controlled by including in the test, as a control, a standard compound such as 4,4'diaminodiphenylsulfone each time unknown compounds are tested and evaluating the results in terms of this as a standard. Improvements in the methods of preparing suspensions and in determining the amount of inoculum would also be helpful. For example, in a limited number of tests, micro-Kjeldahl nitrogen determinations have proved to be far more accurate for the latter purpose than Hopkins vaccine tubes.

The time of the completion of the tests can be reduced to 7-14 days providing only the readings of subsurface growth are recorded and a slightly larger inoculum employed.

Summary. A new technic for rapidly and efficiently testing the growth-inhibiting action of substances on virulent human type tubercle bacilli is described. The order of effectiveness of four compounds in inhibiting the growth of the avirulent acid-fast rapidly growing organism 607 was found to be: Sulfathiazole, sulfadiazine, 4,4'diaminodiphenylsulfone, and sulfanilamide, while for the virulent human strain of tubercle bacillus H37Rv the order was found to be: 4,4'diaminodiphenylsulfone, sulfathiazole, sulfadiazine, and sulfanilamide.

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Subsurface Growth of Virulent Human Tubercle Bacilli in a Synthetic Medium.*

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While there are scattered publications reporting growth of virulent human tubercle bacilli beneath the surface of liquid media, it is generally believed that these organisms will grow only on the surface, and precautions are always taken to float the organisms when subculturing. Drea[1,2] reported that one strain (H37) would grow rapidly in the depths of Long's synthetic medium when suspensions containing as little as 10^{-6} to 10^{-8} mg were inoculated. These findings with H37 have recently been confirmed by Cohn.[3] The present paper reports subsurface growth in a synthetic medium of 5 other virulent human strains of tubercle bacilli and one avirulent rapidly growing acid-fast organism.

Methods. Five virulent human strains were employed: H37Rv, 3637,† 3632,† G-2,‡ and G-5.‡ All 5 strains were pathogenic for guinea pigs. H37Rv, G-2, and G-5 were also pathogenic for mice on intravenous inoculation, and G-2 has been used to produce pulmonary tuberculosis in dogs.[4] No. 607, an avirulent rapidly growing acid-fast organism, was obtained from the National Type Culture Collection.

A synthetic medium was employed containing: Asparagin, 0.5%; monopotassium phosphate, 0.5%; magnesium citrate, 0.25%; magnesium sulfate, 0.05%; glycerol, 2.0%, made up with water redistilled from glass. The pH was adjusted to 7.0 with 40% sodium hydroxide. This medium was tubed in the desired amount, usually 10.0 cc, in 200 × 25 mm new soft glass or pyrex test tubes and sterilized by autoclaving at 10 lb for 20 minutes.

Suspensions of tubercle bacilli were pre-

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1 Drea, W. F., *J. Bact.*, 1940, **39**, 197.

2 Drea, W. F., *J. Bact.*, 1942, **44**, 149.

3 Cohn, Maurice L., *Am. Rev. Tuberc.*, 1944, **49**, 463.

† Obtained from Dr. William H. Feldman, Mayo Clinic, Rochester, Minn.

‡ Isolated from patients with active pulmonary tuberculosis by Dr. Francis D. Gunn, Department of Pathology, Northwestern University Medical School.

4 Mills, Moore A., Barth, E. E., and Gunn, F. D., *Am. Rev. Tuberc.*, 1940, **48**, 28.

pared by grinding aseptically 21-day surface growths from synthetic medium in an agate mortar and suspending them in either sterile double distilled water or in the synthetic medium. No dispersing agent was employed. These suspensions were then standardized by centrifugation in Hopkins vaccine tubes. For inoculation 0.1 cc volumes, containing the desired amount of tubercle bacilli, were used and incubation was at 37°C.

Results. With inocula of 1.0 mg in 10.0 cc of medium, growth was always evident by increased turbidity within 2 days, this rapidly increased and consisted of growth of tubercle bacilli occurring between the medium and the side of the tube. When the tubes were shaken flaky masses of tubercle bacilli could be seen which settled to the bottom where they continued to grow. Six to 10 days following the inoculation the familiar pellicle development begins to take place. With smaller inocula (0.1 mg) subsurface growth may not be evident for from 6 to 10 days and surface pellicle development may not appear until 10 to 14 days following inoculation. With inocula of 0.01 and 0.001 mg subsurface growth usually was not evident until from 2 to 4 weeks and in some tubes did not appear at all within this time.

The degree of dispersion of the organisms in the suspensions employed for inoculation was important in influencing the rapidity with which subsurface growth appeared. The more finely divided suspensions seemed to grow more rapidly than those consisting of larger clumps of bacilli. Whether the actual amount of growth would be different in the two cases, however, has yet to be determined.

