Cholesterol in Seromucoid Fraction of Normal and Pathologic Sera.* (23391)

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Throughout this paper, seromucoid is defined as that fraction of human serum soluble in perchloric acid solutions and precipitable with phosphotungstic acid. Little is known of its properties and of its composition, which is probably heterogenous. It migrates in the greatest part with the α-globulin fraction of serum in the electrophoretic field(1,2). Its content of hexoses and hexosamine is high, while approximately 3.6%(3) to 4.2%‡ of its weight is constituted by materials soluble in hot alcohol, presumably lipids. Winzler *et al.* reported a negative Lieberman-Burchard's reaction in the lipid moiety isolated from mucoproteins of human serum(4). Rimington(5), on the other hand, using entirely different methods, identified cholesterol in a purified mucoprotein obtained from ox serum.

Our interest in the relationship of cholesterol to the seromucoid fraction was also stimulated by previous observations of significant alterations of seromucoid and of cholesterol in the serum of patients with lymphomas(6,7). Accordingly, we determined by chemical technics the cholesterol precipitated with the perchloric acid soluble, phosphotungstic acid insoluble fraction of serum in 6 normal subjects and in 21 patients all suffering from diseases in which significant multiple abnormalities of mucoproteins, seromucoid and cholesterol have been described (6 with Hodgkin's disease, 4 with reticulum cell sarcoma, 1 with lymphoblastoma; 5 with generalized carcinomatosis; 3 with acute leukemia and 2 with parenchymal liver disease).

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Methods. Separation of seromucoid was carried out by a modification of the method of Winzler *et al.*(4), perchloric acid being used at a final concentration of 0.24 M instead of the standard 0.6 M(6). After filtration, the supernatant from the perchloric acid-treated serum (2 ml) was divided in 2 aliquots of 5 ml in glass-stoppered centrifuge tubes for determination of protein and of cholesterol. One ml of 5% solution of phosphotungstic acid in 2 N HCl was added to each test tube. After standing at room temperature for 10 minutes, all test tubes were centrifuged at 2,000 rpm for 10 minutes. Supernatant was discarded. Precipitate was washed once with 5 ml of phosphotungstic acid solution. Precipitate (seromucoid) was then analyzed for protein and for cholesterol. The biuret reaction, with casein as known standard(4), was used for determination of protein; a modification of Zlatkis' method(8) was used for determination of total serum cholesterol. This was as follows: after washing with phosphotungstic acid solution, the seromucoid precipitate was dissolved in 3 ml of aldehyde-free glacial acetic acid. Two ml of a solution of 1% ferric chloride in H_2SO_4 (1.84 s.g.) were added slowly. Tubes were shaken vigorously for 1 minute; 10 minutes later, when tubes had cooled off at room temperature, optical density of samples was read in Beckman spectrophotometer at 560 λ. Preliminary tests showed that phosphotungstic acid would not interfere with spectrophotometric reading for cholesterol at this wave length. Concentration of cholesterol was calculated by comparing the experimental readings with those of a curve showing the values obtained with solutions containing known amounts of cholesterol. A correction formula was used in the final calculations as indicated by Winzler *et al.*(4).

Results. 1. *Cholesterol in seromucoid fraction of normal serum* (Table I). The acetone-

TABLE I. Total Protein, Perchloric Acid Soluble Fraction (Seromucoid), Total Cholesterol and Cholesterol in the Seromucoid Fraction: Health and Disease.

	Total serum protein, g %		Total serum cholesterol, mg %		Seromucoid Protein, mg %		Seromucoid Cholesterol, mg %	
	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ
(a) Healthy individuals	7.18	.356	180.16	27.7	95.16	8.44	2.92	.22
(b) Hodgkins' disease	6.9	.62	164.2	31.4	164.2	49.2	6.1	1.035
(c) Reticulum-cell sarcoma, lymphosarcoma, lymphoblastoma	6.34	.567	159	30.8	189.54	18.46	6.12	1.9
(d) Acute leukemia	6.8	.51	232.3	16.6	122	15.1	2.4	.294
(e) Liver disease	6.1	.5	155	5	155	15	3.3	.2
(f) Metastatic carcinoma	5.84	.28	134	7	215.2	3.5	7.08	.74

X = Arithmetic mean. σ = Stand. deviation (s.d.).

alcohol extract of the seromucoid fraction of normal human serum exhibited positive Lieberman-Burchard's reaction. Untreated seromucoid fractions contained an average of 2.92 mg % cholesterol, as compared to an average total serum cholesterol value of 180.16 mg %. Free cholesterol and cholesterol esters were both found in the seromucoid fractions of normal and of pathological sera as well as in their acetone-alcohol extracts, in an average ratio of 1:4. Pathologic sera were more suitable for this type of study because of their higher seromucoid content. The following were average values in experiments on 3 such sera (metastatic carcinoma): (a) perchloric acid soluble protein (seromucoid), 200 mg %; (b) total cholesterol in this fraction, 14 mg %; (c) free cholesterol, 3.25 mg %. Thus, 81% of the cholesterol found in the seromucoid fraction was combined.

