# PARA-AMINOSALICYLIC ACID WITH STREPTOMYCIN IN TUBERCULOSIS*

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THIS is a preliminary report upon the clinical and laboratory findings at the Toronto Hospital for Tuberculosis, Weston, in a group of patients receiving concomitantly para-aminosalicylic acid (P.A.S.) orally and streptomycin intramuscularly for pulmonary tuberculosis.

Following the interest of Dr. J. C. McClelland, Consultant in Urology, in chaulmoogra oil with streptomycin for renal tuberculosis, the possibilities of combined therapy were discussed with Dr. Philip Greey, Professor of Bacteriology, University of Toronto. After consultations with Merck and Co., Dr. Greey suggested that we investigate the value of P.A.S. in conjunction with streptomycin. The study began in March, 1948, and is continuing.

The early work with P.A.S. as an agent inhibitory to the growth of tubercle bacilli *in vitro* has been reported by others in the literature[1, 2] and will not be further elaborated upon here. Recently, others have made preliminary reports[5, 7] upon the therapeutic and laboratory results of giving P.A.S. in addition to streptomycin to patients under treatment for tuberculosis. These workers report slight, if any, enhanced clinical response from such combined treatment, but draw attention to a delay in the emergence of streptomycin resistant strains of tubercle bacilli among those receiving combined streptomycin and P.A.S. therapy.

In the study being reported, all patients receiving "combined" therapy were placed upon 10 gm. of P.A.S. daily by mouth, or its equivalent as the sodium salt of para-aminosalicylic acid, in addition to 1.0 gm. of streptomycin intramuscularly daily in two equally divided doses. At the beginning of this study, the P.A.S. received in powder form was administered in capsules containing 0.2 gm. of P.A.S. per capsule. Patients received 10 capsules five times daily or a total of 50 capsules containing 10 gm. of P.A.S. daily. Later, a 10% aqueous solution of the sodium salt of P.A.S. was prepared from the P.A.S. powder supplied. This solution was made by adding 120 gm. of sodium bicarbonate to 200 gm. of P.A.S. To this was added 150 c.c. of fluid extract of liquorice, or alternatively, 10 minims of methyl salicylate. In both preparations, solutions were made up to 2,000 c.c. with distilled water. In this manner, solutions of the sodium salt of P.A.S. flavoured with either liquorice or oil of wintergreen were prepared so that 100 c.c. contained the equivalent of 10 gm. of P.A.S. Patients elected to receive one or the other flavouring but all were given 100 c.c. of either solution daily by mouth in three divided doses.

Originally in this study it was desired to determine if the concurrent administration of P.A.S. enhanced the clinical results obtained from the use of streptomycin alone. Alternate patients with exudative tuberculosis considered suitable for treatment with streptomycin were also given P.A.S. The first section of this paper, "Clinical", will deal with the above mentioned cases. The second section of this paper, "Laboratory" will deal with the streptomycin sensitivity of the tubercle bacilli recovered from those patients who received "combined" therapy. To provide material for this latter section (to provide sources of sputum from which tubercle bacilli could be isolated during and after treatment), a number of patients with chronic fibrocaseous pulmonary tuberculosis were given streptomycin and P.A.S.

## CLINICAL

For the comparison of clinical results among patients treated by sanatorium routine, streptomycin and P.A.S. as compared with those receiving sanatorium routine and streptomycin, we have eliminated those who received any other concurrent treatment such as collapse therapy. Also eliminated were any who had received a previous course of streptomycin, and any who required interruption of P.A.S. because of side effects. We have for clinical study 34 patients with pulmonary tuberculosis who received "combined" treatment and where the combined course of streptomycin and P.A.S. had been completed before November 10, 1949. The clinical results among those 34 patients are compared with the results among a similar number who received only sanatorium routine and streptomycin. By clinical results is meant those apparent at the termination of the course of antibiotic treatment under consideration. Comparison of the

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clinical results at any significant period following the cessation of the course of antibiotic therapy would be unreliable because of the wide variety of other treatments applied following the completion of the course of treatment under study.

