Immunochemical Studies of Human Plasma Beta Lipoprotein

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Recently several reports have appeared on the immunochemistry of beta lipoproteins (*1, 2*). The use of the quantitative precipitin technique in these studies required antigens with a high degree of immunological purity. This difficulty may be partly overcome by means of the agar-diffusion technique developed by Ouchterlony (*3*). The present report (*4*) deals with the application of this technique to the immunological properties of human beta lipoproteins of densities less than 1.063 (low-density beta) obtained from several human plasmas.

Antiserums against fraction III were prepared by immunizing two rabbits with 50 mg of lyophilized fraction III (*5*) incorporated in Freund adjuvant, injected intramuscularly. Three weeks later the rabbits were bled, and the serums, which were immunologically identical, were pooled. Two other rabbits were also injected twice with Freund adjuvant to which had been added 2 ml of fresh preparations of beta lipoproteins prepared by the flotation technique (*6*). Both rabbits were bled 3 wk after the first injection, and the serums were used separately.

Low-density beta from normal women and patients with cancer of the breast were prepared by combining Cohn's method 10, as modified by Lever *et al.* (*7*), with the flotation technique of Gofman (*6*). Fractions I + II + III, fractions IV + V, and fraction VI were also prepared by the Lever modification of Cohn's method 10 (*7*). These preparations, as well as the patients' plasmas, were obtained from Marion Barclay (*8*).

Fraction II (*5*) and the saline-soluble material of fraction III (*5*) were also tested. All antiserums and antigens were tested by the agar-diffusion technique of Ouchterlony (*3*), slightly modified for this work (*9*).

Bjorklund had previously shown that antigen-antibody precipitates in agar could be stained if the antigen was a mucoprotein (*10*). We found that precipitates that contained lipoproteins could be stained specifically by using the lipid stains Sudan Black B (*11*) or Oil Red O (*12*).

Several lines were produced when antiserums against fraction III reacted with human plasma or the preparation containing fractions I + II + III (Fig. 1). No lines were obtained with fractions IV + V, fraction VI, or fraction II. Only one line was produced with the low-density beta of healthy women or patients (Fig. 2). The identity of the line caused by lipoproteins could be ascertained by staining the precipitates with either Sudan Black B or Oil Red O.

When saline-soluble material of fraction III was tested no line corresponding to the lipoproteins appeared, indicating that the lipoproteins in this fraction were insoluble. In order to to compare anti-fraction III serums with antiserums against recently prepared lipoproteins, low-density beta was placed in the center reservoir and the various antiserums were placed in the other four reservoirs. Typical reactions of identity were obtained (Fig. 3). This suggests that at least part of the antigenic structure of the beta lipoproteins of fraction III remained unaltered, even though the lipoproteins were insoluble.

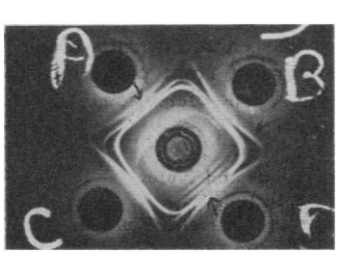
The identity of the proteins responsible for the lines in fractions I + II + III, other than the one caused by low-density beta, is as yet undetermined.

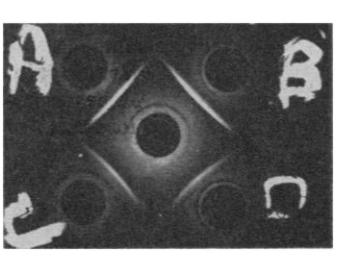
Ultracentrifuge studies have shown that the beta lipoproteins are composed of units with different flotation rates; this may be due to differences in protein-lipid composition (which would affect the size and density of the molecules) rather than to the presence of immunologically heterogeneous lipoproteins (*13*).

Gitlin (*1*) presented data that suggested the immunological heterogeneity of the beta lipoproteins. His studies included Oudin tests that gave rise to 5 or 6 lines with his beta-lipoproteins fraction. However, no evidence was presented that all the antigens responsible for these lines were lipoproteins, and consequently his results may have been due to contaminating proteins. Future work with more potent antiserums may disclose the presence of other beta lipoproteins.

References and Notes

1. D. Gitlin, *Science* **117**, 591 (1953).
2. H. G. Kunkel, *Federation Proc.* (Mar. 1950).
3. O. Ouchterlony, *Acta Pathol. Microbiol Scand.* **32**, 231 (1953).





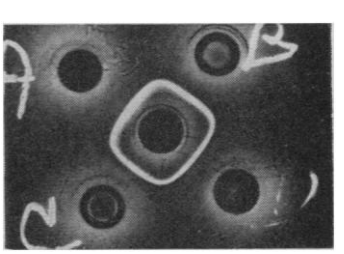


Fig. 1 (left). Center reservoir: antifraction III serum. Peripheral reservoirs: fractions I + II + III from four different plasmas. The arrows indicate the line that was stained by the lipid stains. Fig. 2 (center). Center reservoir: antifraction III serum. Peripheral reservoirs, low-density beta obtained by centrifuging fractions I + II + III at density less than 1.063. Fig. 3 (right). Center reservoir: low-density beta. Top reservoirs: antiserums against low-density beta. Bottom reservoirs: Antiserums against fraction III.

