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Inhibition of intestinal absorption by different samples of cetrimide and the homologous alkyl series C10-C20

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The effects of different samples of cetrimide on the intestinal absorption of D-glucose, sodium butyrate and DL-methionine were compared with those of homologous compounds $C_{(10)}$–$C_{(20)}$ of the same alkyl series, by *in vivo* intraluminal perfusion in the mouse, rat and guinea-pig. Two samples of "cetrimide" had different inhibitory activities in the mouse and rat but were equiactive in the guinea-pig in which both samples caused glucose reversal. None of the homologues, $C_{(10)}$–$C_{(20)}$, paralleled the activity of either of the cetrimide samples exactly. It is concluded that cetrimide should be assayed in the rat before its use in studies of absorption and protein binding.

ABOUT five years ago cetrimide (cetyltrimethylammonium bromide) was shown to have a striking inhibitory effect on the intestinal absorption of nutrients in several species (Nissim, 1960a,b; 1961; 1963). Experiments for the elucidation of the mode of action of cetrimide and related compounds and of their interactions with other drugs such as phloridzin have resulted in a new theory of intestinal absorption (Nissim, 1964).

During these investigations the original sample of cetrimide was used up, and it was found that new batches of cetrimide were less active. The change in activity coincided with a change in the strain of mice from C3H to CBA. It was thus necessary to suspend the studies of the mechanism of action of cetrimide and investigate the activities of the various batches of cetrimide in different species. Two of the new batches of cetrimide were found to have activity similar to that of the original cetrimide and were designated sample A. The other batches were all less active and are referred to as sample B. This paper describes the comparative activities of the two samples A and B, thus defined, on the intestinal absorption of glucose, sodium butyrate and methionine in mice, rats and guinea-pigs. A preliminary report of these findings has been published (Hart & Nissim, 1963). It appeared likely that differences in the activities of the samples were caused by admixtures of homologues of cetrimide (then largely $C_{(16)}$) of different chain length. Accordingly, homologues $C_{(10)}$–$C_{(20)}$, of a high degree of purity, were examined for their potency on the absorption of the three nutrients and for their physical properties. The activities of the two samples A and B of cetrimide were compared with those of the pure alkyl homologues.

Materials and methods

CETRIMIDE SAMPLES

Sample A. The original cetrimide was obtained from the British Drug Houses Ltd., and the new batches which proved active were obtained from Kodak Ltd. and Judex (from B.D.H.).

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Sample B. The batches found to have a low activity were obtained from the British Drug Houses Ltd. and from Hopkin & Williams Ltd.

All batches were supplied as cetrimide, i.e. cetyltrimethylammonium bromide $C_{(16)}$, except those from Hopkin & Williams which were supplied as 'cetrimide B.P.'

The $C_{(12)}$, $C_{(14)}$ and $C_{(16)}$ homologues were obtained through the special courtesy of Printar Industries Ltd., and the $C_{(10)}$, $C_{(18)}$ and $C_{(20)}$ compounds from Glovers Chemicals Ltd.

PERFUSION TECHNIQUE

Except for certain details, the same *in vivo* perfusion method (Nissim, 1965) was used in the mouse, rat and guinea-pig. A known volume of 0·9% saline, containing 0·2% D-glucose, sodium butyrate and DL-methionine, was perfused through the lumen of the small intestine for a known time and the final volume measured. The concentration of each of the three nutrients was then determined and the percentage absorption calculated. The activity of the drug was investigated by dissolving it in the perfusion fluid and comparing the percentage absorption of the nutrients with that obtained in control animals. Some control experiments were always made concurrently with those in which drugs were tested. All concentrations are expressed as w/v.

Mice. Male C3H and CBA mice of either sex bred at Guy's Hospital Medical School and weighing 18–24 g were separated from their food overnight and anaesthetised with sodium pentobarbitone (Veterinary Nembutal, Abbott Laboratories), 60 mg/kg subcutaneously. The whole small intestine was perfused for ½ hr with 25 ml of the nutrient solution.

Rats. Male albino rats (obtained from A. Tuck & Son Ltd. or bred at Guy's), weighing 200–400 g, were separated from their food overnight and anaesthetised with sodium pentobarbitone, 60 mg/kg subcutaneously. The proximal 60 cm of small intestine was perfused for ½ hr with 50 ml of the nutrient solution. In 1 hr perfusion experiments, 50 ml of 0·2% D-glucose alone in 0·9% saline was used.

