centration before the diagnosis could be made and the drug administered.

The data are presented in Tables II and III. The drugs under the conditions of the experiment had no apparent effect on the course of the infection.

11371 P

Growth Promotion of the Tubercle Bacillus by Serum Albumen.

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The author[1] described the growth of single colonies of tubercle bacilli in the depth of coagulated rabbit plasma. It was then observed that rabbit serum + agar was a less favorable medium than coagulated plasma. Only a few colonies developed in "hormone agar", but many more in hormone agar + rabbit serum. The growth-promoting effect of serum was quite variable. Evans and Hanks[2] confirmed the favorable effect of rabbit serum and obtained good growth in the depth of Long's medium after the addition of blood or serum. Kallo and Nathan[3] and Pagel[4] found that some human sera support the growth of tubercle bacilli while others fail to do so. Pagel could not demonstrate the presence of either growth-promoting or specific inhibiting substances. Drea[5] confirmed the inhibiting effect of agar and found that tubercle bacilli grew in the depth of a modified Long's medium when it had been inoculated with varying quantities of a bacillary emulsion.

The addition of human, guinea pig, rabbit, sheep or horse serum all enhance the growth of tubercle bacilli in the depth of synthetic medium The growth appears earlier, is more abundant and takes place after inoculation of smaller quantities. In synthetic medium where the nitrogen is supplied by glycine, growth rarely occurs after inoculation of less than 10^{-1} mg tubercle bacilli, in media with asparagine-ammonium citrate as nitrogen source, growth frequently

1 Boissevain, C. H., *Am. Rev. Tuberculosis*, 1926, **13**, 90.

2 Evans, B., and Hanks, J. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 112.

3 Kallós, P., and Nathan, E., *Z. f. Immunitäts forschung*, 1932, **76**, 393.

4 Pagel, W., *Tubercle*, 1934-35, **16**, 256; *J. Path. and Bact.*, 1940, **50**, 111.

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

occurs after inoculation of 10^{-3} mg tubercle bacilli, but either medium will produce profuse growth after inoculation of 10^{-6} mg bacilli if 5% unheated serum is added to the medium.

The total lipoids extracted from the serum by alcohol and ether are inhibiting and the phospho-lipins are without effect in contrast to what was observed in egg yolk.[6] When the proteins are precipitated by alcohol and redissolved in distilled water, they have the same growth-promoting effect as the original serum, while the filtrate (after removal of the alcohol) is inhibiting.

Separation of the serum proteins in albumen and globulin by half saturation with ammonium-sulfate, showed the albumen fraction to be strongly growth-promoting (after dialysis), while the globulin fraction was inhibiting or without effect. Crystalline horse serum albumen after three recrystallizations was strongly growth-promoting when added in a concentration of 0.1% to synthetic medium; rapid and abundant growth occurred after inoculation of as little as 10^{-7} mg tubercle bacilli. Autoclaving for 10 minutes at 115°C of the synthetic medium containing the serum albumen destroys the growth-promoting effect.

Horse serum albumen after 3 recrystallizations still contains numerous impurities, amongst which are globulins, enzymes, pigments and flavoprotein. Experiments are now in progress to prepare a horse serum albumen completely free of such admixtures.

The growth-promoting activity of crystalline horse serum albumen is much greater than that of the lipoid extracts of egg yolk previously described. Addition of 0.3% egg yolk gave good growth

TABLE I.

No. of Colonies of Tubercle Bacilli Developing in Depth of Media After Inoculation of Varying Amounts of Bacilli.

Mg tubercle bacilli inoculated	Glycine medium	Asparagine-ammonium citrate medium	Glycine medium with 0.1% crystalline horse serum albumen	Asparagine-ammonium citrate medium with 0.1% crystalline horse serum albumen
10^{-1}	7	innumerable	innumerable	innumerable
10^{-2}	0	8	"	"
10^{-3}	0	3	"	"
10^{-4}	0	0	"	"
10^{-5}	0	0	"	"
10^{-6}	0	0	80	"
10^{-7}	0	0	6	30
10^{-8}	0	0	0	1

[6] Boissevain, C. H., and Schultz, H. W., *Am. Rev. Tuberculosis*, 1938, **38**, 624.

after planting 10^{-5} mg bacilli while 0.1% horse serum albumen supports growth after planting 10^{-7} mg bacilli.

Summary. Crystalline horse serum albumen was shown to be strongly growth-promoting for tubercle bacilli grown in synthetic media. Horse serum globulins were without effect or inhibited growth.

11372 P

Association of Tetanospasmin with Hemoglobin in Acute Stages of Tetanus Intoxication of Guinea Pigs.

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The object of this communication is to report on a new observation that tetanus toxin (tetanospasmin) may be found intimately associated with the hemoglobin of guinea pigs during the paralytic stages of the disease. A part of an extensive series of experiments on this problem is briefly summarized, as follows:

Guinea pigs weighing each 350 g were injected subcutaneously in the abdominal region with tetanus toxin* in doses ranging between 20 to 40 M.L.D. and bled from the heart during the stages of complete paralysis and spasmodic contractions (*i.e.*, 24-48 hours following the subcutaneous injection). The pooled blood was defibrinated by shaking with glass beads; filtered through several layers of gauze, and centrifuged at room temperature for one-half hour. The clear, dark red supernatant plasma and the sedimented red blood cells were placed in separate containers.

Washing of red blood cells in 1% NaCl solution was repeated until the supernatant fluid became biuret-negative (usually 4 washings are required). The washed cells were diluted 1:5 in distilled water. When fairly complete laking and some crystallization occurred about one hour later, the solution was centrifuged for one-half hour also at room temperature. This procedure resulted in separation of a dense dark red sediment containing masses of hemoglobin crystals; a superimposed loose whitish precipitate consisting of cellular debris and some hemoglobin crystals; and finally, a clear dark red supernatant fluid.

* Tetanus toxin in powder form obtained from Eli Lilly Research Laboratories (Lilly 27994, 400,000 M.L.D. per g), through the courtesy of Dr. H. M. Powell.