4. This work was supported by U.S. Atomic Energy Commission, contract AT (30-1)-910. We are indebted to Marion Barclay and Mary L. Petermann for the preparation used in this study as well as for valuable discussions and suggestions. We would also like to acknowledge the technical assistance of Richard Clark.
5. These proteins were prepared by Cohn's method 6 and were obtained through the courtesy of J. N. Ashworth of the American Red Cross and E. R. Squibb and Sons.
6. J. W. Gofman *et al., Science* **111**, 166 (1950).
7. W. F. Lever *et al., J. Clin. Invest.* **30**, 99 (1951).
8. M. Barclay *et al., Cancer,* in press.
9. L. Korngold and R. Lipari, *Cancer Research,* in press.
10. B. Bjorklund, *Proc. Soc. Exptl. Biol. Med.* **85**, 438 (1954).
11. B. Swahn, *Scand. J. Clin. & Lab. Invest.* **4**, 98 (1952).
12. E. L. Durrum *et al., Science* **116**, 428 (1952).
13. Evidence for the interconversion of lipoproteins *in vivo* was recently presented by F. T. Pierce, *Metabolism* **3**, 142 (1954).

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Provisional New Type of Group A Streptococci Associated with Nephritis

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During the summer and fall of 1953 an outbreak of streptococcus infections was observed among children at the Red Lake Indian Reservation in Minnesota. Kleinman (*1*) has described the clinical manifestations of sore throat, tonsillitis, scarlet fever, and, particularly, acute glomerulonephritis and pyoderma. The outbreak had reached epidemic proportions by September 1953, at which time the Communicable Disease Center was invited to cooperate with the Bureau of Indian Affairs and the Minnesota Department of Health in an epidemiological investigation. This paper (*2*) deals with the bacteriologic aspects of the resulting study.

Because of limited laboratory facilities at the reservation, the cultures were sent on Loeffler's slants to the Minnesota Department of Health Laboratory in Minneapolis, where the primary isolations were made by one of us (M.S.M.). Isolates of beta hemolytic streptococci were mailed to the Streptococcus Laboratory in Chamblee, Georgia, for serologic identification.

Group A streptococci were isolated from 42 individuals at Red Lake and from six of a "control" group at the Cass Lake Indian Reservation 50 mi away. Each of the 48 cultures was tested with precipitating antiserums for types 1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 15, 17, 18, 19, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 32, 33, 36, 37, 38, 39, 40, 41, 42, 43, 44, 46, and 47 (*3*). Since none of the cultures reacted with these antiserums, rabbits were immunized with two of the Red Lake cultures: DS-C300, from a nose and throat culture of a child with albuminuria but with no clinical nephritis, and DS-C274, isolated in almost pure culture from a skin lesion of a child with acute glomerulonephritis. A satisfactory antibody response to C300 was obtained after three immunization series: the first with a vaccine prepared by the standard procedure (*4*), the second and third with "glow-bead" vaccines (*5*). The data presented here are based on results obtained with antiserum against C300. Preliminary tests with C274 antiserum were confirmatory.

The C300 antiserum reacted with a majority of the 42 Red Lake cultures, including C274, and with three of the six Cass Lake cultures. It did not react with any of the aforementioned types or with two provisional types that were available for study. Results of the tests with the Red Lake cultures are shown in Table 1. Of particular interest is the fact that the C300 antiserum reacted with 12 (92 percent)of the 13 cultures from children with clinical nephritis and with 17 (100 percent) of the cultures from those with pyoderma, the two lesions most characteristic of the epidemic.

In view of the experimental results, we believe that the C300 culture is representative of a serologically specific strain of group-A beta hemolytic streptococci and that its antiserum has characterized the etiological agent of the Red Lake epidemic. Therefore, we propose that any culture reacting with C300 antiserum be referred to as a Red Lake strain until

Table 1. Serologic reactions of group-A streptococci isolated from 42 individuals at Red Lake Indian Reservation.

Diagnosis of individual from which strain isolated	Streptococcal strains reacting with: Antiserum to Red Lake strain (DS-C300) only		Streptococcal strains reacting with: No available antiserums including DS-C300 (untypable*)		Totals	
Nephritis only	3	12	1	1	4	13 nephritis
Nephritis and pyoderma	9	17†	0	0	9	17 pyoderma
Pyoderma only	8		0		8	
Sore throat, tonsillitis, or scarlet fever	2		3		5	
No clinical disease	5		11		16	
TOTAL	27 (64%)		15 (36%)		42 (100%)	

* Tested with antiserums for types listed in text.
† Eleven cultures from skin lesions, six from nose and throat.