The Effect of Antibiotics on the Intestine of the Chick

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During work on the effect of penicillin on vitamin A metabolism in the chick (Coates, Harrison, Kon, Porter & Thompson, 1952) it was observed that the small intestines of birds given penicillin in the diet appeared thinner than those of their controls. A further investigation was therefore undertaken to study the importance of this phenomenon in the mechanism of antibiotic growth promotion.

The experiments were of two types, the first dealing with the quantitative and qualitative changes in the gut and the second with the possible mode of action of penicillin in reducing gut weight. They have accordingly been presented here in two separate sections.

EXPERIMENTAL AND RESULTS

General methods

Management of the birds. Day-old sex-linked Rhode Island Red × Light Sussex cockerels were used in most of the experiments but occasionally pullets were included. Brown Leghorn × Barred Rock sex-linked pullets and cockerels were used in a few experiments. The birds were housed either in Hearson electrically heated brooders (Coates, Kon & Shepheard, 1950) or in small-animal cages inside isolation units (Coates, 1953). Experimental groups consisted of up to thirty-six birds and were sometimes distributed between two or three brooder compartments.

Diets. The basal diet was the chick mash normally used in this laboratory, which supports excellent growth up to 4 weeks of age. It had the following percentage composition: maize 35, wheat 30, miller's offals 8·5, fish meal 10, dried skim milk 7·5, dried grass 3, dried brewer's yeast 3, limestone 1·5, salt mixture* 0·5, arachis oil (containing 64 i.u. vitamin D and 680 i.u. vitamin A/g) 1.

This basal diet was supplemented with antibiotics or other substances according to the experimental requirements. The amounts added were as follows:

Procaine penicillin	45·5 mg/kg diet
Chloramphenicol	25 mg/kg diet
Arsanilic acid	20 mg/kg diet
Raw beef liver	30 g/kg diet

Measurements of the gut. The chicks were killed in the customary way by breaking the neck. The abdomen was opened, and the gut was removed, slit longitudinally and washed in a beaker of water to remove food particles. It was then blotted lightly

* Salt mixture: $MnSO_4.4H_2O$ 6, KI 0·06, NaCl 93·94%.

on botanical drying paper with care not to damage the mucosa. This operation was always performed by one person to eliminate differences in handling. The gut was weighed to the nearest 0·1 g. Its length was measured by suspending it by the pyloric end against a centimetre scale. For the main experiments the whole of the small intestine from the gizzard to the entry of the caecums was taken. On one occasion the gut was divided in two sections: (*a*) the duodenum, from the gizzard to the point of entry of the bile duct, (*b*) the ileum and jejunum, from the point of entry of the bile duct to the ileo-caecal junction.

Determination of fat. The gut was cut into small pieces and digested with 10 ml. conc. HCl in a water-bath until completely disintegrated. This process took about 30 min. After cooling, the digestion mixture was extracted once with a mixture of 25 ml. diethyl ether and 25 ml. light petroleum (B.P. 40–60°) and then twice with 30 ml. of the solvent mixture. The combined extracts were evaporated to dryness on a steam-bath, the last traces of solvent being removed under reduced pressure, and weighed.

Determination of dry matter. Dry matter was determined by heating the sample in an oven for 2 h at 98°, then cooling and weighing. Further heating for ½ h usually sufficed to bring it to constant weight.

Statistical treatment of results. Direct comparison of gut weight between treated and untreated groups could not be made as the birds receiving penicillin were usually heavier than the controls, and it was to be expected that this increase in body-weight would be accompanied by an increased gut weight. Analyses of covariance (Snedecor, 1940) of gut weight on body-weight were made. From these analyses gut weights adjusted to constant body-weight were calculated. The differences between adjusted gut weights were compared by the *t* test of 'Student' (1908, 1925). In the experiments in the isolation units where only two groups were concerned the significance of the difference was found directly from the covariance analysis.

