

Neither is it likely that the disease is due to lack of vitamin K, because it has been observed in chicks receiving 0.1 per cent of petrol ether extract of alfalfa in addition to the basal diet. Such a diet contains 20-30 units of vitamin K per gm., an amount which secures normal blood clotting and prevents the 'ordinary' hæmorrhage of K-avitaminosis. 40 per cent of dried yeast in the diet gives no protection.

The resemblance of the symptom to an allergic reaction might suggest that the protein had been rendered toxic by the extraction process, for example, by being altered in such a way that a trace is absorbed without being broken down by the digestive enzymes.

However, certain observations make it probable that the exudative diathesis is a deficiency disease. Thus the addition of 5 per cent dried alfalfa or 0.5 per cent petrol ether extract or alcohol extract of alfalfa afforded a very material reduction of the percentage of the animals showing the symptom, while 1.5 per cent aqueous alfalfa extract or 0.5 per cent alfalfa ash gave no protection.

These and other observations suggest that the disease is prevented by a specific petrol ether- and alcohol-soluble factor occurring in dried alfalfa, but that this material is no powerful source. It is therefore proposed to attempt to find a richer source of the protective factor and to concentrate and differentiate it in the usual way.

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#### Trimethylamine Formation in Relation to the Viable Bacterial Population of Spoiling Fish Muscle

Suwa<sup>1</sup> and Poller and Linneweh<sup>2</sup> found that the trimethylamine oxide occurring in the muscle of certain sea fishes is reduced to the corresponding volatile amine during its spoilage by bacteria. Recently, Beatty<sup>3</sup> has shown that it is fairly certain that at least 94 per cent of the trimethylamine found in decomposing cod fish muscle arises from the trimethylamine oxide present in this tissue, and not from the other possible precursors. Sterile fish muscle<sup>4</sup> and muscle press juice<sup>5</sup> do not reduce the oxide.

These, and similar observations made by other investigators, have led to the suggestion that the simple determination of the amount of trimethylamine in sea fish muscle might prove a convenient and accurate chemical test by which its stage of decomposition could be accurately judged<sup>6,7</sup>, and attempts have been made to compare the viable bacterial counts (or the logarithms of these counts) with the amount of trimethylamine in cod fish muscle press juice<sup>5</sup> or in spoiling haddock muscle<sup>7</sup>.

Recent work at this station<sup>8</sup> has shown that, while there is undoubtedly an increase in trimethylamine as the viable bacterial population of lightly smoked sea fish fillets increases, there is by no means a constant relationship between these two values. It seemed possible that not all bacteria occurring in spoiling fish muscle are capable of reducing trimethylamine oxide, and that the discrepancy noted above might well be due to an unequal distribution of the reducing and non-reducing organisms in different samples of fish.

In order to investigate this possibility, thirty micro-organisms (15 micrococci, 4 flavobacteria,

6 achromobacteria and 5 yeasts) were isolated at random from fresh halibut and red cod muscle, and from smoked fillets of halibut, red cod and grey cod. Only three of these organisms (2 species of micrococci and 1 achromobacter) formed trimethylamine when grown in dilute aqueous halibut muscle extract sterilized by filtration, or when washed suspensions of their cells were incubated anaerobically in Thunberg tubes in the presence of halibut muscle juice, with or without added trimethylamine oxide, at pH 7.0 for 16 hours at 25° C. Under the last-named conditions, the reduction of the oxide by the three cultures capable of effecting its reduction was found to be linear and practically quantitative, suggesting the presence of a specific dehydrogenase enzyme (or enzymes) catalysing the reaction. By inoculation of aseptically excised cod fish muscle with trimethylamine-forming and non-trimethylamine-forming species of *Micrococcus*, samples of fish with either high or negligible quantities of trimethylamine, but with high bacterial counts in both instances, have been obtained.

From these results, it would seem unlikely that the trimethylamine content alone will prove to be a satisfactory criterion of the degree of bacterial contamination of sea fish muscle, unless the population of trimethylamine-forming to non-trimethylamine-forming bacteria proves to be fairly constant in all cases. As yet the full mechanism of the bacterial reduction of trimethylamine oxide to trimethylamine remains to be determined, though recent work by Watson<sup>9</sup> suggests that the lactic or pyruvic dehydrogenase systems are capable of catalysing this reaction.

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<sup>1</sup> Suwa, A., *Pflügers Arch. Ges. Physiol.*, **128**, 421 (1909).

<sup>2</sup> Poller, K., and Linneweh, W., *Ber.*, **59**, 1362 (1926).

<sup>3</sup> Beatty, S. A., *J. Fish. Res. Bd. Canada*, **4**, 63 (1938).

<sup>4</sup> Brocklesby, H. N., and Biddell, W. A., *Prog. Rep. Pacific Fish Expt. Stn.*, No. 33, 17 (1937).

<sup>5</sup> Beatty, S. A., and Gibbons, N. E., *J. Biol. Bd., Canada*, **3**, 77 (1936).

<sup>6</sup> Boury, M., and Schwintz, J., *Rev. Trav. Off. Pêches Mar.*, **8**, 282 (1935).

<sup>7</sup> Shewan, J. M., *Rep. Director Food Invest. Bd. (London)*, Sec. 4, p. 75 (1937).

<sup>8</sup> Tarr, H. L. A., and Sunderland, P. A., *Prog. Rep. Pacific Fish Expt. Stn.*, No. 37, 7 (1938).

<sup>9</sup> Watson, D. W., cited by Beatty, S. A., *Chem. and Ind.*, **57**, 865 (1938).

#### A Correlation between the Chemical Constituents and Morphological Structure of certain *Thesium* Species

A CHEMICAL examination of *Thesium virgatum* has been carried out, and two characteristic constituents of the plant tissues have been isolated—a phlobatannin, and an amorphous substance which yields glucose and a steam-volatile oil on hydrolysis. These constituents are readily detected in the plant material by a test tube reaction, the phlobatannin with mineral acids yielding a red phlobaphene, and the glycosidic material the free essential oil.

Three of the four sections of the *Thesium* genus mentioned in the "Flora Capensis" are represented in the Cape Peninsula. In general, these sections are well defined; but the boundaries between them are not absolute. In an attempt to follow the gradation from group to group chemically, the above qualitative test has been applied to the vegetative portions of a number of *Thesium* species. Arranging