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CHRONIC COPPER POISONING IN SHEEP

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The long-continued ingestion of salt mixtures containing relatively small percentages of copper sulphate caused chronic copper poisoning among flocks of range sheep on several West Texas ranches during the past year. The salt licks were placed before the sheep as a means of preventing or controlling stomach worm infestation despite a lack of experimental or field evidence that they were of any practical value in this regard. Investigations on seven ranches where the disease occurred revealed practically the same history in each case; the animals had been licking a medicated salt for periods varying from 7 months to several years. Sheep of all ages were affected and the losses were exceedingly heavy on all but one ranch.

Chronic copper poisoning in sheep is characterized by a yellow discoloration of the tissues and brown to black urine, loss of appetite, and weakness. Once the symptoms appear, the disease runs a rapid course and terminates fatally in the great majority of cases. Probably many sheep suffer from a subclinical type of the disease which is manifested principally by loss of condition.

The disease, which has been called icterohemoglobinuria during the past few years, was reproduced experimentally in 12 healthy sheep by feeding the commercial salt mixtures concerned. In some experiments measured amounts of the mixture were administered daily in gelatine capsule, while in others a definite amount was mixed with a handful of cottonseed meal every day and placed in the feed box. (In addition the disease was experimentally reproduced in 14 healthy sheep by feeding other salt mixtures containing copper sulphate). Analysis of the livers of both field and experimental cases of the disease showed definitely toxic amounts of copper to be present in these animals.

An experiment showed that there is no danger of producing the chronic copper poisoning by the routine drenching of sheep to control stomach worm infestation.

Predisposing factors were dry, short range, overstocking, lack of condition in many sheep, and frequent "working" of the animals. There seems to be a definite seasonal variation in the condition, the most severe outbreaks occurring during the spring and fall months.

There is no treatment for the disease. It can be prevented by removing the cause. Losses may continue for at least five months after feeding of the salt mixture is discontinued.

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CHRONIC COPPER POISONING IN SHEEP

I. B. BOUGHTON AND W. T. HARDY

A trouble occurring among sheep on numerous ranches in the Edwards plateau region of Texas characterized clinically by generalized icterus, hemoglobinuria and hematuria, inappetence, and extreme weakness, was found to be chronic copper poisoning. There is an extremely rapid, irregular pulse, accompanied by shallow, hurried respirations in practically all cases but the temperature seldom, if ever, rises above normal. The disease ordinarily terminates fatally within 24 to 48 hours after the first appearance of the characteristic symptoms but occasionally affected animals linger for considerably longer periods of time. Spontaneous recovery is comparatively rare but does occur. The notable lesions at autopsy are a yellowish, friable liver; enlarged, very dark brown to black kidneys; a swollen, "blackberry jam" spleen; generalized icterus; poorly-collapsed, doughy lungs, and a pale flaccid heart.

As a matter of fact the condition is really a cumulative poisoning since the affected animals have ingested small doses of the material containing copper sulphate for weeks or months before there are any clinical manifestations of the disease. Once symptoms appear, the course of the disease usually becomes acute.

During the past several months we have investigated seven different outbreaks of chronic copper poisoning among range sheep caused by the long-continued ingestion of salt mixtures containing varying amounts of copper sulphate in addition to tobacco dust and sodium chloride.

Such salt licks are sold in many parts of the West Texas sheep country as a preventive of stomach worm infestation despite the lack of experimental and field evidence that such mixtures are of any practical value in this regard. Analysis of two of these commercial mixtures fed on ranches where the disease occurred showed powdered copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in amounts varying from 5.3% to 9.9%*. There is little question that some of the commercial mixtures contain even larger percentages of this copper salt.

REVIEW OF THE LITERATURE

Ellenberger & Hofmeister (1) and Baum & Seeliger (2), in the latter part of the past century, proved definitely that chronic copper poisoning, following the continued ingestion of small amounts of various copper salts, is a distinct clinical entity in domestic animals. Ellenberger & Hofmeister (1) describe the typical symptoms and lesions. Their work included studies of the elimination of the ingested copper from the body. This metal disappeared from the urine a few days after feeding of the copper salt was discontinued but small amounts of copper oxide continued to be excreted daily with the feces for weeks or months. They

*All copper analyses reported in this paper were made in the laboratory of the Division of Chemistry, Texas Agricultural Experiment Station.

advanced the theory that part of the fecal copper was reabsorbed in the lower part of the intestines, such prolonged reabsorption having a deleterious effect on the liver and eventuating in chronic copper poisoning. Our observations confirm this idea since we have noted in the outbreaks studied that deaths from this condition occur for as long as five months after the feeding of the salt mixtures containing copper sulphate was discontinued. The authors mentioned above found copper oxide in all the tissues of the poisoned animals, the largest amount (.175%) in the liver and the smallest (.0058%) in the muscles. It should be noted that the largest amount of copper oxide found in the liver, namely, 0.175% was found in a sheep that developed acute copper poisoning five and one-half weeks after the feeding of copper sulphate was discontinued.

Baum & Seeliger (2) in their experiments fed smaller amounts of copper salts than Ellenberger and Hofmeister (1) daily to 15 sheep for periods of time varying from about 50 days to several months. Analyses of the livers of these animals showed a copper content varying from 0.0044% to 0.0525%. According to these investigators the oleate is the most poisonous copper salt with the sulphate following very closely. In their experiments cats, sheep, goats, and dogs are the most susceptible to poisoning in the order named. These authors found icterus and hemoglobinuria to be constantly present in sheep but failed to observe either in their work with goats.

In 1927 Schaper and Luetje (3) observed a fatal disease among sheep characterized by depression, generalized icterus, hematuria, and hemoglobinuria, stormy pulse, increased respirations, edema and eczema of the ears. These animals were grazing in orchards where the trees had been sprayed with Bordeaux mixture two months previously. Autopsies on nine affected animals revealed lesions similar to those mentioned by the present writers except that the enlarged spleens were rose-red in color and firm in consistency. The losses stopped before the cause had been determined.

During the following year (1928) Schaper and Luetje (3) investigated a similar outbreak among sheep grazing in the same orchards which had been sprayed three months previously with Bordeaux mixture. During this investigation some 267 affected sheep were autopsied and the diagnosis of chronic copper poisoning was made. Traces of lead and arsenic were found during analyses of the livers of some of the affected animals but such quantities were always within physiological limits. Cattle grazing in these orchards were not observed to be sick although several owners stated that they had lost cattle manifesting symptoms similar to those shown by affected sheep. Occasional attacks of diarrhea among horses and colts were observed but no fatalities occurred.

The symptoms and lesions observed by Schaper and Luetje during this last investigation agreed very well with those found the previous year. In some of the autopsied animals retinal hemorrhages were seen, but this lesion was never noted in the living animal.

Beijers (4) described chronic copper poisoning in a few sheep which had also been grazing in orchards where the trees had been sprayed with

Bordeaux mixture. He noted the rapid, fatal course of the disease once clinical symptoms appeared, stating that the condition appeared only after the sheep had been grazing in the orchards for several months. Symptoms and lesions similar to those described by previous investigators were noted. In addition, Beijers counted 3,190,000 red blood cells and 26,100 white cells per cubic millimeter in a typical case. These cell counts agree very closely with the results obtained by the present writers in both field and experimental cases.

During parts of the years 1930-1932 a similar condition appeared among sheep on this Station. Schmidt (5) and Schmidt and Hardy (6) investigated this condition, describing the clinical symptoms and the post mortem lesions very thoroughly but did not establish the cause. They were unsuccessful in transmitting the disease to healthy sheep either by contact or through injections of spleen tissue emulsions or blood from typical cases. No blood parasites were found. Schmidt (5) noted the extreme red-cell destruction and remarked upon the difficulty of satisfactory examination of blood smears because of the agglomerations of debris resulting from the cell destruction. Schmidt (5) named the condition icterohemoglobinuria inasmuch as the outstanding symptoms were the generalized icterus and the pronounced hemoglobinuria. It later became known to Schmidt and Hardy (6) that all of these sheep had had free access to a salt mixture consisting of copper sulphate 15%, tobacco dust 25%, sodium chloride 60%, from May, 1929, until September, 1931.

Olafson (7) under the title of icterohemoglobinuria, described a condition occurring in two bands of sheep in New York State, which, from the symptoms, lesions, and blood changes found, seems to be analogous to the subject under discussion. He was unsuccessful in transmitting the disease and did not establish the etiology. In a personal communication, Olafson states that he noted the scarcity of gastro-intestinal parasites in his autopsies. When he remarked on this the owner stated that it was due to the fact that these animals had had access to a salt mixture containing copper sulphate.

The condition in South Africa which DeKock (8) studied in 1928 in one or two bands of sheep brought from the Karroo district to Onderstepoort for blue tongue vaccine production and which he called enzootic icterus, is exceedingly similar to the subject under discussion. DeKock noted the remarkable distribution of the disease and the fact that it did not spread to any of the healthy sheep even after prolonged contact. The symptoms and lesions described by this author agree very well with those we observed. He noted the presence of many "ghost" erythrocytes in blood smears and described a "pigment" cell which he found in the various tissues of typical cases. Olafson (7) mentions this same type of cell. DeKock, in his summary, suggests the dietetic origin of the condition. The present writers know nothing about the diet of the sheep which DeKock observed, but the extreme similarity of the symptoms and lesions suggest the possibility that this author was dealing with chronic copper poisoning.

