Production of Antisera Against Plasma Lipoprotein Fraction.* (21231)

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Interest in the measurement of plasma lipoproteins has recently increased because of the findings that atherosclerotic patients often have elevated contents of these substances. The development of a serological method for this assay would be anticipated because of the protein nature of the molecule. A previous attempt to produce an antisera against lipoproteins involved the injection of dog lipoprotein fraction into rabbits(1). Antisera reacting with the plasma of dogs were developed but no evidence was presented to demonstrate rigorously a true antigen-antibody reaction specific for lipoproteins. The present study represents an attempt to furnish proof of an antigen-antibody reaction specific for lipoproteins.

Methods. Lipoprotein antigen was obtained from the sera of rabbits fed 1% cholesterol and 3% corn oil in addition to regular rations for 30 days. The serum was spun at 30000 rpm in the Spinco model LH preparative ultracentrifuge for 24 hours. The top 2/9 was removed, diluted from 10 to 20 times with saline and injected intravenously into young chickens at intervals over a month's period. The injection schedule suggested by Kabat and Meyer(2) was used. Four to 7 days following the last injection, blood was removed from the chickens and serum or plasma separated. *Antiserum* against human lipoproteins was prepared in the chicken in the same manner. Sera obtained from atherosclerotic outpatients were used as the source of lipoprotein antigen. Titer of antisera was measured by the interfacial precipitin test. Undiluted antisera, 0.1 cc, was placed in the bottom of a 5 mm diameter test tube and 0.1 cc of appropriately diluted plasma containing lipoproteins was layered over it. The development of a white ring at the interfacial zone constituted a positive precipitin test. Tubes were ususally read after 60 minutes. Control tubes containing saline and normal chicken plasma were included. More quantitative information as to the concentration of antigen was determined by measuring the total nitrogen content of the washed precipitate. In this procedure the colorimetric method of Folin and Ciocalteu(2) was used.

TABLE I. Comparison of Titers Obtained by Mixing Sera of Various Animals (Antigen) with Each of 2 Chicken Antisera.

Antigen	Antisera: Chicken anti-rabbit sera	Antisera: Chicken anti-human sera
Rabbit—		
Fat + cholesterol fed	10000-50000	1600
Normal	400- 1600	N at 400
Human—		
Atherosclerotic	800- 1600	800-3200
Normal	400	400
Chicken	N*	N
Rat	400	—
Sheep	40	—
Cow	80	—
Horse	160	—

* N = Negative.

Results. When sera obtained from high fat and cholesterol fed rabbits was titrated against antisera removed from chickens immunized against rabbit liprotein fraction, precipitin titers up to 50,000 were observed. Normal rabbit sera, however, yielded titers up to 1600. Chickens immunized against human lipoproteins yielded antisera capable of differentiating the atherosclerotic sera from that obtained from normal persons. Results of titrations of various animal sera with anti-human and anti-rabbit sera are presented in Table I in summary form. It will be noted that a certain amount of cross-species activity was present. Since the sera of rabbits fed high fat and cholesterol contained from 5 to 10 times the lipoprotein concentration found in the human atherosclerotic patient, it was not surprising that values obtained with rabbit antigen fraction exceeded those obtained with human.

In order to determine the degree of specifi-

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TABLE II. A Positive Correlation (0.69) between the Plasma Lipoprotein Values of Rabbits Measured by Precipitin Test and by Ultracentrifugal Analysis.

Rabbit	Precipitin ppt. mg N	Ultracentrifugal S_f 0-400 mg %
Normal	.03	81
	.12	54
	.14	92
Fat and cholesterol fed	.29	1571
	.33	2181
	.40	2090
	.44	3167
	.45	2908
	.45	3345
	.49	3829
	.49	3855
	.53	1557
	.56	5412

city of the antisera for lipoproteins, the total lipoprotein values (S_f0-400) obtained by ultracentrifugal analysis(3) of rabbit sera were compared with the total nitrogen of the precipitate obtained when the same rabbit sera reacted with chicken antisera. Serum obtained from 3 normal rabbits and 10 rabbits fed 1% cholesterol and 3% corn oil supplements was used. In practice 1 cc of the rabbit serum diluted from 1/20 to 1/80 with saline was mixed with an equal volume of undiluted chicken antiserum. After 1 hour at 38°C, the tubes were placed for about 16 hours in the icebox at 4°C. The washed precipitates were then analyzed for total nitrogen.

The results of this study are shown in Table II. A statistically significant positive correlation coefficient of 0.69 existed between measurements made ultracentrifugally and those measured serologically. A similar correlation was made using the sera obtained from 16 human subjects, 8 of which were diagnosed as atherosclerosis patients. The results in Table III showed a positive correlation of 0.92 which was highly significant.

A convenient method for the demonstration of the antigen-antibody reaction is that described by Ouchterlony(4-6) using the gel diffusion technic. With this method, in Fig. 3, a reaction of identity was established between the lipoproteins of normal human sera and those of the sera obtained from atherosclerotic patients. Although it has been demonstrated that the lipoproteins are quantitatively increased in concentration in the sera of atherosclerotic patients, the gel diffusion plates prove them to be qualitatively identical by serological criteria.

