samples of urine from the last trimester of pregnancy gave positive readings ½ hr after the second dosage of injection. Mention may also be made of the fact that a 2-cc injection of urine, or even 1-cc in a few cases, produced the same result as that obtained by a 5-cc injection, especially in those samples that gave strongly positive results. It appears from these observations that there may be some relation between the gonad-stimulating substances in urine and the liberation of spermatozoa. The results of 130 tests run in this laboratory are summarized briefly in Table 1.

It is interesting to note also that the time required for a positive reaction was only 25 min after the injection of urine in a large number of cases. Records of a few tests from the early trimester showed that a release of spermatozoa continued at intervals for 8 to 10 hr, completely disappearing after that period. The experimental animals were reexamined the next morning, but no spermatozoa were ever released with the voided urine. It cannot be stated with any degree of certainty whether there is seasonal variation, either in the duration or in the rapidity of the release of spermatozoa. Since Wiltberger and Miller (*5*) have stated that "the possibility of seasonal variation is still unknown," we hope to continue our tests during this winter. A detailed account of these and further tests will be published elsewhere.

It is apparent that our observations not only confirm, in the main, those in the most recent paper by Miller and Wiltberger (*4*), but also add some valuable data. Suffice it to say that the use of males of the Salientia as test animals for early pregnancy and certain related conditions will open a new chapter in the investigation of the biologic assay of urine hormones.

We wish to record our sincere thanks to Drs. G. Nandy, Binoy Banerjee, J. Chakravarty, and P. Das of Lake Medical College, Mrs. S. Ghosh of Baldeodas Maternity Hospital, and the authorities of Chittaranjan Seva Sadan, for generously providing us with specimens of urine.

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A Study of Native Species of Male Toads as Test Animals in the Diagnosis of Early Human Pregnancy

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After Galli-Mainini (*2*) in 1947 reported the use of the male South American toad, *Bufo arenarum* Hensel, in the routine diagnosis of early pregnancy, other investigators (*3, 4*) demonstrated that the male frog, *Rana pipiens,* responded to human pregnancy urine in a manner similar to that of the South American toad, namely the rapid release of spermatozoa.

Impressed by the simplicity and economy of this test, we became interested in the possibility that species of male toads native to the Rocky Mountain area might serve as satisfactory test animals. Two species have been tested, *Bufo woodhousii* of Girard, the most widely distributed species of toad in Colorado, and *Bufo americanus,* the common toad of North America. In this region these animals offer the advantages of being more hardy and easier to keep than frogs.

Our technique has been simple, consisting of the injection of 5 cc of untreated urine into the dorsal lymph sac of the animal. We then obtain a specimen of toad urine 3 hr later by the introduction of the tip of a small (1 cc) pipette into the cloacal orifice. The specimen is examined unstained under the low power of a microscope for the presence or absence of the numerous motile, thread-like spermatozoa. If these are absent, specimens are again examined at 5 and 24 hr. Animals may be used again after a rest period of one week.

We have performed a total of 145 tests to this date. Five animals have died as a result of the injection. There were 75 negative results and 65 positive results. In no case did a urine from a nonpregnant patient give a positive result. In 69 Friedman or Aschheim-Zondek and toad tests performed coincidentally, there was one disagreement, a negative toad test and a positive Friedman. The patient could not be followed to ascertain which was correct. Remaining tests were performed on specimens obtained from patients seen in Colorado General Hospital Outpatient Department suspected of early pregnancy. All results in these cases were proven correct by clinical followup. The earliest positive result was obtained only 8 days after the first missed menstrual period. Urine specimens obtained from women past the sixth month of pregnancy gave false negatives in 2 out of 5 specimens tested, confirming the unreliability of this test in late pregnancy as reported by workers using the South American toad (*2*) and the frog (*3*).

A urine specimen from a patient with hydatidiform mole was tested. It gave a positive result in a dilution of 0.05 cc. Basing our calculation on the observation that these animals have responded to a minimum of 10 rat units of a commercial preparation of chorionic gonadotropin, we estimated that there were at least 200,000 rat units of gonadotropin per liter in this specimen, a level consistent with the diagnosis of mole.

