absorption by the colon of the longer chain acids.

Summary. The relative rates of absorption of 7 short chain fatty acids from jejunum and colon of man have been compared with their partition into chloroform from an aqueous solution. The longer the chain length the faster the acids were absorbed. This probably reflected their greater solubility in lipid as shown by their partition into chloroform.

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