THE ROUTINE DIAGNOSIS OF PREGNANCY, HYDATIDIFORM MOLE AND CHORIONEPITHELIOMA USING FEMALE XENOPUS LÆVIS.

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INTRODUCTION

The University of Edinburgh Pregnancy Diagnosis Laboratory (P.D.L.) is nearly twenty-five years of age. The first of many progress reports was published in 1930 by Professor F. A. E. Crew, who was the director and initiator of the laboratory. Eight hundred and forty tests were done in the first year. From this modest beginning has grown an organisation capable of dealing annually with some 25,000 tests. It is calculated that almost 300,000 tests have been performed since 1930, 160,000 of them since 1946. Despite the fact that there are now two Diagnostic Centres in England, the P.D.L. still relies on England for more than two-thirds of its tests. The majority of the Scottish tests come from the South Eastern Region closely followed by Glasgow and the west. Practitioners send in rather more than half the Scottish total, the remainder coming from hospitals, maternity homes and clinics. You will realise that it is one thing to provide a service and quite another thing to get support for it. The early success of the laboratory was in no small part due to the encouragement given by Professor Johnstone, whom I am pleased to say still takes a friendly interest in the unit. Collaboration with the Department of Obstetrics and Gynaecology under Professor Kellar and his colleagues has done much to make us feel that we are something more than an organisation providing a routine service.

It is rather more than twenty-seven years ago since Aschheim and Zondek described the first reliable pregnancy test. In the intervening years more than 2,500 papers have been published on the subject. This considerable literature has repeatedly emphasised that the most reliable tests are those using the rabbit, rat, mouse and some species of amphibia. Due to their lack of precision, other procedures such as skin tests, enzyme reactions, biochemical methods and the like have for the most part failed to gain wide recognition. Before going on to describe the type of biological test used in our laboratory it will be helpful to consider briefly the fundamental physiology upon which it is based.

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Hormonal Basis of Biological Pregnancy Tests

Basically, all the successful pregnancy tests consist of methods utilising laboratory animals for detecting the high levels of gonadotrophin produced during pregnancy. Hence, they rest upon two fundamental facts; that during pregnancy there is an increase either in the production or the excretion of gonadotrophin; and that gonadotrophin produced by the human female will effect demonstrable changes in lower animals. Several hormones are increased above normal levels during pregnancy, but we are only concerned with one of them, namely, chorionic gonadotrophin (C.G.). The name implies that this substance is produced by the chorion and "feeds" or stimulates the gonads. The term chorionic hormone was used by Hamburger (1933) for the gonadotrophin of pregnancy because of its occurrence when living chorionic tissue was present. Evans (1935) suggested the name "chorionic gonadotropic hormone" or "chorionic gonadotropin" to distinguish it from pituitary gonadotrophin. Although the term chorionic gonadotrophin is widely used, available evidence supporting the chorion (a fetal structure) as the source of placental gonadotrophin is doubtful (see Burrows, 1949). Soon after implantation of the fertilised ovum chorionic gonadotrophin is excreted. This appears in the urine at first in small quantities, gradually increasing in amount until a peak is reached about 7th-10th week of gestation. Following the peak there is a very rapid fall to lower levels, this level usually being maintained throughout pregnancy. Occasionally negative results are obtained in the last three months of pregnancy; the more usual picture is for the test to be positive right up to parturition. Chorionic gonadotrophin disappears from the urine twenty-four to forty-eight hours after complete expulsion of the products of conception (Crew, 1936). Because chorionic gonadotrophin is most often found in the body fluids of pregnant women these biological tests are called "pregnancy tests." This term is apt to be misleading because it implies that to get a positive result a foetus must be present. A positive reaction is dependent on the presence of placental tissue and the excretion of detectable amounts of gonadotrophin, and not upon the presence of a foetus. Positive "pregnancy tests" are found in the absence of a foetus in the female when hydatidiform mole or chorionepithelioma is present. In the male certain testicular tumours will also produce a positive response. It would be better to regard these tests as detecting the presence or absence of chorionic gonadotrophin, remembering that this hormone is most usually produced during pregnancy but not exclusively so.

