How can the apparent acceleration of adenoma-formation in "germfree" animals be explained? Can the poorly developed reticuloendothelial system of such mice be related to increased susceptibility to tumorigenesis? Data accumulated thus far indicate that subcutaneously-inoculated MCA induces localized fibrosarcomas more rapidly in germfree than in conventional adult rats and mice(15). Possibly the strains of germfree mouse and rat tested are more uniformly susceptible to MCA than other strains. Do the results here reported suggest that the microbial flora in the conventional animal may induce some relative state of resistance or interference to the carcinogenic mechanism? The germfree animal provides a mechanism for fruitful exploration of carcinogenic stimuli and the alterations which they induce in the host.

Summary. Methylcholanthrene was inoculated subcutaneously into new-born germfree and conventional mice (Swiss-Webster strain). Pulmonary adenomas were thereby induced more frequently and in greater numbers in the germfree animals. Viral agents have not been detected in the adenoma tissues by electron microscopy.

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Cholesterol Content of Human Serum Lipoproteins Obtained by Dextran Sulfate Precipitation and by Preparative Ultracentrifugation.* (28010)

DAVID KRITCHEVSKY, SHIRLEY A. TEPPER, PETAR ALAUPOVIC AND ROBERT H. FURMAN
Wistar Institute of Anatomy and Biology, Philadelphia, Pa., and Oklahoma Medical Research Institute, Oklahoma City

The formation of insoluble complexes between lipoproteins and various sulfated polysaccharides was described by Bernfeld(1), Oncley(2), and Burstein and Samaille(3). The subject was thoroughly reviewed by Cornwell and Kruger(4).

Although it is generally assumed(4) that sulfated polysaccharides precipitate β and lower density lipoproteins ($d<1.063$ g/ml), no comparison of the cholesterol content of the fractions obtained by precipitation and by preparative ultracentrifugation of human serum has been reported. Recently Sakagami and Zilversmit(5) compared the dextran sulfate precipitable lipoprotein in sera from dogs with those obtained by ultracentrifugation and indicated that this method could be used as a means of quantitatively precipitating low density lipoproteins. Advantage was taken, therefore, of an opportunity to compare the cholesterol content of ultracentrifugally isolated α lipoproteins ($d>1.063$ g/ml)

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in sera from human subjects with the cholesterol content of the "dextran sulfate soluble" (supernatant fraction) after dextran sulfate precipitation. The portion of serum cholesterol present as β and lower density lipoproteins may be calculated by difference(6,7,8) or obtained by direct analysis. Data from this study form the basis for this report.

Materials and methods. All sera were prepared from blood obtained at the Oklahoma Medical Research Institute from patients fasted overnight. Preparative ultracentrifugation of serum lipoproteins was carried out according to a modification(9) of the method of Havel, Eder and Bragdon(10). Serum cholesterol was determined according to the Sperry and Webb modification(11) of the Schoenheimer and Sperry method(12). Serum fractions obtained after preparative ultracentrifugation were analyzed utilizing semi-micro adaptations of the above methods. Aliquots of each serum were sent unfrozen in iced containers to the Wistar Institute, Philadelphia, for dextran-sulfate precipitation. Results were compared only after both laboratories had completed all analyses.

The dextran sulfate precipitation method described by Castaigne *et al.*(8,13) was used. Dextran sulfate from 2 different sources (l'Equilibre Biologique, S.A., Commentry (Allier) France, Lot 5438, and Dextran Chemicals, Inc., N. Y.) was used and gave identical results. The precipitation procedure was as follows: To each ml of serum was added 0.04 ml of 5% dextran sulfate and 0.1 ml of 11.1% calcium chloride solution. After 24 hours at refrigerator temperature the mixture was centrifuged and the supernatant taken as "α-lipoprotein solution." The precipitate was redissolved in 1 ml of 0.9% sodium chloride, and 0.1 ml of a 12.7% solution of potassium oxalate was added. After 24 hours at refrigerator temperature the precipitated calcium oxalate was centrifuged and the supernatant taken as "β-lipoprotein solution." Cholesterol analyses were carried out using the procedure of Mann(14).