Part 1. *The effect of penicillin on gut weight*

In all, thirteen experiments were done, lasting 2, 3 or 4 weeks, and the results are shown in Table 1. Invariably the gut weight adjusted to constant body-weight was lower in the penicillin-treated groups, and in all instances this effect was significant. Measurements of length were made simultaneously in many of these experiments, and the results are also given in Table 1. The effect of penicillin on the length of the gut was less striking than on the gut weight; further, the ratio of weight to length of intestine was almost always greater in the untreated birds. It appeared therefore that the decrease in weight of the gut in penicillin-treated birds was not entirely due to a shortening, but also to a thinning of the gut wall. The effect was apparent throughout the entire length of the intestine, as shown by the measurements on portions of gut given in Table 2.

The dry matter of the gut was determined in two duplicate groups of eight birds. The mean content was 18·4 and 18·2% in the controls and 17·7 and 18·8% in the penicillin-treated birds. The difference in weight of the gut was therefore not simply the result of reduced moisture content, but represented a reduction in the total amount of tissue of the intestine.

Table 1. *Effect of penicillin on body-weight, gut weight and gut length in chicks*

				Without penicillin				With penicillin				Adjusted* gut weight			
					Gut				Gut						
Exp. no.	Breed and sex of chick	No. of birds/ group	Age (weeks)	Body-weight (g)	Weight (g)	Length (cm)	Ratio, weight: length	Body-weight (g)	Weight (g)	Length (cm)	Ratio, weight: length	Without penicillin (g)	With penicillin (g)	t†	P‡
1	RIR × LS ♂	10	4	301	13·7	90·4	0·15	339	11·4	86·8	0·13	13·7	10·7	5·7	<0·001
	RIR × LS ♂	10	4	273	13·1	93·6	0·14	302	11·2	83·7	0·13	13·8	11·2	4·9	<0·001
2	RIR × LS ♂	8	4	243	12·6	89·5	0·14	258	10·1	78·2	0·13	12·6	9·6	4·2	<0·001
	RIR × LS ♂	8	4	201	10·7	80·0	0·13	273	11·3	81·3	0·14	12·3	10·2	2·9	0·01<P<0·02
3	RIR × LS ♂	10	4	331	12·5	93·0	0·14	324	10·2	83·0	0·13	12·6	10·5	2·2	0·02<P<0·05
4	RIR × LS ♂	10	4	311	17·2	105·0	0·16	329	10·8	83·0	0·13	17·4	10·5	8·2	<0·001
5	BL × BR ♂ & ♀	20	4	357	14·6	—	—	369	12·6	—	—	14·7	12·5	5·6	<0·001
6	RIR × LS ♂ & ♀	20	4	314	13·5	—	—	334	13·1	—	—	13·8	12·8	2·5	0·01<P<0·02
7	RIR × LS ♂	24	4	270	11·4	—	—	318	11·9	—	—	12·0	11·2	2·0	0·02<P<0·05
8	BL × BR ♂ & ♀	9	3	106	7·3	80·1	0·09	154	7·3	78·0	0·09	8·4	6·2	26·3	<0·001
9	RIR × LS ♂	24	3	177	9·7	88·9	0·11	195	9·1	80·5	0·11	10·0	8·7	3·9	<0·001
	RIR × LS ♂	24	3	186	9·6	89·0	0·11	186	8·6	82·5	0·10	9·6	8·6	2·7	0·01<P<0·02
10	RIR × LS ♂	10	3	192	11·8	85·3	0·11	195	10·1	79·5	0·10	11·9	10·0	2·2	0·02<P<0·05
	RIR × LS ♂	10	3	184	9·2	86·5	0·14	219	8·2	83·8	0·12	9·7	7·7	5·4	<0·001
11	RIR × LS ♂	12	3	221	10·7	—	—	225	9·4	—	—	11·5	9·7	3·6	<0·001
	RIR × LS ♂	12	3	236	11·9	—	—	248	10·8	—	—	11·7	10·2	4·1	<0·001
12	RIR × LS ♂	8	2	131	6·4	79·3	0·08	134	5·3	75·4	0·07	6·5	5·1	4·2	<0·001
13	RIR × LS ♂	20	2	117	7·6	—	—	138	6·9	—	—	8·0	6·5	7·1	<0·001

* For method of adjustment, see p. 111.

