The names "hyperkeratosis" and "X-disease" have been widely used to designate a disease in cattle first described in detail by Olafson (29) in 1947. The condition was first recognized in May, 1941, in New York state and was recorded in 1942 (11). The disease had been reported in 32 states by October, 1948 (42). A cooperative project to study the pathology, cause, and treatment of the disease was initiated in 1949 by 17 agricultural experiment stations, the Bureau of Animal Industry, and the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

No accurate figures are available on the extent of the losses caused by the disease. Estimates (23) range from 2 to 4 million dollars annually for the years 1948-1952. It has also been estimated (7) that cattle worth 4 million dollars have died or been slaughtered because of the disease in the southeastern states alone. These estimates are probably conservative.

The disease has been reported in Germany by Wagener (44), in Morocco by Martin and Hinteman (25), in Australia by Whittem and Blood (48), and in New Zealand by Haughey and Cooper (17).

THE NATURE OF THE DISEASE

The symptoms and lesions of X-disease are numerous, and it should be emphasized that thickening of the skin is only one of the lesions which develop as the disease progresses. Olafson (29) and Olafson and McEntee (30) described profuse lacrimation and salivation, depression, anorexia, and loss of condition in cattle affected with the disease. Severe diarrhea was seen in some of the affected animals. In addition, mastitis, prolonged gestation, dystocia, retained fetal membranes, and metritis were noted in cows with hyperkeratosis. Retarded growth of the horns was noted in younger animals. These authors also noted papillary proliferations on the tongue, oral mucosa, and esophageal mucosa and in the gall bladder and large bile ducts. Cystic dilatation of the renal tubules, as well as of the gastric and intestinal glands, was described. A slight fibrosis was found to accompany proliferation of the smaller bile ducts, as well as fibrosis of the pancreas and proliferation of the ducts. Squamous metaplasia occurred in the interlobular and main ducts of the parotid and submaxillary salivary glands.

McEntee and Olafson (27) have described the pathology of the reproductive tract in cattle with hyperkeratosis. In bulls, squamous metaplasia of the accessory sex glands and excretory ducts of the testes occurred. The pseudostratified columnar epithelium of the epididymis gradually stratified and in some areas the lumen became plugged with keratin. The epididymis became enlarged and hardened so that the condition could be diagnosed by palpation in the living animal. In mature bulls the germinal epithelium disappeared, leaving tubules...
lined chiefly with Sertoli cells, and the basement membranes became hyalinized. Approximately 10 months is required for the reestablishment of normal spermatogenesis once these changes have occurred in a severe case of experimentally produced hyperkeratosis, according to the data of Vlahos et al. (43) and Olson and Skidmore (37).

In cows and heifers metaplasia of the cervix and Gartner's ducts occurred along with moderate dilation of the endometrial glands. The ovaries became small and inactive (27).

The plasma vitamin A in cattle decreased to extremely low levels within 5 days after the substance which produced the disease was fed (13), and the blood levels of ascorbic acid are reported (20, 28) to be elevated in cattle with hyperkeratosis.

Hove (19a) has presented data showing that X-disease in cattle is associated with a decrease in plasma tocopherol.

Jubb (21) has found that the acidophylic cells of the anterior pituitary undergo nearly complete degranulation in cattle with hyperkeratosis, an effect not seen in cattle having a simple vitamin A deficiency.

These symptoms and lesions have been described, in whole or in part, by most investigators working on bovine hyperkeratosis. These include, among others, Bell (1, 2), Sikes and Bridges (38), Olson et al. (35), Miller et al. (28), Sippel (40), Wagener (44), and Kohler (22).

Sheep fed large amounts of the same toxic feed that produced hyperkeratosis in cattle developed somewhat different lesions, including liver cell necrosis, regeneration, and fibrosis, but no bile duct proliferation (27). In the ram some testicular degeneration occurred, but metaplasia of the excretory ducts of the testes and accessory sex organs did not occur. In the ewe squamous metaplasia of the endometrial glands with profuse keratinization developed.

ISOLATION AND IDENTIFICATION OF THE CAUSATIVE AGENT

In the earlier phases of the research on hyperkeratosis some workers believed the disease was caused by a virus. Attempts to find an infectious agent resulted in numerous negative reports (11, 12, 34, 35, 40).

At the outset, one of the most enigmatic aspects of the hyperkeratosis problem was the apparent dissimilarity of the numerous substances which were involved in various outbreaks of the disease. Olson et al. (35) produced the disease in calves by feeding pelleted dehydrated alfalfa and dicalcium phosphate. Olafson and McEntee (30) produced the condition by feeding a processed wheat concentrate. Miller et al. (28) fed a particular lot of timothy hay and produced hyperkeratosis. Wagener (44) and Hansel et al. (13, 14, 31) reported the production of hyperkeratosis by a particular lot of a wood preservative produced in Germany. Bell (1) produced the disease by feeding a lubricant. Washko et al. (46) produced the disease in calves placed on a farm where a natural outbreak had previously occurred. The calves received only pasture and block salt.

