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THE EFFECT OF CETYLTRIMETHYLAMMONIUM BROMIDE AND SOME RELATED COMPOUNDS ON TRANSPORT AND METABOLISM IN THE INTESTINE OF THE RAT *IN VITRO*

BY C. B. TAYLOR

From the Smith Kline and French Research Institute, Welwyn Garden City, Herts

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Nissim (1960*a*, *b*, *c*) has demonstrated that cetyltrimethylammonium and some other surface-active ions inhibit intestinal absorption *in vivo*. This work is of interest first since Nissim has suggested that such substances might be used therapeutically in the treatment of obesity, and secondly because the elucidation of their mode of action may throw light on the physiology of absorption. The purpose of the experiments described here was to investigate further the specificity of action of cetyltrimethylammonium bromide (cetrimide) towards several processes of absorption and metabolism in the intestine and, in addition, to examine the effect of some related substances on absorption. For this purpose it was convenient to use *in vitro* methods since, by means of these, absorption and metabolism can be measured in the same preparation.

METHODS

Animals. Rats of the Wistar strain, weighing between 180 and 220 g were used; they had not been deprived of food.

Anaesthesia. In all the preparations described the animals were anaesthetized with ether before removal of the intestine.

In vitro *intestinal preparations.* For the measurement of intestinal metabolism and the rate of transport of glucose, water and methionine across the intestinal wall, the preparation used was the everted sac of rat intestine, first described by Wilson & Wiseman (1954*a*). The modifications of this method, developed by Parsons, Smyth & Taylor (1958) specifically for studying inhibition of intestinal function, have been closely followed.

Saline. Since considerable importance is to be attached to a comparison of the effect of cetrimide on transport and metabolism in separate experiments, it was thought imperative to use the same kind of saline throughout. Since respiration cannot conveniently be measured in bicarbonate saline, Krebs–Ringer phosphate saline (Krebs, 1933) was used. The gas phase was 100% O_2 and, except where otherwise stated, the concentration of glucose in the saline was initially 500 mg/100 ml.

Measurement of transport. Sacs of jejunum were used, the position of the segment chosen being such that its proximal and distal ends were 10 and 40 cm from the pylorus. The sacs, filled with 3·0 ml. saline (serosal fluid), were placed in 15 ml. saline (mucosal fluid) contained in a 50 ml. conical flask. The flasks were gassed and shaken for 1 hr at 80 c/min in a thermo-

static shaker-bath maintained at 37·5° C. At the end of the incubation period the concentration of glucose, and where appropriate that of methionine, present in the initial fluid and final mucosal and serosal fluids, were estimated. These values, together with the weights of the sacs full and empty at the beginning and end of the experiments, enabled the calculation of the movement of glucose, water and methionine across the intestine. The effect of inhibitors was determined by comparing the transport when inhibitor was present initially at the same known concentrations in both mucosal and serosal fluids, with the transport in control experiments carried out in the absence of inhibitor. In order to provide adequate control of the day-to-day variations in the activity of the preparations, the experiments were carried out in groups of either three or five, containing one or two controls respectively.

Glucose utilization. In these experiments the concentration of glucose in the saline was initially 400 mg/100 ml. The procedure was the same as in the transport experiments until the end of incubation. The flasks were then heated at 100° C for 2 min and the intestine ground up with sand and water. The extract of intestine, together with serosal and mucosal fluids and all washings, was made up to a known volume, and samples taken for the estimation of glucose and lactic acid. Control experiments were carried out in which the initial content of glucose and lactic acid in the intestine was measured.

Trans-intestinal potential. The potential across the intestine was measured by a method similar to that used by Barry, Dikstein, Matthews & Smyth (1961). A segment of everted intestine about 5 cm long was tied off at one end and filled with Krebs–Ringer phosphate saline (serosal fluid), and a polythene agar–0·9 %-NaCl bridge was tied into the other end. The sac so formed was suspended in a Perspex jacketed bath containing saline (mucosal fluid). The bath was maintained at 37·5° C by water circulated through the jacket. The mucosal fluid was mixed and oxygenated by a fine stream of oxygen from a polythene tube let into the floor of the bath. Electrical contact with the mucosal fluid was made with a second agar–0·9 %-NaCl bridge. The two bridges were led into separate flasks of saturated KCl, each containing identical standard calomel electrodes. The potential difference between the two calomel electrodes was measured by means of an E.I.L. 33B Vibron Electrometer. Readings of potential were taken at frequent intervals and, in some experiments, a continuous record was obtained by coupling the output from the electrometer to a Sunvic 10 S chart recorder.

Respiration in everted sacs. Measurements of respiration were made by conventional manometric techniques, using sacs approximately 2 cm in length, incubated in 3·0 ml. phosphate saline containing glucose (500 mg/100 ml.). The side arm contained 0·5 ml. saline with inhibitor at seven times the final concentration required in the main compartment, and 0·2 ml. KOH, 10 g/100 ml., was present in the centre well. The manometers were gassed with oxygen and allowed to equilibrate for 10 min at 37° C. Respiration was measured for 30 min, the inhibitor was then tipped into the main compartment and respiration measured for a further 60 min. Inhibition was estimated by comparing the rate of respiration in the presence of inhibitor (30–90 min) with that of the control period (0–30 min). Each flask, therefore, served as its own control. In the absence of any inhibitor respiration during the period 30–90 min was less than that during the control period by $4{\cdot}1 \pm 1{\cdot}6$ % (35 experiments). The results for inhibition were corrected accordingly. In a few experiments carried out in the same way, inhibition was measured for the periods 30–60, 60–90, and 90–120 min.

Respiration in homogenates. Twenty per cent homogenates of rat liver and intestinal mucosa were prepared in 0·3 M sucrose at 0° C by means of a Potter–Elvehjem homogenizer. The mucosa was scraped from everted intestine with the aid of a pair of angled dental forceps, the yield from the intestine of one rat being about 1·5 g. Respiration was measured manometrically, in the presence of various substrates specified in Table 5 and Text-fig. 4. The medium contained, in addition (M): KH_2PO_4 0·01, $MgCl_2$ 0·01, Na-ATP 0·001 and sufficient sucrose to bring the final osmolarity to 300 milliosmolar, the pH of the medium being adjusted to 7·4. The centre well contained 0·2 ml. KOH 10 g/100 ml. The homogenate was added

directly to the main compartment, the manometers were then gassed with 100 % oxygen and allowed to equilibrate at 37° C for 10 min. Oxygen uptake was measured for 60 min.

When the effect of cetrimide on respiration was to be measured, the inhibitor was present in the main compartment from the beginning of the experiment.

