moisture or low temperature areas, etc.—will compete with molds and insects for toxic gases. The heterogeneous grain bin components, each with its own chromatographic character, assist in altering the vapor composition of multicomponent mixtures after their application to the bin surface. Thus, in a recent trial with a 40-foot bin of oats containing about 20% of screenings, the gases that reached the bottom consisted largely of carbon tetrachloride, small amounts of ethylene dichloride, and no ethylene dibromide (*7*), and insect infestations were not controlled at the bottom of the bin. Variable results in our past experience with field fumigation may now be explained on the basis of differential sorption of the gases. Field tests of such laboratory-developed postulates are planned.

The amount of fumigant residue remaining in or on cereal products after a prescribed treatment with multicomponent mixtures is of practical interest. "Natural" desorption—i.e., after "normal" aeration *in situ*—followed by accelerated desorption of the residues by thermal stripping is used in the analytical determination. Exploratory tests on oats (*7*) show that residue levels after EDC-CT-EDB mixtures are applied are small but, interestingly, in a similar intercomponent ratio as was shown by experimental isotherms described herein. Such work will be reported more fully.

Syringes used as micro fumigation chambers were convenient both for introduction of gas mixtures and in sampling. Gas chromatography enabled rapid, sensitive, and specific measurement of small changes in gas concentration that resulted from the interaction of gas and substrate. The use herein of gas levels considerably smaller than are applied in grain elevators and warehouses expedited the observation of changes in gas concentrations and attainment of equilibria.

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PESTICIDE EFFECTS ON VITAMIN METABOLISM

Carotenoid and Vitamin A Concentrations in Serum and Liver of Steers Fed Forages Treated with DDT or MCPA

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When yearling beef steers were fed for 83 days with forage sprayed at the rate of 1.5 pounds per acre with DDT, terminal liver vitamin A stores (micrograms per gram of liver) were significantly decreased ($P < 0.05$) and blood serum vitamin A levels were increased. No deleterious effect resulted when MCPA-treated forage was fed under similar conditions. In view of the high rate of application of DDT and MCPA and minimal demonstrable effect on vitamin A concentrations, it is unlikely that use of these chlorinated compounds in normal agricultural practice has contributed to the increased incidence of vitamin A deficiency observed in bovines in Canada.

VITAMIN A deficiency of beef cattle occurs in Canada (*9*) and several observers believe the incidence has increased during the past decade (*6*). Changing agricultural practices may contribute to this. Toxic substances in the diet can impose stress conditions that alter nutrient requirements. The use of chlorinated hydrocarbons has increased markedly in North America during the last decade: bovine hyperkeratosis has been produced experimentally through administration of chlorinated naphthalene compounds (*12*) or topical application of an oil-based insecticide carrier (*5*). In both cases an influence on vitamin A metabolism has been demonstrated (*3*). These facts have led us to study the effect of ingested chlorinated agricultural chemicals on vitamin A and carotenoid utilization by animals.

In previous studies with the rat (*11*) it was demonstrated that the feeding of over 10 p.p.m. of DDT in the diet for periods up to 72 days decreased utilization of orally administered carotene and vitamin A. Hepatic storage of vitamin A was also reduced when a diet containing both DDT and carotene or

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Table I. Hepatic Vitamin A and Carotene of Steers Fed Forages Treated with DDT or MCPA

Treatment	No. Treated	0 Carotene	0 Vitamin A	28[a] Carotene	28 Vitamin A	50 Carotene	50 Vitamin A	83[b] Carotene	83[c] Vitamin A
		Days on Experiment; μG. per Gram of Liver							
Control	20	9.0 ± 1	47.9 ± 1.4	7.7 ± 1	64.6 ± 3.7	5.7 ± 0.6	58.8 ± 4.1	8.7 ± 0.5	61.3 ± 4.9
DDT	20	9.0 ± 1	47.6 ± 3.6	8.9 ± 0.4	70.8 ± 5.9	5.1 ± 0.4	49.8 ± 5.5	6.8 ± 0.3	45.7 ± 4.7
MCPA	20	9.0 ± 1	47.5 ± 4.9	7.0 ± 0.5	62.6 ± 6.0	5.1 ± 0.4	51.3 ± 4.1	8.5 ± 0.5	64.3 ± 5.7

[a] DDT group significantly greater ($P < 0.05$) than MCPA, but neither group significantly different from controls.
[b] DDT group significantly lower ($P < 0.01$) than other groups.
[c] DDT group significantly lower ($P < 0.05$) than other groups.
± values are standard errors of the means.

DDT and vitamin A was fed. Since this effect was observed in small animals, we have extended our study to bovines.

