Similar strains of fibroblasts have been established from adult rabbit, spleen, testis and ovary. Epithelial strains have also been isolated from liver and ovary and other tissues containing epithelial cells but not from bone marrow. Epithelial strains were isolated from tissues explanted in plasma clot cultures.

Haff and Swim(1) found that 199 did not support growth of rabbit fibroblast lines. In this laboratory the combination of 199, calf serum and yeast extract has proved an ideal medium for rapid and continuous growth of rabbit fibroblast strains developed from a variety of adult rabbit tissues. In preliminary experiments it was observed that addition of yeast extract increased growth rate and also shortened the lag phase. However, once the lag period was overcome, cell lines derived from marrow and other rabbit tissues proliferate rapidly without yeast extract.

Routine development of fibroblast strains is making possible investigations into possible conversion of normal rabbit fibroblast cells into malignant ones *in vitro*. It is now possible to use the same rabbit for donor tissue and as a recipient after its cells have been grown for any given length of time *in vitro*. The relatively long life span of the rabbit (5 years) makes it another important laboratory animal in which to study mechanisms involved in conversion of normal into malignant cells *in vitro*.

Summary. A simple method is described for routine development of permanent strains of fibroblasts from adult rabbit bone marrow. Marrow particles were grown on glass in medium composed of 1 part calf serum, 2 parts 199 and 0.1% yeast extract. This method is also applicable to development of permanent cell lines from a variety of adult rabbit tissues.

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Non-Specificity of Pituitary-Induced Anuran Ovulation *in vitro*.* (25733)

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Ovulation in *Rana pipiens* induced by injection or implantation of frog pituitary glands is a common laboratory procedure. Implantations or extracts of pituitary glands of a few other vertebrates are also effective in *Rana pipiens, i.e.,* garpike(1), toad(2), newt(3), chicken(4), and sheep(5). Some other anurans, particularly *Xenopus laevis* and several urodeles, ovulate upon exposure to pituitary factors from a wider variety of animals or even pregnant mares' serum and human chorionic gonadotropin(6). In many instances, however, enormous dosages of heteroplastic pituitary tissue are required, suggesting that responses obtained may not be entirely physiological, or that injected material may have merely activated recipient's pituitary gland. Induction of ovulation *in vitro* by addition of extraspecific pituitary factors to Ringer's solution containing ovarian tissue from normal, healthy frogs, rules out participation by the host's pituitary gland, thus providing a very critical test. Routine induction of ovulation *in vitro* with frog pituitary extracts and/or preparations of FSH and LH from sheep pituitary glands has been reported(5,7). It now seems important to record ovulatory responses *in vitro* in a number of interspecific combinations of ovarian tissue and pituitary glands, not only to add data for consideration in the species-specificity controversy, but in view of objection of some(8) that ovulation *in vitro* may

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TABLE I. Ovulation Induced *In Vitro* in Ovarian Tissue from Various *Anura* when Exposed to Homoplastic and Heteroplastic Pituitary Extracts.

Ovarian donors	Pituitary donors					
	Rana pipiens	*Rana clamitans*	*Rana catesbiana*	*Rana sylvatica*	*Pseudacris trilineatus*	*Hyla crucifer*
Rana pipiens	+ Jan.-Dec.	+ June 9	+ June 9 Nov. 14	+ Oct. 26	+ Mar. 31	+ Mar. 31
" *clamitans*	+ June 9	+ June 9	+ June 9			
" *catesbiana*	+ June 9	— June 9	+ June 9			
" *sylvatica*	+ Oct. 26 Feb. 18			+ Oct. 26 Feb. 18		
Pseudacris trilineatus	+ Mar. 31			+ Mar. 30	+ Mar. 30 " 31	
Hyla crucifer	+ Mar. 30 " 31			+ Mar. 30	+ Mar. 30 " 31	+ Mar. 30 " 31

+ = positive response; — = negative response.

not be a wholly normal process.