These native toads have been used up to 8 times with no apparent decrease in accuracy. An effect of repeated injections seems to be the slowing of reaction time. On the first injection all positive results were evident within 3 hr. However, on further injection, spermatozoa did not appear until 5 hr in nine positive tests, and until 24 hr in four such tests out of a total of 50 positive results in animals injected more than once. *Bufo americanus* was used in 18 tests and *Bufo woodhousii* Girard in the remaining 127 tests. The one discrepancy between toad and Friedman tests occurred in *Bufo woodhousii* Girard.

The fact that these two native species of toads, as well as several South American toads and the frog, *Rana pipiens,* respond to the injection of urine from pregnant patients by the rapid release of spermatozoa would seem to indicate that this reaction is generalized in toads and frogs and that it offers a practical means of testing for early human pregnancy.

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Vacuum-Paraffin Infiltrator

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The need for a method which would infiltrate a whole rat brain with paraffin was the basis for development of the vacuum-paraffin infiltrator,[1] an electrical heating unit which contains a glass bottle connected to a vacuum source. The heat in the bottle is controlled by a rheostat which regulates the flow of current into the heating unit.

The procedure followed in developing the infiltrator was first to select a suitable glass bottle. A copper cup was formed into which the bottle was placed so that there was a close sliding fit between bottle and cup. A thin sheet of asbestos paper was wrapped around the metal cup to form a base upon which the high resistant wire was wound. The wire used was 24 gauge chromel "A" asbestos-covered wire. Care must be taken in winding the wire to reduce the hazard of short circuit. A piece of copper tubing was fitted over the coils, and holes were drilled into the tubing so that dissipation of excess heat would be more effective, thereby preventing the contents of the glass bottle from becoming overheated. The wire coil of the heating unit was attached to two contact poles which were incorporated into a wood base facilitating use of a plug to connect the rheostat.

The rheostat, similar to ready-made ones, was designed to be more adaptable to control of the heating unit. Buttons on the control dial of the rheostat were connected at various points of the resistance wire, thereby cutting in or cutting out more or less resistance. A small Christmas tree light was added to the rheostat. It lights up when the switch is on, and dims or brightens as the control knob of the rheostat is turned from button to button; the brighter the light, the higher the temperature in the heating unit. This feature is a valuable aid in indicating whether the circuit is operating properly. If the heating unit wire develops a break, the light will not dim or brighten to the same degree when the control knob is passed over the buttons of the rheostat.

[1] The author extends his appreciation to E. Ehrlich, Sr. for technical assistance in producing this model.

The vacuum source is a conventional water aspirator which is attached to a water faucet. A two-holed rubber stopper is used in the bottle of this model, one hole containing the glass tubing for the vacuum connection and the other containing a thermometer which is used to make an occasional check on the temperature of the paraffin.

The advantage of this vacuum-paraffin infiltrator is that it can be set into operation and left without need for constant attention. It has also been found that resulting paraffin tissue blocks are more compact, due to the low air content of the paraffin. If the clearing agent is a comparatively volatile substance, its removal is expedited by this method. By keeping the paraffin free of the clearing agent, the necessity of having two or three changes of paraffin is eliminated.

The Effect of Supervoltage Cathode Rays on the Nonenzymatic Browning Reaction of Dried Fruits and on Chemical Compounds Pertaining Thereto

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It has been demonstrated previously that ionizing radiations have a lethal action on bacteria and thus may offer a potential means of processing foods (*1, 3*). To determine the feasibility of using these radiations in the preservation of foods, the effects of the radiations on the nutrients in foods, on color components, and on flavor are under investigation in these laboratories.

The experiments described herein relate to some observations made on the effects of high voltage cathode rays, electrostatically produced, on color in dried fruits, extracts thereof, and compounds pertaining thereto. The cathode rays utilized in this work were obtained from a Trump Generator (*6, 7*) operating at 3,000 kv.[1]

Dried prunes were exposed to cathode ray irradiation for dosages up to 10 million roentgen-equivalents-physical (rep). A definite bleaching of the flesh was observed in prunes that were examined immediately after irradiation, but no color changes in the skin were noted. The amount of bleaching increased with greater dosages of radiation. After the prunes had been at room temperature (70°–80° F) for two weeks, the color of the flesh appeared to have returned to normal. No noticeable change was observed in the flavor of the prunes immediately after irradiation or two weeks later.

A similar experiment was carried out with Thompson Seedless Raisins. With raisins, however, bleaching occurred in the skin as well as in the flesh, and a definite off-taste was noted. As in the case of the prunes, the

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