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EFFECT OF ANTIBIOTICS ON THE 5-HYDROXYTRYPTAMINE CONTENT OF THE SMALL INTESTINE AND OTHER ORGANS IN RATS AND MICE

BY

T. J. SULLIVAN

From the Department of Pharmacology and Therapeutics, St. Thomas's Hospital Medical School, London

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Rats and mice were given antibiotics orally and by subcutaneous injection and the effects on tissue levels of 5-hydroxytryptamine and intestinal bacteria were studied. In mice it was found that antibiotics which caused a large reduction in the bacterial flora of the intestine when given orally also caused a significant increase in intestinal 5-hydroxytryptamine. In rats, neomycin caused a reduction in the urinary excretion of 5-hydroxyindoleacetic acid. In both rats and mice, many antibiotics caused a significant reduction in the weight of the spleen.

Stacey & Sullivan (1957) reported that the intestinal content of 5-hydroxytryptamine in mice was raised after oral administration of a combination of streptomycin and chlortetracycline. They found that rectal swabs from treated mice taken on the day of death were sterile, and suggested that sterilization of the gut might have increased the amount of tryptophan available for 5-hydroxytryptamine synthesis by preventing the bacterial metabolism of dietary tryptophan. The present paper reports further experiments undertaken to amplify these results.

METHODS

Groups of adult male mice weighing 20 to 30 g and of rats weighing 130 to 180 g were matched according to weight and age, and were litter-mates where possible. Control and treated groups received a mixed, balanced diet in cubes, and water *ad lib.*

Antibiotics were given twice daily either orally by stomach tube or by subcutaneous injection. The doses used, roughly five times the adult human therapeutic dose in mg/kg, were as follows:

(1) Neomycin, 215 mg/kg, for rats and mice. Solution for rats 50 mg/ml. and for mice 25 mg/ml.
(2) Streptomycin, 70 mg/kg in a solution of 10 mg/ml. for all animals.
(3) Chlortetracycline and oxytetracycline, 70 mg/kg, in a solution of 10 mg/ml. for all animals.
(4) Chloramphenicol, 105 mg/kg, in a solution of 15 mg/ml. for all animals.

When combinations of antibiotics were used, the same doses of the individual drugs were given. Solutions of drugs were made up, when necessary, in normal saline. Controls were given the same volume of normal saline.

Animals were killed by stunning and bleeding. The whole of the spleen and brain was homogenized and aliquots taken for assay of 5-hydroxytryptamine in both rats and mice. The small intestine of mice, from the pyloric sphincter to the ileo-caecal junction, was similarly treated. The mucous membrane of the small intestine of rats was scraped off with a scalpel

and homogenized. This contains all the 5-hydroxytryptamine (Feldberg & Toh, 1953) and is easier to homogenize than the whole small intestine of the rat.

Blood was obtained from mice for estimation of platelet 5-hydroxytryptamine as previously described (Sullivan, 1960). Platelet-rich plasma was prepared as described by Hardisty & Stacey (1955); the platelets were counted in whole blood and platelet-rich plasma by the method of Baar (1948).

The methods of Udenfriend, Weissbach & Clark (1955) and Weissbach, Waalkes & Udenfriend (1958) were used for the fluorimetric assay of 5-hydroxytryptamine in aliquots of intestine and spleen homogenates. In platelet-rich plasma, 5-hydroxytryptamine was assayed by the method of Udenfriend *et al.* (1955) and for brain 5-hydroxytryptamine the fluorimetric method of Bogdanski, Pletscher, Brodie & Udenfriend (1956) was used. Interference by tryptamine and tryptophan was excluded by measuring the fluorescence in 3 N hydrochloric acid, in which these substances do not fluoresce (Udenfriend, Bogdanski & Weissbach, 1955).

The 5-hydroxytryptamine content of blood platelets is expressed as μg/ml. blood, the figures being derived from the results of assays of platelet-rich plasma as described by Hardisty & Stacey (1955). Results of 5-hydroxytryptamine in other tissues are expressed in μg/kg body-weight rather than in μg/g for reasons previously discussed (Sullivan, 1960). Results expressed in μg/g are similar, but in some instances less reliable. There was some variation between the results obtained for control groups because of differences in body-weight between them.

Urinary excretion of 5-hydroxyindoleacetic acid was estimated on pooled rat urine by the method of Marfarlane, Dalgliesh, Dutton, Lennox, Nyhus & Smith (1956), after collection of urine from rats in metabolism cages for 16 to 18 hr overnight. Urinary creatinine was estimated by the method of Folin (1914).

