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INTRODUCTION.

The purpose of this paper is to review the present status of para-aminosalicylic acid (PAS) in the treatment of non-miliary pulmonary tuberculosis.

In 1940, Bernheim at Duke University, observed that benzoic and salicylic acids increased the oxygen uptake of human strains of the tubercle bacillus and concluded that these two acids were actively metabolized by the organism (2). Lehmann (14) confirmed this observation for pathogenic strains and also observed that the oxygen consumption of non-pathogenic strains of the organisms was not affected by these substrates. It was reasoned that derivatives of benzoic acid might compete in this metabolic process, and so, more than fifty such compounds were studied and several proved to be tuberculostatic. Of these, PAS was selected as the most promising.

THE CHEMISTRY OF PAS

PAS, also designated 4-amino-2-hydroxybenzoic acid, is a white to tan crystalline solid with a melting point of about 150 degrees centigrade, with decomposition which is dependent on the rate of heating.

As the free acid, it is very slightly soluble in water, giving a solution of pH 3.5 - 4.5. It is soluble with diffioulty

in dilute mineral acids, such as Hcl, but quite freely soluble in sodium bicarbonate solutions at a pH 7-8. PAS is unstable to heat and especially so when in the form of solutions. Considerable decomposition occurs in 10-15 minutes heating at 100 degrees Centigrade. Loss of carbon dioxide (decarboxylation) is known to occur, and, in addition, the darkening observed when concentrated aqueous solutions stand for prolonged periods at room temperature, suggests oxidative decomposition. Thus, heat sterilization of the solid drug or its solutions must be avoided. The solid material should be stored in a cool, dry place. Protection from the sunlight is also indicated, as light exposure favors darkening of the drug.

Satisfactory methods of determining the amounts of PAS in blood, urine and tissue fluids have been devised by Marshal (19).

THE PHARMACOLOGY OF PAS

IN VITRO STUDIES

Bacillary sensitivity to PAS was tested <u>in vitro</u> in Sauton's medium to which PAS was added in concentrations varying from 0.15 - 15 mg%. The dry weight of the bacilli was determined when the growth of the bacilli covered the surface of the medium. Inhibition was expressed in per cent. The sensitivity of fifteen different strains of tubercle bacilli including the BCG and H37RF strain showed almost complete inhibition of more than 90% by a concentration of 0.15 - 15 mg%. At 0.015 mg% PAS some strains were

inhibited to more than 50%, the average being 56%.

Repeated exposures to PAS *in vitro* using virulent strains of BCG isolated from three month PAS-treated patients in four series of 43 days, showed development of no resistance. In another series of experiments the bacilli were isolated from patients treated with PAS up to eleven months and *in vitro* sensitivity to PAS tested one or more times. In a few cases a slight degree of resistance developed after four to eleven months of treatment with PAS. However, the bacilli were still sensitive to the concentration which prevailed in the blood during treatment. The conclusion was draws that resistance to PAS is not readily acquired by the tubercle bacillus and plays no important role in treatment of tuberculosis with PAS (16). Youmans (25) and Sievers (20) confirmed these results. More recent work, however, has indicated that resistance to PAS does develop.

Vennesland, et.al. completed *in vitro* tests using PAS and streptomycin sensitive human tubercle bacilli, H-37RvNRl. They found that 0.74 gamma per cubic centimeter of streptomycin was completely inhibiting to the *in vitro* growth of streptomycin resistant bacilli. 1.2 gamma per cubic centimeter of PAS almost completely inhibited the same bacilli. An amount of streptomycin or PAS which is only mildly inhibiting alone was found, in combination with a non-inhibiting amount of the other to exert an increased inhibitory effect. This group concludes that the combination of streptomycin and PAS in critical concentration is more effective than either drug if the organism is streptomycin

sensitive (22). Evidence of synergistic action of the two drugs has not been found by other workers (10). It is generally agreed, however, that Streptomycin is more effective than PAS in the bacteriostasis of the tubercle bacilli.

ANIMAL STUDIES.

Lehmann has found that PAS is non-toxic to rats when given as 5% of the food ration for one to two months. A blood level of 37 mg of free acid per 100 cc was found with that dosage. Guinea pigs were more sensitive and showed a decrease in appetite and growth. Blood counts and hemoglobin levels were unaffected in both the rat and the guinea pig (15).

Youmans concluded that "PAS is highly bacteriostatic in vitro for virulent human type tubercle bacilli, including strains resistant to the bacteriostatic action of streptomycin, and is at least moderately effective for the suppresion of tuberculous infections in white mice" (11).

