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Alkali Disease or Selenium Poisoning

A. L. Moxon

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ALKALI DISEASE

or

Selenium Poisoning

Department of

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South Dakota State College of Agriculture and Mechanic Arts

Brookings, S. D.

Foreword

This bulletin has been written as a brief review of selenium poisoning and especially the work done in this department on the "Alkali Disease" project, practically all of which was carried out under the direction of the late Dr. K. W. Franke. The project was started in 1929 and several people have added to the mass of information during the time that they were employed in this department. The following should be mentioned for their contributions: Mrs. John Liska (Miss Florence Marx), Mr. Van R. Potter, Mr. E. Page Painter, Mr. Robert Burris, Mr. Robert Hutton, Mr. Morris Rhian, Mr. Harlan Anderson and Mr. Wesley Ruth.

It is hoped that the material has been presented in such a way as to be of interest to the scientifically trained person as well as to the average reader.

The articles listed in the bibliography should be consulted for a more detailed discussion of the various topics presented herein.

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By Alvin L. Moxon

The term "alkali disease" is a misnomer which has been applied to a livestock disturbance of the semi-arid Great Plains of the United States. The early settlers adopted the term because some of them suspected that the alkali (high salt) waters of the semi-arid area caused the disease, and others associated it with alkali seeps and alkali spots in the soil. Research work carried out at the South Dakota State college in 1912 and 1913 proved that the alkali waters were not the cause of the disease, but the name has remained. Within the last few years it has been proven beyond doubt that the disturbance is caused by grasses, cereals and forages which have absorbed the toxic element, selenium, from the soil. This has brought about some action to change the name of the disease to selenium poisoning. Nevertheless, the term "alkali disease" remains and probably will be retained to designate the specific type of selenium poisoning to which it was originally applied since it is now known that there is more than one type of selenium poisoning.

History—The "alkali disease" has been a problem of considerable importance to the people of the Great Plains since the first homesteaders took up their claims. Probably the first account of the disease on record is the report written in August, 1856, by Madison (1)* an army surgeon stationed at Fort Randall, Territory of Nebraska†. The following is from his report:

> "A very fatal disease manifested itself among the dragoon horses which is supposed not to have been described in works on veterinary surgery. Four companies of the second dragoons arrived at this post about the 10th of August, 1856, one squadron from Fort Lookout and one from the Big Sioux River, the latter accompanied by a number of new or remount horses. The four companies encamped on the east or lower side of the dry ravine separating the dragoon and the infantry camps. About the 20th of August the disease commenced simultaneously in all four companies and many horses died, not, however, until the lapse of weeks and months. The following symptoms were observed: first, among the remount horses . . . . there was a sort of catarrh, or distemper with running at the nose and among all the horses a swelling of the skin of the throat and jaw; also inflammation, swelling and suppuration of sheath, tenderness and inflammation of the feet, followed by suppuration at the joint where the hoof joins the skin, the hoof in instances detaching itself and a new one forming in its place. These were also accompanied by loss of the manes and tails; the appetite was uniformly good; but, from the extreme tenderness of the feet, they were unable to move about in search of food, and it appears that at that time they were entirely dependent upon graz-

† According to the report (1) Fort Randall was located just west of the Missouri River in the Territory of Nebraska, Latitude 43° 18′ N., Longitude 98° 12′ W. This location is now in Gregory County, South Dakota, just north of the Nebraska-South Dakota line.

* Numbers in parentheses indicate articles referred to in Bibliography on pages 88-91.

> ing, there being no forage at the post for issue. Sorrel horses appeared to suffer most but no color escaped. The private horses of the officers shared the fate of the public animals. A few mules and Indian ponies were similarly affected. The acclimated suffered equally with the unacclimated. No treatment was effectual or afforded permanent relief. Bleeding in the feet was tried but its effect was merely temporary. Every case of the disease originated on the lower side of the dry ravine above alluded to. After forage was provided for the horses no new cases occurred, and hence, it is fair to infer that a liberal allowance of forage in the beginning might have rendered the disease much less fatal, or have prevented it. Whether the disease was caused by eating any poisonous herbs, or propagated in any manner by contagion could not be satisfactorily determined, the origin and progress of the malady being something entirely new, even to the Indians and half-breeds of the country . . . "

This reference to the disease is of particular interest at the present time due to the fact that rather extensive livestock losses have been reported from the vicinity of Fort Randall during the past year. Byers (2) gives an interesting discussion of the selenium in soils and vegetation samples taken at Fort Randall in 1934.

At this point it might be well to mention that Marco Polo might possibly have been referring to the "alkali disease" in the story of his travels (3).

The following sentence was taken from his book of travels*:

> " . . . . It is a fact that when they take that road, they cannot venture amongst the mountains with any beasts of burden excepting those accustomed to the country, on account of a poisonous plant growing there, which, if eaten by them, has the effect of causing the hoofs of the animals to drop off; but those of the country being aware of its dangerous quality, take care to avoid it. . . . "

The mountains to which he referred are probably located in a part of the province of Shen-Si on the frontier of China. His observations were evidently made sometime in the latter part of the thirteenth century.

This quotation resembles the testimony given by farmers of the affected areas in South Dakota. Many of them state that they have had horses "alkalied" by allowing them to graze for only one night in certain pastures or by feeding them just one feed of hay or grain raised on certain fields. In connection with the last part of the quotation it is of special interest to note that Beath (4) of the Wyoming Experiment Station has made the following statement as a result of his studies on poisonous plants, and especially seleniferous plants:

> " . . . . In a state of nature, animals, as a rule, avoid plants of a toxic nature, whereas imported stock, and particularly those better bred, are often poisoned because they do not easily acquire the habit of "rustling" . . . . "

Mayo (5) of the Kansas Experiment Station, in 1891, reported the loss of a large number of horses in Kansas during the fall and winter of 1890-91 from a strange disease called "blind staggers," "mad staggers" or just "staggers." His description of the symptoms exhibited by the horses indicates that it is similar to the "blind staggers" which is prevalent in

*Acknowledgment is hereby made to Dr. R. A. Gortner of the University of Minnesota for calling the attention of members of this department to this reference.