Details of techniques used for experiments on the recovery of injected 5-hydroxytryptamine as 5-hydroxyindoleacetic acid, for observation of the rate of loss of 5-hydroxytryptamine from incubated pieces of jejunum and for bacteriological studies, have been described previously (Sullivan, 1960).

Throughout the paper means are shown with their standard errors.

RESULTS

Experiments with mice

Results of 5-hydroxytryptamine assays. Table 1 shows the results obtained when streptomycin and chlortetracycline were given orally and by subcutaneous injection to mice for 9 to 11 days. After oral administration, there was a significant rise of intestinal 5-hydroxytryptamine in treated mice, but there was no difference between treated and control groups after subcutaneous injection, although a smaller number of animals were used. The spleen content of 5-hydroxytryptamine was unaffected in both experiments.

TABLE 1

EFFECT OF STREPTOMYCIN AND CHLORTETRACYCLINE GIVEN FOR 9 TO 11 DAYS ON THE 5-HYDROXYTRYPTAMINE CONTENT OF THE TISSUES OF MICE

Mean results are expressed as μg/kg 5-hydroxytryptamine$\pm$standard error. n.s.=difference not significant ($P>0.05$)

Administration of drugs	Tissue examined	No. of mice		5-Hydroxytryptamine content		
		Control	Treated	Control	Treated	Significance
Orally	Small intestine	17	17	131·5$\pm$9·1	172·0$\pm$11·8	$P<0·02$
	Spleen	10	11	69·0$\pm$6·4	69·0$\pm$5·0	n.s.
Subcutaneous injection	Small intestine	7	9	154·0$\pm$19·7	204·0$\pm$25·2	n.s.
	Spleen	6	7	93·0$\pm$10·9	86·7$\pm$20·0	n.s.

Neomycin was given orally to mice, but not parenterally in view of its severe systemic toxicity (Waisbren, 1956). Table 2 shows the results of two experiments of different duration. In both there was a significant rise of intestinal 5-hydroxytryptamine, while the spleen content was significantly raised in the shorter experiment lasting 9 to 11 days. Brain 5-hydroxytryptamine was unaffected in both experiments. There was a significant rise of platelet 5-hydroxytryptamine after 9 to 23 days of treatment. These assays were spread over a longer period of time owing to the technical difficulties of doing more than a limited number of assays at any one time.

TABLE 2

EFFECT OF NEOMYCIN GIVEN ORALLY ON THE 5-HYDROXYTRYPTAMINE CONTENT OF THE TISSUES OF MICE

Mean values of 5-hydroxytryptamine are expressed as μg/kg $\pm$ standard error, except for platelets, where the results are expressed as μg/ml. blood. n.s.=difference not significant ($P>0.05$)

Duration of treatment (days)	Tissue examined	No. of mice		5-Hydroxytryptamine content		
		Control	Treated	Control	Treated	Significance
9 to 11	Small intestine	10	9	127·5±15·6	209·0±28·9	$P<0.05$
	Spleen	10	9	58·5±7·2	98·0±7·2	$P<0.01$
	Brain	8	8	15·3±0·84	16·8±1·02	n.s.
20 to 21	Small intestine	9	10	196·0±11·3	252·0±12·9	$P<0.01$
	Spleen	9	9	95·5±14·0	144·5±24·2	n.s.
	Brain	8	10	16·5±0·54	15·4±0·57	n.s.
9 to 23	Blood platelets	13	16	3·23±0·17	4·42±0·47	$P<0.05$

Chlortetracycline was given orally for 10 to 20 days. It did not affect the 5-hydroxytryptamine content of the spleen or brain but caused a significant rise of intestinal 5-hydroxytryptamine. The mean values of 5-hydroxytryptamine were 163.0±7.72 μg/kg for 31 control mice and 192.0±9.96 μg/kg ($P<0.05$) for 33 treated mice. Tissue levels of 5-hydroxytryptamine in the small intestine and brain were not significantly affected by subcutaneous injection for 10 to 20 days.

The following antibiotics, singly or combined, were given orally for 10 to 20 days, and all but the last two were also given by subcutaneous injection: streptomycin and chloramphenicol; streptomycin and oxytetracycline; oxytetracycline; streptomycin; chloramphenicol. The 5-hydroxytryptamine content of the small intestine and spleen was not significantly altered by any of these drugs by either method of administration.

Effect upon the weight of the spleen. During the course of these experiments it was observed that the weight of the spleen in mice treated with certain antibiotics, orally and parenterally, was less than in controls. This is shown in Table 3. The weight of the spleen in treated mice was reduced in all experiments except those in which neomycin and streptomycin were given. The weight reduction was significant in all cases except in the experiment in which streptomycin and oxytetracycline were given together orally.

