the outbreaks have subsided either rapidly or very gradually, depending upon other dietary factors. Force-feeding diluted milk, liver juice and small amounts of yeast has brought about recovery of animals in which the disease has progressed to the ataxic or even to the paralytic stage. Recent field trials of synthetic vitamin B_1 as a specific therapeutic agent have produced very favorable results.

Pathologically, the most characteristic lesions are found in the central nervous system, and these have been used as a basis for routine diagnosis in our laboratories during the past five years. The diagnostic lesions occur almost invariably in bilaterally symmetrical locations. They are found ventral to the floor of the fourth ventricle, in the quadrigeminal plate, in certain nuclei just ventral and lateral to the aqueduct of Sylvius, in the thalamus and in certain locations in the cerebral cortex. Histologically, they are characterized by striking vascular changes which affect the smaller vessels. Irregular dilatation or varicose deformity occurs, together with a very marked proliferative reaction involving particularly the endothelial cells, but also, to some extent, the adventitia. These vascular changes result in small, diffuse hemorrhages. A degeneration of nerve cells, with a variable degree of neuroglial reaction, accompanies the vascular changes. The lesions are definitely focal and occur only in certain nerve centers. In their distribution and histologic appearances they are the counterpart of the lesions of Wernicke's polioencephalitis of man.

Alexander[1] has presented convincing evidence, based on animal experimentation, that Wernicke's disease in man is due to a deficiency of vitamin B_1 in the presence of an adequate supply of other vitamins. A study of those diets that bring about a rapid termination of outbreaks of the dietary disease in foxes, as well as therapeutic trials with synthetic vitamin B_1, led us to conclude, independent of Alexander's investigations, that the dietary disease of foxes with which we were dealing with essentially a B_1 avitaminosis. The similarity of the pathologic changes in the brains of foxes with this disease and those in the brains of other animals with a vitamin-B_1 deficiency, as described by Alexander, lends further support to the view that a deficiency of vitamin B_1 is the cause of the disease in foxes. Since it is clear that the disease in foxes is brought about by their eating fish, we are led to the conclusion that the consuming of fish somehow produces a B_1 avitaminosis in foxes. The method by which it is brought about is not known, but that a deficiency does occur seems not unreasonable in the light of experimental and clinical data regarding B_1 avitaminoses in man and lower animals and in view of the chemical instability of thiamin. It seems a definite possibility that some substances in whole fresh fish and in alcoholic liquors are specifically destructive to vitamin B_1. A detailed consideration of facts relative to this point will be presented elsewhere.

SUMMARY

A common and highly destructive dietary disease of silver foxes in captivity is pathologically the counterpart of Wernicke's hemorrhagic polioencephalitis of man. The disease in foxes is caused by feeding fish as 10 per cent., or more, of the diet. It is probable that the fish induce a B_1 avitaminosis which causes the characteristic pathology and the resultant symptoms.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE METHOD FOR PREPARING ANTIGENIC SUBSTANCES FROM THE TYPHOID BACILLUS

IN several laboratories material has been isolated from the typhoid bacillus, *Eberthella typhosa,* which has proved antigenic when injected into small animals and, in some cases, in man. In general, the methods used may be divided into three groups: (1) fractionation of a tryptic digest of the organisms (Douglas and Fleming,[1] Raistrick and Topley,[2] Wakeman[3]), (2) fractionation of a trichloroacetic acid extract of the bacilli (Boivin and coworkers[4]), and (3) dissociation and extraction of the active material by organic solvents such as diethylene glycol (Morgan[5])[6]. An examination of the methods used and the products obtained indicates that the chief problem is the removal of protein from an antigenic complex largely carbohydrate in nature.

We describe here a different procedure, simple and rapid, which has proved useful for the examination of the antigenic constituents of several microorganisms and should be generally applicable to many others.

[1] Leo Alexander, *Am. Jour. Path.,* 16(1): 61–69, January, 1940.

[1] S. R. Douglas and A. Fleming, *Brit. Jour. Exp. Path.,* 2: 131, 1921.

[2] H. Raistrick and W. W. C. Topley, *Brit. Jour. Exp. Path.,* 15: 113, 1934.

