to marked degrees of stimulation. Lactation was scanty or entirely lacking, and litters died 1 or 2 days after birth. The adrenals of some of the animals treated with LCH were somewhat stimulated as indicated by their weights, while others were atrophic.

Summary. Pregnancy in rats hypophysectomized on or before the 9th day of gestation was maintained with lactogenic hormone, pituitary gonadotropic synergist, and whole anterior pituitary extract. Each of these substances maintained pregnancy presumably by its ability to stimulate function of the corpus luteum.

13060 P

A Natural Antibody Reacting with Sedimentable Constituents of Normal Tissues.

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While making serological studies of several transplanted rabbit cancers by the methods that disclosed a distinctive substance in the Brown-Pearce carcinoma,[1] we have noted that the blood serum of normal rabbits will fix complement in mixture with saline extracts of normal rabbit tissues in dilutions far beyond any anticomplementary or summative effects. This phenomenon appears to be due to a natural antibody that reacts with sedimentable constituents of normal tissues.

The antibody can be detected by means of a standardized complement fixation test in which 2 units of complement are employed and 2 hours at room temperature allowed for fixation. Antigens are prepared by extracting normal rabbit tissues (liver, kidney, brain, etc.—either fresh or preserved frozen at −22°C) with sand in a mortar, suspending the ground paste in physiological saline (1:10 to 1:40 or more) and centrifuging at 4400 rpm for 20 minutes. The unheated supernatant liquids, opalescent but free from gross particles, have proved notably effective in the tests.

When the sera of 22 normal adult rabbits were mixed with antigens consisting of 1:20 or 1:40 saline extracts of normal rabbit livers, fixation invariably occurred, and it was complete with many of the sera in dilutions as high as 1:16 to 1:64. No fixation was

[1] Kidd, John G., *J. Exp. Med.*, 1940, **71**, 335, 351.

got when the sera of 13 rabbits less than 4 weeks old from 7 litters were tested concurrently with the same antigens. None of the sera proved anticomplementary in dilutions of 1:2 or more in concurrent tests though a few showed slight anticomplementary effect when a double volume of the 1:2 dilution was tested, and the antigens were not anticomplementary when 4 and 8 times the standard amount were employed in control tests.

Experiments were next undertaken to ascertain some of the properties of the substance present in the serum of normal adult rabbits which is responsible for the fixation. It was found that the substance could be precipitated from sera with ammonium sulfate, like the generality of antibodies. The substance was completely inactivated in sera heated at 65°C for 30 minutes—a procedure that has no noteworthy effect upon the generality of antibodies in rabbit blood.[2] But the fact has long been recognized that natural antibodies are more labile to heat than induced ones;[3] hence the substance under study was compared with 2 natural antibodies present in the serum of normal adult rabbits, *i. e.*, natural anti-sheep hemolysin and natural Wassermann reagin. Tests with the sera of 5 rabbits showed that in general the titers of the 3 serum constituents varied together from individual to individual, and that all were inactivated largely or completely by heating at 65°C for 30 minutes, though not apparently affected at 56°C. Sheep erythrocytes absorbed the natural antisheep hemolysin without affecting the titers of either the natural Wassermann reagin or the unknown substance; absorption with cholesterolized Wassermann antigen removed the natural Wassermann reagin from the sera without affecting their content of the natural hemolysin or the unknown substance; and a saline extract of normal rabbit liver absorbed the unknown substance without removing any of the natural hemolysin and but very little of the natural Wassermann reagin.* The findings as a whole would appear to warrant the conclusion that the substance responsible for the fixation of complement in mixture with a saline suspension of normal rabbit liver is a natural antibody.

Saline extracts of various normal rabbit tissues (kidney, liver, brain, lung, testicle, spleen, muscle) will fix complement regularly in dilutions ranging from 1:10 to 1:1280 in mixture with sera known

2 Jones, F. S., *J. Exp. Med.*, 1927, **46**, 291.

3 Landsteiner, K., and Reich, M., *Z. Hyg. u. Infektionskrankh.*, 1908, **58**, 213; Mackie, T. J., and Finkelstein, M. H., *J. Hyg.*, 1930, **30**, 1; Gibson, H. J., *J. Hyg.*, 1930, **30**, 337.

* Visible flocculations were not observed in the absorption-mixtures, which were kept 2 hours at 37°C and then put into the refrigerator overnight.

to contain the natural antibody. Kidney and liver antigens have invariably done so in high dilutions whereas heart and voluntary-muscle extracts have reacted only in the lower dilutions. Saline extracts of normal rabbit tissues reacted notably well with serum from the same individual. When extracts of normal rabbit kidney (fresh) and liver (frozen) were spun at 20,000 rpm or more for 60 minutes little or none of the effective antigen remained in the supernatant liquids, but the resuspended sediments were fully as potent as the whole extracts. Alcoholic extracts of rabbit liver and heart muscle failed to react with sera containing the natural antibody, a finding which contrasts significantly with the good fixation obtained when these antigens were mixed with the sera of rabbits having experimental syphilis. The antigen reacting with the natural antibody is adversely affected by glycerol as also upon standing in saline suspension for several days in the refrigerator or upon heating at 56°C for 30 minutes—characteristics which account in part for the fact that its effects were not observed in control tests of the previous studies.[1]

Rabbit sera containing the natural antibody also have the capacity to fix complement in mixture with high dilutions of saline extracts of normal tissues procured from heterologous species (rat, mouse, guinea pig, chicken), and this capacity can be absorbed with normal rabbit-liver extract, though rabbit erythrocytes fail to remove it. Further tests are under way to define more clearly the affinities of the natural antibody and the properties of the substances with which it reacts.

13061 P

Distinct Types of Antibodies in the Blood of Rabbits Carrying the Transplanted V2 Carcinoma.

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Rabbits carrying the transplanted V2 carcinoma—a squamous-cell cancer derived originally from a virus-induced papilloma—regularly develop in their blood an antibody capable of neutralizing *in vitro* the papilloma virus (Shope) and capable also of fixing complement in mixture with it.[1] Further studies have now shown that the

[1] Kidd, J. G., and Rous, P., *J. Exp. Med.*, 1940, **71**, 813.