Guinea-pigs. Male and female albino guinea-pigs weighing about 500 g were anaesthetised with sodium pentobarbitone, 45 mg/kg subcutaneously, after a subcutaneous dose of atropine, 1 mg/kg. The whole small intestine was perfused with 100 ml of the required perfusion fluid for ½, 1 or 2 hr. Samples were taken for analysis during the longer experiments, usually at ½, 1 and 2 hr. In some experiments the drug under investigation was dissolved in 0·9% saline and perfused without nutrient to test for leakage of glucose from the blood or, in other words, reversal of glucose absorption. In these experiments the animals were not separated from their food overnight as this procedure lowered the blood sugar in guinea-pigs excessively.

HISTOLOGY

Segments of rat intestine perfused with control or drug-containing solution were fixed in 10% formol saline. Sections were stained with haematoxylin and eosin.

CHEMICAL ESTIMATIONS

Glucose was estimated either by the method of Haslewood & Strookman (1939), or by a modification of the method of Hoffman (1937), using an autoanalyser. Butyrate was estimated by the method of Smyth & Taylor (1958). Methionine was estimated by a modification of the method of McCarthy & Sullivan (1941). The addition of one drop of 0·1% sodium lauryl sulphate gave readings which were more consistent owing to the elimination of unduly low readings apparently caused by some interfering substance from the intestine. Thus previously published figures for the absorption of methionine should be divided by 1·4 to correspond to recent values. Values quoted from previous papers in the series have been modified in this manner.

Results

SAMPLES A AND B OF CETRIMIDE

Mice. In Table 1 the results obtained with the new batches of cetrimide in CBA mice are compared with those obtained with the original sample of cetrimide, sample A, in C3H mice (Nissim, 1962). The inhibitory effect of the drug on the absorption of glucose was significantly smaller. The change in the strain of mice being studied necessitated the examination of sample B in C3H mice. The results in Table 1 show that the difference

TABLE 1. THE EFFECTS OF THE ORIGINAL CETRIMIDE, SAMPLE A, AND THE NEW BATCHES OF CETRIMIDE, SAMPLE B, ON THE ABSORPTION OF GLUCOSE, BUTYRATE AND METHIONINE IN ½-HR INTRALUMINAL PERFUSION EXPERIMENTS IN CBA AND C3H MICE

Treatment	n	Percentage absorption			P
		Glucose	Butyrate	Methionine	
Sample A, male C3H mice					
Controls	27	35·4 ± 1·1	34·0 ± 1·5	33·3 ± 1·1	
Cetrimide, 10^{-3}	4	3·7 ± 1·5	24·9 ± 1·5	22·9 ± 1·6	
Cetrimide, 10^{-5}	10	50·9 ± 2·2	32·9 ± 1·8	30·1 ± 1·8	
Sample B, male and female CBA mice					
Controls	20	41·3 ± 2·9	38·5 ± 2·1	28·1 ± 1·4	<0·1
Cetrimide, 10^{-3}	7	23·6 ± 2·0	22·3 ± 2·6	17·3 ± 2·0	≪0·001
Cetrimide, 10^{-5}	4	40·9 ± 4·1	—	25·1 ± 2·4	
Sample B, male C3H mice					
Controls	15	34·2 ± 1·2	—	—	
Cetrimide, 10^{-3}	4	10·2 ± 0·7	21·4 ± 3·4	18·6 ± 3·1	<0·01

Values are means with standard errors. n = number of experiments. P refers to comparison with glucose values obtained with sample A and C3H male mice.

in activity between the two samples was now far less marked, though still statistically significant with $P < 0.01$. Sample variation and strain variation were therefore both involved.

Rats. The examination of the new batches of cetrimide, which had shown low activity in mice, was continued in the rat, but before this was done the reliability of the assay was given a further check. Cetrimonium stearate had been examined originally in albino rats bred at Guy's (Nissim, 1960b), and at a concentration of 5×10^{-3} had given a reduction of glucose absorption of 37·3%. When the same sample of cetrimonium

stearate was re-examined on the same stock of rats, at a concentration of 5×10^{-3}, 35·8% inhibition of glucose absorption was obtained, giving $P < 0{\cdot}8 > 0{\cdot}7$ between the two sets of experiments. With this confirmation of the reliability of the assay procedure, the new batches of cetrimide were examined in the rat. In two sets of experiments, with four rats in each set, the absorption of glucose in the presence of the new cetrimide, at a concentration of 10^{-3}, was 28·8 ± 2·2% and 27·5 ± 5·2% as compared with 41·5 ± 1·0% in the control. These results indicated that the new batches of cetrimide were comparatively inactive in inhibiting the glucose absorption, for the original cetrimide had reduced this to 5·4 ± 2·4%. These batches of cetrimide showing low activity in both mice and rats were designated sample B. Eventually two new batches of cetrimide were obtained which, in initial experiments in rats, showed activity similar to that of the original cetrimide. One batch, from Judex, was obtained through the courtesy of C. B. Taylor who had confirmed our early results by examining cetrimide in an *in vitro* preparation (Taylor, 1963). The second batch was obtained from Kodak Ltd. The inhibitory activity of these two new batches, designated sample A, was compared with the activity of the other batches, grouped together under sample B, at three concentrations in the rat. The results are summarised in Table 2, where

