and in the absence of glomerular intermittence, the renal vasoconstriction appears to involve all parts of the renal vascular tree uniformly. The arterial blood pressure in these animals is only slightly lowered. The fall in blood volume, which is probably accompanied by a fall in cardiac output, also must be partially responsible for the fall in RPF.

11884

Technic for Demonstrating Antibodies Against Tuberculin in Experimental Animals with Sensitized Collodion Pellets.*

JOHN M. WEIR. (Introduced by E. L. Opie.)

From the Tuberculosis Research Station at Kingston, Jamaica, and the Department of Pathology, Cornell University, New York City.

A method for utilizing suspensions of sensitized collodion pellets for determining the presence of specific antibodies in blood sera has recently been described by Cannon and Marshall.[1] Such a procedure appeared to offer a technic for studying the development of antibodies against tuberculin in tuberculous infection and in experimental procedures in which killed tubercle bacilli were used for immunization. It is the purpose of this paper to report the results obtained in testing the blood sera of such experimental animals with suspensions of collodion pellets sensitized with commercial old tuberculin.

Following the method described by Cannon and Marshall[1] a supply of collodion pellets was prepared. A suspension of these pellets having a turbidity-factor of 244 (Summerson-Klett photoelectric colorimeter with a No. 66 filter) was mixed with an equal amount of a 5% (by volume) solution of commercial old tuberculin. The mixture was allowed to stand overnight at a temperature of 4°C. The pellets were recovered and washed in double-distilled water by centrifuging and resuspending them 3 times. The final suspension of sensitized pellets was diluted to equal a standard suspension having a turbidity-factor of 154. Serial dilutions of the serum to be tested were made in saline solution so that the volume in each tube was 0.5 cc. To each dilution was added 0.5 cc of the suspension of sen-

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation in coöperation with the Jamaican Government.

1 Cannon, P. R., and Marshall, C. E., *J. Immunol.*, 1940, **38**, 365.

sitized pellets. After standing at room temperature for 20 minutes, the serum-pellet mixtures were centrifuged for 4 minutes at 1200 rpm. The result of each test was read after gently resuspending the pellets in each tube. The highest dilution of each serum causing agglutination of the pellets was recorded as the antibody-titer of that serum. Control tests of sensitized pellets in saline solution and of unsensitized pellets in dilutions of the serum being tested were made in the same manner.

To determine that suspensions of sensitized collodion pellets would accurately demonstrate the presence of antibodies against tuberculin in the blood serum of animals inoculated with tubercle bacilli, the following experiment was done. Twelve normal rabbits were bled from the marginal ear vein before inoculation. Six of the rabbits

TABLE I.

Results of Tests on Rabbits before and at Weekly Intervals after Vaccination with Heat-Killed Tubercle Bacilli.

	Before vaccination			1 wk after vaccination		
No. of animal	Pellet titer	Complement-fixation titer	Reaction to tuberculin	Pellet titer	Complement-fixation titer	Reaction to tuberculin
1	0	0	0	1-16	0	12x10x0.5
2	0	0	0	1-16	0	0
3	0	0	0	1-32	0	14x12x0.5
4	0	0	0	1-8	0	22x16x1
5	0	0	0	1-16	0	0
6	0	0	0	1-32	0	0
7	0	0	0	1-16	0	0
8	0	0	0	1-32	0	0
9	0	0	0	1-8	0	0
10	0	0	0	1-16	0	12x10x0.5
11	0	0	0	1-32	0	22x14x0.5
12	0	0	0	1-8	0	0

	2 wks after vaccination			3 wks after vaccination		
No. of animal	Pellet titer	Complement-fixation titer	Reaction to tuberculin	Pellet titer	Complement-fixation titer	Reaction to tuberculin
1	1-128	0	6x6x0.5	1-256	1-5	22x18x1
2	1-128	0	38x22x1	1-256	1-10	35x28x1
3	1-64	1-5	24x22x1	1-128	1-5	40x35x3
4	1-32	1-40	25x22x0.5	1-256	1-20	25x25x0.5
5	1-64	1-5	18x18x0.5	1-128	1-10	0
6	1-64	1-20	0	1-256	1-40	38x32x2
7	1-64	1-5	0	1-128	0	0
8	1-64	0	9x9x0.5	1-128	1-10	16x16x0.5
9	1-32	0	37x25x1	1-256	1-10	32x25x1
10	1-32	0	16x16x0.5	1-128	0	24x24x1
11	1-64	0	0	1-256	1-5	0
12	1-64	0	0	1-128	0	0

Note: Results of tuberculin skin tests represent diameter and depth of edema and hyperemia in millimeters present 48 hr after injection of 0.1 mg O. T.

(Nos. 7 to 12, Table I) received intracutaneous injections in 12 sites of 0.4 mg of heat-killed tubercle bacilli (Ravenel) suspended in saline solution. The remaining 6 animals (Nos. 1 to 6) were inoculated subcutaneously in 12 sites with 0.4 mg of the same preparation of tubercle bacilli, suspended in paraffin oil. Blood samples were obtained from the marginal ear vein at weekly intervals after inoculation for a period of 3 weeks. At the time each blood sample was taken, the animals were tested for skin-sensitivity to tuberculin by intracutaneous injection of 0.1 mg of old tuberculin. In addition to the tests with sensitized pellets, each serum was tested for complement-fixing antibodies, using heat-killed tubercle bacilli as an antigen. All tests were done at the conclusion of the experiment, so that all sera were tested with the same suspension of sensitized pellets. The results are recorded in Table I.