ATPase and alkaline phosphatase activities in microsomes of intestinal mucosa. Twenty per cent homogenates of intestinal mucosa in 0·3 M sucrose were prepared as described above. This and the following procedures were all carried out at 0° C. The homogenates were centrifuged at 10,000 *g* for 15 min to remove cell debris and mitochondria and the supernatant fluid was further centrifuged at 74,000 *g* for 60 min to bring down the microsomes. The pellet of microsomes was resuspended in a volume of sucrose approximately twice the original volume of tissue used. The protein content of this suspension varied in different preparations from 3 to 5 mg/ml. ATPase activity was estimated by determining the liberation of inorganic phosphate from ATP at pH 8·0 and 37° C. The reaction was carried out in tubes containing 1 ml. media consisting of 5 mM ATP, 25 mM TRIS : HCl buffer, pH 8·0, and KCl, NaCl and $MgCl_2$ at various concentrations stated in the results, together with sufficient sucrose to make the final solution 300 milliosmolar. After the tubes had been allowed to equilibrate in a water-bath at 37° C for 5 min, the reaction was started by the addition of 0·1 ml. microsomal suspension and stopped 10 min later by the addition of 1·0 ml. 10 % trichloroacetic acid. Controls were carried out to determine the amount of hydrolysis of ATP in the absence of microsomes and also to determine the amount of inorganic phosphate in the microsomal suspension.

Alkaline phosphatase was estimated at pH 10·0, the substrate *p*-nitrophenyl phosphate being used. The reaction was carried out in tubes containing 0·5 ml. 0·016 M disodium-*p*-nitrophenylphosphate and 0·5 ml. M 2-amino-2-methyl-1;3-propanediol, buffered to pH 10·0 with HCl; this latter solution also contained 0·004 M-$MgCl_2$. The required quantity of cetrimide was added in 0·1 ml. and the same volume of water added to control tubes. The tubes were allowed to equilibrate for 10 min in a water-bath at 38° C. The reaction was started by adding 0·1 ml. microsomal suspension (diluted with 0·30 M sucrose to contain 0·2–0·4 mg protein/ml.) and stopped by adding 4·0 ml. 0·25 N-NaOH. The *p*-nitrophenol liberated was estimated spectrophotometrically at 410 mμ.

Histology. 'Everted sacs' were incubated for 60 min in the presence of various concentrations of inhibitors as described for the experiments on transport. Particular care was taken to avoid any injury to the mucosa in manipulating the tissue at the end of incubation. Sacs were handled only by their ligatures and were not allowed to touch any surface. The sacs were fixed in Bouin's solution immediately after the serosal fluid had been released by cutting through the intestine. The sections prepared were stained with haematoxylin and eosin.

Surface activity. The reduction of the surface tension between air and water was measured at room temperature by means of a Du Noüy tensiometer, the solutions of inhibitors being made up in distilled water.

Chemical estimation. All the chemical estimations were carried out spectrophotometrically by the methods indicated as follows: glucose (Nelson, 1944); lactic acid (Barker & Summerson, 1941); methionine (McCarthy & Sullivan, 1941); phosphate (Fiske & Subbarow, 1925); protein (Aldridge, 1957).

Expression of results. For experiments with everted sacs of intestine the convention of Parsons *et al.* (1958) has been followed, i.e. disappearance of a substance from the mucosal fluid (mucosal transport) and appearance in the serosal fluid (serosal transport) are deemed positive. The term 'glucose loss' is used for the net loss of glucose from both fluids but 'glucose utilization' denotes the loss of glucose from the whole system, the tissue being included in the final analysis. The results are expressed per gram initial wet weight of tissue per hour.

RESULTS

The effect of cetrimide on transport

Glucose and water. Table 1 shows the effect of cetrimide on the movement of glucose and water in the everted sac preparation of rat intestine. The active transport typical of this preparation is clearly demonstrated by the control experiments in which glucose is transported across the intestine from mucosal to serosal fluid against the large concentration gradient produced by this movement. At low concentrations (5×10^{-5} M and 10^{-4} M) cetrimide does not depress glucose transport—in fact the figures suggest that there may be a slight stimulation of this process. At higher concentrations ($2{\cdot}5 \times 10^{-4}$ M and above) cetrimide causes inhibition of

TABLE 1. The effect of cetrimide on transport of glucose and water in everted sacs of rat intestine. Initially the same concentration of glucose (500 mg/100 ml.) was present on both sides of the intestine. Serosal transport (increase of substance in the serosal fluid), mucosal transport (decrease of substance in mucosal fluid) and glucose loss (net loss of glucose from the two fluids) are expressed as ml. or mg/g wet weight of tissue/hr ±S.E. of mean. C.A.F. is used as an abbreviation of concentration of glucose in the absorbed fluid (see text)

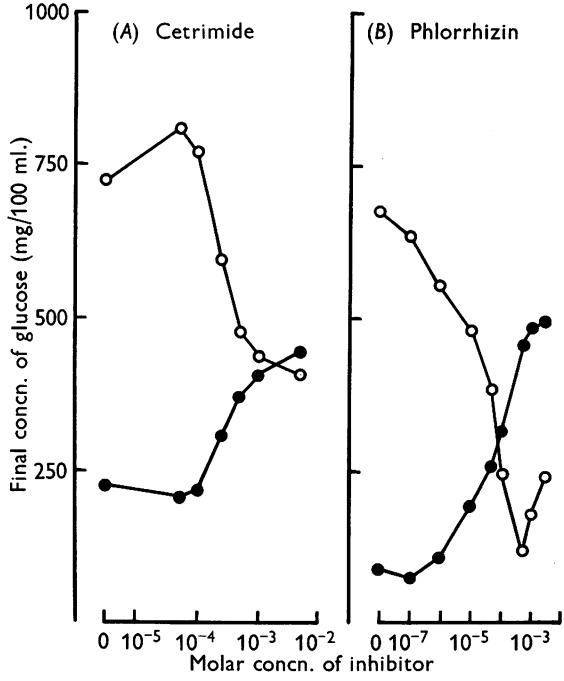
Concn. of cetrimide (M)	No. of expts.	Glucose transport		Glucose loss (mg/g/hr)	Water transport		Final glucose concn.		C.A.F. (mg/ 100 ml.)
		Serosal (mg/g/hr)	Mucosal (mg/g/hr)		Serosal (ml./g/hr)	Mucosal (ml./g/hr)	Serosal (mg/ 100 ml.)	Mucosal (mg/ 100 ml.)	
Nil	15	8·72±0·65	27·65±1·19	18·93±0·78	0·60±0·04	0·95±0·05	725±20	227±10	2910
5×10^{-5}	5	9·96±1·20	28·90±1·90	18·96±0·93	0·55±0·02	0·89±0·05	811±41	207±12	3247
10^{-4}	8	9·40±0·96	29·74±1·35	20·34±0·93	0·55±0·04	0·94±0·06	774±31	220±13	3164
$2{\cdot}5\times10^{-4}$	8	3·99±0·47	19·38±1·31	15·39±0·76	0·39±0·03	0·64±0·07	599±13	309±7	3028
$5{\cdot}0\times10^{-4}$	5	1·00±0·60	13·12±1·64	12·12±1·05	0·24±0·05	0·48±0·10	478±24	370±13	2733
10^{-3}	5	−0·62±0·16	7·68±0·46	8·30±0·67	0·09±0·03	0·21±0·08	439±15	408±7	—
$5{\cdot}0\times10^{-3}$	4	−1·48±0·18	5·30±0·53	6·78±0·66	0·02±0·01	0·10±0·07	411±13	445±4	—

serosal transport, mucosal transport and glucose loss. At any effective concentration of cetrimide serosal transport is depressed to a greater extent than mucosal transport, and at 10^{-3} M serosal transport is negative —that is, glucose is actually lost from the serosal fluid, while mucosal transport is still considerable.