A few of the reports of substances which failed to produce hyperkeratosis may be cited. Thomas and Moss (41) found no evidence of X-disease in young
dairy bulls fed toxic amounts of molybdenum. Gibbons et al. (9) showed that cases of X-disease in Alabama had not been produced by DDT. Miller et al. (28) failed to produce the disease by feeding rancid fat, naphthalene, and pentachlorophenol. In earlier studies, Olafson et al. (32) fed numerous substances without producing the disease. These included a thallium salt, naphthalene, alphachloronaphthalene, many surface-active agents, mineral oil, DDT, 19 different commercial lubricants, and heat-polymerized linseed oil.

In 1951, the nature of the disease was somewhat clarified by the ether extraction and preliminary fractionation of the causative agent from a processed wheat concentrate reported by McEntee et al. (26) and Hansel et al. (13). The substance appeared in the free-fatty acid fraction of the extract but not in the phenolic or neutral steroid fractions. In later fractionations it was found that the active principle itself was not a fatty acid but appeared in the non-saponifiable fraction of the ether extract.

In 1952, evidence from three sources showed highly chlorinated naphthalenes to be the causative agents of X-disease. Bell (2), working in cooperation with the research laboratories of the company which produced the lubricant previously shown to cause the disease, found that highly chlorinated naphthalene which had been added to the lubricant was the causative agent. Sikes and Bridges (38) and Sikes et al. (39) produced the disease with pentachloronaphthalene. Olafson et al. (31) and Hansel et al. (14) isolated the causative agent from the German wood preservative and identified it by infrared and ultraviolet absorption curves and micro-analyses as a highly chlorinated naphthalene. Later, Hansel et al. (15) isolated the causative agent from the processed wheat concentrate and also identified it as highly chlorinated naphthalene. This wheat concentrate consisted of bread crumbs obtained from the floor beneath the slicing and wrapping machine in a bakery. The bread itself was not found to be toxic.

Blickenstaff and Callen (4) isolated the causative agent from a lot of pelleted cottonseed meal and identified it as a highly chlorinated naphthalene by infrared and ultraviolet absorption spectra, microanalysis, and X-ray diffraction patterns.

Bell (3) has studied the relative toxicity of the chlorinated naphthalenes. Naphthalenes containing 36.0 and 46.0% chlorine (corresponding to di- and trichloro derivatives) did not produce the disease. Slight symptoms were produced by tetrachloronaphthalene, and all of the more highly chlorinated derivatives produced the disease. Olafson and coworkers (32) have obtained similar results. Some naphthalenes of higher and lower levels of chlorination were probably present in the purified naphthalenes used in these studies, and in addition each of the above fractions contained several isomers differing in the relative positions of the chlorine on the naphthalene nucleus. For example, there are 14 position isomers of pentachloronaphthalene. The materials isolated from the wood preservative and the processed wheat concentrate were probably mixtures of naphthalenes of various degrees of chlorination. The material isolated from the pelleted cottonseed meal (4) had a chlorine content similar to that of pentachloronaphthalene but probably consisted of a mixture of highly chlorinated naphthalenes. Studies of the effects of each of the various isomers would be of
interest, but the problem of obtaining each isomer in a pure state is an extremely difficult one.

Although the methods used to isolate and identify chlorinated naphthalene from the German wood preservative (14), the processed wheat concentrate (15), and the pelleted cottonseed meal (4) all proved useful for the particular substance being studied, no simple method for measuring the chlorinated naphthalene content of various feeds has yet been devised. The method of Blickenstaff and Callen (4) is likely to be the most useful for feedstuffs containing relatively small amounts of chlorinated naphthalenes, but it does require some special equipment. Engel et al. (8) have developed a colorimetric method for the determination of chlorinated naphthalenes based on the production of a yellow color when petroleum ether solutions of these compounds are combined with dimethyl aniline. This procedure proved applicable to the detection of highly chlorinated naphthalenes in petroleum products but has not been successfully applied to animal feeds.

The removal of chlorinated naphthalenes from products that might be consumed by cattle somewhat deemphasizes the need for a simple method for their determination.

COMMERCIAL USES OF HIGHLY CHLORINATED NAPHTHALENES

Highly chlorinated naphthalenes have been added to lubricants as extreme pressure agents, and the use of such lubricants on feed-pelleting machines has been responsible for many cases of hyperkeratosis. Copenhaver and Bell (6) prepared a pelleted feed, using a lubricant containing 3% chlorinated naphthalene on the pelleting machine and produced hyperkeratosis in calves fed the pelleted feed. A control lot of calves fed pellets made when the mill was lubricated with a lubricant not containing chlorinated naphthalene did not develop the disease. A lesser number of cases of the disease have occurred as a result of cattle licking grease containing highly chlorinated naphthalene from farm machinery. Gregory et al. (10) have reported the production of hyperkeratosis by feeding a concentrate contaminated only by the vapors of octachloronaphthalene that was painted on the walls of a storage room.

Other commercial uses of highly chlorinated naphthalenes that might possibly lead to their accidental consumption by cattle are: (a) additives for motor tune-up oils, (b) electrical insulating compounds, (c) flame resistant agents for paints, floor finishes, plastics, and fabrics, (d) electrical sealing compounds and (e) binders for ceramics.

