trimming back the lower segment. Mouth-feeding began on the second post-operative day, and convalescence was uneventful.

Case 10.—A female infant was admitted on February 5, 1950, when 2 days old. Pregnancy had been terminated at 38 weeks owing to severe pre-eclamptic toxæmia. Birth weight: 4 lb. 11 oz. (2.1 kg.); on admission 4 lb. 4 oz. (1.9 kg.). The lungs were wet; there was no cough reflex; and she was cyanosed and frothing. The case had been diagnosed elsewhere as "atelectasis of the left lower lobe," and a tracheal catheter had been passed for aspiration. Mucus aspirated from the pharynx was blood-stained. Blood transfusion and operation were performed; the fistula was closed and end-to-end anastomosis done after trimming back the lower segment. Her post-operative condition was satisfactory at first, but the cough reflex never returned, and she died 12 hours later with atelectasis of both lungs. Both bronchi were full of blood clot and mucus.

Conclusions

Congenital atresia of the oesophagus is commoner than is generally realized, the incidence probably being at least 1 in 800 births. Cases are missed clinically or at necropsy because they are considered to have developed pneumonia or to have atelectasis.

Diagnosis is easy once the condition is suspected. An infant that brings up an undue amount of mucus, particularly as a fine froth, for several hours after birth should be suspected of atresia, and such an infant *should not be fed by mouth until the condition has been excluded*. Diagnosis can easily be confirmed by passage of a medium-sized rubber catheter.

Operation holds out good prospects of cure if these cases are diagnosed early and operated upon promptly. Operation is hardly worth attempting unless there is available an anaesthetist with special experience in connexion with thoracic surgery, and preferably one who has made a special study of anaesthesia in infancy.

Ten cases treated by operation are recorded, five of whom have survived.

Continual aspiration of mucus from the mouth and pharynx, both before and after operation, is necessary.

The transpleural approach has been used in all five successful cases.

Closure of the tracheo-oesophageal fistula and restoration of bowel continuity by the simplest form of one-layer end-to-end anastomosis is the operation of choice. Attention to the blood supply of the lower segment should reduce considerably the risk of a breakdown of the anastomosis and its attendant disasters.

Feeding by mouth can be resumed 24 hours after operation, or earlier, and a gastrostomy appears to be unnecessary in the uncomplicated case.

Cases 3 and 7 were operated on by Mr. J. L. Griffith, second surgeon to the South-west Regional Thoracic Unit.

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Revised salary scales have been agreed for senior nursing staff in general and fever hospitals and sanatoria. The new scales are part of the general review of nurses' salaries which has been proceeding for some time, and they have retrospective effect from February 1, 1949. The new scales cover all senior staff in these hospitals except tutorial grades, which are now being considered by the Nurses and Midwives Whitley Council. The scales for senior staff in mental and maternity hospitals are also under consideration.

REVIEW OF ONE YEAR'S WORK IN WATFORD PREGNANCY DIAGNOSTIC CENTRE

BY

H. SCHWABACHER, M.R.C.S.

(From the N.W.M.R.H.B., Group 9 Laboratory, Watford)

The Watford Pregnancy Diagnosis Laboratory at Shrodells Hospital, Willow Lane, Watford, was set up in 1949, and was equipped to undertake the Hogben test for pregnancy. This particular test was chosen because it is more reliable, more economical, and more easily adapted for routine work than any other method.

The main requirement for reliable results by the Hogben method is that the test animals (*Xenopus laevis*) are in good condition before being used. The routine followed at Watford is almost identical to that published by Landgrebe and Hobson (1949). When the animals are thought to be suitable they are first tested by the administration of 70 i.u. of chorionic gonadotropin. Only those which respond are used for pregnancy diagnosis. For this purpose a dry stable extract of chorionic gonadotropin was made by a modification of the Katzman and Doisy method (1931).

Preparation of Extract of Chorionic Gonadotropin

All urines yielding a positive Hogben reaction are pooled and filtered the day after receipt. To every litre of urine (A) (acidified pH 4.5) 50 ml. of saturated benzoic acid in acetone is added by volumetric pipette with a stirring movement. This mixture is allowed to stand in the refrigerator overnight before the supernatant fluid is decanted into another beaker (B). The dry deposit is collected on a Buchner funnel, scraped from the filter paper, and stored under acetone in the refrigerator. Fluid in beaker B is again processed as the urine (A). The dried deposits are pooled—30 litres of urine yielding from 1.5 to 6 g. of powder.

The powder is extracted with three consecutive amounts of 100 ml. of water. To this slightly acid extract (C) are added 3 volumes of 95% alcohol and 1 volume of ether (diethyl ether); it is then left in the refrigerator for 18 hours. After decanting most of the alcohol and ether the remaining extract (C) is filtered on a small Buchner funnel, washed three times with acetone, dried quickly in warm air, placed in ampoules, sealed off, and stored in the refrigerator.

Before attempting the biological standardization, one can estimate the probable amount of chorionic gonadotropin per mg., assuming that the original pooled urine contained 20 i.u. per ml. (It is usual to extract about 80% of the active material.)

This powder is standardized by comparison with the international standard powder, using *Xenopus laevis* as test animal (Landgrebe, 1948), and its potency expressed in international units per mg. The potency varies from 100 to 400 i.u. per mg.

Number of Tests Undertaken in 1949.—Once the stock of *Xenopus laevis* reached 200 tested animals, urines from Hertfordshire were accepted. Specimens from London and the South of England were taken as the number of tested animals increased. In February, 41 specimens were received, while the peak number handled was 522 in October; 3,724 tests were performed throughout the year.

