tion. Δ^4-Pregnenediol-17(α),21-dione-3,20-acetate-21 (compound "S" acetate, Reichstein) inhibited the growth of the comb when applied directly or when administered subcutaneously. When 500 μg was applied to the comb a decreased comb ratio of 23% ($t = 3.73$) was found, while one mg administered subcutaneously produced a decrease of 29% ($t = 6.36$).

Summary. Δ^{16}-Dehydroprogesterone exhibited androgenic activity by direct application to the chick's comb. This steroid has approximately 6% of the activity of androsterone. Δ^4-Pregnenetriol-17(α),20(β), 21-one-3-diacetate-20,21, and Δ^4-pregnenediol-17(α),21-dione-3,20-acetate-21 (compound "S" acetate, Reichstein) inhibited the growth of the comb probably by direct antagonism of endogenous androgens. 17(α)-Hydroxyprogesterone produced no consistent effect. By direct comb application 80 and 640 μg levels were without effect while the 320 μg dose produced a 29% comb ratio increment which was statistically significant. When this steroid was injected subcutaneously at a dose of 1000 μg a significant comb inhibition was observed.

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Complement-fixing and Neutralizing Antibody Studies on Humans Vaccinated Against Rabies. (17636)

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Many workers have reported that neutralizing antibodies develop in the serum of animals and of human beings treated with live or killed rabies vaccines(1-5). It also has been shown that complement-fixing antibodies develop to a high titer in rabbits, mice, guinea pigs and dogs hyperimmunized by repeated injections of living virus(6). The somewhat meager evidence to date indicates that the two antibodies are not identical. Thus Habel(7) found that monkeys developing rabies showed no complement-fixing antibodies although virus neutralizing antibodies were present. Similarly, in experiments carried out in guinea pigs, Nachtigal(8) and Bernkopf and Nachtigal(9) showed that complement-fixing antibodies reached a maximal titer about a week after the last injection, diminished to one-eighth the maximal titer after one month, and disappeared completely within three months. On the other hand, neutralizing antibodies reached maximal titers after one month and showed no decrease in titer during the subsequent three months of observation.

This communication reports on the results obtained in determining to what extent neutralizing and complement-fixing antibodies are induced in human beings vaccinated with the Semple-type, phenol-killed rabies vaccine(1).

Materials and Methods. Vaccinations. All vaccinations were made with the Semple-type,

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1. Semple, D., *Sci. Mem. Off. Med. and San. Dept.*, Govt. of India, 1911, No. 44.

2. Pereira da Silva, E., *Arq. Inst. Bact. Camara Pestana*, 1928, v6, 42.

3. Stuart, G., and Krikorian, K. S., *J. Hyg.*, 1929, v29, 1.

4. Stuart, G., and Krikorian, K. S., *J. Hyg.*, 1932, v32, 489.

5. Kubes, V., and Gallia, F., *Canad. J. Comp. Med.*, 1944, v7, 48.

6. Casals, J., and Palacios, R., *J. Exp. Med.*, 1941, v74, 409.

7. Habel, K., *Pub. Health Rep.*, 1945, v60, 545.

8. Nachtigal, D., Summary of thesis presented for Ph.D. degree, submitted to the Senate of the Hebrew University, Jerusalem, May, 1945.

9. Bernkopf, H., and Nachtigal, D., PROC. SOC. EXP. BIOL. AND MED., 1943, v53, 36.

phenol and heat-killed vaccine consisting of 5% rabies-infected rabbit brain tissue prepared by the New York City Department of Health. Subcutaneous injections of 2 ml of vaccine were given each day either for 7 or for 14 consecutive days. The course of treatment was determined on the basis of the criterion set by the New York City Department of Health which stipulates that 14 injections are to be given to those individuals who are bitten by stray animals or by such animals that are known to be rabid. The course of 7 injections is given to those who have lesions above the clavicle if the animal is available for examination, and continued through the whole series of 14 injections if such an animal is proven to be rabid. No injections are given to persons with bites inflicted below the clavicle if the animal is known.

Blood samples. The first blood sample was withdrawn immediately prior to the first injection of vaccine, the second on the day of completion of the treatment, and the subsequent samples at various intervals thereafter. Serum samples were stored in rubber-stoppered vials in the CO_2 box until used, at which time they were inactivated at 56°C for 30 minutes.