† t test of 'Student' (1908, 1925) between adjusted gut weights.

‡ P = probability that a mean difference at least as great as the observed mean difference would have arisen by random sampling from a homogeneous population.

Table 2. *Effect of penicillin on weight and length of parts of the small intestine of chicks at 4 weeks of age*

(Mean values for groups of eight birds. Figures in parentheses are the values for treated birds expressed as percentages of the values for those not given penicillin)

		Without penicillin	With penicillin
Body-weight (g)		243	258 (107)
Gut weight:	total (g)	12·6	10·1 (80)
	duodenum (g)	3·5	2·9 (83)
	ileum and jejunum (g)	9·1	7·2 (79)
Gut length:	total (cm)	89·5	78·2 (87)
	duodenum (cm)	17·5	15·7 (90)
	ileum and jejunum (cm)	73·2	62·8 (86)

Histological preparations of whole intestines, slit longitudinally and then coiled, were made and examined for us by Dr J. M. French of the Medical School, Birmingham University. No morphological differences could be observed between specimens from treated and untreated birds. As the sections were made after embedding in paraffin it was possible that differences in fat content of the cells might have been obscured by this process. Determinations of crude fat in the gut were made on duplicate groups of six birds, with the following results:

Without penicillin			With penicillin		
Body-weight (g)	Gut weight (g)	Fat (%)	Body-weight (g)	Gut weight (g)	Fat (%)
329	15·1	2·65	330	12·3	2·67
323	11·2	2·53	354	10·0	3·08

From these figures it is unlikely that the differences in gut weight could be accounted for by changes in fat content.

Part 2. The mode of action of penicillin in reducing gut weight

Coates, Dickinson, Harrison, Kon, Porter, Cummins & Cuthbertson (1952) put forward the view, endorsed by Bird, Lillie & Sizemore (1952) and by Hill, Branion & Slinger (1952) that one way in which antibiotics increase chick growth is by the suppression of an unidentified, otherwise inapparent but growth-inhibiting 'infection'. They showed that in premises free from such 'infection' chicks derive no benefit from the feeding of antibiotics.

An attempt was therefore made to determine whether the effect of penicillin on gut weight persisted in the absence of 'infection' or whether it only occurred with 'infected' birds. In view of the number of birds continuously present in our usual chick rooms it was impossible to maintain chicks there free from 'infection'. For this part of the work 'uninfected' chicks were taken from Perspex isolation units. These units were only designed to rear chicks up to 2 weeks of age, but earlier experiments in the laboratory had indicated (Table 1) that the effect of penicillin on gut weight could be observed even at this early age. As the figures given in Table 3 show, except on

one occasion penicillin caused no significant depression in the weight of the intestine of chicks reared in the absence of the growth-depressing 'infection'.

Table 3. *Effect of penicillin on body-weight and gut weight of 'uninfected' chicks from isolation units at 14 days of age*

	Without penicillin		With penicillin			
No. of birds in group	Body-weight (g)	Gut weight (g)	Body-weight (g)	Gut weight (g)	F*	P†
9	100	5·3	98	5·5	< 1	—
8	111	6·9	113	7·3	< 1	—
20	100	5·8	106	5·5	3·4	$0·05 < P < 0·1$
10	101	5·4	102	5·9	2·8	$0·1 < P < 0·2$
10	103	4·9	101	5·4	4·8	$0·01 < P < 0·05$
20	111	5·3	111	4·7	6·6	$0·01 < P < 0·05$
10	110	6·4	114	6·3	< 1	—
10	114	5·8	109	6·3	3·2	$0·05 < P < 0·1$
10	120	5·7	120	6·3	2·2	$0·1 < P < 0·2$

* Variance ratio (e^{22}) from covariance analysis.
† See third footnote to Table 1.

