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Toxic Protein Derivatives Causing Aplastic Anemia
A Review

By WAYNE RUNDLES

IN June, 1957, L. L. McKinney and associates reported the climax of a prolonged study of fundamental biologic and medical interest, the synthesis of S-(dichlorovinyl)-L-cysteine, a compound which upon oral administration to calves in doses of only 10 mg./100 lbs. daily, produced a fatal aplastic anemia similar to that produced by feeding trichloroethylene-extracted soybean oil meal.[1]

As early as 1916, Stockman associated a severe hemorrhagic disease in cattle, which broke out in south Scotland, with feeding on soybean meal after extraction of the oil with trichloroethylene.[2] The soya bean, long an important article of human food in the East, was becoming more widely used in the feeding of cattle. The bean was too rich in oil to serve as a good stock food, and, moreover, the oil was far more valuable for other purposes. Soya meal which had been extracted with naptha had become highly regarded by animal growers.

In the Scotland outbreak, 67 cows on 9 different farms had become ill. Fifty-four of these died and 13 recovered. The cattle owners attributed the trouble to the feeding of a new consignment of soya bean meal or cake. Investigation with the manufacturer revealed that the appearance of the cattle disease synchronized with the replacement of naphtha by trichloroethylene as the extracting solvent. Stockman was able to reproduce the disease experimentally by feeding trichloroethylene extracted meal to cattle.

Many cases of the disease were subsequently observed in Germany and Holland in 1923 and 1925, and later in different areas of the United States, Hawaii and Japan.[3]

In the late winter and spring of 1951 an outbreak of fatal aplastic anemia occurred in Minnesota dairy cattle. Field studies showed that all of the involved animals were in herds that had been fed, as part of their ration, trichloroethylene-extracted soybean oil meal (TCESOM).[3] Two extraction plants using this solvent combined with a heating process had begun operation a few months earlier. Calves under six months of age, the most highly bred, best-producing milk cows and those late in pregnancy were particularly susceptible. Those fed the greatest amount of TCESOM were affected first and most severely. Unconfirmed reports appeared in one area that several persons who had consumed milk from cows fed TCESOM developed a hemorrhagic disease.

A systematic study of the relationship of bovine aplastic anemia to TCESOM was undertaken by M. O. Schultze and collaborators at the Minnesota Agri-

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cultural Experimental Station, by L. L. McKinney and associates at the Northern Utilization Research and Development Division, Agriculture Research Service, Peoria, Illinois, and by J. C. Picken, Jr. and associates at Iowa State College, Ames, Iowa.

The Minnesota group found that fatal aplastic anemia could be produced regularly in cattle under experimental conditions by the feeding of toxic TCESOM.[4] Cattle fed 3 lbs. of the meal daily appeared well for 24–28 days, then developed anorexia, fever, serous nasal discharge, abdominal distress, petecchial hemorrhages on the mucous membranes and melena. Death occurred after 35–55 days. Blood studies showed that leukopenia, neutropenia, thrombocytopenia, and anemia with a slightly increased MCV were present a few days before the onset of symptoms. Prothrombin levels were unaffected.

A more chronic form of aplastic anemia, sometimes intermittent in course when animals ate irregularly, was produced by feeding smaller amounts of TCESOM. Heifers fed only 1 lb. per day survived for 10–19 months. Animals with chronic intoxication developed anemia that was characteristically normocytic and normochromic, neutropenia with a relative lymphocytosis and thrombocytopenia. Three of four animals with chronic leukopenia and thrombocytopenia were bred and produced calves. Pregnancy apparently did not aggravate the blood dyscrasia, and the calves were normal at birth and during subsequent suckling. In the original field studies, calves born to affected cows seemed to have suffered prenatal damage.

Serial bone marrow studies in animals maintained on small amounts of toxic TCESOM showed progressive hypoplasia of the bone marrow that particularly affected the neutrophilic and eosinophilic elements. Postmortem examination of animals that died or were slaughtered showed major lesions confined to the bone marrow. If the administration of toxic meal to animals with milder degrees of chronic disease was suspended, they recovered completely.

