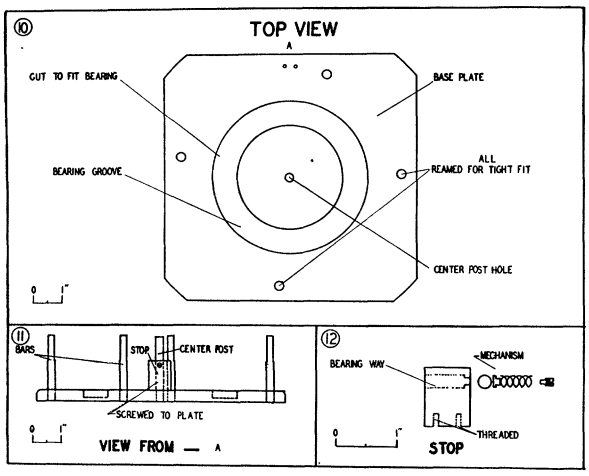

FIG. 3. Views of the base plate upon which the thrust bearing rests and to which is fixed the top plate shown in Fig. 1. The bars on pins (insert 11) are friction-fixed into this base plate but fit loosely into the top plate; the top plate is thus prevented from turning when the turntable or sample receptacle tray is rotated, but at the same time the top plate is permitted to ride freely upon and to seal the top of the turntable. The bearing surfaces between the top plate and sample tray are coated with a thick grease. A seal is further insured by the weight of the counter, top plate, and center post, which compresses a rubber washer against the center of the top plate, pressing it toward the bottom one.

then the preflushing feature is not used. Under such circumstances, the sample is rotated clockwise into the counter; sufficient time is then allowed for flushing and a count is obtained. The sample tray is rotated counterclockwise, so that its receptacle is brought to the outside for changing of samples.

Details of construction of the sample tray and its operation are evident from the illustrations. The plan may be modified to include automatic features.

Male Frogs and Toads as Test Animals for Early Pregnancy and Certain Related Conditions

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Very recently a large amount of evidence has been accumulated to show that males of Salientia can be unequivocally used to test for early human pregnancy (*1–5*). Mainini (*3*) has demonstrated for the first time, that in the males of the South American toad, *Bufo arenarum,* there occurs within 3 hr following injection a release of spermatozoa into the urinary bladder as a result of gonad-stimulating substances in the human pregnancy urine. Wiltberger and Miller (*5*) have confirmed, in the main, and extended Mainini's observations by using the common American leopard frog, *Rana pipiens.* Lima and Pereira (*2*) have also confirmed Mainini's test by using another toad, *Bufo marinus.* Again, while supporting claims made for this test by the above-mentioned workers, Haines (*1*), who used *B. arenarum,* which he received from the originator of the test, observes, "It remains to be seen whether *Bufo bufo* or *Rana temporaria* will be equally suitable." We have also been able to obtain a similar result by using the males of ordinary salientians that are easily available in Calcutta, namely, *Bufo melanostictus, B. stomaticus,* and *Rana tigerina.* Only a few specimens of each of the latter two species were employed for the confirmation of the results obtained in the former, which, however, was used chiefly during the course of our investigation.

In a series of urine tests run during the months of July to September, we obtained only positive results from the first and mid-trimester of pregnancy (Table 1). There was not even a single case of a false negative. Urine tested from the last trimester, however, gave 3 false negatives. This may be due to a low level of gonad-stimulating substances in the urine. Furthermore, both positive and negative tests were obtained from

TABLE 1

URINE TESTS FOR PREGNANCY AND OTHER CONDITIONS

Samples of urine	+	−	Total
Pregnancy—early (up to 90 days) ..	29	0	29
" —mid (from 91–180 days)	11	0	11
" —late (after 180 days) ..	26	3	29
Nonpregnancy	0	14	14
Sterility	0	14	14
Male	0	4	4
Pathological (abortions, mole, etc.)..	9	20	29
Total	75	55	130

some individuals with related pathological conditions, such as hydatiform moles, superinvolutions, abortions, etc. The results of such tests tend to throw some light on the clinical aspects. Neither the control animals (the experimental animals also served as controls; their urine was examined for spermatozoa before they were injected with human urine), nor those injected with urines from nonpregnant or sterile women, or men, ever released spermatozoa.

The technique employed in our series of tests is as simple as that of previous workers (*3, 5, 4*). In spite of our best efforts we could not secure early morning (overnight) urine, except for a few samples. The tests were based chiefly upon urines collected during late mornings and evenings, the identification not being known to us until after the tests. Our findings were afterwards clinically confirmed. Although a fresh toad was used for each urine test, a similar result could also be obtained from the same experimental animal after an interval of 4 to 7 days. One such animal could thus be re-used with success at least four times before it expired. A 5-cc dose of urine was uniformly administered, but if the test turned out to be negative, an additional dosage of 5 cc was administered 2 hr after the first injection, in order to corroborate the first result. In most cases a negative result was obtained even with a double dosage; only three

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.