did appear to reduce aortic atherosclerosis slightly at a dose of 0.10% and under conditions of a lessened intake of dietary cholesterol. 3. The evidence to date is poor that atherosclerosis can be controlled by the use of either polyunsaturated oils in the diet or pharmacologic agents to inhibit cholesterol synthesis as long as significant amounts of cholesterol are absorbed in the body.

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Received June 4, 1963. P.S.E.B.M., 1963, v113.

Production of Mercaptoethanol Sensitive, Slowly Sedimenting Antibody in the Duck.*† (28545)

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Recent studies have indicated the presence of slowly sedimenting, mercaptoethanol sensitive antibody in the antisera of certain non-mammalian vertebrates(1-3). The present study was performed in order directly to compare the mercaptoethanol sensitivity of antibody produced in the duck and rabbit exposed to identical immunization schedules and to ascertain the relation between this antibody and the mercaptoethanol resistant 7S gamma globulin antibody of the mammal.

Materials and methods. Domesticated mallard ducks, both male and female, weighing 1.5 to 2 kg and albino male rabbits weighing 2.5 to 3 kg were used. Groups of 3 ducks and 3 rabbits were immunized with Armour & Co. bovine serum albumin (BSA) by: a) Intramuscular injection of 40 mg BSA in saline, and b) intramuscular injection of 8 mg alum precipitated BSA. All groups were reinjected on day 21 with the same dose and preparation of BSA as was given initially. Serum samples

* Publication No. 49 from Division of Experimental Pathology, Scripps Clinic and Research Foundation.

† This work was supported in part by an Atomic Energy Commission Contract and U.S.P.H.S. grants.

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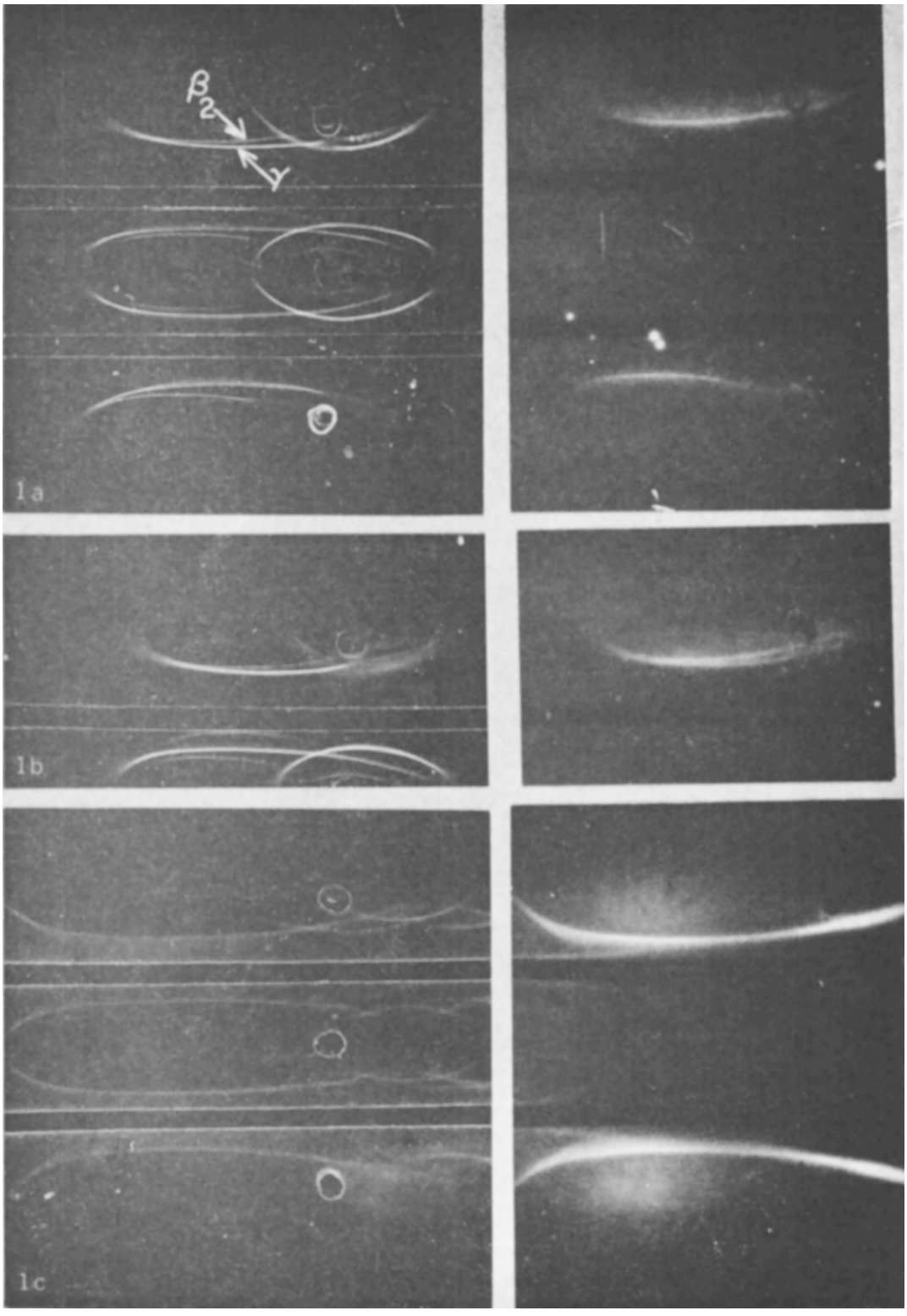
β2
γ
1a
1b
1c

