15 (*B. coli*) did not grow on the solid medium nor did they produce any turbidity in the liquid medium.

15 (*B. acrogenes*) darkened the solid medium and produced a turbidity in the liquid medium.

2 (*B. coli* intermediate) darkened the solid medium and produced a turbidity in the liquid medium.

Comparing the Koser solid medium in which ferric ammonium citrate was substituted for the sodium citrate with the Harder solid medium, it was evident that on the latter the growth and intensity of the red color was much better.

The Harder solid medium offers an easy and brilliant method for the differentiation of *B. acrogenes* and *B. coli* of non-fecal origin from *B. coli* of fecal origin.

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The specific fraction of alcohol soluble specific substance of the tubercle bacillus.

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In a previous paper[1] it was shown that the specific substance of the tubercle bacillus, soluble in lipoid solvents, can be prepared from ether, ethyl and methyl alcohol extracts in a similar grade of purity; the potency and the chemical composition of the preparation is very similar. With the further purification of the product as described in the paper mentioned, and with the separation of it into different fractions by precipitation from warm methyl alcohol, from acetic acid and from chloroform solution, etc., we never obtained markedly more potent preparations. The bulk of the purified substance, which is supposed to contain a large percentage of the specific substance, is composed of fatty acids. Besides these, it contains quite a large amount of P. (2.6 per cent), home reducing sugar (in one case only 4 per cent), and it contains only traces of N. (0.3 per cent).

[1] Dienes, L., and Schoenheit, E. W., *J. Immunol.*, 1925, x, 631.

During further work with this preparation a fortunate chance helped us to separate the antigenically active part of the preparation from the lipoidal part. Quite a large amount of the specific substance was prepared from an alcohol extract of tubercle bacilli as formerly described. The preparation showing the maximum potency was dissolved in ether, and the ether solution was washed with diluted HCl for the elimination of the substances containing N, then several times with distilled water, and kept for 24 hours over anhydrous Na_2SO_4. The perfectly clear solution, which after this procedure has shown unchanged potency for several weeks, first developed a turbidity, then deposited a heavy precipitate and simultaneously with the appearance of the precipitation its potency decreased. The container during this time was opened several times, but after every opening filled with CO_2. The precipitate was freely souble in methyl alcohol and in water, and in the complement fixation test it was

TABLE I.

	Potency in complement fixation Unit	Solubility		Chemical Composition
Specific substance in the original ether solution.	0.0002 mg.	Freely soluble in ether, about 2 per cent in alcohol; is precipitated with aceton. The aceton contains only traces of the specific substance.	It is extracted from a watery emulsion with ether and it is retained by a paper pulp filter.	About 65% fatty acids 2.6% P, and 0.3% N.
Aceton soluble fraction after splitting.	0.00004 mg.	Non-soluble in ether; freely soluble in alcohol, aceton, water.	It is not extracted from a watery emulsion and is not retained by paper pulp; not even if the watery emulsion is made from a mixture of the specific substance and a crude alcohol extract.	15% fatty acids(?) 7.2% P and 0.5% N.
Ether soluble fraction after splitting.	0.02 mg.	Freely soluble in ether, alcohol and aceton.		80% fatty acids(?) 1% P.

5 times as potent as the formerly most potent preparations. By precipitation with aceton the specific substance went into the aceton solution. The aceton solution was evaporated and extracted first with ether, then again with aceton. In Table I are described some properties of the specific substance before and after separation from the lipoids, and of the remaining fatty substances after the separation. In addition the specific substance in the state of the highest purity gave 17 per cent carbohydrates after hydrolysis, and by the combustion (according to Pregl) has shown 8.85 per cent H and 36.0 per cent C. The significance of the carbohydrates is not established, because the aceton non-soluble residue of the precipitate contained 33 per cent carbohydrates without showing any specific activity. It may be that the fatty acids present in the specific substance (the ether soluble substance after hydrolysis) are the result of a contamination with the ether soluble substance.

The changes in the properties of the specific substance and of the remaining solution after their separation are so thorough that we must suppose that it is the result of a chemical splitting. The alcohol soluble specific substance of the tubercle bacillus, as we obtain it in our extracts, consists according to this, of a non-lipoidal specific part containing no, or at least very little, chemically connected with lipoid substances. It may be that the connection with the lipoid substances is the cause of a weak antigenic effect *in vivo,* although the antibody formation has only been observed with non-purified extracts.

In case the specific substance in the original extracts forms a chemical compound with the lipoids we must suppose the existence of a new class of lipoid substances which contains P and no N and the yet undefined specific substance in the molecule.

If the substance described in this paper is the same as the substance found by Laidlow and Dudley[1, 2] cannot be decided. The solubility of the two substances seems to be different.

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[1] Dienes and Schoenheit, E. W., *J. Immunol.*, 1925, x, 631.

[2] Laidlow, P. P., and Dudley, H. W., *J. Exp. Path.*, 1925, vi, 197.