Wyoming at the present time and which has been demonstrated by Beath (4, 6) to be a form of selenium poisoning.

In 1893 the third annual report of the Wyoming Agricultural College and Experiment Station (7) alluded to a peculiar disease of the horses in Carbon county, Wyo. The symptoms mentioned are loss of flesh, partial or whole depilation of manes and tails, elongation and cracking of the hoofs, and erosion of the long bones. Ergot was suspected as being the causal agent.

Peters, (8) in 1904, described the "alkali disease" in Boyd county, Neb., where it had occurred in all kinds of livestock since the settlement of that region in 1891. He attributed the disease to a fungus disease of corn caused by a species of **Fusarium.** Corn from the affected area was shipped to the Nebraska Experiment Station where it produced the typical symptoms when fed to hogs. Peters included in the report testimony from farmers to the effect that corn free from mold, other grains, and grasses also caused the same disturbances.

A legend among the Sioux Indians is of interest in connection with the "alkali disease" (9). According to the legend the drying of the ground produces cracks out of which yellow spiders come to spread poison upon the vegetation. Animals which eat the vegetation are poisoned. This legend indicates that the Sioux Indians suspected the vegetation of causing poisoning of their animals even before the white settlers came in and attributed the responsibility for the poisonings to the alkali waters and to the alkali spots in the soil.

Copies of a large number of letters, concerning the "alkali disease", from homesteaders and ranchers to various state and federal governmental agencies are on file in this department. The first letters are dated 1906 and the latest 1912. Several of the letters are very pathetic and tell about the homesteaders losing all of their horses and cattle from the terrible "alkali disease" and others tell of the horses becoming so lame that they could not be used for farm work or for driving.

On different occasions Federal Veterinarians were sent out on investigations but were unable to determine the cause of the disease. The veterinary report by E. L. Moore in the South Dakota Agricultural Experiment Station Annual Report for the year ending June 30, 1910, indicates that the departments of Botany and Veterinary were investigating the "alkali disease" at that time. The report also indicates that ergot was then suspected as being the causal agent.

Although various surveys and investigations carried out during the period 1906 to 1912 indicated that alkali water was not connected with the "alkali disease" many people continued to believe that alkali water was involved. Larsen, White and Bailey (10) in 1912 and Larsen and Bailey (11) in 1913 carried out experiments which proved that the use of alkali water for dairy cows caused no symptoms of "alkali disease." In fact, they reported that upon post-mortem, examinations and analyses of the vital organs showed nothing abnormal. Furthermore, the feeding of "alkali" water to dairy cows caused no significant changes in the quality of the milk, butter or cheese.

Lipp (12) in 1922 wrote an account of the disease in the west river country of South Dakota. He gave a well written description of the symptoms and discussed the general areas involved.

Hutton, in 1922, made a soil survey of a typical area in an affected locality of South Dakota and came to the conclusion that the "alkali disease" was related to certain soil types. Although the results of his survey have never been published they were reported at the 1931 meeting of the American Society of Agronomy (13).

In 1924, Evans, Bushey and Kuhlman started work on the problem. The following paragraphs taken from a report* made by them are given here. None of their work was published:

> "During the past year the following work has been done on the so-called "alkali disease." This is a disease which has baffled scientists for centuries in various parts of the country. Even in South Dakota much work has been done on it but so far as we are aware, no results of a positive nature either as to the causal substance or the method of obtaining it by animals has ever been discovered. Dr. Marsh of the Department of Agriculture, who is an expert on poisonous plants, came into South Dakota to work on it, but has stated to one of us that it did not lend itself readily to solution so he was forced to give it up. After one year's work on experiment station funds we wish to make the following report:
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> "On May 1, 1924, a trip was made to western South Dakota to observe conditions. Since that time Dr. Evans has made several trips and has collected widely the flora of that region in the hope that there might be some connection. He has also made observations on many farms as to condition of stock, etc.
>
> "He has found this condition to extend over an area of about 250 to 300 square miles. In this territory some farms are free of the disease, others have been troubled severely by it.
>
> **Symptoms of Disease.**—"Hogs, cattle, horses, and chickens are all affected by the disease. Hogs seem to react most easily, sloughing the hair and hoofs very readily when given the proper food. Cattle and horses lose their feet and hair in much the same manner, but more slowly, due no doubt to the greater size of the animals. Numbers of all these animals have been examined which have partially or completely lost their hoofs and nearly all their hair. Tails and manes are first to go with other extremity parts being lost in short order. It is not uncommon to see hogs without any hair whatsoever, appearing much as if they had always been hairless. Cattle lose their hair and hoofs and come to walk on their foreknees, due no doubt to the soreness of their feet. The fertility of the egg of chickens seems to be affected and those eggs which hatch are likely to produce young chickens which are weak and usually do not develop feathers normally. The animals which are "alkalied" usually are thereafter non breeders or are very slow breeders. It seems impossible in trials made, to get a sow with pigs while she is "alkalied." The sow upon which the experiment was conducted later became pregnant, but only after at least partial recovery. The first experiment with the suspected grain was conducted last July, and extended over two months. There were no reactions in that time, although the corn used came from a farm where 13 out of 26 hogs had died and all the others were badly "alkalied" from eating it. The above hogs were fed on the farm and death resulted in late January and early February. Two of the "Alkalied" hogs were bought and shipped to the College with the corn. These hogs, a sow and a boar, never missed a feed from this corn, yet when kept here they recovered and grew new coats and

* Progress report on a cooperative project between Animal Husbandry, Botany, and Agronomy Departments submitted to the Administration at South Dakota State College by Arthur T. Evans, A. L. Bushey and A. H. Kuhlman, May 21, 1925.

hoofs. This led us to believe that the element, if an element were present in the corn, was disappearing. Accordingly we set about to find out. New wheat just threshed was shipped in and a new experiment started. In 14 days positive results were gotten. The hair began to fall and within a short time the majority of pigs were hairless. When put upon other food, these pigs recovered rapidly and grew new coats. Since they received no other feed than the wheat and the check lots showed no reaction, we concluded that the wheat was responsible. Later, corn was shipped in and results were gotten, which were of a positive nature. Recently we fed wheat which gave reactions last summer and found it to be still effective.