TABLE I. Effect of 2-Mercaptoethanol on Duck and Rabbit Anti-BSA.

Days following immunization	γ N I*BSA bound/ml serum					
	Duck			Rabbit		
	1	2	3	1	2	3
7	.03/ .1*	<.01/.05	<.005/ .03	.13/ .11	.05/ .04	.03/ .03
14	.22/ .64	.22/.52	.34 / .91	2.08/2.32	1.18/ 1.25	.78/ .91
21	.14/ .36	—	.31 / .54	1.04/1.28	.82/ .84	.67/ .72
Secondary immunization						
28	1.3 /3.6	.3 /.8	.8 /1.7	2.4 /2.5	20.5 /18.5	14.0 /18.5
35	.8 /2.2	.07/.2	.3 / .6	3.0 /3.4	8.4 / 9.7	8.3 / 7.9

* Numerator represents antigen binding capacity after 2-mercaptoethanol treatment; denominator represents antigen binding capacity of untreated control serum.

were obtained at 7-day intervals after the first injection, for 35 days. Antibody determinations were made by means of the ammonium sulfate salting out procedure of Farr(4) using BSA labeled with I^{131} (I*BSA) as test antigen (5). Treatment of antisera with 2-mercaptoethanol was carried out by placing antisera in cellophane tubing and dialyzing for 18 hours at room temperature against a .001 M phosphate buffered saline pH 7.0 containing 0.1 M 2-mercaptoethanol. This was followed by dialysis against 0.01 M iodoacetamide in saline for 24 hours at 4°C. Control sera were treated identically except that no mercaptoethanol was used in the initial dialysis. All sera were also subjected to ultracentrifugation in a sucrose gradient (6), 0.8 ml fractions being collected by pin puncture of the bottom of the tube and analyzed for the presence of rapidly sedimenting and slowly sedimenting antibody. Gel filtration was carried out using G-200 sephadex with a bed volume of 150 ml. Three ml of a day 14 immune duck serum was fractionated using 0.15 M pH 7.0 sodium chloride solution for elution. Fractions were analyzed spectrophotometrically for protein at 280 mμ, for antibody, and by micro-immunoelectrophoresis(7). Radioimmunoelectrophoresis was performed with duck and rabbit anti-BSA sera by placing the anti-BSA serum in the well and the species specific antiserum in the trough along with I*BSA that had a specific activity of 2 μc/μg and contained 87 γN/ml. The precipitin bands were allowed to develop for 24 hours and the usual procedure for washing, drying and staining carried out. The slides were then placed in contact with Kodak Projection plates for 1 to 3 days and the plates were then developed in the usual manner.

