which occurred during the first hour in the presence of inhibitory amounts of diacetyl may indicate that in addition to its utilization in a normal metabolic pathway the compound may also interfere with the metabolism of an essential substance. Studies concerning this phase of the problem are now in progress.

Summary. Diacetyl prevented the growth of all of a variety of microorganisms in a range of 200-1600 μg/ml. In quantitative studies with a single strain of *E. coli,* diacetyl exerted an inhibitory action at low concentrations (< 129 μg/ml) and lethal action at higher concentrations (> 129 μg/ml). It was demonstrated that diacetyl was utilized during growth of *E. coli* and enhanced final growth when the medium was deficient in glucose.

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Inhibitory Effect of Cortisone Hyperlipemia on Arterial Lipid Deposition in Cholesterol-Fed Rabbits.* (21055)

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Recent experiments on rabbits fed cholesterol and subjected to alloxan(1,2), detergents(3), malnutrition(4,5), and hyaluronidase(6) indicate that the amount of lipid deposited in arterial walls may be markedly at variance with the degree of hypercholesterolemia attained. In some of these experiments hypercholesterolemia was associated with increases of other serum lipid components. It has been suggested that arterial lipid deposition in cholesterol-fed rabbits is reduced if the ratio of these other lipid components, notably phospholipid, to cholesterol is increased. Administration of cortisone to cholesterol-fed rabbits, according to Oppenheim and Bruger(7), and Gordon, Kobernick, McMillan, and Duff(8), retards arterial lipid deposition. Cook *et al.*(9), however, did not find conclusive evidence of retardation of atherosclerosis in 3 cholesterol-fed rabbits that were given relatively small doses of cortisone. Kobernick and More(10) and others have observed rapid development of hyperlipemia in rabbits receiving cortisone. Rich(11) found that in cortisone hyperlipemia the increase was chiefly of fatty acids. The present experiment was designed to determine what role cortisone hyperlipemia plays in retarding arterial lipid deposition in cholesterol-fed rabbits.

Methods. Three groups, each of 6 young adult rabbits, were given daily intramuscular injections of cortisone (Cortone Acetate, Merck) and food supplements of 1% cholesterol in 3 courses according to the following scheme:

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Days of exp.	Group I		Group II		Group III	
	Cortisone, mg	Cholesterol, %	Cortisone, mg	Cholesterol, %	Cortisone, mg	Cholesterol, %
1–14	0	0	0	1	0	1
15–28	5	0	5	1	0	1
29–42	0	0	0	0	0	0
43–56	10	0	10	1	0	1
57–70	0	0	0	0	0	0
71–84	15	0	15	1	0	1

Under this regimen the animals remained in good health throughout the experiment except for one rabbit in Group I that sickened after 8 weeks at the end of the second course of cortisone and was sacrificed along with one animal in each of Groups II and III. The *cholesterol* was dissolved in ether and mixed with rabbit food pellets. The ether was evaporated in warm air for at least several days before use. The body weights and blood pressures by ear capsule (Grant and Rothschild(12)) were recorded for one week prior to and throughout the experiment at 2- or 3-day periods. Blood (8 to 10 cc) was withdrawn by ear vein at weekly periods and analyzed for free and total cholesterol (Schoenheimer and Sperry), phospholipid (Fiske and Subbarow), proteins (Biuret), sugar (Folin and Wu). The bloods were drawn in the morning before the feed cups were replenished but the animals were not in a fasting state. Total serum lipid was determined gravimetrically on the petroleum ether soluble portion of a Bloor extract. Presence of sugar in the urine was tested for with "Clinitest" tablets (Ames Co.) on several occasions. The aortas taken at sacrifice of the animals were stained *in toto* with Sudan IV and photographed. A drawing was made of the grossly visible deposits from tracings of the photographs.

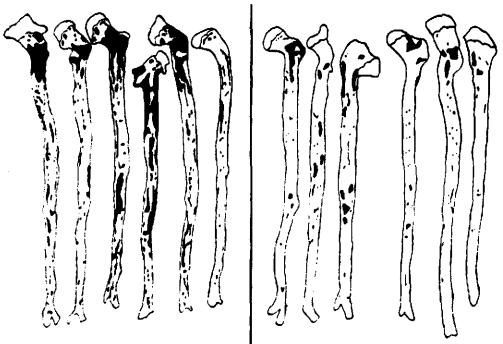

FIG. 1. Distribution of lipid deposits in aortas of cholesterol-fed and cortisone-injected rabbits. The drawings were made from tracings of photographs. The black areas represent lipid deposits. The aorta at the right of each group was from rabbits sacrificed after 8 weeks.

Results. It may be seen (Fig. 1) that lipid deposition was less than one-half as extensive in the aortas of the 6 rabbits that received both cortisone and cholesterol as in the 6 that were only fed cholesterol. Animals receiving only cortisone failed to develop any arterial lipid deposits even though there was a marked elevation in total serum lipid concentration (mean 2.64 g %) and slightly elevated blood cholesterols (mean 156 mg %).

