THE VALUE OF VEGETABLE EXTRACTS IN CULTURE MEDIUMS[1]

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A survey of the literature dealing with the use of various extracts from vegetables and meats as enriching agents in culture mediums shows that certain workers (Davis, 1917 and 1921; Funk and Dubin, 1921; Hosoya and Kuroya, 1923a and b; Kligler, 1919; Lloyd, 1916–17; Thjötta, 1921; Thjötta and Avery, 1921 a and b; Uyei, 1927) have considered that they were dealing with vitamines or growth-accessory substances for bacteria. The organisms which are grown with more than average difficulty are the ones usually spoken of as requiring the presence of vitamines, such as *Diplococcus pneumoniae, Neisseria intracellularis, Neisseria gonorrhoeae, Hemophilus influenzae* and various streptococci. Pacini and Russell (1918) have shown that *Eberthella typhi*, when grown in a synthetic medium, will furnish vitamines which will maintain normal development in rats, but the work of others (Ayers and Mudge, 1922; McLeod and Wyon, 1921; Thjötta, 1924) indicates that the vitamines A and B, effective in animal nutrition, do not influence bacterial growth. Another group of investigators (Cole and Lloyd, 1916–17; Morgan and Avery, 1923; Mueller, 1922 a and b; Rivers and Poole, 1921) has called attention to the value of extracts of meat, blood and various vegetables in the culture of bacteria without suggesting anything regarding the nature of the growth-promoting substance.

Extracts were made of potato, carrot, radish, spinach and beef heart, the latter for comparison. One hundred fifty grams of grated or ground material were added to 200 cc. of distilled

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water. The mixture was stirred for ten minutes and filtered through cheese cloth to remove the coarser particles. The filtrate was then centrifuged at high speed for thirty minutes and the supernatant fluid filtered through Seitz filters and stored in sterile test tubes in the ice box. Table 1 shows the total nitrogen and reducing sugars in these extracts, and indicates approximately what may be expected with this method of preparation, although there are variations between different lots.

When freshly made, the various extracts seemed to be about equal in growth-promoting power, but certain ones, especially those from spinach and beef heart, turned acid on standing and a precipitate was formed, after which their value was greatly decreased. The various extracts were heated to 56°C. for thirty

TABLE 1

EXTRACT	REDUCING SUGAR	TOTAL N
	per cent	*mgm. per cc.*
Potato	0.21	1.23
Carrot	0.86	0.42
Radish	0.36	0.18
Spinach	0.12	0.42
Beef heart	0.04	0.68

minutes, at 100°C. for ten minutes and run in the autoclave at 20 pounds pressure for fifteen minutes. After heating they were tested for growth-promoting power. In general it was found that heat had little effect except as it caused precipitation of the protein content. This was marked in some of the extracts. Potato extract was precipitated least and was found to be the best of those tried, so that most of the following work was done with it. This extract seems to keep for three to four months without losing effectiveness. In order to learn whether all the growth-giving material could be removed by precipitating the protein, a sample of potato extract was treated with 9 volumes of tungstic acid. The resulting precipitate was removed by centrifuging and the supernatant fluid was filtered to insure sterility. Tests showed that approximately half the total nitrogen was

proteid nitrogen. The precipitate was dissolved in weak alkali and made up to original volume. By using volumes of the two fractions which were equivalent to the original, it was found that both the proteid nitrogen and nonproteid nitrogen of potato extract would support equal growth of test organisms, but the growth was less than with the original. In other words, it was found that within certain limits, growth corresponded to the nitrogen content of the extract.

By the addition of increasing amounts of the extracts to tubes each containing 6 to 7 cc. of plain broth, which in this work was used as a routine basic medium, it was found that as little as 0.01 cc. would allow growth of many strains of streptococci which did not grow in the basic medium. An increase in the

TABLE 2

	TUBE 1*	TUBE 2*	TUBE 3*	TUBE 4*	TUBE 5*	TUBE 6*	TUBE 7*	TUBE 8*	TUBE 9*
Potato extract added, cc..	0.01	0.02	0.03	0.05	0.1	0.2	0.5	1.0	None
Growth in twenty-four hours.....	+	++	++	+++	+++	+++	+++	+++	None

* Each with 6 cc. of broth.

luxuriance of growth of streptococci was not noted when amounts greater than 0.2 cc. were added to the same amount of medium. Table 2 shows a typical result.

After the addition of the extract all tubes were incubated for twenty-four hours as a proof of sterility before they were inoculated. Then one capillary drop of a light suspension of hemolytic streptococcus in physiologic sodium chloride solution was added to each tube.

The value of potato extract in promoting growth of streptococci was tested as follows: To tubes containing 6 to 7 cc. of nutrient broth was added 0.2 cc. of the extract. The streptococci were picked from blood agar plates and were kept as stock cultures on blood agar slants. From these stock cultures a small amount was transferred to the test medium with a straight wire, care being taken not to carry over any of the medium. Nineteen

cultures of hemolytic streptococci grew freely within twenty-four hours. Four cultures of *Streptococcus viridans* were tested, three from the blood in cases of subacute bacterial endocarditis and one from chest fluid. All grew well in twenty-four hours, with the exception of one of the strains from endocarditis, which showed fair growth in forty-eight hours.

One culture of a hemophilic organism, corresponding culturally and morphologically with *Hemophilus influenzae*, did not grow in nutrient broth and potato extract, but did grow in brain broth and potato extract. Brain broth alone did not support growth of this organism.

Potato extract, when added to blood agar, has given a somewhat more vigorous growth of *Neisseria gonorrhoeae* than was obtained without it, but the results are not as striking as with the other types of organisms mentioned.

