S-S red blood cells. It is accepted that heme constitutes X growth factor for this organism (4), but the 2 hemoglobins under study (A-A and S-S) are thought to differ only in their globin fraction(5).

As multiple blood donors from unrelated families were used and experiments were run on 2 to 4 separate occasions with similar results, we believe our findings to be highly significant.

Summary. Homozygous sickle cell (S-S) red blood cells were less capable of fostering surface growth of *Hemophilus influenzae*, *Diplococcus pneumoniae*, and *Streptococcus salivarius* in Trypticase soy agar than were normal adult (A-A) red blood cells.

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Cortisone Effect on Exogenous Cholesterol Metabolism: Isotope Observations in Cholesterol-Fed Rabbit. (24801)

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It is firmly established(1-5) that cortisone retards development of atherosclerosis in cholesterol fed rabbits. Although aortal findings were correlated with several kinds of physico-chemical alterations in blood lipid composition, none of these appeared directly responsible for the observed inhibitory phenomenon(1,2,4,5-7). Since cholesterol deposition in aortic intima is primary to development of atheroma in the experimental animal and cortisone exerted an influence upon this process, an investigation was undertaken of the effect of cortisone on cholesterol metabolism. In this paper is reported an experiment comparing fate of meal containing cholesterol-4-C^{14} in untreated and cortisone treated rabbits fed a high cholesterol diet for 2 weeks.

Materials and methods. Eight, female, New Zealand white rabbits of pedigreed stock were used. A morning ration of 35 g of ground food pellets mixed with 1 g of crystalline cholesterol (U.S.P., Merck†) was given daily to each rabbit and 6 hours later an additional 100 g of unground stock chow. The morning meal usually was completely ingested in 3 hours. Tap water was available *ad lib.* At 1:30 daily, 10 mg of cortisone acetate was injected into a thigh muscle of 4 rabbits while others were injected with equal amount (1 ml) of physiological saline. On 12th day a meal containing 1 g of labeled cholesterol (10 μc cholesterol-4-C^{14}) was substituted for the morning ration. After labeled cholesterol meal, heart blood (5 cc) was taken at 6, 10 and 24 hours. A 1 ml plasma aliquot of these blood samples was extracted with 25 ml of 1:1 alcohol-acetone and adequate amounts of extract were taken for cholesterol chemical and radioactivity determinations. A terminal heart blood sample (15 cc) was taken 48 hours after labeled cholesterol meal and the animals killed with an overdose of intravenously administered EVIPAL (n-methyl-cyclo-hexenyl-methylbarbituric acid). The entire liver, the aorta, and 5 g approximately of uppermost section of ileum were excised. The latter 2 tissues were cleaned of adherent fat, opened longitudinally, and rinsed with ice-cold 0.85% saline solution. The lipids of

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TABLE I. Effect of Cortisone in Cholesterol-Fed Rabbits upon Free and Esterified Cholesterol Content of Selected Tissues.

Tissues	Not treated		Cortisone treated*	
	F. C.†	E. C.	F. C.	E. C.
Ileum	3.04 (2.88-3.24)‡	.28 (.23- .42)	2.96 (2.77-3.31)	.32 (.29- .40)
Aorta	1.51 (1.42-1.62)	.07 (0 - .18)	1.52 (1.34-1.75)	.01 (0 - .04)
Plasma	1.07 (.84-1.32)	2.77 (2.28-3.28)	.57 (.46- .62)	1.13 (.88-1.46)
Liver	2.42 (2.12-2.63)	2.14 (1.57-3.31)	1.45 (1.25-1.70)	1.35 (.99-1.94)
Liver content, mg	234.3	205.8	302.0	280.1
Body wt, kg	3.06 (2.49- 3.56)§		2.98 (2.78- 3.20)	
Liver " , g	97.2 (84.9 -109.4)		212.7 (168.6 -251.6)	
" " , g/kg	34.6 (26.9 - 43.9)		71.9 (57.2 - 90.5)	

* See text. † F. C. = Free cholesterol; E. C. = Ester cholesterol ‡ Mean and range of values in group, mg/g of tissue or cc of plasma. § Mean and range of values.

aorta, ileum, 5 g pieces of liver and 5 ml plasma aliquot of terminal blood sample were extracted as follows: twice with 10 volumes of 1:1 alcohol-acetone, twice with 10 volumes of 1:3 alcohol-ether, and once with 10 volumes of 2:1 chloroform-methanol. The combined extracts were taken to dryness under nitrogen on steam bath and the residues redissolved in appropriate volumes of 2:1 chloroform-methanol. Free and esterified cholesterol-4-C^{14} were precipitated as digitonides from suitable aliquots of 1:1 alcohol-acetone solutions according to Swell *et al.*(8). The digitonides C^{14}-activities were measured in windowless gas flow counter and corrected for self-absorption. In some cases unlabelled cholesterol was added to obtain proper plating of digitonides. An attempt was made to plate the digitonide of 2-5 mg of cholesterol. Counting error was held to $\pm$ 3%. Chemical free and total cholesterol were determined colorimetrically on each extract by the method of Sperry and Webb(9).