TABLE 2. THE EFFECTS OF THE TWO SAMPLES OF CETRIMIDE ON THE ABSORPTION OF GLUCOSE, BUTYRATE AND METHIONINE IN ½-HR INTRALUMINAL INTESTINAL PERFUSION EXPERIMENTS IN MALE ALBINO RATS

	Absorption %								
	Glucose			Butyrate			Methionine		
Treatment	Sample A	Sample B	P	Sample A	Sample B	P	Sample A	Sample B	P
Controls	41·5±1·0 (28)			43·0±1·5 (20)			29·0±1·3 (20)		
Cetrimide 10^{-3}	6·4±1·0 (16)	22·8±1·7 (19)	<0·0001	22·3±1·6 (16)	31·8±1·4 (19)	<0·001	11·4±1·1 (16)	26·0±2·0 (19)	<0·001
Cetrimide 10^{-4}	33·3±2·4 (8)	38·1±3·5 (4)	<0·3>0·2	38·4±1·9 (8)	44·0±3·1 (4)	<0·2>0·1	25·2±1·9 (8)	25·3±3·1 (4)	0·99
Cetrimide 10^{-5}	43·0±2·9 (4)	45·9±1·7 (8)	<0·4>0·3	43·7±1·9 (4)	45·7±1·9 (6)	<0·2>0·1	29·9±2·7 (4)	38·0±1·8 (8)	<0·05 >0·025

Values are means with standard errors; the number of animals is shown in parentheses. P refers to comparison between sample A and sample B.

it is seen that the two samples had significantly different effects on the absorption of all three nutrients. This was in contrast to the results in the mouse, in which the differences with respect to methionine and butyrate were negligible. The difference in inhibitory activity was also observed in 1-hr experiments on two groups of four rats. Sample A, at 10^{-3}, reduced the absorption of glucose by 62·3%, whilst the same concentration of sample B reduced it by only 23·8%.

Guinea-pigs. Two types of experiments were made in guinea-pigs, the perfusion fluid being either the usual nutrient-containing solution for the study of absorption, or the nutrient-free 0·9% saline for the study of glucose reversal.

When the original cetrimide was previously examined for its effect on the absorption of glucose in guinea-pigs it gave a steep dose-response curve and, at a concentration of 10^{-3}, a negative value for glucose absorption. This latter observation meant that glucose passed from the blood vessels into the lumen of the intestine, a finding which was most striking when cetrimide was perfused in 0·9% saline alone.

2-hr perfusion experiments with the three nutrients were performed on one control animal, and two animals which had in addition sample B at a concentration of 10^{-3} (Table 3). The glucose reversal obtained with

TABLE 3. THE EFFECTS OF THE TWO SAMPLES OF CETRIMIDE ON THE CONCENTRATION OF GLUCOSE, BUTYRATE AND METHIONINE IN THE LUMEN OF THE GUINEA-PIG SMALL INTESTINE DURING PERFUSION EXPERIMENTS

Treatment	Nutrient	n	Nutrient concentration in lumen			
			Initially	½ hr	1 hr	2 hr
Control nutrient	Glucose	1	200	—	60	10
	Butyrate		200	—	50	50
	Methionine		200	—	80	40
Nutrient solution + sample B, 10^{-3}	Glucose	2	200		211 255	222 261
	Butyrate		200		107	84 44
	Methionine		200		123 99	100 108
Saline solution, 0·9%	Glucose	1	0	—	—	0
	Butyrate		0	—	—	0
	Methionine		0	—	—	0
Saline solution, 0·9% + sample B, 10^{-3}	Glucose	2	0	—	79 81	112 100
	Butyrate		0	—	—	0
	Methionine		0	—	—	0
Saline solution, 0·9% + sample A, 10^{-3}	Glucose	3	0	$72\cdot3 \pm 8\cdot7$	$102\cdot5 \pm 22\cdot5$	
	Butyrate		0	—	0	
	Methionine		0	—	0	
Saline solution, 0·9% + sample B, 10^{-3}	Glucose	3	0	$62\cdot0 \pm 4\cdot1$	$116\cdot8 \pm 7\cdot1$	$168\cdot4 \pm 14\cdot7$
	Butyrate		0	—	0	
	Methionine		0	—	0	

