antibody levels against Col-SK virus could be demonstrated in 28 (21%) of 132 sera from patients, residing in Greater New York, who had been diagnosed as paralytic or non-paralytic poliomyelitis(7). Recent studies of poliomyelitis occurring in certain areas of Mexico revealed an even higher frequency of neutralizing and hemagglutination-inhibitory sera among cases and contacts(8). Barring any other plausible explanation for such differences, the possibility should be considered that geographic variation in the distribution of the virus may be a factor concerned with the selectivity of the serological results.

*Summary and conclusions.* In a series of 57 sera collected from poliomyelitis patients during the acute or subacute stage of their illness, 9 (16%) gave strongly positive reactions, 5 (9%) gave questionable reactions and 43 (75%) gave negative reactions when tested for the presence of hemagglutination-inhibitory substances against Col-SK virus. All of 12 sera from patients convalescing from Jap B encephalitis gave negative reactions. In a control group of 80 sera from healthy individuals 2 (2.5%) gave positive reactions, 4 (5%) gave questionable reactions, and 74 (92.5%) gave negative reactions. Of 14 positive or questionable poliomyelitis sera, 5 contained significant neutralizing antibody levels against Col-SK virus as determined by mouse test. The significance of these findings is discussed.

7. Jungeblut, C. W., *Arch. Ped.*, 1950, v67, 519.

8. Jungeblut, C. W., and Bautista, G., In preparation.

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## Isolation of an Active Polysaccharide Fraction from Plague Organisms.* (18889)

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An attempt to isolate an active polysaccharide from the supernatant of the Haffkine plague vaccine was made by Shrivastava(1). The substance reacted with antiplague horse and rabbit sera but the yield from a large quantity of supernatant (200 litres) was extremely low. The protein-free casein hydrolysate medium of Mueller and Johnson(2) as modified by Seal and Mookerji(3) offered an opportunity to reopen the investigation. The results of the trials to isolate the polysaccharide fractions from 3 sources, namely, (a) supernatant of the Haffkine plague vaccine prepared in the casein hydrolysate broth, (b) bacterial debris of the same vaccine, and (c) specific soluble protein of plague bacilli (Seal) (4) are reported below.

(A) *Isolation from the supernatant of the Haffkine plague vaccine. Strains used:* 1) Virulent plague strains 337/L and 139/L obtained from human plague cases; 2) Avirulent protective plague strain 53H/av originally obtained from a human plague case and made avirulent in the laboratory; 3) Avirulent non-protective plague strain TRU isolated by Schutze(5) from Java human strain Tjiwidej R(4) *Past-pseudotuberculosis* strains, PR/I and PR/IV, obtained from the National Type Cultures Laboratory, London.

*Methods.* Seven liters of bacteria-free filtrate of 3 weeks' growth, at 28°C, of the virulent plague strain 337/L in casein hydrolysate broth at pH adjusted to 7.2-7.3, was re-

* This work was originally carried out in 1942-43 at the Haffkine Institute, Bombay.

1. Shrivastava, D. L., *Annual Rep. Haffkine Inst., Bombay,* for 1938, p. 40.

2. Mueller J. H., and Johnson, E. R., *J. Immunol.,* 1941, v40, 33.

3. Seal, S. C., and Mookerji, S. P., *Ann. Biochem. and Exp. Med.,* 1950, v10, 79.

4. Seal, S. C., *Annual Rep. Haffkine Inst., Bombay,* for 1940-41, p. 48; *J. Immunol.,* 1951, in press.

duced to 1/7 of its volume *in vacuo* over a water bath maintained at 45°-47°C. The material was clarified by centrifuging and the Molisch positive washings of the residue and water were added to bring the volume to 1500 ml, followed by 40 g of sodium acetate, 9 ml of glacial acetic acid and 3 volumes of alcohol and the mixture was left overnight at room temperature. The precipitate thus formed was recovered by centrifuging, taken up in 200 ml water and mixed with 4 ml of glacial acetic acid and 10 ml of saturated solution of sodium acetate. The clarified filtrate of this material, which was strongly Molisch and faintly biuret positive, was precipitated with one half volume of absolute alcohol at room temperature and its supernatant, left after removal of the precipitate, was also similarly treated. The precipitates thus obtained were pulled together and purified by repeated treatment with glacial acetic acid, sodium acetate solution and one half volume of absolute alcohol and finally dried in a desiccator over $P_2O_5$. The total yield was 23.4 mg.

*Serological behavior.* A solution of the substance diluted to contain 1 mg/ml was put up for ring-precipitation test with 12 antisera produced in rabbits against various plague and pseudotuberculosis antigens given I.V. The results are given in Table I.

