TABLE II. Effect of Cortisone (C) and Virus (V) on 8-10 g Swiss Mice Showing % Deaths and % Survivors with Liver Lesions on the 6th Day.

Group	Day of inoc. 1	2	3	4	5	Deaths, no lesions	Deaths, with lesions	Survivors, with lesions
I	C	V	–	–	–	0	28	77
II	C	V	C	C	C	11	50	100
III	–	V	–	–	–	0	0	39
IV	C	–	–	–	–	6	0	22*
V	C	–	C	C	C	22	0	0

* Homogenates of livers with cortisone-induced lesions were not infective for suckling mice.

with the data in Table I shows that all of these survivors in group IV would have lived for at least 12 days. There were no deaths in virus control group III and 39% of the mice had liver lesions. As shown in Fig. 1 and 2, all of the mice in the virus control group III would have survived. Comparison of the percent deaths and percent liver lesions observed in groups I and II with their corresponding cortisone control groups IV and V again shows the effectiveness of cortisone in decreasing host resistance to infection with MHV (Table II).

Summary. A small proportion of mice inoculated with cortisone alone died or developed ascites and/or liver lesions. Bacteria were isolated from some of the livers showing cortisone-induced lesions. Homogenates of such livers were not infective for normal suckling Swiss mice. Cortisone acetate reduced susceptibility of normally resistant weanling Swiss mice to infection with the Gledhill strain of mouse hepatitis virus. The effectiveness of cortisone in decreasing host resistance was enhanced as dosage of the steroid was increased. There was a decrease in this cortisone effect as the time interval between virus and cortisone administration was increased from 1 to 5 days.

1. Gledhill, A. W., Andrewes, C. H., *Brit. J. Exp. Path.*, 1951, v32, 559.

2. Gledhill, A. W., Dick, G. W. A., Niven, J. S. F., *Atti congr. intern. microbiol. Roma, 6th Congr. Rome,* 1953, v3, 219.

3. Gledhill, A. W., *J. Gen. Microbiol.*, 1956, v15, 292.

4. Kass, E. H., Finland, M., *Ann. Rev. Microbiol.*, 1953, v7, 361.

5. Reed, L. J., Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

6. Antopol, W., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 262.

7. Hart, P. D., Rees, R. J. W., *Lancet*, 1950, v2, 391.

8. LeMaistre, C., Tompsett, R., *J. Exp. Med.*, 1952, v95, 393.

9. Gledhill, A. W., Rees, R. J. W., *Brit. J. Exp. Path.*, 1952, v33, 183.

Received July 3, 1958. P.S.E.B.M., 1958, v99.

Response of Germ-Free and Conventionally Reared Turkey Poults to Dietary Supplementation with Penicillin and Oleandomycin.* (24264)

M. FORBES,† W. C. SUPPLEE AND G. F. COMBS
(Introduced by P. Gyorgy)
Germ-free Animal Research Unit, Walter Reed Army Institute of Research, Washington, D. C., and Poultry Husbandry Department, University of Maryland, College Park

The mechanism of the growth-promoting effect of antibiotics in poultry rations has not been clearly established. The prevailing opinion seems to be that antibiotics act mainly by modifying the intestinal microflora(1-4). A basic question is whether antibiotics exert a growth-promoting effect in the absence of an intestinal microflora. Luckey(5) reported that no stimulation of growth was seen when germ-free chickens and turkeys were fed antibiotics at a level of 50 mg/kg diet. In subsequent studies Luckey, *et al.*(6) reported that

* This investigation was supported in part by the Research and Development Division, Office of The Surgeon General, Department of the Army.