In Table I is shown the mean concentration of cholesterol, phospholipid and total lipid at the time of sacrifice. The mean terminal concentration of cholesterol in the animals that received both cortisone and cholesterol was twice as great as in the group that was given only cholesterol even though the latter showed twice as extensive arterial lipid deposits. The total serum lipid content of the "cortisone-cholesterol" rabbits was 3 times as great as in the "cholesterol only" group. Cortisone alone caused sufficient lipemia to make rabbit serum milky after one week of daily injections of 5 mg intramuscularly. The serum of the "cortisone-cholesterol" group at the end of the experiment was almost oily as well as creamy. In both the "cortisone-cholesterol" and "cholesterol only" groups there was from 2.5 to 3 times as much cholesterol as phospholipid in the sera. Inhibition of atherosclerosis in the "cortisone-cholesterol" group cannot therefore be attributed to a lower cholesterol/phospholipid ratio. The lipids other than cholesterol and phospholipid in the sera were probably chiefly neutral fats and

TABLE I. Effect of Cortisone and Cholesterol on Blood Serum Lipids.

	Initial levels	Group I Cortisone	Group II Cortisone and cholesterol	Group III Cholesterol
		mg/% mean		
Total cholesterol	77	156	2809	1420
Phospholipid	121	355	1273	469
Total lipid	633	2640	9420	3230

fatty acids. These increased in greater proportion in the "cholesterol-cortisone" group than in the "cholesterol only" group. The amount of these fatty acids that was derived from cholesterol and phospholipid was not determined. Nevertheless the total lipid is so great compared with the other fractions involved that it is clear that the neutral fat fraction must be a major component. During the 2 periods in which cholesterol feeding was stopped the serum lipids fell rapidly but the relative proportions of the various fractions were the same as at the termination of the experiment.

Cortisone produced the same degree of hyperglycemia and glycosuria in both cholesterol-fed and non-cholesterol-fed rabbits. The mean blood sugar varied from 188 to 370 mg % depending on the dosage of cortisone and fell promptly to mean levels of from 93 to 119 mg % when cortisone administration was stopped. The urine during cortisone administration in 10 and 15 mg doses contained from traces to 4+ sugar but was occasionally negative.

The blood pressures showed no significant variations. There was a mean transient fall of 27 mm Hg shortly after cortisone injections were begun but later in the experiment cortisone injections no longer had any effect on the pressure. The blood pressures of the cholesterol-fed rabbits tended to rise from 10 to 20 mm Hg during the course of the experiment.

TABLE II. Relative Increase of Cholesterol, Phospholipid and Total Lipid in Sera of Rabbits on Cortisone and Cholesterol.

	Group I Cortisone R*	Group II Cortisone and cholesterol R*	Group III Cholesterol R*
Total cholesterol	2.0	36.5	18.4
Phospholipid	2.9	10.3	3.9
Total lipid	4.2	14.9	5.1

* Ratio, final/initial level.

The group receiving only cholesterol weighed more than the other 2 groups at the beginning of the experiment (mean 3457 g), but nevertheless gained weight consistently throughout. Their mean weight gain was 933 g. The groups receiving cortisone gained from 150 to 350 g within 24 hours each time a course of cortisone injections was begun but, consistently, this sudden weight gain was lost during the 2-week period of daily cortisone injections. It was, therefore, thought to be due to transient fluid retention. The original mean weight of the "cortisone-cholesterol" rabbits was 2804 g. Their mean weight after 10 weeks, before the third cortisone course was begun, was only 2922 g, a net gain of 116 g. The final mean weight during the third cortisone course was 3204 g. At necropsy, the cortisone injected animals had wasting of musculature and loss of adipose tissue as compared to the animals receiving only cholesterol.

The rabbits were allowed up to 200 g of food daily. No accurate measurements of intake were attempted because variable and often large amounts of food were scratched from the food cups and lost in the excreta. Nevertheless, both groups of cortisone injected rabbits consistently consumed greater quantities of food and water than did the rabbits fed cholesterol only. This may account for the greater degree of hypercholesterolemia observed in the "cholesterol-cortisone" group. Theoretically, therefore, this group should have developed greater degrees of atherosclerosis than the rabbits that received only cholesterol. The reverse was the case.

Discussion. The above findings are in ac-

cord with those of Oppenheim and Bruger(7) and very similar to those of Gordon, Kobernick, McMillan, and Duff(8). They indicate that not only alloxan but cortisone diabetes is associated with retardation of the development of atherosclerosis in cholesterol-fed rabbits. There is no evidence that any form of diabetes or Cushing's syndrome inhibits the development of atherosclerosis in man. It is becoming increasingly evident, therefore, that conditions that modify the development of atherosclerosis in cholesterol-fed animals would not necessarily have the same effect in man; they might, in fact, yield diametrically opposite results.

