hypophysectomized rats(10). Further studies are in process to attempt to improve the sensitivity by developing better antisera.

The assay devised seems to be a sensitive, specific, and precise assay permitting assays of large numbers of samples with relative ease.

Summary. A radioimmunoassay has been developed for alpha melanocyte stimulating hormone (α MSH). The assay compares favorably with biologic assays in terms of sensitivity and appears to be better in terms of precision and specificity.

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Bone Marrow Composition of Cholesterol-Fed Guinea Pigs.* (31448)

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Dietary cholesterol produces a hemolytic anemia in guinea pigs, which may be either normocytic or macrocytic. The condition is accompanied by enlarged and fatty livers, enlarged spleens and hyperplastic bone marrow (1). The mechanism of this response is unknown. One of the unanswered questions is whether defective RBC are released from the bone marrow of anemic animals or whether the blood cells, as formed, are essentially normal but are damaged by a factor or factors in the peripheral circulation. Pinter and Bailey (2) studied a cholesterol-induced anemia in rabbits. Results from cross-transfusion studies led to the conclusions that much of the trauma to the red cells had occurred during their production and, therefore, that dietary cholesterol had altered the function of the erythropoietic tissue. Our studies (unpublished) with anemic guinea pigs have led us to similar conclusions. A role of lipids not only in the structure but also in the function of cells and subcellular fractions has been recognized(3). It appears plausible that changes in lipid composition may cause disturbances in the cellular metabolism of bone marrow, with subsequent production of damaged red blood cells. We are presenting the results of a study of changes in the lipid composition of bone marrow of guinea pigs in response to dietary cholesterol.

Materials and methods. Groups of young male guinea pigs were fed a semi-synthetic diet containing 30% of casein, 6% of cottonseed oil and amounts of vitamins and minerals adequate for normal growth, with or without addition of 1% cholesterol (Table I). Development of the anemia was monitored by blood counts. When the RBC count had fallen to less than 3 mill/mm^3, the animals and a group of controls were sacrificed. The ends of the tibias and femurs were cut off and preserved for the preparation of marrow sections. Bone marrow smears were obtained from the heads

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TABLE I. Composition of Control Diet.*

Component	g/100 g diet	Component	mg/100 g diet
Casein	30.0	Thiamine • HCl	1.6
Cornstarch	20.0	Riboflavin	1.6
Cerelose	8.4	Pyridoxine	1.6
Cottonseed oil†	10.0	Ca-pantothenate	4.0
Sucrose	6.5	Niacin	20.0
Solka Flok	15.0	Folic acid	1.0
HMW salt mix‡	6.0	Biotin	.05
Choline bitartrate§	.15	Vit B_{12}	.005
Potassium acetate	2.5	Vit A acetate	.6
Magnesium oxide	.5	α-tocopherol	2.0
Zinc carbonate	.013	Menadione	.2
		Vit D_3	.004
		Ascorbic acid	200.0

* For cholesterol-fed group, 1 g cholesterol/100 g diet added.

† 6 g corn oil/100 g diet were fed instead of cottonseed oil for first 8 weeks of experiment.

‡ Hubbell, Mendell and Wakeman, J. Nutrition, 1937, v14, 273.

§ .36 g/100 g diet added for first 8 weeks of experiment.

of the femurs. The remaining marrow was forced out of the shafts with a stream of distilled water from a syringe, frozen on dry ice and lyophilized. The lipids of each marrow sample were extracted with 15 vol. of chloroform-methanol 1:1 (containing 0.1 ml of 1% hydroquinone) and separated by silicic acid column chromatography into fractions containing cholesterol ester (CE), triglycerides (TG), unesterified cholesterol (FC) (containing also free fatty acids and partial glycerides) and phospholipids (PL)(4).

Total lipid (TL)(5), cholesterol(6) and phosphorus(7) were determined in the unfractionated extract and in the appropriate fractions. The difference between TL and the sum of PL and TC was assumed to be the amount of triglycerides. This seems permissible because column recovery in our laboratory has been 95-103% over a period of several years. The fatty acid composition was determined by gas-liquid chromatography (GLC) by means of a Wilkens Aerograph (model 204) with flame-ionization detector. The PL were separated into subclasses by thin-layer chromatography, by means of the

TABLE II. Lipid Composition of Bone Marrow from Control and Cholesterol-Fed Guinea Pigs.*

			Phospholipid		Cholesterol ester		Unesterified cholesterol	
	Total lipid†	Triglyceride‡	Dry wt	Dry, fat-free wt	Dry wt	Dry, fat-free wt	Dry wt	Dry, fat-free wt
	(Dry wt, g/100 g)		(g/100 g)					
Control	68.1 ± 8.4§	65.5	2.1 ±.5	7.1 ±1.5	0	0	.48 ±.1	1.29 ±.35
Cholesterol-fed	21.1 ± 2.7	16.0	4.2 ±.3	5.3 ± .3	.14 ±.03	.19 ±.05	.75 ±.02	.99 ±.06

* 6 animals in control group, 5 in cholesterol-fed group.

