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Precipitable Substances Prepared from Bacilli of the Salmonella Group.

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Extending previous work,[1] soluble specific substances were prepared from a number of types of the Salmonella group which yield large amounts of reducing sugar on hydrolysis, and are resistant to tryptic and peptic digestion and to the action of alkaline hypochlorite solution. The group reactions of these substances correspond in a general way to those of the so-called stable agglutinogens[2] regardless of the method in which the extracts were prepared. (Digestion of the autoclaved bacilli with trypsin, or extraction of the alcohol treated bacilli by heating with saline solution, or dissolving the microbes with alkaline hypochlorite solution.) This parallelism was also seen when the specificity was tested by means of immune sera absorbed with heterologous bacilli of the Salmonella group.

TABLE I. *Precipitation tests.*

Precipitable substances (crude preparations)		Immune sera prepared with bacillary suspensions of the serological types						
Obtained from	Dilution 1	Typhosus	Enteritidis	Paratyphosus B	Stanley	Reading	Newport	Suipestifet
Typhosus	2000	+++	++	0	0	±	±	tr.
	20000	++	+±	0	0	±	0	±
Enteritidis	2000	+++	++±	0	0	0	0	0
	20000	++	++	0	0	0	0	0
Paratyphosus B	2000	++	++	+++	+++	++±	f. tr.	0
	20000	+	++	++	+±	+++	0	0
Stanley	2000	0	++±	++	++	++++	0	0
	20000	0	+±	++±	++	+++	0	0
Reading	2000	tr.	++	+++	+++	++±	0	f. tr.
	20000	0	±	+±	±	+	0	0
Newport	2000	0	0	++	+±	0	++++	0
	20000	0	0	+±	+	0	++	0
Suipestifer	2000	0	0	0	0	0	+++	+++
	20000	0	0	0	0	0	+++	++±

0.2 cc. of the antigen dilutions plus 1 drop of immune serum. The test tubes were kept for 2 hours in the room and over night in the ice box.

[1] Landsteiner, K., and Furth, J., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 379, 602, 771; *J. Exp. Med.*, 1928, xlvii, 171.

[2] White, P. B., Med. Research Council, Special Report Series, 1926, 103.

One striking exception to this was observed with the soluble substance of *B. paratyphosus B* and *Stanley*. The sera for these types when absorbed with bacilli of the strains *Reading* or *Abortus Equi* no longer precipitated the homologous soluble specific substance, although they still agglutinated the homologous bacilli, a reaction in which factor I (White) is involved. It was found that this loss was due to a high sensitivity to alkali of the precipitable substance as originally extracted by saline solution. On the contrary, the other reactions mentioned persisted even after boiling the preparation for several hours with normal sodium hydroxide, whereas hydrolysis with normal hydrochloric acid for a few minutes resulted in liberation of reducing sugar and the destruction of the serological properties. The reactions for factor I were not altered by digesting the substance with pepsin or trypsin at a pH which in itself was not injurious.

It was attempted to establish whether or not by precipitation with immune sera various fractions could be separated from the precipitable substance of one bacillus as one would expect if a special substance would correspond to each single serological factor. In these tests the sera were properly absorbed with heterologous bacilli in order to prepare antibody solutions active for certain factors only. The washed precipitates were dissolved by boiling for a few seconds with dilute alkali. On the whole the results did not indicate a separation of antigen fractions by this method except perhaps in one case. Such a separation, however, could easily be accomplished under the same conditions when a precipitate was made with a mixture of substances derived from different bacilli.

The specific substances of *B. paratyphosus B* and *B. typhosus* reported on previously could be further purified. The preparation recently obtained from *B. paratyphosus B* yielded on hydrolysis with n/2 HCl for 5 h. 77.3% reducing sugar (calculated as glucose) and contained 0.66%. The corresponding substance from *B. typhosus* containing 1.02% N gave 73.2% sugar on hydrolysis. This preparation was precipitated by a common typhoid immune serum up to 1:1,000,000. On hydrolysis of the above substances some insoluble material separated out.

By immunization with small amounts of the preparation described at P2 typhosus[1] immune sera were obtained which agglutinated typhoid bacilli. After removal of the small flaking agglutinins by absorption with typhoid bacilli heated to 100° C. for 2 hours this agglutination had the character of that produced by large flaking agglutinins. Sera with similar properties were produced by injection of typhoid bacilli boiled with alcohol. In both instances

the large flaking agglutinins differ somewhat from those of common typhoid immune sera.

The substance P_2 was found to be highly toxic for rabbits and rats.

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Relation of Cholesterol and Lecithin to Remission in Pernicious Anemia.

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It has been recognized that the cholesterol and lecithin content of the blood are low in severe relapses of pernicious anemia and that these constituents tend to increase with the improvement of the patient's health. Whether the increase of cholesterol and of lecithin bears any definite relationship to the remission of the anemia has not been determined.

Determinations of cholesterol and lecithin in the plasma of 25 cases of typical pernicious anemia have been made, usually every other day over a period of many weeks. Cholesterol was determined by Bloor's method[1] and the lecithin according to the method described by Whitehorn.[2] On admission 4 cases had less than 1.0 million red blood cells, 7 had between 1.0 million and 2.0 million, and the other 14 had over 2.0 million per cu. mm. Of the 4 cases with less than 1.0 million red blood cells per cu. mm., all showed low values for both cholesterol and lecithin; in some instances less than 50% of normal. Two of these 4 cases had a rapid remission, one died soon after entering the hospital, and the fourth is still under observation.

The first case entered the hospital with a red blood cell count of 950,000 per cu. mm. The plasma cholesterol was 93 mg., and the lecithin phosphorus 4.3 mg. per 100 cc. For 17 days the patient was fed a test preparation. During this time the red blood cells fell to 644,000 per cu. mm., the cholesterol fluctuated between 56 and 93 mg. per 100 cc., while the lecithin phosphorus varied between 4.3 to 5.7 mg. per 100 cc. The patient was then given another test preparation of the same cholesterol content. A remission promptly

[1] Bloor, W. R., Pelkan, K. F., Allen, D. M., *J. Biol. Chem.*, 1922, lii, 191.

[2] Whitehorn, J. C., *J. Biol. Chem.*, 1924, lxii, 133.