Nevertheless, it is important to discover why some hypercholesterolemic sera are more closely related to arterial lipid deposition than others. The role of lipids other than cholesterol in the pathogenesis of atherosclerosis is sometimes stressed. It seems remarkable, therefore, that persistent lipemias in which cholesterol does not play a dominant part do not lead to arterial deposits experimentally. In fact, the weight of evidence is in the opposite direction, namely, that when increases of serum cholesterol concentration are associated with even greater increases of other serum lipid components, the tendency for lipid to deposit in arteries is counteracted.

Gordon, Kobernick, McMillan, and Duff (8) found that cortisone reduced the degree of hypercholesterolemia and the ratio of cholesterol to phospholipid in cholesterol-fed rabbits. Our findings and those of Oppenheim and Bruger indicate that cortisone increases the severity of hypercholesterolemia and has little effect on the cholesterol/phospholipid ratio. These differences may depend upon variations in experimental procedure as Gordon *et al.*(8) pointed out. In their experiment fixed amounts of cholesterol were given daily and cortisone injections were continuous over an 8-week period. In our procedure cholesterol-containing food was supplied in larger amounts as noted above. At the end of each 2-week course of daily cortisone injections the rabbits began to lose weight and vigor but recovered during the 2-week rest periods. In any case our findings and those of Oppenheim and Bruger(7) indicate that reduction of cholesterol/phospholipid ratios is probably not the mechanism responsible for retardation of arterial lipid deposition in cortisone-treated rabbits. Our findings and those of Gordon *et al.*(8) are in accord in one particular, namely, cortisone produces a sustained alimentary type of hyperlipemia. If the inhibitory effect of cortisone on experimental atherosclerosis in rabbits depends on changes in blood lipid fractions it seems more logical to attribute this effect to the associated increase in neutral fat that accompanies the hypercholesterolemia. The possibility that cortisone inhibits arterial lipid deposition in some manner independent of its effects on blood lipids cannot be excluded. It should be noted, however, that ACTH does not produce constant or severe lipemias in rabbits(13). Oppenheim and Bruger(7) found that ACTH was less effective than cortisone in inhibiting arterial lipid deposition in cholesterol-fed rabbits. Firstbrook(4) has shown that malnourished rabbits develop less severe atherosclerosis than well-nourished ones when equal amounts of cholesterol are fed to both groups. Since rabbits receiving cortisone as well as cholesterol gained less weight than those on cholesterol alone, it is possible that malnutrition was a factor in inhibiting arterial lipid deposition in the former. McMillan, Whiteside, and Duff(5) found, however, that partly starved rabbits developed higher blood cholesterol levels than adequately fed ones when equal amounts of cholesterol were added to the food. Under these circumstances the underweight rabbits developed as much atherosclerotic change as the well nourished ones with lower blood cholesterol concentrations. The rabbits receiving both cortisone and cholesterol are more comparable to the partly starved ones of McMillan, Whiteside, and Duff(5) since they, too, had much higher blood levels than the rabbits that received only cholesterol. Since, in spite of this, the degree of arterial lipid deposition observed in the cortisone-treated animals was decidedly less than in the controls, it may be concluded that this difference in severity was probably not entirely the result of malnutrition. The variations in blood pressure observed were not sufficiently large to have been a factor in the

inhibition of lipid deposition in the cortisone-treated rabbits.

Summary. Administration of cortisone to rabbits caused hyperlipemia due chiefly to an increase of lipids other than cholesterol and phospholipid. Cholesterol-fed rabbits treated with cortisone developed severer degrees of hypercholesterolemia and hyperlipemia than rabbits on cholesterol alone. The relative proportion of cholesterol in the serum lipids of cholesterol-fed rabbits on cortisone was less than in the serum lipids of rabbits on cholesterol alone. Significantly less lipid was deposited in the arteries of the cortisone-treated, cholesterol-fed animals than in the cholesterol-fed controls. It is suggested that hyperlipemias in which the lipid fractions other than cholesterol undergo the greatest increase, are less likely to be associated with arterial lipid deposition than hyperlipemic states in which cholesterol is the major constituent.

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Copper Metabolism. XII. Influence of Manganese on Metabolism of Copper.* (21056)

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The clinical and pathological similarity between the manifestations of chronic manganese poisoning in man and the changes which occur in hepatolenticular degeneration has been noted several times(1-3). Hepatolenticular degeneration is now known to be characterized by aminoaciduria, hypocupremia, hypercupriuria and an increase in the amount of copper in the tissues(4-9). Studies on the metabolism of copper in man or in animals with manganese toxicity have not been reported, however.

It was, therefore, considered to be of interest to determine whether or not the administration of large amounts of manganese could be shown to influence the metabolism of copper in animals. In this report observations are presented on the level of copper in the plasma, urine and tissues of rats given large amounts of manganese. The influence of manganese on the red blood cells, the urinary excretion of amino acids and the content of iron in the plasma and tissues were also studied. A preliminary report of this work has been published(10).

Methods. Eighty-three male weanling rats of the Sprague-Dawley strain were divided into 4 groups (Table I), housed in individual

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