

A CRITICAL EVALUATION OF RAT HYPERAEMIC PREGNANCY TEST

by

MEERA AIKAT*, D. Phil., M.A.M.S.

The difficulties in the diagnosis of early pregnancy prompted the discovery of a large number of biological, biochemical, chemical and immunological tests. Of all the biological tests Aschheim-Zondek, Friedman, Hogben, Rat Hyperaemic and Male Toad tests are better known and have been extensively used for the diagnosis of pregnancy.

Friedman and Aschheim-Zondek tests are well recognised for their reliability. However, they are costly and require a well-organised animal colony for the regular supply of rabbits and mice to fulfil some of the essential pre-conditions regarding the test animals.

Male Toad test is claimed to give fairly accurate results, is easy to perform and is less expensive. In the hands of the author the test was observed to be unreliable, with a high percentage of false negative results along with a wide seasonal variation.

The present study was undertaken to assess the usefulness of Rat Hyperaemic test, a modified Aschheim-Zondek test, introduced by Berman as a routine pregnancy test. Using this test Farris obtained positive re-

sults in all his cases even as early as the 34th day of gestation.

Material

The Rat hyperaemic test was undertaken in the following obstetrical studies:

- (1) Early suspected pregnancy.
- (2) Bleeding per vaginam after a period of amenorrhoea.
- (3) Hydatidiform mole.
- (4) Follow-up studies after evacuation of mole.
- (5) Follow-up in suspected cases of chorio-malignancy.

Methods

Morning specimen of urine was obtained in a clean vessel and filtered. Urine was preserved at 4°C when not in use. Five ml. of urine was injected subcutaneously on the dorsum of two immature female albino rats of local strain, weighing between 40-50 gms. The injection was repeated after four hours. Sixteen hours after the first injection, animals were killed with coal gas and ovaries were examined. The presence of generalised hyperaemia or a large red follicle even in one ovary was taken as positive.

In cases of hydatidiform mole, in addition to undiluted urine, a second set of animals was injected with urine diluted to 1 in 800.

*From the Department of Pathology, Institute of Post-graduate Medical Education & Research, Chandigarh, Punjab.

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In confirmed cases of chorio-malignancy, the highest titre giving positive result was determined. In the subsequent follow-up period, the test was repeated once in two months.

Observations

1. *Early suspected pregnancy.* The test was performed in 350 cases. In 180 cases, pregnancy was confirmed clinically. In the remaining 170, clinical follow-up and/or curettings confirmed absence of pregnancy. False positive result was observed in three cases of the latter group. Of the three, two were women in premenopausal age (42 and 44 years respectively) and the third had a large uterine myoma. The following Table summarises the result in the confirmed cases of pregnancy at different periods of gestation.

It will be evident that the test was positive in over 98 per cent of cases of pregnancy.

2. *Bleeding per vaginam after a period of amenorrhoea.* There were 102 cases, and in this group also the percentage of accuracy was about 99 per cent. In the negative cases, only one curetting showed chorionic tissue. Table II summarises the result of this group.

3. *Hydatidiform mole.* Out of 42 cases studied, negative results for pregnancy were obtained in two. They were subsequently confirmed as cases of ovarian cyst and of tubo-ovarian mass. Test was positive in all confirmed cases of hydatidiform mole (100%). In 14 out of 40 cases the test was positive in dilution 1/800. Table III summarises the result:

4. *Follow up study after evacuation of mole.*

It will be evident that positive results were observed in seven cases 20 weeks after evacuation. Endometrial

TABLE I
Results in Proved Cases of Pregnancy

Total No. of cases	35-45 days of gestation			46-70 days of gestation			Lactation amenorrhoea			Accuracy
	No. of cases	+	-	No. of cases	+	-	No. of cases	+	-	
180	56	55	1	120	119	1	4	4	-	98.92%

TABLE II
Result of the Test in Cases of Bleeding per Vaginam after varying Periods of Amenorrhoea

Total No. of cases	60-90 days of amenorrhoea				91-above days of amenorrhoea				Accuracy
	No. of cases	+	-	False -	No. of cases	+	-	False -	
102	98	64	34	1	4	4	-	-	99.2%

TABLE III
Result in Confirmed Cases of Mole

No. of cases	Undiluted urine +	1/800 dilution +	Accuracy
40	40	14	100%

TABLE IV
Follow-up Study after Evacuation of Mole

Total No. of cases	Weeks after evacuation					
	Negative in weeks					Positive after 20 weeks
	1-2	3-4	5-8	9-12	13-16	
40	2	6	13	4	8	7

curettage was done in all seven cases. Only in two cases, test was positive after a month of the second curetting. Uteri were removed in both the cases. One proved to be a case of chorio-adenoma destruens and the other chorio-carcinoma, who developed pulmonary metastases.