The spleens of mice treated with the combination of streptomycin and chlortetracycline orally were compared histologically with the spleens of control mice.

TABLE 3

EFFECTS OF ANTIBIOTICS ON THE WEIGHT OF THE SPLEEN IN MICE

Mean weights are given in g±standard error. n.s.=difference not significant ($P>0.05$)

Antibiotics	Method of administration	No. of mice		Weight of spleen		
		Control	Treated	Control	Treated	Significance
Streptomycin and chlortetracycline	Oral	10	11	0·36±0·03	0·19±0·01	$P<0·001$
	Injection	6	7	0·23±0·03	0·13±0·02	$P\simeq0·02$
Streptomycin and chloramphenicol	Oral	12	12	0·20±0·02	0·10±0·01	$P<0·001$
	Injection	8	10	0·31±0·05	0·14±0·02	$P<0·01$
Streptomycin and oxytetracycline	Oral	6	8	0·35±0·02	0·25±0·06	n.s.
	Injection	6	6	0·23±0·03	0·13±0·02	$P<0·02$
Chlortetracycline	Oral	15	14	0·25±0·03	0·16±0·01	$P<0·01$
Chloramphenicol	Oral	9	9	0·45±0·08	0·27±0·03	$P<0·05$
Neomycin	Oral	19	18	0·32±0·03	0·34±0·04	n.s.
Streptomycin	Oral	10	11	0·36±0·03	0·39±0·06	n.s.

TABLE 4

COMPARISON OF THE EFFECTS PRODUCED BY ANTIBIOTICS GIVEN ORALLY ON THE BACTERIAL FLORA, TISSUE 5-HYDRCXYTRYPTAMINE AND SPLEEN WEIGHT IN MICE

† denotes $P<0.05$. The figures for statistically significant results have been given in the text or in Tables 1–3

Bacterial flora	Antibiotics	% change in total content of 5-hydroxytryptamine		% change in weight of spleen
		Intestine	Spleen	
Sterile	Neomycin (2 experiments)	+45·5†	+58·5†	+5
Sterile or partly suppressed	Streptomycin and chlortetracycline	+31†	0	−67†
	Streptomycin and chloramphenicol	+18	−12	−47·5†
Partly suppressed and altered	Chlortetracycline	+18†	+13	−36†
	Streptomycin	+18·5	+22	+9·5
	Chloramphenicol	+4·5	−2·5	−40†
	Streptomycin and oxytetracycline	+1	−11·5	−28
Altered only	Oxytetracycline	+5	—	—

No difference was observed between the two groups, the architecture and the proportion of lymphoid tissue being the same.

Bacteriological studies. Studies of bacteria grown from rectal swabs showed that, compared with controls, the flora grown from treated animals could be classified into four main groups:

(1) Complete sterility; no growth obtained on aerobic or anaerobic plates.

(2) Complete sterility in some treated animals and a partial suppression of bacterial growth in others.

(3) The intestine was not made sterile in any of the animals. The bacterial flora was partially suppressed in some of the treated mice, while in others the flora was altered qualitatively, but there was no obvious reduction in the number of colonies grown.

(4) The bacterial flora altered qualitatively, but no obvious reduction in the number of colonies grown.

Table 4 shows the bacteriological results obtained in mice treated with the various antibiotics orally, and relates these findings to the percentage changes in the 5-hydroxytryptamine content of intestine and spleen, and to the percentage changes in spleen weight. It will be seen that, in general, the more potent the antibacterial action of the antibiotic or combination of antibiotics used, the greater was the effect on tissue levels of 5-hydroxytryptamine. On the other hand, the effect on intestinal bacteria was apparently unrelated to the effect produced on the weight of the spleen.

In Table 5, similar results are shown for those experiments in which antibiotics were administered parenterally. It will be seen that the antibacterial effect was much

TABLE 5

COMPARISON OF THE EFFECTS PRODUCED BY ANTIBIOTICS GIVEN BY SUBCUTANEOUS INJECTION ON THE BACTERIAL FLORA, TISSUE 5-HYDROXYTRYPTAMINE AND SPLEEN WEIGHT IN MICE

† denotes $P<0.05$. The figures for statistically significant results have been given in the text or in Tables 1–3

Bacterial flora	Antibiotics	% change in total content of 5-hydroxytryptamine: Intestine	% change in total content of 5-hydroxytryptamine: Spleen	% change in weight of spleen
Partly suppressed and altered	Streptomycin and chlortetracycline	+32·5	−35	−43†
	Streptomycin and chloramphenicol	−7·5	−39·5	−54·5†
Altered only	Chlortetracycline	0	—	—
	Streptomycin and oxytetracycline	−14	−39	−45·5†
	Oxytetracycline	+1	—	—

less than when the antibiotics were given orally, and so also was the effect on tissue levels of 5-hydroxytryptamine, but again, neither of these factors appeared to be related to the effect upon the weight of the spleen in treated mice.