TABLE I.

| *Pulmonary tuberculosis* | *Min.* | *Mod. adv.* | *Far adv.* | *Total* |
|---|---|---|---|---|
| No. of cases on streptomycin alone | 8 | 15 | 11 | 34 |
| No. of cases on streptomycin plus P.A.S. | 5 | 17 | 12 | 34 |
| Total | 13 | 32 | 23 | 68 |

| | *Streptomycin* | *Streptomycin plus P.A.S.* |
|---|---|---|
| Average no. of days in course of treatment | 67 | 70 |
| Average total dosage of streptomycin in gm. | 67 | 70 |
| Average total of P.A.S. in gm. | 0 | 700 |

Table I shows the sampling of cases in regard to extent of disease, duration and amount of streptomycin given under the two regimens.

TABLE II.

| *Pulmonary tuberculosis* | *Streptomycin alone* | *Streptomycin plus P.A.S.* |
|---|---|---|
| (*a*) Erythrocyte sedimentation rate (elevation signifies greater than 10 mm. fall in first hour—Westergren) | | |
| elevated before treatment | 25 | 26 |
| significantly lowered at end of treatment (reduced by 25% or more) | 19 | 24 |
| (*b*) Conversion of sputum or fasting gastric contents (methods comparable before and after) | | |
| Positive before treatment | 26 | 23 |
| Negative at end of treatment | 18 | 18 |
| (*c*) X-ray clearing—(2 physicians agreeing) | | |
| marked | 1 | 2 |
| moderate | 6 | 8 |
| slight | 19 | 16 |
| none | 8 | 8 |
| (*d*) Cavity changes—(2 physicians agreeing) | | |
| cavity present before treatment | 16 | 17 |
| cavity smaller at end of treatment | 7 | 10 |
| cavity closed at end of treatment | 0 | 0 |
| (*e*) Summary—improvement—all factors considered | | |
| marked | 1 } 6 | 2 } 10 |
| moderate | 5 } | 8 } |
| slight | 20 | 17 |
| none | 8 | 7 |

Table II shows the extent of response in regard to fall in sedimentation rate, conversion of sputum, x-ray clearing, cavity changes as well as a summary of improvement among the two groups. Only those factors which could be measured or assessed accurately have been considered. It will be noted that 10 patients receiving "combined" treatment showed marked or moderate improvement, whereas 6 patients receiving streptomycin alone showed comparable improvement. On the other hand, an almost equal number under each regimen failed to show any significant improvement.

In no patient in this study were toxic manifestations considered to be attributable to streptomycin noted of a sufficient degree to justify discontinuance of treatment. Toxic symptoms thought to be attributable to P.A.S. were encountered in a certain number of those patients receiving "combined" treatment. In order to assess these side effects, one of the authors (M.G.W.J.) studied 50 sanatorium patients not suitable for streptomycin who were given 100 c.c. of a 10% aqueous solution of the sodium salt of P.A.S. daily by mouth. This daily dosage was equivalent to 10 gm. of P.A.S. The solution was administered in 3 divided doses orally before or after meals as the patients preferred. The same author followed each patient with almost daily recording of any symptoms appearing.

TABLE III.

SIDE-EFFECTS NOTED AMONG 50 PATIENTS RECEIVING THE EQUIVALENT OF 10 GRAMS OF P.A.S. BY MOUTH DAILY (ALL BUT 3 RECEIVED THIS TREATMENT OVER A PERIOD OF 60 DAYS)

| | *No. of patients* | *% of total patients* |
|---|---|---|
| Total number of patients studied | 50 | 100 |
| Number of patients showing *no* side effects at any time during this treatment | 26 | 52 |
| Type and incidence of the symptoms noted among the 24 patients who *did* show any side-effects | | |
| Mild nausea | 14 | 28 |
| Occasional diarrhœa | 20 | 40 |
| Mild vomiting | 10 | 20 |
| Marked generalized headache | 5 | 10 |
| Pyrexia of 102° or more by mouth | 2 | 4 |
| Number of patients requiring any discontinuance of the drug | 3 | 6 |