Other substances have been reported to stimulate chick growth, and it seemed of interest to determine whether such substances also reduced the weight of the intestine. The examples chosen were chloramphenicol and arsanilic acid, and their effects on both body-weight and gut weight are given in Table 4. Arsanilic acid consistently reduced the weight of the intestine, even though its effect on body-weight was not always very marked. The effect of chloramphenicol on gut weight was less marked, and a significant depression was noted in only two out of the four trials.

There remained to determine whether an increase in body-weight brought about by some means other than the feeding of an antibacterial substance was also accompanied by a reduced gut weight. We have shown (Coates, Harrison, Kon, Porter, Cuthbertson & O'Sullivan, 1953; Coates, 1953) that preparations of liver could on occasion improve chick growth but that addition of both pencillin and liver to the diet produced no better growth than penicillin alone. In the present experiments, raw whole beef liver was minced, passed through a sieve and added to the diet at the rate of 3%. Table 5 shows the effect on body-weight and on gut weight of supplements of liver with or without penicillin, and in Table 6 are given the results of the t test of 'Student' applied to the body-weight and adjusted gut weight in pairs of treatments. In all four experiments there were the usual marked growth responses to penicillin. On all but one occasion the liver also gave improved growth, but to a lesser extent than the antibiotic, and the two supplements together only once produced better growth than penicillin alone. In general the magnitude of the effect on gut weight was reflected in that on growth. The liver supplement reduced gut weight, but in only one experiment was the reduction significant. It is noteworthy that in the last experiment where liver had no effect on body-weight the gut weight was similarly unaltered.

Table 4. *Effect of penicillin, arsanilic acid, or chloramphenicol on the body-weight and gut weight of 4-week-old chicks*

	Without penicillin			With penicillin					With arsanilic acid					With chloramphenicol				
			Adjusted*			Adjusted*					Adjusted*					Adjusted*		
No. of birds in group	Body-weight (g)	Gut weight (g)	gut weight (g)	Body-weight (g)	Gut weight (g)	gut weight (g)	t†	P‡	Body-weight (g)	Gut weight (g)	gut weight (g)	t†	P‡	Body-weight (g)	Gut weight (g)	gut weight (g)	t†	P‡
10	311	13·7	13·7	339	11·4	10·7	—	—	—	—	—	—	—	325	—	—	—	—
10	308	14·5	14·7	349	12·2	11·7	4·0	<0·001	—	—	—	—	—	301	12·5	12·8	2·5	0·01 < P < 0·02
10	293	15·4	20·1	323	13·8	13·3	8·9	<0·001	340	15·1	11·6	11·8	<0·001	—	—	—	—	—
15	297	—	—	315	—	—	—	—	307	—	—	—	—	326	—	—	—	—
24	267	12·6	13·1	299	11·5	11·1	4·2	<0·001	286	11·7	11·7	3·1	0·001 < P < 0·01	286	13·1	13·1	0·04	> 0·9
24	268	13·3	13·6	297	11·4	10·9	6·3	<0·001	274	11·7	12·0	3·8	<0·001	282	12·5	12·5	2·7	0·001 < P < 0·01
24	308	14·5	14·8	300	12·0	12·6	4·3	<0·001	328	12·7	12·3	4·9	<0·001	333	14·8	14·2	1·0	> 0·9

* For method of adjustment see p. 111.
† t test of 'Student' (1908, 1925) between the adjusted gut weights on this diet and on the diet without penicillin.
‡ See footnote to Table 1.

