TABLE IV.—Hogben Tests in a Case of Hydatidiform Mole

Pre-operative		Ref. No.	Date	Hogben Result in Dilutions	Friedman Test with C.S.F.
1		3308	28/11/49	+ 1 in 400	—
2		4002	30/11/49	+ 1 in 500	
3		4003	1/12/49	+ < 1 in 400	
4		3710	7/12/49	+ 1 in 10	
5		3736	29/12/49	+ 1 in 10	
Operation			2/1/50		+
			3/1/50		
			4/1/50		
Post-operative	Hrs. After Operation				
1	16	4006	5/1/50	+	
2	18	4007		+	
3	42	4008	6/1/50	+	
4	45	4010	"	+	
5	56	4011	"	+	
6	62	4012	7/1/50	+	
7	66	4409	"	+	
8	72	4013	"	+	
9	76	4014	"	+	
10	80	4015	"	+	
11	90	4016	8/1/50	+	
12	93	4017	"	+	
13	97	4039	"	+	
14	100	4042	"	+	
15	104	4040	"	+	
16	108	4148	"	+	
17	109	4149	9/1/50	+	
18	114	4150	"	+	
19	123	4151	"	—	
20	138	4041	10/1/50	—	
21	26 days	4258	30/1/50	—	

One millilitre of cerebrospinal fluid was negative to a Friedman test on November 30. A larger volume, 8 ml., injected on January 3, 1950, produced a strongly positive Friedman reaction. It is emphasized that it is unnecessary to use cerebrospinal fluid for confirmation of hydatidiform mole. A few years ago, to end the habitual procedure of spinal puncture, the Usher Institute, Edinburgh, refused to accept specimens of cerebrospinal fluid.

Pre-operative urine on December 1 yielded a positive Hogben reaction in a dilution of 1 in 500. Tests repeated 28 and 32 days later reacted only in a dilution of 1 in 10. In view of the lessening of chorionic gonadotropin, the absence of evidence of foetal parts by x-ray examination, and the fundus of fourth-month pregnancy it was decided to empty the uterus. This was accomplished by the vaginal route on January 4, 1950.

Twenty specimens of urine were collected in the course of six days, in the hope of detecting the point at which chorionic gonadotropin was no longer being excreted. The test remained positive up to the fifth post-operative day. During this time no specimen caused a reaction in a dilution of 1 in 10. From the sixth post-operative day the test became negative.

Summary

A description is given of the preparation and standardization of chorionic gonadotropin from urine.

The method of using and resting the Watford colony of tested *Xenopus laevis* is recorded.

Some interesting cases are reviewed.

I wish to express gratitude to Dr. F. W. Landgrebe, of the Usher Institute, Edinburgh, for all the helpful interest he has taken in the Watford Pregnancy Diagnostic Centre.

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The Minister of Health recently received a deputation from the Socialist Medical Association, when tuberculosis and its treatment under the National Health Service were discussed. The deputation put forward a number of proposals. The Minister in reply drew attention to the progress already achieved and indicated the lines along which he hoped there would be further advance.

USE OF THE MALE BRITISH TOAD AS A PREGNANCY TEST ANIMAL

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In the last four years various workers have successfully used male toads of numerous species in the Galli Mainini pregnancy test (Galli Mainini, 1947, 1948; Houssay, 1947). It was decided to investigate the possibility of using the British species (*Bufo bufo*) as a pregnancy test animal.

Method

Two male toads of over 15 g. weight each have 1 ml. of filtered first morning urine injected into the dorsal lymph sac. The toad's cloacal urine is taken at the time of injection and the absence of spermatozoa is checked. Further samples are taken three to six hours later, and the presence of spermatozoa in any of these is regarded as a positive result (see Fig.). If no sperms can be detected in either toad's cloacal urine six hours after injection the result is negative.

FIG. 1.—Toad spermatozoa (*Bufo bufo*) ($\times 1,000$). The thick black lines are the heads of the sperms, the tails being much finer. The usual debris found in these smears is also present. (Stained Giemsa.)

The toad's urine is collected by means of a fine glass pipette of 1 mm. diameter inserted into the cloaca and moved gently backwards and forwards. This can be examined, unstained, under a low-power microscope ($\frac{2}{3}$ in.—16 mm.).

Toads can be reused after one week, but no toad should be used for more than four tests, as the reaction is then less prompt, is less pronounced, and sometimes fails altogether.

Results

Eight consecutive tests on 80 pairs of toads gave 53 positive and 27 negative results. Of the 53 positives, 51 were confirmed clinically as pregnancy, one as hydatidiform

mole, and one as a chorionepithelioma of the testis. Of the 27 negatives, 26 were subsequently proved clinically not to be pregnant. The one false negative was an early pregnancy of less than six weeks which a few days later became a threatened abortion.