The inhibition of active glucose transport can also be seen in changes in the final concentrations in the two fluids. Glucose is present initially in both fluids at 500 mg/100 ml. and in the absence of inhibitor the final mucosal and serosal concentrations are 227 and 725 mg/100 ml. respectively. Cetrimide, at concentrations of $2{\cdot}5 \times 10^{-4}$ M and greater, causes a progressive fall in the final serosal concentration. Conversely, the inhibitor causes a rise in final mucosal concentration. In general, therefore, with increasing concentrations of inhibitor the final glucose concentrations tend towards equality, and only at very high levels of cetrimide is the final serosal concentration less than the final mucosal concentration; even then, the reversal of the gradient is only small (34 mg/100 ml.). Since this point

is of considerable importance in distinguishing the action of cetrimide from that of phlorrhizin, comparable curves for the effect of the two substances on final concentrations are shown in Text-fig. 1.

Water transport is inhibited by cetrimide over the same range of concentrations as glucose transport and to approximately the same extent.



Text-fig. 1. The effect of cetrimide (*A*) and phlorrhizin (*B*) on the final mucosal (●) and serosal (○) glucose concentrations, in sacs of everted rat intestine. The initial concentration of glucose was 500 mg/100 ml. in both mucosal and serosal fluids. Here, and in Text-figs. 4, 5 and 6, where concentration of inhibitor is also given in the abscissa, a logarithmic scale is used. The values for phlorrhizin are taken from Parsons *et al.* (1958).

To show this more clearly, and for easier comparison with the results of Parsons *et al.* (1958) for phlorrhizin, the concentration of glucose in the absorbed fluid (C.A.F.) has been included in Table 1. C.A.F., which is calculated as:

$$\frac{\text{amount of glucose absorbed from mucosal fluid}}{\text{amount of water absorbed from mucosal fluid}}$$

and expressed in mg/100 ml., is only given for concentrations of cetrimide up to 5×10^{-4} M, since above this concentration its accurate calculation is precluded by the large percentage variation in water transport. It is a sensitive index of the relative effect of an inhibitor on glucose and water

transport. If both processes are inhibited equally C.A.F. will remain constant, but preferential inhibition of water transport will raise C.A.F.; conversely, preferential inhibition of glucose transport will lower it. The fact that with cetrimide C.A.F. remains constant rules out the possibility of a selective action on the absorption of either glucose or water.

Methionine transport. Cetrimide inhibits active transport of methionine in the intestine *in vitro* (Table 2). Both the appearance of methionine in the serosal fluid and the rise of serosal concentration are strongly inhibited by $2{\cdot}5 \times 10^{-4}$ M cetrimide and abolished by 5×10^{-4} M.

TABLE 2. The effect of cetrimide on methionine transport in sacs of everted rat intestine. Methionine was initially present at the same concentration (100 mg/100 ml.) on both sides of the intestine. Each result is the mean from three experiments

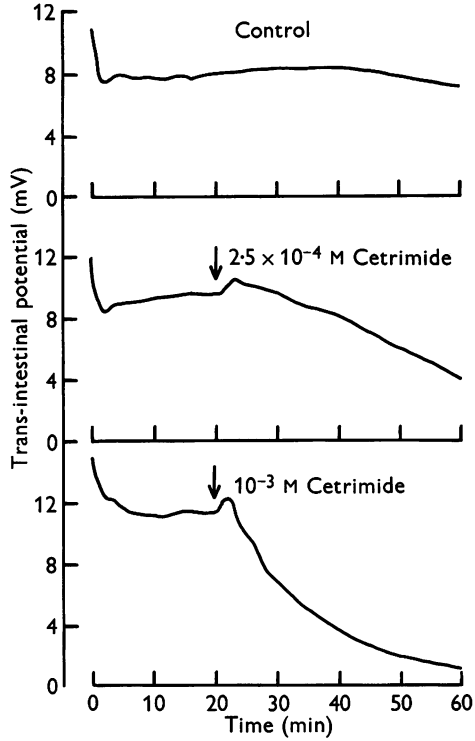
Concn. of cetrimide (M)	Methionine transport		Final methionine concn.	
	Serosal (mg/g/hr)	Mucosal (mg/g/hr)	Serosal (mg/100 ml.)	Mucosal (mg/100 ml.)
Nil	2·82	5·29	163	47
$2{\cdot}5 \times 10^{-4}$	0·80	1·76	118	81
5×10^{-4}	−0·10	1·95	93	80

Trans-intestinal potential. Representative results are shown in Text-fig. 2 for the effect of cetrimide on the potential across the intestine. In the absence of inhibitor the initial potential (serosal side positive) was between 10 and 16 mV. This fell rapidly and within 5 min assumed a relatively constant value of between 8 and 12 mV, which was maintained with little variation for 60 min. When cetrimide was to be tested it was added in a small volume at 20 min. In these experiments the control value was taken as the mean potential during the period 5–20 min. The addition of the inhibitor invariably caused a small increase in potential followed by a fall, the rate of which depended upon the concentration used. The potential was reduced to 50 % of its control value in 37 min by $2{\cdot}5 \times 10^{-4}$ M cetrimide and in 13 min by 10^{-3} M.

The effect of cetrimide on metabolism

Respiration in everted sacs. For the valid comparison of the effect of cetrimide on respiration and transport it is necessary that the tissue should be in contact with the inhibitor for the same length of time in both kinds of experiment. As the duration of the transport experiments was 60 min, the inhibition of the mean rate of respiration was measured during the 60 min following the addition of cetrimide (Table 3). At 5×10^{-4} M cetrimide, a concentration sufficient to cause 88 % inhibition in serosal glucose transport, respiration is depressed by 6·4 %, and even at 10^{-2} M it is only depressed by 20 %.

However, the extent of the inhibition depends upon the duration of the experiment. This may be seen from Text-fig. 3, in which the degree of inhibition during the three 30 min periods following the addition of cetrimide is shown. Inhibition increases with time and even at high concentrations is slow in onset.

