1949, v89, 643.

16. Barron, E. S. G., *Toxicology of Uranium, National Nuclear Energy Series,* 1951, vIV-23, p41, McGraw Hill, New York.

17. Newman, W. F., *Pharmacology and Toxicology of Uranium Compounds, National Nuclear Energy Series,* 1951, vVI-1, p707, McGraw Hill, New York.

18. Spector, W. G., *J. Path. and Bact.,* 1954, v68, 187.

19. Heymann, W., and Lund, H. Z., *Pediatrics,* 1951, v7, 691.

Received July 26, 1956. P.S.E.B.M., 1956, v93.

Genic Control and Hormonal Reversal of Sex Differentiation in Xenopus.* (22688)

C. Y. CHANG AND EMIL WITSCHI.
Department of Zoology, State University of Iowa, Iowa City.

It has long been realized that in the cytodifferentiation of germ cells not only genes and external environmental factors play important roles, but also certain conditions of their immediate milieu(1). The first figure presents diagrammatically the basic facts and their interpretation based on recent work. Germ cells, on arriving in the primitive gonadal cortex, become invested each by a *nurse or follicle cell.* If unopposed, the follicle eventually induces development of the germ cell into an egg. However, if at the critical stage of sex differentiation a germ cell becomes located in the medulla, another inductor system, the *interstitial cells,* upsets the influence of the follicle cells and leads to the spermatogenetic type of differentiation.

How can such an interpretation be integrated with the well known facts of genic control of sex ratios? The present experiments seem to shed some new light on this problem.

Material and methods. Xenopus laevis Daudin has been bred in our laboratory since 1949 without addition of new stock. The experimental larvae were placed in hormone solutions of given concentrations, the solutions being completely replaced every 24 hours. The temperature was kept constant at 20°C.

Experiments. Since in the adult organism follicles and interstitial cells produce estrogenic and androgenic steroids, it was necessary to investigate the possibility that in embryonic sex differentiation the same hormones might first play the role of inductor substances. Experiments with Xenopus gave very interesting results, although not in the sense of a confirmation of the tentative assumption. Small or large doses of androgens added to the aquarium water equally produce what first seemed to be *all premature male cultures.* Given throughout the larval period they yield at metamorphosis little toads that all have well developed dark arm and hand pads and immediately start embracing each other. However, on dissection it is found that only about half of the animals have testes, the other half have normal ovaries (Fig. 2). We shall later come back to this experiment but next must describe the effect of the opposite treatment: exposing the larvae to estrogenic hormones, usually *estradiol.* Here the entire offspring is completely feminized. The original experiments with Allison(2,3) have often been repeated and thousands of offspring have been produced that consisted of females only(4,5). Some recent data are presented in Fig. 3.

These two hormone effects are all the more remarkable, because they are in contrast with the results of parabiosis and gonad transplantation, as described by Chang(6). The simultaneous presence of testes and ovaries in a system with a common blood circulation always results in normal testes and greatly suppressed ovaries. Sometimes even testicu-

* Aided by a grant from the Rockefeller Foundation. The authors are also indebted to Ciba Pharmaceutical Products, Inc., for the generous supply of the steroid hormones.

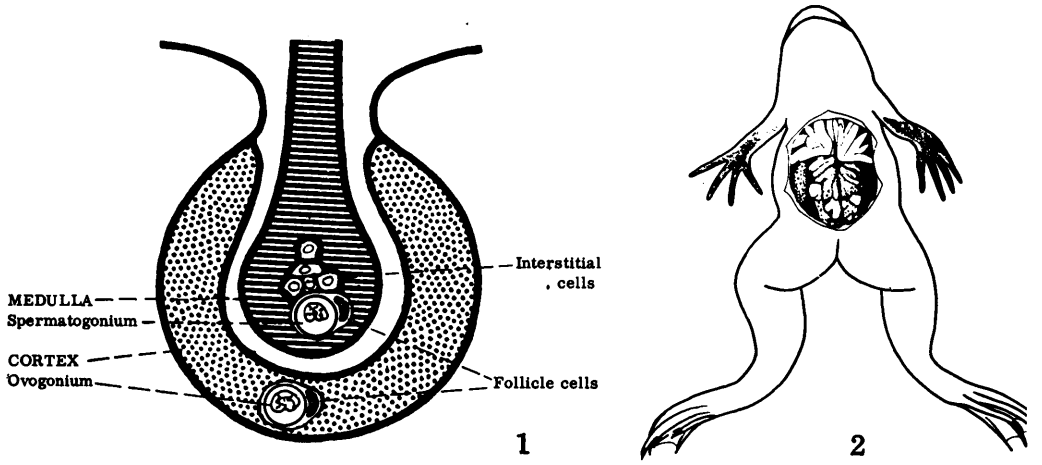

FIG. 1. Diagram of cortical and medullary inductors of sex differentiation.
FIG. 2. Genetic female (ZW) which while under testosterone administrations developed ovaries and male secondary sex characters.

lar nodules appear in the latter.

