jected exceeded the protective range in the dogs used in our experiments.

The mechanism of the toxic action of oleic acid is problematical. Small doses of sodium oleate produced a degree of hemolysis never seen with comparable doses of oleic acid.

While changes in blood serum calcium appeared to be correlated with the degree of hemolysis, the low values of serum calcium may be explained by formation of calcium soaps. Changes of blood pH appeared to be associated with respiratory conditions and with acid and toxic effects of the oleic acid.

Oleic acid does not seem to produce massive edema of the lungs by embolism, but by toxic effects on the capillaries of the lungs. Caution must be used in the preparation of fat emulsions for intravenous alimentation, in order to exclude fatty acids and soaps. Fat embolism *per se* may not be so dangerous unless fatty acids are released in or into the circulation.

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In vitro Effect of Streptomycin and Para-Aminosalicylic Acid (PAS) on the Growth of Tubercle Bacilli.*

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On the basis of Bernheim's[1] observations that the sodium salts of benzoic and salicylic acids specifically stimulated the oxygen uptake of the tubercle bacillus, Lehman[2,3,4] undertook to find a substance of similar chemical structure which might prove bacteriostatic for this organism. Of the 60 derivatives of benzoic acid studied, 4 amino-2-hydroxy benzoic acid (para-aminosalicylic acid or PAS) was found to be the most effective, inhibiting growth of the tubercle bacillus *in vitro* in concentrations as low as 0.15 mg %. This effect was found to be bacteriostatic rather than bacteriocidal. Sievers[5] and Youmans[6] soon confirmed these findings. Youmans reported that 6 streptomycin-resistant strains of tubercle bacilli tested by him were equally sensitive to PAS.

Smith and McClosky[7] and others have reported that a combination of two bacteriostatic agents often is more effective against the tubercle bacillus than either drug alone. It was logical to assume, therefore, that PAS plus streptomycin might behave in a similar manner; in this paper we report the *in vitro* effects on the tubercle bacillus of each of the 2 drugs alone and in combination.

While these experiments were in progress, Youmans[8] reported that the *in vitro* bacteriostatic effect of PAS in combination with streptomycin was no greater than the sum of the individual activities of the two substances.

Methods. Cultures were grown in the liquid medium described by Dubos,[9] and the concentration of tubercle bacilli in mg/cc was determined by turbidimetric readings in calibrated tubes using a blue filter (420 mμ) in a Lumetron colorimeter.

The following 2 test organisms were used: (A) the human strain of tubercle bacilli H-37RV, which is streptomycin-sensitive and (B) the naturally occurring strep-

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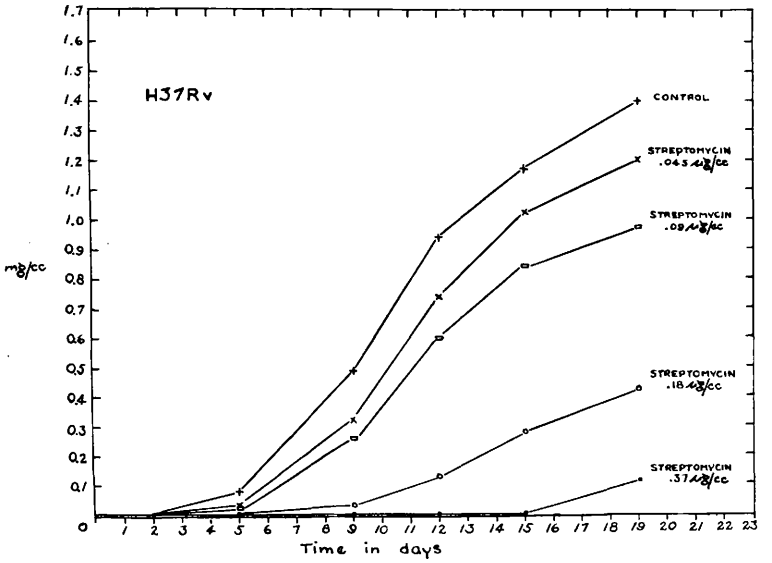

FIG. 1.