One group used 37 guinea pigs experimentally infected and verified by tuberculin sensitivity tests to have tuberculosis. Twenty of these were used as a control and 17 were treated with PAS. Eight (47%) of the 17 treated animals died while 16 (80%) of the control group died. It was concluded that PAS exerts a favorable influence on tuberculosis in the guinea pig (12).

Way, et. al. has established that PAS in rats, dogs and humans is rapidly and totally absorbed and rapidly excreted. According to these workers, 85% of the ingested PAS can be re-

covered from the urine. The recovery is in the form of free and conjugated amines. Ten per cent of the total dosage of PAS has been recovered from rat urine in the form of conjugated amines. Little or no conjugation occurs in the dog (23).

No storage of PAS has been found in the rat, but the largest amounts are temporarily found in lung, liver and plasma protein.

HUMAN STUDIES.

PAS is rapidly absorbed and excreted by the human body. A much higher percentage of conjugation occurs in man than in the rat. Up to 60% of the total dose is conjugated in man, 10% in the rat, and 0% in the dog. Only the free drug is effective therapeutically, and since the high degree of conjugation, and excretion of much of the free drug before it can be used, it is assumed that only a relatively small percentage of any single dose will be effective. After a single oral four gram dose, or, after repeated doses of 2½ grams every six hours in humans, a maximum level of approximately 100 mg per liter is rapidly attained and falls to less than 10 mg per liter by the end of six hours.

Three compounds containing a free amino group and two compounds containing a conjugated amino group were separated from the urine of an individual taking PAS. Of the free amino compounds found, two are believed to be unchanged PAS and p-aminosalicyluric and one of the conjugated amino compounds to be acetylated PAS (23). It was also found that 59% of the urinary

amines were acetylated.

PAS is quite insoluble in acid but becomes much more soluble as the pH increases. This is of possible practical importance for renal damage from crystalluria could occur.

ADMINISTRATION AND DOSAGE OF PAS

PAS has been given via nearly every conceivable route. Among these are oral, intravenous, intramuscular, aerosal, intrathecal, intrapleural, intraduodenal and locally. Since PAS is rapidly absorbed by the oral route it is wise to give it in this manner because patients prefer this route.

The dosage schedule which produces bacteriostatic, free PAS levels with the lowest percentage of conjugated product present is the most efficient. An important problem associated with the determination of PAS blood levels is whether it is necessary to maintain bacteriostatic concentrations constantly, or, whether, as seems to be the case with streptomycin, it is sufficient to build up a tuberculostatic level only at suitable intervals. The answer to this question has not been obtained. At the present time, the actual minimum therapeutic dosage of PAS is not established. Blood levels of 1 mg% free PAS have been found sufficient, at Fitzsimons General Hospital, to inhibit, in vitro, all strains of the tubercle bacillus found there to date (11). Lehmann (21) considers his optimum dosage of PAS for pulmonary tuberculosis to be 14 grams daily, divided. Twelve grams daily, divided, is probably the average dosage used in this country.

At Fitzsimons General Hospital, the drug is given either as a "cocktail" consisting of the powdered form mixed with orange juice, grape juice or chocolate milk and then sweetened to taste, or in the liquid form. Liquid PAS is made by mixing the parted substance with super proportions of sodium bicarbonate so that the sodium salt of PAS is obtained. With proper flavoring the liquid drug is well tolerated (24).

THE TOXICOLOGY OF PAS.

Toxicity from PAS has apparently not been a serious problem. Differences in results obtained by various groups is now agreed to be due to differences in the quality of the substance used. There are not the usual salicylate effects of sweating, tinnitus, deafness and anti-pyresis (15-17). However, these results are not well substantiated by experiment and so definite conclusions cannot be drawn.

There has been a rare report of liver damage and some renal irritation has been reported. Up to 25 grams per day of the drug have been administered with no complaints, and as little as 8 grams per day has caused disturbances. The usual symptoms are of a gastro-intestinal nature such as nausea, vomiting and especially diarrhea, although these have apparently never been of such severe nature as to prohibit use of the drug.

It is the feeling in this country that the sodium salt of PAS reduces the incidence of drug toxicity (23). But one cannot

completely overlook the fact that Lehmann, one of the foremost Swedish workers in this field uses the acid PAS almost entirely because he feels that the sodium form is absorbed and excreted too rapidly (16).

THE PRESENT VALUE OF PAS IN NON-MILIARY PULMONARY TUBERCULOSIS.