The Test Animal

In this laboratory all estimations are done upon the female South African clawed toad Xenopus laevis; this is generally known as the Hogben test. The toad is imported from South Africa and is kept under
laboratory conditions for several months before use. The test is based on the fact that the female Xenopus does not ovulate unless stimulated by the male or is injected with chorionic gonadotrophin. Female toads injected with a sufficient amount of gonadotrophin react positively by extruding eggs within four to twenty-four hours.

**Performance of the Test**

It is usual laboratory practice to ask for a first morning specimen of urine because specimens obtained later in the day are often much diluted. For routine testing it is necessary to insist that the specimens are not collected until ten to fourteen days after the first missed but expected period. Failure to observe this does result in some false negatives.

Before injection the specimen must be prepared and concentrated. There are two reasons for this: (1) the toad is less sensitive than the mouse to chorionic gonadotrophin and more C.G. is required to produce a positive reaction in the toad than the mouse. Therefore unless urines are concentrated false negatives may result. (2) Crude urine is very often toxic to toads and may result in their death. For these reasons a simple extraction method is used. The urine to be tested is acidified to pH 4.0 with dilute hydrochloric acid. To this is added a suspension of kaolin upon which the gonadotrophin is adsorbed. C.G. is extracted from the kaolin by adding dilute sodium hydroxide. A concentrate of 5.0 ml. is produced which is equivalent to 60 ml. of urine. After neutralisation this is ready for injection into the toad. Half the concentrate (2.5 ml.) is injected into the test animal which is then placed in a container and examined about eighteen hours later. As stated previously the toad will deposit eggs within four to twenty-four hours after an injection of pregnancy urine. The speed of the reaction is related to the amount of gonadotrophin injected and the temperature at which the animal is kept whilst under test. Reading the test at eighteen hours after injection is merely convenient and has no special significance. A result is accounted positive when one or more toads lay eggs; recognition of the response does not necessitate killing the toad, so that test animals can be used repeatedly. If, however, no oviposition occurs in eighteen hours a second toad is injected with the remaining 2.5 ml. of the same extract. If both toads are still negative forty-eight hours after the beginning of the test the result is recorded as negative.

**Interpreting Results**

The number of eggs a toad lays in response to a positive injection is related to the amount of chorionic gonadotrophin present in the urine. Positive results can be graded and this may occasionally provide additional information when considered in conjunction with the clinical diagnosis. For example a weakly positive or negative biological reaction, from what appears to be a normal pregnancy, may be the first sign that
all is not as it should be. In a series of 223 cases of confirmed abortion the Hogben test was negative in 75, weakly positive in 29 and positive in 119. Thus in 46 per cent. of tests, the negative and weak positive results provided an informed guess about the state and outcome of the pregnancy (Mathew and Hobson, 1953). It must not be thought that even recurring negative or weak positive results mean the end of a pregnancy. We have on record 4 cases which at no time during pregnancy gave a positive result yet went to full term. It is suggested that these patients produced C.G. in the normal way but that the hormone was destroyed or inactivated, and the products of excretion were biologically inactive.

**Accuracy**

The most essential feature of any biological test for pregnancy is its accuracy and specificity. It is important for clinicians when making a diagnosis that the result of a "pregnancy" test is not misleading due to a low degree of accuracy or lack of specificity. Female Xenopus, unlike mice, are insensitive to menopausal concentrates and only lay eggs when injected with the gonadotrophin of pregnancy. Although we have not discussed the Aschheim Zondek test, since it is no longer used by this laboratory, it is useful to compare the accuracy of the mouse test with the toad test. The figures quoted are based on tests done between January 1949 and June 1951. During this period some 15,000 Aschheim Zondek tests were done in which a correct result was given in 99.5 per cent. of cases and 37,000 Hogben tests with an accuracy of 99.8 per cent. There are no false positive Hogbens, and false negatives are mainly from specimens sent too early after the missed but expected period or not being first morning specimens. On the other hand false positive results are sometimes obtained with the mouse, although many of these may be due to the spontaneous maturation of the test animal's ovaries (Hobson, 1951).