The effect of various concentrations of streptomycin on the growth of a virulent human strain of tubercle bacilli (H-37RV) charted in mg/cc of total growth at various time intervals. (0.74 mg of streptomycin per cc caused complete inhibition of growth and is not shown in the graph.)

tomycin-resistant variant H-37RVNR1.[10] Several other virulent strains of human tubercle bacilli isolated from sputum of patients with pulmonary tuberculosis were tested and behaved in a similar manner. Stock cultures of all strains were kept in Dubos liquid medium and were subcultured at 7- to 14-day intervals.

Commercial streptomycin sulfate was used, and dilutions were made aseptically from a stock solution prepared by adding 10 cc of sterile distilled water to 1 g of the powdered drug. The stock solution was freshly prepared for each experiment.

The PAS, which was supplied by Parke Davis in powdered form, was not sterile. It was accurately weighed for each experiment, dissolved in distilled water or Dubos medium, and sterilized by passing it through a Seitz filter. PAS is unstable at high temperatures and loses some activity if sterilized by heat.[11]

In each experiment a measured inoculum of 0.1 mg of tubercle bacilli per tube was used and the same stock culture was used as the source of the inoculum for all tubes.

Results with H-37RV. Using the strain H-37RV, determinations were made simultaneously on the effect of streptomycin alone, PAS alone, and the two drugs in combination in various concentrations. Eight series were made with double dilutions of PAS ranging from 10 μg/cc in Series 1 to 0.07 μg/cc in Series 8. Series 9 contained no PAS. In each series tubes labeled A through F contained double dilutions of streptomycin ranging from 0.74 μg/cc to 0.023 μg/cc, and tubes labeled G contained no streptomycin. Thus, Series 9 was actually a streptomycin assay; the G tubes in Series 1 to 8 were a PAS assay, and the G tubes in Series 9 were controls since they contained neither drug. Each dilution of drug or combination of drugs was made in triplicate, and turbidimetric readings were averaged.

Streptomycin alone caused complete inhibition of growth of H-37RV after 19 days in concentration of 0.74 μg/cc and almost complete inhibition with 0.37 μg/cc (Fig. 1).

PAS alone inhibited growth almost com-

[10] Vennesland, K., Ebert, R. H., and Bloch, R. G., *Science*, 1947, **106**, 476.

[11] Mimeographed communication from Department of Clinical Investigation, Parke Davis and Company, Oct. 21, 1947.

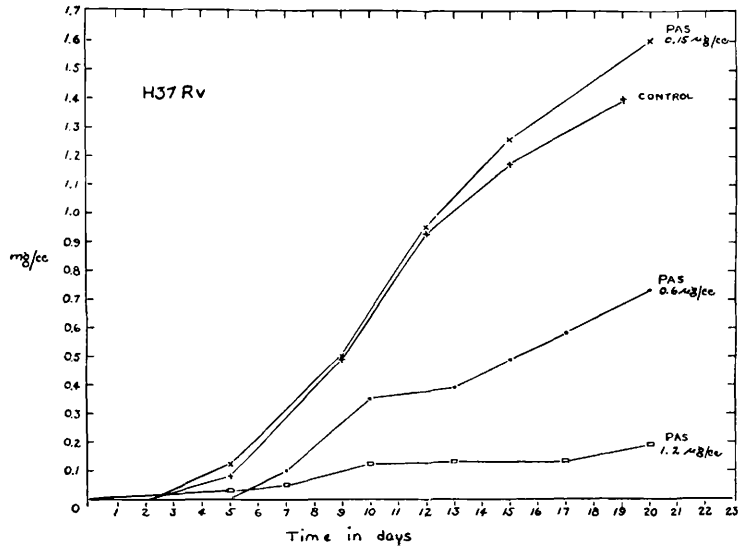

FIG. 2.
The effect of various concentrations of PAS on the growth of H-37RV.

