milk was made by using 86.3 parts of whole milk and 11.7 parts of a chocolate syrup having the following composition:

Glucose	18.0 per cent.
Sucrose	33.5 " "
Invert Syrup	11.0 " "
Water	28.2 " "
Cocoa	9.0 " "
Stabilizer	0.2 " "
Salt	0.1 " "

The final product, therefore, contained 1.05 per cent. of cocoa. Another chocolate milk was prepared in the same manner except that partially skimmed milk (1.5 per cent. fat) was used. Four groups of 21-day-old rats of the Sprague-Dawley strain, averaging 40 to 45 gm, were placed on experiment. One group received mineralized whole milk, a second mineralized whole chocolate milk, a third mineralized partially skimmed milk and a fourth mineralized partially skimmed chocolate milk. The average growth at the end of four weeks for each of these groups is given in Table 1.

TABLE 1

	No. of rats	Average weight in grams after four weeks on milk diets	
		Male	Female
Trial I			
Whole milk	12	172	141
Whole milk plus chocolate syrup	12	182	143
Partially skimmed milk	12	172	137
Partially skimmed milk plus chocolate syrup	12	173	148
Trial II			
Whole milk	6	164	126
Whole milk plus chocolate syrup	6	151	133

The data show that there is no inhibition of the growth of young rats when commercial chocolate milk containing 1 per cent. of cocoa is fed. It is interesting that in Trial I the growth obtained on chocolate milk diets was slightly better than on whole milk. However, in a second trial the males on whole milk grew a little better than those on the chocolate milk. None of the differences are significant. The animals on partially skimmed milk responded as well as those on whole milk. This was undoubtedly due to the fact that sufficient vitamin A was supplied even by the partially skimmed milk when it was consumed at such a high level. The fat supplied in the chocolate probably also aided in the utilization of galactose.

These rats as well as other groups were maintained on the above milks for 16 weeks without any significant difference in growth. When the rats were carried through reproduction normal young were produced in all cases but the mothers on chocolate milk had some difficulty in rearing their young. Further work is now under way to determine the exact cause of this difficulty.

Although man would never subsist on a diet containing only chocolate milk, these results appear to be of some significance since earlier work in our laboratory has shown that the growth response of rats on a mineralized milk is a critical measure of certain changes in the nutritive value of the milk.[4] While these results give no indication of reactions which may be encountered by individual human subjects, they do show that animals may be raised on a diet consisting solely of mineralized chocolate milk without any ill effect. It should also be pointed out that one per cent. of cocoa in liquid milk is equal to about 7 or 8 per cent. of cocoa on the dry basis.

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ANTIBODY RESPONSE IN MAN TO INJECTION OF THE SPECIFIC ANTIGEN OF TYPE V SHIGELLA PARADYSENTERIAE[1]

SEROLOGICALLY specific types of dysentery bacilli are frequently encountered in endemic and epidemic areas. The selection of suitable strains for use in vaccines is but one of the difficulties which arises in attempting to immunize human beings against bacillary dysentery. Furthermore, the inherent toxicity of the organisms themselves is reflected by serious local and general reactions which render the use of vaccines undesirable and often hazardous. It would seem desirable, therefore, to have at hand as a prophylactic agent a material of broad immunological specificity and devoid of many of the toxic elements of the cells themselves. With this in mind we have undertaken the isolation of the specific antigens of certain of the *Shigella paradysenteriae* and have injected human volunteers with one of these chemically purified materials.

Antigens from Gram-negative bacteria can be obtained by a variety of procedures. Thus Boivin and his collaborators[2] used trichloracetic acid for extracting the antigens from a number of different Gram-negative organisms. Topley *et al.*,[3] on the other hand,

[4] C. A. Elvehjem, E. B. Hart, H. C. Jackson and K. G. Weckel, *ibid.*, 17: 763, 1934.

[1] The work described in this paper was done under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Rockefeller Institute for Medical Research.

[2] (a) A. Boivin and L. Mesrobeanu, *Rev. Immunol.*, 1: 553, 1935; 2: 113, 1936; 3: 319, 1937. (b) L. Mesrobeanu, "Les antigènes glucido-lipidiques des bactéries (Etude chimique et biologique)," Paris, Libraires de L'Académie de Médecine, 1936.

[3] W. W. C. Topley, H. Raistrick, J. Wilson, M. Stacey, S. W. Challinor and R. O. J. Clark, *Lancet*, 1: 252, 1937.

obtained both the H and O antigens from the typhoid bacillus by chemical fractionation of enzymatic digests of the organisms. Morgan and Partridge,[4] in their studies on the Shiga bacillus, obtained the specific antigen by extraction with diethylene glycol, and recently Morgan and Schütze[5] reported that this material has been used for the prophylactic inoculation of 12 human volunteers.

