QUANTITATION OF HUMAN CHORIONIC GONADOTROPHIN IN THE MANAGEMENT OF HYDATIDIFORM MOLE

(A Review of the Literature and a Summary of the Singapore Experience with 254 Cases)

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Shortly after the introduction of the Aschheim-Zondek test it was shown that abnormal trophoblastic tissues such as hydatidiform mole and choriocarcinoma were also capable of producing chorionic gonadotrophin. Zondek (1929) found that urine from patients with hydatidiform mole was still capable of giving a positive test after it had been diluted 200 fold. Since such a high titre had never been previously encountered in normal pregnancy, Zondek concluded that it was diagnostic of hydatidiform mole. Almost simultaneously, Roessler and his chief, Robert Meyer (Roessler, 1929) reported a high concentration of urinary gonadotrophin from a single terminal case of chorion-epithelioma. Subsequently their contemporaries applying the test to their own advanced cases of chorionepithelioma confirmed this finding, and for a while it was thought that only a strongly positive biological test was diagnostic of this malignant condition. Mathieu (1939) corrected this erroneous attitude when he pointed out that the quantity of chorionic gonadotrophin produced was related to the amount and vitality of the trophoblast, and it is now well recognised that early choriocarcinoma might produce such low titres of hormone as to give a negative biological pregnancy test.

THE DIAGNOSIS OF HYDATIDIFORM MOLE

A wide range of procedures have been recommended for the investigation of a suspected mole, among which may be mentioned: urine gonadotrophin excretion, serum gonadotrophin titre, vaginal cytology, plain abdominal X-ray, retrograde aortogram, amniocentesis, amniogram, the probe test, ultra sound and placental biopsy. With the exception of the gonadotrophin assay methods, none of these procedures have combined safety with accuracy.

For a few years after Zondek's report of high urinary gonadotrophin excretion in hydatidiform mole, it was believed that the pregnancy test would provide an accurate method for the diagnosis of the disease. Zondek himself repeatedly tried to establish the dictum that a gonadotrophin titre above 200,000 mouse units per litre of urine was diagnostic of hydatidiform mole. If in addition, the pregnancy test was positive with the cerebrospinal fluid, especially in dilution, the diagnosis was indubitable (Zondek, 1937). Erhardt (1931) reported two cases with exceptionally high titres and Aschheim (1931) believed that "values of more than 100,000 mouse units per litre of urine point to the diagnosis of hydatidiform mole with great probability". De Geus (1935) contended that in doubtful cases it was safer to rely on the Aschheim-Zondek test for diagnosis than on the clinical findings. Schuller (1939) thought that if the Aschheim-Zondek test was positive with a 20 times diluted urine there was a 90 per cent probability that the woman had mole.

The demonstration of a transcient rise of gonadotrophin titres in early pregnancy (Browne and Venning, 1936; Evans et al., 1937) and the discovery of high titres in toxaemic pregnancy (Smith and Smith, 1934, 1935) eventually led Zondek to introduce two provisos in the interpretation of a high HCG concentration in urine. They were that (i) the diagnosis of hydatidiform mole was only possible when at least 200,000 mouse units per litre was found on repeated examinations, or when an increase in titre actually occurred; and secondly (ii) only if toxaemia of pregnancy could be excluded (Zondek, 1942). Because of the frequent association of toxaemia in molar pregnancy, to uphold the second proviso, would mean that the pregnancy test would be indecisive in exactly those cases where hydatidiform mole should be diagnosed on clinical grounds.

In the meantime, several workers reported negative tests in some of their cases of hydatidiform mole (Lowenstein; 1929; Phillip, 1931; Koehler, 1935; Remmelts, 1935; Chamorro, 1936; Brews, 1939; Digonnet, 1939). To explain this, Mathieu (1939) coined the term "missed molar abortion", a condition similar to missed

abortion where death of the chorionic tissue was responsible for the negative biological tests. Brindeau (1935) similarly spoke of a "dead mole".

However, there are some workers who do not accept this explanation and who contend that HCG assays are of no assistance whatever in the diagnosis of hydatidiform mole. Ruzicska (1936) stated that the hormone levels in molar pregnancy were often much lower than those found in many publications, and he thought that high titres were the exception rather than the rule. Genell (1946) also found that his patients with molar pregnancy did not have HCG titres above those found in normally pregnant women. So did Hamburger (1944) and Smalbraak (1957).

