Studies on the active transport of D-glucose and L-histidine by rats and golden hamsters fed on a penicillin-enriched diet

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1. The effect of a penicillin-enriched diet (100 mg or 1000 mg penicillin-G/kg food) on the active transport of D-glucose and L-histidine has been investigated by the use of sacs of everted small intestine of normal young adult rats and golden hamsters. The antibiotic was given for up to several weeks.

2. The penicillin made no difference to the final concentration gradients of D-glucose or L-histidine achieved by rat small intestine, although with the hamster these appeared somewhat improved. Water entry into the serosal fluid remained unchanged.

3. The lengths and the dry weights of the small intestines of both species were not altered by the dietary regimen.

The improved growth sometimes seen in animals given an antibiotic in the diet may be due to the curing of disease, alteration of the intestinal microflora, or to more efficient absorption *per se* of limiting nutrients. If the latter change occurs, it may involve augmentation of an active mechanism or easier passive movement or both. As the intestinal uptake of L-lysine has been reported to be increased by the oral administration of penicillin (Draper, 1958), we have carried out experiments to determine whether or not there was enhancement of the active transport of D-glucose and L-histidine when penicillin-G was added to the food of normal young adult rats and golden hamsters. In addition, the dry weight and the length of the small intestine of control and antibiotic-fed animals were measured.

Our preliminary survey suggests that the inclusion of penicillin-G in the diet may result in better active transport of D-glucose and L-histidine by the small intestine of the hamster. In the rat, the antibiotic seemed to have no such effect.

EXPERIMENTAL

Animals and diet. Male albino rats of about 200 g body-weight (aged about 4 months) and male golden hamsters of about 100 g (aged about 3 months) were employed, and were kept in individual cages during the experimental period and for a week or so before. They were inspected daily to ensure an *ad lib.* supply of water and food. The food, for all animals, was made into a stiff mush by blending 700 ml tap water with 1 kg coarsely ground Diet 86, purchased from Oxoid Ltd, Southwark Bridge Road, London, SE 1, its composition being: soluble carbohydrate 53·4%, protein 20%, fat 3·8%, fibre 3·3%, ash (calcium 0·7, phosphorus 0·8) 5·2% and moisture 14·3%. For the penicillin-treated (i.e. experimental) groups, 100 mg or 1000 mg penicillin-G

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were dissolved in the 700 ml tap water to be added to the food and even distribution of the antibiotic throughout the mush was obtained by thorough mixing. This amount of water was readily taken up by the ground food. A watery extract of the mush was slightly acidic (pH 6–7). Although the penicillin activity was found to be present in 4-day-old mush, each batch (stored at 4°) was used for only 3 days.

The mean daily weight gains by the penicillin-fed rats and hamsters were about 3 g and 0·7 g respectively. Similar changes were seen in the control animals.

Preparation of sacs. The animals were killed by a blow on the head, the abdomen and thorax opened by a midline incision, and the small intestine from pylorus to ileo-caecal junction was washed out with bicarbonate-saline (Krebs & Henseleit, 1932) equilibrated with 5 % CO_2, 95 % O_2. The mesentery was then stripped from the small intestine and the latter, minus the duodenum, everted with the aid of a glass rod of about 1·5 mm diameter. The method was that originally described by Wilson & Wiseman (1954) and in greater detail by Wiseman (1961).

The small intestine of both species was divided into six equal lengths. For the hamster, sacs were made from more or less the whole of each of these segments, whereas for the rat, in which the small intestine is very much longer (75 cm in contrast to about 30 cm), sacs were made from the middle 4 cm or so of each sixth. The sacs were moderately distended with a known amount (about 0·4 ml) of bicarbonate-saline (equilibrated with 5 % CO_2, 95 % O_2) in which was dissolved D-glucose (16·7 mM) and L-histidine (2 mM or 20 mM), the volume of this initial serosal fluid being recorded from the 1 ml tuberculin syringe used. The sacs were then put into 150 ml Erlenmeyer flasks containing 20 ml (initial mucosal fluid) of the same solution as was used for filling them, the air was replaced by 5 % CO_2, 95 % O_2, and the stoppered flasks were shaken (80 oscillations per min, amplitude 5 cm) for 1 h in a Warburg bath kept at 37°. At the end of this period each sac was removed from its flask, its surface drained, and its fluid content (final serosal fluid) collected and weighed. This method enables about 96 % of the serosal fluid to be recovered (Wiseman, 1955). Samples of initial and final mucosal and serosal fluids were analysed for reducing sugar and histidine concentrations. A short length of thread ligature left at one end of the sac facilitated the removal of the sac from the flask.