Condition of the mice. In general, the condition of control and treated groups of mice remained satisfactory. In nearly all experiments, the control groups gained up to 10% of their body-weight. The effect of antibiotics on the body-weight of treated mice varied, some treated groups gaining up to 12% during the experiment, others losing a similar amount of weight.

There was no relationship between changes in the body-weight of treated mice and the effect of antibiotics on spleen weight. For instance, mice treated orally with chloramphenicol gained 11.5% of their body-weight during the experiment, their mean body-weight at the end being greater than that of the control group which gained 6% of their body-weight during the experiment. But the mean spleen weight of the chloramphenicol-treated group was significantly lower than that of the control mice.

There was no significant difference in the weight of the small intestine between control and treated groups in any of the experiments.

Experiments with rats

Results of 5-hydroxytryptamine assays. Rats were treated with a combination of streptomycin and chlortetracycline orally for 8 to 9 days and with neomycin orally for 21 to 25 days. No significant effect was obtained on the 5-hydroxytryptamine content of the small intestine in either experiment, although the intestinal 5-hydroxytryptamine content of 7 rats given streptomycin and chlortetracycline showed a mean rise of 36% compared with 5 controls, while that of 6 neomycin-treated animals rose 18% compared with 6 controls.

Effect upon the weight of the spleen. The mean spleen weight of 6 rats treated with streptomycin and chlortetracycline was 0.39 ± 0.03 g and of 5 controls 0.53 ± 0.08 g. Although the spleen weight was lower in treated rats than in controls, the difference was not significant.

Bacteriological studies. In both of these experiments, the antibiotics used caused either sterility or partial suppression of the bacterial flora. Streptomycin with chlortetracycline caused a similar degree of antibacterial action in mice, but the antibacterial effect of neomycin was less potent than in mice, in which it caused sterility in all animals.

TABLE 6

URINARY EXCRETION OF 5-HYDROXYINDOLEACETIC ACID BY 10 CONTROLS AND 10 RATS DURING AND AFTER TREATMENT WITH NEOMYCIN GIVEN ORALLY

Standard errors are given with mean results

Duration of treatment (days)		5-Hydroxyindoleacetic acid μg/kg/24 hr		Creatinine mg/kg/24 hr		Ratio of 5-hydroxyindoleacetic acid/creatinine	
		Control	Treated	Control	Treated	Control	Treated
7		146·0	78·5	28·4	25·1	5·2	3·1
15		228·5	94·5	29·8	24·7	7·7	3·8
17		155·0	84·5	28·2	25·4	5·5	3·3
23		110·0	62·0	32·0	25·0	3·5	2·5
Means		160·0 ±24·6	80·0 ±6·9				
		$P<0·05$					
Days after treatment ended	14	134·0	174·0	30·0	33·2	4·5	5·3
	21	156·0	153·0	29·0	27·6	5·4	5·6
Means		145·0 ±7·7	164·0 ±7·4				

Urinary excretion of 5-hydroxyindoleacetic acid. No difference was found between treated and controls when rats were given streptomycin with chlortetracycline, but neomycin treatment caused a significant reduction of 5-hydroxyindoleacetic acid excretion as shown in Table 6. The level of excretion was similar to that of the control group, 14 and 21 days after neomycin administration had been stopped, however.

Additional experiments in antibiotic-treated animals

Incubation of jejunum. Pieces of jejunum from neomycin-treated and control mice were incubated *in vitro* in Krebs Ringer phosphate solution for up to 2 hr.

At intervals of 30 min the 5-hydroxytryptamine content was assayed to study the rate of release from the jejunum. No difference was found between the groups, and both had lost about 50% of the tissue content of 5-hydroxytryptamine by the end of the 2-hr period.

Recovery of injected 5-hydroxytryptamine. Antibiotic-treated and control groups of rats were given 6 mg/kg 5-hydroxytryptamine by subcutaneous injection and the urinary recovery of the amine as 5-hydroxyindoleacetic acid was studied. There was no significant difference between treated and control groups. The recovery from 7 rats treated with streptomycin and chlortetracycline was 34% and from 5 controls 31%. From 7 neomycin-treated rats recovery was 26.5% and from 6 controls 29.5%.