Out of a total of 50 patients studied carefully for side-effects (see Table III), only 3 required

any discontinuance of the treatment (sodium salt of P.A.S.) because of marked toxic manifestations appearing within the 60 day course. In these 3 cases, it was necessary to discontinue the drug permanently. One of these patients, after receiving the equivalent of 330 gm. of P.A.S. (33 days from start of treatment), developed severe nausea and vomiting with a fever of 104° by mouth. These symptoms continued for 6 days while the drug was continued. The drug was then stopped, whereupon the symptoms and fever subsided within 24 hours. Five days later the drug was recommenced and within 24 hours the same symptoms re-appeared and the temperature rose to 101° by mouth. The drug was then discontinued again (permanently) whereupon the symptoms and fever disappeared within 24 hours. The second patient, after receiving the equivalent of 160 gm. of P.A.S. (16 days from the start of treatment), developed a fever of 104° by mouth with marked nausea and vomiting. The drug was discontinued and the symptoms and fever subsided within 24 hours. The third patient, after receiving the equivalent of 60 gm. of P.A.S. (6 days from the start of treatment), began to show mild nausea and vomiting. The drug was continued but within the next 6 days the symptoms became progressively more marked in spite of the usual remedies. The drug was discontinued and the symptoms disappeared within 3 days.

Among a series of 25 patients receiving streptomycin intramuscularly and the sodium salt of P.A.S. orally also studied for side-effects by the same author, the incidence of toxic manifestations was approximately the same as among those receiving the sodium salt of P.A.S. alone.

No significant difference was observed in the incidence of side-effects among those taking their mixture with liquorice flavouring as compared with those using the oil of wintergreen flavouring.

Each patient in this study received a routine chemical and microscopic urinalysis as well as a total white blood count and a differential white blood count at some time during the course of the treatment. When examined in the above manner, no significant abnormalities were noted to appear in the urine; no significant depression of the total white blood count occurred, and no significant increase in eosinophiles was observed.

In most instances (all but 3) when mild symptoms of nausea or vomiting or diarrhœa occurred, the usual simple remedies such as bismuth and opium mixtures, aluminum hydroxide, etc., were effectual in relieving such mild side-effects without discontinuance of the sodium salt of P.A.S. Frequently, such symptoms when they appeared and were relieved, did not reappear in that patient even after the palliative remedy had been discontinued.

In this study of side-effects, 25 of the 50 patients received a 10% aqueous solution of the sodium salt of P.A.S. prepared in our pharmacy from the P.A.S. powder received from one manufacturer, and the other 25 patients received a 10% aqueous solution of the sodium salt of P.A.S. supplied by another manufacturer. Under the conditions of the study as carried out, no significant difference in the incidence of toxic manifestations was evident between the two preparations.

## LABORATORY

The emergence of streptomycin-resistant strains of tubercle bacilli during and after streptomycin therapy is a phenomenon, the existence and frequency of which continues to weigh heavily in the balance of clinical judgment. The phrases "streptomycin resistance" and "incidence of streptomycin resistance" which are commonly encountered have, without further elucidation, no more than a local significance. In any communication dealing with the subject, it is, therefore, necessary to define both terms, outlining technique, concentrations of antibiotics used, and the criteria of interpretation.

At the Toronto Hospital for Tuberculosis, Weston, organisms were isolated from all patients, where possible, prior to and at monthly intervals during, and for six months following the conclusion of streptomycin therapy.[8] The tubercle bacilli were grown from material initially treated with an equal volume of a 23% solution of tri-sodium phosphate, concentrated, and planted on two diagnostic Loewenstein's slopes, thus avoiding the carrying-over of possible antibiotic in the sputum to the subsequent culture media. Streptomycin sensitivity tests were carried out on Herrold's egg-yolk medium, transferring from Loewenstein's slopes by means of a saline suspension of a cross-section of the isolated bacterial community to a series of Petri dishes in which the concentrations of streptomycin ranged from a control through 0.5, 2, 5, 10, 25 to 50 micrograms of streptomycin per c.c. Only in those instances where growth on the plate containing 50 micrograms of streptomycin per c.c. at the conclusion of a month rivalled in luxuriance the growth on the control plate, was "complete streptomycin resistance" said to have developed.