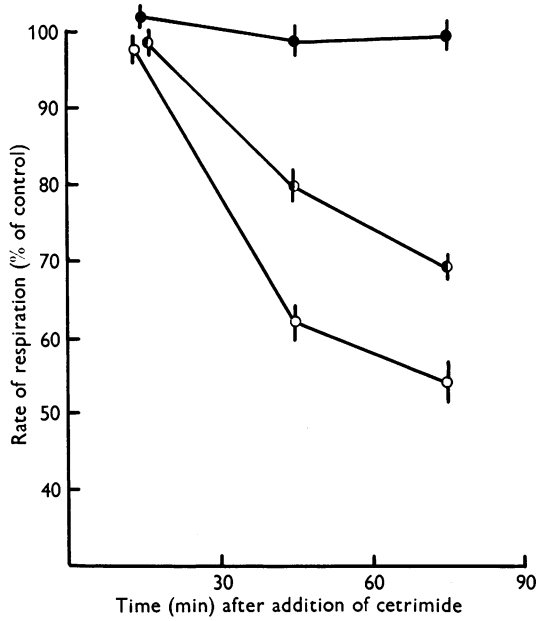


Text-fig. 2. The effect of cetrimide on the electrical potential across sacs of everted rat intestine *in vitro*. The inhibitor was added at 20 min, as indicated by the arrows. The potential is expressed as mV; the serosal side of the intestine is positive.

TABLE 3. The effect of cetrimide on respiration in the intestine *in vitro*. The mean rate of respiration during the 60 min after addition of inhibitor is expressed as percentage of the rate during the initial control period. The number of experiments in each group is shown in parentheses after the standard error of the mean

Concn. of cetrimide (M)	Respiration (% of control)
5×10^{-5}	100·8 ± 4·0 (8)
10^{-4}	97·5 ± 2·8 (8)
5×10^{-4}	93·6 ± 2·7 (8)
10^{-3}	88·2 ± 2·9 (4)
3×10^{-3}	81·3 ± 2·4 (10)
10^{-2}	78·8 ± 2·6 (12)

Glucose and lactate production. Table 4 shows the effect of cetrimide on glucose utilization and lactate production. A concentration of 10^{-4} M cetrimide does not depress glycolysis and there would appear to be a small but significant increase in glucose utilization, similar to that noted in the transport experiments. At 5×10^{-4} M, a concentration which severely inhibits transport of glucose, glucose utilization is not significantly different from the controls and lactate production is depressed by only 7%. Higher concentrations cause more marked inhibition and, for any given



Text-fig. 3. The relationship between duration of contact with cetrimide and inhibition of respiration in sacs of everted intestine. The mean rate of respiration, during the three 30 min periods following the addition of inhibitor, is expressed as a percentage of respiration during the 30 min before addition of inhibitor. Cetrimide 5×10^{-5} M, ●; 10^{-3} M, ◐; 10^{-2} M, ○. The vertical lines represent one S.E. of mean above and below the points.

concentration of cetrimide, glycolysis is inhibited more than respiration and less than transport. In this series of experiments the corresponding values for glucose utilization and lactate production are nearly equal. However, in calculating these results no account has been taken of the values for the initial content of glucose and lactate in the tissue; these were 1·2 and 2·2 mg/g of tissue, respectively. If the control values shown in Table 4 are corrected accordingly the glucose utilization becomes 12·0 mg/g/hr and lactate production 9·0 mg/g/hr. This rate of glycolysis is about 30% lower than that reported by Parsons *et al.* (1958) for similar

experiments carried out in bicarbonate saline, but the recovery of 75% of the glucose disappearing as lactic acid is in agreement with the results of Newey, Smyth & Whaler (1955).

TABLE 4. The effect of cetrimide on glucose utilization and lactate production in the intestine *in vitro*. The standard error of the mean is given for each result

Concn. of cetrimide (M)	No. of expts.	Glucose utilization (mg/g/hr)	Lactate production (mg/g/hr)
Nil	9	$10{\cdot}8 \pm 0{\cdot}32$	$11{\cdot}2 \pm 0{\cdot}33$
10^{-4}	7	$12{\cdot}3 \pm 0{\cdot}42$	$11{\cdot}5 \pm 0{\cdot}53$
5×10^{-4}	6	$10{\cdot}6 \pm 0{\cdot}49$	$10{\cdot}4 \pm 0{\cdot}51$
10^{-3}	3	$7{\cdot}4 \pm 0{\cdot}40$	$8{\cdot}8 \pm 0{\cdot}24$
5×10^{-3}	3	$5{\cdot}2 \pm 0{\cdot}20$	$5{\cdot}3 \pm 0{\cdot}56$

TABLE 5. Respiration in homogenates of liver and intestinal mucosa

Substrate	Concn. (mM)	Q_{O_2} (μl.O_2/mg dry wt./hr) Liver	Intestinal mucosa
α-Ketoglutarate	10	16·7	3·0
Citrate	10	11·7	2·6
Fumarate	10	11·1	2·5
Succinate	10	20·0	3·7
Pyruvate + Fumarate + Acetate	3·3 + 3·3 + 3·3	15·2	2·7

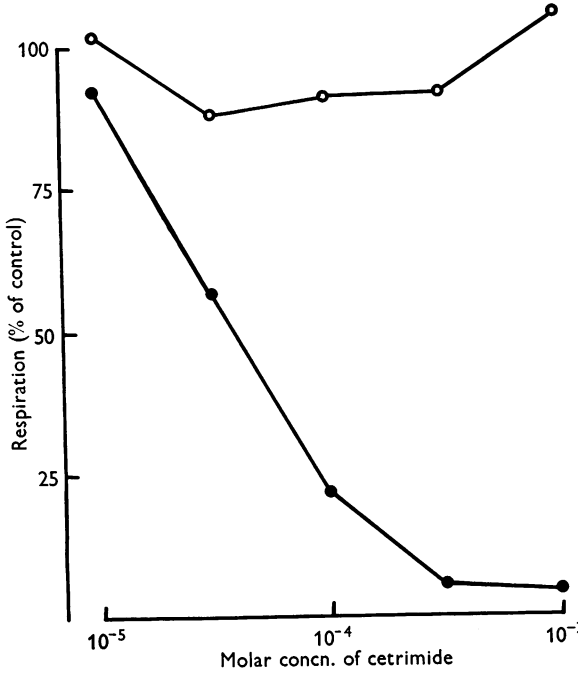
TABLE 6. The effect of homogenate of intestinal mucosa on the respiration of homogenate of liver. The media contained the substrates pyruvate, fumarate and acetate, each at a concentration of 0·002 M. Respiration was measured for 60 min

Volume of 20% homogenate/flask: Liver (ml.)	Intestine (ml.)	Oxygen uptake (μl./flask/hr)
0·3	—	245
0·3	—	240
—	0·3	20
—	0·3	19
0·3	0·1	51
0·3	0·3	60
0·3	0·4	81

The effect of cetrimide in subcellular preparations

Respiration in homogenates. In Table 5 values are given for the rate of respiration of homogenates of liver and intestinal mucosa, in the presence of several substrates. Clearly respiration in mucosal homogenates is relatively slow. Furthermore, it was found that the respiration of liver homogenate is severely depressed by the addition of mucosal homogenate (Table 6). The effect of cetrimide on respiration in homogenates of these two tissues is shown in Text-fig. 4. In liver homogenates respiration is strongly inhibited, 90% inhibition occurring at 3×10^{-4} M. In homo-

genates of mucosa, because of the low Q_{O_2}, the results for respiratory inhibition are not as precise as those obtained with liver. There is, however, no marked depression of respiration even at 10^{-3} M and it is clear that cetrimide is a poor inhibitor of respiration in the mucosal homogenate.