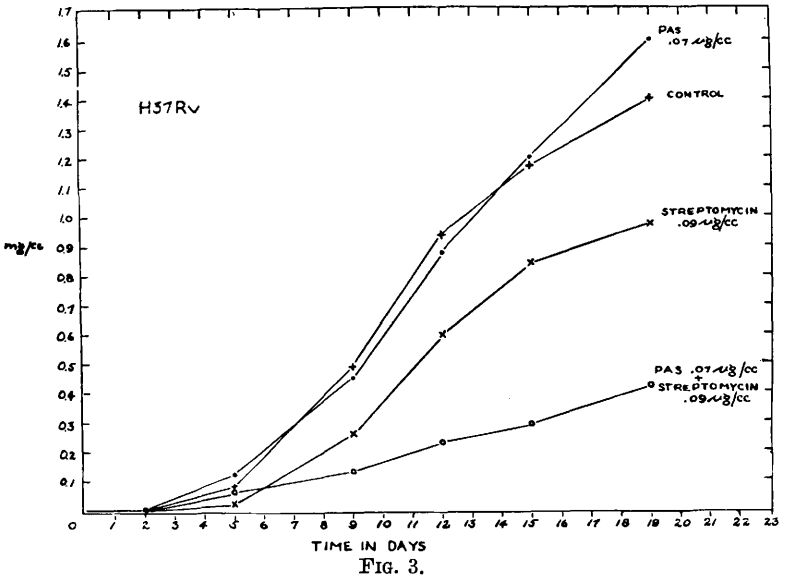

FIG. 3.
A comparison of the effect of various concentrations of PAS, streptomycin, and their combination on the growth of H-37RV.

pletely in concentration of 1.2 μg/cc and 0.6 μg of PAS per cc produced partial inhibition (Fig. 2). It was found in this and other experiments that PAS does not produce the sharp end-point of complete inhibition which is found in the case of streptomycin. Even concentrations of PAS as high as 10 μg/cc did not completely inhibit growth after 20 days of incubation.

The combination of streptomycin and PAS produced a greater inhibition of growth of H-37RV than either drug alone. The most favorable combination of drugs for demonstrating this synergistic effect was a concentration of one drug just below that necessary to produce fairly complete inhibition together with very small concentrations of the other drug. Thus 0.09 μg of streptomycin per cc

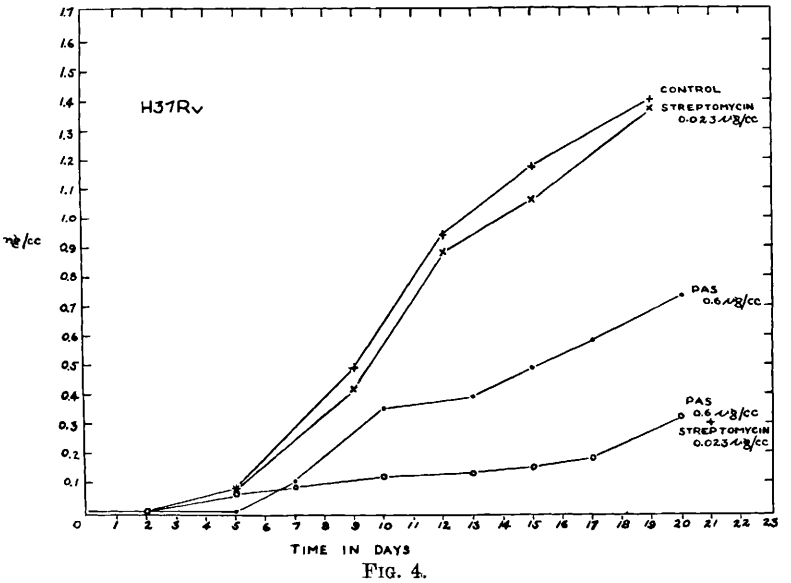

FIG. 4.

A comparison of the effect of various concentrations of PAS, streptomycin, and their combination on the growth of H-37RV.