[3] F. B. Wakeman, *Military Surgeon,* 84: 318, 452, 1939.

[4] A. Boivin and L. Mesrobeanu, *Rev. d'Immunol.,* 1: 553, 1935.

[5] W. T. J. Morgan, *Biochem. Jour.,* 31: 2003, 1937.

[6] The use of concentrated urea solutions to dissociate and extract the antigen has been described recently by J. Walker (*Biochem. Jour.,* 34: 325, 1940).

Concentrated phenols are known to be good solvents for most proteins. This fact, together with our observation that many complex polysaccharides, such as gum acacia, pectic acid, gum karaya, chondroitin-sulfuric acid, etc., are insoluble in strong phenol solutions, suggested the possibility of the use of phenol in the purification of carbohydrates of bacterial origin. We found that the capsular polysaccharides of most of the types of pneumococci are insoluble in concentrated phenol and may easily be separated from associated proteins by extraction of the latter in phenol; the polysaccharides which are soluble in phenol have been similarly purified by fractional precipitation from phenol solutions by alcohol or glacial acetic acid. (The use of this method in the preparation of the type-specific polysaccharides of pneumococci will be the subject of a separate communication elsewhere.) Applying this method to dried typhoid bacilli, we have obtained a substance, probably still crude in nature, which is highly antigenic in mice.

Acetone-dried organisms of the U. S. Army Medical School strain 58 (Panama carrier strain) were repeatedly extracted with U. S. P. liquified phenol (88 per cent.), or better still, with 95 per cent. phenol, then with alcohol to remove phenol. The phenol-insoluble residue was dried with acetone, then extracted with neutral physiological saline (0.9 per cent. NaCl) or with water; the extract was clarified and precipitated with alcohol or alcohol-ether in the presence of sodium acetate. The precipitate was then subjected to a second phenol extraction, and the alcohol-washing, water extraction, clarification and precipitation were repeated. The final product (substance A) after drying with acetone is a fluffy white powder, which readily forms a viscous, faintly opalescent 1 per cent. solution in water or physiological saline.

Phenol extraction may likewise be used on the product obtained by the tryptic digestion procedure. A tryptic digest preparation, made according to Wakeman,[3] gave a weak biuret reaction; one treatment with phenol removed the small amount of material responsible for this reaction. After clarification of the water extract of the phenol-insoluble residue, precipitation and drying, a product (substance B) was obtained which closely resembled substance A in appearance and properties.

Both substances, obtained in yields of 10 to 15 per cent. of the weight of the organisms, give negative or very faint biuret reactions and very strong Molisch reactions. Their nitrogen contents, corrected for ash and moisture, are 3.4 to 3.6 per cent. Hexosamine, if present at all, accounts for less than 10 per cent. of the total nitrogen. Both substances give strong precipitin reactions with antityphoid rabbit serum.

At least 50 per cent. of 88 white mice injected with two doses of 4×10^{-7} mg of either substance A or B were protected from an infective dose of 5×10^{4} live organisms of strain 58 suspended in mucin. At least half a control group of 40 mice were killed by 10 to 100 organisms similarly suspended. The immunizing doses were given one week apart and the infective dose one week after the second immunizing dose; the animals were then observed one week further. All injections were made intraperitoneally. The phenol-soluble material gave equal protection only in doses 10 to 1,000 times greater.

The antigenic power of substance A at least equals that of material we have prepared by Wakeman's method. Solutions containing 1 mg of active material per ml of physiological saline retain their antigenicity upon passing through a Berkefeld N filter.

Like the products described by others, our antigens were found to be toxic. In preliminary determinations of acute toxicity, a single intraperitoneal dose of 0.4 mg of substance A or B killed about 50 per cent. of 40 white mice within 24 hours. The injection of 0.1 mg under the skin of the abdomen of each of 10 guinea pigs caused a slight reddening and induration over the site of injection lasting one day in one animal and two days in another; the other animals showed no reactions. Intravenous doses of 0.1 mg in rabbits caused the death of two of ten rabbits within two hours and an average maximal elevation of temperature in the others of 2.2° F.

Further reports on the chemical, immunological and toxic properties of these substances will appear later.

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