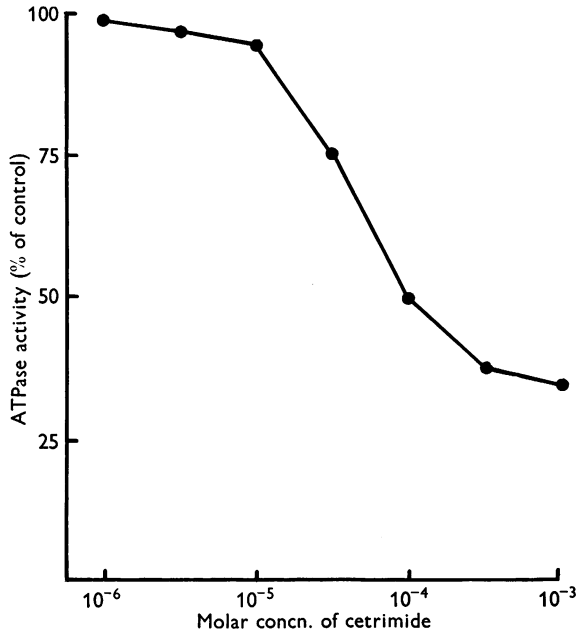


Text-fig. 4. The effect of cetrimide on respiration in homogenates of intestinal mucosa (○) and liver (●). Activity in the presence of inhibitor is expressed as a percentage of control activity. The medium contained pyruvate, fumarate and acetate, each at a concentration of 0·0033 M.

ATPase and alkaline phosphatase. The effect of cetrimide on ATPase activity of microsomes prepared from intestinal mucosa has been determined in a medium containing 5 mM-$MgCl_2$, 50 mM-KCl and 10 mM-NaCl (Text-fig. 5). Slight inhibition occurs at concentrations as low as 10^{-5} M, and at 10^{-3} M the activity is inhibited by 70%. Further investigation revealed that the ATPase activity observed in the presence of sucrose and TRIS buffer alone is stimulated to a very marked degree by the addition of magnesium, potassium or sodium, and that in this respect magnesium is particularly effective (Table 7, cols. 1 and 3). Furthermore, the extent of the inhibition caused by cetrimide depends upon the cation present (Table 7, cols. 2, 4 and 5). At a concentration of 2×10^{-4} M cetrimide has little effect upon the unstimulated activity, or on that elicited by sodium or potassium. On the other hand, ATPase activity observed in the presence

of magnesium is depressed by 36 % at 2×10^{-4} M cetrimide and, as may be seen from the table, that part of the activity apparently due to magnesium stimulation is inhibited by 60 %.

The alkaline phosphatase activity of mucosal microsomes was measured. In the absence of inhibitor the activity was 20·6 μmole *p*-nitrophenol liberated/mg protein/hr. In the presence of 2×10^{-3} M and 10^{-2} M cetrimide, the activities were 20·9 and 18·1, respectively. Even these very high concentrations, therefore, caused no more than 13 % inhibition.



Text-fig. 5. The effect of cetrimide on ATPase activity of microsomes of intestinal mucosa of rat. The incubation medium contained 10 mM-Na^+, 50 mM-K^+ and 5 mM-Mg^{2+}.

TABLE 7. The effect of cetrimide on cation-stimulated ATPase activity in microsomes from intestinal mucosa of rat. The results are the means from experiments carried out in triplicate. Activity is expressed as μmoles inorganic phosphate liberated in 60 min by 0·2 ml. microsomal suspension

	(1) No cetrimide	(2) 2×10^{-4} M cetrimide	(3) Cation stimulation (%)	(4) Inhibition of total activity (%)	(5) Inhibition of stimulated part of activity* (%)
Unstimulated	9·85	9·31	—	5	—
K^+ (100 mM)	16·9	16·0	+ 72	5	5
Na^+ (100 mM)	17·1	16·1	+ 74	6	6
Mg^{2+} (5 mM)	22·5	14·3	+128	36	60

* Obtained by subtracting the unstimulated from the stimulated values.

Histology

Histological sections were made from sacs of everted jejunum, which had been incubated for 1 hr in phosphate saline containing 500 mg/100 ml. glucose (Pl. 1*a*). This shows that the experimental procedures of eversion and incubation cause little damage to the tissue. The villi have a flattened appearance, but the epithelium is continuous and for the most part closely attached to the stroma of the villi. The nuclei of the epithelial cells are elongated and deeply stained.

Incubation with 10^{-4} M cetrimide gives sections similar in appearance to the controls, but 5×10^{-4} M and 10^{-3} M cause severe disorganization of the mucosa (Pl. 1*b* and *c*). At these two concentrations the general outline of the villi is lost and only fragments of epithelium can be seen.

Anaerobic conditions yield sections in which the villi are swollen (Pl. 1*d*). The epithelium is discontinuous and detached from the stroma. The nuclei of the epithelial cells are round, and lightly stained.

Dinitrophenol at 2×10^{-4} M also causes severe damage to the mucosa (Pl. 1*e*). The villi, where they can be discerned, are swollen and the epithelium is ruptured.

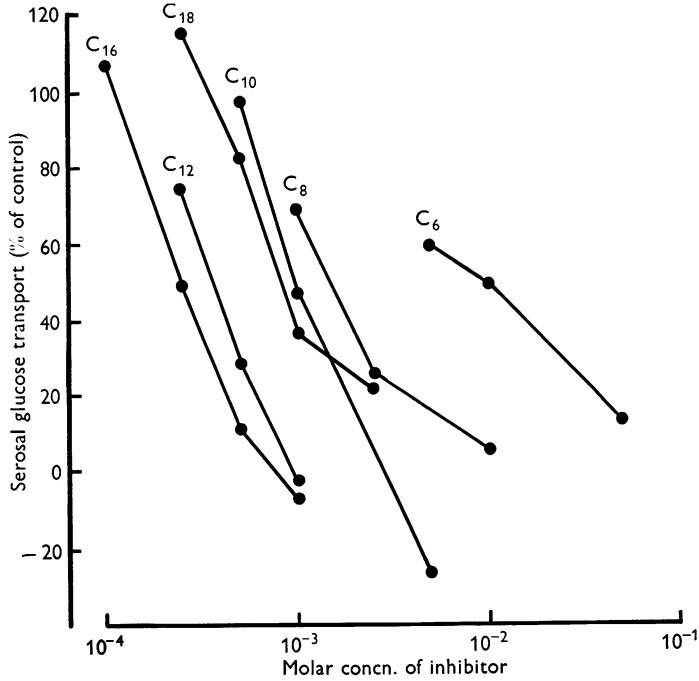
TABLE 8. The effect of quaternary ammonium compounds on serosal glucose transport and surface tension. Compounds of the series $C_nH_{2n+1}N(CH_3)_3.Br$ are designated by their alkyl chain length. (50 dyn/cm corresponds to a fall of 22·4 dyn/cm in surface tension)

