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Brief Communication

DISTURBANCES OF VITAMIN A METABOLISM IN ANIMALS

The liver represents the main storage organ for vitamin A. Vitamin A is mainly present in the liver as retinyl esters. In plasma, however, it is in the form of retinol and is bound to a specific protein, retinol-binding protein (RBP). Detoxication of exogenous compounds such as PCB (*Innani et al.* 1976) seems to result in increased vitamin A utilization, with a subsequent reduction in the hepatic vitamin A concentration. An exception to this are the long-acting sulphonamides (*Bravo et al.* 1977). Sulphomethoxypyridazine causes a significant fall in the plasma vitamin A level and an increase in the hepatic vitamin A concentration in rats. It is possible that some other toxic compounds interfere with the retinol-binding protein mechanism, thus inhibiting the mobilization of vitamin A from the liver to the plasma and producing symptoms of A-avitaminosis.

About 1½ year ago an apparent increase occurred in the number of animals submitted to the laboratory because of stillbirths, disturbances of the central nervous system and unfertility. The phenomenon is still continuing. Chemical investigations of the liver extracts of these animals has shown a common factor, the presence in their livers of a large amount of an unknown compound. The analytical methodology was the same as that used for the last 15 years in the investigation of chlorinated hydrocarbons: fats extraction, purification of the sample by preparative thin layer chromatography, elution and gas chromatographic analysis (GLC) with electron capture detector (ECD). Several hundred liver extracts have been analyzed in the past 15 years, but his unknown compound was not present in the gas chromatograms. The unknown compound was analyzed by capillary GLC combined with a mass spectrometer (MS) using the electron impact and the chemical ionization mode. The high resolution mode was used to determine the elemental composition of the compound. The mass spectrum indicated an intense molecular ion with a value of m/z 268 and the elemental composition was found to be $C_{20}H_{28}$. The sample was compared with a standard of retinyl acetate and found to give identical results. We got, in fact, a thermal decomposition spectrum of the retinyl

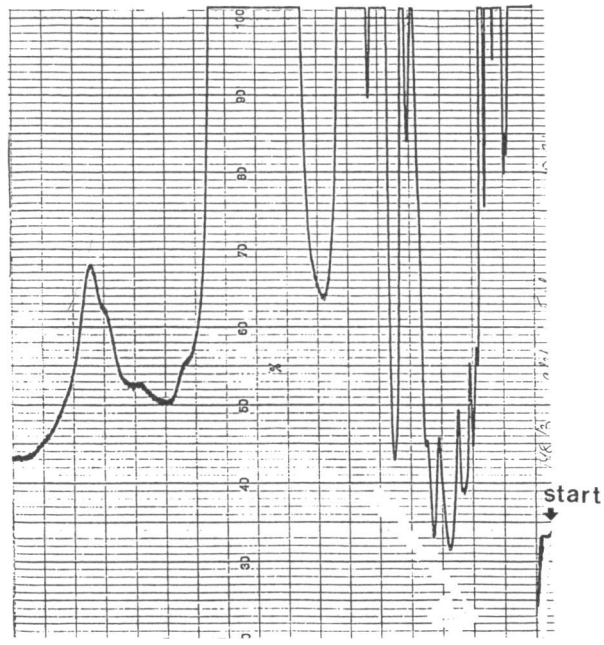

Figure 1. Gas chromatogram of a mink-liver extract containing 8533 USP/g of retinol.

ester to anhydroretinol, as reported (*Dunagin et al.* 1964), which has a molecular ion of m/z 268. The retinyl acetate standard gave the same peak as the sample by GLC, too. Moreover, when the amount of vitamin A in the samples was very high, transretinal was also detected.

The liver samples were analyzed for the presence of vitamin A by the method of Carr-Price (*Wanntorp* 1947). When the amount of vitamin A was within the concentration range of 153 USP/g to 357 USP/g (1 USP = 0.30 μg retinol), no abnormal peaks were present in the gas chromatogram. On the other hand, the gas chromatogram of a liver sample with an A vitamin content of, e.g. 2333 USP/g, showed very intense peaks. The gas chromatogram of a mink-liver extract which had vitamin A content of 8533 USP/g is shown in Fig. 1. The mass spectra of vitamin A (Retinyl Acetate) "a" and the liver extract sample "b" are compared in Fig. 2. It is interesting to note that we found a high concentration of vitamin A in liver samples from animals which were dead or ill, exhibiting symptoms of A-avitaminosis. The animals affected are mink, calves, chicken and several other

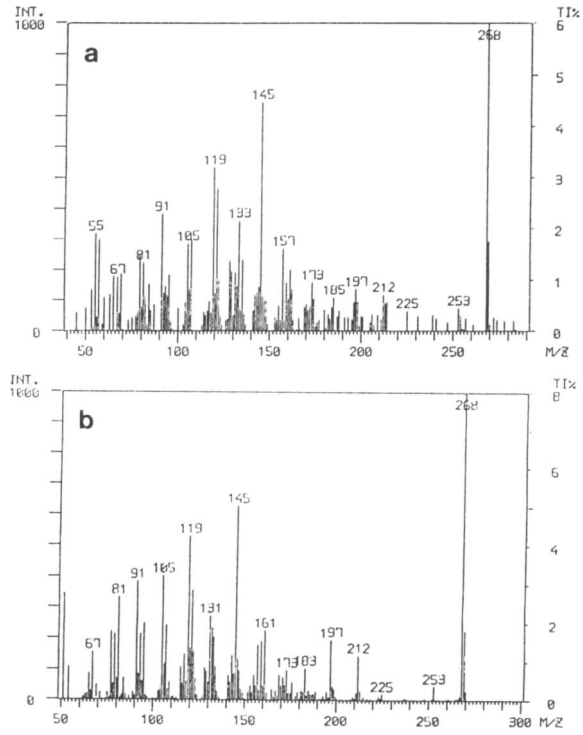

Figure 2. Mass spectra of retinyl acetate "a" and of the mink-liver extract "b".

species. So far we have not detected high concentrations of vitamin A in pig livers, but this phenomenon has been noticed in wildlife, too. We can thus assume that the mechanism which regulates the utilization of vitamin A is altered, affecting for instance the synthesis of RBP, or that there is competition for the same receptor with another compound. It is not possible to say with any certainty what is causing such a problem, but we suspect in the first place that mycotoxins may be the cause. This problem began suddenly in the summer of 1982 when a large quantity of raw material was imported for the production of animal feed in Finland. Only traces of aflatoxins at a concentration below 1 mg/kg, have been detected following the analysis of animal feed. Sterigmatocystin and other compounds with similar chemical properties have been found on a number of occasions in feed. These compounds have been tested with a cell culture and have been found to be cell toxic.

We have detected aflatoxin B_1 and G_1 at levels of 0.28 µg/kg and 0.34 µg/kg, respectively, in the liver of a dead calf. We have not found any references concerning the influence of mycotoxins on vitamin A metabolism, but trace amounts of aflatoxins in the feed decrease plasma carotenoids significantly (*Tung et al.* 1973).

We have reason to believe that mycotoxins or moulds that produce mycotoxins, which were not present earlier in Finland on such a wide scale, have been introduced in Finland along with imported materials or else through some other channel. Anyhow, the problem is still open and further investigations need to be done since there may be many other explanations for this phenomenon.

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