fiable into the same 4 groups.

Thirty-nine "atypical" strains of viridans streptococci were examined, 30 of which came from plates exposed to the street air, and 9 from plates exposed to the classroom. Six or 15% gave reactions with one of the 4 groups, 3 falling into group I, none into group II, I into group III, and 2 into group IV. One of the 6 reacting strains was recovered from the classroom air, the remaining 5 from the street air.

Discussion. More than half of the "typical" viridans streptococci isolated from the air were classifiable antigenically by the use of antiserums obtained by immunizing rabbits with strains of streptococci from bacterial endocarditis. Immunologically therefore they resemble the viridans streptococci obtained from human sources, 50% of which are classifiable antigenically with the same group antiserums. The "atypical" streptococci obtained from the air less frequently show antigenic relationship with the human strains.

It has been suggested that the presence of viridans streptococci in the air may be used as an index of human contamination in the same manner as the presence of *E. coli* is used as an index of fecal contamination. A method has been offered by which it is possible to identify many viridans streptococci serologically. The procedure may possibly be applied in epidemiological studies in the identification of viridans streptococci of human origin.

Summary. Twenty-six of 50 "typical" streptococci isolated from the air resemble immunologically the viridans streptococci obtained from human sources, whereas only 6 of 39 "atypical" streptococci from the air exhibit a similar relationship.

The epidemiological application of the data is discussed.

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Complement Fixation Antigen in *Entamoeba histolytica* Infection.

JOHS. BÖE. (Introduced by Konrad Birkhaug.)

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Although infection with *Entamoeba histolytica* produces specific antibodies, the serological diagnosis is scarcely employed in this disease because of the ease with which ameba are demonstrated microscopically, thus rendering a serological diagnosis superfluous. However, the main obstacle to an extended use of a serological reaction has been the extreme difficulty of preparing a useful antigen.[1]

Izar[2] demonstrated specific antibodies in *E. histolytica* infection and obtained positive complement-fixation reactions during the disease as well as in those whom he considered to be healthy carriers. As antigen he employed an aqueous extract of ameba-containing feces and the contents of a liver abscess. Craig[3] however first put the serological diagnosis in amebic infections upon a rational basis. He made use of an alcoholic extract of ameba-cultures as antigen. But this extract was quite weak and had to be used undiluted. Craig found that the serological reaction was quite often positive in *E. histolytica* infection and exhibited a high degree of specificity. But the serological diagnosis often failed in cases where it would be most useful, namely in ulcerative colitis. The findings by Craig have since been confirmed by many, including Tsuchyia[4] and Weiss and

1 Manson-Bahr, P., *The Dysenteric Disorders*, Cassell & Co., London, 1939.

2 Izar, G., *Arch. Schiffs. u. Tropenhyg.*, 1914, **18**, Beiheft II.

3 Craig, C. F., *Amebiasis and Amebic Dysentery*, Chas. F. Thomas, Balto., 1934.

Arnold[5] who attempted to improve the antigen preparation. Otherwise, the serological reaction was conducted as the Wassermann reaction or with slight modifications.

Paulson and Andrewes[6] made a comparative study of various amebic antigens by sending sera to 3 different laboratories, after a thorough parasitological examination of the patients. The serological findings in these 3 laboratories showed wide variations. Several sera gave positive reactions in cases with negative fecal findings and negative serological reactions in cases with positive amebic findings. But in the main there was agreement between the serological and parasitological findings.

Besides being a diagnostic aid, the serological test may be of some value during the treatment of amebic dysentery. Thus Craig demonstrated that the serological reaction turned negative following successful treatment and turned positive when relapse occurred. But the serological reaction has also certain theoretical interest in the biology of *E. histolytica*. Thus if the "healthy carrier" shows antibody production, then we may suppose, according to our knowledge about other infections, that the ameba has invaded the tissue. Thus Dobell[7] may be correct in considering the *E. histolytica* as an obligate tissue parasite, as opposed to the view of Reichenow[8] and Westphal[9] that the ameba occurs normally only in the intestinal lumen.

In a study of the distribution of pathogenic protozoa in a group of Norwegians[10] we made a special search for intestinal *E. histolytica* and found that approximately 3% of 1100 healthy persons had this parasite in their feces. These ameba proved virulent in cats which presented the characteristic histopathological picture with ameba in the intestinal wall. Our study included also a number of sera for detection of specific amebic antibodies. At first we found it expedient to simplify the antigen preparation, which hitherto appeared to be the main stumbling block in the serological diagnosis of amebic disease. Inasmuch as our Norwegian material is very scanty, we trust that others having greater access to clinical material will repeat our work. Hence we shall detail the preparation of our antigen.