Laboratory.—"The first work in the laboratory seemed to indicate that the substance we were dealing with was ergot. Examination of grasses and of the ground revealed literally millions of sclerotia in various stages of decay. The grasses showed many also. Many residents of the area testify to the fact that the grass many seasons blows black with them.

"Our first laboratory work yielded what appeared to be very heavy tests for ergotoxine, ergotinine and cornutine by regular approved methods. In checking over our work, we became convinced there must be something else coming out in our precipitations. Later we isolated a number of types of proteins and found that the approved methods of extractions for sclerotia would not hold for extractions made where other ground substances were present. This required a completely new technique, which we have proceeded as rapidly as time permits to work out. Although we are not as convinced that it is ergot as we were at one time, yet we have not entirely abandoned our original assumption.

"In the meantime we have proceeded to eliminate one by one the other possibilities which may be considered as animal poisons carried in plants. Our first attack was on glucosides, which we have eliminated, we believe, with surety. At the same time, we have run a complete analysis of the corn from the region which gave positive results on hogs for K, P, Fe, S, Ca, Na, Mg, checking it against normal corn, but find no striking difference which would account for the poisonous effects we are getting. We are checking these results again to be positive. But how can one be sure the corn he tests is affected? One might readily pick the wrong sample to test, which would seriously affect results. In fact we have spent much time on some corn that we had every reason to believe was capable of producing the ailment only to find later that it was nontoxic. It must be remembered that hogs eat large composite samples and it is only when they receive these over many day periods that results accrue, indicating an accumulating poison.

"Our quickest results have come from feeding hogs our residues after extraction. During the past winter we have extracted hundreds of pounds of corn supposed to carry the disturbing element. In no instances have we ever gotten it into the extract. We have extracted with anhydrous ether, alcohol, water, acidulated alcohol, basic alcohol, and in a number of other ways. In all cases the experiments have been conducted over four weeks unless results were gotten sooner. In one case the hogs reacted in 10 days.

Following is an annotated list of points covered in our work:

1. Fed 48 hogs on West River corn for two months. No reaction.
2. Fed 12 calves and one hog on West River corn for two months. No reaction.

3. Fed 18 hogs on new West River wheat 14 days. Gave characteristic reaction of "alkalied" hogs.
4. Fed three miscellaneous lots of hogs to prove presence or absence of factor in corn.
5. Fed 30 rats West River corn. Produced no ill effects.
6. Fed 30 rats West River wheat with varying results.
7. Fed two pigs three weeks on alcoholic extract. No symptoms.
8. Fed two pigs three weeks on residue after extraction with alcohol. "Alkalied" pigs.
9. Fed two pigs three weeks on water extract. No symptoms.
10. Fed two pigs three weeks on residue after extraction with water. "Alkalied" pigs.
11. Fed two pigs three weeks on basic alcoholic extract. No symptoms.
12. Fed two pigs three weeks on residue after extraction with basic alcohol. "Alkalied" pigs.
13. Fed two pigs three weeks on acidulated alcohol extract. No symptoms.
14. Fed two pigs three weeks on residue after extraction with acidulated alcohol. "Alkalied" pigs.
15. Fed one pig on portion of wheat which gave reactions last summer. Pigs "alkalied" in 14 days, May 22, 1925.
16. Fed five rats basic alcohol extract. No reaction.
17. Fed five rats ether extract of corn. No reaction.
18. Fed five rats on acid alcohol extract. No reaction.
19. Fed five rats on water extract. No reaction.
20. Fed five rats on West River corn. Gave symptoms.
21. Fed five rats on West River wheat. Gave symptoms.
22. Made several hundred tests for alkaloids. Doubtful results.
23. Made numerous tests for glucosides. No positive results.
24. Made complete analysis for Mg, S, Fe, Ca, P, K, Na. No appreciable variation from normal corn.
25. Made determinations on percentage of amides, albumens, globulins, prolamines, and glutelins. No apparent differences from normal.

"This is a brief outline of the work as it has been carried out to date. We have found so many complicating factors entering in that it seems largely a matter of eliminating all that we can and reducing it to its simplest terms before solution is possible. Even then we are not intimating or promising solution. We are fully aware that investigators in various other institutions, working on plant poisons, notably Wyoming, have worked for a number of years and have not then discovered the cause. The loco weed has been under investigation for 15 years and it has been impossible to find out just what does the damage. The problem on which we are working may lend itself quickly to solution when a number of necessary points are cleared up and then again it may take years."

Available reports indicate that the cooperative project was terminated soon after the above report was made. However, close friends of Professor Bushey have stated that he continued some chemical investigations on the "alkalied" grains until the time of his death, but unfortunately, he left no record of his investigations.

In August, 1928, the late Dr. K. W. Franke came to South Dakota State College to begin his duties as Station Chemist and early in 1929 began active work on the "alkali disease" project. The author had the pleasure of working on the problem under his direction from January, 1930, to the time of his death in September, 1936.

The work on the project during the fiscal year, 1928-29, was of a preliminary nature. Samples of "alkalied" grains were analyzed qualitatively for Silica, Iron, Aluminum, Phosphorus, Chromium, Zinc, Manganese, Nickel, Cobalt, Alkaline Earths, Barium, Strontium, Calcium, Magnesium, Sulfur, Potassium, and Sodium. The same samples were analyzed quantitatively for Silica, Calcium, Iron, Aluminum, Magnesium, Phosphorus, Sulfur, Sodium, and Potassium. No significant results were obtained from the above analyses.