The toxicity of different lots of TCESOM varied considerably. Meal prepared from recently harvested beans proved to be much more toxic than that made from beans which had been stored for some months. The precise manufacturing conditions required to produce a toxic meal are still not understood. A heat treatment step, used to remove solvent and to destroy antinutritional factors present in raw meal, which were known to cause digestive disturbances, diarrhea, unpalatability, etc., may be a factor in producing a toxic meal. Soybean meals that were extracted with other solvents like hexane were well tolerated.

Trichloroethylene administered to calves for long periods in amounts far exceeding the residues present in TCESOM produced no clinical or metabolic evidences of toxicity,[5] and it was readily metabolized.[6] The toxicity of the meal could not be ascribed to auto-oxidation products of trichloroethylene or to triethylamine used as a stabilizer.[7,8]

Different animal species varied in their susceptability and reaction to the feeding of toxic TCESOM. Two horses were fed meal of known toxicity at levels of 4 and 6 lbs. daily. Aplastic anemia identical with that observed in cattle developed in 112 and 168 days. Death occurred after 198 and 287 days. Bracken fern intoxication, another relatively common but still unexplained cause

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of aplastic anemia in cattle, was known to be poisonous to horses and rats by virtue of thiaminase activity which could be destroyed by steaming the plant.[9] In the aplastic anemia resulting from feeding toxic TCESOM, evidence of thiamin deficiency was lacking and there was no therapeutic response to the administration of thiamin, vitamin K, ascorbic acid, antibiotics, etc.

Sheep proved irregularly susceptible but more resistant than cattle to toxic TCESOM. Emaciation and infection were conspicuous terminal features. Rats, mice and rabbits were not affected. Guinea pigs, hamsters and dogs developed a chronic wasting disease with progressive weakness, debilitation and emaciation. Blood and bone marrow changes were absent or mild. Occasionally there was evidence of disturbed renal functoin.

Swine, chickens and turkeys, the species that consume most of the soybean oil meal produced in the United States, were not notably diseased during the bovine epidemic. Feeding these species toxic TCESOM in amounts used in normal agricultural practice produced no clinical or hematologic abnormalities.

Using the calf bioassay developed by Schultze and associates, McKinney and his collaborators at Peoria fractionated toxic TCESOM and found that the marrow-damaging agent was associated with the purified protein component of the meal.[8,10] Chemical analyses showed that toxic TCESOM contained 0.5 mole less sulfhydryl groups per 10^6 Gm. and about 20 parts per million more chlorine than did hexane-extracted meal from the same beans. Evidence was obtained showing that trichloroethylene would react on heating with cysteine and the sulfhydryl group of reduced glutathione.[1]

S-(*trans*-dichlorovinyl)-L-cysteine was then synthesized (fig. 1). When given orally or intravenously to calves in doses of 10–20 mg./100 lbs. daily it produced the complete clinical, hematologic and postmortem picture of severe aplastic anemia in the bovine.[1,11] The injection of S-(dichlorovinyl)-L-glutathione produced similar effects with about 2/3 the toxicity of the cysteine derivative on a mole basis.[12]

Despite the resistance of mice to the oral ingestion of toxic TCESOM the injection of S-(dichlorovinyl)-L-cysteine daily at a dosage of 100 mg./Kg. mouse weight was lethal in two days. At 25 mg./Kg. 50 per cent of the mice died on the sixth day, and at 15 mg./Kg. all lived for at least eight days.[12] Both rats and mice seem to be much more resistant to the compound than calves.[11]

Little is known about the toxicity of these compounds in man. Three of the five chemists working on the synthetic project developed severe swelling about the eyes and nose, nasal symptoms and dermatitis. They also observed harmful effects in guppies, bean sprouts and drosophilia.[12] We have given to patients with metastatic cancer, acute leukemia and uncontrollable polycythemia vera the cysteine derivative intravenously in doses up to 30–40 mg. to a total of 4 mg./Kg. during the course of 2–4 weeks without observing definite clinical or hematologic effects. Further studies of the biochemical and pharmacologic actions of these compounds, their possible anti-neoplastic effect in experimental leukemia and in human neoplastic disease are being undertaken.