During 1961 Hidiroglou and Knutti (*4*) observed a number of unthrifty calves raised on pastures treated with the herbicide MCPA (4-chloro-2-methylphenoxyacetic acid). These animals, which had dead-looking hair coats, inflamed watery eyes, cloudy corneas, and excessive lacrimation, responded to vitamin A treatment. In a controlled experiment with beef cows, some evidence was found that MCPA or some constituents in the MCPA-treated forage interfered with the conversion of carotene to vitamin A by the animal. Phillips (*10*), however, found no detrimental effect of MCPA per se on carotene or vitamin A metabolism in the rat. During the 1963 pasture season, forages treated with either DDT or MCPA were fed to beef steers and the investigation of concentrations of carotenoids and vitamin A in liver and blood is reported here.

Materials and Methods

Sixty yearling Hereford steers, average weight 542 pounds, were assigned to one of three groups of 20 animals each on the basis of body weight. Sufficient standing forage for one week of feeding was sprayed at the rate of 1.5 pounds per acre with either DDT or MCPA. The forage for feeding during the following week was mechanically harvested 24 hours after spraying and fed *ad libitum*. The chopped forage comprised the entire ration of the steers.

Liver biopsy samples were taken at approximately monthly intervals by the technique of Erwin *et al.* (*1*) and blood samples were collected at approximately 2-week intervals. Vitamin A and carotenoids were determined in the liver samples by the method of Gallup and Hoefer (*2*). Blood samples were analyzed by a modification of the method described by Moore (*8*). An analysis of variance was made of the data (*13*).

Results

Changes in concentration of liver vitamin A (Table I) are of prime importance. There was no difference in this concentration between groups of animals when assigned at the beginning of the experimental period. After 28 days of feeding, neither experimental group differed significantly from the control group, but there was a significant difference ($P < 0.05$) between the two experimental groups. This disappeared at the third biopsy (50 days of feeding). At the end of the experimental period, the concentration of liver vitamin A was significantly less ($P < 0.05$) for the DDT treatment than for controls or MCPA treatment. Over the 83-day feeding period net gain of vitamin A (micrograms per gram of liver) was 13.4 μg. per gram for the control group and 16.8 μg. per gram for MCPA treatment. Although these net gains are minor, they are in contrast to the net loss of 1.9 μg. per gram for the DDT treatment.

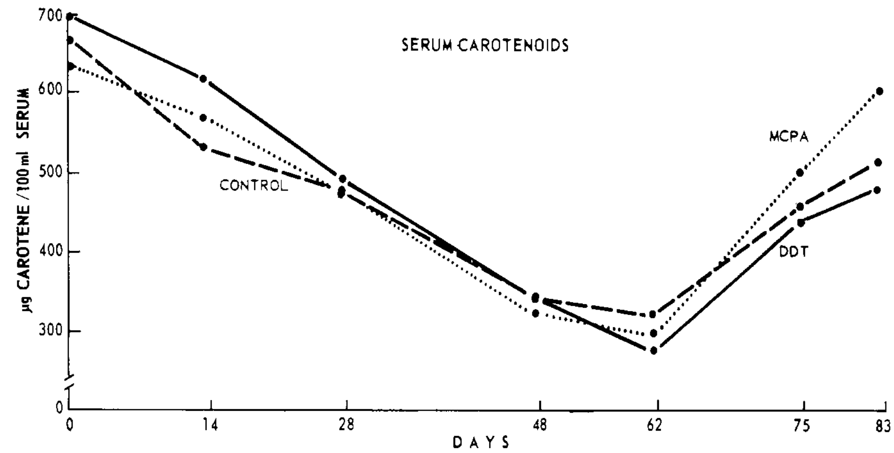

Figure 1. Serum carotenoids of steers fed DDT- or MCPA-treated forage

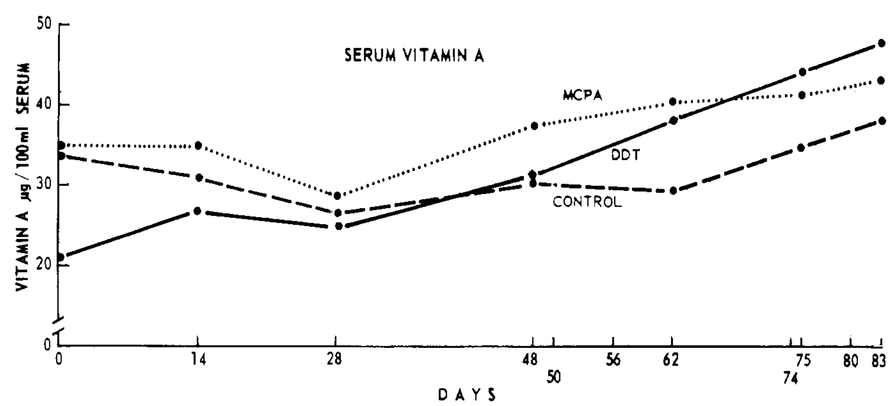

Figure 2. Serum vitamin A of steers fed DDT- or MCPA-treated forage

Serum carotenoids (Figure 1) were extremely high at the initiation of the experimental period (June 23, 1963) and for the first 62 days decreased similarly for all groups. At day 14 the mean serum carotenoids level for the control group was significantly lower ($P < 0.05$) than for the other two groups. This was probably fortuitous, because a similar difference did not occur again until day 83. At this time the serum carotenoid level of MCPA-treated animals was significantly higher ($P < 0.05$) than that of the other two groups.