Human lipoproteins of the S_f6 class were isolated in purified state by Dr. Ray A. Brown of these Laboratories. This preparation gave strong precipitin tests with chicken anti-human antisera. Used in the gel diffusion plate S_f6 lipoproteins gave the reaction of identity with the lipoproteins contained in unfractionated human sera.

In an attempt to eliminate certain nonspecific and non-antigenic reactions, cholesterol and the cephalin-cholesterol reagent were added to immunized and normal chicken sera. Cholesterol alone had no visible effect while the cephalin-cholesterol reagent (Wilson Laboratories) produced an equal precipitation in both immunized and normal chicken sera.

Discussion. In order to establish the reaction described in this study as a true antigen-antibody precipitin reaction specific for plasma lipoprotein, the following observations are listed: 1) Rabbit and human sera containing lipoproteins will not generally react with normal chicken sera. Chickens must first be immunized with the appropriate foreign lipoprotein fraction. 2) The activity of the unfractionated chicken antisera was present in the redissolved precipitate obtained after ½

TABLE III. A Positive Correlation (0.92) between Human Plasma Lipoprotein Values Obtained by Precipitin Measurement and Ultracentrifugal Analysis. Sixteen subjects.

Precipitin ppt. mg N	Ultracentrifugal S_f 0-400 mg %
.16	202
.17	270
.18	362
.21	389
.21	544
.22	405
.22	594
.24	445
.24	598
.25	615
.27	661
.30	755
.31	770
.39	648
.40	837
.51	1357

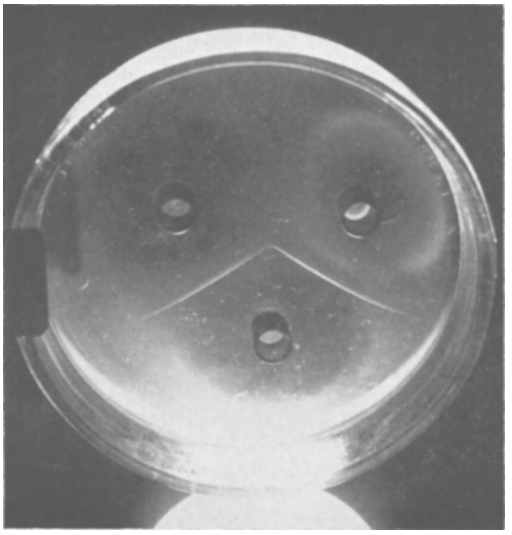

FIG. 1. Gel diffusion plates. *Left cup*: Normal human plasma. *Right cup*: Atherosclerotic plasma. *Center cup*: Chicken anti-human lipoprotein serum. The smooth blending of the lines of individual precipitates indicates the reaction of identity.

saturation with ammonium sulfate. 3) Chicken anti-human sera reacts with purified S_f6 lipoproteins. The reaction of identity (Ouchterlony) was given between purified lipoprotein and those present in atherosclerotic plasma. 4) A high degree of positive correlation existed between the total lipoproteins as measured by the ultracentrifuge and the quantity of precipitate produced by chicken antisera.

Summary. The injection of a lipoprotein fraction from the sera of cholesterol-fed rabbits or human atherosclerotic patients into chickens brought about the formation of antibodies specific for either rabbit or human lipoproteins. A positive correlation existed between the amount of precipitate produced by mixing antisera with sera containing lipoproteins and the lipoprotein content measured ultracentrifugally.

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1. Baker, S. P., Ogden, E., and Riddle, J. W., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 119.

2. Kabat, E. A., and Meyer, M. M., *Exp. Immunochemistry,* C. C. Thomas, Springfield, Ill., 1948.

3. Gofman, J. W., Lindgren, F., Elliott, H., Mantz, W., Hewitt, J., Strisower, B., and Herring, V., *Science,* 1950, v11, 166.

4. Ouchterlony, O., *Arkiv. Kemi. Mineral Geol.,* 1949, v26B, 1.

5. ———, *ibid.,* 1950, v1, 43.

6. ———, *ibid.,* 1950, v1, 55.

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Inhibitory Effects of Normal Guinea Pig Serum and Immune Rabbit Serum on Transplanted AKR Lymphomas.* (21232)

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When normal guinea pig serum is given intraperitoneally in relatively large amounts to C3H mice carrying the Gardner 6C3HED lymphosarcoma or to A mice carrying Lorenz's Lymphoma II, the growths regularly regress, as a recent study has shown(1); furthermore, the potency of the normal guinea pig serum against 6C3HED lymphoma cells *in vivo* is considerably enhanced when a small quantity of an immune serum, made by injecting the lymphoma cells into rabbits, is given together with it(1). In the work now to be reported normal guinea pig serum and immune rabbit serum, separately and in combination, have been tested for inhibitory effects *in vivo* against the cells of 8 transplanted lymphomas of AKR mice. As will be shown, the findings with the AKR lymphomas differ in at least one respect from those obtained with the C3H

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