

years with Dr. Dochez in attempts at the experimental transmission of colds. We were successful in a large proportion of cases, tried not only with chimpanzees but also with human beings. It is true that we did not have the air conditioning but we had thermostatic control. There is one question which I should like to ask which is rather interesting in the light of Dr. Kerr's statement that one has no chilling, under the conditions of his experiments, and therefore gets no colds. It is a fairly well known fact that explorers who have gone to the north pole for as long as two or three years, in spite of exposures to cold, to fatigue, and to practically every type of elemental exposure, never get colds. The minute they hit civilization, where colds are present, back come colds again.

A NEW BIOLOGIC TEST FOR HORMONES IN PREGNANCY URINE

PRELIMINARY REPORT

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CHICAGO

It has been known that male fish of certain species undergo color changes during the breeding season and develop what is known as the "wedding dress." These chromatophore reactions have been developed artificially by stimulation with various hormone preparations.¹ Following the same principle it was thought that analogous reactions might be obtained on female fish of species that have an externally visible oviduct. Tozawa² found that males of the Japanese bitterling (*Acherlognathus intermedium*) developed a chromatophore reaction to hormones injected only during the breeding season, April 15 to July 1. In 1932 Fleischmann and Kann³ reported that female bitterlings show an enlargement of the ovipositor following injection of an estrogenic preparation (Progynon) in doses of from 40 to 120 mouse units, while physiologic extract of sodium chloride and an anterior pituitary extract yielded no reaction. These authors claim a lengthening of the oviduct with estrogenic substance during the quiescent period. In 1933 Szusz⁴ tested female bitterlings with boiled and unboiled urine from pregnant women, male urine, estrogenic substance (Progynon and Hovigral), and anterior pituitary extract (Glandulin), 10 cc. of each to 1 liter of water in which the fish are placed. During the breeding season forty-eight fish were tested and all showed lengthening of the ovipositor in from thirty-six to seventy-two hours with both the boiled and the unboiled urine. Six fishes tested with anterior pituitary extract showed no changes. Urine from twenty nonpregnant females gave twelve positive and eight negative reactions. Male urine caused some lengthening in seven of the thirty-seven tested, greater lengthening in twenty-two instances, and no reaction in eight. This author tested twenty-two fish

from July 1 to August 15 and all gave negative reactions. Szusz does not know what is responsible for the reaction. He concluded that full lengthening of the ovipositor takes place in seventy-two hours, that the test can be positive only during the breeding season, that male urine may cause a positive reaction, and that a negative reaction during the breeding season is absolutely indicative of the absence of pregnancy.

The senior author felt that the possibilities of the female bitterling test deserved further investigation. After sporadic attempts at testing on the various problems associated with this research, the results became so confusing that it was felt tests could not be conducted along scientific lines until the fish had been standardized; i. e., it was necessary to know in advance whether each fish was capable of responding positively to urine from a known pregnant woman and that the same fish would not react to the urine from a patient who was not pregnant. Although a great number of tests have been carried out, only those run subsequent to Aug. 1, 1934, at which time our supply of fish was standardized, are included in this report. In this process of standardization it was found that some fish did not respond to pregnancy urine because of immaturity or stunted size, while others of apparently mature size were refractory to the active principle for some unknown reason. Because of the number of fish that fell in this classification, it is imperative that this standardization process be carried out. After a positive test it requires from fourteen to twenty days for the ovipositor to return to its normal length, during which time the fish cannot be used for testing purposes.

TECHNIC

A previously standardized fish is put in a two quart bowl which contains one quart of water at the proper temperature, about 75 F. The fish is observed to make certain that the oviduct is not beyond normal limits. Four cc. of the urine to be tested is put into this water. The fish is observed at twenty-four hour intervals. If the test is found to be positive after the first twenty-four hours the test is discontinued; if negative, it must be carried out for seventy-two hours before a definitely negative report may be given, in spite of the fact that about 80 per cent of our positives were positive at the end of the first twenty-four hours. Normally the oviduct is about 2 mm. in length and reaches less than half the distance to the end of the ventral fin. With a positive reaction the ovipositor reaches past the edge of the ventral fin or to a length of 15 to 25 mm. After the test is completed the fish is put into a tank for recovery and left for from two to three weeks in order to allow for the regression following the positive reaction. It may then be used for other tests.

RESULTS

At the outset it was deemed advisable to run the fish tests parallel to Friedman tests in order to determine the relative merits of the two. The laboratories of the Presbyterian and Mount Sinai hospitals graciously cooperated with us in this work by giving us urines and reports on their Friedman tests. To date we have thirty-one such tests, with twenty-seven absolute checks and four discrepancies between the two tests. The four cases in which there were differences of result are as follows:

1. An ectopic pregnancy, ruptured, proved at operation. Fish test positive, Friedman test negative.

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2. Woman, aged 51; three months amenorrhea with subsequent menstruation. Fish test positive. Friedman test negative.
3. Patient four days beyond expected date for menstruation. Sterile five years. Fish test negative. Friedman test positive. Pregnancy still improved. Later fish test positive.
4. Patient four days past expected menstruation. Fish test negative. Aschheim-Zondek test (commercial laboratory) negative. Six days after expected menstruation, fish test negative, Friedman test positive. Twelve days after expected period, fish test negative. Histologic studies of curettage material revealed the presence of a very early pregnancy.

