

specific activity will be somewhat increased rather than decreased or unaffected. Such moderate increases in specific activity, unaccompanied by increases in total activity but usually associated with a loss of enzyme units, indicate that some enzyme has in fact been destroyed but rate of destruction has been exceeded by rate at which other nitrogen has been lost. These factors should be borne in mind in any comparison of response to irradiation of the same enzyme in a radiosensitive tissue and in a relatively radioresistant one.

Finally, it may be of interest that according to Smith and Low-Beer(8), 2 spleen enzymes involved in pyrimidine metabolism, uridine phosphorylase and cytidylic acid phosphatase, exhibited a slight increase in specific activity, and no increase in total activity (*i.e.*, case 3a) in rats exposed to 650 r, while a third enzyme, uridylic acid phosphatase, showed decreases in both specific and total activities (*i.e.*, case 2).

Summary. Adenosine deaminase and guanase activities were studied in homogenates and supernatant fluid fractions, respectively, prepared from spleens of control rats and animals given 600 r of whole-body X-ir-

radiation. Specific activities of both enzymes were increased slightly in irradiated animals but total organ activities of both enzymes were decreased as compared to levels obtained in control rats. Interpretation of these and similar findings is discussed, and it is concluded that they do not represent direct or indirect enzyme activation or enzyme synthesis but rather reflect loss of nitrogen content and weight which are concomitants of splenic involution.

The author is grateful to Josef Bukovsky for expert technical assistance with some experiments.

1. Eichel, H. J., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 155.
2. Roth, J. S., Eichel, H. J., *Radiation Research*, 1959, v11, 572.
3. Eichel, H. J., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 38.
4. Kalckar, H. M., *J. Biol. Chem.*, 1947, v167, 461.
5. ———, *ibid.*, 1947, v167, 429, 445.
6. Feinstein, R. N., *Radiation Research*, 1956, v4, 217.
7. Eichel, H. J., Roth, J. S., *ibid.*, 1960, v12, 258.
8. Smith, K. C., Low-Beer, B. V. A., *ibid.*, 1957, v6, 521.

Received April 1, 1960. P.S.E.B.M., 1960, v105.

Maintenance of Gonads of *Xenopus laevis* in Organ Cultures.* (26025)

CHARLES L. FOOTE AND FLORENCE M. FOOTE

Dept. of Zoology, Southern Illinois University, Carbondale

Some *in vivo* and *in vitro* effects of testosterone on gonads of larvae of *Rana catesbeiana* and *Xenopus laevis* have recently been reported(1). The present studies were made to determine whether gonads of *Xenopus*, placed in organ cultures at varying stages of development, would differentiate and respond to treatment with estrogen and chorionic gonadotropin in the same manner as *in vivo*.

Materials and methods. Larvae were made bacteriologically sterile(2) and cultures were set up as previously described(3). One part

of glass distilled water in the medium was replaced by the hormones, estradiol (Progyron, Schering Corp.) or chorionic gonadotropin (Antuitrin-S, Parke, Davis and Co.). Developmental stages of larvae are numbered according to Nieuwkoop and Faber(4).

Results. Controls. Undifferentiated gonads from larvae of stages 49-50, attached to mesonephroi, showed no development in culture. In sectioned material no gonads nor germ cells could be positively identified. However, in gonads from larvae of developmental stage 52 and above, ovaries and testes could be distinguished. Some of these showed pro-

* Aided by grants from U.S.P.H.S. and Graduate School, Southern Illinois Univ.

TABLE I. Results of *In Vitro* Treatment of Gonads of *Xenopus* with Hormones.

Stage	Medium	Hormone	Days treated	Total animals	Males	Females	Undiff.	Undet.
49-55	Standard	None	5-56	49	15	18	4	12
51-60	"	Ch. gonado., 100 I.U.	11-59	36	11	10	0	15
55-65	"	Estradiol, 20 μ g	14-56	44	17	15	0	12
56	"	" , 10 μ g	22	16	5	4	0	7
Adult σ	"	" , "	22	8	8	0	0	0

gressive development comparable to control animals *in vivo*. The sex ratio of gonads placed in culture was 1:1 as in intact control animals (Table I). In cultured gonads it was difficult definitely to determine the sex in every case since cells of the organs migrated to such an extent that the form of both cells and organs was modified.

Estradiol. Gonads from larvae in stage 55 were the youngest placed in cultures containing estradiol. Maintenance of cultured gonads on this medium was equally as good as in those kept in culture on standard medium without added hormone. Neither gonads of this stage nor those from older larvae or adults showed any modification of sex ratio.

Chorionic gonadotropin. The response of gonads to this hormonal substance was similar whether gonads were in culture or in intact animals. There was no evidence of sex transformation in either situation. In older male larvae the seminiferous tubules were more distended than in controls. There was hyperplasia of the rete apparatus in both ovaries and testes.

Discussion. Recent reports(1,5) give results following *in vitro* treatment of larval gonads of *Rana catesbeiana* with chorionic gonadotropin, estrogen, and androgen. In comparing these results with responses of *Xenopus* gonads it was found that chorionic gonadotropin caused hyperplasia of rete cells in both genera. No modification of gonads was observed in either group of larvae following estrogen administration. Androgen caused reversal of ovaries to testes in *Rana*, but in *Xenopus* there was only a retardation of development(1).

Gallien(6,7) and Chang and Witschi(8,9) found that testes were reversed to ovaries in *Xenopus* larvae treated with estrogens, but we found no similar change in gonads maintained in cultures with estradiol. The critical

time in development when estradiol has its most pronounced effect corresponds to stages 48 through 55 when sex differentiation occurs according to Chang and Witschi(9). They state that at this time estradiol interferes with gene action, depressing the medullary inducator system and causing genetically male larvae to develop permanently into females. The fact that testes grown *in vitro* retained their male characteristics in the presence of estrogen may perhaps be due to the inability of gonads to be successfully maintained as typical organs prior to stages 58-60. Estradiol had little effect on the medulla of an already well-differentiated gonad *in vitro*.

Summary. Our study indicates that ovaries and testes, from larvae of *Xenopus laevis*, placed in organ cultures show progressive development, but gonads removed from larvae prior to or during time of sex differentiation do not differentiate *in vitro*. Older larval testes retain their normal male characteristics even on a medium containing estradiol. Chorionic gonadotropins stimulate rete apparatus of both ovaries and testes in organ cultures as well as in intact animals.

1. Foote, C. L., Foote, F. M., *Arch. Anat. Micro. Morph. Exp.*, 1959, v48, 71.
2. Wilde, C. E., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1948, v69, 374.
3. Foote, C. L., Foote, F. M., *Anat. Rec.*, 1958, v130, 553.
4. Nieuwkoop, P. D., Faber, J., *Normal table of Xenopus laevis (Daudin)*, Amsterdam, North Holland Pub. Co., 1956.
5. Foote, C. L., Foote, F. M., *Proc. XVth Inter. Cong. Zool.*, 1958, 602.
6. Gallien, L., *C. R. Acad. Sci.*, 1953, v237, 1565.
7. ———, *Bull. Biol. France et Belgique*, 1956, v90, 1.
8. Chang, C. Y., Witschi, E., *Proc. Soc. Exp. Biol. and Med.*, 1955, v89, 150.
9. ———, *ibid.*, 1956, v93, 140.

Received May 31, 1960. P.S.E.B.M., 1960, v105.