Effect of antibiotics on intestinal absorption in mice

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1. The effects of dietary antibiotics (penicillin, neomycin or terramycin) on the absorption of D-glucose, D-galactose, L-arginine or L-histidine by the mouse were investigated by using sacs of entire everted ileum.

2. Compared with the controls, there was generally an increased absorption of all these solutes. Tissue uptake of the solutes remained unaltered. The inward movement of water into the sacs was increased but was generally independent of solute transport.

3. The body-weight decreased slightly and caecal weight increased with penicillin only. The weight of the small intestine decreased with the different antibiotics, and the gut wall became thinner. Faecal fat increased slightly, but not significantly, with neomycin only. Water intake decreased with the different antibiotics.

There is unequivocal evidence that supplementing the diet with antibiotics increases the growth rate of the majority of farm and laboratory animals, but evidence on the mechanism of action of the antibiotics is lacking. Antibiotics act either indirectly on animals by effecting changes in the microbial population of the alimentary tract, or act directly by effecting enzymic and/or endocrine changes and hence modifying cellular metabolism (Luckey, 1959; François & Michel, 1968).

The purpose of this work was to determine whether antibiotics increase intestinal absorption in growing mice, since generally growth responses resulting from dietary antibiotics might be associated with increased intestinal absorption of essential nutrients. The antibiotics selected, (a) K penicillin-G (benzylpenicillin), (b) neomycin sulphate, and (c) terramycin (oxytetracycline hydrochloride), affect the composition of indigenous micro-organisms by generally eliminating either (a) Gram-positive micro-organisms, or (b) Gram-negative micro-organisms, or (c) both groups of micro-organisms (Garrod & Scowen, 1960). When given orally, both penicillin and terramycin are largely absorbed by the small intestine and excreted in the urine; neomycin is not readily absorbed and is excreted in the faeces. Penicillin is also partly destroyed by the acidity of the stomach and by penicillinase in the digestive tract.

Dubos, Schaedler & Stephens (1963) showed that penicillin, terramycin or chloramphenicol (0·1–1·0 g/l.) in the drinking water of mice rapidly eliminated Gram-positive lactobacilli from the alimentary tract which became replaced by Gram-positive enterococci and Gram-negative bacilli. When the treatment was stopped and the animals were given a balanced diet, the original micro-organisms progressively returned to their previous level, but on an imbalanced diet re-colonization was retarded. Little work has been done on the action of antibiotics on intestinal absorption in mammals and birds and the results obtained, which will be briefly discussed later, are sometimes conflicting.

Some other effects (water intake, body-weight, weights of small intestine and caecum; histology of the small intestine and faecal fat content) resulting from antibiotic treatment are also briefly described.

EXPERIMENTAL

Animals and diet

The animals were kept in a constant temperature room at 21° (± 1·5°). Mice (Q strain) of both sexes, weighing about 30 g each and aged 3–6 months, were used. The sexes were kept separately in pairs in standard mouse cages, which were initially disinfected. The animals were provided with a standard pelleted diet (41 B; supplied by Oxoid Ltd, Southwark Bridge Road, London, SE 1); each antibiotic was dissolved in the drinking water (0·3 g/l.) and given separately *ad lib.* The water intake was measured daily and a fresh supply provided. Food intake was not measured because the pellets were frequently crumbled by the mice and lost. Observations suggest, however, that the quantity of food taken remained unchanged in different experiments. The bedding (Vermiculite; supplied by Pan Britannica Industries Ltd, Waltham Abbey, Essex) was changed every other day and cages were cleaned.

The three antibiotics used were given to batches of ten to twelve mice (both sexes) in separate experiments for 14 consecutive days and the mice were then killed. A further untreated batch served as controls. The mice were weighed before and after treatment.

