present in sufficient amounts in the total adrenals to imply greater amount of cellular material in the female adrenals.

Halberg(2) showed that eosinophil counts of female mice were lower at all times of the day than those of males. The present findings are in agreement with this observation. Adrenal cortical steroids are known to depress eosinophil counts(11). This suggests that steroid activity may be higher in female than in male mice. The finding of more cholesterol in the female mouse adrenal than in the male suggests that female mice may have higher stores of steroid precursors and possibly of steroids themselves in the cortex. The zona fasciculata is thought to elaborate glucocorticoids which are most effective in depressing circulating eosinophil levels(11). It is of interest that the sharpest differences between male and female adrenal glands exist in the zona fasciculata.

Summary. The zona fasciculata and to some extent the zona glomerulosa of the adrenals of female STR/N mouse stained more intensely and uniformly for lipid with oil red O than those of the males. The female adrenal was considerably larger than that of the male although body weights of the male were larger than the female. The absolute amounts of total lipid, cholesterol and phospholipid in the adrenals in female mice were higher than in males. Eosinophil count of female mice was lower, but not significantly so, than male counts.

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Antigenicity of Cholesterol Induced Chicken Atheroma.* (26346)

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These experiments were undertaken to determine whether or not the lipid or lipoprotein complex comprising the central mass of an experimentally produced arterial atheroma could stimulate specific antibody formation, and to clarify the serologic characteristics of such an antibody. It was also planned to investigate the *in vivo* effects of such anti-atheroma antibody on lesions of experimental atherosclerosis. Antigenic properties of experimental atheroma and normal intima were to be compared.

Methods and materials. The chicken was chosen as the experimental animal. The necessary dietary conditions for atheroma induction in the chicken have been well defined and are easily reproducible(1).

Eight-week-old HYLINE leghorn cockerels (Corn Belt Hatcheries, Joliet, Ill.) were

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placed on a diet of 2% cholesterol, 5% cottonseed oil and 93% chicken maintenance mash. When necessary, by addition of sucrose the protein content of the commercial mash was adjusted to 12-13%, a level optimal for atheroma production(2). The birds exhibited gross fatty lesions after 6-10 weeks and many plaques showed calcification at 16 weeks.

Antigens. Material from atheroma to be tested for antigenicity was obtained from birds which had been on the diet for between 8 and 16 weeks. Material from normal arterial vessels to be tested for antigenicity was obtained from 16 week or older leghorn cockerels which had been fed commercial chicken maintenance mash. The test material was removed from the thoracic aorta and brachiocephalic arteries.

Atheromatous material was scraped from diseased vessels with a razor blade and suspended in saline to a concentration of 0.3-0.4 mg protein/ml (micro-Kjeldahl analysis). Microscopic sections of scraped vessels showed that only atheromatous intima had been removed. Normal intima was removed by placing the intimal surface of normal vessels on a glass plate and scraping away the adventitia and most of the media. This antigen, therefore, contained some normal media in addition to normal intima and might more properly be called normal intima-media antigen, but for the sake of simplicity will be referred to as intima antigen. It was suspended in saline to a concentration of 0.8-1.0 mg of protein/ml. Both suspensions were prepared in a glass tissue homogenizer. All preparations were carried out at 0-5°C. Atheroma antigen was used the day of preparation and intima antigen was used up to one week following preparation.

Various normal chicken organs were prepared for use in studies on antibody specificity. The organ was homogenized in saline with a Waring® blender and the suspension centrifuged 10 min at 3000 rpm. The sediment was washed twice with saline and homogenized in a glass tissue grinder. This suspension was centrifuged 45 min at 30,000 rpm. The resulting sediment was resuspended to a concentration of approximately 1 mg of protein/ml when used for adsorption studies. The entire procedure was carried out at 0-5°C.