2. *Status of cholesterol in perchloric acid soluble fraction (seromucoid) of serum.* Normal and pathologic human sera were studied to establish the "chemical status" of cholesterol precipitated with the seromucoid fraction: (a) dilution of serum up to 1/10 with water and lowering of concentration of the perchloric acid reagent below a critical level of 0.225 M caused parallel and predictable reduction in yield of seromucoid and of cholesterol recovered from this fraction; (b) in a second set of experiments, however, cholesterol was extracted first by adding 5 ml of mixture of equal volumes of acetone and ethanol to 2 ml of both normal and pathologic serum. The seromucoid fraction was then precipitated and its cholesterol content determined. Only 40% of the expected cholesterol content could be found. Also, the seromucoid fraction was precipitated first from 2 ml of normal or pathologic serum and 5 ml of acetone-alcohol mixture were then added, to extract cholesterol. Again only 30 to 40% of the original cholesterol could be recovered in all instances from the washed seromucoid residue; (c) the precipitated seromucoid fraction contained only 15% of the expected cholesterol, after serum had been treated with 2.5 volumes of cold ethyl acetate; (d) cholesterol could be extracted entirely by treating the precipitated seromucoid fraction with 2.5 volumes of boiling alcohol; (e) in a final experiment, the seromucoid fraction was prepared from 50 ml of normal serum. After treatment of serum with perchloric acid, the supernatant (containing the seromucoid) was dialysed against concentrated dextrin. Fluid was thereby concentrated 6 times; pH was finally adjusted to 6.5. The solution, exhibiting a specific gravity of 1.10, was centrifuged for 22 hours in a Spinco Model L ultracentrifuge at 40,000 rpm (average 105,000 g).§ Five fractions of equal volume were collected from top to bottom. The bottom fraction had a distinct yellow color. In addition, there was a well defined solid residue. Total seromucoid and cholesterol were determined in each fraction (Table II). Tendency of cholesterol to accumulate in the heaviest fraction was obvious.

The results of the various experiments excluded the existence of a true chemical bond-

§ By Dr. Peter Bernfeld, Cancer Research Unit, Tufts University Medical School.

TABLE II. Composition of Various Ultra-centrifugation Fractions from Seromucoid Fraction of Normal Human Serum (See Text).

	Protein, mg %	Cholesterol, mg %
Total seromucoid	98	2.5
Fraction 1 (top)	20	0
" 2	18	0
" 3	15	0
" 4	15	.5
" 5 (bottom)	15	1.3
Residue, mg	15	.7*

* Actual amount from 50 ml of normal serum.

age between mucoprotein and cholesterol.

3. *Cholesterol in seromucoid fraction of patients with lymphoma* (Table I). Total seromucoid was considerably increased in 9 of 11 cases, while total serum cholesterol was slightly decreased in this series. The cholesterol found in the seromucoid fraction, however, was significantly increased, in excess of the elevation that one would expect because of the larger content of seromucoid.

4. *Cholesterol in seromucoid fraction of serum of patients with miscellaneous diseases* (Table I). Sera of 5 patients with disseminated carcinomatosis showed a low total cholesterol content but uniformly high seromucoid content. Cholesterol found in the seromucoid fraction was also high. These findings were similar to those found in lymphoma, although, perhaps, of greater magnitude. Moderate elevation of the seromucoid fraction was observed in 3 patients with acute leukemia, who did not show anemia at the time of study. Serum was also obtained from 2 patients with parenchymal liver disease showing considerable increase of seromucoid‖ and decrease in total cholesterol. Amount of cholesterol found in the seromucoid fraction, however, was within normal limits.

