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.

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

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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

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TABLE I. Fecal Excretion of Cholesterol-4-C^{14} after 72 Days of Feeding Fat-Free, Corn Oil or Lard Diets.

		Diet			Wt (g)			% administered dose recovered as:			
			Avg daily intake				Avg daily	Neutral sterols			
Group	Rat #	Fat content	g	Cal.	Begin.	End	dry wt of feces (g)	Non-dig. precip.	Dig. precip.	Bile acids	Total recovery
1	1	None	19.3	71.8	182	354	1.29	1.43	9.00	14.82	25.25
	2	30% corn oil	12.7	66.8	175	284	.92	20.21	9.81	22.46	52.48
	3	30% lard	12.1	63.6	173	337	.86	1.17	14.71	28.63	44.51
2	4	None	17.9	66.6	169	284	1.12	1.34	11.06	12.88	25.28
	5	30% corn oil	12.5	65.8	191	315	.92	21.96	8.43	33.80	64.19
	6	30% lard	12.4	65.2	154	273	.86	7.00	13.69	38.80	59.49
3	7	None	15.3	56.9	148	259	1.01	1.08	11.59	13.56	26.23
	8	30% corn oil	11.1	58.4	174	325	.90	17.77	7.43	28.49	53.69
	9	30% lard	10.9	57.3	172	288	1.06	6.60	21.73	34.89	63.22
4	10	None	18.4	68.4	195	333	1.21	1.95	7.33	20.51	29.79
	11	30% corn oil	12.6	66.3	192	300	.81	25.08	11.11	23.34	59.53
	12	30% lard	12.9	67.9	166	330	.89	3.66	16.28	35.70	55.64

fat, resulted in marked acceleration in metabolism of cholesterol-4-C^{14} to *non-digitonin precipitable* neutral sterols(1,2). The primary purpose of the present series of experiments was to examine the excretory products of cholesterol-4-C^{14} in rats subjected to long term feeding of diets containing no fat, 30% lard or 30% corn oil. Initial attempts to characterize the non-digitonin precipitable neutral sterol fraction in the feces of these animals are also reported.

Materials and methods. Male rats of the Long-Evans strain, weighing 160-195 g were pair-fed isocaloric quantities of diets containing no fat, 30% corn oil, or 30% lard† for a period of 72 days. They were then anesthetized with ethyl ether, and a solution of cholesterol-4-C^{14}, containing 1.12 x 10^6 cpm and prepared with the aid of Tween 20(3), was injected into the tail vein. Feces were collected at intervals for 14 days.

The samples of feces were dried and extracted continuously for 24 hours with ethanol in Soxhlet extractors. The methods for separating neutral sterols and bile acids have been described(2). In addition, the supernatant portions and the washes which remained following precipitation of the sterol digitonides were combined, taken to dryness, and redissolved in acetone-ethanol. Digitonides were again precipitated from this fraction by the method of Sperry and Webb(4) in order to assure that all digitonin precipitable sterols had been removed.

The supernatant fractions and washes which remained after digitonin precipitation of the neutral sterols from feces of rats fed the experiment diets for short periods (2), were combined, taken to dryness, and dissolved in pyridine. Excess digitonin was precipitated in ethyl ether(5), and the supernatant was again taken to dryness. Ketonic and non-ketonic fractions of this non-digitonin precipitable residue were separated by precipitation with Girard's Reagent "T" as follows(6): The residue was dissolved in 10% acetic acid in ethanol and refluxed for 30 minutes in the presence of an excess of Girard's Reagent "T". This solution was brought to pH 6 with a cold $NaHCO_3$ solution and extracted 3 times with ethyl ether. The bicarbonate-acetic acid solution was then acidified with 10 N HCl and extracted 3 times with ethyl ether. Each ethyl ether fraction was then taken to dryness, dissolved in methyl alcohol, added to 0.4% diphenyloxazole in toluene and assayed for C^{14} in a liquid scintillation counter.

After assaying for C^{14} in these experiments, an internal standard of cholesterol-C^{14} was added to each sample. The C^{14} of the standard was determined, and, when necessary, corrections for quenching were made.

Results. In Table I are listed the results

† The composition of the diet has been described (2).

TABLE II. Distribution of Non-Digitonin-Precipitable Sterol-C^{14} between Ketonic and Non-Ketonic Fractions.

Group	No. of rats	Diet	% administered dose recovered as non-digitonin precipitable neutral sterols	% non-digitonin precipitable sterols in	
				Ketonic fraction	Non-ketonic fraction
1	4	Fat free	9.61 ± 1.82	8.46 ± 4.68	91.54 ± 5.62
2	4	30% corn oil	34.75 ± 2.42	6.62 ± 4.02	93.38 ± 4.03
3	4	30% lard	11.64 ± 3.68	8.82 ± 5.25	91.18 ± 5.27

from pair-fed rats receiving diets containing no fat, 30% corn oil or 30% lard for 72 days. Average daily caloric intake of the various rats was similar, and there was no difference in weight gain of the animals fed the various diets. (Average weight gain was 134 g for the animals fed fat-free diets, 123 g for the animals fed corn oil, and 141 g for the lard group). Furthermore, average daily dry weights of the feces were comparable.

