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PROSTIGMINE AS A DIAGNOSTIC TEST  
OF PREGNANCY

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## INTRODUCTION

The clinical use of prostigmine (neostigmine) as a diagnostic test of pregnancy has appeared in the medical journals relatively recently. In this paper I shall report the findings in all the available articles and also present my suggestions of a new clinical procedure involving the use of prostigmine in the diagnosis of early pregnancy before other signs and symptoms of the condition present themselves.

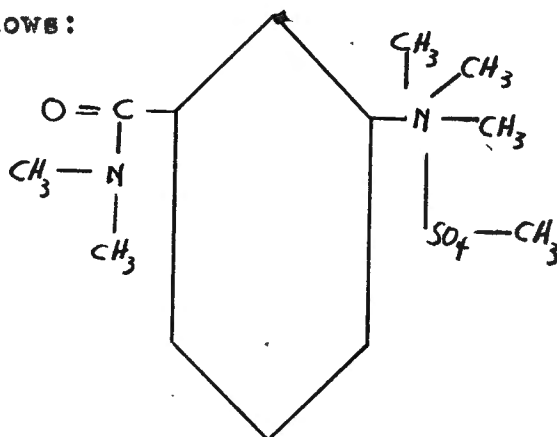
## HISTORY OF PROSTIGMINE

Prostigmine is a synthetic alkaloid belonging to the physostigmine group of drugs and is similar to but ten times as strong as ganeserine which is derived from the calabar bean. Prostigmine was first introduced into therapeutics in 1931 for its stimulant action on the intestinal tract and four years later it was found to be effective in the symptomatic therapy of myasthenia gravis (Goodman and Gilman);.

## CHEMISTRY AND PREPARATION OF PROSTIGMINE

The drug is available only in the form of its methylsulfate or bromide salt (Hoffmann-La Roche).

The structural formula of the compound in its methyl salt is as follows:



di-methylcarbamic ester of 3-hydroxy-phenyl-trimethyl-ammonium methyl sulfate

The salts are white crystalline powders which are odorless, bitter and freely soluble and stable when in aqueous solution.

#### TOXICOLOGY OF PROSTIGMINE

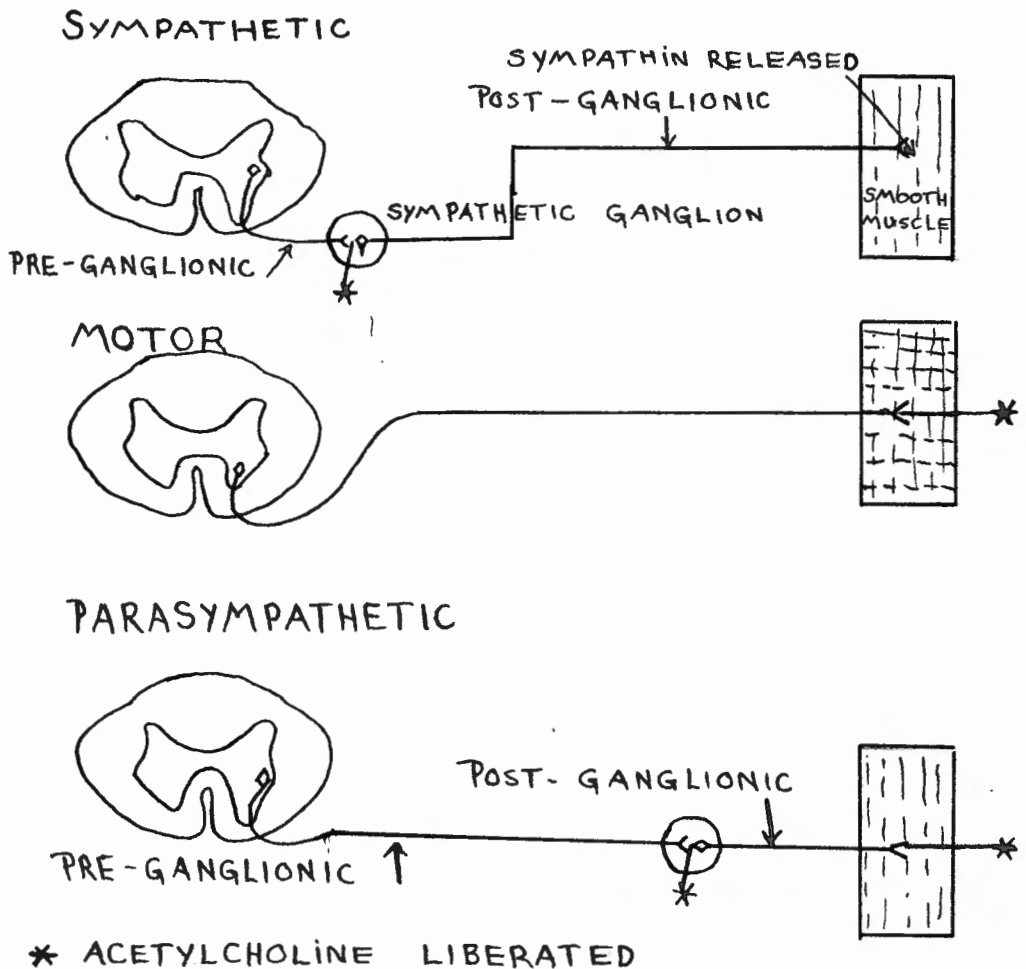
The signs and symptoms of prostigmine poisoning (usually due to an excess of the drug) are mostly those of extreme shock; spasticity of the bowels with nausea and vomiting, colic, generalized muscle twitching, restlessness, weakness, pin-point pupils, nystagmus, rapid, thready pulse, fall in blood pressure, and ashen-grey skin with perspiration. Death may ensue within one-half to two hours after symptoms are first noticed. Treatment consists of intramuscular or intravenous adminis-

