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A preliminary report on the chemical composition of residue antigen.

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In a number of preparations of residue antigen obtained from various micro-organisms, chemical analyses have been carried out to find whether or not the presence of proteins, etc., could be definitely excluded. To this end, total nitrogen has been determined, together with amino-nitrogen before and after acid hydrolysis of the preparation.

The bacteria were grown in large agar plates, washed off with saline and treated in the manner previously described in detail,[1] and the resulting material dried in the dessicator.

Specific precipitin tests were given by saline solutions of the dry material with immune rabbit sera, but insufficient quantities were available to attempt to correlate the dilution at which the test became negative, with the chemical composition. For analysis, the entire specimen was carefully weighed, brought into solution in a few cc. of water, and diluted to a definite volume, usually in a 10 cc. volumetric flask, aliquot portions being taken for separate determinations.

Total nitrogen was determined by the micro-kjeldahl method, titrating with 0.02 N solutions, and the amino-nitrogen determinations were carried out by the micro-Van Slyke method.

Phosphorus was determined by Tisdall's[2] method, after wet ashing.

In those preparations in which sufficient material was obtained for duplicate analyses, agreement was fairly uniform. However, we do not believe that absolute accuracy can be claimed for all the determinations, particularly those of amino-nitrogen after

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1 Zinsser and Parker, *J. Exp. Med.*, 1923, xxxvii, 275.

2 Tisdall, *J. Biol. Chem.*, 1922, l, 329.

hydrolysis, because of the small quantity available for use, and the relatively low content of nitrogen. The values for total nitrogen are probably the most accurate.

It will be observed that there is a rather considerable variation in each type of nitrogen in the different lots. Considering the results as a whole, one sees that the low and variable total nitrogens point to the presence of a nitrogenous substance associated with a non-nitrogenous compound. Concerning the nature of the former, it may perhaps be peptone or polypeptid, since an increase in amino-nitrogen is regularly found after hydrolysis. In connection with the probable non-nitrogenous fraction, of interest is the recent work of Avery and Heidelberger,[3] in which an undoubtedly similar substance prepared by them from pneumococcus broth cultures was shown to be largely a complex carbohydrate. The presence of phosphorus introduces a further possibility of complication, suggesting as it does the presence of nucleic acid derivatives or carbohydrate-phosphate combinations.

The usual color test for proteins and their split-products; *i. e.*, biuret, Millon's, Hopkins-Cole, salicylic acid, ninhydrin and xanthoproteic, were carried out on small quantities of the material in fairly strong solution. Results were uniformly negative, except for an occasional biuret and almost regularly a faint xanthoproteic test.

For the separation and identification of the components of such a mixture, considerable quantities of material will probably be required, quantities practically out of the question if one must depend on micro-organisms of the types used in this study as a source. Since all bacteria which have been investigated thus far, have yielded substances having the same general properties, it is not unreasonable to suppose that the phenomenon may be still more general, and that yeast may produce similar residue antigens, opening the way to a continuation of the work with material which may readily be procured on a large scale. Experiments in this direction have clearly shown that extracts prepared from Fleischmann's yeast will yield a specific precipitate with sera of rabbits immunized to a strain of yeast isolated from this

[3] Avery and Heidelberger, *J. Exp. Med.*, 1923, xxxviii, 73.

material. A further study of the properties of this extract is now under way.

	Wt. of samples	Total nitrogen	Animo nitrogen	Animo nitrogen after hydrolysis	Phosphorus
	mg.	per cent	per cent	per cent	per cent
Tubercle Bacillus	25.32	2.83	.19	1.91	
	22.18	4.50	.31	1.43	
	21.96	1.96	.035	1.53	
Pneumococcus Type I.	18.17	6.23 6.07	0.57 0.53	2.06 2.10	1.24
	14.2	4.45	.34	1.82	—
	28.36	1.45	.16	.47	—
	18.08	3.81	.12	.69	—
	20.8	3.28	.32	1.10	—
	20.7	4.20	—	—	—
Meningococcus	18.86	3.69 3.76	0.41 0.40	3.38 3.49	2.2
	25.1	5.21	—	—	—
	18.06*	2.44	.16	.19	—
	14.88*	2.25	—	—	—
	16.8 *	1.64	.16	.47	—

*Represent successive stages of an attempt to purify a single preparation.

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The preserving effect of alkali on the blood cells of Limulus.

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1. According to Leo Loeb (1910) alkali may under certain conditions have a preserving effect on the blood cells of *Limulus*.[1] If we add a drop of *Limulus* blood to an excess of an isotonic sodium chloride solution, those amebocytes which come into direct contact with the solution soon send out sharp pseudopodia and lose their granules and become hyaline. Similarly in a watery solution of very weak alkali the cells soon lose their granules and are dissolved; but if both substances are combined, a small

[1] Loeb, L., *Pflüger's Arch.*, 1910, cxxxi, 465.