Neutralization tests. The Lederle strain of mouse-brain fixed virus was made up in a 20% suspension and serial tenfold dilutions were made from the centrifuged supernate in buffered saline solution containing 10% inactivated normal rabbit serum. Each virus dilution was mixed with an equal volume of undiluted human serum, incubated for 2 hours at 37°C, then inoculated intracerebrally into albino Swiss mice 4 to 5 weeks old. Fifty per cent lethal endpoints were determined. Inactivated and undiluted normal human serum was used for controls.

Complement-fixation tests. Mouse brain tissue infected with the Lederle strain of fixed rabies virus was used as specific antigen after being benzene extracted from the dried state by the method of DeBoer and Cox(10). Normal non-infected mouse brain tissue was similarly processed and used in all tests as control antigen. The method used was essentially the overnight ice-box technique of Kolmer and Boerner(11), as modified for use in our laboratory(10,12). The customary serum and antigen controls were included in each test. A 2 + fixation, or higher, was taken as the endpoint.

Experimental. From the total number of 2,841 individuals who presented themselves for antirabies treatment at the Manhattan Clinic between January 1, 1946, to December 31, 1948, random blood samples were taken from approximately 100 persons. Of these, serial specimens were tested from 18 individuals who had received a series of 7 injections and from 69 who had received 14 injections of vaccine. Of those who received 7 injections of vaccine, only one individual had received antirabies treatment approximately 18 months previously. Of those who received 14 injections, antirabies treatment had been received by one individual 6 months previously, and by two others 2 years previously. The 4 individuals who had had previous antirabies treatment were excluded from all calculations concerning the comparative geometric mean antibody titers.

Table I shows the comparative geometric mean of neutralizing indices and complement-fixation (C-F) titers in patients who received 7 injections of vaccine (Series A), whereas Table II shows similar data for those who received 14 injections of vaccine (Series B). It is quite apparent from the data of these two tables that both neutralizing and C-F antibodies were induced in the two groups of vaccinated individuals, but that the neutralizing antibodies reached higher levels and persisted longer than did the C-F antibodies—with the highest antibody levels being attained in the B series of patients. Thus, in the A series (Table I) both types of antibodies began to appear by the first week after treatment, and reached their maximum 4 to 5 weeks after treatment. The C-F antibodies had disappeared entirely by the 4th month, whereas the neutralizing antibodies persisted in

10. DeBoer, C. J., and Cox, H. R., *J. Immunol.*, 1947, v55, 193.

11. Kolmer, J. A., and Boerner, F., *Approved Laboratory Technic*, D. Appleton-Century Co., New York, 3rd ed., 1941, pp. 616-646.

12. Van der Scheer, J., Bohnel, E., and Cox, H. R., *J. Immunol.*, 1947, v56, 365.

TABLE I.
A Series: Course of 7 Injections of Vaccine.
Comparative Geometric Mean of Neutralizing and Complement-fixing Antibody Titers.

Blood samples	Neutralization index*	Complement-fixation titer*
Pre-treatment	2.2 (13)	.1 (16)
At completion of treatment	3.3 (14)	.1 (16)
1 week after treatment	46.7 (16)	2.9 (15)
4-5 " " "	429.5 (9)	3.4 (9)
6-7 " " "	83.9 (4)	3.0 (5)
17-25 " " "	303.4 (11)	.0 (10)
29-32 " " "	301.3 (5)	.0 (5)
33-46 " " "	91.2 (5)	.0 (5)

* The figures in parentheses represent the number of serum specimens comprising the sample used for calculation of geometric mean titer.

TABLE II.
B Series: Course of 14 Injections of Vaccine.
Comparative Geometric Mean of Neutralizing and Complement-fixing Antibody Titers.

Blood sample	Neutralization index*	Complement-fixation titer*
Pre-treatment	3.8 (50)	0.1 (61)
At completion of treatment	175.8 (66)	2.6 (63)
1 week after treatment	5445 (64)	6.1 (59)
4-5 " " "	7780 (45)	4.6 (44)
6-7 " " "	2576 (17)	4.0 (23)
17-25 " " "	981.7 (56)	1.4 (56)
29-32 " " "	576.8 (36)	1.0 (36)
33-46 " " "	914.1 (13)	1.1 (14)

* Same as in Table I.

TABLE III.
Comparative Antibody Titers of 4 Individuals Who Had Had Previous Antirabies Treatment.