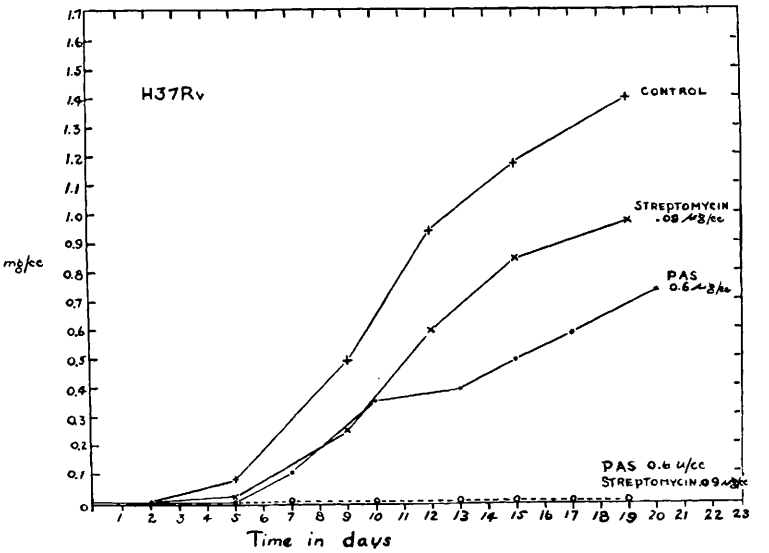

FIG. 5.

A comparison of the effect of various concentrations of PAS, streptomycin, and their combination on the growth of H-37RV.

produced only slight inhibition of growth, although 0.18 μg/cc produced fairly marked inhibition (Fig. 1). PAS alone in concentrations of 0.3, 0.15, and 0.07 μg/cc produced no inhibition. The combination of 0.09 μg of streptomycin per cc with each of these small concentrations of PAS, however, produced marked inhibition (Fig. 3).

Similarly, a concentration of 0.6 μg of PAS per cc produced only moderate inhibition of growth, and a concentration of 0.023 μg of streptomycin per cc produced none, but a combination of drugs in these concentrations caused increased inhibition (Fig. 4). The combination of 0.09 μg of streptomycin per cc and 0.6 μg of PAS per cc resulted in com-

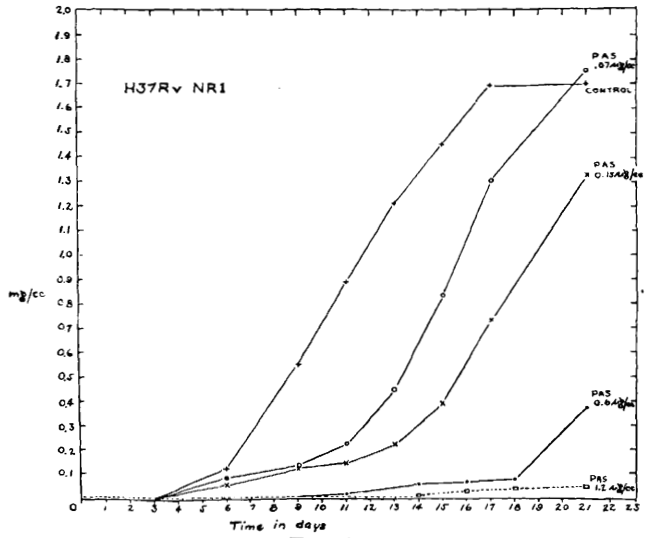

FIG. 6.

The effect of various concentrations of PAS on the growth of a streptomycin-resistant strain of virulent human tubercle bacilli (H-37RVNR1).

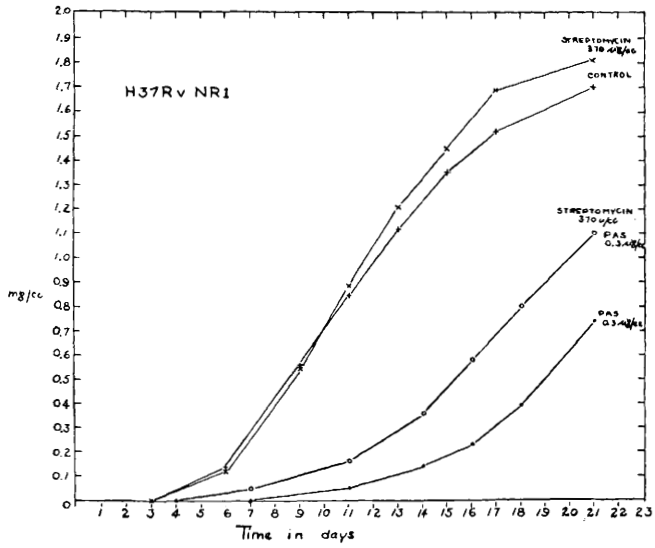

FIG. 7.