Newsom and Cross (9) in 1921 reported several outbreaks of icterohematuria among bands of sheep in Colorado. The similarity of the

symptoms and lesions described together with the fact that these authors were unable either to find any blood cell parasites or to transmit the disease to healthy sheep suggests the possibility that they may have been dealing with chronic copper poisoning. In 1931 the same authors (10) gave a general survey of the disease as it occurred in their state and remarked that newly-introduced sheep seemed to be most susceptible. Newsom, in a personal communication, states that they found no gastro-intestinal parasites among autopsied animals.

Williams (11) and Johnson (12) (13) in Montana, reported an icterohematuria in sheep, both authors believing that the condition was a true piroplasmiasis (babesiosis) which had been previously described from Roumania by Babes (14). Neither of these authors was successful in transmitting the disease to healthy sheep by injection of blood or tissue emulsions from affected animals. They reported the presence of small blue-staining bodies in the red cells of typical cases but it is worthy of note that, according to Olafson (7), "Nuttall states the recorded presence of ovine piroplasmiasis in North America is due to errors of observation." The symptoms and lesions described by both William and Johnson coupled with the regional nature of the outbreaks described is suggestive of chronic copper poisoning.

FIELD OBSERVATIONS OF CHRONIC COPPER POISONING

In August, 1933, one of the writers (W. T. H.) autopsied twelve registered Rambouillet sheep on a ranch approximately 150 miles north of this Station. None of these animals had been dead longer than eight hours.

The owner stated that 38 sheep (36 ewes and 2 yearlings) had died within the past 36 hours, all of them showing a yellowish discoloration of the tissues and voiding dark-brown to black urine during the course of the disease. The autopsies revealed generalized icterus, yellowish, friable liver; enlarged "blackberry jam" spleen; enormously swollen, black, soft kidneys; bladder full of dark-brown urine; the blood chocolate colored; gastric and intestinal mucosa showing brownish stains; the heart musculature pale and flabby and the lungs doughy. The absence of the stomach worm (*Haemonchus contortus*) was noted in all animals autopsied. This band of sheep had been treated for gastro-intestinal parasites about 45 days previously with a commercial drench containing copper sulphate and nicotine sulphate. All the registered sheep on this ranch had been licking a salt mixture containing copper sulphate for the past 2½ years, while the grade sheep had had access to plain white salt only. No losses had occurred among the grades.

On October 5, 1933, a ranchman brought two sick sheep to the laboratory for examination, stating that he had lost approximately 700 head from the same condition during the past nine months. Both of the animals presented symptoms identical with those observed in the outbreak reported above. The gross lesions seen at the autopsy of these animals were also the same. Stomach worms were not found in these animals. According to this owner his animals had shown evidence of a heavy stomach worm infestation and had been treated with a commercial copper sulphate

and Black Leaf 40 drench several times during the past year. This flock had been licking a medicated salt mixture (the same type of mixture as that mentioned in the first outbreak) for the past twelve months.

This man was accompanied by a neighbor from an adjoining ranch who stated that he too had lost and was still losing sheep from the same condition. The history of his flock, both as regards drenching and the feeding of a similar medicated salt mixture, was about the same. His losses started about the same time and he estimated that so far 500 sheep had died from the same condition. In both of these outbreaks, by far the largest loss had been among adult sheep, only a relatively few lambs (6 months or more of age) having been affected.

The sick animal which was left at the laboratory showed extreme paleness of visible mucous membranes, weakness, inappetence, and hemoglobinuria. The temperature was normal (102.4° F.) but an erratic pulse of 120 and hurried shallow respirations were noted. A count showed only 4,384,000 red blood cells and 16,200 white blood cells per cubic millimeter. A differential count gave: neutrophiles 66.5%, lymphocytes 27.5%, monocytes (large lymphocytes) 5.5%, and basophiles 0.5%. Examination of stained blood slides showed marked anisocytosis, numerous "ghost" red cells, punctate basophilia, and polychromasia. Much red cell debris on every slide made detailed observation difficult. Thorough search of many slides failed to reveal the presence of organisms either in or between the cells. This animal died on the third day, presenting the same lesions as those observed in the first outbreak reported above. Six stomach worms (*Haemonchus contortus*) were found in the abomasum. A detailed search for intestinal parasites was not made.

Chemical analyses of the livers from two typical field cases of the disease in sheep occurring on the ranch from which the case just described originated showed 14.7 milligrams (0.0147%) and 53 milligrams (0.053%) of copper respectively in 100 grams of tissue (Sheep No. 771 and Sheep No. 801 in Table 8).

Personal investigation on these two ranches revealed practically the same range conditions as those obtaining on the first ranch visited in August. The grass and weeds were very short and dry as a result of the prolonged drouth. The sheep did little grazing, remaining around the water tank much of the time; the salt troughs were all placed next to this tank. It was observed in several instances that the sheep, after licking the salt went immediately to the tank and drank. This observation had also been made on the first ranch where chronic copper poisoning was found. The ranchmen concerned had noted that their sheep stayed around the watering places much more than they usually did during seasonable years. There is little doubt that the sheep were licking more salt than they would during times of good range.

About ten days later, at the time of our second visit to the two ranches mentioned above, a neighbor from an adjoining ranch said that he had lost approximately 25 head of grown ewes and a good many lambs (about 7 months of age), all of which showed the same symptoms as those described above. Autopsy of a typical case on this ranch revealed the

characteristic lesions observed in the other outbreaks. Medicated salt (a similar mixture to that used on the two adjoining ranches) had been before his sheep for five months when his losses started.

Later, chemical analysis of the liver of a ewe from this ranch dead from the typical condition showed 28.3 milligrams (0.0283%) of copper in 100 grams of tissue (Sheep 802 in Table 7).

While the greatest loss was among pregnant and suckling ewes many fatal cases occurred in lambs (6-7 months old) and rams. The majority of the animals died within 36-48 hours after symptoms were noted by the ranchmen. Spontaneous recovery had been noted in a few cases.

Thorough search of several animals revealed the spinose ear tick, *Ornithodoros megnini*, as the only external parasite present.

Tests maturing on December 12, 1933, in which experimental feeding of a salt mixture containing copper sulphate to healthy sheep produced symptoms and lesions indistinguishable from the field cases described in the foregoing pages, definitely proved that the condition we were dealing with was a chronic copper poisoning. Previous to this time we had called the condition icterohemoglobinuria. With this information we investigated on December 28, 1933, losses among sheep on another ranch located some 50 miles northwest of this Station and found precisely the same condition as that observed on the ranches previously mentioned. At the time of our visit the owner stated that some 45 head of sheep had died within the past two weeks.

Clinical symptoms and autopsy lesions in two typical cases were the same as those observed in previous outbreaks. The absence of stomach worms was again noted. Later, examination of stained blood slides showed changes similar to those reported above. Analysis of the liver of a typically-affected lamb (approximately 8 months old) showed 48.8 milligrams (0.0488%) of copper in 100 grams of tissue (Sheep No. 807 in Table 7).

A commercial medicated salt mixture containing copper sulphate had been constantly before the sheep on this ranch since the previous April (8 months). On another ranch this man had been feeding plain white salt only and had not lost any sheep from this condition. The condition of the range on both ranches was very poor with the grass short, dry, and dead.

On January 4, 1934, the same condition was found on a ranch 25 miles north of this Station, four ewes having died during the preceding months. Autopsy of a clinically-sick ewe revealed the characteristic lesions and the absence of gastric parasites. This ranchman had kept the medicated salt mixture before his sheep almost continuously since 1927.

On February 7, 1934, another outbreak of the same condition was investigated on a ranch some 80 miles west of this Station. The history, as regards the range condition, feeding of a commercial medicated salt mixture, and animals lost were typical. Medicated salt had been in the troughs for the past 2½ years. The owner estimated a loss of 250 sheep (mostly ewes) during the past six months. While we did not see any affected sheep the symptoms and lesions described by the owner left no doubt as to the true condition.

The last outbreak of the condition investigated by us on February 8, 1934, occurred on a small ranch about 125 miles north of this Station. Sixty head of registered ewes had died during the past four months. Autopsies of two typically-affected sheep showed the characteristic lesions; stomach worms were not found in either of these animals. Analysis of the liver taken from a typically-affected grown sheep showed 24.8 milligrams (0.0248%) of copper in 100 grams of tissue (Sheep 115 in Table 8).

A mixture containing 4% of copper sulphate in addition to tobacco dust, bone meal, and salt had been in the troughs almost continuously for the past twelve years. This man usually sold many of his sheep (weaned lambs and ewes) as breeding stock every year. Apparently none of the animals sold had developed the condition since none of the buyers had ever complained of any unusual death loss.