The results given in Table I show that the polysaccharide fraction isolated from the virulent plague bacillus reacted with antisera raised against both virulent and avirulent protective plague strains *viz.*, 337/L and 53/Hav respectively. This activity was retained by these sera even after absorption with the non-protective plague strain TRU or the pseudotuberculosis strain PR/I, but the substance gave completely negative reaction with antisera produced against 337/L boiled suspension, TRU, PR/I and the protein fraction P(½-⅓). Thus the substance was related to the specific soluble protein or Antigen I of plague bacillus (corresponding to the envelope antigen of Schutze)(5), and not to its somatic part or Antigen II of plague bacillus which corresponds with the antigens used for producing the antisera giving negative reactions

5. Schutze, H., *Brit. J. Exp. Path.*, v13, 284.

TABLE I. Results of Ring-Precipitation Test of Plague Polysaccharide (1 mg/ml) Against Various Absorbed and Unabsorbed Antiplague and Anti-pseudotuberculosis Rabbit Sera.

| Antisera | Result |
|---|---|
| 1. 337/L (live)* | 2+ |
| 2. " abs with TRU | 1+ |
| 3. " " " PR/I | 1+ |
| 4. " (boiled ½ hr) | 0 |
| 5. TRU (live) | 0 |
| 6. PR/I (live) | 0 |
| 7. 53/Hav (live) | 2+ |
| 8. " abs with TRU | 1+ |
| 9. " " " PR/I | 1+ |
| 10. Lauto P⅓ (plague protein) | 2+ |
| 11. $L_5$ P⅓ ( " " ) | 1+ |
| 12. $L_5$ P½-⅓ ( " " ) | 0 |

* Notes in parentheses are description of antigens used for the production of antisera, No. 10-12 being the specific soluble protein fractions of plague bacillus (Seal)(4).

(Seal)(4). Consequently attempts to isolate this substance from the non-protective plague and pseudotuberculosis strains which are related antigenically to the somatic part of plague bacillus, failed.

(B) *Isolation from the bacterial debris of the Haffkine plague vaccine.* Fifty ml of bacterial debris, obtained by centrifuging 3½ weeks' growth, at 28°C, of virulent plague strain 337/L in casein hydrolysate broth, was hydrolysed with 6% $H_2SO_4$ over water bath under reflux for 5 hours. The solid part of the hydrolysate was removed in the centrifuge and the liquid portion neutralized to Congo-red paper with sodium acetate, boiled with animal charcoal and filtered. Osazone crystals were prepared from this filtrate and purified according to the method used by Linton *et al.*(6). The crystals, as seen under the microscope, resembled *Arabinose* osazone (Fig. 1) and gave a melting point of 166°-168°C, the same that of known *Arabinose* osazone. Similar osazone crystals were also prepared from the debris of another virulent plague strain 139/L and avirulent protective plague strain 53/Hav, while TRU strain showed only a few stray crystals but none could be isolated from the debris of pseudotuberculosis, PR/I and PR/IV.

(C) *Isolation from the specific soluble pro-*

6. Linton, R. W., Mitra, B. N., and Seal, S. C., *Ind. J. Med. Res.*, v23, 610.

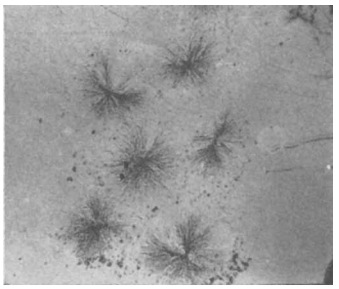

FIG. 1. Microphotograph of osazone crystals obtained from the virulent plague strain, 337/L.

*tein of plague bacilli.* The same method as used in the case of bacterial debris was employed for preparing osazone crystals from the specific plague proteins, L auto P1/3 and $L_5$P1/3. The same type of crystals in a purer form was obtained in both of them, the melting point being 166°-168°C.

*Comments.* In the attempt to isolate polysaccharide from the supernatant of the Haffkine plague vaccine prepared in the enriched casein hydrolysate broth a very small quantity (23.5 mg) could be isolated from 7 liters of the vaccine. From the nature of its chemical and serological reactions it seems that the substance is intimately associated with the specific soluble proteins for the protective plague strains. This has been confirmed by isolating an arabinose-like osazone (melting point 166°-168°C) from the plague proteins, $L_5$P1/3 and Lauto P1/3. The same osazone crystals were found in the protected plague strains 139/L and 53/Hav but not in the avirulent non-protective plague strains TRU or in the pseudotuberculosis strains, PR/I and PR/IV. These findings lend support to the view that the specific protective substance of plague bacilli is a polysaccharide-protein complex, probably a nucleoprotein.

*Summary.* A polysaccharide yielding osazone resembling that of *Arabinose* with a melting point of 166°-168°C has been isolated from the supernatant of the Haffkine plague vaccine as well as from the specific soluble protein and, to a less extent, from the bacterial debris of both virulent and avirulent protective plague strains. It is absent in non-protective avirulent plague and pseudotuberculosis organisms. The protective substance of the plague bacillus is probably a polysaccharide-protein complex.

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## Serum Iron and Hemoglobin Values of 275 Healthy Women.* (18890)

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The importance of serum iron in the intermediary metabolism of iron is recognized. Many investigators have reported serum iron values for women and these values range from a low of 30 μg % as reported by Eckerström (1) to a high of 210 μg % as reported by Vahlquist(2). There is some variation in the range of values which is considered by different investigators to be normal for women. Dahl(3), who has studied the greatest number of subjects recently, suggests 70 μg to 140

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1. Eckerström, S., *Acta Med. Scand.*, 1944, v118, 69.

2. Vahlquist, B. C., *Acta Paediat.*, 28, suppl. 5, 1941.

3. Dahl, S., *Brit. Med. J.*, 1948, v1, 731.