Preparation of intestinal sacs and experimental procedure

Each mouse was first lightly anaesthetized with ether and then killed by a blow on the head. The abdominal cavity was immediately exposed by a midline incision; the small intestine was severed at the pyloric-duodenal and ileo-caecal junctions and removed by carefully dissecting it from mesentery. The lumen of the entire small intestine was flushed with oxygenated 0·9% saline solution, turned inside out with a thin steel rod (Wiseman, 1961), placed in a long, narrow Perspex trough containing oxygenated 0·9% saline solution, lightly stretched and measured, and the duodenum was removed (the ileo-duodenal junction was arbitrarily taken as the point where the intestine is closely attached to the posterior abdominal wall). The everted ileum was drained. and after removal of excess surface moisture by laying it on to filter paper, the tissue was weighed. The ileum was made into a sac by ligaturing the distal end, moderately distending it with a known volume (1·5–2·0 ml) of oxygenated bicarbonate-saline solution (Krebs & Henseleit, 1932) containing the dissolved solute (*serosal fluid*) by means of a 1 ml tuberculin type syringe and blunt needle, and the proximal end was ligatured. The sac was then laid on to dry filter paper to ensure that it was not perforated (small perforations were ligatured; if large, the sac was discarded), re-weighed and transferred to a 150 ml Erlenmeyer flask containing 25 ml of the same test solution (*mucosal fluid*). The flask was gassed (95% O_2 and 5% CO_2) for 3 min (3 l./min) and stoppered. The entire procedure took 12–14 min to complete. The flask was then transferred to a metabolic incubator (Companstat; manufactured by Gallenkamp Ltd, London) and shaken for 1 h (80 oscillations/min; amplitude 2 cm) at a

temperature of 37°. A small device on the stopper allowed the air pressure inside the flask and that of the atmosphere to reach equilibrium quickly without allowing the gas inside to escape.

After incubation, the sac was removed and washed by dipping in a large volume of 0·9% saline solution, then dried and weighed before and after emptying its contents. The serosal fluid increased slightly in weight (volume) in all the experiments. The tissue was washed again and then homogenized in distilled water with a glass homogenizer. The mucosal, serosal and homogenate fluids were diluted appropriately and analysed for sugar or amino acid.

Variations in the results were minimized by standardizing the procedure given above in all experiments, which were routinely started at the same time of day to ensure that digestion had proceeded to the same extent in all animals.

The following determinations were also made. (1) The ileum was removed, trimmed of adhering mesentery and fat and slit open; the tissue was washed in 0·9% saline solution, blotted and weighed. It was then dried at 120° for 6 h and weighed again to determine oven-dry weight. The tissue fat from dried samples was removed by ether extraction, heated at 120° for 2 h and re-weighed. (2) The fresh weight of the caecum was found after it had been opened, and the tissues washed and blotted. (3) Faecal pellets were collected during the last 24 h before killing the mice, and faecal fat was analysed by the colorimetric method of Duncombe (1963). (4) Histological examination of the small intestine of mice in different experiments was made by filling everted sacs with Bouin's fixative, preparing and sectioning the tissue at 8–10 μm, and staining sections with Ehrlich's haematoxylin and eosin and Masson's trichrome. Preparations of non-everted sections were also examined.

Chemical analyses

D(+)-glucose (BDH), D(+)-galactose (BDH), L-arginine monohydrochloride (Koch-Light) and L-histidine monohydrochloride (Koch-Light) were commercial samples of chemically pure grade and all were used without further purification. The initial concentration of each test substance in the mucosal (incubation) and serosal (gut sac) fluids was 5·0 m-moles in all experiments. The sugars were estimated by the colorimetric method of Nelson-Somogyi (Somogyi, 1952) and the amino acids by that of Macpherson (1946).

Calculations

The terms used in expressing the results are those given by Barry, Matthews & Smyth (1961). The final concentration of the mucosal and serosal fluids and gut homogenates are expressed in m-moles/g initial wet weight of sac.

The serosal fluid transfer (net water influx) was calculated from the net increase in original serosal volume (weight) and the initial, not final tissue weight, because after incubation the empty sac sometimes decreased slightly in weight owing to slight damage to the mucosal surface. The final concentration of sugar or amino acid in the gut homogenates is therefore probably slightly higher than that found; also, it was not possible to estimate gut tissue fluid uptake.

The standard deviations of the means were calculated by using the formula for small samples. Results in different experiments were compared by using Student's t test, variances assumed unequal (Bailey, 1959).

RESULTS

Although results were generally slightly higher with females than with males ($P < 0.05$), combined values for both sexes are given.

Water intake

The intake of drinking water with penicillin, neomycin, terramycin and controls was 5.9 ± 1.1, 6.2 ± 0.9, 6.4 ± 1.1 and 9.4 ± 1.4 ml/30 g mouse per day. Hence, the average amount drunk with the supplementary antibiotics was approximately similar; controls drank rather more (ratio 1·6:1). Therefore, the total intake of different antibiotics during 14 days was 24·8 mg for penicillin, 26·0 mg for neomycin and 26·9 mg for terramycin.