EXPERIMENTAL INVESTIGATIONS

Transmission Tests

Although no organisms had been found either in the blood or organs of affected animals, several attempts to transmit the disease were made before the true cause of the condition was determined.

A healthy mutton was given intravenously 5 cc. of whole blood taken from the jugular vein of a typical field case of the disease. This animal remained healthy and was released on the 41st day.

Three healthy sheep were given intravenously 2 cc. each of a heavy emulsion of spleen tissue from a typical field case of the disease. These animals likewise remained healthy and were released on the 35th day.

In the outbreaks studied the only external parasite found on any of the sheep was the common spinose ear tick, *Ornithodoros megnini*. There was nothing to indicate that the tick played any part in the causation or transmission of the disease. Despite this fact we injected two healthy sheep subcutaneously with a saline emulsion of the mashed bodies of ear ticks collected from field cases of the disease with negative results.

Seven live first-stage nymphs of the common spinose ear tick, *O. megnini*, were collected from the ears of a typical field case and placed in one ear of a healthy ewe, where they attached. This animal was held under daily observation for 32 days and then released, having remained healthy. Seven live third stage nymphs of *O. megnini*, were placed in one ear of another ewe. This animal also remained healthy and was released on the 32nd day.

Previous to the studies reported here Schmidt and Hardy (6) tried to transmit the disease by placing the common sheep tick, *Melophagus ovinus*, collected from a typically-affected animal, on a healthy mutton sheep. The ticks attached and fed on this animal, which was released as healthy after the observation period of 100 days.

Copper Sulphate Feeding Tests

The history of the flock and the limitation of the disease to the registered sheep, on the first ranch where the condition occurred led us to

suspect the commercial salt mixture containing copper sulphate as being the cause of the trouble. The histories in the subsequent outbreaks coincided largely with the first one.

In a preliminary test three pregnant ewes were given daily, in gelatine capsules, a measured amount of a mixture composed of:

Copper sulphate	25%
Sodium chloride	60%
Tobacco dust	15%

The average daily dose was 14.8 grams of this mixture. All three of these animals succumbed within less than 40 days.

The symptoms were very much the same as those observed in range sheep except that the animals voided greenish-black soft feces during the course of the disease. The autopsy lesions were practically identical; a severe gastritis and a severe enteritis noted in these animals were evidently due to the local irritant action of the mixture, which contained a much larger percentage of copper sulphate than any of the commercial mixtures.

In another test a pregnant ewe which ate 15 grams daily of a similar mixture (to which was added a handful of cottonseed meal to mask the salt mixture) succumbed after 86 days, exhibiting symptoms and lesions practically identical with those observed in the field cases.

In view of the results of these preliminary trials with mixtures containing a large percentage of copper sulphate, feeding tests of the commercial salt mixtures which had been used on the ranches where the condition occurred were made. For this purpose two different mixtures, containing 9.9% and 5.3% of copper sulphate respectively, were secured from the owners.

Administered in Gelatine Capsules: Five healthy sheep were used in a feeding test with the mixture containing 9.9% of copper sulphate; in this experiment the mixture was administered daily in hard gelatine capsules.

These animals were placed in individual pens and fed a maintenance ration consisting of chopped stalks and heads of hegari and cottonseed cake.

Sheep 786 consumed 410 grams of the mixture or 41 grams of copper sulphate in 28 days and died on the 29th day. The first symptoms, icterus and brownish urine, were noted on the 25th day, and a red cell count of 5,008,000 per cubic millimeter was obtained on the 27th day. At this time stained blood slides showed anisocytosis, "ghost" erythrocytes, and punctate basophilia. The animal exhibited marked weakness accompanied by a rapid stormy pulse and hurried shallow respirations. The temperature remained normal, never exceeding 103.1° F.

The autopsy revealed: generalized icterus, a yellowish, friable, slightly enlarged liver; the spleen swollen, soft with the parenchyma about the color and consistency of blackberry jam; the kidneys greatly swollen, almost black in color and very friable; the heart musculature pale and flabby with an icteric tinge; lungs doughy, poorly collapsed; spots of brownish stain

scattered over the abomasal and intestinal mucosa; chocolate-colored blood; dark-brown urine in the bladder. The serum separated from the blood cells by centrifuging was a port-wine color.

The symptoms and lesions observed in this animal demonstrate clearly that it died from chronic copper poisoning. Further, the condition produced

Table 1. Feeding commercial salt mixture containing 9.9% copper sulphate (administered in gelatine capsules)

Sheep	Sex	Age	Weight	Average daily dose of mixture	Copper sulphate in salt mixture eaten daily	Total amount of mixture fed	Total amount of copper sulphate in mixture eaten	Days fed	First symptoms observed after	Died of chronic copper poisoning after	Copper in liver
786	M	3	77	14.12	1.39	410	41	28	25	29	No analysis
784	M	3	81	14.21	1.40	412	41	29	27	30	do
785	M	3	100	13.44	1.33	498	49	37	37	38	.0452
794	F*	aged	101	16.85	1.66	590	58	35	35	36	.0812
793	F*	do	126	15.82	1.56	649	64	41	42	42	.0195

*Pregnant at time of experiment.

in this animal by the feeding of a commercial salt mixture containing copper sulphate was indistinguishable from the disease observed in the various field outbreaks reported above.

Sheep 784 was given 412 grams of the mixture, or 41 grams of copper sulphate in 29 consecutive days. Icterus and hemoglobinuria appeared on the 27th day, persisting until death from the typical disease on the 30th day. The temperature the day before death was 102.1° F., the pulse 50, weak, irregular, and the respirations 36. Autopsy lesions were characteristic; no stomach worms were found and the microscopic blood picture was typical.

Sheep 785 received 498 grams of the mixture, or 49 grams of copper sulphate, in 37 consecutive days. Slight icterus was noted on the 36th day. The next day the animal carried a temperature of 102.4° F., respirations 116, very fast and shallow, pulse 140, weak, irregular. Pronounced hemoglobinuria and hematuria were noted while the characteristic mucosanguineous nasal discharge almost occluded the nostrils. A count of 2,024,000 red blood cells per cubic millimeter was made. Blood slides revealed many nucleated red cells, marked anisocytosis, stippling, a few "ghost" erythrocytes, and slight polychromasia. The animal died during the morning of the 38th day of the experiment, showing typical lesions of chronic copper poisoning at autopsy. A thorough search failed to reveal any stomach worms. Analysis of this animal's liver showed 45.2 milligrams (0.0452%) of copper in 100 grams of tissue (Sheep 785 in Table 2).

Sheep 794 consumed 590 grams of the mixture, or 58 grams of copper sulphate in 35 consecutive days. The appetite was capricious from the 32nd day until death four days later. Slight conjunctival icterus first appeared on the 35th day; no other symptoms were noted. The next morning (36th day) this animal was found dead. Autopsy revealed characteristic lesions of chronic copper poisoning and no stomach worms were noted. A normal

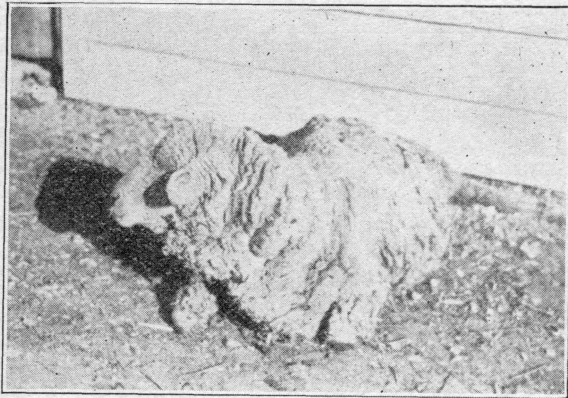


Fig. 1. (Sheep 793 in Table 1) Experimental chronic copper poisoning 24 hours after the first symptoms appeared. (Animal is unable to stand).

fetus, approximately three months old, was found in the uterus. Analysis of the liver revealed 81.2 milligrams (0.0812%) of copper in 100 grams of tissue (Sheep 794 in Table 8).

Sheep 793 received 649 grams of salt mixture or 64 grams of copper sulphate in 41 consecutive days. Anemia, as evidenced by exceeding paleness of the skin and visible mucous membranes, appeared on the 37th day. A greenish-black diarrhea persisting until death, occurred on the 39th day. On the 42nd day the pulse was 116, very weak and irregular, respirations 100, shallow and labored, and temperature 103.4° F. A red cell count showed 3,708,000 per cubic millimeter and examination of blood cell count showed 3,708,000 per cubic millimeter and examination of blood slides revealed marked anisocytosis and many stippled cells. Clinical symptoms were typical. Death occurred on the 42nd day. Autopsy lesions were characteristic of chronic copper poisoning; stomach worms were not found. Analysis of 100 gms. of liver from this animal showed 19.5 milligrams (0.0195%) of copper. (Sheep 793 in Table 1).



Fig. 2. Same animal as above, photographed three hours later, moribund. Note nasal discharge.

On January 5, 1934, a feeding experiment with the commercial mixture containing 5.3% of copper sulphate was started. Two healthy sheep were penned individually, given a maintenance ration consisting of chopped heads and stalks of hegari and cottonseed cake, and started on a salt mixture which was fed in hard gelatine capsules.

