The Male Frog, *Rana pipiens,* as a New Test Animal for Early Pregnancy

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In a series of tests run during recent months we have found the male frog, *Rana pipiens,* to have high diagnostic value as a test animal for early pregnancy. These animals are easily obtainable in the United States, are not killed for the test, and may be used over again. The test is of relatively little value in the last months of pregnancy, but after the first trimester any test for pregnancy is, after all, principally of academic interest.

Ever since Ascheim and Zondek first proved that the gonad-stimulating substances in pregnancy urine were capable of stimulating the ovaries of immature rodents, subsequent modifications of their test have been made on female animals. It seems that the male gonads had been quite forgotten until Mainini (*1*) showed in March of 1947 that these substances would also cause the release of spermatozoa in the South American toad, *Bufo arenarum* Hensel. Siegler and Fein (*2*) have presented a graph showing the concentration of the chorionic gonadotropic hormones in the blood and urine of pregnant women. This graph indicates that the concentration begins about the 15th day, rises rapidly till the 30th day, and drops rapidly till the 90th day. It remains at a low level throughout the remainder of the pregnancy and drops to zero after the delivery of the complete placenta.

In our pregnancy tests with male frogs we have found that our results tend to correspond to this graph. Urines tested from women in the last trimester gave us nearly 50% false negative results, whereas those urine specimens from the *first trimester* so far have given entirely positive results. There have been no false negatives. In our series we have used well over 200 test animals with the urines of pregnant women, nonpregnant women, and men. Check animals were untreated frogs and frogs injected with Ringer's solution (cold). No check animal has ever produced spermatozoa. The possibility of seasonal variation is still unknown.

The technic of the test is simple. A first morning (overnight) specimen of urine is obtained and 5 cc carefully injected subcutaneously into the dorsal or lateral lymph sacs of the frog. Two or more frogs are used, as there may be a difference in sensitivity to the test. Each frog is placed in a separate, clean, dry glass jar with a perforated lid and set aside for 2–4 hrs at room temperature. At the end of this time any urine that has been voided by the frogs is examined microscopically. If spermatozoa are not present, the urine is carefully drained from the jar without disturbing the frog. The frog is then seized in the hand while still in the jar. This pressure usually induces another urination. The new specimen of urine is then examined for spermatozoa. The frog's sperms are easily identifiable. When spermatozoa are present, the test is positive; when they are not present, the test is negative. The fact that there are no intergrades eliminates all subjective interpretations. The test animals are not killed and may be used for another test after 4 or 5 days.

A more detailed summation of our work will be published later.

References

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Chromosome Breakage in Plants Induced by Radioactive Phosphorus (P^{32})

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That X-rays, radium, and certain other rays emitted by a source external to living cells induce chromosome breaks and rearrangements is well established (*1*). Consequently, radioactive elements emitting beta or gamma rays within living cells may be expected to produce similar results. When the source of radiation is external, it is usually most convenient to give the radiation treatment in one or a few doses of rather short duration. With radioactive elements introduced into the tissues, low dosage per unit of time may be continued over a long period. The fact that some radioactive elements may be incorporated in the chromosomes may also have effects of special interest. The recoiling nucleus will almost certainly have enough energy to break any chemical bond (*2*). Comparable initial dosages of different radioactive elements may show somewhat different effects, since there may be differential localization of the active element, the rays may differ in hardness, and the rates of decay may be very different.

Preliminary experiments on mutation induction by radioactive phosphorus (P^{32}) have been carried out, using the following plants for test organisms: *Triticum aestivum* L., variety *Thatcher* (n = 21), *T. durum* Desf., variety *Pelissier* (n = 14), *T. monococcum* L. (n = 7), and *Hordeum distichon* L., variety *Hannchen* (n = 7).

In the most complete series of tests undertaken, the radioactive phosphorus was made available to the germinating seeds and young seedlings. Seeds to be treated were divided into two equal lots supplied, respectively, with .18 and .018 microcurie of P^{32} for each seed (P^{32} in the form of Na_2HPO_4). Forty-eight seeds of each species were treated; 6 untreated plants of each species were used as controls. The seedlings were transferred from the test tubes to 1-gal crocks containing untreated soil 13 days after seed germination began. At this time it was found that $90 \pm 4\%$ of the activity in the original solution had entered the plants.

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