That the growth evident in the cultures actually consisted of tubercle bacilli is indicated by the fact that stains made from them consistently showed only masses of acid-fast bacilli, and subcultures made on a variety of media incubated both aerobically and anaerobically were consistently negative for any organisms other than tubercle bacilli. Contamination, which occasionally occurred, was always grossly evident, and on stain and subculture revealed the usual laboratory contaminants. That the organisms after growing as indicated above are still virulent was demonstrated by the fact that a 3-week-old subsurface growth of H37Rv produced progressive tuberculosis when inoculated into guinea pigs.

The same phenomena of subsurface growth can be observed in 250 cc flasks containing 100 cc of medium; in fact, if the inoculum which is usually floated so carefully on the surface of the medium is allowed to sink and the flask than shaken vigorously to disperse the organisms, subsurface growth is usually evident within a few days. If the flask is not shaken further the usual surface pellicle growth will eventually appear. However, if the flask is shaken daily pellicle development will be prevented, and only subsurface growth will occur.

All 5 virulent strains of human type tubercle bacilli grew as described above and while differences have been noted between strains in the rate of subsurface growth the significance of this is not known since individual strains varied in their rate of growth at different times.

When 0.1 mg of a suspension of the avirulent strain No. 607 was inoculated into 10.0 cc of synthetic medium, surface pellicle growth usually appeared within 2 to 3 days. However, if prior to this time the tubes were shaken subsurface growth could be detected. In our experience with this strain subsurface growth has always appeared before surface growth.

The above results show that many, if not all, human type tubercle bacilli are capable of rapidly developing beneath the surface of liquid synthetic medium provided a sufficiently large inoculum is used. In fact, subsurface growth may be the more normal type of growth since, as pointed out by Drea,[1,2] it is more analogous to the type of growth which takes place *in vivo* than is pellicle development.

The ease and rapidity with which tubercle bacilli will grow in this manner in a synthetic medium should remove many of the technical difficulties which have confronted investigators working with these organisms. It should provide a far more rapid and accurate, and a far less cumbersome, method for studying the metabolism of human type tubercle bacilli,

and for the *in vitro* testing of growth promoting or growth inhibiting substances.

Summary. Suspensions of five virulent strains of *My. tuberculosis var. hominis* were found to rapidly produce subsurface growth when inoculated into synthetic medium. One avirulent rapidly growing strain grew in a similar manner.

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Response of Rats to Diets Containing Propylene Glycol and Glycerol.

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The present paper is a brief preliminary report regarding some of the principal observations in connection with experiments which were started in 1941 in an attempt to compare the physiological effects of propylene glycol and glycerol when fed to rats at different levels of intake.

The first series of growth experiments covered a period of 20 weeks. The basal ration consisted, in parts per 100, of casein 20, salt mixture 4, Cell-U-Flour 2, butter fat 6, cod liver oil 2, brewer's yeast 6, and corn starch 60. Each experimental group consisted of 6 to 10 young rats. The basal ration was modified by replacing corn starch with glycerol and propylene glycol, respectively, at levels of 1, 3, 6, 10, 15, 20, 30, 40, 50, and 60 parts per 100 of total ration. Consequently, the first series of experiments comprised 21 dietary groups.

In a second series of experiments, groups of young rats were fed 4 basal diets identical with that of the first series with the exception that in 3 of these starch was replaced by glucose, sucrose, or dextrin, respectively. In this series, propylene glycol and glycerol were fed only at a 30% level, replacing, in each case, 30 parts of the carbohydrate.

A third series of feeding trials was conducted in which propylene glycol and glycerol were fed in isocaloric amounts at a level approximating 30% of the diet.

In the first series of experiments, no marked differences in growth response could be detected until the alcohols were fed at the 30% level. At this level the glycerol-fed animals grew at an approximately normal rate, while the propylene glycol animals grew less rapidly. Young rats, starting at 21 to 28 days of age, lost weight and died in a few days when the diet contained 40, 50, or 60% of propylene glycol. When young rats received diets containing comparable amounts of glycerol, growth was depressed and some animals eventually died but the majority of the animals survived the experimental period. Older (half grown) animals were able to tolerate the higher levels of propylene glycol but their growth responses were never equal to those of the animals receiving comparable amounts of glycerol.

When rats received propylene glycol and glycerol diets during alternate weeks (at the 30% level), growth responses were generally increased during glycerol periods and depressed during propylene glycol periods. While glycerol diets were consumed more rapidly because of palatability, calculations show that the glycerol-fed rats made greater gains per caloric unit of food intake. No differences could be noted when other carbohydrates replaced corn starch in the basal ration. Livers from glycerol-fed rats were invariably richer in glycogen than livers from rats receiving comparable amounts of propylene glycol.

A series of metabolism studies was also conducted in which groups of half-grown rats were fed comparable diets containing increasing amounts of glycerol or propylene glycol during alternate weeks. Without exception, rats excreted in their urine the greatest percentage of the ingested water, the greatest percentage of the thiamine intake, and the greatest percentage of the polyhydric alcohol