By the use of such antisera we have obtained precipitin graphs which suggest a 200% to 400% increase in hypothetical "hybrid" proteins in the canine circulation by the end of 18 days, with a 25% retention of these hypothetical intermediary products at the end of 3 months.[1] That the intermediary substances thus demonstrated are partially caninized horse proteins or their equivalent and not mere toxic increases in some normal non-specific canine factor is shown by our failure to obtain similar increases and retentions on intravenous injection of heterologous proteins (*e. g.,* beef serum).

Parallel with these tests we are following the parenteral history of the undenaturized residue of the injected horse proteins by means of antisera from which the canine and "hybrid" factors have been "absorbed". To prepare this altered precipitin 2 cc. crude antiserum is diluted with 7 cc. Ringer's solution, and 1 cc. normal canine serum then added. The mixture is incubated 2 hours, stored in the ice-chest over night, then passed through a Berkefeld filter. The "reduced precipitin" thus obtained gives no demonstrable reaction with normal canine proteins and presumably no reactions with proteins of dominant canine characteristics.

Our results to date indicate that undenatured horse proteins decrease rapidly in the canine circulation. But 12.5% to 25% of the routine initial dose is demonstrable at the end of 15 days, and but 1% to 3% by the 25th day. From 0.01% to 0.1% is present at the end of three months.

These figures agree with the orthodox conception of the parenteral history of chemically unaltered foreign proteins.

4632

A Flagella and Capsule Stain for Bacteria.

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The following method of flagella and capsule staining is offered as rapid, simple, and dependable. The method has been especially designed for staining *Bacillus proteus, Bacillus subtilis,* and the various members of the colon-typhoid group.

The procedure is as follows: 1. Make a thin smear of a 15-24

[1] For technic and typical graphs see PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 14.

hour agar growth of the bacteria in a loopful of water on a clean slide. Air-dry. Do not heat. 2. Cover with mordant (5% tannic acid, 3 parts; 10% ferric chloride, 1 part) for 2 minutes. 3. Put 7 drops of mordant in a watch crystal or other small receptacle and add one drop of Ziehl-Neelson carbol fuchsin stain. Mix. Add one drop of concentrated hydrochloric acid. Mix. Add one drop of formaldehyde. Mix. 4. Pour off mordant from slide and cover smear with the mixture prepared in paragraph 3. Apply 7 minutes. 5. Wash in running water. 6. Cover with Ziehl-Neelson carbol fuchsin stain (Basic fuchsin, 10 gm.; 95% ethyl alcohol, 100 cc.; phenol, 5%; water, 1000 cc.) and steam gently for one-half minute. 7. Wash in running water.

The following observations are in order. A. Remove a small portion of the youngest marginal growth from an agar culture. The medium must not be dry. Add to droplet of water upon a slide. Allow this to stand a few minutes. Then transfer a loopful to a second slide. Work with this second slide. B. The mordant will keep indefinitely and thus can be prepared in quantity. The mixture prepared in No. 3 should be used fresh. C. The action of the mordant usually requires 2 minutes. D. Steaming of the stain must not be too severe. Instead of using the recommended stain in No. 6, the more powerful mixture of anilin gentian violet and carbol fuchsin may be preferred by some. E. Filtered mordant and stain yield better preparations than unfiltered materials.

The flagella stain described is a capsule stain as well. It shows the capsules of such organisms as *Diplococcus pneumoniae, Streptococcus fecalis,* and Friedlander's bacillus when these are grown in liquid protein rich media. It is a valuable routine capsule stain for the peritoneal exudate of white mice that have been killed by Pneumococci. The following procedure is recommended: 1. Add a loopful of the exudate to a loopful of water and spread thinly upon a slide. 2. Apply mordant as described in No. 2 for 10 seconds. 3. Wash in running water. 4. Apply cold diluted carbol fuchsin stain for 10 seconds. 5. Wash with water, blot, and examine.

The undiluted exudate, stained likewise, shows the capsules well.