Occasionally the serosal volume of a sac decreased during the incubation: such sacs were rejected.

Dry weight. After being emptied, the sacs were laid on Whatman no. 50 filter paper and the tissue beyond the ligatures, together with the ligature thread, cut off and discarded. Excess surface fluid was then blotted and the empty sacs were dried for 2 h at 120°, after which time the dry weights were 20–35 mg.

For the values given in Table 5, the whole of each sixth of intestine was dried for 4 h at 120°, then extracted with ether and redried.

D-glucose and L-histidine monohydrochloride. These were commercial samples of chemically pure grade. The sugar was estimated by the colorimetric method of Nelson (1944) and the amino acid by the colorimetric method of Macpherson (1946).

Concentration ratios. The final concentration ratio was the ratio of the D-glucose or L-histidine concentration in the serosal (inner) fluid to that in the mucosal (outer)

fluid at the end of the 1 h incubation. The initial concentration ratio for both D-glucose and L-histidine was 1:1.

Rates of transport of D-*glucose*, L-*histidine and water.* The amounts of D-glucose and L-histidine transported into the serosal fluid during an experiment were calculated (the initial and final concentrations and serosal fluid volumes being known) and the transport rates expressed as μmoles D-glucose or L-histidine entering the serosal fluid per 100 mg dry weight of sac per h.

The rate of transport of water into the serosal fluid during the incubation is given in m-moles water per 100 mg dry weight of sac per h.

The standard deviations of the means were obtained by using the formula for small samples.

RESULTS

Transport by hamsters

Table 1 shows the D-glucose concentration ratios which could be achieved in 1 h by sacs of everted small intestine of golden hamsters on ordinary diet with or without added penicillin-G. Also given are the rates of entry of the sugar into the serosal fluid. It can be seen that in this species (unlike the rat) the ability to actively transport D-glucose was as pronounced in the lower ileum (sac 6) as in the upper jejunum (sac 1) and that there was no mid-intestinal peak. It should be noted that the mucosal and serosal fluids in these experiments contained 20 mM L-histidine, which partially inhibits the active transport of D-glucose, and the values recorded were therefore less than would have been obtained with amino acid-free incubation media (Hindmarsh, Kilby & Wiseman, 1966*a*, *b*). The concentration ratios for L-histidine and its rates of entry into the serosal fluid of these sacs are given in Table 2. In contrast to D-glucose, L-histidine could be concentrated better by the upper than by the lower small intestine of the hamster.

It seems that the dietary penicillin caused a small improvement in the active transport of both D-glucose and L-histidine. More of the individual results in the antibiotic-fed hamsters were above than below their respective mean control values. In addition, the means over the feeding period were, in general, higher than the respective control means. The absorptive behaviour of each region of the intestine may vary depending on its anatomical position, as with L-histidine, and, possibly, on the length of time of administration of the penicillin. The means over the six sacs give a valuable overall evaluation of possible trends. The entry of water into the serosal fluid was unaltered in the experimental animals, falling within the control range of 10–65 m-moles water per 100 mg dry weight of sac per h. Unlike in the rat, the transport of water by the hamster was of the same magnitude throughout the length of the small intestine.

Transport by rats

With the rats, penicillin-G in the food appeared to have no influence on the active transport of D-glucose or L-histidine (Tables 3, 4). This was observed when the dosage was either 100 mg or 1000 mg penicillin-G per kg diet, the lower dose being given for up to 13 days and the higher dose for up to 18 days. The results for the higher level

Table 1. *Effect of* 100 *mg penicillin-G/kg diet on* D*-glucose active transport by sacs of everted small intestine of the hamster*

Initial mucosal and serosal fluid contained 16·7 mM D-glucose and 20 mM L-histidine; initial mucosal volume 20 ml; initial serosal volume about 0·4 ml; length of sacs about 4 cm. Experimental period 1 h. Temp. 37°. Values for controls are means with standard deviations; number of sacs in parentheses. For penicillin-fed hamsters, one animal was used at each time interval, except at 4 weeks