These differences in opinion stem from various causes—small series, inaccurate tests, unstandardised techniques, the failure to correlate the findings with the period of amenorrhoea, the inclusion of aborted cases and most important of all, the failure to appreciated the vagaries of urine investigation. As Smith and Smith (1938) have pointed out, random or early morning urine has no quantitative significance.

In the two studies with serum (Payne, 1941; Delfs, 1957) it was found that all patients with intact hydatidiform mole (totalling 29 patients) had titres of HCG which were in excess of the amount produced in normal pregnancy at the corresponding period of amenorrhoea. The serum test is therefore much more reliable than random urine assays, and if a high serum titre is maintained longer than a week, it is especially diagnostic. Multiple pregnancy may show an initial abnormally high titre but this falls to the normal range within a few days just like in normal pregnancy (Delfs, 1957).

Unfortunately the Delfs' uterine weight gain method requires the maintenance of large animal colonies and is expensive and time consuming. Perhaps for this reason the test has not enjoyed popularity outside her own department and it is not quite suitable for centres in the Far East where large numbers of moles are seen and where there are insufficient laboratory facilities and a limit to the funds available.

Recently a number of cheap, simple and accurate immunological methods have been introduced for the quantitative assay of HCG (Brody and Carlstrom, 1960; Wide and Gemzell, 1960). However, Wide (1962) found that immunological tests measure a broader spectrum of "gonadotrophins" than biological tests, and this reopened the question of the usefulness of the pregnancy test (this time an *immunological* pregnancy test) in the diagnosis of hydatidiform mole and its eventual follow-up.

In a study of 600 patients with normal pregnancy in whom the serum HCG was assayed with the haemagglutination-inhibition test, it was found that there was a peak of gonadotrophin production in the first trimester where up to 480,000 I.U. per litre may be found (Teoh, 1966). In a similar study of 30 patients with intact hydatidiform mole (Teoh, 1967a) the range of HCG titres was found to vary between 320,000 I.U. per litre and 1,920,000 I.U. per litre, i.e. the titre was always high. Before the 14th week of amenorrhoea, there was some overlap with the high titres found in normal pregnancy (Fig. 1) but after the 100th day of amenorrhoea, a titre of 320,000 I.U. per litre was 2.5 times higher than the highest level found in normal pregnancy beyond this time. Twins showed normal titres, and although toxaemic patients tend to have high titres the levels were within the normal range. The immunological assay of serum can therefore be employed to distinguish between a molar and a normal pregnancy.

The haemagglutination-inhibition test was originally designed for urine on which the test can be performed without prior extraction unlike serum. It was decided to test whether a simpler urine test could be used instead. In a comparison of 4 hourly urine and serum estimations, it was found that whereas the serum titre remained constant throughout 24 hours, the urine concentration showed wide fluctuations (Fig. 2). Urine assay was thus found to be unsuitable (Teoh, 1967a).

THE FOLLOW-UP OF HYDATIDIFORM MOLE

It has been known for a long time that choriocarcinoma most frequently arises from hydatidiform mole and this has been re-emphasised by several recent publications (Hobson, 1955; Chun et al., 1964; Tow, 1964). In Singapore, Tow (1964) found that 26 out of 200 cases of hydatidiform mole developed malignant sequele.

Before the introduction of the pregnancy test, the diagnosis of choriocarcinoma usually rested on the presence of profuse uterine bleeding, extensive metastases, and the discovery of choriocarcinomatous tissue in the

