paragine (134.2), in spite of a higher calculated m.v., penetrates the intestinal wall far more rapidly than malonamide (104.4), the percentages of absorption being 61.6 and 16.8 respectively. In another experiment, in which succinamide (126.4), asparagine (134.2) and xylose (153.4) were alternately placed in the same loop, the percentages of absorption were found to be 9.2, 46.2 and 7.7. Finally, when a mixture of malonamide plus xylose is compared with asparagine plus xylose, the following percentages of absorption are observed: malonamide plus xylose 11.7 and 13.3 respectively, asparagine plus xylose 65.6 and 10.7 respectively.

These results as well as others suggest that the amino acids, like some hexoses, exhibit preferential intestinal absorption. It may be that, in both cases, specific cellular mechanisms favoring the entrance of particularly useful substances into the body are involved. This also seems to be true of the reabsorption of substances by the kidney tubules, which, at least in the case of the frog, have been shown to retain selectively Cl ions, some hexoses (chiefly glucose and galactose) and amino acids.

The next step in our investigation will be directed towards the elucidation of the mechanism effective in the selective absorption.

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A New Rapid Method for Direct Typing of Pneumococcus in Sputum of Pneumonia Cases.

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The diagnostic laboratory is not lacking in reliable methods for the typing of pneumococcal pneumonias.[1, 2, 3] Most noteworthy, perhaps, is the Neufeld method[4] which affords direct typing of pneumococci in the sputum by a "Quellung" or swelling of the bacterial capsule in the presence of the type-specific antiserum. In this preliminary report we shall briefly outline the technique and the merits of a new method.

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1 Avery, O. T., *J. Am. Med. Assn.*, 1918, **70**, 17.

2 Kohn, L. A., *J. Am. Med. Assn.*, 1925, **84**, 1733.

3 Rosenthal, L., and Sternberg, B., *J. Lab. and Clin. Med.*, 1929, **15**, 78.

4 Cooper, G. M., and Walter, A. W., *Am. J. Pub. Health*, 1935, **25**, 469.

Technique: The method involves a serological test for precipitinogen in the sputum. Freshness of the sputum sample is not obligatory. A few drops of sputum suffice for the test; ½ to 1 cc. is optimal for reasons of convenience. The sputum is treated with an equal volume of a 10% solution of saponin (Merck) in 0.9% saline. The mixture is then shaken and incubated at 37°C. for one-half hour. To aid in clarification approximately ¼ volume of a stock suspension of aluminum hydroxide* is added to the mixture with brief stirring. This is then promptly centrifuged at high speed for 15-20 minutes. The supernatant, which is clear, or at worst homogeneously opalescent, is then tested for type-specific precipitinogen by the routine "ring-test" method.

For type-specific antibody, horse antipneumococcus serum has been found satisfactory. It is important that the antiserum be clear. The undiluted type-specific antiserum is pipetted into narrow-bore precipitin tubes (2.5-3 mm. inside diameter) with a capillary pipette and is carefully overlaid with about ½ volume of the sputum-antigen. In the homologous type antiserum, ring formation at the interface often begins to appear immediately, or after a few minutes. The tubes are incubated at 37°C. and re-observed after one-half and one hour. With the technique described above, a specific ring-test was always confirmed by the later formation of specific precipitate upon mixing antigen and antiserum in the precipitin tube. For this purpose the tubes may be re-incubated for one hour after shaking and then refrigerated over night.

It is advisable to set up a control ring-test with saponin solution in place of antigen (saponin-treated sputum). If the antiserum is turbid, non-specific rings are occasionally obtained with saponin in 1:20 dilution. This precipitation at the interface is hazy and somewhat diffuse and is readily differentiated from specific "rings." Furthermore, such precipitated material in the control tube disappears on shaking, and in no case does a precipitate reappear on prolonged incubation. These control rings have been encountered in only 3 of 54 tests. With clear antiserums, this occasional complication is entirely absent; such serums never give false "rings."

Sputum samples from 22 unselected cases of lobar pneumonia

* The $Al(OH)_3$ is prepared as follows: to a 10% solution of potassium alum an excess of dilute NH_4OH is added drop by drop with constant stirring until the resultant milky suspension is distinctly alkaline to litmus. The precipitate is then washed several times with water until neutral to litmus, and is then resuspended in water to about the volume of the original alum solution. This stock suspension is stable for the present purpose; however, it must be shaken before use.

have been tested by this new method. With the first 8 samples, the $Al(OH)_3$ treatment was omitted. Correct typings by means of specific "rings" were obtained within one-half hour in 20 of the 22 cases. Of the 20 successful typings, only 3 failed to give a specific precipitate after further incubation. All these 3 were sputums which had not been treated with $Al(OH)_3$. All typings were confirmed by the mouse method of Sabin.[5]† The positive results include cases of Pneumococcus Types I, II, III, V, VI, VII, VIII, IX, XVIII, XXII. Two sputums failed to give a typing by this method. One of these, an atypical case of Type III lobar pneumonia, showed so few pneumococci in the sputum that the failure to detect specific precipitinogen was readily understandable. This sputum failed to type by the Neufeld as well as by the mouse method: the pneumococcus could be typed only after isolation in pure culture. The other sputum which failed to give a typing was a Type VII lobar pneumonia. This sputum was obtained after crisis, on the sixth day of the disease, but contained a "moderate" number of pneumococci.

Perhaps the chief objection to all other methods for detecting precipitinogen in the sputum lies in the difficulty of obtaining an antigen of sufficient clarity for a precipitin test. In the method described here, this difficulty seems to have been successfully overcome by the use of aluminum hydroxide as a clarifying agent. It may be pointed out, furthermore, that the authors believe the new test is less subjective, more easily mastered, and not significantly more time consuming than the Neufeld test. The ultimate proof of the value of this test must await a more extended series of cases.

Summary: A new rapid method for the direct typing of pneumococci in sputum is outlined. In this method, saponin is used to dissolve the bacteria[6] and liberate the type-specific antigen. Aluminum hydroxide was employed for clarifying the sputum.

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[5] Sabin, A. B., *J. Infect. Dis.*, 1930, **46**, 469.

† For the results of the mouse typings we are indebted to the personnel of the bacteriology diagnostic laboratory of this institution.

[6] Klein, S. J., *J. Bact.*, 1935, **30**, 43.