Period of penicillin feeding	Sac 1	Sac 2	Sac 3	Sac 4	Sac 5	Sac 6	Mean (sacs 1–6)
	Final D-glucose concentration ratio (serosal:mucosal)						
0 (controls)	2·30 ± 0·42 (14)	2·15 ± 0·23 (10)	2·04 ± 0·23 (17)	2·12 ± 0·23 (8)	2·23 ± 0·18 (14)	2·02 ± 0·23 (7)	2·14
7 days	2·97	2·48	2·04	2·40	2·25	*	2·39
9 days	2·52	2·09	2·15	2·27	2·15	2·21	2·23
4 weeks	2·40, 2·85	2·16, 2·30, 2·45	1·94, 2·21, 2·28	2·03, 2·40, 2·48	2·18, 2·69, 2·91	2·07, 2·24, 2·54	2·37
10 weeks	2·42	1·82	1·78	2·31	2·62	2·06	2·17
12 weeks	2·45	2·22	1·82	1·86	2·34	2·22	2·15
Mean (7 days–12 weeks)	2·60	2·18	1·99	2·23	2·39	2·19	
	Serosal fluid D-glucose gain (μmoles per 100 mg dry wt sac per h)						
0 (controls)	50·1 ± 18·2 (14)	48·4 ± 7·5 (10)	44·0 ± 12·4 (17)	51·1 ± 11·5 (8)	46·4 ± 9·9 (14)	42·7 ± 12·2 (7)	47·1
7 days	65·9	55·3	38·4	44·7	43·6	†	49·3
9 days	48·4	48·0	41·6	47·1	49·8	45·6	46·8
4 weeks	59·4, 72·7	61·6, 62·9, 63·4	48·5, 54·7, 56·7	62·0, 70·0, 71·1	72·5, 74·8, 76·0	59·6, 65·3, 68·4	64·8
10 weeks	46·7	39·9	40·4	49·9	51·2	41·4	44·9
12 weeks	45·3	29·9	13·5	28·1	44·8	39·4	33·5
Mean (7 days–12 weeks)	54·5	47·1	37·4	47·5	52·8	47·7	

* 2·19 assumed for this missing value. † 47·7 assumed for this missing value.

Table 2. *Effect of* 100 *mg penicillin-G/kg diet on* L*-histidine active transport by sacs of everted small intestine of the hamster*

For penicillin-fed hamsters, one animal was used at each time interval, except at 4 weeks. Other details as in Table 1

Period of penicillin feeding	Sac 1	Sac 2	Sac 3	Sac 4	Sac 5	Sac 6	Mean (sacs 1–6)
	Final L-histidine concentration ratio (serosal:mucosal)						
0 (controls)	1·51 ± 0·22 (8)	1·49 ± 0·12 (10)	1·33 ± 0·11 (11)	1·29 ± 0·07 (8)	1·19 ± 0·12 (8)	1·19 ± 0·08 (8)	1·33
7 days	1·71	1·49	1·47	1·29	1·24	1·03	1·37
9 days	1·60	1·46	1·35	1·23	1·12	1·18	1·32
4 weeks	1·47, 1·52	1·49, 1·55, 1·66	1·25, 1·37, 1·38	1·19, 1·23, 1·40	1·15, 1·17, 1·36	1·10, 1·14, 1·29	1·35
10 weeks	1·86	1·63	1·47	1·48	1·35	1·23	1·50
12 weeks	1·72	1·63	1·43	1·34	1·37	1·30	1·47
Mean (7 days–12 weeks)	1·68	1·59	1·41	1·32	1·26	1·18	
	Serosal fluid L-histidine gain (μmoles per 100 mg dry wt sac per h)						
0 (controls)	28·3 ± 10·8 (8)	30·7 ± 8·0 (10)	27·9 ± 9·3 (11)	28·8 ± 11·5 (8)	18·4 ± 10·6 (8)	19·6 ± 8·7 (8)	26·6
7 days	48·4	39·5	29·0	25·0	21·4	10·2	28·9
9 days	33·8	40·4	32·8	29·9	26·0	25·2	31·4
4 weeks	28·8, 34·3	41·7, 44·8, 45·6	27·7, 38·9, 41·9	33·1, 39·0, 43·7	27·9, 33·8, 37·4	24·8, 30·5, 34·4	35·6
10 weeks	42·0	43·8	41·8	36·9	26·8	18·6	35·0
12 weeks	40·4	29·0	12·8	25·8	32·4	26·4	27·8
Mean (7 days–12 weeks)	39·2	39·3	30·5	31·2	27·9	22·1	

Table 3. *Effect of* 100 *mg penicillin-G/kg diet on* D*-glucose active transport by sacs of everted small intestine of the rat*

Initial mucosal and serosal fluid contained 2 mM L-histidine. For penicillin-fed rats, one animal was used at each time interval. Other details as in Table 1