Table 5. *Effect of liver, penicillin, or liver and penicillin on gut weight of 4-week-old chicks*

Exp. no.	No. of birds in group	Without penicillin: Body-weight (g)	Without penicillin: Gut weight (g)	Without penicillin: Adjusted* gut weight (g)	With penicillin: Body-weight (g)	With penicillin: Gut weight (g)	With penicillin: Adjusted* gut weight (g)	With raw liver: Body-weight (g)	With raw liver: Gut weight (g)	With raw liver: Adjusted* gut weight (g)	With raw liver and penicillin: Body-weight (g)	With raw liver and penicillin: Gut weight (g)	With raw liver and penicillin: Adjusted* gut weight (g)
1	18	306	13·9	14·9	352	11·5	11·0	344	14·0	13·9	336	11·3	11·4
2	20	309	12·9	13·6	337	12·5	12·4	337	12·8	12·8	360	12·3	11·6
3	36	296	13·4	14·1	330	12·0	11·6	316	13·2	13·2	331	12·4	12·0
4	36	282	12·2	12·5	310	10·9	10·6	284	12·1	12·4	312	11·3	11·0

* For method of adjustment see p. 111.

Table 6. *Comparison by 'Student's' t test of body-weights and adjusted* gut weights quoted in Table 5*

Exp. no.	Basal and penicillin: Body-weight: t†	Basal and penicillin: Body-weight: P‡	Basal and penicillin: Gut weight: t†	Basal and penicillin: Gut weight: P‡	Basal and liver: Body-weight: t†	Basal and liver: Body-weight: P‡	Basal and liver: Gut weight: t†	Basal and liver: Gut weight: P‡	Penicillin and (liver and penicillin): Body-weight: t†	Penicillin and (liver and penicillin): Body-weight: P‡	Penicillin and (liver and penicillin): Gut weight: t†	Penicillin and (liver and penicillin): Gut weight: P‡	Liver and (liver and penicillin): Body-weight: t†	Liver and (liver and penicillin): Body-weight: P‡	Liver and (liver and penicillin): Gut weight: t†	Liver and (liver and penicillin): Gut weight: P‡
1	2·2	0·02 $<P<$ 0·05	8·2	$<$0·001	1·8	0·05 $<P<$ 0·1	2·2	0·02 $<P<$ 0·05	1·0	0·3 $<P<$ 0·4	0·9	0·3 $<P<$ 0·4	0·5	0·6 $<P<$ 0·7	6·4	$<$0·001
2	2·1	0·02 $<P<$ 0·05	2·5	0·01 $<P<$ 0·02	2·1	0·02 $<P<$ 0·05	1·8	0·05 $<P<$ 0·1	1·6	0·1 $<P<$ 0·2	1·7	0·05 $<P<$ 0·1	1·6	0·1 $<P<$ 0·2	2·4	0·01 $<P<$ 0·02
3	2·9	0·001 $<P<$ 0·01	4·7	$<$0·001	1·7	0·05 $<P<$ 0·1	1·7	0·05 $<P<$ 0·1	0·1	$>$0·9	0·7	0·4 $<P<$ 0·5	1·3	0·1 $<P<$ 0·2	2·3	0·01 $<P<$ 0·02
4	2·5	0·02 $<P<$ 0·05	4·9	$<$0·001	0·2	0·8 $<P<$ 0·9	0·3	0·7 $<P<$ 0·8	0·1	$>$0·9	0·8	0·4 $<P<$ 0·5	2·5	0·01 $<P<$ 0·02	3·8	$<$0·001

Diets compared

* For method of adjustment see p. 111.
† t test of 'Student' (1908, 1925).
‡ See footnote to Table 1.

DISCUSSION

The finding that penicillin added to the diet of chicks decreases the weight of their intestines has already been reported by Gordon (1952). Pepper, Slinger & Motzok (1953) found a similar effect with aureomycin. In germ-free conditions, Gordon (1952) did not observe a difference in intestinal weight on treatment with penicillin, although the gut of germ-free chicks was in general lighter than that of conventionally kept birds. Reyniers (private communication) has suggested that the thickening of the gut in conventionally kept animals is a defence mechanism against the absorption of bacterial toxins or other detrimental microbial products. Similarly, it is possible that inclusion of penicillin in the diet eliminates some undesirable micro-organisms, the 'normal' pathological reaction is lessened and the gut is therefore thinner. In germ-free chicks lymphatic tissue was reduced (Gordon, 1952). It is conceivable that a lower bacterial burden in the intestine might result in the formation of less lymphoid material, but in our experiments with antibiotics no histological changes were apparent. In any event, bacteriological studies, reviewed by Braude, Kon & Porter (1953) have shown no evidence for a consistent reduction in numbers of intestinal micro-organisms in chicks given antibiotics, so that it is unlikely that the lowered weight of the intestine is an indirect result of a lesser microbial burden.