The striking difference between these reactions proves that the inductor substances are not steroid hormones; for even if the male parabiont were producing an androgen, our first experiment shows that it could only stimulate secondary sex characters but not inhibit ovarial development. On the other hand, if the female partner were liberating small amounts of estrogens, the genetically male co-twin should develop ovaries—which again is not so. What then is the nature *of the inductor substance?* Further analysis of this problem must be preceded by a clarification of the role played by the *sex genes* (Fig. 3).

In Xenopus normally the female is heterozygous (ZW) and the male homozygous (ZZ). Therefore, treating part of the lay of a normal pair with estradiol should give an all female offspring in which half are of ZZ constitution. This had to be verified in breeding tests, for both ZZ and ZW animals are of the same appearance. Of 43 tested in this way, 22 gave an F_1 generation with the normal 1:1 ratio (ZW females): but 21 produced entirely male offspring and thereby proved to be of ZZ constitution. Evidently we can now dispense with the heterozygous sex type. Breeding homozygous stock only, one may produce any desired number of females by estradiol treatments at the larval stage. The possibility of controlling the sex ratio at the will of the experimenter is often a great convenience.

These breeding experiments with sex reversed toads spread some revealing light on the problem of the nature of sex determining genes. Since estradiol need only be administered during a short larval period, it is evident that the entire process of ovogenesis and the development of female secondary sex characters, including sex behavior, are no longer under the control of the sex genes. Apparently the genes play an active role only during a short and definite period, starting a chain reaction which later runs off independently. In trying to locate this period we first treated several 100% ZZ (male) cultures for 1-week periods with estradiol, and soon found that the critical time lies at the transition of stages 26 and 27(7), which actually is the time of initial embryonic differentiation (Table I). Attempts to pinpoint more exactly the time of interference with gene action led to surprisingly clear results. The primitive undifferentiated gonad of Xenopus consists of a series of about 15 pseudosegments or gonomeres. In accordance with general vertebrate development, they form and mature in craniocaudal sequence. If 2-day administrations of estradiol are given at the end of stage 26, the tops of the gonads differentiate into ovary, but the lower part remains unaffected and develops according to the testicular type (Fig. 4b, 5b). If, however, treatment is delayed until the

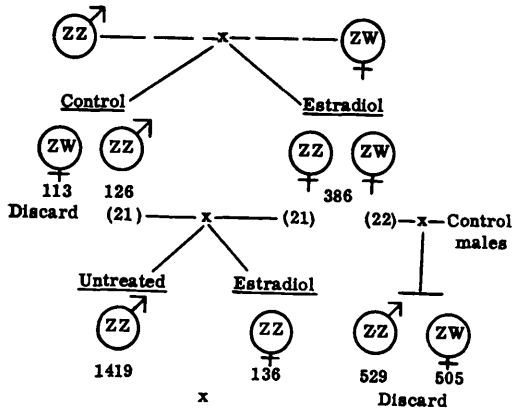

FIG. 3

FIG. 3. Experimental breeding record of Xenopus.

start of stage 27, the lower gonomeres become ovarial lobes. One may even produce triple sequences with ovary in the center and testis at both poles (Fig. 4a, 5a). In average cultures complete feminization requires close to 3 days. This means that about every 5 hours a new gonomere enters the responsive phase.

These experiments help in clarifying some aspects of the problem of *gene action*, recently discussed by Curt Stern(8). In general, the breeding of sex-reversed males in successive generations gives proof that the upset of genic expression neither induces, nor depends upon perceptible readjustments in the genic pool. This conclusion is unavoidable here, because the very cells that develop into eggs instead of sperms carry the genes into the subsequent generations. Also it becomes evident that the results of gene action are in time and quality co-determined by preadjusted factors of the internal milieu. In the present case the internal milieu is represented by inductor cells and substances. These are mostly proteins while the genes are DNA compounds. Delbrück has presented some interesting thoughts regarding the possible relationships between these widely different chemical classes(9). For a more penetrating discussion much more precise knowledge is needed especially regarding the inductor substances. In sex differentiation of gonochorists (*i.e.*, species with separate male and female sexes) there are at least two classes separately recognizable. Cortex and medulla each produces an agent, *corticin*

TABLE I. Series of All-Male (ZZ) Larvae, Each Treated during One of 4 Successive Weeks, with Estradiol, 50 μg/l.