Period of penicillin feeding	Sac 1	Sac 2	Sac 3	Sac 4	Sac 5	Sac 6	Mean (sacs 1–6)
	Final D-glucose concentration ratio (serosal:mucosal)						
0 (controls)	1·46 ± 0·35 (7)	1·34 ± 0·42 (7)	2·03 ± 0·52 (7)	2·04 ± 0·55 (8)	0·80 ± 0·21 (8)	0·49 ± 0·07 (7)	1·36
4 days	1·48	1·67	2·35	1·52	0·71	0·52	1·38
5 days	1·80	1·68	2·00	1·48	1·09	0·80	1·48
6 days	1·49	1·38	1·78	1·29	0·95	0·38	1·21
7 days	1·64	0·93	1·24	1·73	0·76	0·67	1·16
9 days	1·18	1·27	1·33	1·62	0·80	0·61	1·14
12 days	1·52	1·19	1·76	1·77	1·24	—*	1·34
13 days	1·10	1·04	1·45	1·22	0·87	0·44	1·02
Mean (4–13 days)	1·46	1·31	1·70	1·52	0·92	0·57	
	Serosal fluid D-glucose gain (μmoles per 100 mg dry wt sac per h)						
0 (controls)	16·9 ± 13·1 (7)	14·8 ± 15·2 (7)	23·4 ± 10·0 (7)	26·8 ± 10·9 (8)	0·4 ± 4·8 (8)	−4·7 ± 1·7 (7)	12·9
4 days	26·9	40·5	67·0	28·8	6·6	−4·3	27·6
5 days	27·2	29·5	31·2	9·3	7·5	1·7	17·7
6 days	15·9	12·4	25·6	16·4	3·6	−5·8	11·4
7 days	22·0	0·7	5·1	21·2	−1·0	−2·7	7·6
9 days	7·8	9·0	9·0	21·4	1·3	−0·6	8·0
12 days	32·5	15·2	45·7	35·4	14·7	—†	23·6
13 days	11·9	13·3	25·3	17·1	7·8	−3·9	11·9
Mean (4–13 days)	20·6	17·2	29·8	21·4	5·8	−2·2	

* 0·57 assumed for this missing value. † −2·2 assumed for this missing value.

Table 4. *Effect of* 100 *mg penicillin-G/kg diet on* L*-histidine active transport by sacs of everted small intestine of the rat*

Initial mucosal and serosal fluid contained 2 mM L-histidine. For penicillin-fed rats, one animal was used at each time interval. Other details as in Table 1

Period of penicillin feeding	Sac 1	Sac 2	Sac 3	Sac 4	Sac 5	Sac 6	Mean (sacs 1–6)
	Final L-histidine concentration ratio (serosal:mucosal)						
0 (controls)	2·09 ± 0·31 (7)	2·03 ± 0·30 (7)	2·47 ± 0·32 (7)	2·72 ± 0·71 (8)	2·02 ± 0·48 (8)	1·45 ± 0·49 (7)	2·13
4 days	2·03	2·25	2·79	2·09	2·28	1·87	2·22
5 days	1·65	1·90	1·72	2·01	1·84	1·64	1·79
6 days	2·94	2·84	2·74	3·01	2·64	2·04	2·70
7 days	1·97	1·35	1·72	2·26	1·77	1·86	1·82
9 days	1·65	1·84	1·99	2·48	2·24	2·17	2·06
12 days	2·28	2·18	2·92	2·98	2·08	2·06	2·42
13 days	1·56	1·76	2·04	1·85	2·22	1·74	1·86
Mean (4–13 days)	2·01	2·02	2·27	2·38	2·15	1·91	
	Serosal fluid L-histidine gain (μmoles per 100 mg dry wt sac per h)						
0 (controls)	3·9 ± 1·8 (7)	3·7 ± 2·3 (7)	4·2 ± 1·1 (7)	4·9 ± 1·5 (8)	2·3 ± 1·2 (8)	0·9 ± 0·9 (7)	3·3
4 days	5·6	7·1	9·7	5·9	6·6	3·9	6·5
5 days	3·8	5·2	3·7	2·5	2·9	2·5	3·4
6 days	5·6	4·8	6·5	6·4	3·1	4·3	5·1
7 days	3·8	1·0	1·8	4·5	3·0	2·0	2·7
9 days	2·5	2·5	2·7	4·9	2·7	2·4	3·0
12 days	7·3	4·5	11·2	7·7	4·6	3·3	6·4
13 days	3·3	3·3	5·8	4·8	4·9	1·6	4·0
Mean (4–13 days)	4·6	4·1	5·9	5·2	4·0	2·9	

of the antibiotic have not been included in the tables as they were so similar to those for the lower penicillin dosage. Water transport, which was less in sacs 5 and 6 than in the upper small intestine, also remained within the control range during intake of the diet containing penicillin.