The physiological effects of giving antibiotics in the diet might be brought about by an alteration in the metabolic activity, rather than the numbers, of organisms in the gut. The demonstration of changes in bacterial metabolism presents many difficulties, and we have so far not attempted to investigate this aspect of the problem.

The point of immediate importance to consider is whether or not the thinning of the gut wall is an essential part of the growth-promoting action of penicillin. Experimental evidence is available to show that the feeding of antibiotics improves absorption of nutrients. For instance Common, Keefe, Burgess & Maw (1950) reported that in certain circumstances the calcium and riboflavin contents of the serum were raised in chicks treated with aureomycin, and Migicovsky, Nielson, Gluck & Burgess (1951) came to the conclusion that penicillin enhanced the absorption of calcium. We ourselves (Coates, Harrison, Kon, Porter & Thompson, 1952) as well as Burgess, Gluck, Brisson & Laughland (1951) found higher storage of vitamin A in the livers of chicks given penicillin. These findings lend support to the view that thickening of the gut is part of a defence mechanism, for if the absorption of harmful toxins is reduced, the absorption of essential nutrients may of necessity be impaired as well. For this reason we were interested to establish whether the weight of the chick's intestine bore any relation to the degree of 'infection' that we believe to account in part for the growth-stimulating properties of antibiotics. Although the results reported here are by no means conclusive, there are indications that the weight of the intestine is correlated with the degree of the uncharacterized 'infection' counteracted by antibiotics. 'Uninfected' chicks from the isolation units showed no effect of penicillin on intestine weight. It would be desirable to repeat this part of the investigation on older 'uninfected' chicks in which changes in weight, if any, would be greater, but that has not so far been possible on our premises.

Our experiences with arsanilic acid were on the whole in accordance with those with penicillin. Chloramphenicol had less consistent effect on the gut weight. It is not known whether these drugs exert their growth-promoting effect by the same means as penicillin, hence for the time being it is difficult to account for the failure of chloramphenicol on two occasions to reduce gut weight. Possibly some pharmacological action may have complicated the picture.

The finding that supplements of raw liver also reduced gut weight was somewhat unexpected, but not inconsistent with our hypothesis of the mode of action of penicillin. If the postulated 'infection' implies simply the establishment of an intestinal microflora competing with the host for certain unidentified growth factors, then a supply of these factors, in the form of fresh liver, might reduce the effect of 'infection'. Alternatively, even if the 'infective' organisms do not require such nutrients, the addition of liver to the diet might encourage the establishment of a more fastidious microbial population and thereby reduce those organisms responsible for the 'infection'. Such a change in the microbial population should be detectable, but we have not yet made bacteriological examination of the gut contents of chicks given liver supplements.

More work with 'uninfected' chicks and a fuller knowledge of the microbial population of the chick gut would help much to assess the significance of the lowered intestine weight in birds treated with antibiotics.

SUMMARY

1. Procaine penicillin added to a normal mash accelerated the growth of chicks but reduced the weight and to a lesser extent the length of the small intestine.

2. No histological changes were observed to account for the lowered weight. The moisture and fat content of the gut were not substantially altered by the penicillin treatment.

3. Chicks kept in isolation units, and hence showing no increased body-weight when penicillin was fed, showed no decrease in gut weight.

4. Arsanilic acid and occasionally chloramphenicol similarly reduced the weight of chick intestines.

5. Supplements of raw liver had an apparent small, not significant, effect similar to that of penicillin on both body-weight and intestine weight.

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