

A SUBSTITUTE FOR POTATO AS A CULTURE MEDIUM.*

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THE appearance of bacterial cultures on potato, which in the early days of bacteriology was considered of diagnostic value, has been shown to be subject to considerable variation. The cause of this variability is sometimes due to differences in composition and reaction of potatoes of various origin. The "invisible" film of *B. typhosus* is an example of the unreliability of potato as a substratum. On potatoes of slightly acid reaction there is a hardly visible growth; if the reaction is alkaline, however, there is abundant growth, which resembles that of other bacteria. The "honey" appearance of *B. mallei* also remains indistinct, if the potato is more acid than usual. Still, most bacteriologists adhere tenaciously to the use of potato as a culture medium, because certain characters develop better on this medium than on any other. Pigments especially often develop abundantly on potato, and frequently appear on this medium only. The secretion of an amylolytic enzym can be demonstrated only on a starch-containing medium. A substratum which enables microorganisms to exhibit these faculties to advantage must, therefore, be of some value.

Media have been prepared by expressing the juice of potato and then adding other ingredients. Although this method permits the adjustment of the reaction, there is nothing gained in knowledge of the chemical composition. I have been able to find only one attempt to substitute a synthetic medium for potato, the "starch jelly" of E. F. Smith.[†] This is prepared by filling 2 grams of starch, freed from impurities by repeated washing in water, into dry culture tubes and adding 10 c.c. of a solution of ammonium sulphate, sodium asparaginate, magnesium sulphate, sodium chloride, dipotassium hydrogen phosphate, calcium chloride, sodium sulphate, or in place of this solution, Uschinsky's medium, omitting the glycerin. The tubes are then heated in a Koch inspissator in a slanting position for two or three

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hours on each of five or six consecutive days at a temperature of 75° to 85° C. The starch swells and forms a jelly. By this long exposure to heat the water evaporates and has to be replaced. The resulting medium breaks easily and has a rough surface.

This paper came to my attention after I had experimented for several months with a medium, the preparation of which is simpler than the one described above. Starting with a composition suggested by the results of chemical analyses of potato, it was found by a series of cultures that some constituents were of no value, and that the quantity of the necessary constituents might be reduced. The following method of preparation has been found the most advantageous and permits considerable latitude for varying the composition. Fifteen grams of agar are dissolved by heat in about 600 c.c. water. A solution of the following salts in 200 c.c. water is then prepared:

Asparagin	5 gr.
Dipotassium hydrogen phosphate	2
Disodium hydrogen phosphate	2
Magnesium sulphate	2
Calcium chloride	2
Ammonium lactate	2

This solution, in which a fine precipitate is formed, is added to the hot agar solution, 10 grams of peptone are added, and the whole mixture filtered after the reaction, which is about 5 per cent acid, is brought to the neutral point with phenolphthalein as an indicator. To the hot filtered solution a suspension of 30 grams of washed starch, made perfectly homogeneous in a mortar, is gradually added with constant stirring. The mixture is then brought to near the boiling-point and finally weighed. The total should weigh 1,000 grams. The medium is tubed and sterilized in the autoclav for five minutes at 120°, and is cooled in a slanting position. The salts used in the medium are the principal ones contained in potato according to chemical analysis.

This mode of procedure may be varied in several ways. By the mode of preparation described a jelly is obtained which is slightly opaque, homogeneous, smooth, and of sufficient firmness to retain the slant desired. If the salt solution is added after filtration of the agar, the white precipitate will be held in suspension by the swelling starch, and, if the starch has not been heated too long, a whitish, opaque medium results, which shows pigments to great advantage.

The addition of glucose increases its nutritive value, but brings out no amyolytic activity of the microorganisms.

I claim the following advantages for this medium over ordinary potato:

1. The composition is always the same, and the reaction may be adjusted to suit the purpose of the study.
2. The chief nutrient substances of potato, especially starch, are in a highly assimilable form.
3. The disadvantage of potato—i. e., variability in composition—is avoided. The advantages—i. e., pigment formation, amyolytic action, and gas formation—are not only preserved, but are more pronounced than on potato.
4. Diffusible pigments are readily differentiated from non-diffusible ones.
5. The preparation is simple, and the cumbersome potato tube is rendered superfluous.