trations of atropine sulfate milligrams two.

Prostigmine is readily absorbed via the gastro-intestinal tract, subcutaneous tissues and mucous membranes. It is destroyed partly in the liver but the major part is excreted by the kidneys at the rate of one milligram in two hours.

#### PHARMACOLOGICAL ACTION OF PROSTIGMINE

The relationship between the chemical structure and the action of the compound was pointed out by Stedman and his collaborators and confirmed by Aeschlimann and Reinert. The methylcarbamic ester group  $\text{C H}_3 \text{NHC O O}$  is the essential feature determining the activity of physostigmine, prostigmine and all related compounds. As to the actual mechanism, so far as is known at present, it is the same for the whole group of drugs. That is, they produce their effects by inhibiting the action of esterase which normally hydrolyzes and inactivates acetylcholine. This, of course, allows the acetylcholine to produce its effects at the places where it is physiologically released by nerve impulses. This is true with very few exceptions. Following is a series of schematic drawings which depict the points of liberation of acetylcholine.



#### THERAPEUTIC USES OF PROSTIGMINE

From the diagrams and previous explanations one can readily understand the basic pharmacology for the following major applications of the drug:

1. Stimulation of the gastro-intestinal tract in intestinal ileus.
2. Treatment of atony of the urinary bladder.
3. As symptomatic treatment of myasthenia gravis.
4. To initiate menses in simple, non-complicated, functional amenorrhea.

5. As a diagnostic test of pregnancy.

A REPORT OF THE LITERATURE ON PROSTIGMINE  
AS A DIAGNOSTIC TEST FOR PREGNANCY

It was in 1939 that Reynolds published his paper which confirmed the experiments of Markee and Pompen who had concluded that the administration of estrin to ovariectomized rabbits caused hyperemia of the uterus beginning sometime between a few minutes and an hour after injection. This phenomenon was found even after severance of all central nervous system connections with the uterus. The acetylcholine content of the uterine tissue was found to be increased in proportion with the hyperemia.