It is well appreciated that many other media are presently in use and that growth at different concentrations of antibiotic than those mentioned above is assumed to indicate the acquisition of "resistance" by the isolated bacilli. This does no more than serve as a reminder of the necessity for full definitions where the above phrases are used.

Table IV indicates the pre-therapy level of resistance in over 95% of some 225 cases investigated. The remaining 5% of cases showed pre-treatment growth of varying degrees on the 5 and on rare occasions on the 10 micrograms per c.c. plate.

TABLE IV.

PRE-STREPTOMYCIN SENSITIVITY

| Micrograms streptomycin per c.c | | | | | | |
|---|---|---|---|---|---|---|
| Control | 0.5 | 2 | 5 | 10 | 25 | 50 |
| Extent of growth | | | | | | |
| ++++ | ++++ | ++ | 0 | 0 | 0 | 0 |

Table V indicates the incidence of emergence by local definition of strains completely and less markedly resistant, in patients who received streptomycin therapy.

Having determined arbitrarily the incidence of the emergence of streptomycin-resistant

TABLE V.

INCIDENCE OF STREPTOMYCIN RESISTANCE AMONG PATIENTS RECEIVING STREPTOMYCIN ALONE FOR 60 TO 90 DAYS

| | *No.* | % |
|---|---|---|
| "Completely resistant" (up to 50 micrograms per c.c.) | 37 | 42 |
| Less marked increase in resistance (up to 10 to 25 micrograms per c.c.) | 18 | 20 |
| No significant increase in resistance | 33 | 38 |
| Total cases | 88 | 100 |

strains of tubercle bacilli among patients receiving streptomycin therapy in the usual dosage (*i.e.*, 1 gram daily in two equally divided doses, over a period of 60 to 90 days), the same methods and criteria were employed to investigate 19 cases receiving concurrently one gram of streptomycin daily, plus 10 grams of para-aminosalicylic acid over an equal period of time. In these 19 cases, the para-aminosalicylic acid was administered orally as an aqueous solution of the sodium salt flavoured with liquorice or methyl salicylate. The drug was administered usually in three or five equally divided doses over a 12 hour period. Blood levels of P.A.S. determined during the course of the day showed an initial rise fifteen to thirty minutes following the first treatment of the day and rising to levels of 5 to 12 mgm. % at the conclusion of 60 to 90 minutes, falling away gently as the next dosage hour was approached. Blood specimens taken immediately before the first morning dose, failed to show the presence of the drug.

Table VI contrasts and demonstrates in percentage form the delay in emergence of "completely resistant" strains of tubercle bacilli from those patients receiving combined streptomycin and P.A.S. for 60 to 90 days when compared with patients receiving streptomycin alone for a similar period.

TABLE VI.

COMPARISON OF RESISTANCE APPEARING AFTER
(*a*) Streptomycin alone
(*b*) Streptomycin and P.A.S.

| | (*a*) *streptomycin alone* | | (*b*) *streptomycin and P.A.S.* | |
|---|---|---|---|---|
| | *No.* | % | *No.* | % |
| "Completely resistant" (up to 50 micrograms per c.c.) | 37 | 42 | 0 | 0 |
| Less marked increase in resistance (up to 10 to 25 micrograms per c.c.) | 18 | 20 | 4 | 21 |
| No significant increase in resistance | 33 | 38 | 15 | 79 |
| Total cases | 88 | 100 | 19 | 100 |

The above effect has been obtained in patients receiving the equivalent of 10 grams of P.A.S. daily and who showed maximum P.A.S. blood levels varying between 5 and 12 mgm. % of P.A.S. Investigations suggest that much lower concentrations of P.A.S. (0.005 mgm. %) give a similar delaying action *in vitro*. This might indicate that the delay in emergence of resistant variants might be obtained in patients on combined therapy with P.A.S. dosage considerably less than that mentioned above.

