because of its association with the water in the digesta, was cleared from the reticulo-rumen much more rapidly than Cr_2O_3, giving rise to wider variations in the concentration of PEG in the dry matter of faeces.

The two animals used in this investigation were kindly made available to us by Dr A. T. Phillipson. One of us (J.F.D.G.) is in receipt of a Drummond Junior Fellowship for Research in Nutrition.

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The effect on chick growth of inactivated penicillin, mineral sulphates or furazolidone supplements

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The growth-promoting effect of antibiotics for chicks, under certain conditions, has been firmly established for several years, and the literature on this subject has been extensively reviewed (Taylor, 1957; Braude, Kon & Porter, 1953).

Other materials have also been tested for growth-promoting activity. Thus, claims have been made that inactivated penicillin or its degradation products (Jukes, 1956; Wacker, Heyl, Büechl & Holthoff, 1955, 1956), copper sulphate or potassium sulphate (Wacker *et al.* 1956), sodium sulphate (Gordon & Sizer, 1955) or furazolidone (Mellen & Waller, 1954; Berg, Hamilton & Bearse, 1956) can stimulate the growth of chicks. We report here experiments in which we have investigated these claims.

EXPERIMENTAL AND RESULTS

Management of chicks

Day-old Rhode Island Red × Light Sussex cockerels were used in all experiments but one, for which only pullets were available. The birds were housed in electrically heated tier brooders, in a room where a growth response to penicillin had been obtained regularly for many years. The birds had free access to food and water. The experimental groups consisted of from twelve to twenty chicks. The groups were duplicated when possible and the tests finished at 4 weeks.

Statistical analysis

Variance analyses were done on all the values in order to establish the significance of differences between mean weights. The analyses were based on group means whenever possible, but in the absence of replicate groups individual body-weights were used.

During these experiments two groups of chicks grew very poorly from the beginning and looked unthrifty. It seemed likely that they had been affected by chilling or some other factor unconnected with the experimental treatment. We thought it reasonable therefore to omit the figures for these groups from the statistical analysis.

Part 1: experiments with inactivated penicillin

The materials tested in these experiments were samples of penicillin inactivated by either penicillinase or heavy mineral salts or by autoclaving; they were kindly supplied by Dr E. Lester Smith, F.R.S., of Glaxo Laboratories Ltd. They had been shown to have no antibiotic activity when tested against a range of organisms. D-Penicillamine and phenylacetic acid were also tested, as they are breakdown products likely to arise from the inactivation of penicillin. The sample of D-penicillamine was prepared by Dr E. Lester Smith; in his opinion no appreciable racemization during its preparation is likely to have occurred to give the L-form, which has been reported as having a growth-depressing effect on rats (Wilson & du Vigneaud, 1948, 1950) and on chicks (Jukes, 1956).

Diets. In the first experiment the basal diet was a chick mash that had been used in this laboratory for some years and was known to give excellent growth up to 4 weeks of age. It had the 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 (NaCl 93·94, $MnSO_4.4H_2O$ 6, KI 0·06%) 0·5, arachis oil (containing 64 i.u. vitamin D_3 and 680 i.u. vitamin A/g) 1.

To this diet was added one of seven substances at a level equivalent to 25 mg benzylpenicillin/kg, the level usually employed in this laboratory:

Procaine penicillin	Benzylpenicillin
Benzylpenicillin inactivated by penicillinase	Benzylpenicillin inactivated by mineral salts
Benzylpenicillin inactivated by autoclaving	D-Penicillamine
	Phenylacetic acid

In the second experiment we used an all-vegetable-protein diet having the percentage composition: maize 37·8, barley 20, defatted soya grits 35, dried grass 3, bone meal 1·5, limestone 1, arachis oil (containing 64 i.u. vitamin D_3 and 680 i.u. vitamin A/g) 1, salt mixture (NaCl 0·672 and $MnSO_4.4H_2O$ 0·028 parts) 0·7. The diet was supplemented with vitamins (mg/100 g): biotin 0·022, folic acid 0·083, thiamine 0·33, pyridoxine 0·44, riboflavin 0·77, calcium pantothenate 1·65, and nicotinic acid 5·5.