When undiluted serum from markedly hyperglyceridemic subjects was studied, precipitation of lower density lipoproteins usually was incomplete. In working with sera from hyperlipidemic dogs, Sakagami and Zilversmit(15) found that dilution of the serum (1:5) prior to precipitation with dextran sulfate was necessary in order to achieve consistent results. Sera from patients whose triglyceride levels were higher than 400 mg % were treated in this fashion.

TABLE I. α Lipoprotein and Total Serum Cholesterol Levels (mg %) of Normal Human Sera (Triglyceride Level < 400 mg %) after Dextran Sulfate Precipitation or Ultracentrifugation.

	α lipoprotein cholesterol (21)*		Total serum cholesterol (21)	
Laboratory	Avg	Stand dev†	Avg	Stand dev
Wistar‡	49	13.6	231	75
OMRI§	48	13.0	240	86

* No. of sera.

† $\sigma = \sqrt{\frac{\Sigma(X^2)}{N} - \bar{X}^2}$.

‡ Analyses carried out at Wistar Inst. after dextran sulfate precipitation.

§ Analyses carried out at the Oklahoma Med. Research Inst. after ultracentrifugation.

Results and discussion. Cholesterol values obtained in the 2 laboratories for the α lipoprotein fractions and for whole serum in 27 subjects are presented in Table I. The mean α lipoprotein cholesterol values were 49 mg % (W) and 48 mg % (OMRI) respectively, and mean total serum cholesterol values were 231 mg % (W) and 240 mg % (OMRI). It is noteworthy that the difference between the individual α lipoprotein cholesterol values from the 2 laboratories averaged 15.7% and that between indivdiual serum total cholesterol determinations, 9.2%. These differences are relatively small in view of the large differences (100% in some instances) reported in interlaboratory studies of serum cholesterol levels(16) and in view of the differences between the analytical methods employed. When the same serum was analyzed in the same laboratory by 6 different methods, Morris(17) found a 52.5% difference between the highest and lowest values and a 9.4% difference between the second highest and lowest values.

Table II presents cholesterol values for α lipoproteins from hyperglyceridemic patients. These sera were diluted (1:5) with saline prior to dextran precipitation. To obtain

TABLE II. α Lipoprotein and Total Serum Cholesterol Levels (mg %) of Hyperglyceridemic (> 400 mg %) Human Sera after Dextran Sulfate Precipitation or Ultracentrifugation.

	α lipoprotein cholesterol (8)*		Total serum cholesterol (8)	
	Avg	Stand dev†	Avg	Stand dev
Wistar‡	26	9.4	367	137
OMRI§	24	10.3	378	118

* No. of sera.

† $\sigma = \sqrt{\frac{\Sigma(X^2)}{N} - \bar{X}^2}$.

‡ Analyses carried out at Wistar Inst. after dextran sulfate precipitation.

§ Analyses carried out at the Oklahoma Med. Research Inst. after ultracentrifugation.

consistent results it was necessary to use 1 ml of serum for dilution. Average values were 26 mg % (W) and 24 mg % (OMRI) for α lipoprotein cholesterol and 367 mg % (W) and 378 mg % (OMRI) for total cholesterol, respectively.

Despite apparently total precipitation of oxalate in preparation of the β-lipoprotein solution there was evidence of interference with color formation. By colorimetric analysis, Sperry and Schoenheimer(18) found 15% less cholesterol in oxalated than in heparinized plasma and Kenny(19) reported a similar discrepancy. Addition of 0.1 ml of 12.7% potassium oxalate solution to aliquots of some of the sera utilized in this study showed an average drop in cholesterol concentration of 14.3% (Table III). Cholesterol is known to form a complex with oxalic acid which may, under the conditions employed in this study, prevent precipitation of the oxalate by calcium and also interfere with the color reaction. This discrepancy was not observed by Sakagami and Zilversmit(5) who could account for all of the serum cholesterol in the α and lower density lipoprotein fractions of canine serum. When the authors utilized the Sperry-Webb(11) cholesterol assay which entails digitonide precipitation, the sum of the cholesterol in the α and lower density lipoprotein fractions equalled serum total cholesterol.

