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A broth cholera vaccine

It has become customary to prepare prophylactic vaccines against bacterial infections from growths of organisms on agar. As recently as 1940, the Cholera Advisory Committee of the Indian Research Fund Association¹ recommended that 'the (Cholera) vaccine should consist of a suspension of the vibrios obtained by washing off the growth from a 24-hour agar culture with 0.85 per cent saline solution'. This recommendation was in accordance with the practice of the majority of laboratories preparing cholera vaccine in large quantities. The reason for the preference for growths on agar over growths in broth, must be due to the anxiety of workers to obtain their suspensions of organisms as free as possible from extraneous proteins.

The acute shortage of the supplies of agar in the country, brought about by the

outbreak of war against Japan, led us to investigate the possibility of preparing an effective cholera vaccine from growths in a liquid medium. The success of this effort needed the fulfilment of two conditions: (1) the availability of a liquid medium as free as possible from proteins and yet yielding good growth of the vibrio, and (2) the development of a reliable method of testing the protective power of the vaccine in experimental animals. In the acid hydrolysate of casein of Mueller and Johnson², we have found an excellent liquid medium for the purpose. It gives a profuse growth of the vibrio, is easy to prepare, does not give biuret reaction, and what is more, costs less than half to prepare than the usual laboratory nutritive broths. We have been able to develop a protection test in white mice which gives repeatable results within narrow limits. Our mouse protection test determines the minimal dose of the vaccine required to protect 50 per cent of the immunised mice against an infective dose of 10 mld's administered intraperitoneally with mucin³.

In the several experiments we have performed so far the vaccine prepared from cultures in the liquid medium incubated at 23°C for seven days, killed and preserved with phenyl mercuric nitrate, 1 mg per 100 ml, gave a mouse protective dose of 0.00003 ml. Against this, the custom-

ary cholera vaccine made from 24-hour agar cultures of the same strain containing 8,000 million organisms per ml, gave a mouse protective dose of 0.0004 ml. Further our vaccine has a lower toxicity, as much as 1.5 ml per mouse (18–20 gm) produced no deaths. However, we are working to still further detoxicate it by the additions of formalin. 0.8 ml of agar vaccine killed four out of five mice.

The new cholera vaccine we have described is about ten times as potent as the customary agar culture vaccine, has low toxicity and has the great merit of being easier to prepare in large quantities than the agar culture vaccine.

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2. Mueller, J. Howard and Johnson, Everett, R., *J. Immunol.*, 1941, **40**, 33–38.
3. Griffiths, James, J., *Pub. Health Rep.*, 1942, **57**, 707–710.

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