Treatment	Stage (7)	N1*	N2†	♂	♀̂	♀
1st wk	24–25	47	38	38		
2nd	25–26	47	41	38	3	
3rd	26–27	72	65		4	61
4th	27–28	47	41	41		
Continuous	24–29	80	69			69
None		292	278	278		

* Original No. of tadpoles.
† No. of tadpoles survived.

and *medullarin* respectively, which induce ovogenesis or spermatogenesis. While in primitive hermaphrodite species the inductions by these substances may proceed side by side, even within one and the same gonad, in gonochorists the agents assume the character of antigens, so that the cortex now produces also an *anti-medullarin* and the medulla an *anti-corticin*. These "antibodies" have a wider range of action than the primary inductor substances and, as parabiosis experiments prove, they are proteins of high specificity that often are carried by the blood stream. These conclusions are partly a reinterpretation of previously reported experiments, but they are also based on the following new observations on Xenopus.

It was mentioned above that in male-female parabiosis, the ovary becomes extremely reduced. The same is the case if a testis is implanted into the body cavity of an early female larva. Interestingly, the same inhibition is not exerted by the testicular lobes of partially feminized males. Quite to the contrary, the ovarial lobes grow at about the same rate as ovaries of completely feminized larvae (Fig. 6). The full spermatogenic development in the testicular segment remains unaffected by the proximity of ovarial, also normally maturing lobes (Fig. 7). This is new evidence of the separate existence of the inductor of male differentiation (medullarin) and the inhibitor of ovarial differentiation (anti-corticin). Estradiol administration blocks the former only during actual treatment but the latter more generally and lastingly.