Body-weight

Body-weight changes after 14 days on antibiotics averaged -1.5 g/30 g mouse for penicillin, $+2.1$ g for neomycin, $+1.9$ g for terramycin and $+1.8$ g for controls. Thus, the body-weight remained unaffected by neomycin and terramycin treatment, and like the controls, increased by 6–7%, but with penicillin it decreased by 5%.

Intestinal weight and length

The fresh intestinal weight after treatment with antibiotics was 0.97 ± 0.32 g/30 g mouse for penicillin, 1.14 ± 0.31 g for neomycin, 1.16 ± 0.37 g for terramycin and 1.19 ± 0.34 g for controls; the oven-dry weights were 0.18 ± 0.06 g, 0.19 ± 0.07 g, 0.20 ± 0.08 g and 0.23 ± 0.05 g/30 g body-weight respectively. Therefore, the oven-dry weight of the small intestine decreased in mice receiving any of the antibiotics, with penicillin by -22%, with neomycin by -17% and with terramycin by -13% compared with untreated controls. The fat-free (oven-dry) intestinal weight also fell proportionately. The lightly stretched small intestine averaged 50 cm in length; the duodenum was 10 cm long (range 8–13 cm) and the ileum 40 cm long (range 31–50 cm). Although the mice in all experiments were alike in weight (about 30 g each) the ileal length in different individuals varied considerably, and no significant differences in length were found between treated and control animals.

Histological examination

Histological examination of the ileum in different groups showed that in the mice given antibiotics there was a slight, generalized atrophy of the different regions compared with untreated controls. The villi appeared slightly flatter in mice given penicillin than in those given the other antibiotics. Thus, as in most animals treated with antibiotics (Madge, 1969), or in germ-free animals, the weight of the small intestine of the mice was reduced, resulting in a decrease in its thickness, but probably not in its length.

Caecal weight

The fresh caecal weights were $0{\cdot}33 \pm 0{\cdot}06$ g, $0{\cdot}275 \pm 0{\cdot}07$ g, $0{\cdot}275 \pm 0{\cdot}07$ g and $0{\cdot}28 \pm 0{\cdot}06$ g/30 g body-weight for mice given penicillin, neomycin or terramycin and the controls respectively. Hence, compared with that of the controls the fresh caecal weight remained unaltered with neomycin or terramycin treatment, but increased by 15% with mice given penicillin. The caecal content was more liquid with mice fed on neomycin than with those given either penicillin or terramycin. The caecum of males was slightly heavier than that of females (results not statistically significant).

Faecal fat

Faecal fat content averaged $19{\cdot}4 \pm 2{\cdot}9$, $22{\cdot}9 \pm 3{\cdot}6$, $20{\cdot}7 \pm 4{\cdot}1$ and $20{\cdot}2 \pm 2{\cdot}3$ μmoles/g dry weight of faeces for mice given penicillin, neomycin or terramycin and the controls respectively. These differences were not statistically significant, although faecal fat content increased slightly in mice given neomycin.

Intestinal absorption

Sugar transfer and uptake (*Fig.* 1)

Glucose. Since the serosal to mucosal transfer ratio of both sugars after incubation was greater than 1·1 in all experiments, the sugars were transported against a concentration gradient (Wiseman, 1955). There was a slightly greater ($P = 0{\cdot}05$) transfer of glucose in mice treated with penicillin or terramycin compared with the controls (transfer ratios 5·4, 5·5 and 4·8 respectively), but uptake was substantially greater with neomycin treatment (transfer ratio 10·3; $P = 0{\cdot}01$–$0{\cdot}001$). Glucose uptake by the gut tissues during incubation was $2{\cdot}3 \pm 0{\cdot}08$ m-moles/g wet gut weight for penicillin, $2{\cdot}8 \pm 1{\cdot}0$ m-moles for neomycin, $2{\cdot}2 \pm 0{\cdot}9$ m-moles for terramycin treatment and $2{\cdot}5 \pm 0{\cdot}7$ m-moles for the controls; these differences were not statistically significant.