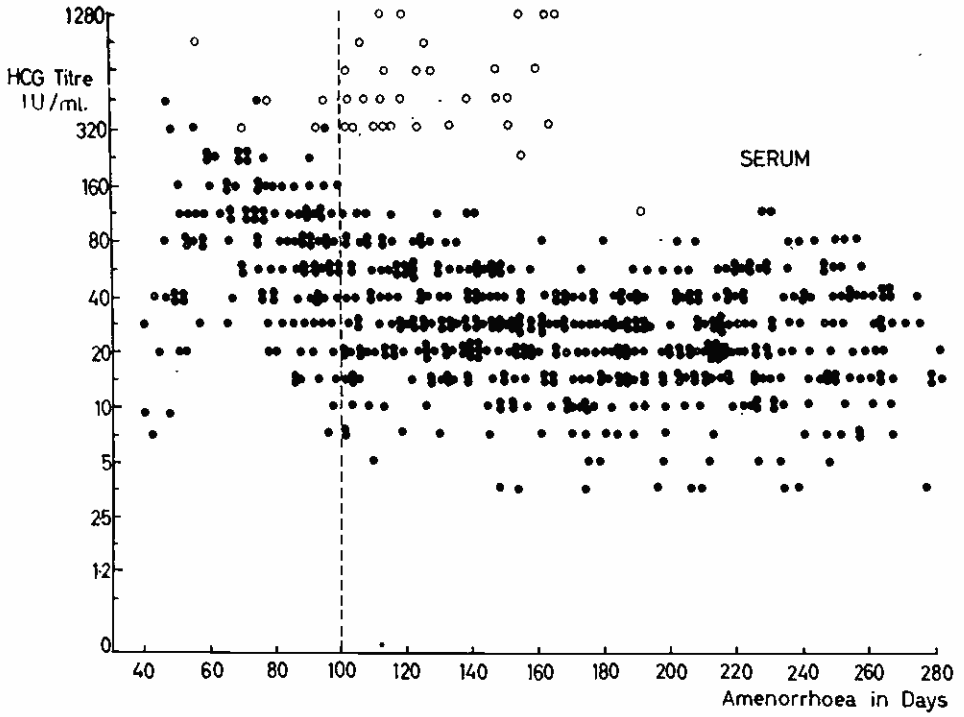

Fig. 1. Immunological HCG activity in the serum of 600 cases of normal pregnancy (black dots) and 37 patients with hydatidiform mole (clear circles).

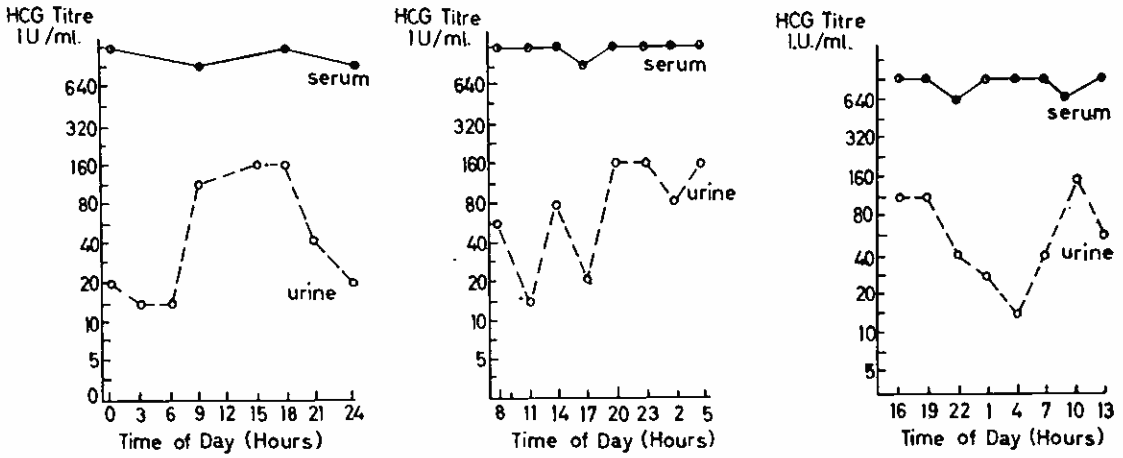

Fig. 2. Parallel assays of the immunological HCG activity in serum and urine samples collected from 3 patients with hydatidiform mole show wide fluctuation in the urine titres while the serum titres remained constant.

curettings. To ensure a cure in all cases, early diagnosis is imperative and this is dependent on the detection of HCG during the follow-up of patients who have had hydatidiform mole. Assays of either urine or serum for HCG therefore constitute an essential part in the long term management of all patients with hydatidiform mole.

The immediate post-evacuation follow-up of hydatidiform mole poses 4 important questions:

1. What is the interval between evacuation of a mole and the complete disappearance of the hormone from the tissues of the patient?
2. At what stage does a positive test indicate remnant molar tissue?
3. When does it indicate the presence of choriocarcinoma?
4. Is a persistent negative test a guarantee against malignant trophoblastic disease?