A progress report was submitted on June 30, 1930, for the fiscal year. This report included the results of feeding trials on about 60 rats. Photographs included in the report showed severe degeneration of the livers of the rats which were fed toxic grains. (See Fig. 17). Other typical symptoms such as the loss of hair, inhibited growth, restricted food intake, etc., were discussed. The possibility that arsenic, thallium, or some of the other less common elements might be causing the disease was discussed. Autopsy reports on one "alkalied" horse and four "alkalied" pigs, which were shipped in from western South Dakota, were also included.

In March, 1932, the second progress report in the so-called "alkali disease" project was submitted to the college administrative officials. This was a rather detailed report, 187 pages in length, in which all of the work done between June 30, 1929, and March 14, 1932, was summarized. The report was divided into two general sections: Section I, Experimental Feeding Trials with Rats; Section II, An Investigation on the Effect of "Alkalied" Grain on the Hatchability of Eggs and the Effect of "Alkalied" Grain on Growing Chicks. The details of this report will be discussed more fully in other parts of this bulletin.

In March, 1931, Dr. Henry G. Knight, Chief of the Bureau of Chemistry and Soils, visited this laboratory and became interested in the selenium problem to the extent that he called a conference in Washington, D. C. on April 29, 1931. This conference was attended by Director J. W. Wilson and Dr. K. W. Franke from the South Dakota Experiment Station, Dr. Henry G. Knight from the Bureau of Chemistry and Soils, and representatives of the Bureaus of Plant Industry, Animal Industry, Home Economics, and Dairy Industry of the United States Department of Agriculture. As a result of this conference a memorandum of understanding, effective July 1, 1932, for cooperative work relative to the cause and geographical localization of osteo-oedimic or "alkali" sickness, was drawn up between South Dakota Agricultural Experiment Station and the Bureaus of Animal Industry, Home Economics, Dairy Industry, Chemistry and Soils, and Plant Industry of the United States Department of Agriculture. The possibility that thallium, arsenic, or some of the other less common elements were causing the disturbance, as discussed by Franke in his progress report, submitted on June 30, 1930, is said to have been discussed at this conference.

On May 14, 1931 an interbureau committee meeting was held for the purpose of discussing research on the problem (2). At this meeting Dr. Munsell of the Bureau of Home Economics reported that symptoms produced in rats by feeding compounds of fluorine, lithium, and oxalic acid were different from those resulting when toxic grain was fed. A discussion followed in which Dr. H. G. Knight, Chief of the Bureau of Chemistry and Soils, is said to have suggested that the possibility of selenium

being present in the toxic grain be investigated. Dr. W. O. Robinson of the Bureau of Chemistry and Soils was furnished with a sample of toxic wheat on May 25, 1931.

A report of a committee meeting held on September 21, 1931, contained the following paragraphs:

> "Mr. W. O. Robinson, of the Bureau of Chemistry and Soils, has been making tests for selenium and has found that selenium is present in the protein to several times the extent in the whole grain. He has been making tests upon samples of soil from South Dakota presumed to carry the toxic principal but has not yet been able to detect selenium in his samples.
>
> "Miss DeVaney, of the Bureau of Home Economics, reported upon tests made with selenium and other compounds added to the grain. With selenium, similar symptoms as reported by Dr. Franke for the toxic grain were obtained."

A cooperative preliminary field survey was conducted between July 18, 1931 and August 17, 1931, by Dr. K. W. Franke from this department; Mr. T. D. Rice, Bureau of Chemistry and Soils, U. S. D. A.; Dr. A. G. Johnson, Bureau of Plant Industry, U. S. D. A.; and Dr. H. W. Schoening, Bureau of Animal Industry, U. S. D. A. The results of this preliminary survey were published in the form of a U.S.D.A. Circular (14).

In July of 1933 an interbureau committee of the U. S. D. A. met with the Secretary of Agriculture and took up the need for a special grant for work on the selenium problem. On September 5, 1933, a presidential order authorized the Secretary of Agriculture to set aside $35,000 for the work (2).

In October, 1933, T. D. Rice of the Bureau of Chemistry and Soils, K. W. Franke from this department, and H. G. Byers of the Bureau of Chemistry and Soils conducted a preliminary soil survey of the affected area. They were accompanied on a portion of the survey by D. R. Ready of the Bureau of Chemistry and Soils and R. L. Piemeisel of the Bureau of Plant Industry. The survey was made for the purpose of determining whether or not any topographical, botanical, or other criterion could be used as a guide in the locating of seleniferous areas. A large number of soil and vegetation samples were collected and analyzed, the results of which have been published as a U. S. D. A. Bulletin (2).

The Cooperative Agreement, as of July 1, 1932, was terminated by the U. S. D. A. on June 30, 1934. Since that time research on the problem has been carried out independently by this department and by some of the interested bureaus, especially the Bureau of Chemistry and Soils of the U. S. D. A. in Washington.

During the spring of 1936 Dr. M. I. Smith of the United States Public Health Service and Dr. K. W. Franke were in the field for six weeks investigating human cases of selenium poisoning. The results of their survey have been reported (15) and will be discussed further under Public Health on page 83.

Beath and associates at the University of Wyoming have been engaged in studies on the selenium problem for some time. In 1921, the Department of Veterinary Science at the Wyoming Station (16) drew up a project for the study of " An Obscure Disease of Cattle on the Range." According to Beath, Eppson, and Gilbert (6) the project was continued intermittently until 1930, and during the same time interval, the Chemistry

Department of the Wyoming Station was carrying out investigations on suspected water, moldy forages, and other feedstuffs that might be involved in cases of the livestock poisoning. In 1931, they made a special study of the chemical and physiological properties of certain native **Astragali.** During the next year, 1932, they published a summary (17) of their work. In the same year, 1932, M. Taboury (18) published the results of his researches on the occurrence of selenium in certain vegetation. One of the plants in which Taboury found selenium was a species of **Sium (S. latifolium).** Since **Sium cicutaefolium** was known to occur at a certain area where "blind staggers" had persisted for many years, the work of Taboury led Beath, Eppson, and Gilbert to examine **Sium cicutaefolium** for selenium. This work was the beginning of the experiments in Wyoming which later included other types of vegetation growing particularly upon the so-called gumbo soils.