Galactose. Galactose was transported at a higher rate than was glucose, resulting in transfer ratios of 6·9 for penicillin, 7·9 for neomycin, 7·0 for terramycin and 6·0 for the controls. Galactose transport was significantly increased in mice given any of the antibiotics compared with untreated controls, neomycin treatment again being more effective ($P = 0{\cdot}001$) than either penicillin or terramycin ($P = 0{\cdot}05$–$0{\cdot}001$). Tissue uptake of galactose during incubation was $7{\cdot}0 \pm 1{\cdot}6$, $7{\cdot}5 \pm 1{\cdot}5$ m-moles, $6{\cdot}8 \pm 1{\cdot}2$ m-moles and $7{\cdot}3 \pm 0{\cdot}9$ m-moles/g wet gut weight for mice given penicillin, neomycin or terramycin and for controls respectively (results not statistically significant).

Amino acid transfer and uptake (*Fig.* 1)

Arginine. Like the sugars, the amino acids were transferred against a concentration gradient, although at an appreciably reduced rate. With arginine, the transfer ratio for mice given penicillin was 2·2, for those given neomycin 1·6, for those given terramycin 1·6 and for the controls 1·25. The concentrations of arginine in the tissues after incubation were $10{\cdot}8 \pm 2{\cdot}1$ m-moles, $11{\cdot}0 \pm 2{\cdot}3$ m-moles, $12{\cdot}0 \pm 1{\cdot}9$ m-moles and $11{\cdot}4 \pm 0{\cdot}9$ m-moles/g wet weight for mice given penicillin, neomycin or terramycin and the controls respectively (differences not statistically significant). The serosal

transfer in all experiments was low, suggesting that the relatively great accumulation of arginine in the tissue during incubation interfered with the transport mechanism.

Histidine. Histidine was also transferred against a concentration gradient (transfer ratios: penicillin 2·8, neomycin 3·1, terramycin 2·5 and the controls 1·6). In treatment with neomycin or terramycin, serosal transfer was slightly greater than in the controls ($P = 0{\cdot}05$), but with penicillin it was substantially greater ($P < 0{\cdot}001$). Tissue uptake

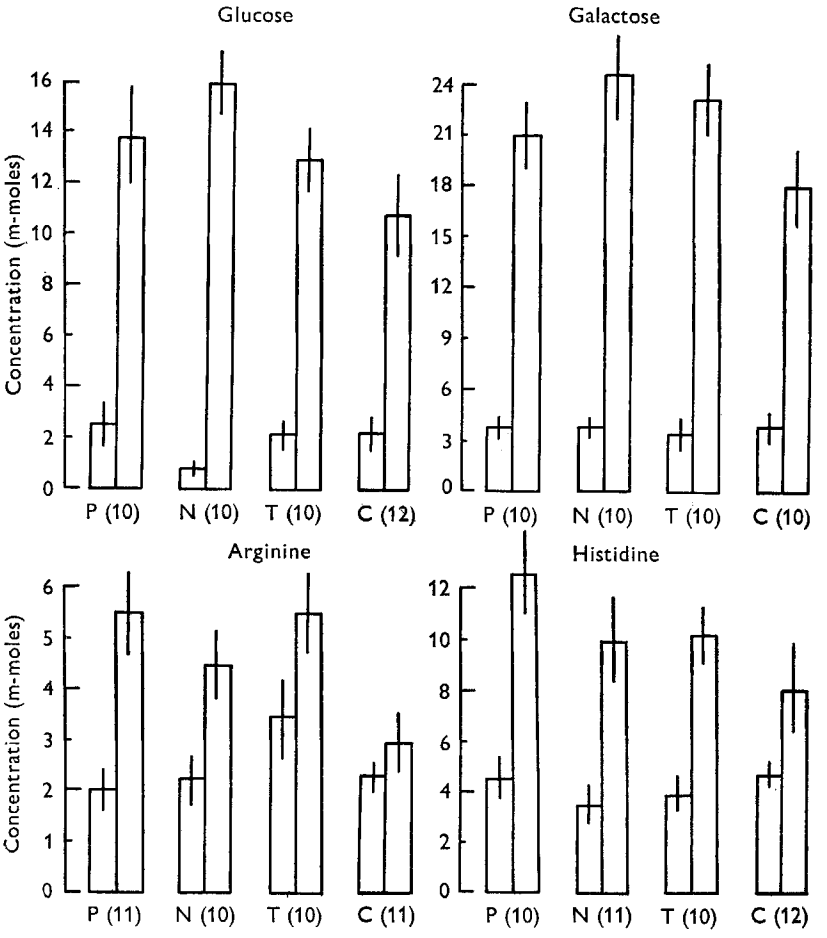

Fig. 1. Intestinal transfer of D-glucose and D-galactose, and L-arginine and L-histidine in mice given a dietary supplement of penicillin, neomycin or terramycin, and in control mice without supplement. Each histogram shows (left) the mucosal transfer and (right) the serosal transfer. The vertical lines give standard deviations. Initial concentration of each substrate, 5·0 m-moles; number of sacs in parentheses. P, penicillin; N, neomycin; T, terramycin; C, controls.