The lower ileum of the rat was incapable of transferring D-glucose against its concentration gradient (confirming the work of Barry, Matthews & Smyth, 1961), although it could do this for L-histidine. For both test materials, active transport was best in the region of sacs 3 and 4. The values in Table 3 would have been somewhat greater if the mucosal and serosal fluids had been free of amino acid (Hindmarsh *et al.* 1966*a*, *b*).

Table 5. *Effect of* 100 *mg penicillin-G/kg diet over a* 10*-day period on the fat-free dry weight of the small intestine of the rat and golden hamster*

Each small intestine was divided into six equal lengths. Values (mg fat-free dry weight/sixth of intestine) are means with standard deviations; the numbers of animals are given in parentheses

Animal	Length 1	Length 2	Length 3	Length 4	Length 5	Length 6
Control rats (8)	194 ± 50	210 ± 48	200 ± 39	187 ± 40	173 ± 43	171 ± 44
Penicillin-fed rats (8)	184 ± 46	191 ± 44	192 ± 35	190 ± 41	161 ± 31	162 ± 24
Control hamsters (8)	51·1 ± 7·9	59·2 ± 10·5	66·9 ± 9·9	61·9 ± 12·7	50·2 ± 9·9	44·1 ± 9·3
Penicillin-fed hamsters (8)	54·0 ± 8·3	59·2 ± 10·1	63·9 ± 9·5	56·4 ± 7·9	43·9 ± 6·6	42·9 ± 8·0

Intestinal weight and length

The fat-free dry weights of the small intestines (divided into six equal lengths) of control and penicillin-fed rats and hamsters are presented in Table 5. The experimental diet eaten for 10 days produced no change in weight per sixth.

Cutting the intestine into six equal parts required gentle stretching of the organ, and this opportunity was taken to measure its length. Despite the low degree of accuracy of this method, the average lengths (mean ± standard deviation) of the small intestines of the control rats (74·5 ± 6·4 cm) and of the experimental ones (73·5 ± 6·9 cm) were remarkably close, as were those of the control (31·0 ± 2·8 cm) and penicillin-fed hamsters (27·6 ± 8·0 cm). These results would suggest that there was no thinning of the intestinal wall in the animals treated with antibiotic.

DISCUSSION

It appears that the dietary penicillin did not improve the active transport of D-glucose or L-histidine by the small intestine of the rat, although it probably did so to a small extent in the hamster. It is possible, therefore, that Draper's (1958) finding that ingestion of penicillin by chicks caused a faster disappearance of L-lysine from the small intestine and caecum might have been due to better active as well as to enhanced passive movement of the amino acid through the intestinal wall. According to Ferrando, Bost & Brenot (1953), penicillin and chlortetracycline increased the rate of absorption of nitrogen from casein hydrolysate introduced into lengths of ileum in anaesthetized rats by altering the epithelial permeability. Also, Baumann's (1956) experiments with isolated intestine of rats and chicks given antibiotic suggested that there might be

more rapid diffusion of xylose. Such an effect would, of course, have had no influence on active transport by the sacs used in the present investigation, where movement of D-glucose and L-histidine was against the concentration gradient.

An alternative explanation for the enhanced uptake of L-lysine by Draper's treated intact chicks is that change in the intestinal microflora was in some way responsible. Carroll, Hensley, Sittler, Wilcox & Graham (1953) have stated that chlortetracycline promoted amino acid absorption by intact rats (from unheated soya-bean meal, an inferior protein source) by virtue of its action on the microflora, and Sauberlich (1954) has claimed that antibiotics may extend the availability of the limiting amino acids when the dietary protein is of poor quality. On the other hand, Kwong, Barnes & Fiala (1962) detected no greater utilization of nitrogen or methionine from unheated soya-bean when given with enough penicillin to produce better growth.

The absence of evidence of decrease in length or dry weight of the small intestine in our rats and hamsters given penicillin was possibly a reflection of there being no 'hypertrophy due to infection' under our control conditions. Thinning of the small intestine of chicks consuming penicillin (Coates, Davies & Kon, 1955) and pigs given chlortetracycline (Braude, Coates, Davies, Harrison & Mitchell, 1955) has been recorded, the phenomenon being more marked in the former species. Whether or not the total thickness of the intestinal wall really limits active, or even passive, absorption of nutrients is, in any event, not certain.

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