The following paragraphs are taken from the same bulletin by Beath, Eppson, and Gilbert (6).

> "Early in 1933 the director of this Experiment Station referred the authors to a confidential report, issued jointly by the United States Bureau of Chemistry and Soils and the South Dakota Agricultural College and Experiment Station, that outlined in considerable detail a disease of livestock known as the "alkali disease." The areas most involved were reported to be parts of western South Dakota and the extreme eastern part of Wyoming. This disease had been investigated for many years from a variety of angles. While no definite proof was advanced in this report as to the fundamental cause, a footnote carried this reference to selenium: "Through the kindness of Dr. H. G. Knight, Chief of the Bureau of Chemistry and Soils, U.S.D.A., some preliminary tests were made that showed a possibility that selenium might be involved."
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> "Since this time the Bureau of Chemistry and Soils of the United States Department of Agriculture has published on selenium and the "alkali disease" of livestock.* The original reference to Knight's work, therefore, is no longer a confidential matter, hence its inclusion in this bulletin."

In 1934, Beath et al (4) published a bulletin pointing out the definite relationship between plants containing selenium and the acute poisoning of livestock and also the danger that would result if selenium bearing plants were plowed under and the soil sowed to crops such as wheat, oats, etc.

Since that time several more articles concerning the selenium problem have been published by Beath and others from the Wyoming Station, the results of which will be discussed in various sections of this bulletin.

A large number of articles concerning the selenium problem has also been published from this laboratory and from the various bureaus of the U. S. D. A. at Washington, D. C. These will be discussed likewise in various sections of this bulletin. The various phases of the selenium problem will be discussed separately in the remainder of the bulletin. For a list of these phases see Table of Contents.

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* Nelson, E. M. et al (1933). Selenium as an Insecticide. Science 78 124 Williams, K. T., and Byers, H. G. (1934). Occurrence of Selenium in Pyrites. Analytical Edition, Ind. Eng. Chem., 6 296.

Selenium Poisoning in Livestock

Selenium poisoning in livestock may be divided into two general classes. The chronic type, "alkali disease," which is the predominant type in the greater part of the seleniferous area of South Dakota, and the acute type, "blind staggers", which is the predominant type in Wyoming. Of course, there are all degrees of poisonings between these two extreme types, which are referred to by ranchers as one or the other of the above types, or possibly by other terms such as "mineralized animals," "bobtailed disease," "locoed animals," etc. Recently it has been shown by Fraps and Carlyle (19) that loco in livestock is produced by locoine, the poisonous principle of the loco weed, Astragalus earlei, and has no relation to the disturbances caused by selenium. It is, however, probable that locoine and selenium exist in the same plants in seleniferous areas.

In general it can be said that the "alkali disease" (chronic selenium poisoning) results when an animal consumes feed containing a rather low concentration of selenium (5 to 40 p.p.m.) for a period of several days or weeks. Many ranchers, however, have expressed the belief that horses may, by grazing for only one night in a seleniferous pasture or by eating one feeding of seleniferous grain, become "alkalied" to the extent that they will lose the hair from their manes and tails and in some cases lose their hoofs or become so lame that they can walk only with great difficulty. Likewise, the acute form ("blind staggers") results when an animal consumes plants which have taken up relatively large quantities of selenium (hundreds or even thousands of parts per million).

It is the author's opinion that the chronic type ("alkali disease") is caused by selenium which is bound in the proteins of plants and is relatively insoluble in water while the acute type ("blind staggers") is caused by organic selenium compounds which are soluble in water and may be readily extracted from plants.

"Blind Staggers".—Although we have had reports of cases of "blind staggers" in South Dakota, we have not had the opportunity to investigate any of the cases. There is no doubt but what many cases occur, especially in the southwestern part of South Dakota, and plans are being made to investigate some cases during the next year.

Draize and Beath (7) and Beath, et al (4, 6) have discussed the disturbance in great detail. Their discussion of the disease will be briefly summarized in the following paragraph.

The term "blind staggers" is rather misleading because the afflicted animals may not become blind nor do they necessarily stagger about. In the first stages of the disease cattle may have a tendency to stray from the herd. At this stage there is a slight impairment of vision and the animal will have difficulty in judging distances of objects in its path. In the next stage there is usually a more pronounced blindness accompanied in most cases by a depraved appetite and the desire to chew wood, bone, metallic objects, etc. There is a greater tendency to wander, often aimlessly in circles, and in case the animal encounters a solid object it makes an effort to push the body forward rather than to turn to the side and go around the object. In the last stage, characterized by various degrees of paralysis, there is evidence of abdominal pain, grating of the teeth, sali-

vation and grunting. Death usually results from failure of respiration. The pathology of "blind staggers" has been adequately described (4, 6, 7) and will not be discussed here.

"Alkali Disease".—Symptoms observed in a large number of "alkalied" animals on farms and ranches in the affected areas:

Dullness and lack of vitality is a general symptom of the "alkali disease." The animals become emaciated, their coats become roughened and they fail to respond well to feeding, even when moved to a selenium-free locality and given good feed and water. The heart and liver are severely damaged, especially in advanced cases. Many cases of atrophy of the heart (dish-rag heart) and atrophy and cirrhosis of the liver have been observed by Draize and Beath (7). Anemia is common, especially in advanced cases. Anemia is also prevalent in rats fed on selenium-containing grains (20). Erosion of the bones, especially the joints of the long bones, which causes the animals to become very stiff and lame, is accompanied by a disturbance of the calcium phosphorus metabolism. Experimental dogs fed "alkalied" grains showed a decrease in blood phosphorus with relatively little change in blood calcium (unpublished data).