THE INTERPRETATION OF POSITIVE PREGNANCY TESTS

The early workers who employed biological tests noted that gonadotrophin persisted in decreasing quantities for two months following a mole and if a test was still positive after the second month following evacuation, it was an indication of trouble (Mathieu, 1939). A repeat curettage will remove remnant molar tissue and if a positive test were due solely to an incomplete evacuation, the test would rapidly become negative. When choriocarcinoma is the cause of the positive pregnancy test, a small growth in the uterine cavity may also be removed by curettage and result in the pregnancy test becoming negative. The correct diagnosis is provided by histological examination. More frequently, the growth is situated outside the uterine cavity and in such cases the HCG titre would be unaffected by the repeat curettage. The occurrence of a positive pregnancy test after a repeat curettage calls for cytotoxic chemotherapy. If the uterus is enlarged, hysterectomy should be considered.

However, a number of investigators have obtained positive tests up to a year after removal of the mole (Mack and Catherwood, 1930; Delfs, 1957), and the patients concerned have remained well. For this reason, conservation of the uterus was stressed even in the face of positive tests after 2 months, provided there was no rise in the titre. This attitude has failed to gain support because workers with wide experience have seen too many cases of choriocarcinoma with low or even negative titres. (Chan and Pang, 1964; Tow, 1966).

Eighty-two patients with hydatidiform mole were followed with the H-I test at the Kandang Kerbau Hospital to find out when the test would become negative following the evacuation of the mole. In the women who recovered with primary treatment alone, the test took an average of three weeks to become negative. In the majority of cases the test was negative by the end of the fourth week (Figs. 3, 4, 5). In 17 patients the test was still positive after the end of the fourth week, and of these 12 were found to have choriocarcinoma. In some patients a subsequent rise in titre was encountered probably due to an active proliferation of the trophoblast, but in others the titre merely remained a low positive beyond the normal period of 4 weeks (Figs. 6, 7, 8).

In the local experience, a second curettage is indicated if a rise in titre interrupts the fall following evacuation, or if the test remains positive beyond the fourth week. The efficacy of the repeat currettage and the cytotoxic chemotherapy that is usually instituted will be shown by the drop in HCG titre leading event-

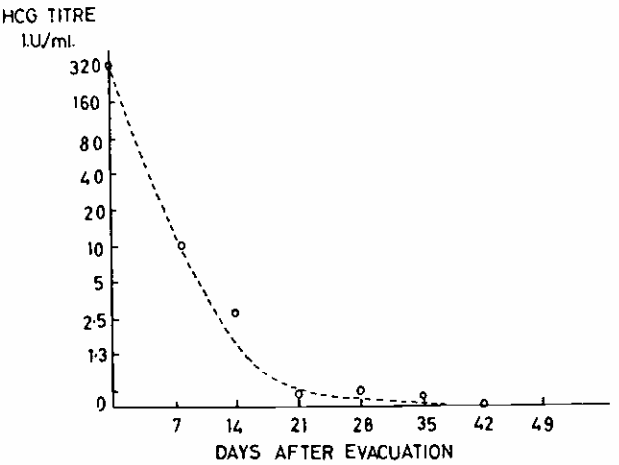

Fig. 3. The rate of disappearance of HCG in benign hydatidiform mole.

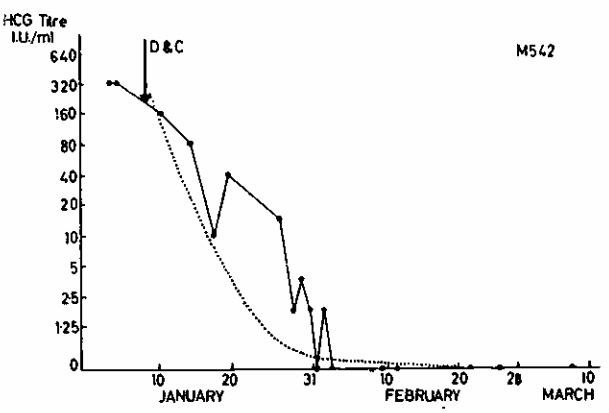

Fig. 4. Post D & C titres of HCG in benign mole.