It will be noted in Table I that suspensions of collodion pellets sensitized with tuberculin uniformly demonstrate the presence of antibodies against tuberculin in the blood serum of the inoculated animals. All of the preinoculation sera failed to cause agglutination of the sensitized pellets, as did control tests with saline and unsensitized pellets. The method accurately indicates a rise in antibody production with increasing intervals of time after vaccination. It is interesting to note that the animals exhibiting a low degree of antibody production in the first postvaccination sera continue at a level lower than the other animals. The presence of antibodies against tuberculin is demonstrated by the use of sensitized pellets in sera, in which complement-fixing antibodies are not demonstrable and, in some instances, when skin-sensitivity to tuberculin is absent. There was no demonstrable difference in the degree of antibody production between the animals injected intracutaneously with saline suspensions of the tubercle bacilli and those injected subcutaneously with suspensions in paraffin oil.

The previous experiment indicates that suspensions of sensitized pellets differentiate between pre- and postvaccination blood sera. To check this observation further, 90 sera from other laboratory animals were obtained. These sera were tested with suspensions of sensitized pellets without knowledge of the previous history of exposure of the animals to tubercle bacilli. Tests for complement-fixing antibodies and tests for skin-sensitivity to tuberculin had been carried out at the time the bleedings were done. After the completion of the pellet tests, the animal records were consulted, and it was learned that 12 of the sera had come from animals which had never had a known exposure to tubercle bacilli. The remaining 78 sera were

TABLE II.

A Comparison of Tests on Inoculated and Normal Animals with Sensitized Collodion Pellets, Complement-Fixing Antigen, and Intracutaneous Tuberculin.

Type of tuberculous material inoculated	Animal	Sol. used to suspend inoculum	No. animals tested	No. of sera exhibiting positive reaction with sensitized pellets	No. of sera exhibiting positive complement fixation	No. of animals exhibiting positive tuberculin test
B. C. G.	Rabbit	Saline	5	5	2	4
Living Ravenel	"	"	2	2	1	2
Dry Jamaica 22	"	"	1	1	0	0
Heat-killed Ravenel	"	"	13	13	8	9
Dry Jamaica 22	"	Oil	17	17	8	7
Heat-killed Ravenel	"	"	33	33	23	21
Phosphatide Extract of Bacillus	"	Saline	5	5	3	0
Heat-killed Ravenel	Monkey	"	1	1	0	0
Heat-killed Ravenel	Baboon	"	1	1	1	1
None (Controls)	Rabbit	—	12	0	0	0

Note: The inoculated animals were bled for tests and were skin-tested at varying intervals after the tuberculous material was received.

obtained from animals which had received injections of tuberculous material. The results of the tests on these sera and the results of the tuberculin tests done at the time the sera were obtained from the animals are recorded in Table II.

All the blood sera from the animals of the nonexposed group failed to cause agglutination with sensitized pellets. Of the sera obtained from animals exposed to tubercle bacilli or to injections of phosphatide extracts of the tubercle bacillus, all exhibited positive reactions with suspensions of sensitized pellets, the height of the antibody titer varying with individual sera. Control tests with saline and normal serum gave negative results. In contrast to these results, the complement-fixation tests demonstrated the presence of complement-fixing antibodies in only 46 of the 79 sera positive to the pellet test. Skin-sensitivity was absent at the time the sera were obtained in 39 animals.

Such results suggest the use of this technic with human sera. The results of such studies are now being analyzed.

Summary. A method of utilizing suspensions of sensitized collodion pellets for the study of antibody-formation in animals during experimental studies with the tubercle bacillus is described. Suspensions of pellets sensitized with old tuberculin uniformly demonstrate

the presence of antibodies against tuberculin in the blood serum of animals which have received injections of tubercle bacilli. The test clearly distinguishes sera of normal animals from those obtained from animals infected with tubercle bacilli. This method demonstrates the presence of antibodies against tuberculin in many of the animals tested at a time when the complement-fixing antibodies are not demonstrable, and at a time when many of the animals fail to exhibit skin-sensitivity to tuberculin.

.11885

Isolation of Isoandrosterone from Urine in a Case of Virilism.

H. HIRSCHMANN. (Introduced by D. W. Wilson.)

From the Department of Obstetrics and Gynecology and the Gynecean Hospital Institute of Gynecologic Research, University of Pennsylvania, Philadelphia, and from the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York.

Adrenal virilism of women is frequently associated with an abnormally high titer of urinary androgens. So far two androgenic compounds, dehydroisoandrosterone and isoandrosterone, have been isolated from urine of this type. Dehydroisoandrosterone has been found in several cases of virilism (mostly tumors of the adrenal cortex),[1] but isoandrosterone has been encountered only in one instance (a case of adrenal hyperplasia)[2] and has as yet not been found in any other type of human urine. We, therefore, wish to report the isolation of isoandrosterone from the urine of a woman suffering from severe hirsutism.

S.T., a 27-year-old woman was admitted to the Urological Clinic of the Presbyterian Hospital, New York, in 1937. Examination showed a marked hirsutism of body, face, and limbs, a slightly enlarged clitoris and a hypoplastic uterus. Aerograms taken in 1937 and 1939 gave no evidence of an adrenal enlargement. On biopsy (1940) both ovaries were found to be polycystic. The clinical data do not seem to permit a diagnosis as to the gland responsible for the

1 Crooke, A. C., and Callow, R. K., *Quart. J. Med.*, 1939, **32**, 233; Callow, R. K., *Proc. Roy. Soc. Med.*, 1938, **31**, 841; Dorfman, R. I., Wilson, H. M., and Peters, J. P., *Endocrinology*, 1940, **27**, 1.

2 Butler, G. C., and Marrian, G. F., *J. Biol. Chem.*, 1938, **124**, 237; Marrian, G. F., and Butler, G. C., *Nature*, 1938, **142**, 400.