reversal in the experimental lot was induced by the cortisone. Fig. 2 to 4 show how the degenerative processes in the ovarian cortex are immediately followed by thickening of the walls of the ovarian sacs, proliferation of numerous medullary cords, and differentiation of seminal tubules. The ovarian sacs become gradually reduced to seminal and reticular tubules of the testis.

The cortisone experiments are being continued, in order to obtain more information about the range of dosages, that will result in sex reversal; however, the fact that this substance like the other tested androstanes interferes with normal ovarian development and eventually causes sex reversal in females of *Rana sylvatica*, is now safely established. This result is somewhat surprising because cortisone has not, so far, been known to have androgenic functions. As a matter of fact, the results of our experiment should neither be considered as a demonstration of an androgenic character. This term should rather be restricted to designate the stimulation of male secondary sex characters. In our experiment cortisone evidently acts entirely by interfering with normal ovarian development. The development of the gonadal cortex becomes inhibited, and later the differentiated ovocytes are caused to degenerate. The transformations that lead to the development of the testicular structure probably are not induced directly by the cortisone. Since constitutionally the female larvae are bisexual, with the female determiners epistatic over the male factors, the inhibition or destruction of the ovarian cortex automatically creates the possibility for a compensatory, medullary-testicular development(3). The action of cortisone on the sex glands is, therefore, best characterized as antagonistic to the development of an ovarial cortex or, shortly, as *anti-ovariogenic.*

Summary. Cortisone, like 3 other tested alkyl substituted androstanes, causes the transformation of ovaries of frog larvae into testes. Its action is initially anti-ovariogenic, and sex reversal follows by a compensatory development of the medullary rudiment. The dosages that produce sex reversal are high in comparison with the threshold values of testosterone and methyltestosterone.

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Antigenic Similarity of Five Human Strains of Pleuropneumonia-like Organisms. (18318)

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Serologic studies of the pleuropneumonia-like organisms (hereafter referred to as PPLO) have been hampered by the difficulties encountered in isolating and maintaining strains. Cultural and morphologic characteristics of human strains have been studied, but relatively few observations employing serologic methods have been recorded. Warren and Sabin(1) compared one human strain with 5 mouse and 2 rat strains and found no serologic cross reactivity, confirming previous findings that strains from different animal species are antigenically diverse(2,3).

The PPLO have attracted attention as possible etiologic agents in non-gonococcal urethritis. Further information concerning this possible relationship may be gained by the application of serologic methods. Therefore, available strains were studied serologically

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1. Warren, J., and Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1942, v51, 24.

2. Sabin, A. B., and Johnson, B., PROC. SOC. EXP. BIOL. AND MED., 1940, v44, 569.

3. Sabin, A. B., *Bact. Rev.*, 1941, v5, 1.

with rabbit as well as with human sera. The preliminary phases of this study are reported herewith.

Methods and materials. Six strains of PPLO from the male and female human genital tracts were included in this study and will be designated as follows: 225† (female), L-50† (female), Douglas† (female), Campo‡ (male), N3‡ (female) and J-"L"§ (male). Each of these strains conformed to the morphologic and cultural characteristics of the group. Cultures were maintained by weekly transfers to the cystine trypticase semi-solid medium previously described from this laboratory(4). This medium was employed in broth form (*i.e.* without agar) in preparing antigens for injecting rabbits and for testing antisera. Approximately 15 transfers at 4- to 6-day intervals were made into the broth in order to obtain a smooth homogeneous growth. For the final mass production of antigen, 15 ml of a 4-day broth culture were inoculated into 1 liter of the same medium. After 4 to 6 days incubation at 35°C, the culture was centrifuged at 4200 rpm for one hour. The resultant sediment was then washed in sterile physiologic saline 4 times at 4200 rpm for one hour and resuspended in sterile saline to match the opacity of a No. 3 MacFarland nephelometer tube. Those suspensions used for testing antisera were preserved with 1:5000 aqueous merthiolate and stored at 4°C. Approximately 30 to 50 ml of test antigen could be obtained from each liter of broth culture. Uninoculated broth also produced a sediment which, when diluted to match the No. 3 nephelometer tube, yielded about 10 to 20 ml from each liter.

Antisera were produced by progressive daily intravenous injections into rabbits of 1, 2, 3 and 4 ml of viable, *i.e.*, unpreserved antigen. Five days after the last injection (9th day), a trial bleeding was performed, after which the rabbits were given an additional injection of 4 ml, then bled from the heart on the 12th day. Control rabbits were injected similarly with the suspended sediment from the uninoculated broth. For the *agglutination test*, sera were inactivated at 56°C for 20 minutes and serial dilutions made in physiologic saline. An equal quantity (0.5 ml) of antigen was added to each tube. After thorough mixing, the tubes were incubated at 52°C for 18 hours, then centrifuged at 800 rpm for 5 minutes. Readings for degree of agglutination were made with the aid of a microscope mirror while the tubes were gently shaken. Complete agglutination was recorded when a floccular sediment appeared in a clear supernate. Tests showing a finely granular sediment were considered negative. Serum controls included sera from normal rabbits as well as from those injected with suspended sediment from the uninoculated medium. Saline-antigen mixtures and suspended sediment from uninoculated medium were used as antigen controls.