† Dept. of Microbiology, School of Medicine, University of Pennsylvania. Present address: Temple University School of Medicine, Philadelphia.

lower levels of antibiotics (oxytetracycline 25 mg/kg, procaine penicillin 11 mg/kg) caused slight growth increments in germ-free chicks and that procaine penicillin (46 mg/kg) produced a statistically significant increase in the growth rate of germ-free poults. Recently Gordon, *et al.*(7), repeating Luckey's first studies, found no evidence of increased body weight in germ-free chicks fed procaine penicillin at a level of 50 mg/kg diet. These reports were based on comparisons of such limited numbers of birds that the significance of the observed differences is not clear. In more extensive experiments with germ-free and conventional chicks, it has been shown that the growth rate of chicks on an adequate diet is not affected by dietary supplements of penicillin (45 mg/kg) unless penicillin-sensitive organisms which depress growth are present (Forbes and Park, to be published). Experimental results were also obtained suggesting that *Clostridium welchii* type A is one such penicillin-sensitive growth depressing organisms(8, Lev and Forbes, to be published).

In the present paper a series of experiments with turkey poults is described in which the effect of dietary supplementation with procaine penicillin or oleandomycin was compared under germ-free and conventional rearing conditions.

Methods. Maryland Medium White poults hatched from eggs supplied by the Poultry Department, University of Maryland, and Beltsville White poults from eggs obtained from the Agricultural Research Center, Beltsville, Md., were used in these studies. The procedure followed in rearing germ-free birds§ was patterned after that of Reyniers *et al.*(9). Twenty-day embryonated eggs were immersed for 2 minutes in a detergent solution, then for 12 minutes in a 2% $HgCl_2$ solution maintained at 37°C, then were passed through a tube into a previously steam sterilized Reyniers germ-free unit where incubation was completed. Usually, 24 eggs were incubated in each unit and yielded 14 to 16 poults. The poults were weighed and distributed in 2 groups of 7 to 8 birds of approximately equal average weight. In each unit the 2 groups were kept in wire cages (approximately 11″ wide, 22″ long, 13″ high), one group serving as control while the other received the antibiotic supplemented diet.

§ In this report the term germ-free is intended to mean free of bacteria and fungi.

TABLE I. Basal Diet I.

	Per 100 g diet
Corn starch, g	41.40
Washed protein‡	44.44
Corn oil	4.04
DL-methionine	.71
Glycine	.51
Choline chloride	.46
Diphenyl-p-phenylene-diamine	.0125
Minerals*	7.45
Vitamins†	.89

* Minerals, g/100 g diet (all c. p. or reagent grade) — 1.846 $CaCO_3$, 3.313 $Ca_3(PO_4)_2$, .808 K_2HPO_4, .455 $MgSO_4 \cdot 7H_2O$, .606 NaCl, .051 $FeSO_4 \cdot 7H_2O$, .002 $CuSO_4 \cdot 5H_2O$, .031 $MnSO_4 \cdot H_2O$, .0002 $CoSO_4 \cdot 7H_2O$, .004 KI, .01 K AL (SO_4) 2.12 H_2O, .001 $Na_2MoO_4 \cdot 2H_2O$, .005 $Na_2SiO_3 \cdot 9H_2O$, .001 HBO_3, .012 $ZnCl_2$, .303 KCl.

† Vitamins, mg/100 g diet—12 alpha tocopherol acetate (from Myvamix), 2 Menadione, 60 thiamine, 10 riboflavin, 32 calcium pantothenate, 40 niacin, 6 pyridoxine HCl, .2 biotin, 2.4 folacin, .012 cyanocobalamin, 101 inositol, 1.0 p-aminobenzoic acid, 40 ascorbic acid. Also—5656 I.U. vitamin A (from Nopcay "20") and 529 I.C.U. vit. D (from Nopdex "15").

‡ Drackett C-1 Assay protein, washed once with tap water and 3 times with distilled water at pH 4.6, then dried at 50-60°C.

Eggs used for the conventional (non germ-free) tests were similarly sterilized but were transferred from the $HgCl_2$ solution into a standard incubator. After hatching the birds were transported to the Univ. of Maryland and distributed in electrically heated wire floored battery brooders. Lights were left on continuously both in the germ-free units and in the room where the conventional tests were carried out. The temperature inside the germ-free unit was 37-38°C when the birds hatched, being gradually decreased to 25-27°C over a period of 10 days and maintained there until the end of the experiment. Food and water were supplied *ad libitum*. Individual weights were taken initially and after 7 and 14 days on test.

