Adjuvant Action of Phosphorylated Hesperidin. (20626)

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Experimental attempts to stimulate antibody formation and to promote the persistence of an effective titer have been undertaken ever since Pasteur and Joubert published their work in 1877. Since that time a sizeable body of information has accumulated in the literature on the use of adjuvants of widely diverse natures as exemplified by reports of the activities of various chemical agents, foreign proteins and non-related bacteria. Perhaps the most widely investigated adjuvant is that which consists of water and mineral oil with an emulsifying agent. Successful results with this adjuvant have been ascribed to "the slow prolonged absorption of the active antigen"(1) or "to the direct contact of the oil with the tissues eliciting the gathering of phagocytes that not only attempt to carry off the oil but are also effective in antibody formation"(2).

It has been shown that hyaluronidase can be effectively inhibited *in vitro*(3) and *in vivo*(4) by phosphorylated hesperidin. Application of this principle has been extended to experiments designed to determine if this hesperidin derivative will delay absorption of parenterally administered substances. Work in this laboratory has proven, in fact, that trypsin(5), heparin(6), vitamins, antibiotics, pressor amines, and local anesthetics(7) have a more prolonged blood level when administered with phosphorylated hesperidin. If a delay in absorption of a particulate antigen from the injection site will produce an adjuvant action, then the addition of a compound such as phosphorylated hesperidin to a vaccine should be attended by an increase and prolongation of antibody titer. Verification of this has been achieved in animal experiments as follows:

Three groups of 6 rabbits each were immunized with single subcutaneous injections of formalinized typhoid organisms suspended in a different medium for each group. The

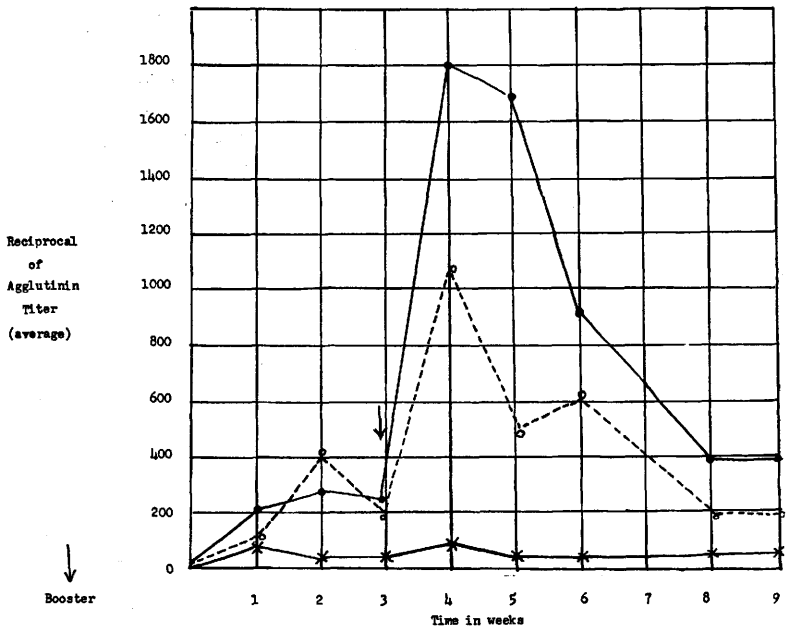

FIG. 1. Graph showing antibody titer in rabbits after administration of antigen in various vehicles. —×— Typhoid in saline. —●— Typhoid in 4% phosphorylated hesperidin. ---○--- Typhoid in water in oil emulsion.

suspending media were saline, 4% phosphorylated hesperidin solution and a water-in-oil emulsion. In each case the amount injected consisted of one cc of material containing 500 million organisms/cc. The water-in-oil emulsion was composed of 2 parts saline suspension of organisms, 2 parts paraffin oil and one part G 1096 Atlas emulsifier. A booster injection was given at the end of the 3rd week. Blood samples were collected each week and the antibody titration carried out by means of the standard agglutination test.

The results are pictured graphically in Fig. 1 which shows the unusual effectiveness of phosphorylated hesperidin as an adjuvant. Figures are averages for the 6 rabbits in each group. In view of the current interest in gamma globulin for passive immunization against poliomyelitis, and the relative shortness of the period of effectiveness, it is intended to determine if the use of phosphorylated hesperidin might extend the period of protection now offered by gamma globulin.

Summary. An adjuvant action of phosphorylated hesperidin in the induction of the antibody response to typhoid vaccine has been demonstrated.

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Influence of Low Tryptophan Diets Containing 6-Methyltryptophan on Oral Infection with Poliomyelitis Virus.* (20627)

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A study of the relation of the nutritional state of the host to susceptibility to Lansing (Type II) poliomyelitis in mice has established the fact that the development of Lansing infections is altered in animals suffering from a number of nutritional deficiencies (1). Tryptophan deficiency is particularly effective in modifying poliomyelitis in mice (2,3) and mice may be partially protected from Lansing virus by feeding the tryptophan analogue, 6-methyltryptophan(4). Preliminary experiments indicating that low tryptophan diets containing 6-methyltryptophan also exert some protective action against oral infection with poliomyelitis virus in monkeys are described here.

Toxicity of 6-methyltryptophan. Our studies in mice(4,5) had shown that the greatest protection against Lansing infections was observed in the animals fed diets containing a small amount of tryptophan (0.05%) and 10 times that amount of 6-methyltryptophan. The tolerance of 1.2 to 2.2 kg Philippine cynomolgus monkeys‡ for 6-methyltryptophan§ (6-MT) was approximated by feeding 6-MT in a diet consisting of: Corn grits,‖ 89%; corn oil¶ (fortified with oleum percomorphum** 25 ml/liter), 5.5%; Salts "IV" of Phillips and Hart(6), 4%; 1-lysine HCl, 1.0%; and DL methionine, 0.5%. Water soluble vitamins

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‡ Obtained from Okatie Farms.

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‖ Quaker "Hominy Grits".

¶ Mazola.

** Mead Johnson and Co.