DISCUSSION

With the exception of neomycin, antibiotics only affected the 5-hydroxytryptamine content of the intestine in mice. The absence of any effect on 5-hydroxytryptamine in other tissues indicates that this was a local effect only, due possibly to increased storage of 5-hydroxytryptamine. Neomycin caused a rise of 5-hydroxytryptamine in the spleen and platelets, in addition to the intestine. This might have been due to the fact that the capacity of the intestine for storing 5-hydroxytryptamine was exceeded in neomycin-treated mice, resulting in the release of excess of the amine into the circulation. The results of incubation of jejunum *in vitro,* from which about the same amount of 5-hydroxytryptamine was lost in neomycin-treated and control mice, do not support this explanation, but these findings may not apply *in vivo.* Another possibility is that neomycin caused an increase in 5-hydroxytryptamine synthesis in the tissues. Since very little neomycin is absorbed from the lumen of the intestine, this is unlikely to have occurred.

The results suggest that the effect on intestinal 5-hydroxytryptamine was mediated by the effects the antibiotics produced on intestinal bacteria. After oral administration, the more effective the antibacterial action, the greater was the effect on tissue levels of 5-hydroxytryptamine. When these antibiotics are given by injection, only a small proportion reaches the intestine via the biliary tract (for references see Sollmann, 1957), and it was found that the effect of antibiotics on intestinal bacteria was much less potent after subcutaneous injection than when they were given orally. The antibiotics also had a greater effect on tissue levels of 5-hydroxytryptamine after oral administration than after injection

If this explanation of the mechanism of action of these antibiotics is correct, it is analogous to the effects of antibiotics in promoting growth in various animal species, which is believed to be due to alterations in the bacterial flora of the intestine (Stokstad, 1954). Results obtained from growth experiments suggest that inhibition of bacteria might release essential nutrients for the use of the host, which would otherwise be metabolized for use by bacteria. This mechanism might also account for the rise of tissue 5-hydroxytryptamine in antibiotic-treated mice. It is suggestive that Linkswiler, Baumann & Snell (1951) found that inhibition of intestinal bacteria in rats by chlortetracycline prevented destruction of dietary pyridoxal. A similar, tryptophan-sparing, action was found by Jones & Combs (1951) in chlortetracycline-fed chicks.

There is evidence that some antibiotics are capable of producing pharmacological effects (Nakatsuka & Matsumoto, 1952; Rokos, Burger & Procházka, 1958; Sobek, 1959), and the possibility that the effects on tissue 5-hydroxytryptamine were due to the direct pharmacological actions of antibiotics cannot be excluded. This seems unlikely, however, especially since neomycin, which produced the greatest effect, is absorbed only in negligible amounts from the intestine.

The effect of antibiotics upon the spleen weight in mice occurred after both oral administration and subcutaneous injection. It was unrelated both to the effect on tissue levels of 5-hydroxytryptamine and to the antibacterial effects of the drugs. The latter makes it unlikely that the effect was mediated by alterations in the intestinal bacterial flora, and since it has been shown that the size of the spleen is unchanged in germ-free animals compared with controls (Stokstad, 1954), it is unlikely that the phenomenon was due to the prevention or suppression of systemic infections. The most likely explanation is that the reduction in spleen weight was due to direct action by the antibiotics used, and this is supported by the fact that those antibiotics which are not absorbed after oral administration, namely, neomycin and streptomycin, did not reduce the spleen weight.

In rats, the effects of antibiotics on intestinal 5-hydroxytryptamine were similar to those in mice, but the results were not significant at the 5% level. This may have been because the numbers of rats used were too small. Alternatively, if alteration of intestinal 5-hydroxytryptamine is dependent upon changes in the intestinal flora involving specific strains of bacteria, this species variation may be accounted for by differences in the occurrence of these strains in rats and mice.

Anderson, Ziegler & Doeden (1958) reported that when neomycin was given to monkeys in sufficient doses to produce sterilization of the intestine, urinary excretion of 5-hydroxyindoleacetic acid was unaffected. In the experiments described above, neomycin caused a significant reduction in the urinary excretion of 5-hydroxyindoleacetic acid in rats, although streptomycin with chlortetracycline did not affect excretion of this metabolite. Since no significant difference was found in the percentage of injected 5-hydroxytryptamine recovered as 5-hydroxyindoleacetic acid between neomycin-treated and control rats, it is unlikely that neomycin caused any alteration in metabolism.

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