mobility of 4 components (Fig. 1). One of these could be considered impurity because it was also found in the prothrombin preparations from which the thrombin was derived. The other 3 components have their isoelectric points at about pH 4.7, 4.1 and 3.6. One of these values is near that found for prothrombin which, under the same conditions, has an isoelectric point of about pH 4.2. The latter value is somewhat uncertain because prothrombin is not sufficiently soluble at its isoelectric point to permit accurate measurement. The value represents the result of extrapolating a curve rather than precise determinations. Such solubility difficulties are not encountered with thrombin preparations. The protein with an isoelectric point of pH 4.1 is quite soluble at that pH. It is thus different from the protein found in the prothrombin preparations. Of the other 2 proteins, one has an isoelectric point at pH 4.7 and the other at pH 3.6. Tentatively we are referring to these proteins by the terms 4.7-Thrombin; 4.1-Thrombin; and 3.6-Protein. This conveys the idea that two possess thrombic activity and that the third one does not.

Analytical solubility curves described by Seegers and McGinty(6) and repeated again later indicated the possibility that thrombin activity might be possessed by 2 different proteins. In electrophoretic separation experiments this idea received further confirmation. However, the work could not be done as nicely as we had hoped. Consequently it is only possible to say that it is quite likely that thrombic activity resides in 2 proteins, that the question is not satisfactorily answered, and that there are no indications to the contrary. Work on the 3.6-protein was somewhat more encouraging. This component is present in low concentration but has a high mobility and it was possible to separate material which most probably represented this protein. It was free of thrombic activity and it contained much less tyrosine than the thrombin preparation as a whole. It contained carbohydrate. The separations in several experiments were considered to be technically satisfactory and it seems justifiable to conclude that this protein is inert with respect to thrombic activity.

Summary. Results of electrophoresis experiments with purified thrombin preparations indicate that thrombic activity is probably associated with 2 proteins. Under the conditions of the experiments one has an isoelectric point of pH 4.1 and the other at pH 4.7. Another substance is derived from prothrombin and is always found in the thrombin preparations. It has a high electrophoretic mobility and its isoelectric point is 3.6. This is called 3.6-protein, and it is very likely devoid of thrombic activity.

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Cortisone-induced Transformation of Ovaries into Testes in Larval Frogs.* (18317)

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In continuation of earlier experiments with standard androgenic and estrogenic substances, a study was made of the effects of some alkyl substituted androstanes on the process of sex differentiation in larval frogs *(Rana sylvatica)*. The 4 investigated compounds all brought about inversions in genetic females. However, the required dosages are high in comparison with testosterone and its derivatives(1).

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TABLE I. Effect of Pregnenolone on Sex Differentiation in *Rana sylvatica*.

Dosage µg/l	Sex distribution ♂	♂	♀
500	41		
100	41	1	
25	27		21
Controls	72		99

TABLE II. Effect of Progesterone on Sex Differentiation in *Rana sylvatica*.

Dosage µg/l	Sex distribution ♂	♂	♀
500	58	10	
250	36	30	10
100	39	13	40
20	37	7	48
Controls	45	1	48

TABLE III. Effect of Desoxycorticosterone Acetate on Sex Differentiation in *Rana sylvatica*.

Dosage µg/l	Sex distribution ♂	♂	♀
1000	65	14	
500	67	14	7
200	41	2	47
Controls	45	1	48

The substance to be tested is first dissolved in absolute alcohol, at near saturation. The desired concentrations in the water of the aquaria containing the larvae are obtained by "injecting" from a graduated syringe adequate amounts of this concentrate or of properly prepared alcoholic dilutions. Controls show that the small amounts of the solvent (0.05 to 0.25 ml per l of water) thus introduced, are without effects. Treatments usually are started at stage 25, when the gill sacs have closed and the gonads are still morphologically indifferent; they are continued until metamorphosis (stages 30-33).

Pregnenolone at 100 µg and 500 µg per liter causes the complete masculinization of all genetic females, while 25 µg/l remain without effect (Table I). It may be concluded that the critical or threshold concentration is at about 50 µg/l.

Progesterone has considerable anesthetic and toxic qualities, especially if administered at early stages. Partial masculinization of the female begins at 250 µg/l. At 500 µg/l, the sublethal concentration, the masculinization of all females is practically complete (Table II).

Desoxycorticosterone acetate is less toxic than progesterone though, as in the latter, masculinizing effects appear only at concentrations that approach the lethal limit. At 500 µg/l not all ovaries are definitely affected; but at 1000 µg/l all females have become transformed into hermaphrodites or males by the time of metamorphosis (Table III). This result is not in agreement with a report by Vannini(2) who contends that solutions of 400 µg/l in *R. dalmatina*, effect a reduction of the medullary component of the ovaries. According to our observations on *R. sylvatica* one should not expect any pronounced reaction with this dosage.