The difference between groups was greater for serum vitamin A (Figure 2) than for serum carotenoids. By chance the initial level for animals being assigned to DDT treatment was lower than the mean for other animals but by day 48 it was the same as for the controls. Except for this, at no time did blood vitamin A levels of animals consuming treated forage drop below control levels. From the 62nd day on, vitamin A levels of animals consuming DDT- or MCPA-treated forage were significantly higher than those of controls, but no differences were observed between the herbicide or pesticide treatment.

Discussion

The environmental stress due to DDT to which these animals were subjected was purposely much greater than would be encountered under normal agricultural practices. Forage was harvested 24 hours after spraying (at 1.5 pounds per acre), so animals were consuming continually over the entire experimental period a diet containing as determined by analysis 40 to 60 p.p.m. of *o,p*-, *p,p*-DDT dry weight. In agreement with previous studies in the rat (*11*), this rate of feeding significantly decreased liver storage of vitamin A and carotenoids. The decreases, however, were not of sufficient magnitude to decrease blood levels of either constituent. The stress of DDT ingestion at high levels did not produce nor approach an avitaminotic state in the steers. It is reasonable to assume that the stress (if any) imposed on bovines by ingestion of DDT-contaminated herbage under practical conditions would not appreciably affect vitamin A utilization and metabolism, and hence should not contribute to increased vitamin A deficiency of beef steers in North America.

Studies conducted by Mitchell, Hodgson, and Gaetjens (*7*) have not demonstrated any harmful effects to sheep or cows grazed on pasturage treated with 2,4-D, or to a cow fed over 5 grams daily of the herbicide. Although there was no gross effect of 2,4-D on performance of the animals, some case reports (*4*) have suggested that vitamin A utilization may be impaired by MCPA in suckling calves. In a previous study with rats, Phillips (*10*) demonstrated that the feeding of relatively large amounts of MCPA did not impair hepatic storage of vitamin A following oral administration of carotene or vitamin A, nor did it alter the rate of decrease of hepatic vitamin A stores. Results of the present experiment with bovines are in agreement with those observed in the rat. Following continual feeding of MCPA-treated forage, there was no evidence that any detrimental or abnormal influence was exerted on the utilization of carotenoids. Serum carotenoids were significantly greater at the termination of the experimental period in the group consuming MCPA-treated forage; however, this did not result from decreased conversion of carotenoids to vitamin A as shown by liver vitamin A concentrations similar to the control group. Hidiroglou and Knutti (*4*) have stated that the indirect hazard to cattle by eating MCPA-sprayed forage in the fall is negligible, because there is a lower proportion of plants absorbing the herbicide and the possible effect of herbicide on carotenoid utilization in older cattle may be less than in calves, because of the vigorous activity of the rumen which rapidly eliminates the plant hormone.

It is concluded that under the conditions of this experiment the feeding of MCPA-treated forages does not influence carotenoid utilization in bovines.

Acknowledgment

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ANIMAL GROWTH STIMULANTS

Metabolism of Labeled Diethylstilbestrol in Ruminants

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The results of an earlier study on the metabolism of tritium-labeled diethylstilbestrol (DES-T) in lambs and steers have been confirmed and extended. The "free phenolic" material found in the tissues and excreta after DES-T administration was identified by carrier crystallization and chromatographic methods as stilbestrol, and the "conjugated" material excreted in the urine as its glucuronide. It was shown that no other forms of DES are present in appreciable quantities in alcohol-ether extracts of the tissues and excreta. Rumen microflora were shown to be capable of hydrolyzing the glucuronide in vitro and it was proposed that the excretion of the free form in the feces is partially the result of degradation of the conjugate by intestinal bacteria.

ALTHOUGH the administration of diethylstilbestrol (DES) to ruminants as a growth stimulant has become a widespread commercial practice, little is known of the metabolic fate of this compound in these species. Most of the information currently available pertains to monogastric animals, in which the physiological effects of DES (and therefore possibly its catabolism) are known to differ in certain important respects from those in ruminants. The study described here is an extension of an earlier investigation by Mitchell, Neumann, and Draper (*17*) into the metabolism of DES in cattle. It was under-

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