There have also been tests run on five ectopic pregnancies proved at operation, which have all been positive but which had not been checked against the Friedman test.

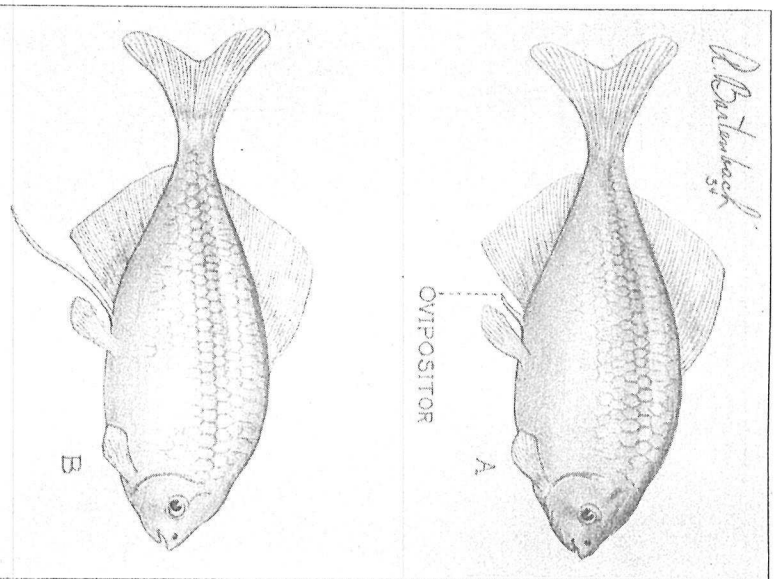
COMMENT

We believe that we have a test for some element which exists in excess in the urine of pregnant women, most probably estrogenic substance. From a study of the literature and its correlation with our observations such

Our results will probably be more consistent and better classified when we have developed our technic both for handling the fish and for the actual carrying out of the tests. This test will prove of clinical value with advantages over the Aschheim-Zondek and the Friedman test. The fish are primarily cheaper than rabbits or mice, are easier to maintain because of the small amount of space, food and care required in their handling, and at the end of the test it is not necessary to operate on the experimental animal and destroy it, as the fish may be used repeatedly for testing purposes. Another point in favor of this test is the fact that most positive results are obtained in twenty-four hours, while the other tests must run from forty-eight to seventy-two hours.

Should our predictions prove correct, the fields of research that may be opened by such a test are virtually boundless. To cite a few problems that we contemplate attacking as soon as our supply of test animals becomes sufficiently numerous: quantitative determinations for estrogenic substance at periods of physiologic and pathologic variation (menopause, puberty, various stages in the menstrual cycle, so-called functional amenorrheas, menorrhagias and metrorrhagias, and feminizing and defeminizing tumors); quantitative tests in hydatidiform mole and chorionepithelioma; tests for the presence of excess hormones associated with fibroid tumors and malignant conditions; methods of assay for the retention of potency and standardization of commercial hormone products; tests for the differential diagnosis of ectopic pregnancy. It is very important that we determine at an early date the time during pregnancy when the positive test first appears and the time during the puerperium when the test becomes negative.

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Japanese hatching (*Acheilognathus intermedium*): *A*, normal, or negative, test; *B*, positive test.

a conclusion can be reached. First, the authors referred to all had uniformly positive results with the use of estrogenic hormones and fairly consistent negative results with anterior pituitary extracts. Boiling destroys the gonadotropic factor of pregnancy urine, yet boiled urine gave results identical to those obtained with unboiled urine. At certain times in the life of a woman there is a physiologic excess of the follicular hormone, particularly during the onset of the menopause, and we take the liberty of citing case 2 as being illustrative of this, explaining the negative Friedman test on the basis that that test is dependent on the presence of an excess of anterior pituitary-like gonadotropic hormone rather than estrogenic substance. Several tests that we have carried out with some commercial products have given positives with estrogenic preparations and negatives with gonad-stimulating products.

Clinical Notes, Suggestions and New Instruments

SUPERIOR LARYNGEAL NEURALGIA RELIEVED BY OPERATION

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True neuralgia of the superior laryngeal nerve is a distinct clinical entity comparable to trigeminal and glossopharyngeal neuralgia. It is characterized by paroxysms of unilateral, lancinating pain, which radiates from the side of the thyroid cartilage to the angle of the jaw and sometimes to the ear. There is a trigger zone at the plica of the nerve in the pyriform sinus. The pain is ordinarily initiated by swallowing but can be produced by touching the trigger zone with a probe. The other objective finding is a pressure point on the skin surface just above and lateral to the thyroid cartilage, where the sensory branch of the nerve pierces the hyothyroid membrane. Between the seizures, which may last from a few seconds to a minute, the patient is free from discomfort. As in other cranial nerve neuralgias, the attacks usually appear after middle age and increase in frequency and intensity. As a rule there is no disease of the pharynx or larynx present. Treatment directed at a coexisting tonsillitis or laryngitis is as futile as the extraction of teeth for the douloureux. Superior laryngeal neuralgia should not be confused with the more or less constant pain due to a malignant condition or tuberculosis of the larynx and epiglottis. The laryngeal crises of tabes must also be ruled out. It is most important to differentiate it from glossopharyngeal neuralgia, in which syndrome the pain radiates to the ear from the tonsillar region, which is the trigger zone.

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