Immunization. Three adult, male albino rabbits were immunized with atheroma antigen and 3 similar rabbits were immunized with normal intima antigen. Approximately 0.1 g (wet wt) of antigen (representing the material from 2 or 3 chickens), suspended in 3 ml of Freund's adjuvant (Difco), was injected subcutaneously into the back of each animal. Booster injections were given after 3 weeks, 11 weeks, and 9 months. Rabbits were bled following the eleventh week and ninth month of booster injections and the sera stored at −20°C until used. Sera, prior to their use in complement fixation studies were heated at 56°C for 30 min.

Serology. Antisera were assayed by complement-fixation(3). In the presence of two-100% hemolytic units of guinea pig complement, 0.1 ml of a dilution of antigen was added to 0.2 ml of various dilutions of antiserum. Mixtures were incubated for 30 min at 37°C. Residual complement was detected by addition of 0.2 ml of a 2.5% suspension of amboceptor-sensitized sheep rbc. After incubation for 30 min at 37°C, the titer was recorded as the lowest serum dilution showing complete hemolysis. Appropriate anti-complementary controls were run with each experiment. To study the behavior of the antigen-antibody systems in the zones of antibody excess, equivalence and antigen excess, isofixation curves were derived by plotting the antiserum titer obtained during block titration with varying concentrations of antigen(4).

Results. Antibody response. Complement-fixing antibodies against homologous antigens were obtained in about equal titer following immunization with either atheromatous or normal intima. Antisera obtained at 9 months and used for these studies all had titers of 1/512. The activity of suspensions of both atheromatous and normal intima in complement-fixation was found primarily in the sediment following centrifugation at 30,000 rpm for 1 hr in a Spinco ultracentrifuge (#40 rotor).