Results. The results obtained were similar, whether animals were immunized with soluble or alum precipitated BSA, and therefore no distinction will be made as to the immunization procedure in the presentation of results. Table I presents the results of mercaptoethanol treatment on 3 duck and 3 rabbit antisera at different times following immunization. The antibody titers in the sera from the 3 ducks were reduced 45 to 80% by 2-mercaptoethanol treatment. The sensitivity of duck antibody remained relatively constant throughout the 35-day observation period. The antibody titers in the treated rabbit sera, on the other hand, showed relatively minor fluctuations from the untreated control sera. Most rabbit antisera treated with mercaptoethanol were within 10% of the control sera; the greatest deviation observed was a 24% depression in titer. Sucrose gradient ultracentrifugation was performed on all sera and in no duck or rabbit antiserum was more than 5% of the total antibody rapidly sedimenting.

FIG. 1a. Upper well—day 14 duck anti-BSA. Middle well—normal duck serum. Lower well—mercaptoethanol treated day 14 antiserum. Troughs—rabbit anti-duck globulin plus I*BSA. Right hand photograph shows position of anti-BSA by localization of I*BSA autoradiographically.

FIG. 1b. Upper well—day 28 duck anti-BSA. Lower well—normal duck serum. Trough—rabbit anti-duck globulin plus I*BSA.

FIG. 1c. Upper well—day 14 rabbit anti-BSA. Middle well—normal rabbit serum. Lower well—mercaptoethanol treated day 14 antiserum. Troughs—sheep anti-whole rabbit serum plus I*BSA.

TABLE II. Gel Filtration of Duck Anti-BSA Antiserum.

Fraction No.	Effluent vol	ABC*	Immunoelectrophoresis	
			γ globulin	β_2 globulin
10	37	.00	—	—
13	48	.05	—	—
14	52	.14	+	±
15	55	.30	+	++
16	59	.18	++	++
17	63	.10	++	—
18	67	.07	+++	—
19	70	.05	++	—

* Antigen binding capacity (μg N I*BSA bound/ml).

The day 14 antiserum of duck No. 3 was fractionated on a G-200 sephadex column and the effluent fractions analyzed for protein, antibody and presence of γ or β_2 globulin by immunoelectrophoresis. Table II presents the results of this fractionation. Fraction 10 was from the first protein peak and probably contained 19S proteins. There was no antibody activity in this fraction which confirmed the data obtained by ultracentrifugation. The peak antibody titer occurred on the ascending side of the globulin peak and coincided with the presence of a β_2 globulin by immunoelectrophoresis. Although gamma globulin was also present in the fractions with peak antibody activity, the strongest precipitin bands for gamma globulin occurred in fractions that had very little antibody.

To characterize further the species of protein associated with the mercaptoethanol sensitive antibody immunoelectrophoresis was performed on antisera from duck 3 using rabbit anti-duck globulin in the troughs together with I*BSA and comparing the I*BSA-anti BSA precipitin band as demonstrated by autoradiography with the serum protein pattern. The results are presented in Fig. 1. Fig. 1a represents the serum protein of day 14 duck antiserum and beside it the localization of antibody by autoradiography. Normal Peking duck serum was placed in the center well and untreated day 14 antiserum and 2-mercaptoethanol treated serum in the upper and lower wells respectively. The arrows indicate the positions of the proteins with the immunoelectrophoretic characteristics of mammalian 7S gamma globulin and β_2 globulin. The antibody was localized to the β_2 globulin band which migrated toward the well side of the 7S γ globulin line. These results were obtained with 3 other day 14 duck antisera as well. Figure 1b shows a day 28 duck antiserum in the upper well and normal duck serum in the lower. At this time antigen is localized to both 7S γ globulin and to the β_2 globulin as evidenced by the split line in the autoradiograph. Figure 1c depicts a day 14 rabbit antiserum. All the antigen was localized to the 7S γ line. The diminution of the intensity of the autoradiograph which is evident with the mercaptoethanol treated duck sera was not seen with the rabbit antisera. It is also to be noted that the β globulin which localized around the well of the untreated duck sera disappeared on treatment with mercaptoethanol. The significance of this band is not known.