Diets. The composition of the purified type washed Drackett protein starch basal diet is shown in Table I. All feed was sterilized by placing one kg portions in gauze bags in a layer not exceeding one inch in thickness in the autoclave of each germ-free unit.

Steam was allowed to flow through the autoclave for 10 minutes, the pressure was then increased to 17 p.s.i. (252-255° F), maintained for 25 minutes and gradually returned to normal. The feed was then either transferred into the germ-free unit or removed for use in the concurrent tests with conventionally reared poults.

Weighed quantities of procaine penicillin G or oleandomycin (Pfizer) mixed with starch were sealed in glass ampules, sterilized by irradiation, introduced into the germ-free unit *via* a germicidal trap and mixed into the sterile diet. Similarly sterilized antibiotics were used in the conventional tests. Irradiation‡ (1,800,000 rep) did not modify antibacterial activity of the antibiotics as measured by the tube dilution method using *Micrococcus pyogenes var. aureus H* as the test organism.

Sterility tests. Moistened swabs were used weekly to take multiple anal samples or fresh fecal specimens from all animals. The swabs were inoculated in thioglycollate broth, in cooked meat medium and in trypticase soy broth. Other swabs were streaked on brain-heart infusion agar containing 5% blood, on lactobacillus selection agar (Baltimore Biological Laboratory) and on Sabouraud agar. Replicate cultures were incubated at room temperature and at 37°C. Some blood plates were incubated anaerobically at 37°C. Cultures were kept for 2 weeks and examined periodically. Gram stains were made of samples of the broth cultures. At the conclusion of most of the experiments, a bird was killed inside the unit and samples of the intestinal contents were cultured in the various media.

Statistical analysis. The growth data were subjected to the Fisher F test, results from replicate tests being combined. To simplify the calculations, the numbers in the various comparisons were equalized by selection using a table of random numbers. No discrepancy was found between total data as compared to selected data.

‡ Performed through the courtesy of Dr. Howard Andrews, Radiation Branch, Nat. Cancer Inst. and Mr. John Hickey, Sanitary Engineering Branch, N.I.H., Bethesda, Md.

TABLE II. Effect of Procaine Penicillin (45 mg/kg) on Growth of Germ-Free and Conventional Poults.

		Avg wt in g on 14th day				
		No supplement		Penicillin		
Exp.	Status	No. birds	Wt	No. birds	Wt	Diff.
1	GF	6	221	7	198	−23
	Conv.	10	182	10	212	+30
2	GF	7	217	7	227	+10
	Conv.	12	181	15	219	+38
3	GF	10	179	6	186	+ 7
	Conv.	11	138	9	192	+54
1, 2 & 3	GF	23	202	20	207	+ 5
combined	Conv.	33	170	34	212	+42

TABLE IIA. Statistical Analysis of Randomly Selected Data of Combined Experiments Shown in Table II.

	Avg wt in g on 14th day (20 birds/group)		
Status	No supplement	Penicillin	Diff.
GF	201	207	+ 6
Conv.	166	207	+41

Analysis of Variance

		Source of var.		
Main effects	DF	MS	F	p
Status: GF *vs* conv.	1	6,187	1+	n.s.
Treatment: Pen. *vs* no pen.	1	10,732	1+	"
Interaction Treatment *vs* status	1	5,993	5.50	<.05
Error	76	1,089		

Results. The conventionally reared poults fed the penicillin supplemented ration gained an average of 42 g more than their controls receiving the unsupplemented ration (Table II). In contrast, the germ-free birds fed the basal diet grew at a rate comparable to the conventional birds receiving penicillin and growth was not improved by addition of penicillin. Statistical treatment of the data (Table IIA) indicates that response to penicillin in the conventional environment was significant ($p<0.05$).