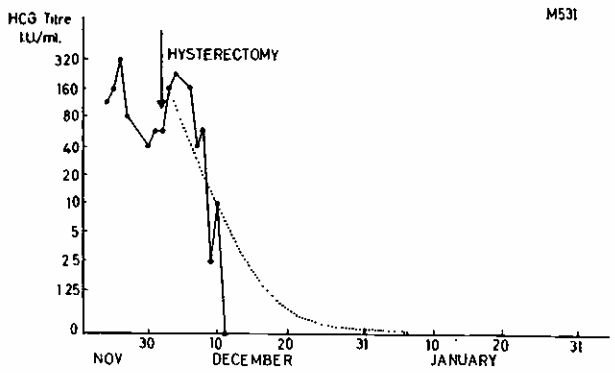

Fig. 5. Post hysterectomy titres of HCG in benign mole.

ually to negative tests (Figs. 6, 7, 8). The quantitation of HCG can thus be further used as an index of cure. Persistence of HCG in the serum or urine, especially a rise in the titre after treatment, is an ominous sign.

The commonest cause of a positive assay after a period of negative tests is pregnancy, but in two of the 254 patients studies, it was due to a late development of choriocarcinoma. Unfortunately the assay does not distinguish the two conditions. The two patients concerned already had a hysterectomy so that ruled out pregnancy and after chemotherapy the assay soon became negative. They are both well.

DURATION OF FOLLOW-UP

The risk of choriocarcinoma in patients with hydatidiform mole is greatest in the first two years following the mole, and in order that the maximum benefit may be derived from following them with HCG assays, they should be put on an oral contraceptive so that a possible pregnancy will not complicate the diagnosis of choriocarcinoma when this disease is suspected. After two years, the risk still exists but becomes considerably smaller, and many workers are of the opinion that the patients need not be followed beyond this time. In the present studies, 13 patients developed choriocarcinoma in the first year, 3 in the second, one in the third and none between the fourth and sixth years. Delfs (1959) and Hobson (1955) did not encounter any patient with choriocarcinoma developing more than two years after a mole in their large follow-up study. Tow (1966) in Singapore has seen a case of choriocarcinoma where the mole-malignancy interval was 13 years, and in another the interval was seven years. These are exceptional cases and the early detection of the two cases would necessitate the follow-up of more than a thousand patients a year, considering that an average of 80 new cases of mole are seen annually. Given adequate facilities this can be done, but when the facilities are limited the most good will be achieved by limiting the care to possibly three years, each patient to be carefully screened at monthly intervals.

CONCLUSION

From the Singapore experience, it has been shown that the quantitation of HCG by the haemagglutionation-inhibition test of Wide and Gemzell is a simpler, faster, cheaper and more

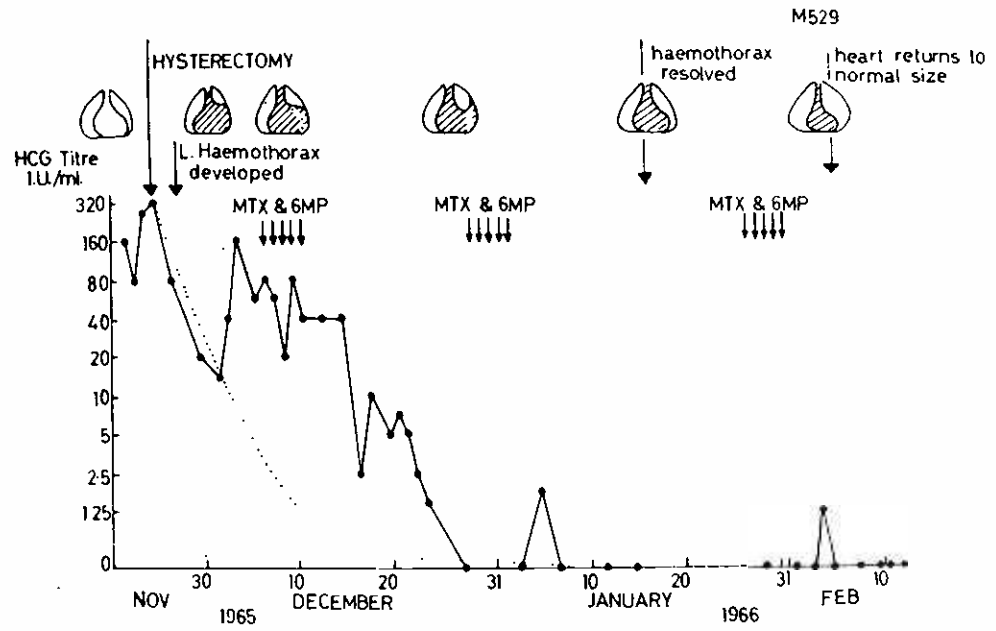

Fig. 6. Prolonged Excretion of HCG in a patient with concurrent choriocarcinoma accompanying hydatidiform mole.