Discussion

A suitable quantity for injection was 1 ml. of first morning urine, this being the most concentrated specimen of the day. Positives could then be obtained when afternoon specimens gave negative results. Some of Klopper and Frank's (1949) false negatives may be due to this, despite the injection of 2 ml. a toad. Cerquiglini's (1948) injection of 10 ml. a toad is far too much for toads around 20 g. in weight.

The weight of the toads should be over 15 g. This was shown by analysis of the first 25 positive tests (see Table). Of the toads heavier than 15 g. 97% were positive after six hours, while only 75% of the lighter toads were then positive.

TABLE.—Difference in Response Between Light and Heavy Toads

Time in Hours	No. of Toads First Giving Positive Result	
	Over 15 g.	15 g. or Less
After 2	24	5
" 4	7	6
" 6	2	1
" 24	1	2
Not in 24	0	2

Pairs of toads are used for every test, and the result is regarded as positive even if only one responds. Thus in our series of 53 positive tests (that is, 106 toads), three toads gave a negative result. With single toads the accuracy would then have been 97%, but the use of pairs brings it to over 99.9%.

In considering the stage of pregnancy after which the toad test becomes positive one has to realize that this, as well as the Aschheim-Zondek, Friedman, and Hogben tests, is essentially a test for gonadotrophins; so the result will depend on the concentration of gonadotrophins excreted in the urine. In normal pregnancy the urinary gonadotrophin level shows a marked rise about the fourth to sixth weeks; before this time it is generally too low for there to be sufficient gonadotrophin in the injection mass to produce a positive result in the various biological pregnancy tests.

As the thresholds of different test animals—mice, rabbits, *Xenopus*, *Bufo*—are not the same, and as in various tests different amounts of urine are injected, it is obvious that not all of the pregnancy tests will become positive at the same time. It has been tentatively put forward (Frazer and Wohlzogen, 1949), and since confirmed (Frazer and Wohlzogen, 1950), that in the British toad the threshold (ED 50) is about 8 i.u. for standard chorionic gonadotrophin. Despite the results of Bach and Szmuk (1949) on frogs, this threshold in *B. bufo* has not varied significantly at different times of the year in toads kept in a warmed animal-house. Use of the same standard gonadotrophin has shown a threshold for the Friedman test of 15 i.u. approximately; for the Aschheim-Zondek 10 i.u. approximately, and for the *Xenopus* 70 i.u. approximately (Frazer and Wohlzogen, unpublished). With the usual amounts of urine injected in these tests—namely, Friedman 15 ml., Aschheim-Zondek 2 ml., *Xenopus* 7 ml., and *B. bufo* 1 ml.—this means roughly that the gonadotrophin levels above which positive results are obtained with unconcentrated urine will be: Friedman, 1 i.u./ml.;

Aschheim-Zondek, 5 i.u./ml.; *B. bufo*, 8 i.u./ml.; and *Xenopus*, 10 i.u./ml.

From this it becomes apparent that the Friedman test will give a positive result earlier in pregnancy than the others. The toad test, however, can be brought up to the same level of sensitivity as the Friedman test by concentrating the urinary gonadotrophins eight times by the method of Scott (1940).

Conclusion

The series of positive results with unconcentrated morning urine were obtained from cases ranging from the fourth week (eight cases) to the seventh month of pregnancy. Over this period there would seem to be no bar to the use of the toad test; but when negative results are obtained in what may be the third to sixth weeks of pregnancy it would be advisable, for 100% accuracy, either to concentrate the urine eightfold or tenfold and repeat the test, or to perform a Friedman test. If neither is feasible the test should be repeated after a fortnight. Which alternative is adopted may depend upon the laboratory facilities available.

Summary

The use of the male British toad (*Bufo bufo*) for pregnancy tests is described. In a series of 80 consecutive cases no false positive and only one false negative result was obtained.

The results are discussed, and suggestions are made for the assessment of the results of pregnancy tests in what may be the first six weeks of pregnancy.

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Medical Memoranda

Inversion of the Uterus

Inversion of the uterus is a very rare condition. The incidence is variously estimated at figures varying between 1 in 16,000 and 1 in 200,000 deliveries. The most searching article on the subject is that written by P. Das (1940). He studied the literature from the earliest times and found records of 391 cases, of which 297 were puerperal. I have found references to a further 122 cases in the journals available to me, while many more are to be found in the *Index Medicus*.

CASE REPORT

The patient, aged 34, was in her fourth pregnancy. Her last menstruation was on October 10, 1946, and her expected date of confinement was July 28, 1947. Her three previous pregnancies had gone to term and her labours had been rapid—the longest three hours. All the children are alive and in good health. Throughout pregnancy she was very well and had no abnormalities of any kind.

Two weeks before her expected date she felt crampy pains which she attributed to indigestion. These persisted for about 24 hours, when they became stronger and were followed by a "show," after which delivery was accomplished in about 50 minutes. The perineum was intact. The third stage was completed in 20 minutes, with a total blood loss of about 10 oz. (284 ml.).