Methods. Specimens of *Rana pipiens* were obtained periodically from collectors in Vermont and Wisconsin. *Hyla crucifer, Pseudacris trilineatus,* and *R. sylvatica* were collected late in fall or at least 2 weeks before onset of breeding in the vicinity of Ann Arbor, Mich. All preceding species go into hibernation with a fully mature ovary and normally breed immediately upon emerging in spring. Fully mature eggs are present in ovaries, from Sept. until mid-April. *R. clamitans* and *catesbiana,* also obtained locally in Michigan, emerge from hibernation with only partially mature ova, considerable increase in ovarian weight occurring between emergence and breeding late in June. These species are suitable for ovulatory experiments, then, only during about the first half of June. Procedure was essentially that previously described (7), *i.e.,* suspension of ovarian fragments on cotton thread in shell vials containing 10 ml Ringer's fluid with added pituitary extract. For some smaller animals a whole ovary was placed in small Petri dish with 20 ml pituitary-Ringer solution. Pituitary dosages were the equivalent of 1/16 of gland/10 ml of fluid, and were made by triturating fresh tissue in small mortar, adding 1 ml of distilled water to lyse any remaining cells, then diluting to desired volume with Ringer's solution. Controls were always assembled for each species of ovarian tissue on given day, and consisted of ovarian fragments or whole ovaries suspended in Ringer's fluid without pituitary extract. None of these controls ever ovulated spontaneously, indicating that in no instance was ovarian tissue already stimulated to point of ovulation by donor's pituitary gland.

Results (Table I) show uniform responsiveness of ovaries used and lack of specificity of pituitary factors among species represented. Percentages of ovulation were approximately as expected, *i.e.,* 60% or more, if breeding season was imminent and somewhat less (15-50%) in the fall. Ovulation in excess of 95% was obtained with *Pseudacris* ovary when stimulated by either *Pseudacris* or *R. pipiens* pituitary extract; *Hyla* ovary shed about 90% of its eggs when exposed to *R. pipiens* pituitary factors but only about 25% when exposed to its own hypophyseal hormones. The failure of *R. catesbiana* ovary to respond to *clamitans* pituitary would seem more than accidental, since the same tissue responded to pituitary extract from *pipiens* and *catesbiana* on the same day. On the other hand, different areas of the same ovary (at least in *pipiens* work) have been unequally responsive, and a particularly resistant section of *catesbiana* ovary may have been selected inadvertently for exposure to

clamitans pituitary extract. Undoubtedly, many factors enter into potential capacity of any ovary to ovulate on being stimulated by pituitary hormones, and without extensive study, a meaningful evaluation is difficult. It is for this reason that individual percentages of ovulation have been omitted from Table I.

It should be noted particularly that *R. pipiens* ovary has now been induced to ovulate *in vitro* during every month of the year. The ovary of a freshly-caught specimen ovulated Aug. 29th, and September ovulation has been induced on several occasions in freshly-collected frogs. Animals kept in forced hibernation in the laboratory may be used routinely from mid-October into early June (no ovulation in Ringer controls) and on 2 occasions ovulation was induced *in vitro* in mid-July from specimens held in cold room since the previous April.

Negative results have been obtained with *R. pipiens* ovary *in vitro* when pituitary extracts from the following animals were added: sexually immature *Ambystoma maculatum* (Nov. 19), 2 sexually mature turtles (*Pseudemys scripta troosti* and *Chrysemys picta*, Mar. 29), and normal and castrated laboratory white rats of both sexes (Dec. and Mar., on several occasions). Human chorionic gonadotropin (Antuitrin-S, Parke-Davis) and combinations of this urinary factor with mammalian FSH (Synapoidin, Schering) failed to induce ovulation either *in vivo* or *in vitro* in repeated assays, nor have they increased significantly the responses obtained with frog pituitary extract. In every instance potential responsiveness of ovarian tissue was verified simultaneously by exposure of other ovarian fragments to *R. pipiens* pituitary extract.

Summary. Ovulation *in vitro* has been induced in a variety of anurans using homoplastic and heteroplastic pituitary extracts, indicating (1) that the reaction as induced in excised ovarian tissue is truly physiological and normal, and (2) that there is a general lack of specificity of pituitary hormones for this response.

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Enzyme Distribution in Human Brain. Lactic and Malic Dehydrogenases.* (25734)

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Studies on distribution of enzyme activity in mammalian brain have been carried out by histochemical and microchemical methods. These technics have proved suitable for detailed study of small portions of the nervous system, *i.e.*, Ammon's horn(1) and limited areas of cerebral cortex(2). There have been no systematic studies of normal enzymatic activity on the human central nervous system. The present work was undertaken to define normal enzyme distribution in human gross material and to serve as a baseline for similar studies of various neuropathological entities. Lactic dehydrogenase and malic dehydrogenase activities were chosen because they offered the possibility of comparing information obtained by this method with histochemical

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