Substance	Concn. for 50 % inhibition serosal transport (M)	Relative molar potency (%: cetrimide = 100 %)	Concn. depressing surface tension to 50 dyn/cm (M)
C_{18}	$8{\cdot}2 \times 10^{-4}$	28	10^{-4}
C_{16}	$2{\cdot}3 \times 10^{-4}$	100	$8{\cdot}1 \times 10^{-4}$
C_{12}	$3{\cdot}6 \times 10^{-4}$	64	$2{\cdot}8 \times 10^{-3}$
C_{10}	$9{\cdot}6 \times 10^{-4}$	24	$1{\cdot}1 \times 10^{-2}$
C_8	$1{\cdot}5 \times 10^{-3}$	15	$> 10^{-1}$
C_6	10^{-2}	2	$> 10^{-1}$
C_5	Inactive at 10^{-2}	—	—
C_1	Inactive at 10^{-2}	—	—
Benzalkonium	$3{\cdot}3 \times 10^{-4}$	70	$2{\cdot}6 \times 10^{-4}$
Hyamine 1622	$3{\cdot}2 \times 10^{-4}$	72	8×10^{-4}
Choline	Inactive at 10^{-2}	—	—

Iodoacetate at 5×10^{-4} M caused complete disorganization of the mucosa (Pl. 1*f*). The epithelium is detached and broken, and no villi, as such, can be seen.

Surface activity and inhibition by substances related to cetrimide. A series of substances of the general formula $C_nH_{2n+1}N(CH_3)_3.Br$ has been tested for inhibition of intestinal absorption *in vitro*. The individual members of the series are subsequently referred to by their alkyl chain lengths. The effect of the compounds C_1, C_5, C_6, C_8, C_{10}, C_{12}, C_{16}, C_{18} on serosal transport

is illustrated in Text-fig. 6 and Table 8. For each substance the residual transport at any concentration is expressed as a percentage of the mean control value for that substance. The short-chain compounds (C_1 and C_5) are without effect even at a concentration of 10^{-2} M; potency increases with chain length from C_6 to C_{16}. Compound C_{18} is, however, considerably less active than C_{16}. The optimum chain length would appear, therefore, to be that of cetrimide.



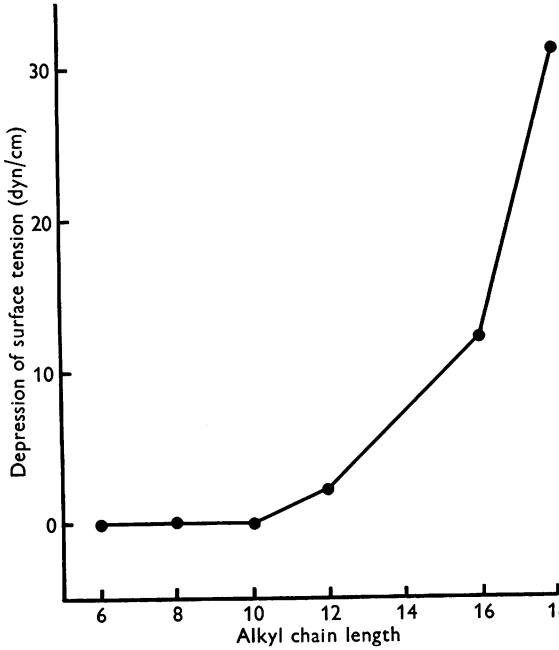
Text-fig. 6. The effect of a series of compounds $C_nH_{2n+1}N(CH_3)_3.Br$ on serosal glucose transport in sacs of everted intestine. Each individual member of the series is denoted by its alkyl-chain length. Activity in the presence of inhibitor is expressed as a percentage of control activity.

Hyamine 1622 ((di-*iso*-butylphenoxyethoxyethyl)benzyldimethyl-ammonium chloride, Rohm and Hass, U.S.A.) and benzalkonium (alkylbenzyldimethylammonium chloride) were both about 30% less effective than cetrimide. Choline was inactive at 10^{-2} M.

The general pattern of inhibition with all the active compounds was similar to that described in detail for cetrimide, transport of glucose and water being inhibited to approximately the same extent.

It is seen from Table 8 that the surface activity of the members of the series increases with chain length. In particular it is noted that, contrary to its effect on the intestine, C_{18} is a more potent surface-active agent than

C_{16}. The relationship between surface activity and inhibition of transport is made clearer if we consider the surface activity of the solutions of these compounds at concentrations which cause the same degree of intestinal inhibition. In Text-fig. 7 the surface activities of solutions giving 50 % inhibition of serosal transport are plotted against alkyl chain length. If the effect of these compounds on the intestine was correlated with surface activity it would be expected that the surface activity of all the solutions



Text-fig. 7. Surface activity and inhibition of serosal glucose transport by a series of compounds $C_nH_{2n+1}N(CH_3)_3 \cdot Br$. For each member of the series the depression of surface tension (air–water), caused by the concentration which inhibits serosal glucose transport by 50 % is plotted against alkyl-chain length.

bringing about 50 % inhibition would be approximately the same. This is clearly not so, since the short-chain compounds give this degree of inhibition with no detectable lowering of surface tension, whereas such inhibition with long-chain compounds is accompanied by a very strong surface activity. In fact cetrimide causes marked reduction in surface tension at concentrations which do not depress intestinal absorption.

DISCUSSION

The experiments described here show that, in the rat intestine *in vitro*, the transport of glucose, water and methionine, together with the transintestinal potential—which is probably a measure of active sodium trans-

port—is inhibited by cetrimide at concentrations of $2{\cdot}5 \times 10^{-4}$ M and above. This is in good agreement with the *in vivo* results of Nissim (1960*c*), who has shown that cetrimide inhibits the disappearance of glucose, water, methionine and butyrate from the lumen of segments of rat intestine perfused *in situ*. The substances studied are all known to be transported actively and, although the transport of some of these may be linked, nevertheless there must be several separate mechanisms. The poor specificity of cetrimide for transport processes in the rat suggests either that all enzymic and carrier mechanisms involved are directly inhibited over the same range of concentrations of inhibitor or—and this seems more probable—that cetrimide interferes with a requirement common to all the processes. The results are therefore discussed in the light of two such requirements, first, the provision of an adequate supply of metabolic energy in a suitable form, and secondly, the maintenance of a relatively impermeable epithelial membrane.