Table 1. *Effect on the growth of chicks of inactivated penicillin,* D-*penicillamine or phenylacetic acid in a diet containing animal protein*

Addition to the diet†	No. of birds	Mean weight at 4 weeks (g) Group	Total	
None	20 20	303 309	306	Standard error ±7·7 (8 degrees of freedom)
Procaine penicillin	19 20	318 328	323	
Benzylpenicillin	20 19	350 331	341*	
Benzylpenicillin inactivated by autoclaving	20 20	294 307	301	
Benzylpenicillin inactivated by penicillinase	20 20	283 312	298	
Benzylpenicillin inactivated by mineral salts	20 20	309 304	307	
D-Penicillamine	19 20	312 296	304	
Phenylacetic acid	19 20	316 305	311	

* Significantly different from controls at $P < 0.05$.
† Each addition was equivalent to 25 mg benzylpenicillin/kg.

Table 2. *Effect on the growth of chicks of inactivated penicillin and* D-*penicillamine in a diet containing vegetable protein*

Addition to the diet: Substance	Benzyl-penicillin equivalent (mg/kg)	No. of birds	Mean weight at 4 weeks (g) Group	Total	
None	—	20 19	222 219	221	Standard error ±4·7 (7 degrees of freedom)
Procaine penicillin	40	19 20	243 248	246**	
Benzylpenicillin	10	20 18	252 260	256**	
Benzylpenicillin	40	19 16	245 253	249**	
Benzylpenicillin inactivated by penicillinase	10	18 20	220 223	222	
Benzylpenicillin inactivated by penicillinase	40	20 20	222 216	219	
D-Penicillamine	10	20 19	214 234	224	

** Significantly different from controls at $P < 0.01$.

To this diet was added one of the four substances

Procaine penicillin	Benzylpenicillin
Benzylpenicillin inactivated by autoclaving	D-Penicillamine

Benzylpenicillin was added at 10 mg and 40 mg/kg, and inactivated benzylpenicillin at equivalent levels. Procaine penicillin was added at the 40 mg equivalent level only and D-penicillamine at the 10 mg equivalent level only.

Results. The mean weights of chicks at 4 weeks of age are given in Tables 1 and 2. It is clear that with neither basal diet did inactivated benzylpenicillin or its degradation products show any growth-promoting activity. On the other hand, both benzylpenicillin and procaine penicillin exhibited a growth response of an order frequently observed in this laboratory, although the response to procaine penicillin did not reach significance with the animal-protein diet.

Part 2: experiments with mineral sulphates

Experiments 1 and 2

Diet. The effect of adding various levels of copper sulphate to the animal-protein ration used in part 1 was studied. Some groups of chicks also received the diet supplemented with procaine penicillin, either with or without copper sulphate.

Results. Mean weights of chicks at 4 weeks of age are shown in Table 3. In Expt 1 no significant increase in weight was observed, but in the groups receiving copper sulphate agreement between replicates was not very close. Similarly in Expt 2, over the whole range of doses from 0·025 to 0·2%, no significant increase in weight resulted. The highest level of copper sulphate significantly depressed the weight of chicks.

Experiment 3

Diet. According to Wacker *et al.* (1956) the growth of chicks is enhanced by the addition of copper sulphate or potassium sulphate to a diet low in animal protein. In view of our failure to obtain any significant growth response with supplements of copper sulphate in the normal animal-protein diet, this diet was modified to contain only 2% fish meal and 3% dried skim milk, the difference being made up with maize. To this new basal mash was added either 0·1% copper sulphate or an equimolar amount of potassium sulphate.

Results. The mean weights of chicks at 4 weeks of age are shown in Table 4, from which it can be seen that no increase in growth rate resulted from the supplements. Indeed, on this occasion there was a significant growth depression with the normal animal-protein diet.

Experiment 4

Diet. Gordon & Sizer (1955) reported that supplementation of a semi-synthetic diet with sodium sulphate failed to improve the growth of chicks. However, they found that, if the mineral sulphates included in the diet were replaced by chlorides,

Table 3. *Effect on the growth of chicks of copper sulphate in a diet containing animal protein*

Addition to the diet	No. of birds	Mean weight at 4 weeks (g): Group	Total	
	Experiment 1			
None	15	298	291	Standard error ± 9·3 (4 degrees of freedom)
	15	283		
$CuSO_4.5H_2O$, 0·05 %	15	319	307	
	15	295		
$CuSO_4.5H_2O$, 0·1 %	15	293	296	
	15	298		
Procaine penicillin, 45·5 mg/kg	15	339	339*	
	15	(265)†		
Procaine penicillin, 45·5 mg/kg, and $CuSO_4.5H_2O$, 0·1 %	15	330	318	
	15	306		
	Experiment 2			
None	20	291	302	Standard error ± 7·9 (2 degrees of freedom)
	17	313		
$CuSO_4.5H_2O$: 0·025 %	20	—	288	
0·05 %	20	—	308	
0·1 %	19	—	309	
0·2 %	20	—	251*	
Procaine penicillin, 45·5 mg/kg	19	334	336	
	20	338		

† Omitted from calculations (see p. 346).
* Significantly different from controls at $P < 0.05$.