The rule that androgens have no sex-reversing effect on female larvae becomes

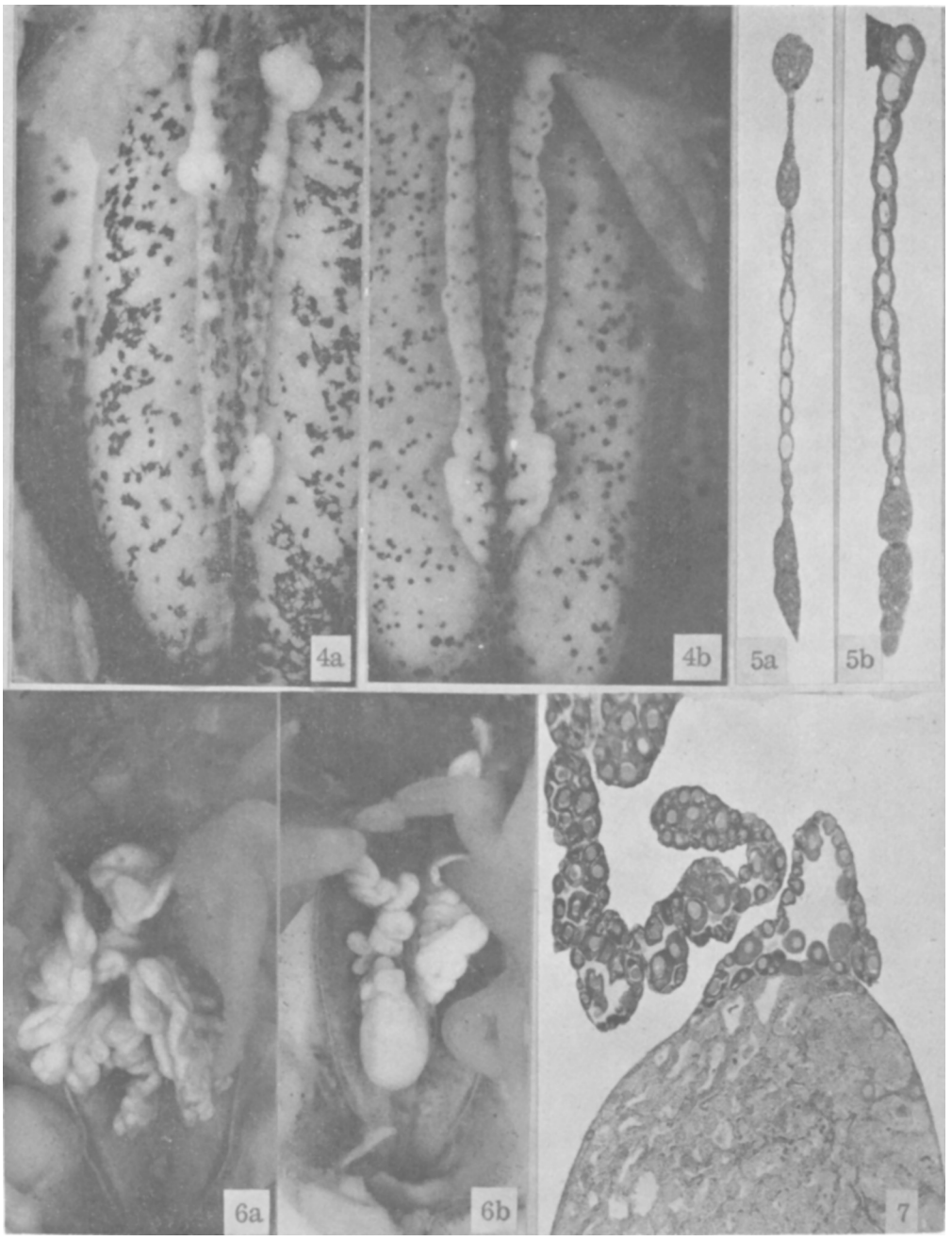

FIG. 4. Newly metamorphosed hermaphrodites of ZZ stock treated for 3 days with estradiol; a. ovarial gonomeres in the center of the sex glands; b. ovarial gonomeres above the (broader) testicular parts; × 19.

FIG. 5. Frontal sections through one sex gland each of 4a and 4b. Ovarial gonomeres with central cavities; ×27.

FIG. 6. ZZ males treated with estradiol, preserved 4½ mo. after metamorphosis; a. completely feminized; b. with a testicular segment in the lower right gonad; × 5.

FIG. 7. Longitudinal section through lower right gonad of 6b showing hermaphrodite mosaic; × 40.

TABLE II. Double Treatment with Estradiol, 50 μg/l, Followed by Testosterone, 50 μg/l, until Metamorphosis.

	Treatment	♂	♀	♀
A.	Control	55	—	—
	Estradiol (7 days)	—	1	17
	Estradiol followed by testosterone	16	5	—
B.	Control	72	—	63
	Estradiol (7 days)	1	5	18
	Estradiol followed by testosterone	7	4	10

slightly modified in the following experiment. Genetic males (ZZ), treated at the proper time with estradiol, become changed into females, if reared later in pure water. However, if the estradiol treatment is immediately followed by administration of testosterone, the animals revert back to males. Table II presents 2 experiments with identical procedures. But in the first one (A) the mother was a sex reversed male (ZZ) while in the second (B) she was of normal ZW constitution. Both experiments show that estradiol treatments feminize offspring of the ZZ type; but if it is immediately followed by testosterone, the reversion is wiped out, all ZZ individuals become males again (or intersexes). Under exactly the same conditions the ZW individuals develop normally into females. This abolition of initiated sex reversal is the only indication that androgens may inhibit the cortical inductors in a similar though much less effective way as estrogens are suppressing the medullary system.

Conclusions and summary. Continuation of experiments on sex determination, particularly with Xenopus, led to the conclusion that the primary inductors, corticin (of follicle cells) and medullarin (of interstitial cells) are antigens. The corresponding *anti-corticin* and *anti-medullarin,* suppressing the contrary inductor system, are responsible for the unisexual (gonochoristic) development of each individual. Normally the decision of cortical (female) or medullary (male) prevalence depends on the quantitative relationship between sex determining genes. Experimental reversal of this influence does not produce, or depend on, any perceptible temporary or permanent change in gene composition or function. If administered at a short critical period of sex determination (St. 26/27), estradiol causes ZZ larvae to develop permanently as females. This effect is brought about by depression of the medullary inductor system. The possibly ensuing development of hermaphrodite glands indicates that selectively the capacity of antibody (anticorticin) formation is more generally affected than that of the antigen (medullarin).

1. Witschi, E., *A. mikr. Anat.,* 1914, v86, 1.
2. Witschi, E., and Allison, J., *Anat. Rec.,* 1950, v108, 589.
3. Witschi, E., *A. Anat. micr. et Morph. exp.,* 1950, v39, 215.
4. Chang, C. Y., and Witschi, E., PROC. SOC. EXP BIOL. AND MED., 1955, v89, 150.
5. Gallien, L., *C. rend. Acad. Sci.,* 1953, v237, 1565.
6. Chang, C. Y., *J. Exp. Zool.,* 1953, v123, 1.
7. Witschi, E., *Development of Vetebrates,* Saunders, Philadelphia & London, (1956).
8. Stern, C., *Analysis of Development,* Saunders, Philadelphia and London, (1955), 151.
9. Delbrück, M., *Angew. Chem.,* 1954, v66, 391.

Received July 27, 1956. P.S.E.B.M., 1956, v93.

Differential Distribution of Enzyme Hydrolyzing Acetylsalicylic Acid (Aspirin) in Certain Fluids of the Rat.* (22689)

HARRY BECKMAN.
Department of Pharmacology, Marquette University School of Medicine, Milwaukee.

It appears likely that degradation of salicylic acid occurs principally, though probably not exclusively, in the kidneys since metabolites appearing in urine are not recoverable from the blood(1). The process is probably a complex one involving filtration at

* Partially supported by a grant from the Wisconsin Heart Assn.