**Dilution Tests**

In addition to performing many thousands of "pregnancy tests" annually we do about 1,000 dilution tests. Dilution tests are usually requested when a pregnancy is not behaving normally and a hydatidiform mole or chorionepithelioma is suspect. Dilution tests are essentially the same as pregnancy tests except that the urine or concentrate is diluted with distilled water before injection. Dilutions in common use are 1/10 and 1/100. In our laboratory if a test is positive in a dilution of 1/100 further dilutions are done until no positive response is obtained. The rationale of the dilution test as a means of differentiating between a pregnancy, a mole or epithelioma is due to the observations of Zondek (1929) and Aschheim (1930) that women with hydatidiform moles excrete abnormally large amounts of chorionic gonadotrophin. Unfortunately this was followed by a statement (Zondek, 1931) to the effect that a level of 50,000 mouse units or more
of gonadotrophin per litre of urine, an amount normally sufficient to produce a positive reaction when injected in a dilution of 1/100, was suspicious of either a mole or chorionepithelioma. Despite evidence to the contrary, the impression created was that urines from patients with a mole or epithelioma would always give a positive "pregnancy test" in dilution of 1/100; a view still held by some clinicians and pathologists. Because experience showed that this was far from being true, the use of dilution tests as a means of differentiating between pregnancy, hydatidiform mole and chorionepithelioma declined. It is now well established that weakly positive and even negative reactions may be obtained with undiluted urines from such cases (Hobson, 1952). Work done in this laboratory shows that a positive reaction in a dilution of 1/100 or more is given by 48 per cent. of molar cases, 35 per cent. of chorionepitheliomas and 6 per cent. of normal pregnancies.

There is no doubt that the greatest caution must be exercised when interpreting the results of dilution tests. Blindly accepting a positive result in a dilution of 1/100 or even 1/150 as evidence that a mole is present has led to the termination of several pregnancies. Positive reactions in high dilutions obtained after the fourteenth week of pregnancy are much more reliable, the peak excretion of gonadotrophin in normal pregnancy having passed. It is the experience of this laboratory that the greatest contribution dilution tests can make is in the follow-up of a case after the removal of a mole or epithelioma. A positive result will not indicate whether the level of gonadotrophin is rising or falling unless several tests are done. It must be emphasised that a single post-operative estimation, though better than nothing, may do more harm than good. This will be particularly true when the first post-operative test is negative, indicating that viable chorionic tissue is no longer present. This is not always so and in some cases merely denotes a period of building up of new tissue until enough is present to secrete a detectable amount of gonadotrophin. The new tissue is often far removed from the original nidus; the lungs, liver, bone and brain being favourite sites for the development of secondaries.

These growths are often difficult to control and may result in the death of the patient. From our own data on the post-operative excretion of gonadotrophin cases of hydatidiform mole and chorionepithelioma, it would seem advisable to do weekly tests for the first month followed by a monthly test for the next six months. In a series of more than 70 moles the time taken for the test to become negative ranged from six to 129 days after removal. Of the 15 cases of chorionepithelioma so far investigated, 9 of these developed after a previous mole. If, in such cases, use had been made of the biological test it would have been possible to show an increasing gonadotrophin output, indicating without a doubt that chorionic tissue was present somewhere in the body. This allows for some form of treatment to be introduced at a much earlier date than if clinicians have to wait for the
appearance of unmistakable clinical signs. It is not unreasonable to suppose that an earlier diagnosis of choriocarcinoma would affect the ultimate outcome.

**Summary**

All the reliable biological tests for pregnancy detect chorionic gonadotrophin. Chorionic gonadotrophin is not only produced during pregnancy but also, often in large quantities, when a hydatidiform mole or choriocarcinoma is present. Weakly positive or negative tests may be used to predict an impending abortion. Generally speaking dilution tests must be regarded as only providing additional information when attempting to differentiate between pregnancy, hydatidiform mole and choriocarcinoma. The greatest use of dilution tests after the removal of a mole is advocated. In the majority of cases these tests will indicate the presence of retained or new chorionic tissue in the body long before the condition is detectable clinically.

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