

found a distinct decrease in the output of inorganic sulfur on the day after feeding these pyrimidines. In some cases this decrease amounted to one-half of the amount ordinarily excreted. The writer is investigating this point further.

## 4630

**Effect of Protein Heat-Denaturation on the Precipitin Graph.**

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If 1 cc. horse serum is added to 19 cc. normal dog serum and the mixture is heated to 60° C. for several days, parallel titrations of the resulting products by means of ice-chest ripened anti-horse rabbit precipitin show a gradual flattening of the precipitin graph,<sup>1</sup> without appreciable change in the end reaction to "titer". The flattening of the graph is, therefore, taken as an index of specific protein denaturation. The stability of the end point suggests the relative reliability of the end reaction as a quantitative test.

The above tests are preliminary to an attempted interpretation of the altered precipitin graphs obtained with parenterally injected alien proteins.

## 4631

**Parenteral Retention of Undenatured Foreign Proteins.**

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Ice-chest ripened anti-horse rabbit precipitin contains: (a) a high-titer, highly active specific precipitin for horse proteins, and (b) a low-titer, relatively inert "non-specific precipitin" for canine proteins. Acting together these two precipitins presumably function as (c) a precipitin for hypothetical proteins of intermediary, "hybrid," or horse-canine specificity.

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<sup>1</sup> For technic and typical graphs see PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 14.

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.<sup>1</sup> 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 undenaturated 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

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<sup>1</sup> For technic and typical graphs see PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 14.