Table 4. *Effect on the growth of chicks of copper sulphate or potassium sulphate in a diet low in animal protein (A) or in a diet of normal animal-protein content (B)*

Addition to the diet	No. of birds	Mean weight at 4 weeks (g): Group	Total	
	A			
None	11	160	164	Standard error ± 7·8 (4 degrees of freedom)
	12	167		
$CuSO_4.5H_2O$, 0·1 %	12	153	158	
	12	162		
K_2SO_4, 0·07 %	12	160	173	
	12	185		
Procaine penicillin, 45·5 mg/kg	11	207	200*	
	12	192		
	B			
None	12	308	308	Standard error ± 3·6 (4 degrees of freedom)
	12	308		
$CuSO_4.5H_2O$, 0·1 %	12	290	289*	
	12	287		
K_2SO_4, 0·07 %	12	284	284**	
	12	283		
Procaine penicillin, 45·5 mg/kg	12	330	337**	
	12	344		

* Significantly different from controls at $P < 0.05$.
** Significantly different from controls at $P < 0.01$.

then the addition of sodium sulphate had an effect. Therefore it seemed reasonable to suppose that, assuming the growth-promoting effects of copper sulphate and potassium sulphate reported by Wacker *et al.* (1956) to have depended on the sulphate ion, our failure to elicit any growth-promoting effect with either compound may have been due to the presence of manganese sulphate in the salt mixture of the basal diet. For a detailed study of this problem, knowledge of the sulphur content of the diet and the requirement of chicks for sulphur would be necessary. However, as a preliminary indication it was thought worth while to substitute manganese chloride for the sulphate in the normal diet and in the diet low in animal protein. The low-protein diet with manganese sulphate was used as a control. All these diets were tested with and without the addition of 0·034% copper sulphate, which provided the sulphate equivalent of manganese sulphate.

Table 5. *Effect on the growth of chicks of* 0·034% *copper sulphate in a diet of low or of normal protein content, with or without the replacement of mineral sulphate by chloride*

	Without coppper sulphate			With copper sulphate			
		Mean weight at 4 weeks (g)			Mean weight at 4 weeks (g)		
Basal diet	No. of birds	Group	Total	No. of birds	Group	Total	Standard error of the mean
Normal protein, chloride replacing sulphate	14 15	337 361	349	14 14	359 354	357	±8·7
Low protein	15 12	168 182	175	15 13	170 174	172	±5·1
Low protein, chloride replacing sulphate	15 15	181 170	176	15 14	182 161	172	±8·4

Table 6. *Effect on the growth of chicks of* 0·07% *potassium sulphate in a diet of low or of normal protein content, with or without the replacement of mineral sulphate by chloride*

	Without potassium sulphate		With potassium sulphate		
Basal diet	No. of birds	Mean weight at 4 weeks (g)	No. of birds	Mean weight at 4 weeks (g)	Standard error of the mean
Normal protein, chloride replacing sulphate	16	281	16	290	±15·6
Low protein	16	175	15	162	± 9·1
Low protein, chloride replacing sulphate	16	171	16	174	± 9·1

Results. The mean weights of chicks at 4 weeks of age are shown in Table 5. No improvement in growth resulted from the addition of copper sulphate to any of the diets, nor did the replacement of manganese sulphate by the chloride cause any lessening in growth rate, when compared with that of chicks given the normal diet containing manganese sulphate in another experiment running concurrently.

Experiment 5

Diet. There remained the possibility that any growth-promoting effect of sulphate was masked by copper toxicity. Therefore Expt 4 was repeated, but this time the diets were supplemented with potassium sulphate instead of copper sulphate. The potassium salt was added at the level used in Expt 3.

Results. The mean weights of chicks at 4 weeks of age are shown in Table 6. No differences were observed as a result of adding potassium sulphate to either the low- or the normal-protein diets.

Part 3: experiments with furazolidone

Diets. The chick mash described on p. 346, containing adequate amounts of animal protein, was used in all tests. The drug was added to the diet at the rate of 7·5 mg/kg. Procaine penicillin was added to the diet of some groups, at a level equivalent to 25 mg benzylpenicillin/kg, to serve as a basis for comparison of growth rates. Five experiments were done and on two occasions the antibiotic and furazolidone were both added to the diet.