Results. In both groups average body weight was the same while in cortisone injected rabbits, livers weighed twice that of untreated animals (Table I). In liver and plasma terminal concentrations of free and esterified cholesterol of cortisone treated rabbits were approximately 50% of respective tissue values in the untreated animal. Cortisone injection resulted in retarded development of hypercholesterolemia in this period of high cholesterol ingestion. The difference between the 2 groups of animals with respect to concentrations of liver cholesterol fractions was more than compensated by the increased weight of liver in cortisone injected animals. In the latter free and ester cholesterol content in the entire liver were approximately 30% more than in the entire organ of untreated rabbits. Concentrations of cholesterol fractions in ileum region and aorta of both groups were the same, respectively.

Specific activities of plasma free and esterified cholesterol as a function of time after fed labeled meal, are plottd in Fig. 1. Growth of activity in free and ester cholesterol fractions of plasma of cortisone injected rabbits proceeded at different rates from that of the untreated. In the latter, free and ester cholesterol specific activity rose with successive blood samples during experimental period. In cortisone injected rabbits ester cholesterol specific activity was high in the 6-hour blood sample, and fell precipitously between 10 and 24 hours, while at the same time the specific activity of free cholesterol rose only slightly.

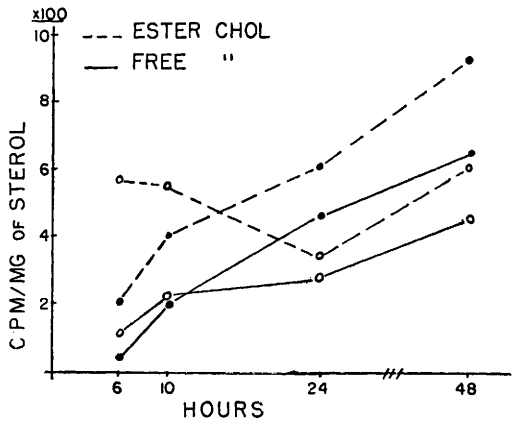

FIG. 1. Plasma free and esterified cholesterol specific activity-time relations after fed 1 g cholesterol meal containing 10 μc cholesterol-4-C^{14}. Untreated (●), cortisone (○).

TABLE II. Specific Activities (cpm/mg) of Free and Esterified Cholesterol in Tissues of Cholesterol-Fed Rabbits 48 Hr after Ingestion of Meal Containing Cholesterol-4-C^{14}.

Groups	Ileum		Aorta		Plasma		Liver	
	F. C.*	E. C.	F. C.	E. C.	F. C.	E. C.	F. C.	E. C.
Not treated	852† (444-1046)	1702 (1006-2250)	107 (61-147)		666 (339-965)	929 (468-1332)	608 (317-915)	700 (347-1269)
Cortisone treated‡	561 (312-792)	756 (374-952)	71 (53-84)		418 (327-637)	611 (346-701)	400 (287-476)	353 (238-420)

* F. C. = Free cholesterol; E. C. = Ester cholesterol. † Mean and range of values of group.
‡ See text.

In cortisone injected rabbits 2 days after feeding labeled cholesterol meal, specific activity of free and esterified cholesterol fractions in ileum region, liver, plasma, and aorta free cholesterol specific activity were lower than specific activity values of respective cholesterol fractions in untreated animals. A trace C^{14}-activity was found in aorta ester cholesterol of the 2 groups (Table II).

Discussion. These results showed that the behavior of ingested cholesterol was modified in cortisone injected, cholesterol-fed rabbit compared to the untreated animal. Studies are in progress to elucidate the mechanism of action of cortisone upon influx of dietary cholesterol. It is not certain whether cortisone caused changes in removal and catabolism of cholesterol ingested by the rabbit, and or altered the biosynthetic rates of endogenous cholesterol. Either one or the combination of these altered states of cholesterol metabolism could account for the findings in the C^{14}-activity data. Several possible mechanisms of cortisone inhibition of experimental cholesterol atherosclerosis have been postulated—change in serum macromolecular lipoproteins(1), altered cholesterol:phospholipid ratio and effect of cortisone on tissue permeability(3), and a role of elevated neutral fat in serum(2). All these were suggested on the basis of inhibited atherosclerosis correlated with some "exaggerated" quantitative chemical finding in blood of long-term cortisone treated, cholesterol fed rabbits. The findings of this experiment are suggestive of cortisone exerting control upon some aspect of cholesterol metabolism. Whether there exists a close relationship between role of cortisone and inhibited development of atherosclerosis in rabbits needs to be studied.

Summary. The fate of a cholesterol meal containing 10 μc of cholesterol-4-C^{14} was compared in untreated and cortisone treated, cholesterol-fed rabbits on their regime for 14 days. Plasma free and ester cholesterol specific activity were followed during 48 hours after the fed labeled cholesterol meal, and then animals were killed. Terminal concentrations of free and ester cholesterol in plasma and in liver of cortisone treated, were significantly lower than the respective values in untreated. The free and ester cholesterol specific activity of plasma, liver, ileum and aorta of cortisone treated were 40-60% lower than that of untreated animals. It was concluded that cortisone injection of cholesterol-fed rabbits caused a change in behavior of ingested cholesterol. Further studies will be necessary to elucidate the mechanism of cortisone action.

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