Since 1944 when Lehmann first adminstered PAS to man in the treatment of tuberculosis, many humans have taken the drug. Much work is currently being done in this country and a good share of it is found in the Veterans and Army hospitals.

Generally speaking, the results of the various workers indicate that PAS is useful as a therapeutic agent against pulmonary tuberculosis although less effective than streptomycin. Both drugs are under similar limitations when it comes to the indications for chemotherapy of tuberculosis, which is a giant problem in itself. The important point is that better drugs are needed.

Streptomycin, though the drug of choice, has the very serious objection in that bacterial resistance soon developes to it. PAS has the same undesirable characteristic (8, 5, 18).

Fortunately, PAS shows some promise of being useful in a slightly different way. Demerec (24) postulated that "the most effective way of theoretically preventing origin of resistant strains of bacteria is the use, in clinical treatment of a mixture

of two antibiotics....that affect the same pathogen but are independent in their actions." A group at the Mayo Clinic (29), looking for a way to prevent the emergence of resistant strains of bacilli to streptomycin, used a combination of PAS, streptomycin and Promin. This method did not increase the toxicity of any one of the drugs and results showed fewer resistant strains of the tubercle bacilli than after a similar period of streptomycin treatment alone.

The work at the Mayo Clinic seemed to bear out Demerec's theory for preventing the origin of resistant strains, and, so, with this principle in mind, a treatment program combining daily PAS with intermittent streptomycin was begin at Fitzsimons General Hospital. Sixty-seven cases were used on the combined regimen of streptomycin - 2 grams every third day and 12 grams daily of PAS for 120 days. This regimen has been compared to a regimen (500 cases) of daily streptomycin, a regimen (100 cases) of intermittent streptomycin (q 3 day streptomycin) and a regimen (50 cases) of daily PAS. The information being sought was the incidence of emergence of bacterial resistance to the drugs on the different regimens. Knowing that different research methods may cause considerable error in final results, which may render comparison of various reported results impossible, the following principles were adhered to: In order to compare properly the effect of different dosage regimens on the emergence of resistant strains of tubercle bacilli, it is necessary (1) to employ a statistical-

ly significant number of patients on each regimen, (2) to perform the in vitro sensitivity determinations by the same method, preferably in the same laboratory, (3) to use the same criterion of resistance, and (4) to compute the results by the same method." There was no attempt made to correlate clinical response with the development of bacterial resistance. The results of the various regimens have been tabulated in Table I., and have been expressed on a percentile basis.

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Days of therapy	Daily SM (500 cases)	q 3 d. SM (100 cases)	Combined SM & Daily PAS (67 cases)	Daily PAS (50 cases)
	%resistant to SM		% resistant to combined SM & PAS	Resistant to PAS
28	1.5	0	0	0
42	8.1	0	0	0
60	20.0	4.1	0	0
75	30.0	4.4	0	14.2
90	41.6	19.7	0	19.2
120	69.0	33.3	0	53.1

Table I shows the comparison of the incidence of bacterial resistance in patients treated with different streptomycin and para-aminosalicylic acid dosage schedules.

TABLE I. (10).

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According to the reported results, carried out to the 120th day, the combined therapy regimen compares very favorably

with the other regimens studied. This appears to be good evidence that the use of a combined regimen will, at least, greatly decrease the incidence of the emergence of bacterial resistance to these drugs in the treatment of tuberculosis. Subsequent verification of these findings will be quite encouraging, and, may establish the value of PAS in pulmonary tuberculosis which will be in a combination with intermittent streptomycin therapy and the ability of this combination to prevent, or greatly curtail, the emergence of resistant strains of tubercle bacilli.

SUMMARY.

It is generally agreed that PAS is tuberculostatic in relatively small amounts, but to a lesser degree than streptomycin. It has been proven effective against human tuberculosis as well as experimental tuberculosis in laboratory animals.

PAS, especially the sodium salt of the drug, is believed to be not significantly toxic in therapeutic quantities. There are instances of general gastro-intestinal upsets following use of the drug but none of the usual "salicylate reactions." The average dose used in this country is 12 grams daily divided.

PAS, used alone causes emergence of resistant strains of the tubercle bacilli similar to the number found in streptomycin therapy. PAS, 12 grams daily in a combined regimen using 2 grams of streptomycin every three days, for a period of 120 days, has apparently prevented the origin of bacterial resistance

to either drug. If this trend continues PAS will certainly become a valuable adjunct in the treatment of tuberculosis, and will do this primarily because it prolongs the usefulness of streptomycin in the chemotherapy of tuberculosis.

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