† Mean size of total lipid samples extracted: control 234 ± 65 mg, cholesterol-fed 34 ± 3.5 mg.

‡ Triglyceride estimated as TL − [PL + TC]. Recovery of lipid from columns averaged between 95 and 103%.

§ Means ± S.E.

TABLE III. Major Fatty Acids of Bone Marrow Lipids from Control and Cholesterol-Fed Guinea Pigs.*

	Weight (%)						
	12:0† + 14:0	16:0	16:1	18:0	18:1	18:2	20:4
Total							
Control	3.1 ± .76	27.6 ±.93	1.5 ± .65	4.7 ± .35	26.5 ±2.3	35.8 ± 2.4	0
Cholesterol-fed	3.3 ±1.3	27.3 ±.17	1.8 ±1.1	10.1 ±1.7	26.8 ± .39	24.0 ± 3.0	0
Phospholipids‡							
Control	2.2	21.6	.1	16.4	18.4	20.0	7.5
Cholesterol-fed	2.2	23.0	1.7	19.0	23.7	14.8	7.3

* Minor amounts of other fatty acids have been omitted from Table. The concentration of fatty acids higher than C18 in the unfractionated extract was too low for detection.

† No. of carbons : No. of double bonds.

‡ PL of 2 and 3 animals of control and anemic groups, respectively, were analyzed.

TABLE IV. Major Classes of Phospholipids of Bone Marrow from Control and Cholesterol-Fed Guinea Pigs.

Origin	Phosphorus, % of total*								
	Lysophosphatidyl choline†	Sphingomyelin	Phosphatidyl choline	Phosphatidyl inositol	Phosphatidyl serine	Phosphatidyl ethanolamine	Solvent front	Recovery of P (%)
Control	1.3 ± .15	7.2 ± 1.0	36.1 ± 2.9	5.9 ± 1.8	11.9 ± 3.0	7.2 ± 1.4	27.0 ± 4.8	67
Cholesterol-fed	.7 ± .2	5.0 ± .8	40.7 ± .8	5.3 ± .6	10.4 ± .4	14.4 ± 1.3	12.6 ± 2.2	69

* % of phosphorus is in terms of total amount of phosphorus found on the plate. Values are means ± S.E. of 5 animals in each group. The PL of each marrow sample were separated on one plate. Phosphorus was determined on each band in triplicate.

† Identifications by comparison of chromatographic mobility with commercial standards.

solvent system described by Skipski *et al*(8). After visualization by dichlorofluorescein (0.2% in methanol) the individual components were eluted with the developing solvent, followed by 3 portions of methanol, and transmethylated by controlled heating with 1% H_2SO_4 in methanol. Fatty acid composition of each PL class was determined by GLC after extraction of the methyl ester with petroleum ether. The phosphorus content was determined in the remaining aqueous layer.

Results and discussion. Table II shows that, in the control guinea pig, more than half of the dry weight of the bone marrow was lipid, of which over 90% was TG. Converted to fresh weights, these figures agree very well with those of Dietz(9) for normal guinea pig marrow. The anemic animals had marrow with less than half as much total lipid (as dry weight). Decreases in the TG fraction accounted for the differences. The apparent increase in PL and unesterified cholesterol became nonsignificant when computed to a TG-free, dry-weight basis.

This finding indicates that the ratio of PL and cholesterol to protein did not change significantly in the rapidly proliferating bone marrow. Cholesterol ester was absent in the marrow of control guinea pigs, but increased to measurable amounts in that of the anemic animals. Table III shows the major fatty acid components of the unfractionated lipid extract and of the PL fraction. The fatty acid distribution in what is essentially TG was not unlike that in mammalian adipose tissue(10). It resembled closely that reported by Evans *et al*(11) for rabbit marrow. Feeding cholesterol resulted in an increase in saturated acids at the expense of linoleic acid. This is similar to the decrease of polyunsaturated fatty acids reported by Evans and Oppenheimer(12) in the hyperplastic marrow of anemic rabbits.

The same general trend was apparent in the fatty acids of the unfractionated PL. Proportions of arachidonic acid were the same in control and anemic groups. Results of the separation of the PL into subclasses are shown in Table IV. The major components were the phosphatidyl cholines (PC), with smaller amounts of phosphatidyl ethanolamines (PE) and phosphatidyl serines (PS), some sphingomyelin and phosphatidyl inositol (PI). This distribution of phosphorus among the different PL classes is not too different from that reported for the marrow of rabbit, cow, sheep and pig(13,14). The limitations of the method together with the variability in age and the degree of anemia manifested by the cholesterol-fed guinea pig make it necessary to regard differences between anemic and control groups as semiquantitative. Nevertheless, the trends toward increases in PC and PE and the very considerable decrease in the phosphorus

TABLE V. Fatty Acid Composition of Phospholipid Classes of Bone Marrow from Control and Cholesterol-Fed Guinea Pigs.*