Hechter, Soskin and Lev conducted important research which acted as the basis for most of the work in this field. Due to its importance the article will be discussed at length. Their results in experiments with laboratory animals showed that the effects of estrin may be divided into two groups; one, those specific actions of estrin which do not depend upon hyperemia and two, those actions which are secondary to the production of hyperemia, and which are associated with the acetylcholine liberating action of estrin. Distention seemed to be greatly dependent upon hyperemia, since the affect

was inhibited by atropine and duplicated by the action of yohimbine. The accumulation of tissue water appeared to be due mainly to the specific action of estrin, although hyperemia played a role in this water accumulation. What was perhaps the most important clinical application of their results was the fact that such a large proportion of the estrous phenomenon in animals was dependent upon hyperemia. "It may be assumed that the role of hyperemia is probably even more important in the phenomenon of periodic menstrual bleeding in the human female. Since the hyperemia is presumably also under the influence of at least the parasympathetic nervous system, it is not difficult to understand the well-known profound influence of mental, emotional and physical strain upon the menstrual history of women. It is possible that certain cases of primary amenorrhea and other functional disturbances which are now generally ascribed to endocrine deficiencies may in fact exist despite normal hormone secretion and be due to an abnormal decrease or increase in vascular responsiveness." They concluded that, if this be so, our therapeutic approach in such cases of functional amenorrhea might better be along pharmacological rather than along endocrine lines.

Almost simultaneously, Soskin, Wachtel and Hecter



applied the above theory that menstrual bleeding depended upon the role of uterine hyperemia in normal females. Following very careful control studies on rabbits and rats, volunteer human females were selected for the experiments. Because prostigmine was able to produce the hyperemia of the uterus by allowing acetylcholine to be liberated at the parasympathetic nerve endings in the uterus, it was chosen as the drug rather than estrin or acetylcholine ( see PHARMACOLOGICAL ACTION OF PROSTIGMINE). One to two cubic centimeters of a 1:2000 dilution was injected intramuscularly once a day for three days; discontinuing injections if flow resulted before three days elapsed. They found that with the exceptions stated below, the women invariably menstruated from within one-half to seventy-two hours (averaging about twenty-eight hours) from the time of the last injection. The women who did not flow with this routine were; first, those with a history of marked past menstrual irregularities; second, those with local pelvic pathology; third, those with clinical endocrinopathies; fourth, those in the menopause, and fifth, those who were pregnant --- regardless of the length of time elapsed after their first missed period. In their experience the drug did not alter the course of pregnancy at any time. It presented no

toxic manifestations in the amount given. No undesirable side effects were recorded. Within the limitations above set forth the test proved to be exceptionally accurate. It was a logical conclusion that the test might be used in the diagnosis of pregnancy if the other exceptions were ruled out.

For all practical purposes, all students of this topic used about the same procedures in their experiments. The subjects were females who entered the offices or clinics complaining of amenorrhea. A history was taken to determine previous menstrual difficulties and a physical examination was done with special attention being paid to endocrine disorders, pelvic pathology and pregnancy. Each woman who was given the prostigmine injections gave a sample of urine. A parallel series of Friedman tests were run as confirmation. Whenever possible, "follow-ups" were carried out, especially if pregnancy was suggested. These cases were confirmed later by absolute signs of pregnancy. Settel duplicated the results of Soskin et al. and stated that the use of prostigmine is an excellent test because it is both therapeutic and diagnostic at the same time. That is to say, if flow results, the patient not only has the assurance of the physician that she is not preg-

nant, but observes her own menstrual flow, which is proof beyond question to the mind of a concerned female. With thirty patients between the ages of nineteen and forty-six, Grossmann concluded that, with the known exceptions, this test was most accurate and had no undesirable side effects. Parrella reported the same results on sixty cases in one series and two hundred in another. Douglas reported on sixty-two cases, Carapetyan on fifty-seven cases, Corber on thirty-four cases and Sneider on twenty-seven. They all essentially agreed and confirmed the findings of Soskin, Wachtal and Hechter. The common opinions of these authors, all of whom were in favor of this test were: the test is accurate, effective, easy, safe, and productive of a psychological satisfaction if the patient flows. Each experimenter found the limitations to the test to be those enumerated earlier in this paper. Douglas suggested the logical theory that the parasympathetic nervous system plays a larger part in simple amenorrhea than was formally believed and states as his proof the number of patients who respond to the prostigmine administration by having menses. Grossmann, in another series of one hundred cases, found that with the selection of his patients so as to exclude the groups of women who will

not respond to the test (see the above limitations) the procedure was one hundred per cent accurate in the diagnosis of pregnancy. It was better than the results of Friedman tests run on the same patients at the same time. Lately, Natenshon is quoted as saying, "It is often difficult to diagnose pregnancy even at two or three months because of such factors as obesity, malposition of the uterus, etc., and thus this test can be an ideal office procedure performed with great ease, safety, economy and accuracy."