Technique of Test

The chorionic gonadotropin was extracted from the urine according to Scott's method (1940). Half of the eluted gonadotropin—that is, 2.5 ml.—was injected into one toad; the other half was kept at 2° C. overnight and injected into a second toad if the first had failed to ovulate after 18 hours.

Over a series of 103 specimens it had been noticed that, when two toads were injected with the extract on the same day, only in three cases did one toad ovulate without the other responding. Hence it was decided to inject the second toad only if the first had yielded a negative result after 18 hours. On the evidence obtained from the Pregnancy Diagnostic Centre, Edinburgh, about 50% of our tests would be positive, and we were thus able to increase the possible number of tests undertaken with the same number of test animals by 25%. Table I demonstrates that on 55 occasions out of a total of 1,220 tests the *first injected* toad yielded a false result when "read" at 18 hours, in that it had failed to ovulate until 24 to 48 hours, or failed to ovulate at all, whereas the *second toad*, inoculated with the same extract a day later, yielded a positive result.

TABLE I.—*Demonstrating the Need to Inject a Second Animal When the First Animal Failed to React*

No. of Cases	Delayed or Negative Reaction in Toad I		Positive Reaction in Toad II from Identical Extract
	No Reaction After 48 Hours	Positive Reaction within 18 to 48 Hours	Positive Reaction within 18 Hours
55 (4.5% out of 1,220 tested)	35 (63.6%) 2.86%	20 (36.4%) 1.64%	55 (100%) 4.5%

The recording of delayed positive or false negative results is being continued, as it is thought we may have here a means of evaluating the sensitivity of the animals in our colony.

Distribution of Toad Population in the Tanks.—Negative reactors (A) are placed in tanks (A1) and positive reactors (B) in tanks (B1) until there are 50 animals in each tank. These animals are rested for one month. Toads from tank A1 are used for the fresh tests, and are rested in tank A2 or tank B2. All A3 toads (reacting negatively to three injections) are tested by injection of 70 i.u. of extract. We have had to reject 2.7% of our toads. Thus the subnormally reacting toads are continually being excluded from use. "Positive" toads in B tanks are used regularly for the second injection of a negative Hogben reaction.

An Analysis of the Tests Undertaken

We have recently attempted to encourage the doctors to fill in the data asked for on our request forms by refusing to submit the result of the Hogben test until they return

TABLE II.—*Reasons for Submitting Urine for the 3,724 Hogben Tests Performed*

Clinical Condition	
Abortion	12.6%
Hydatidiform mole	5.7%
Abnormal implantation	1.9%
Tuberculosis (pulmonary)	1.5%
Assumed to be normal pregnancies	39.6%
Menopause	3.2%
Fibroid or tumour	3.2%
Unmarried mother	0.35%
Testicular carcinoma	6.4%
Hormonal amenorrhoea (i.e., obesity, anaemia, etc.)	0.0%
Heart lesions	15.3%
No information given	100.00%

the amended form. We now receive a wealth of information.

In Table II are shown the reasons for which the urines were sent to our laboratory for test.

Confirmation of Laboratory Findings

Printed cards, asking whether the Hogben reaction agreed with clinical findings, are posted two months after the Hogben test is carried out. This enables the practitioner to report to us with certainty.

False positive Hogben reactions do not occur. The animal cannot ovulate unless it is injected with the active substance. When, at our request, the doctors were asked to investigate the laboratory's two false positive reports, it was elicited that the patients had concealed their abortion (Table III, D).

TABLE III.—*Interesting Cases*

Group	Evidence			
	Histological Report	Ref.	Hogben Test	Doctors' Confirmation
A. Testicular	Teratoma Seminoma Malignant	557 562 2,560	— — +	
B. Pregnancy with menstruation	Confinement May Menstruation August to October	2,159 2,728	+1/10 +1/10	Pregnant
C. Pregnancy with delayed Hogben reaction	L.M.P. 10 weeks	1,099	—	X-ray foetal parts present
	" 12 "	1,291	—	
	" 20 "			
	L.M.P. 8 weeks	796	—	
	" 10 "	1,163	—	
D. Pregnancy	" 11 5/7 weeks	1,360	+	Neg. reinvestigated; patient had aborted Neg. reinvestigated; patient had aborted
	L.M.P. 5 weeks	Edinburgh	—	
	" 9 "		—	
	" 11 "	2,151	+	
		891	+	
		1,386	+	

False negative Hogben reactions may occur when the chorionic gonadotropin in the urine is deficient because the urine has been sent too early, or the urine is not a concentrated specimen—that is, of too low a specific gravity—or if the patient is a poor secretor or excretor, or destruction of the chorionic gonadotropin by an unknown factor has occurred.

Owing to the small number of tests undertaken and to the insufficient confirmatory returns in 1949, figures for the evaluation of the accuracy of the work at Watford will be pooled with this year's results and published in the report for 1950.

Some interesting cases are recorded in Table III. There has been only one case of testicular carcinoma: this we were able to confirm (Group A). Group B represents one patient in whom we were able to allay the fear of malignancy and predict a second conception within three months of delivery of a first baby. The three cases in Group C in which the Hogben test became positive two months after probable conception are examples of failure to send early morning specimens of urine of high specific gravity.

Case of Hydatidiform Mole

This case is recorded (Table IV) by permission of Professor F. J. Browne.

According to dates, this patient was 8 to 10 weeks pregnant when first seen, yet the uterus showed a four-months pregnancy. The uterus failed to increase in size during the four weeks of the investigation.

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

BY

J. F. D. FRAZER, B.M., B.Ch.

AND

F. X. WOHLZOGEN, M.D. Vienna

(From the Department of Physiology, St. Mary's Hospital Medical School, London)

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