Table 2. Feeding commercial medicated salt mixture containing 5.3% copper sulphate (administered in gelatine capsules)

Sheep	Sex	Age	Weight	Average daily dose of mixture	Copper sulphate in salt mixture eaten daily	Total amount of mixture fed	Total amount of copper sulphate in mixture eaten	Days fed	First symptoms observed after	Died of chronic copper poisoning after	Copper in liver
		Yrs.	Lbs.	Grams	Grams	Grams	Grams	Days	Days	Days	Per Cent
3	M	2	67	12.18	.645	500	26	40	20	41	.0712
4	F	aged	85	12.18	.645	500	26	41	22	41*	No analysis

*Pneumonia and chronic copper poisoning.

Sheep 3 received 500 grams of the mixture, or 26 grams of copper sulphate, in 40 consecutive days. A very slight icterus and a capricious appetite were noted on the 20th day; these symptoms persisted until death from chronic copper poisoning on the 41st day. This animal lost weight gradually from the 20th day forward, becoming increasingly weaker. A count on the 22nd day showed 3,952,000 red blood cells per cubic millimeter, while blood slides revealed many nucleated red cells, punctate basophilia, and slight anisocytosis. On the 34th day a hemoglobin estimate (Talquist) of 50% and a red cell count of 6,002,000 per cubic millimeter were obtained. On the 40th day we noted pulse 100, respiration 100, temperature 102.8° F. The chocolate color of the blood made a colorimetric hemoglobin estimate impossible. There were 1,732,000 red blood cells per cubic millimeter and slides showed fragmented and "ghost" red cells, marked anisocytosis, and polychromasia. The animal died on the next day presenting typical lesions at autopsy; no stomach worms were found. In 100 grams of liver from this animal analysis showed 71.2 milligrams (0.0712%) of copper (Sheep 3 in Table 8).

The other animal in this experiment, Sheep 4, received 500 grams of the salt mixture, or 26 grams of copper sulphate, in 41 consecutive days. On the 22nd day 7,728,000 red cells per cubic millimeter were counted; the hemoglobin (Talquist) amounted to 55%. Slight icterus, remaining static until death, developed on the 22nd day. A capricious appetite persisted during the last 16 days, while hemoglobinuria and hematuria appeared on the 39th day. The animal gradually became weak, lying down most of the time during the last ten days. Symptoms of pneumonia developed on the 34th day. On this date we found a count of 6,080,000 red blood cells per cubic millimeter and estimated the hemoglobin at 60%

(Talquist). The animal died on the 41st day; autopsy revealed the lesions of chronic copper poisoning, complicated by lobar pneumonia. Again there was a complete absence of stomach worms.

Administered in Feed: Feeding experiments, in which the 15 grams of the commercial salt mixtures were masked with a handful of cottonseed meal and fed daily to healthy sheep, were carried out concomitantly with the capsule feeding tests. The animals were penned individually and allowed a maintenance ration of cottonseed cake and chopped hegari heads and stalks. In no cases were the animals given additional salt mixture until the previous day's dose had been consumed.

Three healthy sheep were fed 15 grams of the commercial mixture containing 9.9% copper sulphate.

Table 3. Feeding commercial medicated salt mixture containing 9.9% copper sulphate with ground feed

Sheep	Sex	Age		Weight		Average daily dose of mixture	Copper sulphate in salt mixture eaten daily	Total amount of mixture fed	Total amount of copper sulphate in mixture eaten	Days fed	First symptoms observed after	Died of chronic copper poisoning after	Copper in liver
		Yrs.	Lbs.	Grams	Grams	Grams	Grams	Grams	Days	Days	Per Cent		
790	M	3	88	15	1,485	645	64	49*	50	50	.0259		
789	M	3	88	15	1,485	1665	165	113**	84-89†	115	No analysis		
792	M	3	84	15	1,485	1290	128	85	85-101	—‡	do		

*No salt mixture for 7 days.

**No salt mixture for 2 days.

†Recovered; symptoms reappeared on 113th day.

‡Recovered.

Sheep 790 consumed 645 grams of the mixture or 64 grams of copper sulphate in 49 consecutive days and died of typical chronic copper poisoning on the 50th day. The animal remained healthy, so far as clinical signs were concerned until the 49th day, when the skin and visible mucous membranes were noticed to be extremely pale; the hemoglobin (Talquist) was 70%, the red cell count 9,888,000 per cubic millimeter, and a stained slide showed a few nucleated red cells and slight anisocytosis. The next day (50th) this sheep was off feed, showed the typical mucosanguineous nasal discharge, pronounced icterus, black urine, arched back, extreme weakness, pulse 120, respiration 100, and temperature 99.4° F. Death occurred later in the day, the autopsy showing the characteristic lesions of chronic copper poisoning and no stomach worms were found. Analysis showed 25.9 milligram (0.0259%) of copper in 100 grams of liver tissue from this animal (Sheep 790 in Table 8).

Sheep 789 ate 1665 grams of the mixture, or 165 grams of copper sulphate, in 113 consecutive days, dying of typical chronic copper poisoning on the 115th day. Hemoglobin (Talquist) was 70% on the 3rd day of the test; on

the 49th day it was 65%, and 9,184,000 red blood cells per cubic millimeter were counted. The 64th day showed hemoglobin (Talquist) 75% and red cells 12,828,000 per cubic millimeter. On the 84th day hemoglobin (Talquist) had increased to 80%; the red cells had fallen to 8,768,000 per cubic millimeter, while the white cells numbered 7,320 per cubic millimeter. At this time a faint icteric tinge, lasting for six days, was noted. On the 113th day this animal showed a slight icterus, deeply yellow urine, and normal pulse, respiration, and temperature. The next day the typical symptoms were exhibited; hemoglobin (Talquist) was 55%, red cells numbered 5,652,000, and white cells were 17,600 per cubic millimeter. Slight anisocytosis and much cellular debris were noted on several slides, while neutrophiles constituted 85% of the white cells. Death occurred during the night and autopsy revealed typical lesions of chronic copper poisoning; no stomach worms were found.

Sheep 792 consumed 1290 grams of the mixture, or 128 grams of copper sulphate, in 85 days, showed typical symptoms of chronic copper poisoning from the 85th to the 101st day, refused further salt mixture, and gradually recovered, being released as healthy on the 137th day. Hemoglobin (Talquist) was 65% on the 2nd day and 60% on the 49th day with a red cell count of 8,048,000 per cubic millimeter. The 84th day, hemoglobin (Talquist) showed 70%, red cells 7,608,000 per cubic millimeter, white cells 6,900. The animal was off feed, showed a faint icteric tinge in the conjunctiva, faintly colored urine, and a pulse of 108, but the respirations and temperature were normal. Two days later we counted 8,704,000 red blood cells and 7,250 white blood cells per cubic millimeter. Twenty-four hours later, pronounced hemoglobinuria and icterus appeared; red cells were 8,768,000 per cubic millimeter, white cells 9,850, pulse 120, weak and irregular, respirations 40, and temperature 104.4° F. A few stippled cells and slight anisocytosis were noted in slides. For the ensuing fourteen days this animal exhibited hemoglobinuria, pronounced icterus, inappetence, irregular pulse, hurried respirations, and a normal temperature. On the 98th day the blood showed 4,360,000 red cells and 11,410 white cells per cubic millimeter, and 60% hemoglobin (Talquist). Blood slides showed polychromasia, some stippling, slight anisocytosis, and increased neutrophiles. By the 101st day the symptoms were abating rapidly. A cell count on the 114th day showed 6,704,000 red blood cells and 4,000 white blood cells per cubic millimeter; the hemoglobin was estimated at 65% (Talquist), while blood slides revealed only normal cells. A differential white cell count showed normal percentages. Recovery was uneventful. When released on the 136th day, red blood cells amounted to 7,432,000 and white cells to 6,650 per cubic millimeter, while the hemoglobin had mounted to 80% (Talquist).

The history of this animal demonstrated that spontaneous recovery from chronic copper poisoning does occur under experimental conditions but such recoveries are relatively rare on the range.

The feeding test of the commercial mixture containing 5.3% copper sulphate was carried out with two healthy mutttons. These animals received 15 grams per day of the preparation mixed with a handful of

cottonseed meal; in addition they were fed a maintenance ration of chopped hegari (heads and stalks) and cottonseed meal.

Table 4. Feeding commercial medicated salt mixture containing 5.3% copper sulphate with ground feed

Sheep	Sex	Age	Weight	Average daily dose of mixture	Copper sulphate in salt mixture eaten daily	Total amount of mixture fed	Total amount of copper sulphate in mixture eaten	Days fed	First symptoms observed after	Died of chronic copper poisoning after	Copper in liver
		Yrs.	Lbs.	Grams	Grams	Grams	Grams	Days	Days	Days	Per Cent
1	M	2	68	15	.795	1530	81	102	102	103	No analysis
2	M	2	61	15	.795	1170	62	82*	79	82	do

*No salt mixture for 2 days.