Antigen. E. histolytica was grown on a modified Cleveland-Collier medium. Ten heavy cultures were transferred to a centrifuge tube and centrifuged at low speed. The supernatant fluid was decanted, the residue suspended in saline and re-centrifuged. This process was repeated usually about 8-10 times until the supernatant fluid became clear. The bacteria-poor residue now consisted mostly of ameba plus traces of starch from the medium. The residue was dried thoroughly in a desiccator, pulverized finely in a porcelain mortar and kept dry in ampules. This powder was resuspended in saline and titrated for its anticomplementary dose, one-fourth of which was employed in the actual tests.

Other reagents: The hemolytic system contained a 2.5% suspension of sheep red blood cells and an anti-sheep-erythrocyte-amboceptor with a titre of 1:4000, produced in the rabbit. Fresh guinea pig serum served as complement. The sera to be tested were inactivated at 56° C for one-half hour.

Complement-fixation reaction: The serological technic was identical with the usual Wassermann test. We employed 0.2 cc of each reagent and the total amount of liquid measured 1 cc. Four units of amboceptor and 2 units of complement were used, with the necessary controls. As positive control we employed serum from the rabbit immunized with the saline suspension of the *E. histolytica* antigen. This rabbit serum had a very high titre and gave negative reactions with the contaminating fecal bacteria. After having mixed serum, antigen and complement, the tubes were placed on the waterbath at 37° C for one-half hour. At this

4 Tsuchyia, H., *J. Lab. and Clin. Med.*, 1934, **19**, 495.

5 Weiss, W., and Arnold, *Am. J. Digest. Dis. and Nutr.*, 1934, **1**, 231.

6 Paulson, M., and Andrewes, J., *Arch. Int. Med.*, 1938, **61**, 562.

7 Dobell, C., *Med. Res. Comm. Spec. Rep. Ser.*, 1918, No. 15.

8 Reichenow, E., *Arch. Schiffs. u. Tropenhyg.*, 1937, **41**, 257.

9 Westphal, A., *Ibid.*, 1938, **42**, 441.

10 Böe, Johs., *Acta Med. Scand.*, 1943, **113**, 321.

time we added amboceptor and erythrocytes. After 10 minutes on the waterbath we read the reaction and controlled this after 20 minutes at room temperature. The complement-fixation reaction was also done at ice-box temperature. Our impression was that the reaction proceeded more rapidly and in higher titre. Otherwise our results were essentially the same. We compared these results with those obtained with an antigen prepared exactly after Craig's method. The 2 antigens gave in our hands identical results. A total of 121 sera were tested with the 2 antigens. In only 5 of these patients could we demonstrate *E. histolytica* in the feces. The serological reaction was positive in only one of the healthy amebic carriers as well as in one patient who showed no ameba in the feces. This latter patient had an uncertain liver disturbance, but our numerous fecal examinations remained negative for ameba. Our sole purpose for presenting these data is to induce investigators having access to greater clinical material, to repeat the serological reaction in amebic dysentery with the saline suspension antigen of *E. histolytica.*

Summary. A saline suspension of *E. histolytica* was employed as antigen in the complement-fixation test which gave identical results with those obtained with the Craig alcoholic extract antigen of *E. histolytica.*

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Protection of Animals Against *Cl. welchii* (Type A) Toxin by Injection of Certain Purified Lipids.*†

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(Introduced by Joseph C. Aub.)

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This communication reports evidence that purified total lipid extracts of certain animal organs protect mice and dogs against the lethal action of the toxin of *Cl. welchii* (type A).‡ In the case of hog liver, this protective action has been traced to the acetone insoluble, alcohol soluble (lecithin) fraction. Wüth[1] reported that hemolysis of red cells *in vitro* by *Cl. welchii* could be inhibited by lecithin. Since bacterial toxins have been inhibited *in vitro* in a non-specific way by a number of substances, including soaps,[2] olive oil,[3] lanolin,[4] and cholesterol,[5] this observation received little attention, other than mention by van Heyningen[6] that the effect might

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1 Wüth, O., *Biochem. Z.*, 1923, **142**, 19.

2 Larson, W. P., Halvorson, H. O., Evans, R. D., and Greene, R. G., *Colloid Symposium Monographs*, 1925, Vol. 3, p. 152, New York Chemical Catalogue Co. (Reinhold Publishing Corp.).

3 Myers, G. N., *J. Hygiene*, 1934, **34**, 250.

4 Weinberg, M., and Guillaumie, M., *C. R. Soc. Biol.*, 1935, **120**, 936.

5 Degkwitz, R., *Erg. d. physiol.*, 1931, Bd. 32, S. 821.

6 van Heyningen, W. E., *Biochem. J.*, 1941, **35**, 1246.