Discussion. It has been known for some time that cholesterol and some of its esters form complexes with the albumin and globulins of serum(10). Serum lipoproteins also are said to contain as much as 30% of cholesterol(11), although it has been postulated that cholesterol does not form a definite complex with lipoproteins(12). It would appear that no chemical bondage is involved in the case of the cholesterol found in the seromucoid fraction of serum by our technics. Rather, the finding is probably due to phenomena of co-precipitation, of adsorption, or of splitting of some cholesterol-containing fragments from other serum components by the action of perchloric acid and then co-precipitation with the seromucoid by phosphotungstic acid.

Although the distribution of cholesterol between protein and seromucoid fraction may, at times (Fig. 1), follow a "typical pattern,"

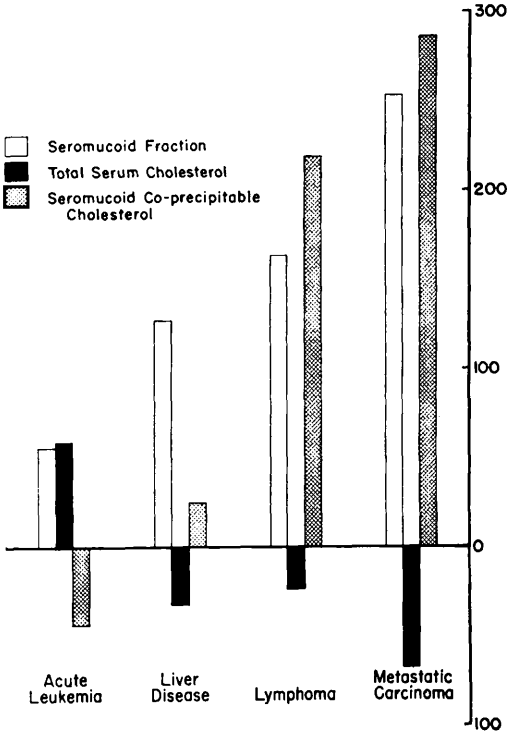

FIG. 1. Graphic illustration of changes in seromucoid, total cholesterol and cholesterol recovered from seromucoid fraction of serum of patients with various pathologic conditions. Horizontal line is avg normal value. Height of columns indicates % deviation from normal values. These data are those of single cases. Included is a selected case of liver disease, primarily to show the striking difference between this condition and disseminated malignancy. In both cases, seromucoid fraction is increased. Cholesterol recovered from this fraction, however, is low in liver disease, high in disseminated malignancy.

‖ This is unusual(9). Cases were purposely selected to allow better comparison with findings in the serum of patients with lymphoma and metastatic carcinoma. In other cases of liver disease, when seromucoid is decreased, amount of cholesterol in this fraction is too low for accurate determinations.

these data are hardly of diagnostic value, since significant changes are found usually in patients with clinically obvious disease. Our study, however, by apparently showing the effects of perchloric acid upon cholesterol-containing components of serum, different from normal to pathologic conditions, may eventually bring a new approach in the investigation of disease states with abnormalities in cholesterol metabolism. Such possibility is being studied.

Summary. 1. A fairly fixed amount of free cholesterol and cholesterol esters is found in the perchloric acid soluble (seromucoid) fraction of human serum. Phenomena of co-precipitation or of adsorption are probably responsible for this finding. 2. Seromucoid fraction, total serum cholesterol, cholesterol found in the seromucoid fraction were studied quantitatively in pathologic sera obtained from patients with lymphoma, disseminated malignancies, acute leukemia and from selected patients with liver disease. Seromucoid fraction was elevated in all diseases. Cholesterol found in the seromucoid fraction was relatively increased in sera of patients with lymphomas and metastatic carcinomas; relatively or absolutely decreased in acute leukemias and liver disease. 3. The significance of these findings consisted in the indication of possible effects of perchloric acid on cholesterol-containing components of serum. Such effects seemed to vary from normal to pathologic conditions, thus suggesting the possible future value of this study in the investigation of abnormalities of cholesterol metabolism in disease.

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Pseudorabies Virus in Tissue Culture: Differentiation of Two Distinct Strains of Virus by Cytopathogenic Pattern Induced.* (23392)

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Scherer and Syverton(1) described the cultivation of pseudorabies virus in HeLa cells. They pointed out the rounding and granulation of the infected cells, and the presence of intranuclear inclusion bodies. The present paper describes the effects of pseudorabies virus on monkey kidney cells, and the separation of 2 strains of virus on the basis of their effects on the monkey kidney cells.

Materials and methods. Virus. The rabbit brain adapted Aujeszky strain of pseudo-

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