Sheep 2 consumed 1170 grams of the salt mixture, or 62 grams of copper sulphate, in 82 consecutive days. The 32nd day we counted 5,824,000 red blood cells per cubic millimeter and estimated the hemoglobin at 55% (Talquist); the 47th day red cells were 7,752,000 per cubic millimeter with

hemoglobin at 70% (Talquist); the 68th day red cells numbered 6,600,000 per cubic millimeter, white cells 10,800, and hemoglobin 65% (Talquist). Slight conjunctival icterus appeared on the 79th day accompanied by a pulse of 144, respirations 20, and temperature 104.4° F. On the 81st day the pulse was 140, respirations 30, temperature normal. Typical symptoms of chronic copper poisoning were manifested on this date. A count showed 2,472,000 red

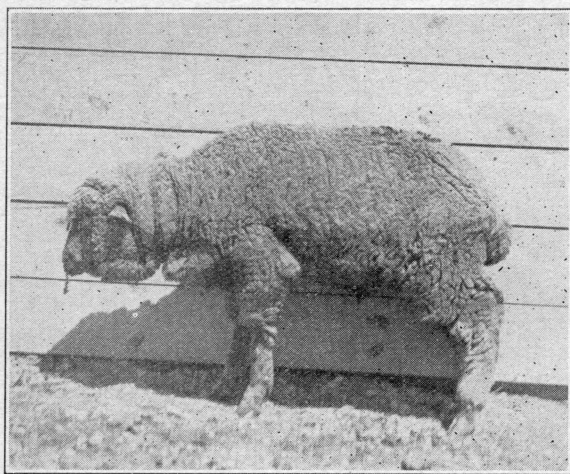


Fig. 3. (Sheep 2 in Table 4) Experimental chronic copper poisoning resulting from ingestion of a medicated salt.

blood cells and 18,400 white blood cells per cubic millimeter, with hemoglobin approximately 40% (Talquist). Slides showed anisocytosis, marked polychromasia, "ghost" and many fragmented red cells, and stippling (average of 17 stippled cells per field).

Approximately 10% of the red cells were young, nucleated normablasts. Urine sediment showed whole and fragmented red cells and free hemoglobin. Death occurred on the 82nd day and at autopsy typical lesions of chronic copper poisoning were found. Stomach worms were absent.

Sheep 1 received 1530 grams of the mixture or 81 grams of copper sulphate in 102 consecutive days, dying on the 103 day of typical chronic copper poisoning. On the 32nd day the blood showed 7,776,000 red blood cells per cubic millimeter and hemoglobin 50% (Talquist). On the 68th day the blood showed 7,624,000 red cells and 8,500 white cells per cubic millimeter with hemoglobin at 65%.

This animal was off feed and evidently sick on the 102nd day and 24 hours later typical symptoms were manifest. Pulse 149, respiration 58, and temperature 103.7° F. The blood changes in smear slides were typical. Autopsy lesions after death were characteristic of chronic copper poisoning. No stomach worms could be found.

Fed Free Choice in Trough: In a field experiment started on November 28, 1933, nineteen healthy, grown sheep were placed in a 118-acre pasture

Table 5. Feeding medicated salt mixture containing 13.2% copper sulphate in trough in pastures, free choice

Sheep	Age in years	Status of the health of the animal on			
		5-29-34 clinical examination	6-20-34 Hemoglobin (Dare)	6-25-34 clinical examination	7-15-34 clinical examination
			Per Cent		
703	3	Healthy	78	Healthy	Healthy
708	aged	do	81	Dead; chronic copper poisoning	—
711	do	do*	58	Healthy	Healthy
712	do	do	94	Dead; chronic copper poisoning	—
713	2	do	95	Healthy	Healthy
715	2	do	80	do	do
716	3	do	—	do	do
717	2	do	93	do	do
719	2	do	63	do	do
720	2	do	96	do	do
721	2	do	120	do	Dead; chronic copper poisoning
723	aged	do	100	do	Healthy
724	2	do	60	do	do
725	2	do	84	do	do
726	2	do	Dead; chronic copper poisoning	—	—
727	1½	do**	88	Healthy	Healthy
728	2	do	75	do	do
729	2	do	80	do	do
731	2	do	87	do	do

*Red blood corpuscles 8,299,000 per cmm., hemoglobin (Talquist) 70%.

**Red blood corpuscles 9,144,000 per cmm., hemoglobin (Talquist) 75%.

and allowed free access to a mixture containing 13.2% copper sulphate in addition to tobacco dust and plain salt. The salt mixture was placed in the salt trough near the watering tanks, where the animals had free

access to it at all times. The range in this pasture was in fair condition as regards grazing.

From the beginning of the experiment the animals were rounded up at intervals of two weeks and examined clinically for signs of disease.

Six months after the start of the experiment, all animals were clinically healthy. Two animals were chosen at random: one showed 8,299,000 red blood cells per cubic millimeter, hemoglobin (Talquist) 70%; the other showed 9,144,000 red blood cells per cubic millimeter, hemoglobin 75% (Talquist).

One month later these animals were rounded up and examined. They were held in the pen without food or water for 24 hours. All of the animals but one were in good condition so far as clinical examination showed. Hemoglobin (Dare) estimates at this time showed only normal percentages. Sheep 726 was noticed to be showing the typical symptoms when found in the pasture and died in the pen from chronic copper poisoning. The lesions found at autopsy were typical of the disease and no stomach worms were found.

Five days later two sheep, Nos. 708 and 712, succumbed to chronic copper poisoning after presenting the typical symptoms for 48 hours before death. Autopsy lesions were characteristic and we could not find stomach worms in either of the two animals.

Fifteen days later the remaining animals were penned over night without food or water and released in the morning. The afternoon of the same day they were rounded up and examined. One sheep, No. 721, was showing the clinical symptoms of chronic copper poisoning. It was held in the pen and died the next morning; autopsy lesions were typical and no stomach worms were found. The rest of the animals were in apparently good condition but it was noted that all of them showed a distinct paleness of the visible musous membranes.

The animals were held without food or water on the two occasions mentioned because it had been observed during the outbreaks on this Station and on the various ranches that cases of the disease usually appeared shortly after the animals were rounded up and starved for 12 to 24 hours.

Administered As a Drench: There is no reference in any of the available literature to chronic copper poisoning following the routine use of aqueous solutions of copper sulphate as a vermifugal drench. Wright and Bozicevich (15) drenched two sheep with 100 cc. of a 1% aqueous solution of copper sulphate at weekly intervals for 42 and 52 weeks respectively. Analysis of the livers of these two animals showed more than twice the amount of copper than was found in the liver of a control animal, yet neither of the treated sheep showed any indications of chronic copper poisoning.

Hardy and Schmidt (16) reported the regular monthly drenching of one flock of sheep over a period of four years without causing chronic copper poisoning. In this work a 1¾% aqueous solution of copper sulphate in doses of 100 cc. for adult sheep and 50 cc. for lambs was used.

Chronic copper poisoning depends upon the amount of some copper salt ingested and the rate at which this metal is excreted. Small amounts of copper, normally derived from the feed, are found in the animal body, principally in the liver, in all healthy animals. When the intake of copper is increased there is an increase in the amount of the metal deposited in the tissues of the body, especially in the liver, such increase in deposition depending upon the rate of excretion and the time elapsing between repeated ingestions of the copper salt. Since in routine drenching of sheep with copper sulphate in the control of stomach worms the animal is forced to ingest an unusual amount of this salt, usually at definite intervals, the question arose as to whether sufficient time was allowed to elapse between drenchings to permit the excretion of enough copper to prevent the occurrence of chronic copper poisoning.

In order to determine the rate at which copper is excreted following the usual doses of this metal used in routine drenching Schmidt (personal communication) administered orally 100 cc. of an aqueous solution containing 1¾% copper sulphate and 0.8% Black Leaf 40 to each of two healthy sheep. Both these animals, Sheep 3 in Figure 4 and Sheep 4 in Figure 5 were placed in separate metabolism crates for one week prior to treatment. At the end of this time they were drenched as stated and all feces and urine excreted were collected separately daily for 15 and 27 days respectively. The rate of copper excretion, as determined by analysis of these materials, is shown in the following graphs (Figures 4 and 5).

The sharp increase in copper excretion in the urine of both animals on the second day after drenching is followed the next day by a sharp decline to a low daily level of excretion, which continued throughout the test periods. The same is true as regards the fecal excretion of copper except that the amount was much greater and the rate of excretion began to increase on the third day and reached its maximum on the fourth and sixth day subsequent to drenching. These graphs show definitely that the largest part of an unusual dose of copper is excreted in the urine and feces within a few days after administration of such a dose. In other words, the amount of copper retained in the body subsequent to routine administration of the usual drench containing copper sulphate in the control of stomach worms is very small and cannot reach toxic proportions.

Two healthy sheep were drenched by the writers with 100 cc. of a 1% aqueous solution of copper sulphate daily until they succumbed to chronic copper poisoning. These animals were housed in individual pens and fed a maintenance ration of cottonseed cake and alfalfa hay during the experiment.