Values are concentrations in mg/100 ml of either individual experiments or means and standard errors; n = number of animals. P between glucose values for two samples at ½ hr $<0\cdot4$ $>0\cdot3$ and at 1 hr $<0\cdot7$ $>0\cdot6$ in last two experiments.

sample B indicated clearly that in the guinea-pig it was no less active than the original cetrimide, for the dose-response curve obtained with the original cetrimide showed that no reversal would have occurred at a concentration of sample A as high as $0\cdot9 \times 10^{-3}$.

Confirmation of the glucose reversal was obtained by perfusing sample B, at a concentration of 10^{-3}, in 0·9% saline containing no nutrient. Preliminary experiments had shown distinctly that the degree of glucose reversal depended on the blood sugar level. Animals perfused with nutrient-free saline were not therefore separated from their food overnight. In this way a blood sugar level between 200 and 300 mg/100 ml was assured. In two 2-hr experiments a final concentration of 100 mg% or more was observed (Table 3) with, of course, no trace of either methionine or butyrate. Finally, the potency of the two samples of cetrimide in causing glucose reversal was compared in two groups of three guinea-pigs in which the cetrimide was perfused at a concentration of 10^{-3} in

nutrient-free 0·9% saline. The results in Table 3 show that both samples produced a similar degree of glucose reversal and thus had equivalent activity in the guinea-pig, in marked contrast to their distinctly different activities in the mouse and rat.

PHYSICAL AND HISTOLOGICAL OBSERVATIONS

During the present experiments, the surface-activities of the two cetrimide samples, as assessed by the degree of frothing of the perfusate in the apparatus, appeared to be similar. On the other hand, differences in the solubilities of the two samples were quite marked. Sample A dissolved easily at 10^{-3}, in the nutrient solution, and did not precipitate when stored at a low temperature (0·5°). Sample B had to be warmed to about 35–40° before it dissolved completely to give a concentration of 10^{-3}, and it reprecipitated when stored in the cold. Both samples remained in solution at room temperature.

The perfusion fluid collected at the end of the experiment was always inspected for cloudiness and mucosal debris. At concentrations of 10^{-3}, both samples produced some damage to the intestinal mucosa, no difference in the amount of debris being noted. Sections of the small intestine from such experiments were examined histologically, but again it was impossible to distinguish between sections perfused with sample A and those perfused with sample B. At concentrations of 10^{-3}, both caused some breakdown at the tips of villi with comparable accumulation of cellular debris in the lumen.

HOMOLOGOUS COMPOUNDS

Pure members of the homologous series of chain length $C_{(10)}$–$C_{(20)}$ were examined for their activities at a concentration of 10^{-3} in ½-hr perfusion experiments in rats. The results are shown in Table 4, where

TABLE 4. THE EFFECTS OF THE TWO SAMPLES OF CETRIMIDE COMPARED WITH THOSE OF HOMOLOGOUS COMPOUNDS AT A CONCENTRATION OF 10^{-3}, ON THE ABSORPTION OF GLUCOSE, BUTYRATE AND METHIONINE IN ½-HR INTRALUMINAL INTESTINAL PERFUSION EXPERIMENTS IN MALE ALBINO RATS

Treatment	n	Absorption, %		
		Glucose	Butyrate	Methionine
Controls	20	41·5 ± 1·0	43·0 ± 1·5	29·0 ± 1·3
Cetrimide				
Sample A	16	6·4 ± 1·0	22·3 ± 1·6	11·4 ± 1·1
Sample B	19	22·8 ± 1·7	31·8 ± 1·4	26·0 ± 2·0
$C_{(10)}$	4	33·6 ± 1·3	45·3 ± 0·7	19·3 ± 1·2
$C_{(12)}$	4	7·3 ± 2·2	31·5 ± 1·1	14·6 ± 0·9
$C_{(14)}$	4	10·7 ± 1·5	30·7 ± 3·1	14·8 ± 3·2
$C_{(16)}$	4	14·3 ± 2·8	29·2 ± 0·7	8·2 ± 2·2
$C_{(18)}$	4	23·9 ± 1·7	29·6 ± 1·5	25·1 ± 2·0
$C_{(20)}$	4	26·0 ± 2·1	30·9 ± 0·8	17·9 ± 1·9

Values are means with standard errors; n = number of animals.

they are compared with those obtained at the same concentration of the two samples of cetrimide. Compounds $C_{(10)}$–$C_{(16)}$ dissolved easily in the nutrient solution, whilst $C_{(18)}$ and $C_{(20)}$ had to be warmed to 50° before they dissolved, though once dissolved they remained in solution at

room temperature. The inhibitory effect on glucose absorption was maximal with $C_{(12)}$, and decreased thereafter with increasing chain length. There were no significant differences in the effects of the five compounds, $C_{(12)}$–$C_{(20)}$, on butyrate absorption, all producing about 30% inhibition, while $C_{(10)}$ had no significant effect. A peak in the inhibitory activity on methionine absorption occurred at $C_{(16)}$.