Antibody characterization. Adsorption of

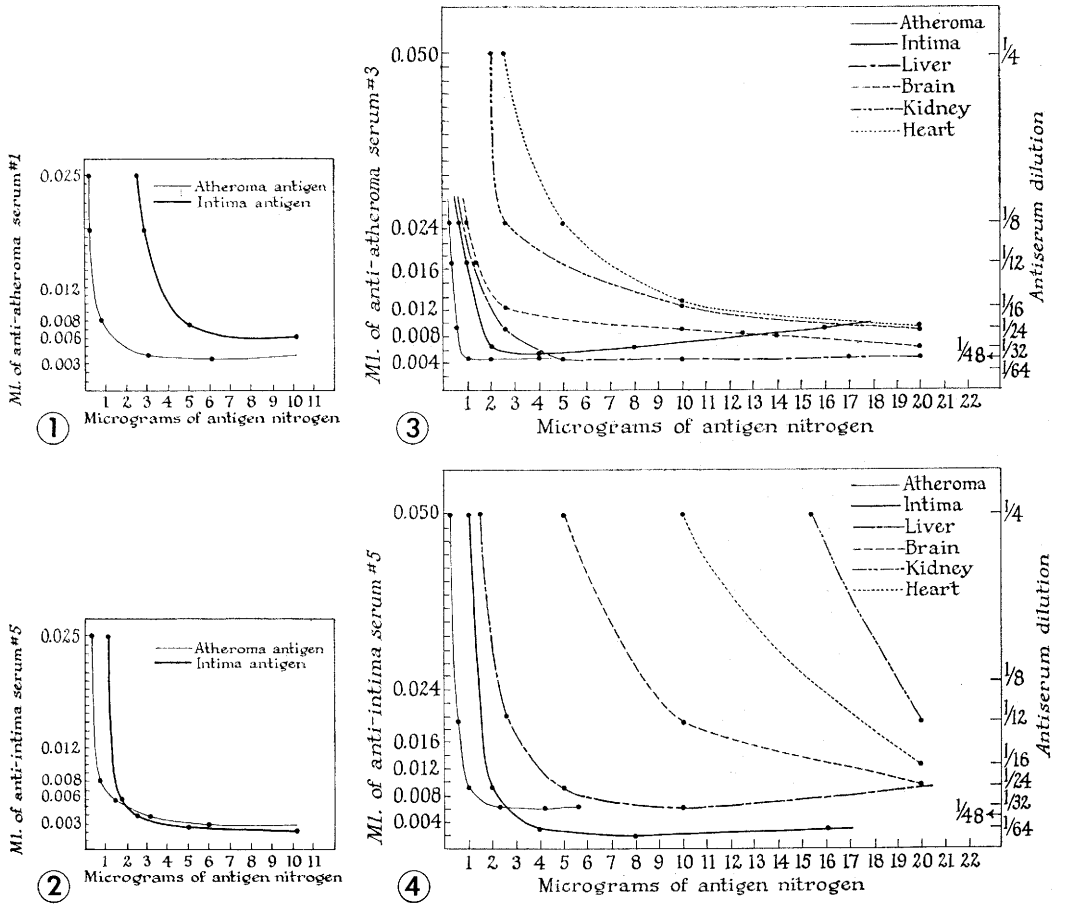

FIG. 1. Isofixation curves for an anti-atheroma serum *vs* atheroma antigen and normal intima antigen.

FIG. 2. Isofixation curves for an anti-intima serum *vs* intima antigen and atheroma antigen.

FIG. 3. Isofixation curves for an anti-atheroma serum *vs* various tissues.

FIG. 4. Isofixation curves for an anti-intima serum *vs* various tissues.

anti-atheroma or anti-intima rabbit sera with sheep or chicken erythrocytes or with guinea pig kidney suspensions resulted in marked reduction of antibody titer in complement fixation assays. All studies on specificity and cross-reactivity were therefore carried out with heterophile adsorbed antisera (equal volume of washed, packed sheep erythrocytes; room temperature; 45 min.; 2 adsorptions).

By means of isofixation curves a suggestion of antigenic specificity could be obtained with anti-atheroma but not anti-intima sera. The anti-atheroma sera exhibited higher titers when reacted with homologous atheroma antigen rather than intima antigen (Fig. 1). The reverse situation was not true, that is, anti-intima sera exhibited nearly identical titers when reacted with either atheroma or intima (Fig. 2). The suggested specificity of anti-atheroma serum was further supported by adsorbing both anti-atheroma and anti-intima sera with normal intima sediment. Following 2 adsorptions for 20 min at room temperature, the titer of the anti-intima serum was significantly reduced from 1/59 to 1/22 but the anti-atheroma serum titer remained constant at 1/29. Adsorption of antisera with atheroma suspension was technically impossible.

Both anti-atheroma and anti-intima sera were found to cross react with other tissues, particularly liver. To see if the antisera had specificity for their homologous antigens,

they were adsorbed twice with liver sediment, and the resulting titers *vs.* liver or homologous antigen were assayed.

Neither anti-atheroma nor anti-intima serum reacted with liver after the second adsorption. Two adsorptions led to a marked decrease (1/48 to 1/8) in titer of anti- atheroma serum *vs.* atheroma and a lesser decrease (1/64 to 1/32) in titer of anti-intima serum *vs.* intima.

These experiments indicated that anti-intima serum was made more specific for its homologous antigen by adsorption with liver, whereas anti-atheroma specificity was not increased. Adsorption studies with kidney antigen also showed anti-atheroma serum to lack specificity. To rule out non-specific adsorption by these organs, rabbit anti-diphtheria toxin serum was adsorbed similarly with liver and kidney. No change in titer was observed.

The reactivities of anti-atheroma and anti-intima sera with chicken atheroma, intima, liver, kidney, brain, and heart were further analyzed by means of isofixation curves (Fig. 3 and 4). Comparison of the two figures indicated that anti-atheroma serum cross-reacted with these organs to a greater degree than anti-intima serum. In the zone of antibody excess, anti-atheroma serum reacted moderately only with liver. These experiments therefore correlated in part with the previous adsorption studies suggesting anti-atheroma serum to have little tissue specificity.

In vivo studies. Three ml of anti-atheroma serum was injected intravenously into six 17-week-old atherosclerotic cockerels which had been on the atherogenic diet 7 weeks. Three of the animals were sacrificed after 5 days and the remainder after 8 days. A similar experiment using anti-intima serum and normal 17-week-old cockerels was performed. Organs were fixed in 10% formalin and sections of aorta, heart, liver, and kidney were examined for evidence of histologic change. No consistent histologic differences were observed in the tissues of the experimental and the 17-week-old noninjected controls.