5. *Follow up in suspected cases of chorio-malignancy.* There were seven cases in this group. In five it was preceded by a molar pregnancy, in one after abortion and in another after normal delivery. The last case presented itself for the first time with a metastasis in the vagina.

Case No. 1

P₅+₁, aged 35 years, was a case of molar pregnancy. She continued to have positive test in undiluted urine five months after evacuation. She was re-admitted after a bout of haemoptysis and pulmonary metastases were detected radiologically. Hysterectomy was performed and chorio-carcinoma was confirmed histologically. Even after hysterectomy the test remained positive till she received 100 mg. of Methotrexate.

Case No. 2.

P₃+₀, aged 32 years, was admitted with vaginal bleeding for two months after molar evacuation. The test was positive in 1/400 dilution. X-ray chest revealed multiple metastases. Hysterectomy was performed and chorio-carcinoma was confirmed histologically. The urine became negative, after 150 mg. of Methotrexate.

Case No. 3.

P₇+₀, aged 38 years, had a persistent positive test even after seven months of evacuation of mole. She had no other complication. Uterus was removed and histological examination revealed chorio-adenoma destruens. Ten days after operation the test was negative.

Case No. 4.

P₂+₁, aged 31 years, had irregular bleeding per vaginam after evacuation of mole. The test continued to be positive in undiluted urine even after second curettage. Hysterectomy was performed and a small invasive growth was seen in the uterus which was diagnosed histologically as chorio-adenoma destruens. The test was negative 10 days after the operation.

Case No. 5.

P₄+₁, aged 28 years, was admitted with bleeding per vaginam of three

months' duration after an abortion. The test was positive in 1/800 dilution. No metastases were detected. Curettings showed abnormal trophoblastic activity. Total hysterectomy was performed. A haemorrhagic invasive growth was confirmed to be chorio-carcinoma. Test became negative two weeks after removal of the uterus.

Case No. 6.

P₁ +₀, aged 22 years, was admitted with a history of irregular bleeding per vaginam. Routine examination revealed a haemorrhagic growth in vagina and an enlarged uterus. She gave history of a live birth four years prior to onset of symptoms. Test was positive in 1/400 dilution. The vaginal tumour and the uterus were removed and a diagnosis of chorio-carcinoma was confirmed histologically. She continued to have positive test and only after 200 mg. of Methorexate the test became negative. However, she was readmitted after a month with metastases in liver and the test was found to be positive.

Case No. 7.

P₁ +₁, aged 27 years, was admitted in poor general condition with bleeding per vaginam since one year after expulsion of mole. Uterus was 12 weeks' size and x-ray of chest revealed metastases in both lungs. Test was positive in 1/800 dilution. She expired on the third day of admission. At autopsy, chorio-carcinoma was confirmed histologically.

Discussion

The Rat Hyperaemic test was found to be reliable in diagnosis of early pregnancy, hydatidiform mole and in suspected cases of chorio-malignancy. Frank and Berman, Farris and Gillissen obtained 100% results using different strains of albino rats. In the present series the test was positive in 98.8 per cent of cases of confirmed pregnancy. False positive was recorded in two women

in their pre-menopausal age of 42 and 44 years respectively. It is likely that the test animals were sensitive to excess of pituitary gonadotropin in these two cases.^{1,3,7} In the third positive case, there was a large uterine myoma. False positive result has also been reported in myoma due to high level of oestrogen.

The test was found to be a very reliable index in molar pregnancy, with amenorrhoea of 8 weeks or more. Titres of 1/800 in such cases appear to be of diagnostic significance, since this was recorded in all such cases. A titre 1/500 was seen in a case of twin pregnancy. A persistent test even with undiluted urine after evacuation of mole, should be regarded as a warning signal. In one case where chorio-carcinoma was ultimately confirmed, a decrease in titre was observed even when there were pulmonary metastases. Persistence of positive titre with undiluted urine after the fifth month of evacuation appears to have same significance as a rising titre. A titre of 1/400 or more, one month after evacuation of mole, abortion or delivery should also be regarded as danger signal. This test can be used to follow up cases treated with cytotoxic drug.

Summary

1. The Rat Hyperaemic test, using local strain of albino rats, was found to be a reliable test for diagnosis of pregnancy in approximately 99% of cases.
2. It is equally sensitive in cases of suspected abortion.
3. Positive result in dilution of

1/800 or more is almost diagnostic of molar pregnancy.

4. The test can be a useful parameter for the diagnosis of chorio-malignancy in suspected cases and is particularly useful in the follow up cases of abortion and molar pregnancy.

5. It can also be utilised to assess the effect of cytotoxic drug in the treatment of chorio-malignancy.

This test is simple and can be undertaken even with limited facility.

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