Oleandomycin also caused a marked growth improvement in the conventional poults but none in the germ-free birds (Table III, IIIA). As with penicillin, supplementation with oleandomycin increased growth in the conventionally reared birds to a level comparable to

TABLE III. Effect of Oleandomycin (30 mg/kg) on Growth of Germ-Free and Conventional Poults.

		Avg wt in g on 14th day				
		No supplement		Oleando		
Exp	Status	No. birds	Wt	No. birds	Wt	Diff.
1	GF	7	229	7	258	+29
	Conv.	9	171	7	193	+22
2	GF	8	219	7	193	−26
	Conv.	19	175	19	226	+51
3	GF	12	173	11	176	+ 3
	Conv.	9	159	10	179	+20
1, 2 & 3 combined	GF	27	201	25	199	− 2
	Conv.	37	170	36	207	+37

TABLE IIIA. Satistical Analysis of Randomly Selected Data of Combined Experiments Shown in Table III.

	Avg wt in g on 14th day (25 birds/group)		
Status	No supplement	Oleando	Diff.
GF	199	199	0
Conv.	178	208	+30

Analysis of Variance

		Source of var.		
Main effects	DF	MS	F	p
Status: GF *vs* Conv.	1	1,584	1	n.s.
Treatment: Oleando *vs* no oleando	1	7,856	1	"
Interaction				
Treatment *vs* status	1	13,537	8.47	$<.01$
Error	96	1,599		

that of the germ-free birds.

The results of these experiments are in agreement with the theory that growth response to antibiotics is due to their action on the microflora.

Summary. Maryland Medium White and Beltsville White poults reared under germ-free conditions showed excellent growth on a purified type washed Drackett protein starch basal diet. Supplementation of the diet with procaine penicillin (45 mg/kg) or with oleandomycin (30 mg/kg) did not improve growth. In concurrent tests, conventionally reared poults fed the basal ration grew at a lower rate than the germ-free birds while those fed the basal ration supplemented by either penicillin or oleandomycin grew at a rate comparable to that of the germ-free birds.

1. Jukes, T. H., Williams, W. L., *Pharmacol. Rev.*, 1953, v5, 381.
2. Stokstad, E. L. R., *Physiol. Rev.*, 1954, v34, 25.
3. Jukes, T. H., *Antibiotics in Nutrition*, 1957, Medical Encyclopedia, Inc., New York, N. Y.
4. Combs, G. F., *Proc. First Internat. Conf. on the Use of Antibiotics in Agric.* 1956, *Nat. Acad. Sci. N.R.C.*, pub. 397, 107.
5. Luckey, T. D., *Colloquium held at Univ. of Notre Dame,* June 4, 1952.
6. Luckey, T. D., Gordon, H. A., Wagner, M., Reyniers, J. A., *Antibiotics and Chemother.*, 1956, v6, 36.
7. Gordon, H. A., Wagner, M., and Wostmann, B. S., Antibiotics Annual 1957-1958 Medical Encyclopedia, Inc., New York, N. Y., p248.
8. Forbes, M., Park, J. T., Lev, M., 1958, *Ann. N. Y. Acad. Sc.*, in press.
9. Reyniers, J. A., Trexler, P. C., Ervin, R. F., Wagner, M., Luckey, T. D., Gordon, H. A., *Lobund Rep.* no. 2, 1949, Univ. of Notre Dame, Ind.

Received July 8, 1958. P.S.E.B.M., 1958, v99.

Influence of Long Term Fat-Feeding on Excretion of Cholesterol-4-C^{14} Metabolites.* (24265)

JEAN D. WILSON AND MARVIN D. SIPERSTEIN
(Introduced by Donald W. Seldin)
Department of Internal Medicine, The University of Texas Southwestern Medical School, Dallas

Studies in this laboratory have recently been concerned with the influence of variations in the type and quantity of dietary fat on metabolism of cholesterol-4-C^{14}. We have previously reported that a 4-day period of feeding rats isocaloric diets containing corn oil, as compared to diets containing lard or no

* This work was supported by grants from the American Heart Assn., the Dallas Heart Assn., and by Research Grant of Nat. Heart Inst., U.S.P.H.S.