Horses.—In most cases the first prominent symptom to appear in horses is loss of the long hair from the tail and mane. For this reason the "alkali disease" has been called the "bobtailed" disease in certain localities. The horse shown in Figure 1 lost the long hair from its tail six weeks after being moved to an affected ranch. The loss of hair from the tail and

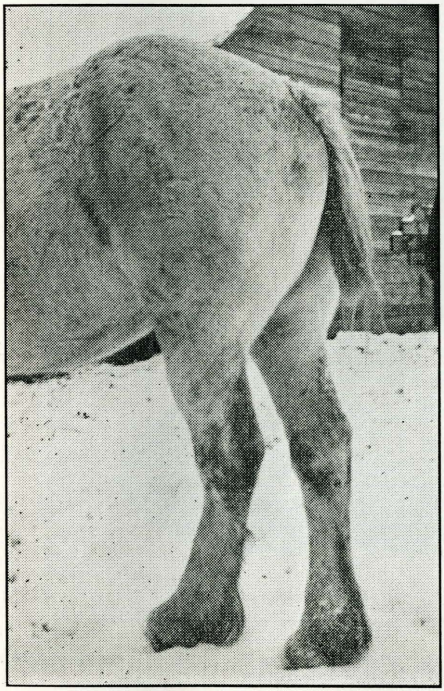


Fig. 1—This photograph shows the loss of the long hair from the tail after the horse had been on an affected ranch for only six weeks.

mane is usually accompanied by, or followed shortly by, soreness of the feet. A ring begins to appear on the wall of the hoof below the coronary band. In mild cases no further change may take place, although the animals may be lame for some time. In severe cases a gradual separation of the wall of the hoof occurs at the ring below the coronary band, and a new growth of hoof starts near the coronary band. In the less severe cases the new hoof grows at an apparently normal rate and pushes the old part of the hoof ahead leaving a break in the surface as shown in Figure 2. In the more severe cases the old hoof separates from the new growth and clicks against the new hoof as the animal moves. As the new hoof grows the old one is gradually pushed down until it is finally sloughed off. Franke, et al (14) show a picture of a horse which had lost the hoofs from both front feet and one hind foot. During the time that the animals are shedding the old hoofs and growing new ones they are very lame and in severe pain. Usually they will not move and unless they are given food and water will often die of starvation and thirst. The separation of the hoof wall from the foot takes place quite rapidly in some cases. Figure 3 shows the hoof of a horse which died two months after being moved to an affected ranch.

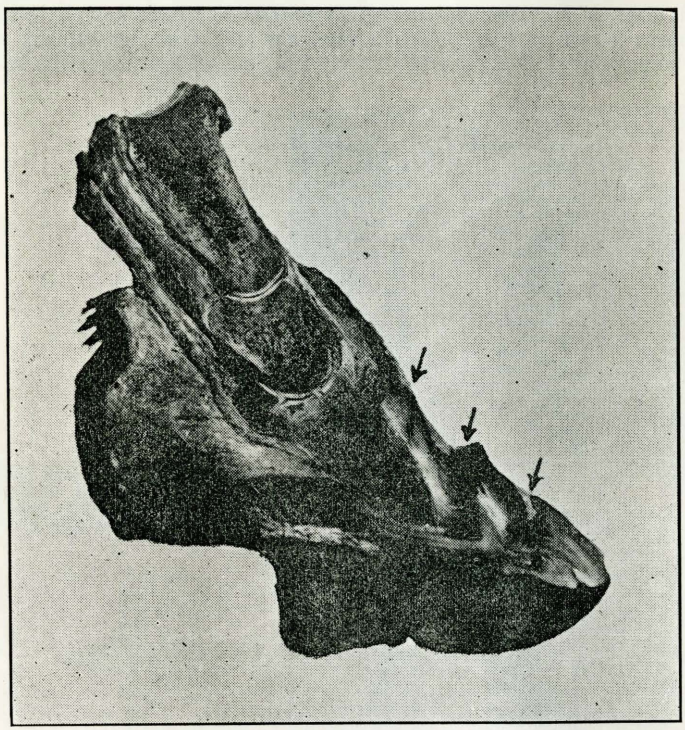


Fig. 2—Cross section of a hoof from an "alkalied" horse. The arrows indicate points at which growth has been interrupted.

Colts are sometimes born with deformed hoofs and other symptoms of the "alkali disease." Figure 4 shows the hoof of a 14-day-old colt that was born with abnormally developed hoofs. One of the hoofs came off when the colt was only a few days old and consequently its feet were so sore that it could hardly stand.

Fig. 3.—This photograph shows the separation of the hoof from the foot of a horse which died after it had been on an affected ranch for only eight weeks.