of histidine was $8{\cdot}4 \pm 1{\cdot}6$ m-moles/g wet gut weight for mice given penicillin, $7{\cdot}2 \pm 1{\cdot}3$ m-moles for mice given neomycin, $7{\cdot}5 \pm 1{\cdot}4$ m-moles for mice given terramycin and $8{\cdot}0 \pm 1{\cdot}0$ m-moles for the controls; these differences were not statistically significant. As with arginine, accumulation of histidine in the tissues decreased serosal transfer during incubation.

Water movement (Fig. 2)

Generally, the net serosal fluid transfer was appreciably lower than the net increased serosal solute transfer, indicating that the solute transfer was relatively independent of water influx. Thus, for experiments using sugar as substrates, the water transfer

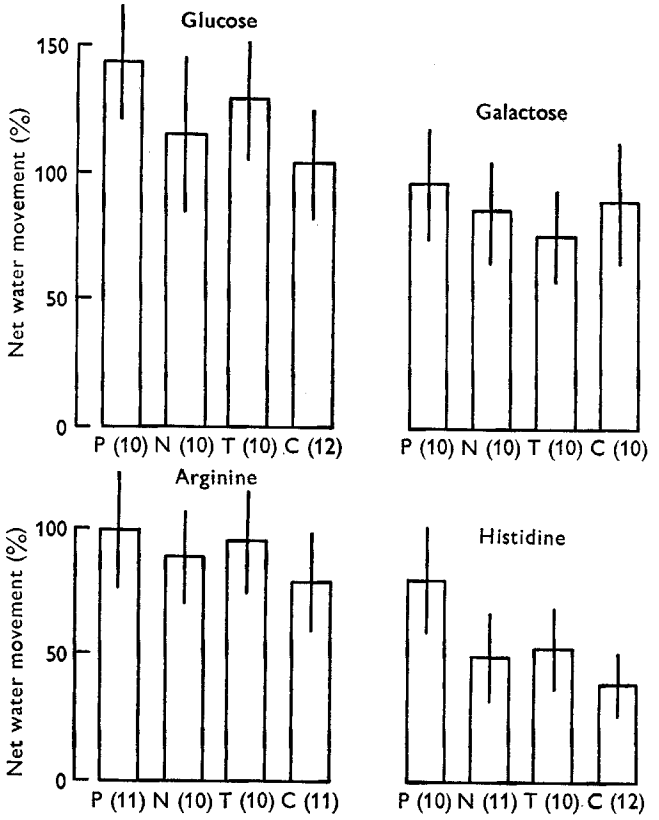

Fig. 2. Serosal fluid transfer in the small intestine of mice given a dietary supplement of penicillin, neomycin or terramycin, and in control mice without supplement. The vertical lines give standard deviations. Number of sacs in parentheses. P, penicillin; N, neomycin; T, terramycin; C, controls.

was 1–1·5 times whereas sugar transfer was 2·5–5 times. With amino acids as substrates, however, the increased solute transfer was lower owing to accumulation in the tissues. Furthermore, there is little correlation between the transfer of either sugar or of either amino acid and the corresponding inward water movement. For example, the serosal transfer of glucose in different experiments was lower than the serosal transfer of galactose, yet the net serosal fluid transfer using glucose was higher than with galactose. Also, the serosal transfer of arginine was lower than that of histidine, yet the net serosal fluid transfer using the first amino acid was higher than using the second.