CHLORINATED NAPHTHALENES AND VITAMIN A METABOLISM

The effect of chlorinated naphthalenes on vitamin A metabolism has been repeatedly demonstrated. Hansel et al. (13) found that plasma vitamin A levels declined rapidly when substances which produced hyperkeratosis were fed, and this effect proved useful in subsequent fractionation and isolation studies. The low plasma vitamin A levels persisted for at least 1 month beyond the period
during which the toxic agents (later shown to be chlorinated naphthalenes) were fed. Hoekstra et al. (19) reported similar findings and noted partial, temporary alleviation of the hyperkeratosis syndrome as a result of vitamin A therapy over 9-12 day periods. In general, the use of vitamin A therapy for hyperkeratosis has been disappointing. Calves fed highly chlorinated naphthalene will develop hyperkeratosis despite the concurrent administration of massive daily doses of vitamin A, although the appearance of the symptoms is slightly delayed (32, 47). Plasma vitamin A returns to low levels after cessation of the vitamin A therapy in affected animals (13, 19). The question of whether vitamin A deficient animals are more susceptible to chlorinated naphthalene poisoning than are normal animals has not been satisfactorily settled.

Goats, sheep, swine, mice, chickens, and rats, in contrast to cattle, all appear resistant to chlorinated naphthalene poisoning (19, 32). In recent experiments Warner (45) has shown that dairy calves depleted of their vitamin A stores will succumb to the disease and exhibit low vitamin A plasma levels when a total of 500 mg. of chlorinated naphthalene is fed with either vitamin A or carotene. With similar treatment vitamin A depleted goats appeared normal in all respects and maintained normal plasma A levels. The relationship of this species difference in susceptibility to the well-known species differences in carotene-vitamin A metabolism is not yet understood. The highly chlorinated naphthalenes may prove useful research tools in studies of vitamin A metabolism in various species.

EFFECTS ON GROWTH, REPRODUCTION, AND LACTATION

Decreased growth rate or loss of weight in cattle with hyperkeratosis has been mentioned by many workers (6, 19, 23, 28, 30). Since growth rates are influenced by so many nutritional and hormonal factors, it is useless to speculate at present on the mechanism of this action of the chlorinated naphthalenes. It is not unlikely that a reduced feed intake, the anti-vitamin A effect, and the degranulation of the acidophyls in the anterior pituitary are all factors in the adverse effects of chlorinated naphthalenes on growth rates.

Failure of the reproductive processes in cattle with hyperkeratosis is almost a necessary sequel to the lesions previously described (27) of the reproductive tracts of both bulls and cows. Spermatogenesis ceases in bulls with experimentally produced hyperkeratosis, and normal sperm production is not reestablished for approximately 10 months (43). Abortions are likely to occur in cows pregnant at the time of the outbreak of the disease (23, 29, 39). Olson et al. (36) have shown that reproduction is not permanently impaired by the disease. Ninety-four heifers that recovered from an outbreak of the disease eventually produced normal calves. In general, those most severely affected with the disease, were the slowest to conceive.

A decreased milk flow and a decreased percentage of fat in the milk occur in cows with hyperkeratosis (23, 32, 39). Olson (33) and Olafson and McEntee (30) have reported the development of hyperkeratosis in calves receiving milk from cows with the disease. Indications are that the causative agent appears in the milk for a considerable period of time after it is withdrawn from the feed.
OTHER SUBSTANCES POSSIBLY RELATED TO HYPERKERATOSIS

Although many of the outbreaks of X-disease have now been traced to highly chlorinated naphthalene ingestion, some outbreaks of the disease are still unexplained. At the same time, there is no convincing evidence that substances other than highly chlorinated naphthalenes produce the disease. Hoekstra et al. (18) produced skin lesions in calves by spraying them with mineral seal oil, but these authors point out that the condition produced differed from that produced by their hyperkeratosis-producing feed, or by highly chlorinated naphthalene. Lesions were not observed in the gastrointestinal tract, gall bladder, or kidneys. Harshfield and Rehfeld (16) also have reported a chronic dermatosis in cattle as a result of oil applications. No symptoms other than those on the skin were noted.

Carll et al. (5) isolated three species of aspergilli from the same processed wheat concentrate used by other workers to produce hyperkeratosis. Bread on which one of the aspergilli had been cultured was toxic when administered in an aqueous slurry by stomach tube. The lesions produced, however, were not characteristic of hyperkeratosis, and this same lot of processed wheat concentrate has also been shown to contain highly chlorinated naphthalenes (15). In addition to the material isolated and identified as highly chlorinated naphthalene, the German wood preservative (14) also contained a second active fraction, having an odor similar to that of trichlorobenzene. This fraction was contained in the steam distillate from the wood preservative. Fractional distillations indicate that this material consists largely of a mixture of dichloronaphthalenes (32).

It is noteworthy that no new outbreaks of the disease have been reported during the past year (21). The cooperation of oil companies and feed processors in eliminating chlorinated naphthalenes from products used on the farm has been an important factor in the reduction of the incidence of the disease.

REFERENCES

BOVINE HYPERKERATOSIS: A REVIEW