Cortisone became available to us late in spring, when the youngest of this year's sylvatica larvae had already entered the stage of sexual differentiation (stage 25/26). A group of 20 was placed in a concentration of 1 mg/l. The slightly late start of the treatment may account for the initial establishment of the normal sex ratio (11 ♀ + 9 ♂). When after 25 days of the treatment 3 larvae were preserved, it was found that 2 of this group were females. Their ovaries show first, but unmistakable traits of sex inversion (Fig. 1). Some ovocytes undergo degeneration; mitotic divisions of gonia are not occurring, and there is a loosening of the normal stratification of the cortex. Some gonia are leaving the surface layer and drift toward the medulla of the gonad. At the same time the ovarian sacs decrease in size and the entire medulla (sac epithelia and ovarian rete) becomes more massive. In the case represented by Fig. 1 the presence of the melanophores in the hilum and along the sac epithelium indicates a renewed immigration of medullary blastema cells (of mesonephric origin). Melanophores are often seen in testes but never in normal

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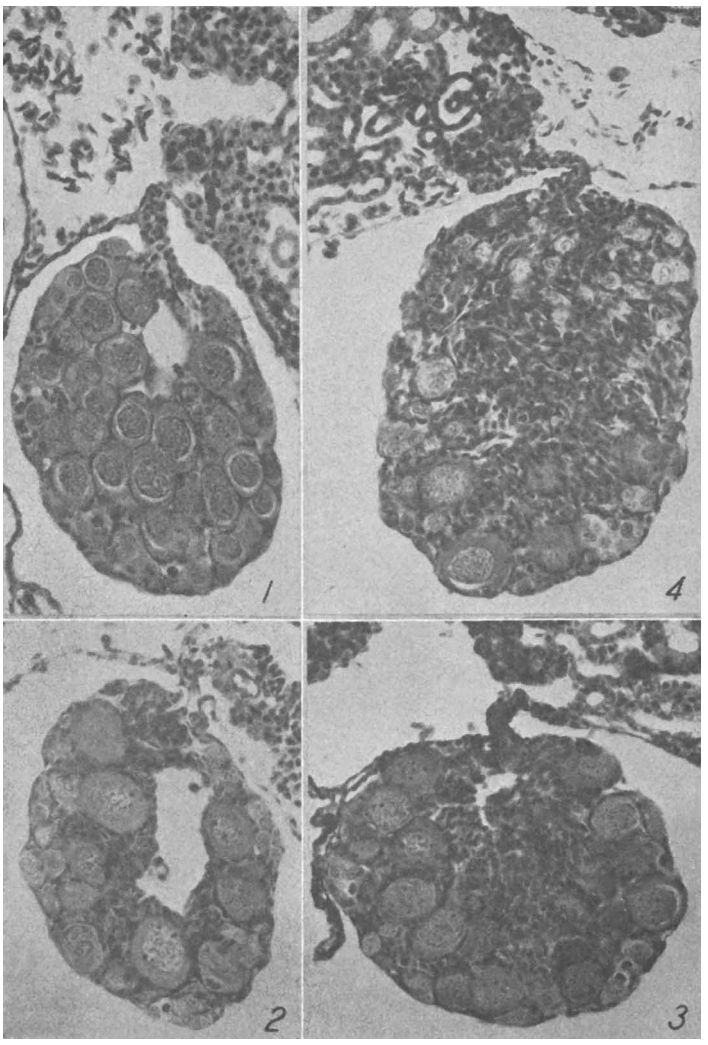

FIG. 1. Effect of cortisone on ovarian development; 1 mg/l, 25 days treatment. Reduction of the lumen of the ovarian sac; shifting of undifferentiated gonia toward the medulla; melanophores within the gonad. Ovarian character still predominant. ×167; larval stage 29.

FIG. 2-4. Effect of cortisone on ovarian development; 1 mg/l, 46 days treatment. Cross-sections through three sex glands illustrating successive steps in the transformation of ovaries into testes. The degenerative reduction of the cortex is followed by massive medullary proliferation. While the cavities of the ovarian sacs shrink, medullary cords grow peripherally and absorb the remaining undifferentiated gonia, which thus become spermatogonia. In Fig. 4 one notices the formation of seminal tubules and of an efferent ductule. All Figs. ×167; larval stages 30-32.

ovaries of this species; in the present case they constitute unequivocal evidence that sex reversal has become initiated.

The remaining 17 specimens were preserved during metamorphosis (stage 30) after 46 days of treatment. In this group normal or near normal ovaries are found no longer; it consists of 8 typical males and 9 hermaphrodites. The latter have ovaries in various stages of testicular transformation, up to near-completion of the process (Fig. 2 to 4). Since 171 controls consisted of 99 females and 72 males, and exhibited no tendency toward intersexuality, it is evident that sex

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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