A comparison between the effect of cetrimide on transport and metabolism is complicated by the fact that, for respiration at least, the degree of inhibition depends upon the duration of the experiment. For this reason comparison is made between experiments in which both metabolism and transport were measured for a period of 60 min contact between tissue and inhibitor. It was found that for any concentration of cetrimide, metabolism as measured by glucose utilization, lactate production and respiration was inhibited to a less extent than transport. At a concentration of 5×10^{-4} M, which causes severe inhibition of serosal and mucosal transport, metabolism is scarcely affected and, even at twenty times this concentration, respiration is inhibited by only 20 % Depression of glycolysis and respiration in this preparation may give an over-estimate of the direct effect of the inhibitor on the metabolic enzymes, since interference with the active entry of glucose into the cells may of itself reduce metabolism by deprivation of substrate. In contrast to cetrimide, the substance triethyltin, which is known to interfere directly with both respiration and the efficiency of oxidative phosphorylation, causes strong inhibition of respiration at all concentrations that inhibit glucose transport (Parsons, 1959). The results of experiments with everted sacs suggest, therefore, that the action of cetrimide is not primarily one on respiration and glycolysis.

Measurements of metabolic inhibition in homogenates may be expected to give a more reliable estimate of the direct effect of cetrimide on the processes of metabolism, since in homogenates the inhibitor has free access to all the cellular components, and since the complicating factor of secondary inhibition due to interference with the entry of substrate is absent.

The interpretation of the effect of cetrimide on respiration in mucosal homogenates is difficult, since these preparations yield Q_{O_2} values of about

3·0, whereas values of between 10 and 20 have been reported for everted sacs of rat intestine (Parsons *et al.* 1958; Wilson & Wiseman, 1954*b*). The reason for this is not clear, but the finding that respiration of liver homogenate is depressed by addition of mucosal homogenate may indicate that the cause lies in high proteolytic activity rather than in inadequate provision of co-factors. In intestinal homogenate cetrimide is a poor inhibitor of respiration at concentrations up to 10^{-3} M. It seems unlikely, therefore, that cetrimide could have any direct effect on respiration at the intracellular concentrations attained in the experiments with everted sacs. Respiration in liver homogenate is, however, very strongly inhibited by cetrimide. The inhibitor would, therefore, appear to have a greater intracellular effect in liver than in mucosa, but since only a small fraction of the respiratory activity of the mucosa can be obtained in homogenates, it is possible that the true intracellular effect of cetrimide in the mucosa is the same as that indicated by the experiments with liver homogenates. In general, however, the results of the experiments with homogenates support the view that cetrimide has little direct effect on respiration in everted sacs at the low concentrations sufficient to inhibit transport.

This conclusion must be accepted with some caution since metabolism supplies energy for a number of cellular functions of which transport is but one, and it is probably an over-simplification to regard all these processes as drawing energy from the same single metabolic pool. There may be several separate metabolic units providing energy for separate functions and associated with individual cellular structures. That part of the metabolism concerned with transport may account for a relatively small proportion of the whole, so that even strong inhibition of this fraction might be difficult to detect from measurements of total metabolic activity.

It is possible that cetrimide interferes with the transfer of energy, currently assumed to be by way of energy-rich phosphate, from the cellular respiratory system to the transport mechanisms. In this connexion the effect of cetrimide on ATPase is of particular interest in view of recent evidence (Skou, 1957; Dunham & Glynn, 1961; Wheeler & Whittam, 1961) that there is close coupling of ATPase and certain transport mechanisms, and since these coupled systems are thought to be present in the cell membranes. Microsomal ATPase of the intestinal mucosa is inhibited by cetrimide, in the presence of magnesium, potassium and sodium, and the inhibitor shows some specificity towards that part of the activity stimulated by magnesium. The specificity is emphasized by the fact that cetrimide has little effect on alkaline phosphatase even at very high concentrations. The significance of this inhibition of ATPase in relation to inhibition of transport is difficult to assess, since the role of ATPase in transport is not yet clearly understood. Also, it is by no means certain

that the system studied here is analogous to that which has been shown to be concerned in the transport of sodium in erythrocytes, nerve and kidney, for although it has some similarities to those systems, namely, that it is stimulated by sodium, potassium and magnesium and has an optimum at pH 8·0, the ATPase of rat mucosal microsomes is little affected by high concentrations of ouabain. (Unpublished observations.) While the inhibition of ATPase and transport by cetrimide may be related, the results presented here do not provide any evidence of causal relationship.

The histological studies showed that in the everted sac preparation damage to the mucosa occurs at all concentrations of cetrimide which inhibit absorption. Clearly, if the primary action of the inhibitor is to cause a direct physical effect on the epithelium and to break down the cell membranes, one of the main prerequisites of active transport, namely, a barrier to diffusion, is lost and the question of whether or not individual processes of transport and metabolism are inhibited becomes largely irrelevant. However, the coincident damage to the epithelium is not necessarily the cause of the inhibition of active transport, since the structural integrity of the mucosa itself may be dependent on the energy of aerobic metabolism. This seems probable since dinitrophenol, mono-iodoacetate and anaerobic conditions also cause epithelial damage. It is likely that the processes responsible for maintaining the structural organization of the cell require the composition of the intracellular fluid, which is to a large extent dependent upon active transport, to be kept within narrow limits. Any non-specific inhibitor of transport may therefore be expected to cause injury to the epithelium.

In this discussion the importance of the integrity of the cell membrane, as a condition of active transport in the intestine, is stressed, since it has not previously been stated explicitly. This concept has some relevance to the theory, proposed by Matthews & Smyth (1960) and Smyth (1961), that the transfer of glucose across the intestinal epithelium takes place in two stages. This has been deduced from differences in the effect of various inhibitory conditions on the movement of glucose in the *in vitro* intestine. Phlorrhizin abolishes mucosal transport at concentrations which still permit considerable metabolism of glucose derived from the serosal fluid, giving rise to the characteristic inversion of the final concentration gradient noted in Text-fig. 1. This is due to specific inhibition of the mechanism transporting glucose across the mucosal pole of the cell. Matthews & Smyth have called this mechanism stage one. In contrast, dinitrophenol and anaerobic conditions abolish active transport of glucose, as estimated by serosal transport, while still permitting the entry of glucose into the cells from the mucosal fluid. Matthews & Smyth postulate, therefore, that dinitrophenol and anaerobic conditions inhibit a second stage which is

dependent upon aerobic metabolic energy. According to this hypothesis cetrimide would be classified as a second-stage inhibitor, as would monoiodoacetate and phloretin, since these substances also permit the entry of glucose from the mucosal fluid at concentrations which abolish serosal transport (Taylor, 1956). However, the interpretation of Matthews & Smyth assumes that the permeability of the cells is unchanged by any of the inhibitory conditions. An alternative explanation is that dinitrophenol and anaerobic conditions inhibit the transport processes, including active entry of glucose, by depriving them of the energy of aerobic metabolism, and, at the same time, increase cell permeability, so that the substrate glucose may enter the cells freely from the mucosal fluid. The second-stage inhibition proposed by Matthews & Smyth, therefore, may be loss of the impermeability of the cellular membranes.