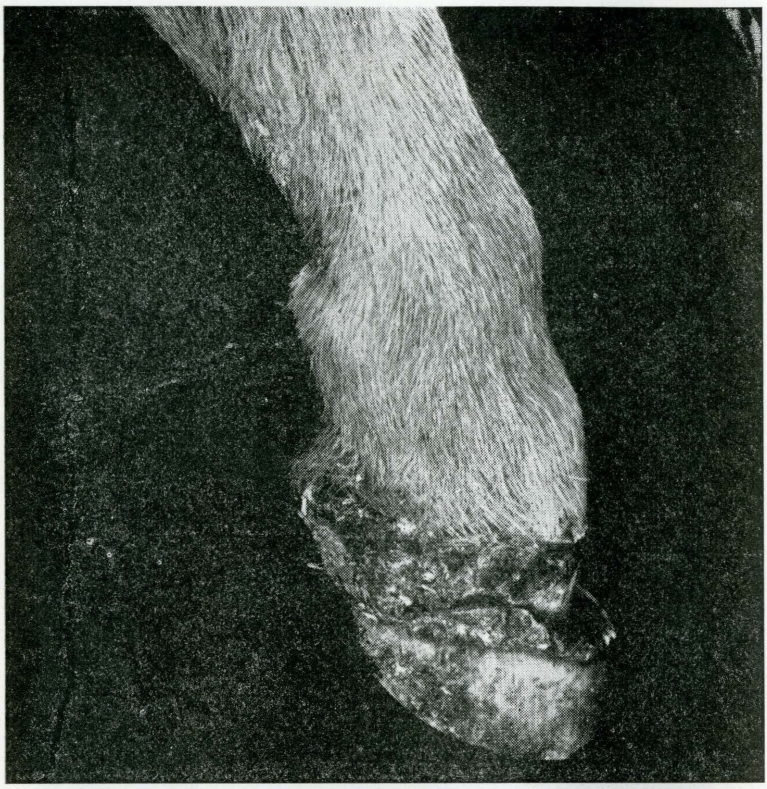


Fig. 4.—Photograph showing the separation of the hoof from the foot of a 14-day old colt. The colt was born with its feet in this condition.

Cattle.—The chief early symptom in cattle is lameness and loss of the long hair from the switch. The hoofs are involved in much the same way as the hoofs of the horse. At first there is a tenderness, followed by lameness, which becomes so pronounced that the animals do not move because of the pain. Many of the animals will rest on their knees while grazing. There is a separation of the old hoof from the foot followed by the growth of a new hoof. Usually the old hoof does not come completely off but remains attached to the new growth and oftentimes cattle will have ragged hoofs 8 to 10 inches long growing upwards at the end. Examples of this

are shown in Figures 5 and 6. Extreme emaciation is also common and often results in death. In some cases cattle with horns will show distinct ridges comparable to the ridges on the hoof after they have been severely "alkalied" (Figure 7). The deformed hoofs of "alkalied" cattle are sometimes called "frozen feet" by cattlemen. Erosion of the joints of the long bones causes considerable stiffness of the legs. In some cases of the less severe type there may be very little indication of the "alkali disease" shown by the condition of the hoof, but the cattle will make very slow gains or in many cases will lose weight.

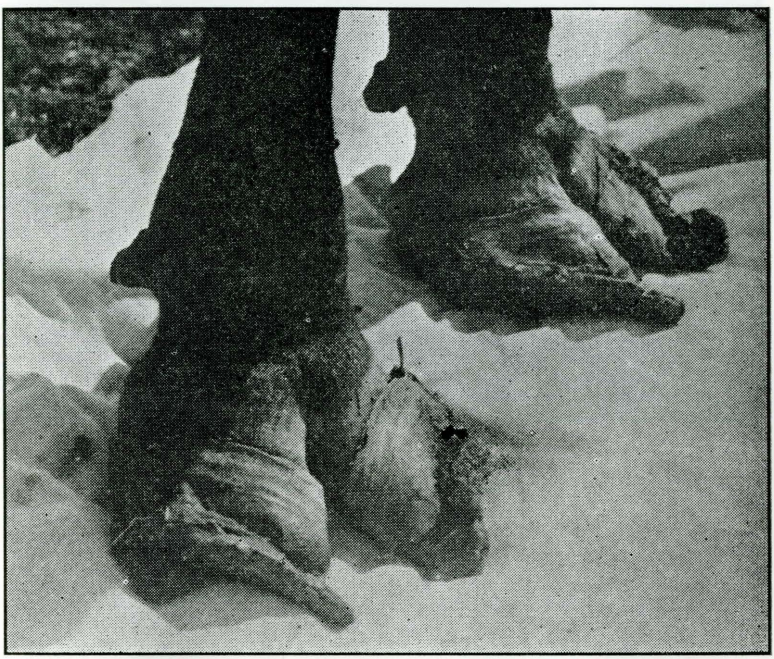


Fig. 5.—Fore feet of a severely "alkalied" cow.

Nursing calves often show all of the prominent symptoms of the disease. In some cases the calves are born with the symptoms and in other cases they get enough selenium in the milk from their dams to become affected. Milk from "alkalied" cows has been analyzed and found to contain considerable quantities of selenium.

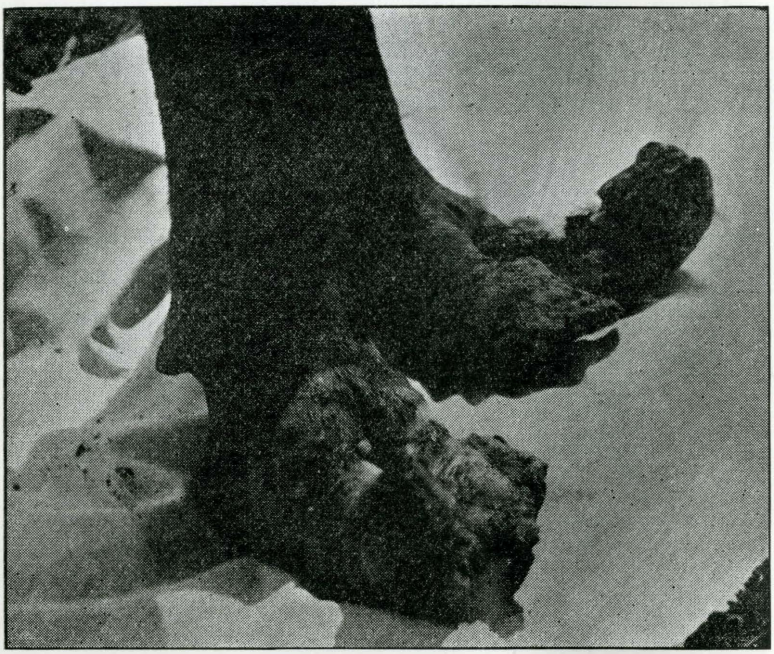


Fig. 6.—Hind feet of a severely "alkalied" cow.