Discussion. Since much of the rabbit anti-atheroma antibody was in response to a heterophile antigen, goats and guinea pigs were also immunized in a separate study (unpub). Both species had a prompt antibody response to chicken atheroma but no heterophile antibodies were detected. The decrease in titers of rabbit antisera following adsorption with Forssman positive tissues and lack of heterophile antibody response during immunization of Forssman positive animals indicated Forssman antigen is present in chicken atheroma and normal chicken intima. This reaffirms the known distribution of Forssman antigen(5,6).

The identity of substances responsible for antigenicity of chicken atheroma was not determined. Lipids such as cholesterol, cholesterol esters and phospholipids are among the principal chemical constituents of chicken atheroma. Many of the lipids may be free but some are probably in the form of protein complexes. The antigenicity of free lipids has not been demonstrated but ample evidence is available that lipids may function as haptenes and confer antigenic specificity when coupled to a suitable protein carrier (7,8). In addition to the possible lipid-protein antigens the cellular components of atheroma material may be antigenic.

A suggestion of anti-atheroma specificity in relation to normal intima was shown with rabbit antisera. Similar specificity was demonstrated with goat anti-atheroma sera but could not be shown with guinea pig anti-atheroma sera (unpub). Atheroma antigen is antigenically related to the stroma or parenchyma of other organs, particularly liver and brain. Lipoproteins may be the important common antigens since they are found in cell walls and in parenchymal cell nuclei of many organs(9). The marked cross-reactivity of anti-atheroma sera suggests the absence of antigens unique for chicken atheroma.

Lack of observable *in vivo* activity of antisera may be related to their titers, specificities and the poor opportunity for mechanical contact between injected antisera and aorta. Modification of the course of experi-

mental atherosclerosis by injection of anti-atheroma antibodies remains to be demonstrated.

Summary. Rabbit antibodies were produced in response to cholesterol induced chicken atheroma and chicken normal intima. Properties of these antisera were studied by complement fixation and described by isofixation curves. It was found that anti-atheroma sera reacted best with atheroma antigen and in addition reacted well with a wide variety of other chicken tissues. Anti-normal intima sera reacted well with either intima or atheroma antigen and had a limited spectrum of reactivity with other tissues. *In vivo* activity of these antisera could not be demonstrated.

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Effect of Potassium on Membrane Current of Single Ranvier Node During Excitation. (26347)

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When a frog nerve fiber is immersed in Ringer solution, containing 30-90 millimolar potassium chloride, and the resting membrane potential of the node maintained by an applied voltage, a peculiar prolonged response may be initiated in the node by superimposing a depolarizing stimulus on the applied voltage. This response consists of a spike followed by a prolonged depolarization which remains for a considerable period of time and decays slowly to the resting membrane level(1,2). Although other explanations have been put forward(1,2), the Ionic Theory of nerve activity actually predicts such a response to be elicitable under these abnormal conditions(3,4).

The Ionic Theory postulates 2 "carrier" systems: one which reacts rapidly with sodium, the other more slowly with potassium during excitation. These systems both depend for activation upon the magnitude of the electrical potential across the cell membrane. At the resting level, the systems are relatively inactive. With reduction of the membrane voltage by depolarization, carrier activity is initiated. The sodium carrier causes the membrane to become selectively permeable to sodium while the potassium carrier actively increases membrane permeability to potassium. Consequently membrane depolarization results in ionic current flow of each specie of ion along its electrochemical gradient. Initiation of sodium carrier activity produces a rapid regenerative depolarization by inward sodium current flow. The initial membrane potential level, –80 millivolts, tends to shift rapidly toward the sodium equilibrium potential, +40 millivolts. This large polarization change inactivates the sodium system, and the outward potassium current flow along its electrochemical gradient drives the membrane potential back toward the normal potassium equilibrium potential, –90 millivolts, and to the original resting level.

What happens in the abnormal situation