TABLE III. Effect of Oxalate* on Serum Cholesterol Determinations.

	Serum total cholesterol†	
	Avg	Stand dev
No oxalate added	224 (100 %)	66
Oxalate "	192 (85.7%)	56

* 0.1 ml of 12.7% potassium oxalate added to 1 ml serum.

† 10 sera analyzed.

The analyses of 3 aliquots of serum using dextran sulfate from different sources is shown in Table IV. It would appear that any good grade dextran sulfate with a molecular weight over 20,000 may be used.

It was found that the full 24-hour period is required for complete precipitation of β and lower density lipoproteins, as was noted in respect to dog serum(5). No further precipitation occurs beyond this time, even after 48 hours. Thus, for one particular sample, serial α and β lipoprotein cholesterol values were as follows: one hour, 87.4 and 75.0 mg %; 6 hours, 78.6 and 96.6 mg %; 24 hours, 46.8 and 109.0 mg %; 48 hours, 46.5 and 111.2 mg %.

TABLE IV. Comparison of Cholesterol Content of α and β Lipoproteins Precipitated by Dextran Sulfate from Different Sources.

	Lipoprotein cholesterol, mg %			
	Dextran A*		Dextran B†	
Sample	α	β	α	β
I	61.5	173.1	60.3	163.4
II	64.9	219.3	63.3	213.2
III	37.6	156.8	37.6	155.9

* l'Equilibre Biologique, S. A., France.

† Dextran Chemicals, Inc., U. S. A.

Dextran sulfate precipitation provides a relatively simple method for separation of high density α lipoproteins from β and lower density lipoproteins. The method is applicable to large numbers of sera and permits partition of the high density and lower density lipoprotein sterol levels in human sera. Determination of α lipoprotein and serum total cholesterol permits calculation of β and lower density lipoprotein cholesterol by difference. Leveille and co-workers(20) recently employed this measure to determine the α lipoprotein cholesterol in the sera of a number of animal species. This method for α lipoprotein cholesterol determination is less troublesome than the extraction method of Katchalsky *et al.*(21).

Summary. A comparison of α and β lipoprotein cholesterol levels of lipoproteins of human serum obtained by dextran sulfate precipitation compares well with analyses of lipoproteins obtained by ultracentrifugation. Comparison of dextran sulfate from 2 different sources gave identical results. The presence of oxalate may interfere with the analysis of β lipoprotein cholesterol, but this can be avoided by using analytical methods which entail digitonide precipitation.

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Tissue Distribution and Storage Forms of Vitamin B_{12} Injected and Orally Administered to the Dog.* (28011)

CHARLES ROSENBLUM, PETER G. REIZENSTEIN,† EUGENE P. CRONKITE AND HENRY T. MERIWETHER

Merck Sharp & Dohme Research Laboratories, Rahway, N. J., and Brookhaven National Laboratory, Upton, L. I., N. Y.

Previous attempts by the present authors (1,2,3) to compare the behavior of orally administered Vit. B_{12} with that of injected material have been inconclusive. Thus, 30 days after administration to normal humans, 0.19% of a tracer amount (0.5 μg) of injected radioactive Vit. B_{12} is excreted(1) per day; and in an independent study(2), the eventual daily output was found to be 0.23% of a 3 μg dose. By contrast the total excretion rate of normal Vit. B_{12} from body stores (3) appears to be only $\simeq$ 0.03% per day. Such a divergence may result from incomplete mixing of radioactive Vit. B_{12} and body stores of the vitamin. Alternatively it may actually reflect the functioning of different compartments attending the several modes of administration involved. The possibility of degradation or transformation of vitamin within tissues and organs must also be considered. Because of the apparent discrepancy, experiments were initiated in humans

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† Present address: Karolinska Institut, Stockholm, Sweden.