DISCUSSION

The causes for growth responses following supplementary antibiotics are unknown. There is evidence, however, that antibiotics may suppress unfavourable micro-organisms in the alimentary tract, since antibiotics fail to stimulate growth in either germ-free animals or animals in a clean environment, and since poorly absorbed antibiotics (neomycin, bacitracin) sometimes stimulate growth. Because antibiotics sometimes decrease the thickness of the intestinal wall, in particular the lamina propria of the villi, the intestinal lining is normally in a state of mild physiological inflammation caused by bacterial irritation (Sprinz, 1962; Dubos, Savage & Schaedler, 1967). Moreover, when germ-free animals were experimentally infected with micro-organisms the mucous membrane became thicker and the intestine increased in weight (Gordon & Bruckner-Kardoss, 1961).

Although several workers have investigated the effects of antibiotics on intestinal absorption some of the results have been either inconclusive or contradictory, owing probably to differing experimental methods, to differing concentrations of antibiotics and substrates or to both factors. Generally, however, dietary antibiotics increase intestinal absorption. Thus, the thinning of the intestinal wall resulting from antibiotic treatment appears to be associated with an increase in the absorptive efficiency of the intestine in the utilization of nutrients, although the physiological mechanisms causing increased absorption remain obscure. The increased weight in chicks fed on supplementary antibiotics can be associated with an increase in intestinal transfer of nutrients. However, antibiotics seem to have little effect on the growth rate of rats on a normal diet, but on an imbalanced diet antibiotics improve growth. Absorption experiments with rats fed on a balanced diet with supplementary antibiotics have proved confusing, for absorption may become increased, or decreased or remain unchanged (work on chicks and rats summarized by Madge, 1969). The effects of antibiotics in an imbalanced diet on intestinal absorption have not been investigated, although Suda & Shimomura (1964) found an increased absorption when rats were fed on a protein-deficient diet. Antibiotics are highly lethal to guinea-pigs, and the increased serosal transfer following treatment with antibiotics may be partly attributed to starvation and partly to the effects of the antibiotics (Madge, 1969).

Present investigations using mice showed that different antibiotics generally increased intestinal transport and cell permeability, although penicillin decreased body-weight while neomycin and terramycin had no effect on body-weight compared with untreated controls. Thus, increases in intestinal absorption in mice following supplementation of diet with different antibiotics are not necessarily associated with changes in body-weight. The elimination of normal Gram-positive micro-organisms by treatment with penicillin is not mainly responsible for increasing intestinal absorption since elimination of Gram-negative organisms with neomycin or elimination of both groups of micro-organisms with terramycin had similar effects. Furthermore, intracellular sugars or amino acids appeared to remain unchanged after incubation, suggesting that the action of the antibiotics was mainly limited to increased permeability of the intestinal mucosa and not to changes in cellular metabolism.

Some other effects of antibiotics found in these investigations will also be briefly compared with previous work, mainly with mice. The water intake with different antibiotics in the present experiments was less than in the controls. In contrast, Mills (1960) found that mice drank more with supplementary penicillin, and Barnett & Munro (1968) showed that water intake remained unchanged with supplementary terramycin.

Relatively low doses of oral penicillin slightly decreased the body-weight of mice, but neither neomycin nor terramycin had any effect on body-weight compared with normal controls. Both Basil, Ireland, Thorn, Tomich & Somers (1955) and Mills (1960) found that relatively high doses of oral penicillin decreased body-weight in mice, and Mills (1960) also noted that relatively low doses increased body-weight. Dubos, Schaedler & Costello (1963) showed that oral penicillin decreased weight gain in mice reared under sanitary conditions but increased it in mice obtained commercially. Mirone (1953) noted that the growth of mice increased with supplementary aureomycin on a casein-rich diet, but terramycin had little effect.

Following dietary supplements of antibiotics, the caecum of different animals sometimes increased in size. In this work, the caecum of mice receiving supplementary penicillin became markedly distended and increased in weight, but remained unchanged in those receiving either neomycin or terramycin. Caecal enlargement in mice following antibiotic treatment was also found by Meynell (1963) and Dubos *et al.* (1967).

There was little change in faecal fat content of mice on dietary antibiotics, although it increased slightly with neomycin. Faloon, Noll & Prior (1953) and Faloon (1954) were unable to detect changes in faecal fat content following treatment with either aureomycin, terramycin or chloramphenicol, but it increased in patients following doses of neomycin (Jacobson, Chodes & Faloon, 1960). Eyssen & De Somer (1963) concluded that a temporary loss in body-weight in growing chicks fed on a basal sucrose diet with supplementary virginiamycin was associated with a temporary rise in faecal fatty acids.

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