Results. The mean weights of chicks at 4 weeks of age are shown in Table 7. The diets with furazolidone or penicillin significantly ($0·05 > P > 0·02$) increased the growth rate over that of the controls. In Expts 4 and 5, when the two drugs were given together, the growth increase was significantly ($0·05 > P > 0·02$) greater than that due to either substance alone. However, it should be noted that the response to penicillin was smaller than is usual in our laboratory and did not reach significance ($0·2 > P > 0·1$).

DISCUSSION

Contrary to the report of Wacker *et al.* (1956) we have found no evidence of growth-promoting activity of copper sulphate added to the diet of chicks.

It is well known that copper-sulphate supplements stimulate the growth of pigs. Braude, Mitchell, Barber & Allen (1957) also reported that copper carbonate had a growth-promoting effect similar to that of copper sulphate, thus indicating that the activity is due to copper and not to the sulphate moiety. Wacker *et al.* (1956) also reported growth increases with 0·05% $CuCl_2$. We have found (Coates, Davies & Harrison, 1956) 0·5% copper sulphate to be toxic to the chick, although 1% merely retarded the growth of pigs (Barber, Braude, Mitchell, Rook & Rowell, 1957).

Our results with potassium-sulphate supplements do not confirm the findings of Wacker *et al.* (1956). It is significant that Gordon & Sizer (1955) could obtain no growth response to sodium sulphate when the diet was supplemented with a mineral mixture containing the sulphates of magnesium, manganese and copper. When these sulphates were replaced by chlorides or oxides, sodium sulphate showed growth-promoting activity. These workers concluded that the chick can satisfy a small part of its total sulphur requirements from inorganic sulphates.

Machlin & Pearson (1956) suggested that the increased growth rate observed with diets supplemented with sodium sulphate may be due to a physiological requirement

Table 7. *Effect on the growth of chicks of addition per kg diet of 7·5 mg furazolidone or 45·5 mg procaine penicillin* or both*

	Addition to the diet											
	None			Furazolidone			Procaine penicillin			Furazolidone and procaine penicillin		
		Mean weight at 4 weeks (g)			Mean weight at 4 weeks (g)			Mean weight at 4 weeks (g)			Mean weight at 4 weeks (g)	
Expt no.	No. of birds	Group	Total	No. of birds	Group	Total	No. of birds	Group	Total	No. of birds	Group	Total
1	18	288	289	20	308	317	19	316	312	—	—	—
	19	289		20	325		18	307				
2	19	310	314	19	320	326	20	320	350	—	—	—
	19	318		20	332		20	379				
3	19	319	322	20	335	338	20	347	346	—	—	—
	19	324		19	340		18	344				
4	19	300	298	19	302	302	19	299	309	19	337	333
	19	296		17	(273)†		20	318		20	329	
5	20	274	272	18	317	316	19	273	290	17	349	338
	20	269		17	314		20	306		19	327	
Mean	—	—	299	—	—	322	—	—	321	—	—	336

* Equivalent to 25 mg benzylpenicillin.
† Omitted from calculations (see p. 346).

for sulphate *per se*, which could be satisfied by dietary sulphate or by sulphate obtained from the oxidation of sulphur-containing amino acids. Since our low-protein diet contained 3% dried skim milk it may have provided a source of organic sulphur not available in the diet of Wacker *et al.* (1955, 1956), which might account for our failure to confirm their results. However, the diet used by Gordon & Sizer (1955) contained 15% casein and 10% gelatin, which would provide appreciable quantities of sulphur-containing amino acids, yet added sodium sulphate stimulated growth on this diet.

Thus no clear picture emerges from these conflicting results. No values have been given for the copper and sulphur content of the diets used by the different workers, or for the optimal requirements of the chick for both elements. Until such knowledge is available, it is impossible to evaluate the reported growth-stimulating effects of either copper or sulphate.

We have been unable to demonstrate any growth-promoting effect of inactivated penicillin or of the two degradation products, D-penicillamine and phenylacetic acid. Elam, Gee & Couch (1951) similarly failed to establish a growth response with autoclaved penicillin, and Jowsey, Cook, MacGregor & Blakely (1957) could find no increase in growth rate of turkey poults given inactivated penicillin. Fell & Stephenson (1953) failed to obtain a significant growth response with penicillamine. However, Jukes (1956) with D-penicillamine, and Wacker *et al.* (1955, 1956) with D-penicillamine or inactivated penicillin, could demonstrate growth stimulation, as did Elam *et al.* (1951) and Fell & Stephenson (1953), who injected inactivated penicillin into chicks. Taylor & Gordon (1955) also showed in pigs a growth stimulation with supplements of inactivated penicillin.