Rats fed the corn oil or lard diets uniformly excreted more C^{14} than did rats fed diets containing no fat. Excretion of bile acid-C^{14} and digitonin precipitable neutral sterol-C^{14} tended to be higher in the rats fed lard. As in the short term experiments, however, the most striking difference among the various animals was the marked increase in excretion of non-digitonin precipitable neutral sterols in the rats fed corn oil. This accounted in these rats for 18-25% of the administered dose of C^{14}.

The results of preliminary attempts to identify the nature of the non-digitonin precipitable neutral sterols in previously reported experiments on rats fed the experimental diets for 4 days(2) are listed in Table II. Ketonic sterols were separated by Girard's Reagent "T" precipitation, and radioactivity of both ketonic and non-ketonic fractions was assayed. It is evident from these studies that the marked acceleration in excretion of non-digitonin precipitable neutral sterols in rats fed corn oil is accounted for primarily by the C^{14} recovered in the non-ketonic sterol fraction.

Discussion. The mechanisms by which dietary fat influences cholesterol metabolism both in man(7) and rat(8) remain unknown. The previous finding of a qualitative difference in metabolism of cholesterol-4-C^{14} in rats fed either corn oil or lard has suggested that, in part, the effect of unsaturated fats may be secondary to acceleration of excretion of non-digitonin precipitable neutral sterols(1,2). This concept is supported by the finding of Swell, Trout, Hopper, Field and Treadwell (9) that as much as 20% of an injected dose of cholesterol-4-C^{14} may be converted to non-saponifiable, non-digitonin precipitable sterols within 24 hours in rats fed oleic acid. The present studies in rats fed for 2 months diets which varied in fat content confirms the finding that isocaloric substitution of 30% corn oil diet for either fat-free or 30% lard diets results in marked acceleration in excretion of non-digitonin precipitable neutral sterol-C^{14}, accounting in these rats for 33-42% of excreted C^{14}. Furthermore, this alteration in cholesterol-C^{14} metabolism is no more pronounced after 72 days than after 4 days of corn oil feeding(2). As yet neither the site of transformation nor the nature of this non-digitonin precipitable neutral sterol is known. Since no 3-alpha-hydroxy neutral sterols have yet been isolated in nature, it was thought that excretion of Δ^4-cholestene-3-one or cholestane-3-one might account under these circumstances for the non-digitonin precipitable fraction. However, these findings clearly demonstrate that the ratio of carbonyl to non-carbonyl fractions in the non-digitonin precipitable neutral sterols is unchanged by feeding of corn oil, indicating that ketonic sterols can account for only a negligible portion of the non-digitonin precipitable sterols. Thus, the sterol, which is excreted in increased quantities under the influence of corn oil feeding, is presumably a 3-alpha-hydroxy neutral sterol, the nature of which remains to be determined.

Summary. Effects of long-term feeding of various types and amounts of fat on fecal cholesterol excretion are reported. Diets con-

taining 30% corn oil produce marked acceleration in excretion of C^{14} as non-digitonin precipitable neutral sterols in rat feces, accounting in these animals for 17-25% of the administered C^{14}. This non-digitonin precipitable neutral sterol does not contain a ketonic group and is presumably a 3-alpha-hydroxy sterol.

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Characteristics of the Asian Strain of Influenza A.* (24266)

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Besides the marked antigenic variation found in the Asian strain of influenza A, several other characteristics differentiate it from influenza A viruses isolated in recent years (1,2). Included were growth behavior in the embryonated egg, and reaction with various erythrocytes, with antibody and with non-specific inhibitors. Although the virus propagated in monkey kidney cultures tended to minimize such differences, even in this case hemagglutination behavior set it apart from earlier strains. Pertinent features of Asian virus isolated and propagated in monkey kidney cultures and in the chick embryo are here described.

Materials and methods. Cultures (TC) were prepared with 0.25% trypsin (Difco, 1-250) digested Rhesus monkey kidneys, grown at 35°C in stationary tubes with 0.5% lactalbumin hydrolysate (Nutritional Biochemicals) and 2% calf serum (Microbiological Associates) in Earle's solution. After 7-12 days they were washed and maintained at 35°C with Mixture 199 (Microbiological Associates), and pH held between 7.0 and 7.6 during experiments by adding $NaHCO_3$ solution or allowing escape of CO_2. Ten day White Leghorn embryonated eggs were used for virus isolation in the amniotic sac or propagation in the allantoic sac (E). Throat garglings were obtained with Mixture 199, 0.5% lactalbumin hydrolysate in Hanks' solution or boiled skimmed milk and were collected from July through Dec. 1957 in Louisiana. Except where references are made to observations on several strains, experiments were done with Asian virus isolated in New Orleans from a Boy Scout on board a train from the 1957 Jamboree, Valley Forge, Pa. Separate passage lines were derived from the original specimen and maintained in the egg and in TC. The egg-ferret-mouse-egg line ($E_4F_1M_3E_{12}$) of A/Japan/305/57 (FE) was obtained from the Communicable Disease Center, U.S. Pub. Health Serv. Erythrocyte (rbc) suspensions for hemagglutination (Ha) were based on packed cell volume in a 15 ml conical tube after centrifugation in an International No. 1 at 1500 rpm for 10 minutes. A final concen-

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