separating the two halves of the electrolysis cell,[4] since the diffusion current of oxygen increased from 0.79 microampere to 1.10 microamperes when the solution was allowed to stand for 10 minutes with no nitrogen bubbling, but the electrode rotating. By using a large silver anode in a simple cylindrical electrolysis cell, it was possible to reduce the diffusion current of oxygen to 0.60 microampere using unpurified tank nitrogen and to 0.20 microampere using the purified nitrogen.

Assuming that the residual current in an entirely air-free solution was given by the current obtained in the sulfite experiment described below (0.15 microampere at 0.8 V vs. S.C.E.), the oxygen content of the unpurified tank nitrogen is calculated to be 0.15 per cent., and that of the purified nitrogen 0.02 per cent.

Heyrovsky[5] and Hohn[6] describe the use of sodium sulfite to remove dissolved oxygen, but give no quantitative data regarding its efficiency, or the rate of the reaction between oxygen and sulfite in neutral or alkaline medium. In the present study, 0.1 gm of sodium sulfite was added to 100 ml of 0.1 N potassium chloride, allowed to stand for 10 minutes, and a current voltage curve was determined with the rotating electrode. No trace of oxygen was detectable in the solution. The current-voltage curve is shown as the lowest curve in Fig. 3.

The stationary and the rotating wire microelectrodes are also useful for the voltammetric determination of other constituents which can be electro-reduced or electroöxidized. These applications and experimental details will be discussed in forthcoming papers.

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A DEFICIENCY DISEASE OF FOXES

A DIETARY disease that occurs in animal populations and is associated with the feeding of fish has been found in our investigations to be a vitamin deficiency and to be the counterpart of the alcoholic polioencephalitis of man described as Wernicke's disease. This condition, which we have for years termed "Chastek paralysis," is of considerable economic importance in the fox-raising industry. Clinically, it presents a well-defined syndrome characterized by a preliminary period of anorexia lasting a week or ten days, followed by a rapid development of weakness, ataxia and spastic paralysis. Death usually occurs within 48 to 72 hours after the onset of neurologic disturbances. Up to the present time we have observed outbreaks of this disease on fox ranches located in five different states. Most of them have occurred during the months from November to May, inclusive, and no large outbreaks have been noted during the summer. The mortality has varied from 1 to 37 per cent. of the total herd, according to the type of treatment instituted. Small ranches with as few as 17 pairs of foxes and large ranches having several thousand foxes have been affected.

The clinical characteristics of the disease have been essentially the same in all outbreaks. They have, without exception, occurred on ranches where fish or fish products were fed as 10 per cent., or more, of the diet. The first evidence of disease among the foxes usually appears from three to six weeks after fish have been added to the diet. The foxes show only anorexia for a week or 10 days and then a few animals at a time begin to exhibit signs of neurologic disturbances. These include weakness and unsteady gait, ataxia, hyperesthesia and spastic quadriplegia. Convulsions may occur shortly before the animal dies. The symptoms are always progressive and lead to death within a few days.

Repeated attempts to demonstrate an infectious agent as the cause of the disease have been consistently negative. A total of about 190 red foxes, 20 dogs, 7 ferrets, 6 mice, 6 squirrels and 12 rabbits and guinea pigs have been inoculated with suspected material by intraperitoneal, subcutaneous, intramuscular or intracranial routes or have been fed the material by stomach tube in attempts to transmit the disease. In no case has the disease been successfully transmitted.

Dietary experiments show that this disease can readily be produced by feeding foxes a ration poor in vitamin B_1 and containing fresh fish.

Carp has been the most common species of fish used on ranches where this disease has appeared. However, quillbacks, mullets and suckers, and Atlantic whiting have also been incriminated. The fish causing this disease have come from a variety of sources, including the Atlantic Ocean, fresh-water lakes in Utah, Minnesota and Canada, and the Cedar River in Iowa.

It is clear that the course of all the outbreaks has been determined by the dietary management on the ranch. In all cases the disease has been progressive, involving an increasing number of foxes as long as fish remained in the diet. In most instances the owner of the fox farm has suspected fish to be a cause of the disease and has eliminated it from the diet within a short time after the first death occurred. On one ranch, however, the feeding of fish was not discontinued until three weeks after the first death, and the result was a mortality of 34 per cent. in a herd of over 200 foxes.

After fish has been eliminated from the fox ration,

[4] J. J. Lingane and H. A. Laitinen, *Ind. Eng. Chem.*, Anal. Ed., 11: 504, 1939.

[5] J. Heyrovsky, "Polarographie," in W. Böttger, "Physikalische Methoden der Analytischen Chemie," Bd. 2, Akad. Verlagsgesellschaft, Leipzig, 1936.

[6] H. Hohn, "Chemische Analysen mit dem Polarographen," Verlag von Julius Springer, Berlin, 1937.

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).