Some authors do not advocate the prostigmine test as a good diagnostic test of pregnancy. However, these men are in a definite minority, at least as far as the reports in the literature are concerned. Also, the basic features of their findings agree with those of the other authors mentioned above. That is, following about the same procedures, they produced almost identical results and each confirmed that the limitations given earlier were correct. It is on these limitations that the camps of authors are divided. Some men conclude that, with these restrictions to the test, it cannot be of practical clinical value. Greenhill said, "Since a negative" (positive) "test" (no flow) "does not prove pregnancy, because of the possibility of endocrine

dysfunction, pelvic pathology, history of past menstrual difficulties or menopause, the test would have to be confirmed by physical examination, if possible, or biological laboratory tests (as the Friedman)."

Winter, with four cases, Winklestein with ninety cases, Wiesstien with sixteen cases, and Weisman with no cases, all agree with Greenhill that the test is of little value in diagnosing pregnancy because of its limitations as to the patients.

#### COMPARISON WITH OTHER TESTS OF PREGNANCY

Although there are many laboratory tests for pregnancy, either the Aschheim-Zondek, or one of its modifications, is accepted almost universally as the best. It therefore behooves one to compare a contemporary test with the already accepted one. The Friedman modification is probably the procedure in most common usage today. It entails the injection of urine from a pregnant woman into a vein of a non-pregnant female rabbit. The ovaries of the rabbit are examined after forty-eight hours and if corpora lutea or corpora hemorrhagica is observed the test is considered positive. Most errors in the Friedman test are due to technical difficulties, factors which are usually out of the hands of the doctor, since most

physicians do not perform their own tests. However, in the prostigmine procedure the difficulty is in the selection of patients. This can be controlled by the physician. Errors in the handling of the urine specimen, difficulties and expense in obtaining the proper rabbits, the obvious difficulty of preventing the animals from becoming pregnant, the space and expense in housing, feeding and caring for the rabbits, the inconvenience of having the animals become sick or die and the chance of error in the interpretation by the technician are some of the more obvious objections to and reasons for mistakes in the Friedman procedure. As for the prostigmine test, all of these difficulties are avoided. It is an examination that can be carried out in the office with little expense involved on the part of either the patient or the physician. One cubic centimeter ampules of prostigmine retail at three dollars a dozen. On patients who flow, the diagnosis of non-pregnancy is indisputable. As to those who do not flow, a diagnosis of pregnancy has been proven to be exceedingly accurate where the patients were properly chosen. On the other hand, it is true that the Friedman test has been proven to be ninety-seven to ninety-eight per cent correct where the technical obstacles were properly controlled.

It gives false positives only in a small number of cases as when the female is taking estrogen or has productive growths such as chorioepithelomas and hydadiform moles. It is thus indisputable that where the diagnosis is positive, the Friedman test is more accurate.

#### SUGGESTIONS FOR A NEW CLINICAL PROCEDURE

If prostigmine is a good test of early pregnancy, why hasn't it been accepted? I believe this is because it has previously been considered only as a substitute for the Friedman test. I agree, it would seem impracticable to replace the already successful, universal and accurate Friedman test with a test of limited applicability, but that does not finish the problem. It is most surprising that there appears to be no reported suggestions as to the advisability of the use of the two tests in conjunction.

I suggest the following procedure to be used for women with amenorrhea in whom no definite signs or symptoms can be found as to its etiology.

- A. Inject one cubic centimeter of a 1:2000 dilution of prostigmine intramuscularly once a day for three days, terminating injections if menstrual flow results.

- B. Instruct the patient to return to the office seventy-two hours after the last injection, if menses is not theretofore produced.
- C. Order a Friedman test run on a sample of her urine if she has not menstruated at the end of the seventy-two hour period.

A possible modification would be to have the patient leave a sample of her urine on the day of the last injection with instructions to call the office if she flows within seventy-two hours.

The normal limitations to this procedure would usually resolve themselves into cases where the cause of the amenorrhea was discovered in the examination of the patient or where the physician is reasonably certain that the prostigmine test would be ineffective or was otherwise inadvisable.