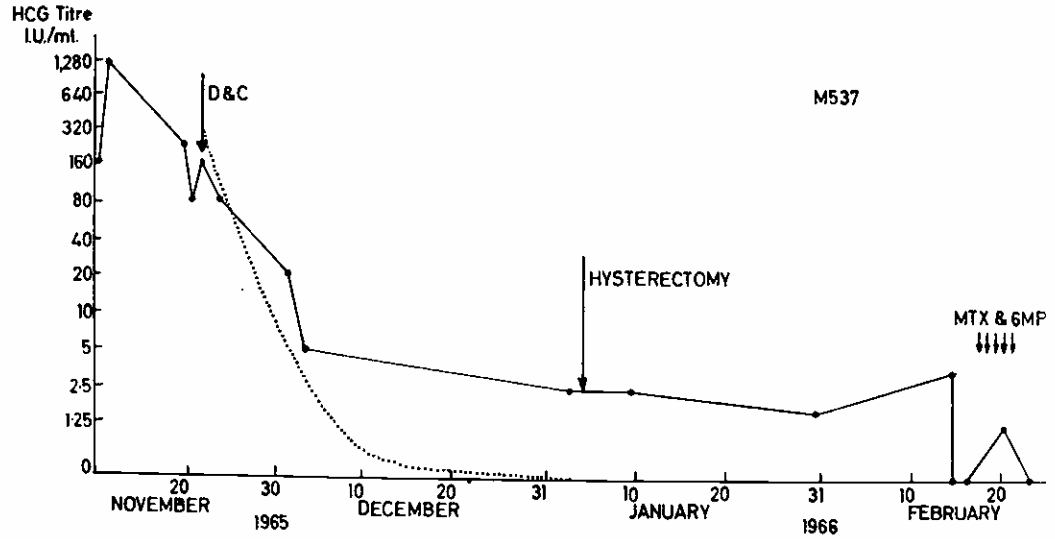

Fig. 7. Persistence of HCG in a patient with choriocarcinoma. Note the response to chemotherapy.

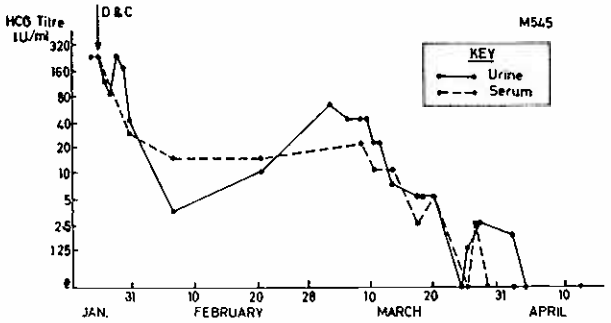

Fig. 8. Another pattern of HCG production in a patient with choriocarcinoma. Note the rise in titre after the initial fall.

accurate procedure than the biological pregnancy tests. The assay of serum in a patient suspected of hydatidiform mole may assist the diagnosis, a titre of 320,000 I.U. per litre after the 100th day of amenorrhoea being diagnostic of the disease. The test is also extremely useful in the follow-up of patients with mole. It will indicate remnant molar tissue by remaining positive beyond the fourth post-evacuation week. A positive test after a period of negative tests and in the absence of pregnancy again provides the diagnosis of choriocarcinoma.

Although a persistent negative test is not an absolute guarantee against malignant trophoblastic disease, the prognosis is good as long as the test remains negative. If choriocarcinoma is present, sooner or later, a positive test will be produced.

The recovery of all the patients who received prompt treatment following the appearance of a positive H.I. test demonstrate that, provided the patients are frequently screened, the H.I. test is sufficiently sensitive to detect a case of choriocarcinoma in time for effective therapy to produce a cure.

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