Discussion and conclusions. The above data indicate the presence of mercaptoethanol sensitive, slowly sedimenting anti-BSA antibody in the duck which by gel filtration and radioimmunoelectrophoresis did not behave like a mammalian 7S γ globulin. The gel filtration experiment suggested that the antibody was somewhat larger in size than the 7S proteins and the radioimmunoelectrophoretic patterns localized the antibody activity to a protein which migrated separately from the 7S γ globulin line. This antibody bears a striking resemblance in some of its characteristics to the β_2A (γ1A) immunoglobulin described in the mammal. Although the evidence is still not complete, data obtained with ragweed skin-sensitizing antibody indicate that β_2A (γ1A) antibodies are a) larger than 7S(8), and b) mercaptoethanol sensitive(9).

Mercaptoethanol sensitive, slowly sedimenting antibody has been recently observed in non-mammalian vertebrates such as the chicken(2,3), frog and goldfish(1), and turtle. The finding of this class of antibody in inframammalian species to the apparent exclusion in the cold blooded vertebrates at least of 7S mercaptoethanol resistant antibody suggests that this class of antibody represents a stage in the evolution of the immune response which in the mammal has become largely superceded by mercaptoethanol resistant 7S γ globulin.

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Received June 11, 1963. P.S.E.B.M., 1963, v113.

Effects of Intrahypothalamic Implants of Reserpine on Lactation and Pituitary Prolactin Content in the Rabbit.* (28546)

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Lactation is induced in the estrogen primed rabbit by systemic or intraventricular treatment with the tranquilizing agent, reserpine (1), apparently by antagonizing the inhibitory control exerted by the central nervous system over the release of pituitary prolactin (2). Similar effects on lactation are observed following the placement of small electrolytic lesions in the basal tuberal region of the hypothalamus(3) and such lesions have been found to deplete the hypophysis of prolactin (4). If a lesion has effectively activated lactation, subsequent treatment with reserpine neither activates the mammary glands nor depletes the hypophysis of prolactin(2). The results indicate that reserpine may exert its lactation inducing effect at this critical hypothalamic site. It is therefore of interest to apply reserpine directly to this site as well as directly to the hypophysis and study the effects of these treatments on the mammary glands and pituitary prolactin content.

Materials and methods. Twelve multiparous New Zealand white rabbits were ovariectomized on day 0 and treated with 0.1 mg estradiol benzoate subcutaneously daily for 10 days. On day 11 or 12 a minute amount of reserpine phosphate (<0.1 mg) was stereotaxically(5) implanted into the basal hypothalamus or hypophysis; 3 of the 12 rabbits were implanted with reserpine on day 0.

Reserpine implants were prepared as follows: the tip of a piece of 24-gauge tubing was carefully heated with reserpine phosphate powder and the melted reserpine was drawn into the tube. The outside of the tubing was carefully cleaned with ether, leaving only a small amount of solid reserpine visible on the tip. The recrystallized reserpine was hard and only very slowly soluble in physiological saline, but was effective in inducing a general depressant effect when homogenized, suspended in saline and injected subcutaneously. After stereotaxic implantation, the tube was fixed securely to the skull of the rabbit with dental cement and 2 small anchoring screws.

Autopsy was usually performed on day 18 or 19, from 6 to 8 days after reserpine implantation; but the 3 rabbits implanted on day 0 were autopsied 15 days later. The mammary glands were examined and graded by the Gardner-Turner classification(6) on a scale of 0 to 4 on days 0, 11 or 12 at implantation and autopsy on day 15, 18 or 19, and confirmed by histological study of biopsy and autopsy specimens. Histological methods of locating implantation sites in the brain and pituitary gland have been described earlier by Kanematsu and Sawyer(7). Prolactin content of each pituitary gland was assayed intradermally on 12 White King squabs approximately 4 weeks of age weighing 400-600 g(7). Standard samples of purified prolactin were assayed throughout the course of the investigation.

Results. In Fig. 1, the mammary glands of

* Supported by USPHS grant.