Sheep 746 received a total of 3,200 cc. (32 grams of copper sulphate) of the 1% solution at the rate of 100 cc. daily in 36 consecutive days. This animal first showed typical symptoms on the 34th day and died two days later. Autopsy lesions were typical of chronic copper poisoning. No stomach worms were found.

Sheep 747 received 3,000 cc. (30 grams of copper sulphate) of the 1% solution in 34 consecutive days. This animal succumbed to typical chronic copper poisoning during the afternoon of the 34th day, 24 hours after the

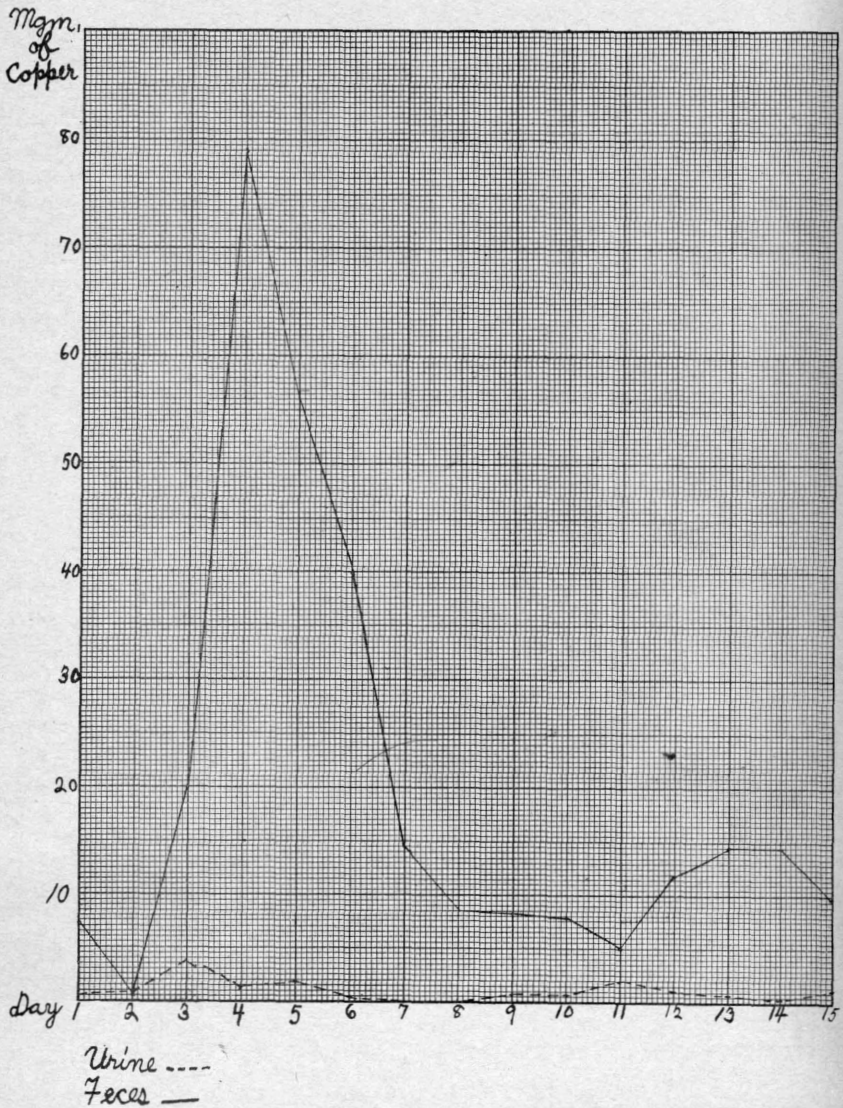


Fig. 4. Analysis of urine and feces for copper (Sheep 3 drenched with 100 cc. of an aqueous solution containing $1\frac{3}{4}$ per cent copper sulphate and .8 per cent Black-leaf 40).

first appearance of typical symptoms. The autopsy of this animal revealed generalized icterus, characteristic kidney and spleen changes but the liver changes were grossly those encountered in pregnancy diseases. A diagnosis of chronic copper poisoning complicated with pregnancy disease seemed to

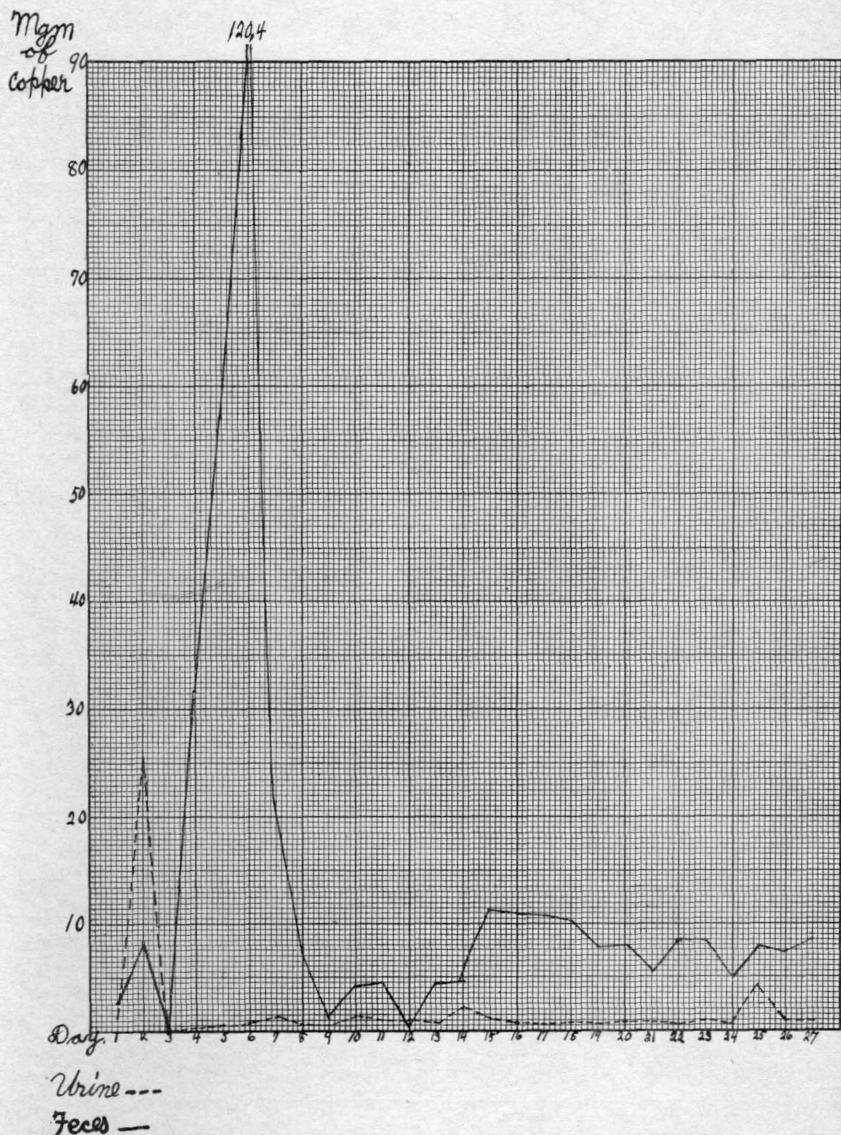


Fig. 5. Analysis of urine and feces for copper (Sheep 4 drenched with 100 cc. of aqueous solution containing 1 3/4 per cent copper sulphate and .8 per cent Blackleaf 49).

be warranted. In 100 grams of liver from this animal, analysis showed 45.6 milligrams (0.0456%) of copper (Sheep 747 in Table 8).

The continuous daily drenching of these animals far exceeds the drenching treatment to which sheep are routinely subjected in stomach worm control.

The fact that neither of these animals manifested any signs of chronic copper poisoning earlier than the 30th day of consecutive drenching indicates that the danger of chronic copper poisoning from routine drenching is negligible, to say the least. Obviously the possibility of producing the

Table 6. Daily drenching of sheep with 100 cc. of a 1% copper sulphate solution

Sheep	Sex	Age	Weight	Number of days drenched	First symptoms on	Died from chronic copper poisoning after	Total amount of drench given	Copper in liver
			Lbs.			Days		Per cent
746	M	8 mo.	70	36	34th* day	36	3200 cc.	No analysis
747	F	3 yrs.	80	34	33rd* day	34**	3000 cc.	.0456

*No drench given on four days.

**Complicated with pregnancy disease.

condition in sheep which are drenched only three or four times a year is non-existent since copper is eliminated in the urine and in the feces daily. The results of daily analyses of urine and feces for copper of the two sheep shown in Figures 4 and 5 illustrate definitely the truth of the foregoing statement.

There was no field evidence that suckling lambs were affected by the milk from ewes that had developed chronic copper poisoning. In the literature, the excretion of copper in the milk seems to be a controversial subject, Hess, Supplee and Bellis (17) finding it in the milk of mothers and cows. Froehner (18) states that copper taken by way of the mouth

Table 7. Medicated salt mixture containing 15% copper sulphate fed to lactating ewe in gelatine capsules

Animal	Sex	Age	Weight	Daily dose of mixture	Days Fed	First symptoms on	Milk flow stopped	Results	Copper in liver
		Yrs.	Lbs.	Grams					
Sheep 114	F	3	66	12.46	56	48th day	28th day	Died on 56th day; chronic copper poisoning	No analysis
Suckling lamb 114A	M	3 weeks	16½	—	—	none	—	Remained healthy Autopsied; normal	

is either not excreted in the milk or is so excreted only in traces and that such milk is not dangerous for the suckling young.