The usual indications for following this routine is with a patient concerning whom the etiology of the amenorrhea is not found upon examination. Examples of some of these are early (1-2 month) pregnancies, sub-clinical endocrine disorders, questionable beginning of menopause and the inability to demonstrate pelvic pathology.

There would seem to be five major advantages to the



adoption of this suggested procedure: first, it would often save money; second, in cases where the patient flowed, it would be an important treatment as well as a diagnosis; third, in cases of pregnancy it would constitute a worthwhile check on the Friedman test; fourth, in cases of a discrepancy in the results of the two tests the physician would usually obtain an otherwise unavailable but important indication that the cause of the amenorrhea was not merely functional; and fifth, it would very frequently have an advantageous effect upon the physician-patient relationship.

Whenever a laboratory test for pregnancy is indicated or desired the following of my suggested procedure is economically advisable. The cost of three prostigmine ampules is only seventy-five cents. The cost of a Friedman test to the physician is normally seven dollars and fifty cents. Even if only one-half of the patients flowed, eliminating the necessity of a Friedman test, the average savings to the doctor and his patients would be considerable. Of course, there is more time consumed in my suggested procedure than in simply taking a urine specimen. Nevertheless, the physician's time consumed in the original visit would be essentially the same in both tests and there is no real reason that the

office nurse could not handle such subsequent visits as proved to be necessary.

If the patient is not pregnant, the resumption of menstrual flow is unquestionably of great psychic benefit. The very fact that she flows, after having missed a period, is proof of an underlying functional disturbance which needed correction. Some functional disturbances, such as fear of pregnancy, would actually be cured, and even in more complex functional disturbances, reestablishment of menstruation would be a beneficial symptomatic treatment. Its advantages are not restricted to psychological therapy. There are some cases when it would be a physiological treatment, as in a sterility problem. If the patient so desires pregnancy that she has functional amenorrhea, the reestablishing of her periods is necessary before the clinical routine of aiding conception can be continued.

The errors in the Friedman test are almost solely the result of technical difficulties. In the prostigmine test when used singly as a test for pregnancy, the only false results occur when the patients are not properly chosen. It follows, that when the two are combined as suggested, and both yield positive results, the diagnosis of pregnancy would be practically infallible.

If under the present established procedure, the physician has been unable to find any etiology for his patient's amenorrhea and yet she yields a negative Friedman test, the physician would probably assume a functional disorder. In reality, it is always possible that the patient could have unascertained endocrine disorders, pelvic pathology, or menopause. That such a patient should be discharged merely as a functional problem, could lead to an unfortunate situation. The suggested procedure would be advantageous in this connection. If the prostigmine failed to produce a flow and yet the Friedman test was negative, the physician would have the immediate indication that the amenorrhea was not functional, even though theretofore he had failed to find signs or symptoms of endocrine or organic pathology.

The suggested procedure would very frequently have an advantageous affect upon the physician-patient relationship. I am not at all referring to any imagined benefit. The physician would have carefully explained to his patient that prostigmine would in no way interfere with pregnancy. The woman who did not desire pregnancy and flowed with prostigmine would obtain great psychological relief which would often show itself in the

physician-patient relationship. Also, it would avoid the embarrassing situation of telling a patient (on the basis of a Friedman test) that she was not pregnant when she had flowed in the meantime.

The disadvantages to the suggested procedure are mainly those due to the length of the patient's time which is consumed. It would sometimes require three daily visits, a seventy-two hour wait for results and then another forty-eight hour wait for the results of the Friedman test. Also, there is always the remote possibility of the patient who, refusing to accept non-pregnancy, would transfer the responsibility of her menses to the doctor even though the test had been carefully explained.

#### CONCLUSIONS

Normally, ovarian hormones produce uterine hyperemia by increasing its acetylcholine content. Acetylcholine causes vasodilatation. The suddenness of decrease of ovarian hormones produces relative ischemia which causes a sloughing of the endometrium. Prostigmine causes an increase in acetylcholine by inhibiting acetylcholine-esterase and its sudden withdrawal produces the same effect as in normal menstruation. With certain limitat-

ions, prostigmine has been found to be a good diagnostic test of pregnancy. Because of such limitations, it is my opinion that it would be a poor substitute for the Friedman test. I believe that by using it in conjunction with the Friedman test it has real value.

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