Certain experiments have been made to test the suitability of potato extract as an enriching substance in sugar fermentation tubes by adding 0.1 cc. of the extract to each tube containing 5 cc. of the sugar broth used (nutrient broth and 0.5 per cent sugar). The typical fermentation reactions of *Eberthella typhi* were unchanged by the addition of the extract. As a further test two strains of streptococci were chosen, one *Streptococcus viridans* which grew in the sugar broths without the addition of any enriching fluid, and the other a hemolytic streptococcus which would not. With each organism two series of tubes were set up, the first series with the extract added and the second series without it. The *Streptococcus viridans* fermented identical sugars in each series, thus proving that the addition of extract did not affect the results. The hemolytic streptococcus fermented certain of the sugars in the series of tubes containing the potato extract and failed to grow in the other series.

Attempts to grow *Diplococcus pneumoniae* in nutrient broth and potato extract have shown that growth may be obtained by using more of the extract than seems necessary with streptococci. The growth so obtained in two instances was used as an antigen in the typing test with clear-cut results, while the brain broth

cultures did not agglutinate. Further attempts are being made to determine the value of potato extract for this purpose.

The growth-promoting substance in vegetable extracts has been called a vitamine or growth-accessory factor by most workers. As previously stated, some have found that vitamine B does not aid the growth of bacteria, and have suggested that a special vitamine is necessary for bacteria. It is difficult to devise experiments which will show conclusively that a substance of unknown composition is or is not a vitamine without resorting to well established experiments in animal nutrition. I believe that the substance in fresh plant and animal tissues, which are water-soluble, may be better considered as food substances for bacteria than as food-accessory factors or vitamines. The following experiments and observations are made in support of this statement.

A tube of potato extract was used as a medium for growing *Salmonella schottmülleri*. After seven days' incubation the culture was heated to 80°C. for ten minutes and centrifuged at high speed to throw down the bacteria. The clear supernatant fluid was added to tubes of plain broth in varying amounts up to 0.2 cc. After these tubes were incubated to prove sterility they were inoculated with a culture of hemolytic streptococcus which was known to grow well when 0.2 cc. of potato extract was used. Growth was not obtained after the addition of the extract which had been used as a medium for *Salmonella schottmülleri*. Evidently the growth-promoting material had been exhausted by the paratyphoid organism. It is not surprising that a medium containing such small amounts of nitrogenous and carbohydrate substances should be exhausted by an actively growing organism in seven days. This experiment indicates that the growth-promoting substance is a food rather than a food-accessory factor, and it also shows that the growth of *Salmonella schottmülleri* of itself did not produce a food-accessory factor for the streptococcus used.

When the foregoing experiment was repeated with one change only, that is, the substitution of plain broth for potato extract, growth of the streptococcus was obtained. The paratyphoid

organism evidently does not use up all of the nitrogenous material when as much as 1 per cent peptone is supplied, but it does exhaust the nitrogenous material in potato extract if only 1 to 2 mgm. for each cubic centimeter is present. As a further test a plain broth containing 0.1 per cent peptone was used instead of the usual 1 per cent and the experiment was repeated. It was found that, with the smaller amount of peptone, growth of the streptococcus did not occur when the supernatant fluid of the paratyphoid culture was added to plain broth as before. To make sure that this was not a chance result the experiment was repeated several times, using six different Gram-negative bacilli for the treatment of the 1 and 0.1 per cent peptone broths. The cultures so obtained were tested for power to stimulate growth of ten different cultures of streptococci, with the results mentioned. It is understood, of course, that the streptococci used did not grow perceptibly in nutrient broth with the rather light inoculations employed. From these experiments it appears that, when a larger amount of nitrogenous material is used, the growth of the Gram-negative bacilli comes to an end before the food is exhausted and that the residual end-products furnish a suitable pabulum for the growth of streptococci. But when a small amount of nitrogenous material is employed, as in 0.1 per cent peptone or in potato extract, the residual material is not sufficient. This is taken as evidence that the significant factor in accelerating growth of streptococci is proper food rather than the presence of food-accessory factors.

In regard to the effect of the carbohydrate in the various extracts used, it was noted that there was no correlation between the amount of reducing sugar present and the power to stimulate growth. Extract of beef heart which contained only 0.04 per cent was slightly superior to carrot extract which contained twenty times that amount of reducing sugar.

SUMMARY

Aqueous extracts of potato, carrot, spinach, radish and beef heart were sterilized by filtration and added to nutrient broth aseptically. It was found that as little as 0.01 cc. of these

extracts, when added to 6 to 7 cc. of nutrient broth, would promote growth of many streptococci which did not grow in the broth alone, while 0.2 cc. was sufficient to give vigorous growth of most streptococci. Potato extract was found to be the most satisfactory, because of its high nutritive value and because it keeps well without change in reaction or precipitation of protein.

Heat was found to have an effect on the extract which was proportional to the precipitation, those media giving the most precipitate being the least useful in accelerating growth.

Both the proteid and nonproteid nitrogen fractions of the potato extract serve to stimulate growth of streptococci, but not in as marked a degree as the whole extract.

Potato extract may be used in place of fresh blood in many instances where it is not essential to observe hemolysis. It is helpful in getting a growth of streptococci and pneumococci free from cells and precipitated fractions of medium. As an enriching substance in ordinary fermentation tubes it allows growth of streptococci without interfering with the action on the sugar.

The substance in potato extract responsible for promoting growth is thought to be nitrogenous material which furnishes suitable food for the bacteria rather than food-accessory substances.

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