In an experiment designed to ascertain whether a lamb would develop copper poisoning while nursing a mother suffering from this condition, a salt mixture containing 15% copper sulphate was fed to a lactating ewe with a lamb at her side. In this test the ewe was fed measured amounts of the mixture daily in gelatine capsules and she and her lamb were

housed in a small pen in the barn. The maintenance ration fed this ewe consisted of chopped heads and stalks of hegari and cottonseed cake.

It will be noted in Table 7 that the mother (Sheep 114) received a total of 698 grams of the mixture (105 grams of copper sulphate) in 56 consecutive days. She developed typical symptoms of chronic copper poisoning on the 48th day and died eight days later. The lamb (114A) nursed its mother regularly until the milk flow ceased on the 28th day and remained healthy throughout the test. It was killed and autopsied after the death of the ewe and no lesions of copper poisoning were found. Analysis of the liver showed a physiological amount of copper.

Very minute quantities of copper are normally present in the animal body and seem to be essential to the building of hemoglobin according to the recent work of several investigators. But a search of available literature failed to reveal any definite quantity of this metal as a normal standard. Obviously, a rather wide variation in the amount of copper present in the body is to be expected inasmuch as it is found in-practically all the forages which constitute the ration of sheep.

The analyses of the livers of both field and experimental cases of chronic copper poisoning together with those of four normal lambs are tabulated in Table 8. The average amount of copper found in experimental cases, 48.1 milligrams per 100 grams of liver, is appreciably greater than the average amount of 33.92 milligrams per 100 grams of liver, found in the

Table 8. Copper content of livers from sheep fed medicated salt and from normal sheep

Sheep	Origin	Copper sulphate content of salt mixture	Amount of salt mixture eaten	Copper sulphate in salt mixture eaten	Copper in 100 grams of liver	Copper in liver
		Per cent	Grams	Grams	Milligrams	Per cent
771	Field case	9.9	—	—	14.7	.0147
801	do	9.9	—	—	53.0	.053
802	do	9.9	—	—	28.3	.0283
807	do	5.3	—	—	48.8	.0488
115	do	4.0	—	—	24.8	.0248
793	Experimental	9.9	649	64	19.5	.0195
785	do	9.9	497	49	45.2	.0452
794	do	9.9	590	48	81.2	.0812
3	do	5.3	500	31	71.2	.0712
790	do	9.9	645	64	25.9	.0259
747	do	1% solution as drench	3000 cc.	30	45.6	.0456
A	Normal	—	—	—	.48	.00048
B	do	—	—	—	.8	.0008
C	do	—	—	—	1.36	.00136
D	do	—	—	—	4.19	.00419

field cases. This is to be expected since the experimental animals received probably larger daily amounts of the salt mixtures over a shorter period of time than the sheep would lick under range conditions. It is interesting to note the very small amounts of copper found in the normal lambs as compared to the amounts found in both the field and experimental cases of copper poisoning. The quantities found in the field and experimental cases are definitely of toxic proportions exceeding as they do by many times the amounts found in the normal lambs.

The variation in the amounts of copper found in the normal lambs is to be expected since the conditions under which sheep are kept, character of forage and grazing (feed lot, farm, or range) undoubtedly play a very important part in the amount of copper in the tissues and organs of the body. The two lambs which showed 1.36 and 4.19 milligrams, respectively, per 100 grams of liver were ordinary range yearlings. They had never had access to any mixture containing copper sulphate and had not been treated with a copper sulphate drench for about 8 months.

SYMPTOMS AND PATHOLOGY

Once the symptoms become apparent the course of the condition is rapid, the affected animal usually succumbing within 24 to 48 hours. On the range the animal is noted to be listless and dull, lagging behind the band. Closer examination shows the conjunctival membranes stained a dirty yellow. The skin carries the same yellow color with a peculiar greenish cast. Hemoglobinuria, manifesting itself in a urine port-wine to black in color and rather copious in quantity, is a characteristic symptom in sheep and often the first one noted by the owner. A fairly profuse mucosanguineous nasal discharge which strings from the nostrils appears shortly after the onset of the last stage of the disease and continues until death. This discharge is characteristic but may not always be present. The pulse is fast, 120 to 160, weak and irregular, and the heart beat is tumultuous. The respirations are hurried, 40 to 60, shallow and may be labored. The temperature remains approximately normal throughout the disease, 102 to 104.4° F. We have never found any temperatures which could be classified as more than high normal in any of our cases.

Ordinarily the animal remains standing during the greater part of the disease, becoming recumbent a short time before death. Typically-affected sheep show a peculiar "humped" position of the lumbar region, probably resulting from the pain caused by inflamed kidneys. Trembling and extreme weakness are often seen during the last few hours of life. As a general rule there is no evidence of either diarrhea or constipation, the feces being normal both in appearance and quantity.

Autopsies of a few affected sheep showed a pronounced dryness of the fecal material in the anterior portion of the colon, evidencing the existence of a mild constipation. The greenish-black soft feces characteristic of acute copper poisoning were observed in a few experimental sheep which were capsule-fed approximately 15 grams daily of a mixture containing 25% copper sulphate, 15% tobacco dust, and 60% sodium chloride. But this type of feces was never seen in either field or experimental cases where the commercial salt mixtures were fed.

Distinct paleness of visible mucous membranes and skin is seen in animals when such lack of color is not masked by the characteristic dirty-yellow discoloration. This paleness of the mucous membranes resembles to some extent the "porcelain" membranes of sheep heavily infested with the common stomach worm (*H. contortus*) and is always noted in experimental cases a few days before the appearance of icterus.

The appetite, capricious at the onset of symptoms, is usually completely absent for several hours before death. On the range the sick animal grazes spasmodically, if at all.

In many cases the wool assumes a harsh, dry appearance. Loosening of the wool, as evidenced by the ease with which it can be pulled out, and spontaneous slipping have been observed in many cases. The ranchmen, where the condition occurred, stated that their wool clip was considerably less than normal and that much dead wool dragged from the sheep by brush and weeds, could be found in the pastures.

In none of our field cases have we noted the edema of the head and ears (followed by eczema) described by Schaper and Luetje (3) and Beijers (4).

At autopsy the generalized icterus, involving all the tissues, presents a striking picture. The characteristic dirty-yellow color often becomes brighter upon exposure to the air.

The liver is slightly enlarged, with rounded edges, very friable, and has a yellowish (tawny) color. On cross section the parenchyma has a decidedly greenish cast. The gall bladder is usually distended with a thick, often flocculent bile of a greenish-brown color.

The kidney changes are practically constant. They are enlarged, usually enormously, and show a black, marbled surface when the capsule is removed. On cross section it is often difficult to distinguish between the cortex and the medulla. The tissue is friable and tears easily if pressed with the point of the finger. Invariably the hilus shows the characteristic icteric discoloration.

As a rule the bladder is distended with a brown to black urine and the mucous membrane may show yellowish to brown spots of discoloration. Microscopic examination of the centrifuged urine sediment shows much free hemoglobin and many red cells, fragmented and whole.

The spleen is always enlarged, sometimes twice its normal size, with the parenchyma about the color and consistence of blackberry jam. In cases where the disease runs a course of several days the parenchyma may be fairly firm but shows the characteristic deep brown to black color.

The peritoneal surfaces of the stomach usually have an icteric tinge while the mucosa of the abomasum very often shows brownish stains spotted over the surface. This same brownish stain is noted throughout the intestinal tract. A slight swelling of the mucosa of the cecum was noted in one experimental case.

As a rule the contents of the digestive tract are normal in quantity and consistency although the fecal matter in the colon is sometimes noted to be dryer than normal. The mesenteric and gastric lymph glands have a watery appearance and may show an icteric tinge.

The lining of the abdominal cavity presents the characteristic yellow color. The pannicular part of the diaphragm shows a decided icteric tinge which may extend into the muscular portion.

The lungs are very poorly collapsed and doughy in consistency; the edges are rounded and the entire organ may show a dirty-yellowish to brownish color.

The heart musculature is pale and flabby with an icteric tinge. The pericardial fluid is increased in quantity and is characteristically a deep-yellow color with a reddish tinge.

The blood characteristically has a chocolate color and a greasy feel. When drawn from the jugular vein in the final stage of the disease, it coagulates very slowly or not at all in the tube when left over-night in the ice-box. During autopsy it is observed that the blood stains the hand. After venous puncture it is noted that blood continues to drip from the small wound for hours.

The blood serum is always tinted a light-red to brown color due to the presence of hemoglobin liberated through the destruction of red blood cells. Whether the serum be allowed to separate naturally from the cells in the drawn blood or whether it be separated by centrifugalization, it always shows the presence of hemoglobin.