Blood sample	Neutralization index Patient No. 1	2	3	4	Complement-fixation Patient No. 1	2	3	4
Pre-treatment	794	1580	100	5	0	0	0	0
At completion of treatment	7940	>31600	>31600	7940	32	32	128	8
1 week after treatment	>25100	>31600	>31600	7940	32	32	32	4
4-5 " " "	—	20000	>63100	—	—	32	16	—
6-7 " " "	>31600	—	—	—	8	—	—	—
17-25 " " "	20000	10000	>15800	—	4	8	8	—
29-32 " " "	—	—	>31600	—	—	—	4	—
33-46 " " "	—	1580	—	—	—	8	—	—

Patient No. 1: Received 7 inj. in present series; had rabies vaccine 18 mo. before.
Patient No. 2: Received 14 inj. in present series; had rabies vaccine 6 mo. before.
Patients Nos. 3, 4: Received 14 inj. each in present series; had rabies vaccine 2 years before.

significant titer through the 8th to 12th month. The same general picture was true also for the B series (Table II) although here the antibodies began to be apparent at time of completion of treatment. Furthermore, the maximal level of the neutralizing antibodies of the B series was approximately 18 times that of the A series.

Table III shows the results obtained with serum specimens from the 4 individuals who had had antirabies vaccine 6 months to 2 years prior to the present course of treatment. Patient No. 1 received 7 injections of vaccine during the present series, while the remainder all received 14. Two rather interesting points are apparent in the table. First, it is seen that 3 of the 4 pre-treatment serums showed appreciable amounts of neutralizing substances with the titers apparently being inversely proportional to the time interval elapsing after vaccination. This, of course, indicates how antibodies of this type persist for fairly long

periods of time in the blood. Secondly, it is seen that quite a marked antibody rise or "booster" effect resulted following the second series of vaccine injections.

Discussion. The results shown here with serum specimens obtained from individuals who had received antirabies treatment are in full accord with those of Habel(7), Nachtigal(8), and Bernkopf and Nachtigal(9) who carried out similar tests on somewhat more limited number of serum specimens from vaccinated animals and showed that the virus neutralizing antibodies are quite distinct from the complement-fixing antibodies on the basis of the marked differences noted in their comparative titration endpoints and time intervals of maintenance. No attempt has been made in this study to determine the nature or properties of the 2 types of antibodies, nor is it intended to imply that there is any correlative relationship between either or both of these antibodies and the degree of immunity attained by the vaccinated host. In this connection it is of interest to mention briefly the work of others in this field. Nachtigal(8) reports that 2 independent and different antigens are involved in eliciting neutralizing and complement-fixing antibodies. This was proven by ultracentrifugation experiments with infected mouse-brain suspensions and comparison of the infective and the complement-fixing titers of the sediment and the supernatant fluid. By use of the ultracentrifuge it was possible to sediment the bulk of the virus without decreasing the original complement-fixing titer of the supernate. The virus-containing sediment did not fix complement at all. Thus the rabies antigen specifically involved in the production of complement-fixing antibodies is not pathogenic, is an entity separate from the infective virus itself, and is apparently of smaller particle size. This of course indicates that rabies has a "soluble" antigen similar to those known to exist in many other virus infections.

Finally it is evident from the literature that there is divergence of opinion as to whether serum neutralizing antibodies indicate resistance of the host to infection or not. However, the great bulk of evidence indicates that there is no essential or necessary parallelism between the neutralizing antibody titer of the serum and the degree of resistance of the host to infection (see 4,5,13,14,15,16). The studies of Kubes and Gallia(5) are worthy of mention here. These workers, while agreeing that the serum neutralization test does not give an accurate index of the immunity status of the host, claim that the brain-tissue neutralization test carried out with brain tissue suspensions of immunized mice gives a true picture of the antirabies immunity of the organism. While this type of study is worthwhile and feasible from the laboratory point of view, obviously it could not be carried out with human beings.

Summary. 1. Blood samples taken prior to vaccination and at rather frequent intervals after completion of antirabies vaccination were tested from 18 individuals who received 7 injections of vaccine and from 69 individuals who received 14 injections of vaccine.

2. Both neutralizing and complement-fixing antibodies were induced in the two groups of vaccinated individuals, but in all instances the neutralizing index reached higher levels and persisted longer than did the complement-fixation titer. The highest antibody levels were attained in those patients who received the greater number of vaccine injections.

3. Four individuals had had antirabies vaccine 6 months to 2 years previously. Three of the 4 still showed an appreciable neutralizing index prior to being revaccinated, and all 4 showed a marked antibody rise or "booster" effect after the second series of vaccine injections.

13. Jonnesco, D., *Ann. Inst. Pasteur*, 1932, v49, 332.

14. Habel, K., *Pub. Health Rep.*, 1941, v56, 692.

15. Webster, L. T., *Am. J. Pub. Health*, 1936, v26, 1207.

16. Casals, J., *J. Exp. Med.*, 1940, v72, 453.

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