In the overwhelming majority of patients with aplastic anemia the etiology of the disease, whether endogenous or exogenous, is never satisfactorily estab-

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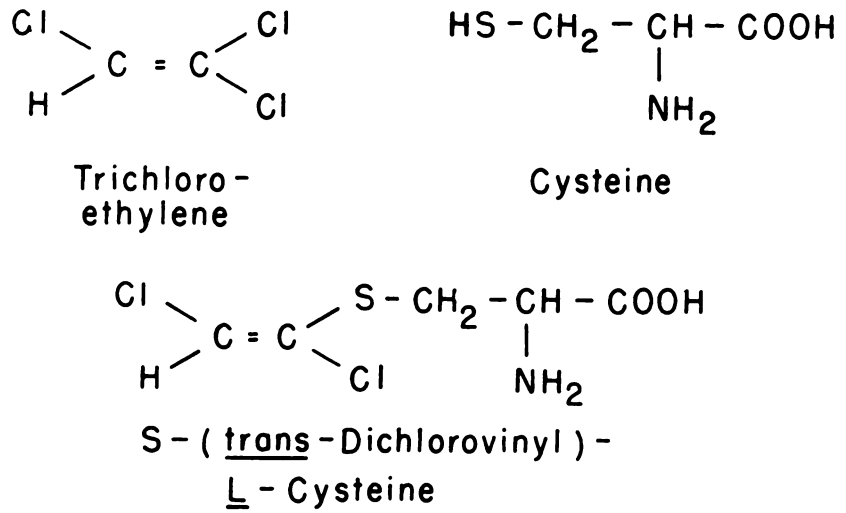

FIG. 1.—Reaction of trichloroethylene and cysteine to form toxic derivative S-(*trans*-dichlorovinyl)-L-cysteine.

lished. Exposure to drugs and chemicals seems to be universal. The effect of trace amounts of various compounds, conditioned as it may be by individual idiosyncrasy, seems almost impossible to evaluate.

Soybean oil meal is not a current hazard to man as far as we know, even via foods ingested by animals, since it is no longer extracted with trichloroethylene commercially. Trichloroethylene has been used in the past to extract scrap and fish meal and to remove caffeine from coffee.[12] Meat products processed with trichloroethylene for animal food have a distinct but relatively low degree of toxicity for the bovine.[11] The factor causing aplastic anemia can be produced under laboratory conditions by heating not only soybean meal but also zein, lactalbumin, casein and gelatin with trichloroethylene.[13] Trichloroethylene is widely used as a solvent and cleaner as well as an anesthetic. There may be hazards related to the preparation or processing of foods and drugs of which we are not now aware.

SUMMARIO IN INTERLINGUA

Es reportate studios relative al production experimental de letal anemia aplastic in varie animales per medio del administration oral de S-(dichlorovinyl)-L-cysteina, un composito primo synthetisate per L.L. McKinney e su associatos in junio 1957. Le anemia assi evocate es simile a illo observate sporadicamente depost 1916 in vaccas recipiente un dieta que include torta oleaginose de soja resultante del extraction del oleo per medio de trichloroethyleno.

Es delineate le historia del occurrentias de anemia aplastic in pecore assi alimentate. Es notate que varie animales reage variemente, que trichloroethyleno per se non pare esser toxic, e que le reaction del animales a S-(dichlorovinyl)-L-glutathiona es simile a lor reaction a S-(dichlorovinyl)-L-cysteina.

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Le periculo del generation de iste o simile agentes in le processage de nutrimentos pro humanos es signalate. Es notate que trichloroethyleno ha essite abandonate como agente de extraction industrial.

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