No attempt was made in this study to demonstrate the acquisition of P.A.S. resistance in the tubercle bacilli isolated from any of the patients on combined therapy.

## SUMMARY

1. The clinical responses of 34 patients with active pulmonary tuberculosis treated by sanatorium routine, streptomycin and P.A.S. have been compared with the clinical responses of a similar number and type of patients treated by sanatorium routine and streptomycin for similar periods.

2. When the summations of clinical responses among the two groups were compared, there appeared to be a slightly greater therapeutic response in the group receiving "combined"

treatment, but this difference was neither marked nor constant.

3. The incidence of toxic or side effects appearing among patients receiving P.A.S. or the sodium salt of P.A.S. orally in conjunction with streptomycin intramuscularly was discussed. With the dosage and method of administration used in this study and using the preparations now available, approximately 6% of patients receiving "combined" treatment by streptomycin and P.A.S. will probably require discontinuance of the P.A.S. because of side effects, within the first 60 days of this treatment.

4. In 19 cases on "combined" therapy from which tubercle bacilli could be isolated before and after treatment, the incidence of the emergence of "completely resistant" variants was seen to be markedly delayed when compared with patients receiving streptomycin alone.

5. Reference has been made to the blood levels of P.A.S. obtained with a dosage of 10 gm. of P.A.S. or its equivalent in 3 or 5 divided doses daily, and attention has been drawn to the possibility that a lower P.A.S. blood concentration might perhaps serve equally well from the aspect of delaying the onset of streptomycin resistance.

We are indebted to Merck and Company Limited of Canada for supplies of para-aminosalicylic acid and streptomycin which made this study possible. We are also indebted to the Nivea Pharmaceuticals Ltd. for a supply of the sodium salt of para-aminosalicylic acid used in the study of side effects. We also acknowledge the financial aid given in the laboratory aspects of this study by the Ontario Department of Health through the Federal Health grants.

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## CERVICAL SCRAPINGS TEST

### A New Method for the Early Detection of Carcinoma of the Cervix

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DURING the past five years the attention of gynæcologists has been focused on the early detection of cancer of the genital tract, particularly that of the cervix. We, of the Women's College Hospital, herewith present a simple, accurate method for the detection of carcinoma of the cervix which is new in the way the test is made, but adheres to the usual procedures of pathological diagnosis. We call it the cervical scrapings test.

We followed the work of Papanicolaou and others in this field with great interest and profit, but it was impossible for us to have a trained cytologist and the necessary laboratory. However, compelled by the desire to find some way of discovering the lesion before symptoms manifested it to the patient, we were led to investigate the possibility of early diagnosis by the pathologists using their ordinary methods.

#### CLINICAL PROCEDURE

To obtain the necessary material, various types of instruments were used, such as wooden spatulæ, metal, blunt and pointed curettes, and finally the use of an aspiration syringe. This material was immediately dropped into fixative and sent to the laboratory. By January, 1948, at the suggestion of Dr. W. L. Robinson, Professor of Pathology and Consulting Pathologist to the Women's College Hospital, a new technique was evolved which has now been laid down as our laboratory routine. It will be described later. Serial sections of the material are carefully screened and finally checked by Professor Robinson for diagnosis. The patient is put in the lithotomy position, examined bimanually, and the cervix well exposed by a bivalve speculum. No lubricant or antiseptic is used. The Ayre notched wooden spatula is the instrument of choice, which Dr. Ayre uses for his cervical smear method. The notch of the spatula is placed in the external os and firmly held there while the spatula is rotated three or four times in a circular motion. Then the flat end of the spatula is used to wipe the posterior surface of the cervix and posterior