Since D-penicillamine is a sulphur-containing amino acid it may be thought that its reported growth-promoting activity might be explained by its sulphur content. However, at the level of 10 mg/kg at which both Wacker *et al.* (1956) and Jukes (1956) obtained growth responses, it contributed only about 2 p.p.m. of sulphur to the diet. This quantity could hardly account for the observed increases in weight. Residual antibiotic activity is unlikely to be the source of growth stimulation by inactivated penicillin, since several workers tested the materials they used for this activity. We can offer no explanation for these inconsistent results.

The results of our experiments with furazolidone indicate that it is an effective growth-promoting agent for chicks under the conditions tested. Coates, Dickinson, Harrison, Kon, Porter, Cummins & Cuthbertson (1952) and others have shown that antibiotics stimulate growth by suppression of an 'infection' present in premises regularly housing chicks, but absent from specially cleaned ones. Berg *et al.* (1956) found that the growth rate of chicks was enhanced by the addition to the diet of 55 mg or 110 mg furazolidone/kg. The increase in growth rate occurred in the presence of penicillin and with birds kept either in batteries or on litter. These workers suggested that the activity of furazolidone might be due to its effectiveness in combating a minor infection present in the environment. In contrast, Jacobs, Elam, Anderson, Gee, Fowler & Couch (1953) found no increase in the weight of chicks when their diets were supplemented with furazolidone, but these birds were housed in premises where no chicks had been kept for 10 weeks and which had also been thoroughly cleaned. In this experiment the several antibiotics tested were without effect. Libby & Schaible (1955) reported enhanced growth of chicks given 7·5 mg furazolidone/kg diet, whether penicillin was present or not. In the absence of the antibiotic, contamination of a 'clean' environment with used litter from a deep-litter house retarded growth, which was restored to normal by supplements of furazolidone. Francis & Shaffner (1956) tested furazolidone at several levels and obtained no response, but unfortunately they did not describe the environment in relation to 'infection'.

Our chick house in which the tests with furazolidone were done has been in continuous use for several years, during which a consistent growth response to penicillin has been obtained. We have been unable to test the effect of furazolidone on chick growth in 'uninfected' premises.

From the literature quoted and our own few observations, it seems likely that the mode of action of furazolidone in stimulating chick growth is similar to that of penicillin. Even if it is, there is insufficient evidence so far to settle the question whether both drugs are affecting the same organisms.

SUMMARY

1. Rhode Island Red × Light Sussex cockerels were used in duplicate groups, of from twelve to twenty birds, to test several materials for growth-promoting activity.

2. When tested in premises where a growth response to penicillin was obtained, inactivated penicillin, D-penicillamine or phenylacetic acid failed to stimulate the growth of chicks.

3. No growth response resulted from the addition of copper sulphate or potassium sulphate to a diet containing a normal level or a low level of animal protein, even when the usual supplement of inorganic sulphate was omitted from the diets.

4. The significance of these findings is discussed.

5. Furazolidone stimulated the growth of chicks to the same extent as did penicillin. When the drugs were given together, the response was greater than to either alone, although in this experiment an unusually small response to penicillin was obtained.

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The metabolism in sheep of the alkaline earth products of fission

The absorption and excretion of ^{45}Ca and ^{89}Sr by Blackface wethers

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Strontium-90, which is one of the products of nuclear fission, is deposited in the United Kingdom roughly in proportion to the rainfall in any given area. Its uptake by vegetation is maximal in the permanent natural hill pastures where the soil is acid and deficient in many nutrients including calcium and phosphorus. For these reasons hill sheep retain more ^{90}Sr than other animals which are important as sources of food for man (Bryant, Chamberlain, Morgan & Spicer, 1957). Grazing sheep are therefore very sensitive indicators of the pollution of a given area by radioactive fallout, and it is important that the metabolism of strontium in sheep be studied.

Strontium behaves very similarly to calcium, both chemically and physiologically. However, during the passage of the two elements through the animal body various quantitative differences in their behaviour may be observed. These amount to a 'discrimination' by the animal against strontium and in favour of calcium. The chief sites where this discrimination occurs in the non-lactating animal appear to be the alimentary canal during absorption and the kidney during the excretion of calcium and strontium in the urine (Comar, Russell & Wasserman, 1957).

Discrimination against strontium during absorption from the alimentary canal has been reported in rats (Comar, Wasserman & Nold, 1956; Harrison, Jones & Sutton,

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