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A Cultural Method for Classifying Staphylococci as of the "Food Poisoning" Type.

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Staphylococci isolated from sporadic cases of food poisoning are a problem in any health department since so much is dependent upon feeding tests for experimental proof of their etiological significance. Often such evidence is lacking or difficult to obtain. Los Angeles County has experienced difficulty with this problem many times. Conditions, therefore, favored the gathering of a representative collection of strains of staphylococci. Investigation of these strains has led to the development of a valuable cultural test which separates enterotoxic strains from the non-enterotoxic within the collection. So far as we know, this is the first time that a cultural characteristic common to the enterotoxic strains has been demonstrated.

Owen[1] isolated staphylococci from dried beef which had affected 19 persons. Twenty-eight years later, the American Medical Association[2] published an editorial on staphylococcus food poisoning that dealt with the successful feeding tests made in human beings by Dack, Cary, Woolpert, Oram and Wiggers.[3] This test was a revival of the one used by Barber,[4] who suffered gastro-enteritis after drinking a culture of staphylococcus. Jordan and his coworkers[5] confirmed the results of feeding tests made in human beings and with McBroom[6] introduced feeding tests conducted in monkeys. Woolpert and Dack[7] described tests in monkeys that seemed to be more successful than those conducted by Meyer[8] and ourselves. Other work, including that of Dolman[9] warrants the conclusion that the reaction of human beings is the most dependable physiological test of

1 Owen, *Physician and Surgeon,* 1907, **29**, 289.

2 Editorial, *J. A. M. A.*, April 13, 1935.

3 Dack, Cary, Woolpert, Orem, and Wiggers, *J. Prev. Med.*, 1930, **4**, 167.

4 Barber, *Phil. J. Sc., Sect. B*, 1914, **9**, 515.

5 Jordan and co-workers, *J. A. M. A.*, 1930, **94**, 1648.

6 McBroom, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 161.

7 Woolpert and Dack, *J. Inf. Dis.*, 1933, **52**, 6.

8 Meyer, Sonderabdruck aus Zangger-Festschrift Seite 278-289, 1934, Rascher and Cie, A.-G. Verlag, Zurich.

9 Dolman, *J. Inf. Dis.*, 1934, **55**, 172.

food poisoning. Meyer[8] mentions the fact that alarming symptoms have developed in human volunteers. Therefore, for various reasons routine human tests are impractical.

Since the report of Dack, *et al.,* we had isolated staphylococci from sporadic outbreaks of food poisoning involving from one to 12 people. Tabulations of cases in California[8] for the 3-year period, 1931-1933, totaled 186 in 13 episodes. In a 6-month period (September, 1934, through February, 1935), Los Angeles County experienced 4 heavy outbreaks involving at least 600 people. We resorted to the feeding of milk cultures of staphylococci to kittens with fairly consistent results. Such work only identified the responsible organism after the outbreaks had occurred. Until a practical and fairly specific test was devised, control measures that might detect possible sources and thus prevent future outbreaks were impossible.

Attempts to develop a satisfactory test have been made by various workers and the results are summarized by Stritar and Jordan[10] who state, "Neither in biochemical, hemolytic nor agglutinative characters is there evidence of homogeneity." This was aggravatingly true in the experience of many laboratory workers. Our first attempts were no exception.

Results obtained in repeated outbreaks gradually provided material for statistical analysis. All foods involved were listed in relation to the predominance in them of carbohydrate, hydrocarbon or protein components with the striking fact that *protein was the only consistent major ingredient of the food involved in every outbreak.* Accordingly we concentrated upon experimental work involving protein. Among tests made were the usual observations for liquefaction of gelatin. However, we soon abandoned the 20°C. incubation and resorted to incubation at body temperature (or over) for the series of tests that followed. Here, again, we met with the experience of others,[10] *viz.,* that liquefaction caused by the same strain varied in degree, incidence and rapidity. Attempts were made to speed up liquefaction.

The basic ingredients of nutrient gelatin, beef extract, peptone, gelatin, were varied in different batches of the medium. Plantings were made from 70 strains of staphylococci isolated from different sources and include pyogenic, septicemic, saprophytic, dermotoxic, hemolytic, lethal and enterotoxic strains. The medium used at present is so specific that it yields positive results with the entero-

10 Stritar and Jordan, *J. Inf. Dis.,* 1935, **56**, 1.

toxic strains and negative ones with the non-food poison cultures in our own collection.

The majority of the strains were tested several times by feeding kittens, in order to determine whether they were enterotoxic or non-enterotoxic. The test in kittens together with epidemiological findings established the characteristics of the cultures studied.

Beef Extract Gelatin. Dry Difco granular gelatin in an open, wide evaporating dish in an incubator for at least 5 days. In the actual medium use 15% gelatin and 3.0% Difco beef extract. In a double boiler melt these together with the necessary volume of distilled water. In setting of gelatin, variation is influenced by several factors other than the percentage of gelatin. So far we have used the plan described in Wadsworth's Standard Methods in New York State under "Heat Sterilized Gelatin," (page 97). Heat the gelatin gradually until it dissolves. Do not adjust pH. Let it stand without stirring until cooled to 45°C. Raise to 95° to 100°C. for 20 minutes and stir occasionally. Make up the lost weight. Filter through paper. Place 4 cc. in tubes (4x½ inch). Plug tubes with cotton. Sterilize at 15 pounds for 15 minutes. After removal from the autoclave, place at once in ice box. When the gelatin has set, cork and paraffin the tubes to prevent evaporation. 25°C. is the lower limit for melting of the medium ready for use.

When planting, insert loop through the medium to the bottom of the tube. Incubate at 37.5°C. (at this temperature the gelatin naturally becomes liquid). Read results at the end of exactly 24 hours' incubation by placing all tubes in water at 21°C. until the control tubes set.

Any degree of liquefaction evidences a food poison staphylococcus. No liquefaction evidences a negative strain. Some strains completely liquefy while others only slightly affect the surface of the medium. Peptone, mineral salts and carbohydrates in usual amounts seriously retard liquefaction. We hope to definitely establish the essentials in the beef extract necessary for the test. If this is accomplished, then the variables which can occur in different beef extracts as causes of experimental error may be avoided.