Blood cell changes are characteristic. If the cell count is made after the appearance of symptoms, it may show red cells decreased to 2,000,000 per cubic millimeter and the white cells increased to 26,000 per cubic millimeter. The hemoglobin content of the blood remains relatively high, figuring the great decrease in the number of red cells.

Stained blood slides show enormous red-cell destruction. In many cases these slides show so much red-cell debris that satisfactory examination is rendered difficult. Marked anisocytosis, polychromasia, and punctate basophilia (stippling) are present in the red cells, although we have examined blood slides from typical, fatal cases where neither polychromasia nor stippling was noted. Many "ghost" erythrocytes are noted; sometimes the cell wall is intact enclosing an empty space, while in other cells only a portion of the wall remains to indicate the presence of a red cell devoid of hemoglobin.

Slides obtained in the later stage of the disease usually show many nucleated red blood cells, principally young normablasts and a few megaloblasts. In one case normablasts constituted approximately 10% of the intact red cells.

In most of the blood slides we have examined from typical cases, the ratio of white cells remains approximately normal. In some cases an increase of granular neutrophiles is noted.

Microscopically, many liver cells show vacuolation near the central vein; in some functional cells the nucleus is gone and the cytoplasm is much shrunken. Many of the cells show atrophy, which begins at the center of the lobule. A few large mononuclear granular cells staining a reddish-pink with hematoxylin-eosin were seen in some of the liver sections. Small yellow-brown deposits of what is evidently copper are sometimes seen in liver tissue.

The kidneys show degenerative changes. Red cells (whole and fragmented) and hemoglobin are seen blocking the tubules and in many cases the tubular epithelial cell nucleus is very hard to see. Bowman's capsule is often distended with a homogenous material which has a brownish-pink color when stained with hematoxylin-eosin. The spleen tissue shows

degenerative changes. The organ is crowded with whole and fragmented red cells, which probably explains the enlargement of this organ.

The results of experimental feeding of a copper-sulphate-containing salt mixture to goats agrees with the results obtained by other investigators. In two goats, fed the mixture in gelatine capsules, a fatal chronic copper poisoning was induced but neither of these animals exhibited the characteristic icterus and hemoglobinuria. A slight icterus was noted at autopsy but the urine, both that voided before death and that found at autopsy, remained clear and of normal color.

Schmidt, in a personal communication, reports the appearance of marked generalized icterus and hemoglobinuria, prior to death from chronic copper poisoning, in two goats fed experimentally one with 10 grams daily of a commercial medicated salt mixture containing 5.3% copper sulphate and the other with 1½ grams copper sulphate mixed with 10 grams of stock salt, both mixtures being administered in hard gelatine capsules. The goat receiving 10 grams of the commercial salt mixture daily, died after 10 months while the other animal, receiving 1½ grams copper sulphate daily, died after 5½ months. Both animals showed typical lesions at autopsy.

TREATMENT

Naturally there is nothing in the way of specific treatment of the condition. Removal of the salt mixture and thorough cleaning of the troughs should be carried out immediately. Losses from chronic copper poisoning persisted on all the ranches for at least five months after the salt mixtures were removed. The appearance of the flocks in general five months after the salt mixture had been removed was very noticeably improved; the animals were in better flesh and decidedly more active. Two of the ranchmen consulted at this time stated that their wool clip, per sheep, was much increased over the individual clip last year during the occurrence of the disease. There is a probability that many of the animals suffer from a subclinical type of chronic copper poisoning which is manifested principally in the lack of condition and the character of the wool.

DISCUSSION

The foregoing experiments in which commercial medicated salt mixtures containing copper sulphate were fed to healthy sheep on maintenance rations show conclusively that such mixtures are capable of inducing a fatal copper poisoning if fed over sufficiently long periods of time.

Experimentally there was no indication that males or lambs are more resistant to chronic copper poisoning than are pregnant or suckling ewes. There was some evidence to show that the strain of parturition is sometimes sufficient to precipitate a fatal poisoning which might otherwise have appeared only at a later date.

Few suckling lambs manifest symptoms of the disease in the range outbreaks. In the experiment shown in Table 7 there is certainly no indication that the lamb derived any ill effects from the milk it received from

its mother which died of chronic copper poisoning. There is little question but that the appearance of chronic copper poisoning in suckling lambs results from the continued ingestion, other than through the milk, of small amounts of some salt of this metal.

In the experiments in which the salt mixture was added to a small amount of cottonseed meal the blood of some of the sheep showed a decided increase in both hemoglobin content and number of red blood cells about three weeks after the feeding started. How much of such increase was due to the maintenance ration received and how much was due to the blood-stimulating effects of the ingested copper sulphate is a debatable question. It has been the experience of the writers that the red cell content of blood in range sheep is greatly influenced by the presence or absence of internal and external parasites and by the ration. We consider 7,000,000 to 12,000,000 red blood cells per cubic millimeter a normal count.

As stated in the foregoing pages we found the common sheep stomach worm, *Haemonchus contortus*, in only one animal which was suffering from chronic copper poisoning. In controlled field experiments conducted at this Station with salt mixtures containing copper sulphate, careful search of the stomach and intestinal tract of sheep which had access to the mixtures for several months showed no appreciable difference between the number of stomach worms in these animals and the number found in the control sheep which had free access to plain white salt only. The explanation of the scarcity or total absence of stomach worms in the animals dead from copper poisoning probably lies in the fact that the concentration of the copper in the animals becomes so strong that most or all of the worms are killed. But the fact that some of these parasites were found in one fatal case of poisoning shows that the stomach worm can survive even in such cases. And the field observations of the writers and many other people interested in the sheep industry have been that clinical stomach worm infestation is common among sheep which have been licking salt mixtures containing copper sulphate for weeks or months. In none of our autopsies did we make a detailed search for the small intestinal parasites by washing the contents of the gut into containers. Gross examination of the lightly-scraped intestinal mucosa at the time of autopsy failed to reveal any parasites adhering thereto in any of the cases cited above. Schmidt, in a personal communication, states that he found many of the small intestinal nematodes (*Trichostrongylus sp.*) in a goat dead from chronic copper poisoning.

In all probability the exceedingly dry condition of the range during the past year favored the occurrence of chronic copper poisoning on the various ranches. The grazing was short, most of the pastures overstocked, and many of the animals were not in top condition. There is little question that the animals licked more of the salt mixture than they would during a time of good range.

Observations, both on this Station and on the ranches where medicated salt mixtures containing copper sulphate were fed and where outbreaks occurred, indicate that "working" the sheep usually is followed within a

short time by the appearance of several cases of the disease, especially when the animals are held away from feed and water for 12 to 24 hours. This was particularly evident on one of the ranches where the sheep had been penned at three-week intervals during a period of about three and one-half months. The owner was treating the sheep at these intervals with a copper sulphate drench for stomach worms. Many of the animals died in the pen before they were treated and many others died shortly after they were turned into the pasture. In the latter case the appearance of the disease was probably due to the combination of an additional dose of copper sulphate and the lowered resistance of the animal as a result of crowding in the pen and the lack of food and water.

During the outbreaks there seems to be a seasonal occurrence of the disease; it occurs mainly in the spring and fall months, checking to a large extent during the summer and winter. The appearance of many more cases in the spring and fall months can be explained to some extent by the fact that the animals, under range conditions, are "worked" more during these months for the purpose of marking, shearing, weaning, etc.

There is no evidence to lead one to believe that the tobacco dust in the salt mixture plays any part in the causation of chronic copper poisoning. That this material has any favorable effect on the sheep, either as regards prevention of tapeworm or increase in wool production, is highly questionable. Mixtures of sodium chloride and tobacco dust, in varying proportions, have been placed before sheep in many localities and for many years but no deleterious effects have ever been reported. We conducted one experiment in which a healthy two-year-old mutton received 14 grams daily in hard gelatine capsules of a mixture containing sodium chloride 85% and tobacco dust 15% for 42 consecutive days. Altogether this animal received 575 grams of the mixture, or 86.25 grams of tobacco dust. During this time this sheep was penned and fed a maintenance ration of chopped heads and stalks of hegari and cottonseed cake. Both the hemoglobin content and the number of red cells remained at a normal level. The animal had gained two pounds at the time of its release on the 42nd day of the test. This animal was under daily observation during the next 30 days. It remained healthy.

SUMMARY AND CONCLUSIONS

So-called icterohemoglobinuria, as it has occurred in West Texas in the past few years, is in reality chronic copper poisoning.

This condition resulted from the long-continued ingestion of commercial salt mixtures which contained relatively small percentages of copper sulphate in addition to sodium chloride and tobacco dust.

Typical cases of the condition, as it occurred on the range, were produced experimentally by the feeding of two such commercial mixtures to healthy sheep.

There is no treatment for the condition since the animal dies within a day or two after it manifests the characteristic symptoms. Because copper is eliminated very slowly from the body many animals die from

the poisoning for weeks or a few months after the source of the copper has been removed.

Experiments with a weak aqueous solution of copper sulphate as a drench show that danger of chronic copper poisoning resulting from the average routine use of this agent in controlling stomach worms is negligible, if not impossible.

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