		Weight (%)					
		12:0 + 14:0	16:0	18:0	18:1	18:2	20:4
Origin	Control	11.6	29.5	12.2	12.2	4.6	0
	Cholesterol-fed	18.5	30.0	10.5	14.4	0	0
Lysolecithin	Control	9.4	27.5	8.6	12.7	23.4	0
	Cholesterol-fed	4.8	29.9	12.6	15.3	24.0	0
Sphingomyelin	Control	13.4	46.7	13.6	11.5	0	0
	Cholesterol-fed	8.3	40.9	19.5	6.8	4.5	0
Lecithin	Control	7.1	34.3	16.0	14.1	25.4	2.2
	Cholesterol-fed	5.0	29.2	16.3	17.1	25.3	4.2
PI	Control	14.0	17.6	24.3	15.3	13.7	0
	Cholesterol-fed	8.0	13.0	41.8	17.1	17.9	0
PS	Control	2.3	5.5	44.7	11.8	9.4	23.5
	Cholesterol-fed	1.2	5.8	45.4	11.8	10.7	23.6
PE	Control	2.9	9.6	23.2	14.5	24.3	17.9
	Cholesterol-fed	.4	7.7	23.8	12.2	22.4	25.3
Solvent front	Control	3.3	18.4	17.9	14.4	24.3	8.5
	Cholesterol-fed	4.0	20.8	16.2	17.6	24.9	9.4

* Total percentages of fatty acids do not add to 100% because amounts of minor fatty acids and of unidentified GLC peaks have been omitted from Table.

in the bands near the solvent front (probably cardiolipins and phosphatidic acid) in the anemic guinea pig may be worth further investigation.

The fatty acid composition of the PL was further investigated because the anemic animals showed increased fragility of the circulating RBC, and it seemed possible that changes in the proportions of the different fatty acids in individual PL classes might be associated with this change in osmotic fragility(3) .

Table V shows the fatty acid composition of the major PL classes of the marrow of one typical anemic and one control animal. Data resemble those for PL classes in other mammalian tissues(3). Sphingomyelin contained predominantly saturated fatty acids and was especially high in palmitic acid. The lecithins, lysolecithins and "neutrals" were rich in linoleic acid, while most of the arachidonic acid of PL was found in PE and PS. Differences between the data for the anemic and the control animals were slight and generally not significant, although there was a trend toward more stearic acid in the lysolecithins, sphingomyelin and PI fractions and possibly of arachidonic acid in PE of the anemic marrow.

The changes we have observed in the overall composition of guinea pig marrow in response to a cholesterol-induced anemia are similar to those observed in other species and in other types of anemias. Anemias produced by bleeding(12,15), by injection of acetylphenylhydrazine(16), and by benzene poisoning(17) have been reported to cause a decrease of TL in the marrow of rabbits and cats. Krause(15), however, also reported increases in the proportion of PL and cholesterol, expressed on a fresh-weight basis in cat marrow.

The biochemical changes we have described were reflected by histological changes. The large, fat-laden cells present in marrow of normal guinea pigs were replaced by large numbers of nucleated cells, primarily of the erythroblastic series (unpublished).

Our results show that the marrow from anemic guinea pigs has an abnormal lipid composition. No conclusion can be drawn as to whether these changes reflect only hyperactivity of the marrow or whether they indicate the production of abnormal RBC. The decision must await further elucidation of the effects of changes of lipid composition on cellular function and of the factors controlling the changes.

Summary. The anemia produced in guinea pigs by dietary cholesterol led to a stimulation of erythropoietic activity with a prolifera-

tion of marrow cells which displaced the fat globules normally occupying the femoral marrow cavity. The triglyceride content decreased to half of its original level while the relative proportion of PL and cholesterol, on a dry, fat-free basis, remained unchanged. The proportion of linoleic acid in the remaining portion of TG decreased. Fractionation of the PL showed changes in their composition, particularly a decrease of the less polar components. The relationship of these changes in marrow to the changes observed in the peripheral blood requires further study.

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Changes in Serum Proteins and Glycoproteins in an Acute Controlled Infection. (31449)

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Changes in serum proteins following infection have been noted for some time. Leutscher(1) in reviewing the medical application of moving boundary electrophoresis noted that a characteristic of several acute infections was a decrease in the serum albumin and an increase in the serum alpha protein. This reviewer stated that these changes were part of a nonspecific response to injury or infection. Jencks *et al*(2), reporting on 1,516 general admissions to a large general hospital, demonstrated an albumin decrease and α_2-globulin increase in infectious disease. However, similar changes occurred in other illnesses as well. Graham *et al* (3) cited several others and reported their own results which were similar to those already noted. The most recent review was that of Peterman(4). Although the cited data were in accord as to the changes found, they were obtained from patients who had presented themselves to the hospital when infection was well established. When do changes in serum proteins appear and what is their relation to the febrile period? These questions are the subject of this research which involves subjects studied in a prospective manner.

Methods. As part of a larger program to develop and test vaccines, it became possible to study serum protein changes in a group of 22 male volunteers. These men, all members of the Seventh Day Adventist Church, were all in excellent health as evidenced by repeated normal physical and laboratory findings. Exposures to the organism, *Pasturella tularensis*, were carried out by aerosol methods routinely used in this unit. The men received a total infective dose of 2.2×10^3 or 2.2×10^4 viable organisms. Two men were

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