Shigella paradysenteriae Type V has been chosen for study because this organism gives rise in experimental animals to antisera which cross react broadly with other types of the Flexner group. The antigen is obtained from the cells[6] either by direct extraction with diethylene glycol or by the enzymatic degradation with trypsin of acetone-killed cells. In either case, subsequent separation from serologically inert bacterial products is accomplished by means of dialysis, precipitation of nucleic acid as a heavy metal salt followed by electrodialysis, and finally fractionation of the antigen from solution with acetone or alcohol. The material obtained by these procedures is a lipo-carbohydrate-protein complex which appears to be quite homogeneous when examined by electrophoresis. The antigen contains 4.5 per cent. nitrogen, 1.5 per cent. phosphorus and 15 per cent. phospholipid. On acid hydrolysis some 50 per cent. of reducing sugars are liberated, the antigen is broken down, and its immunological properties are destroyed. The protein-like moiety of the antigen is characterized by marked acetic properties and an unusually high tyrosine content.

The material isolated from Type V bacilli is a potent antigen which is highly toxic. Three injections of 50 micrograms given intravenously to rabbits evoke antibodies which agglutinate the homologous organisms in dilutions as high as 1:6400, and in lower dilutions agglutinate Types W, Y, Z and VZ microorganisms as well. Quantities as small as 0.5 mg invariably kill mice when injected intraperitoneally. Repeated attempts to detoxify the material without destroying its antigenic efficacy by a variety of chemical and enzymatic means have thus far been unsuccessful.

Despite the toxicity of the material, its unusual antigenic properties have enabled us to use sufficiently small doses for the production of antibodies in human beings without encountering untoward reactions. A group of 20 volunteers were injected intradermally with a total of 22.5 micrograms. The first dose of 2.5 micrograms was followed within a week by a second dose of 7.5 micrograms. The third and final dose of 12.5 micrograms was given one week later. There was little or no systemic reaction resulting from administration of these small amounts. The initial dose was in all instances followed by a local reaction which began 2-3 hours after the inoculation. The reaction consisted of swelling, redness and tenderness associated with transient lymphangitis and lymphadenopathy. The local reactions disappeared after 24–36 hours. Administration of the second dose of 7.5 and of the final one of 12.5 micrograms was unaccompanied by local or systemic reactions.

The volunteers were bled 2½ weeks following the last injection. The agglutination titer of these sera and of those collected before injection was determined. In all instances there was a marked increase in titer following inoculation, some sera agglutinating in dilutions as high as 1:800 to 1:600. These titers compare favorably with those obtained following the use of typhoid vaccine. Several antisera when tested against heterologous strains agglutinated microorganisms of Types W, Y, Z and VZ as well as those of the homologous type. The titers varied with the type and in all instances were higher than those of the pretreatment sera. That the antibodies evoked by the Type V antigen are not of a transitory nature is evidenced by the fact that the sera of several volunteers taken 6 months after injection showed no pronounced diminution in titer. When compared with the pooled pre-treatment serum, the post-treatment pooled serum showed a tenfold increase in mouse protective antibodies. A challenging dose of 1500–2000 M.L.D. of homologous Type V organisms required 0.20 cc of the former and only 0.02 cc of the latter serum to protect 50 per cent. of the animals. Furthermore, the post-treatment pooled serum showed a moderate increase in protective antibodies against virulent heterologous Type Z organisms.

A desirable agent for the prophylactic immunization of human beings against dysentery bacilli infections is one that is polyvalent and relatively non-toxic. The antigen prepared from Type V *Shigella paradysenteriae* gives rise in human beings to antibodies which are broadly cross reactive. The toxic properties of the antigen are, to be sure, undesirable, yet because of the small quantities necessary it can be used for human administration. Whether the injection of human beings with these specific antigens will afford protection against bacillary dysentery must, of course, await trials in the field.

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[4] (a) W. T. J. Morgan, *Biochem. Jour.*, 30: 909, 1936; 31: 2003, 1937. (b) W. T. J. Morgan and S. M. Partridge, *Biochem. Jour.*, 34: 169, 1940; 35: 1140, 1941.

[5] W. T. J. Morgan and H. Schütze, *Lancet*, 2: 284, 1943.

[6] We are indebted to Dr. W. A. Jamieson, of the Eli Lilly Company, Indianapolis, Indiana, for his generous cooperation in furnishing us with dysentery bacilli.