Absorption and cross-absorption studies were performed with the lyophilized antigens of the Douglas and Campo strains of PPLO, and their corresponding antisera. The dried powder from 1 ml of lyophilized antigen was resuspended in 1 ml of physiologic saline, the suspension centrifuged at 3200 rpm for 15 minutes, the supernate discarded and 1 ml of antiserum (diluted 1:5) added. The antigen-antiserum suspensions were well mixed and incubated at 52°C for 1 hour then centrifuged at 3200 rpm for 30 minutes. The supernates were diluted serially and the agglutination titers were determined by the same procedure used for the unabsorbed serum.

Results. Pre-immunization sera did not agglutinate 5 of the 6 strains of PPLO. Similarly, 12 other sera from normal rabbits and antisera for various *Salmonella* and *Shigella* strains failed to agglutinate suspensions of these 5 strains. The sixth strain, L-50, was agglutinated by both normal human and normal rabbit sera, thus was considered unsatisfactory for serologic study.

Antisera for all six strains agglutinated the homologous antigens in titers varying from 1:320 to 1:1280. Marked cross reactivity

† Obtained from Lt. C. D. Graber, Third Army Area Medical Laboratory.

‡ Obtained from Dr. J. Warren, Army Medical Dept. Research and Graduate School.

§ Obtained from Dr. L. Dienes, Mass. General Hospital, Boston, Mass.

4. Norman, M. C., and Kuhn, L. R., *J. Bact.*, 1949, v58, 270.

TABLE I. Agglutination Titers of Antisera with Homologous and Heterologous Strains of Human Pleuropneumonia-like Organisms.

	Antigens							
Sera	225	L-50	Douglas	Campo	N3	J-"L"	Medium control*	Saline-antigen control
225	1:1280	1:320	1:640	1:1280	1:1280	1:320	0	0
L-50	1:1280	1:1280	1:640	1:640	1:1280	1:320	0	0
Douglas	1:1280	1:1280	1:1280	1:1280	1:640	1:640	0	0
Campo	1:1280	1:640	1:640	1:1280	1:1280	1:1280	0	0
N3	1:1280	1:640	1:1280	1:320	1:1280	1:640	0	0
J-"L"	1:1280	1:1280	1:1280	1:320	1:1280	1:1280	0	0
Control†	0	0	0	0	0	0	0	0
Normal rabbit	0	1:320	0	0	0	0	0	0

* Resuspended sediment from uninoculated medium.

† Serum from rabbit which was injected with the resuspended sediment of the uninoculated medium.

occurred between the strains, as can be seen in Table I. The homologous and heterologous sera agglutinated at the same dilutions, or differed in titer by only one or two dilutions. This would suggest that the individual organisms were antigenically similar. A recently isolated strain of *Salmonella typhimurium* grown in the same medium used for the PPLO, was not agglutinated by any of the antisera for PPLO.

Antisera from rabbits injected with the Douglas and Campo strains were tested after absorption with the Douglas and Campo antigens. The antibodies for the Douglas, Campo, N3 and J-"L" strains were removed completely from these two antisera by absorbing with either the Douglas or Campo strains. (Insufficient antigen from strain 225 was available at the time of these tests.) The results of the cross-absorption tests further indicate the antigenic similarity of the human strains of PPLO studied.

Sera obtained from 18 cases of non-gonococcal urethritis failed to agglutinate the five test strains, although PPLO had been isolated from only 2 of these patients. Thirty samples of human sera submitted for routine serological tests also failed to agglutinate any of the 5 human strains. These latter results are contrasted with the complement-fixation studies of Beveridge, Campbell and Lind(5) who found that a high percentage of normal human sera reacted positively with human strain antigens.

The time consumed in preparing antigens prompted an investigation into the possibility of using reconstituted suspensions of pleuropneumonia-like organisms which had been lyophilized and stored at 4°C for 3 months. The results with the lyophilized antigens were the same as those obtained with freshly prepared antigens.

Summary. 1. Suspensions of PPLO suitable for rabbit immunization and for use in agglutination tests were produced in relatively large quantities from cystine trypticase broth. 2. Five human strains of PPLO were closely related antigenically, as measured by agglutination titers with homologous and heterologous rabbit sera. These strains failed to agglutinate in the presence of normal rabbit and human sera. A sixth strain was agglutinated in all normal human and rabbit sera. 3. Further evidence was obtained for the antigenic homogeneity of the human PPLO studied by cross-absorbing 2 antisera and 2 antigens. After such absorption no antibody activity remained for any of the 4 strains tested. 4. Sera from 18 cases of non-gonococcal urethritis failed to agglutinate suspensions of 5 human strains of PPLO. 5. Stable suspensions were produced from antigens lyophilized for at least 3 months.

5. Beveridge, W. I. B. *et al., M. J. Australia,* 1946, v1, 179.

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