The optimum chain length for inhibition of serosal glucose transport in the series $C_nH_{2n+1}N(CH_3)_3.Br$ is C_{16}. A similar optimum has been found by Shelton, van Campen, Tilford, Lang, Nisonger, Bandelin & Rubenkoenig (1946) for the bactericidal activity of this series. The parallelism between the potency of surfactants as inhibitors of intestinal absorption and as bactericides may be extended beyond this series of compounds, since hyamine 1622 and benzalkonium chloride, which are shown here to be strong inhibitors of glucose transport, are also powerful bactericides. Furthermore, it is known that anionic surfactants have bactericidal properties but that non-ionic surfactants have not (Glassman, 1948); and Nissim (1960*b*) has shown that sodium lauryl sulphate given orally to mice causes loss of weight and gastro-intestinal damage similar to that observed with cetrimide, but that the non-ionic surfactants sorbitan monostearate and polyoxyethylene sorbitan mono-oleate are without effect. The comparison of the efficacy of the alkyltrimethylammonium bromides as surfactants and inhibitors of glucose transport suggests that the two properties are not simply correlated, first, since C_{18} is a stronger surfactant than C_{16} but a weaker inhibitor of transport, and secondly, since 50 % inhibition of serosal transport can be obtained with or without lowering of surface tension, according to the chain length of the compound. However, the absence of a correlation between intestinal inhibition and surface activity, as measured by depression of surface tension, does not preclude a mode of action dependent simply upon the affinity of the substances for the surface of the epithelial cells and such affinity might be expected to depend upon chain length and ionic charge.

SUMMARY

1. The effect of cetyltrimethylammonium bromide (cetrimide) and some related compounds on transport and metabolism in the small intestine of the rat has been studied *in vitro*.

2. In sacs of everted intestine transport of glucose, methionine and water are inhibited by cetrimide at concentrations of $2{\cdot}5 \times 10^{-4}$ M and above. The electrical potential across the everted intestine is inhibited over the same range of concentrations.

3. Glucose utilization, lactate production and respiration in sacs of everted intestine are only slightly inhibited by 5×10^{-4} M cetrimide, a concentration sufficient to abolish active transport. Higher concentrations do depress metabolism in this preparation.

4. Respiration of mucosal homogenates is little affected by cetrimide, but respiration of liver homogenate is strongly inhibited (90 % at 3×10^{-4} M). Mucosal homogenate respires slowly (Q_{O_2} about 3·0) and respiration of liver homogenate is depressed by the presence of intestinal homogenate.

5. ATPase activity of mucosal microsomes is stimulated by sodium, potassium and magnesium. Cetrimide inhibits ATPase activity when magnesium is present, but has little effect on the activity elicited by sodium and potassium in the absence of magnesium.

6. Cetrimide at a concentration of 10^{-4} M caused no histological damage to the sacs of everted intestine, but 5×10^{-4} and 10^{-3} M caused considerable injury to the mucosa. Anaerobic conditions, dinitrophenol (10^{-4} M) and iodoacetate (5×10^{-4} M) also damage the preparation.

7. A series of substances of the general formula $C_nH_{2n+1}N(CH_3)_3.Br$ has been tested for inhibition of glucose transport. Measurements were also made of the surface activity of these substances. Compounds C_1 and C_5 were without effect on absorption but compounds C_6, C_8, C_{10}, C_{12}, C_{16} and C_{18} all inhibited glucose transport, the optimum chain length being C_{16} (cetrimide). The ability of these substances to inhibit absorption does not appear to be correlated with their surface activity, since strong inhibition can be obtained with or without depression of surface tension, depending upon the chain length of the compound.

8. The mode of action of cetrimide is discussed.

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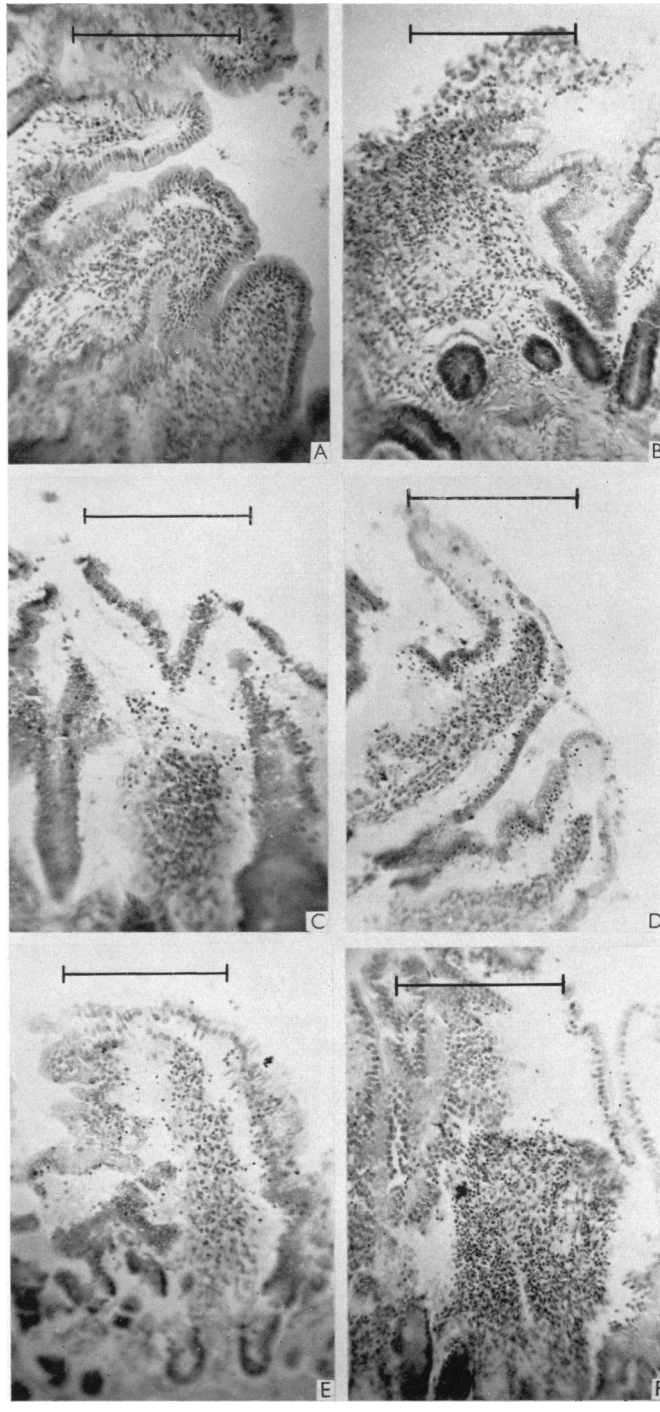
EXPLANATION OF PLATE

Histological sections made from sacs of everted rat intestine, after incubation for 1 hr at 37·5° C in Krebs–Ringer phosphate saline containing glucose 500 mg/100 ml. The tissue was fixed in Bouin's solution and the sections were stained with haematoxylin and eosin. Calibration, 0·20 mm. *a*. Control. *b*. 5×10^{-4} M cetrimide. *c*. 10^{-3} M cetrimide. *d*. Anaerobic conditions. *e*. 2×10^{-4} M dinitrophenol. *f*. 5×10^{-4} M mono-iodoacetate.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