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MICROBIAL METABOLISM AND AGRICULTURE[1]

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THIS paper is designed to point out certain relationships between fundamental studies on microbial metabolism and the great basic science and art of agriculture, and that these advances in physiology, enzymology and physiological chemistry are significant not only in terms of human nutrition and human physiology and curative and preventive medicine, but also in farm production.

Obviously I am not called upon on an occasion of this kind to explore to the limits all the ramose diverticula of modern findings and theories relative to the uptake, synthesis and breakdown of substances useful or harmful to the microbial cell. Rather I should indicate those studies and advances which seem particularly promising in their application to non-microbial organisms. There is a great practical significance to the present and mounting knowledge on microbial metabolism.

Studies on microbial metabolism have stimulated a greater appreciation of the real unity of the basic characteristics of all protoplasm and the wide-spread uniformity of many of its activities. First an example: Until comparatively recently the ability of plants to fix carbon dioxide was conceived to be quite dependent upon the presence of chlorophyll. Then Winogradsky and others found that certain microbes were able to fix carbon dioxide without chlorophyll, and so living things became segregated into those which could not use carbon dioxide and those which could—the latter being subdivided into those which required chlorophyll and light (the photosynthetic forms) and those which utilized reduced inorganic

[1] Address of the retiring vice-president of Section O—Agriculture, American Association for the Advancement of Science, Cleveland, Ohio, September 14, 1944.

of the serum. The turbidimetric assay method, because of the erratic readings obtained, was abandoned in favor of the serial dilution method with a lengthened period of incubation.

Various methods for estimating the concentration of penicillin in body fluids have appeared in the recent literature. The method which seemed to possess the most merit was that of Rammelkamp,[1] or modifications thereof, in which a beta hemolytic streptococcus is employed in preference to staphylococci because of its greater sensitivity to penicillin. In order to increase the sensitivity, however, as well as the ease of determining the endpoint, washed red blood cells were used in the test. This necessitated establishing a source of washed cells and the use of young, freshly prepared cultures of the organism to prevent the addition of preformed lysin which interferes with the endpoint.

A large number of various species of bacteria were studied in an effort to determine whether an organism existed which was highly sensitive to penicillin yet could be grown and maintained on common laboratory media with a minimum of effort and which would give sharp and reproducible endpoints. The organism finally selected was a strain of *Bacillus subtilis*[2] obtained from the Northern Regional Research Laboratory. This organism grows luxuriantly at 30° C with a diffuse turbidity, while at 37° C growth consists of a pellicle with the medium below the pellicle remaining clear. Its resistance to penicillin is of the same order as most strains of hemolytic streptococci requiring as little as 0.0085 units per ml to inhibit a 1:100 dilution of a broth culture. This sensitivity is maintained over long periods of time without the necessity of repeated transfers. Cultures maintained at room temperature in screw-capped bottles were tested over an interval of six months with no demonstrable loss of sensitivity. Since then thousands of determinations have been made with satisfactory results.

Technique of the test: One-half ml amounts of broth[3] are placed in Wassermann-tubes and serial dilutions by halves made by adding one-half ml of the fluid being tested to one of the tubes and carrying one-half ml in serial dilution for as many tubes as necessary. The first tube in the series contains one-half ml of the material under test only. A standard is prepared for comparison by diluting a known potency penicillin (reference standard) to one-unit per ml in broth. This one-unit standard is diluted exactly as above in serial dilution by halves. One and one-half ml of a 1:100 dilution of the test organism in broth is then added and all tubes incubated at 37° C over night. The last tube in which no growth occurs is taken as the endpoint. This is usually very sharp, inasmuch as one tube will be absolutely clear while the next one in the series will have the typical pellicle of *B. subtilis* on the surface of the media.

The concentration of penicillin in the unknown is then determined by comparing the endpoint of the unknown with that of the standard. An example is given in Table 1.

TABLE 1

Fluid	Tube No.								
	1	2	3	4	5	6	7	8	9
Standard	0	0	0	0	0	0	+	+	+
Serum	0	0	0	0	0	0	0	+	+
Urine 1:10 ...	0	0	0	0	+	+	+	+	+

In the example in Table 1, the standard caused complete inhibition in the sixth tube. Since this represents one unit, the serum tested contains twice this amount, or two units, while the urine which caused complete inhibition in the fourth tube and had a primary dilution of 1:10 contains 0.25 units × 10 or 2.5 units. The test as described here can be used to determine potencies as low as 0.03 unit per ml. To determine potencies lower than this it is necessary to vary the dilution series of both standard and unknown.

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[1] C. H. Rammelkamp, *Proc. Soc. Exp. Biol. and Med.*, 51: 95, 1942.

[2] Some other strains of *B. subtilis* have been found to be considerably less sensitive.

[3] Mimeograph: Methods Used by the Food and Drug Administration for the Assay of Penicillin—Revised, January, 1945.

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