Histological examination of sections from intestine perfused with the pure and active $C_{(12)}$ and $C_{(14)}$ homologues showed greater damage to villi than was obtained either with sample A or B of cetrimide. This difference was reflected in the greater amount of debris observed in the naked-eye inspection of the perfusion fluid.

Discussion

Commercial samples of cetrimide, even from the same manufacturer, were not equivalent in effect. This finding is of importance on account of the value of this drug as a tool for research in the study of the mechanisms involved in intestinal absorption. Previous evidence suggests that cetrimide inhibits absorption by binding to the mobile intracellular proteins which constitute the normal carriers for active nutrient absorption (Nissim, 1964). The results obtained with the two samples of cetrimide may indicate that species and strain differences exist in the protein carriers themselves or in the factors controlling the association-dissociation constants between nutrients and proteins.

Both sample A and sample B, at a concentration of 10^{-3}, caused the same degree of damage to the mucosa, while they exerted significantly different effects on the absorption of nutrients. This fact constituted yet another reason for concluding that the inhibitory effects of cetrimide were not simply due to some non-specific action on absorption, an action closely related to the mucosal damage it produced, as believed by Taylor (1963). It also suggested the interesting possibility that these two properties of cetrimide could be separated in some compound which might in future be synthesised. Such a compound, which would be devoid of any damaging effect on the intestinal mucosa and which would reduce the absorption of carbohydrates, fats and proteins, might prove of value in the treatment of obesity or of atherosclerosis.

The results obtained with the quaternary ammonium homologues indicate the importance of the length of the alkyl chain in determining activity. Other changes in the molecule, however, must also be considered (Nissim, 1960c). In the present experiments, not all the differences between the two samples of cetrimide could be explained on the basis of differences in chain length. A comparison of the activities of the two cetrimide samples with those of the pure homologous series would suggest that sample A may be composed largely of the shorter chain compounds, and sample B of chain lengths $C_{(10)}$–$C_{(20)}$. At least one significant anomaly would remain unexplained, however, according to this hypothesis. None of the pure homologous compounds were as active in inhibiting butyrate absorption as sample A of the so-called 'cetrimide', and the

difference between the percentage absorption of 22·3 $\pm$ 1·6 for 16 animals and the percentage absorption of any of the homologues, $C_{(10)}$–$C_{(20)}$, gave P $<$ 0·05. Furthermore, the damage to intestinal villi produced by the pure $C_{(12)}$ and $C_{(14)}$ homologues was appreciably greater than that produced by the no less active sample A of cetrimide. It is quite probable that differences exist in the molecule, such as the number of methyl groups, which could also partly contribute to the differences in activity. Further, the long chain quaternary ammonium compounds would be expected to form micelles in solution, and this may also be associated with reduction in activity.

According to the specification of the British Pharmacopoeia 1953, cetrimide consisted largely of the $C_{(16)}$ compound with only small amounts of the other homologues and was soluble in 10 parts of water. In the 1958 specification, cetrimide B.P. contained a mixture of the $C_{(12)}$, $C_{(14)}$ and $C_{(16)}$ compounds, which on drying gave 94–100% of bromide calculated as the $C_{(14)}$ homologue, and this was soluble in only two parts of water. Lastly, according to the British Pharmacopoeia 1963, cetrimide contains mainly the $C_{(14)}$ compound with only small amounts of the $C_{(12)}$ and $C_{(16)}$ homologues. This gradual replacement of the $C_{(16)}$ by the $C_{(14)}$ compound in cetrimide B.P. and the resulting increase in solubility was noted by Jones (1963), for these changes affected the formation of the cetrimide salts of nucleic acids during their isolation. The present investigation shows that preparations supplied as $C_{(16)}$ have differed, and indicates that differences beside chain length are involved.

Finally, since the difference in the chemical nature between samples A and B has not yet been elucidated, it seems essential that the identity of samples of cetrimide, intended for absorption and other biological studies involving protein binding, should be determined by prior biological assay in the rat.

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