A comparison of the effect of 0.3 mg of PAS, of 370 mg of streptomycin, and of their combination on the growth of H-37RVNR1.

plete inhibition of growth (Fig. 5).

Results with Streptomycin-Resistant Strain. In this experiment using the streptomycin-resistant variant H-37RVNR1 the procedure was the same as that outlined above except that 10 × dilutions of streptomycin were made in Tubes A through F in all series in concentrations ranging from 3700 μg/cc to 0.037 μg/cc.

Streptomycin alone produced no inhibition of growth of this resistant strain even in the highest concentrations. It appeared that the streptomycin-resistant strain was slightly more sensitive to PAS alone than H-37RV, although

the inhibiting concentrations were in the same general range (Fig. 6).

The combination of streptomycin and PAS caused no increase in the amount of inhibition on this resistant strain; in fact, there was slightly less inhibition with the combination of 370 μg of streptomycin per cc and 0.3 μg of PAS per cc than with PAS alone (Fig. 7).

Discussion. Relatively low concentrations of para-aminosalicylic acid (PAS) inhibit the growth of virulent human tubercle bacilli *in vitro*. It is worth noting that the concentration of drug necessary to inhibit growth varies considerably with the size of the inoculum. Thus more than 100 $\times$ the amount of drug is necessary to cause inhibition if an inoculum of 1.0 mg of tubercle bacilli per 10 cc of medium is used instead of 0.1 mg. This is not true of streptomycin. In experiments simultaneously carried out with both drugs concentrations of 1.48 μg of streptomycin per cc produced almost complete inhibition of growth using a large inoculum, whereas only slight inhibition was obtained with 100 μg of PAS per cc.

There is no question that the combination of streptomycin and PAS in critical concentrations is more effective than either drug alone if the organism is streptomycin-sensitive. These *in vitro* studies agree with the results of animal experiments, which will be reported elsewhere.

It appears that there is no increase in the inhibitory effect of the combination of streptomycin and PAS on the growth of a streptomycin-resistant strain if the streptomycin alone is ineffective (Fig. 7). If, however, a high concentration of streptomycin causes some inhibition, a combination of this amount of streptomycin with PAS will cause greater inhibition than either drug alone. Thus a streptomycin-resistant strain of virulent human tubercle bacilli, isolated from the lung of a patient on autopsy, was partially inhibited by 370 μg of streptomycin per cc. Using this strain the inhibitory effect of PAS was enhanced by the addition of 370 μg of streptomycin per cc.

Summary. 1. The *in vitro* growth of a virulent strain of human tubercle bacilli, H-37RV, is inhibited by 0.74 μg/cc of streptomycin. 2. The *in vitro* growth of H-37RV is inhibited almost completely by a concentration of 1.2 μg/cc of PAS. 3. A moderately inhibiting concentration of either streptomycin or of PAS when combined with a completely non-inhibiting concentration of the other drug produces a marked enhancement of inhibition of the *in vitro* growth of H-37RV. 4. *In vitro* growth of a resistant strain of human tubercle bacilli, H-37RVNR1 is inhibited by 1.2 μg/cc of PAS. 5. The inhibition of growth of this resistant strain is not enhanced by the addition of streptomycin.

We gratefully acknowledge the generosity of Parke Davis and Company, who supplied the para-aminosalicylic acid.

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Vitamin A and Thyroid Iodine after Reticuloendothelial Cell Block.*

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The apparent antagonism between vitamin A and thyroid activity has stimulated investigations designed to clarify further the problem, and to determine the nature of this antagonism. It has been shown[1,2] that excess

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† Special Fellow.

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