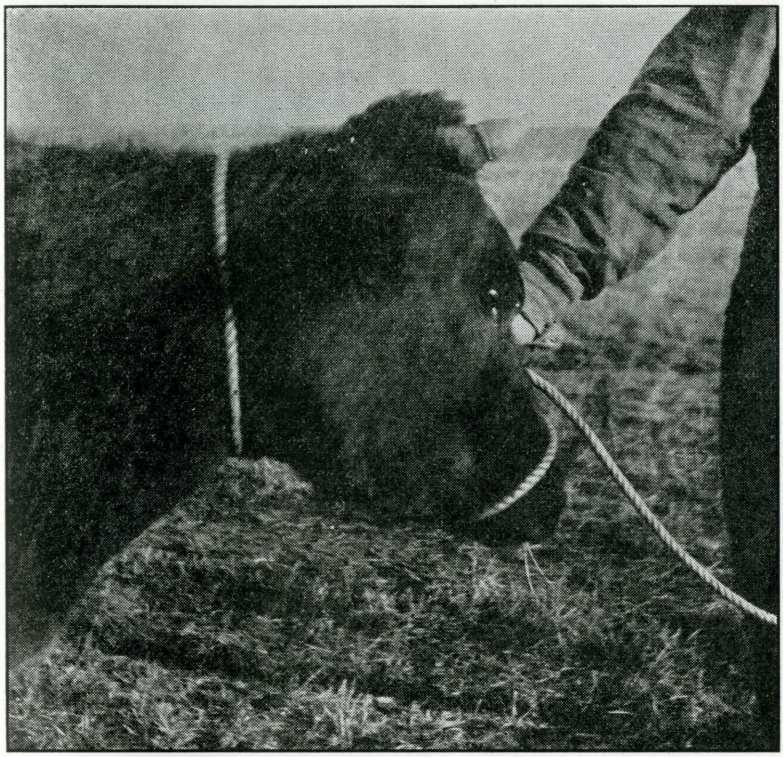


Fig. 7.—Photograph of "alkalied" cow's head showing a ring on the horn which marks an interruption in the growth of the horny material similar to the interruptions in growth of hoof in Fig. 2.

Fig. 8.—A grazing scene showing an "alkalied" steer which has assumed a kneeling position, while grazing, to avoid standing on its fore feet which were badly diseased.

Hogs.—The symptoms of "alkali disease" in hogs are lameness, loss of hair from the body, and irregular growth of the hoofs with occasional shedding of the hoofs similar to the condition in horses and cattle. Hogs which have been "alkalied" usually do not respond well to feeding and are difficult to fatten. When fed toxic corn young hogs will usually lose most of their hair in from two to three weeks. Suckling pigs are often "alkalied" and lose their hair because of the transmission of selenium through the milk. An "alkalied" pig is shown in Figure 9.

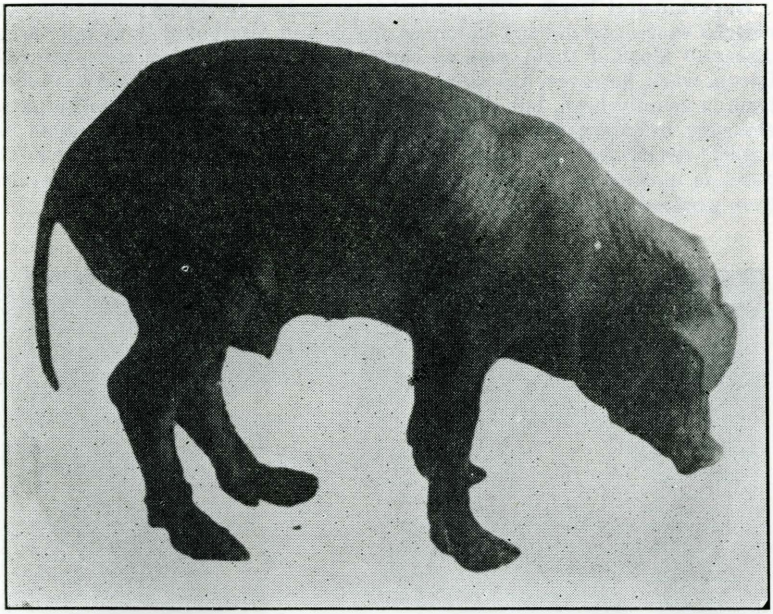


Fig.. 9.—An "alkalied" pig. Notice the general run down condition, thinness of hair, and diseased feet.

Control Measures for "Alkali Disease" in Livestock.—At the present time there is no approved treatment for the "alkali disease" in livestock other than moving the diseased animals to unaffected areas and feeding them on selenium-free feeds. Grains and forage which contain appreciable amounts of selenium should not be used for feeding livestock. Grazing animals should be allowed as much free range as possible so that they will have an opportunity to avoid the toxic vegetation.

Poultry.—Growing chicks and full grown poultry are apparently affected very little by toxic grains, but eggs produced by hens fed "alkalied" grain usually do not hatch well. The eggs are fertile but produce deformed embryos. The chicks which do hatch are usually weak and have an abnormal wirey condition of the down as shown in Fig. 10. The "alkali disease" in poultry will be discussed further in the next section.

Poultry*

Experimental Work. The early homesteaders in the affected area soon noticed that eggs did not hatch normally and attributed it to the "alkali disease." Most of them were of the opinion that the eggs were infertile. Recent work, however, has shown that the fertility of eggs from "alkalied" hens is usually high, but that they fail to hatch because of the deformed embryos. In most cases the chicks which do hatch from the eggs from "alkalied" flocks are very weak and have a rather peculiar appearance. The down is usually of a wirey nature and the chicks appear as if they had been greased as shown in Figure 10.

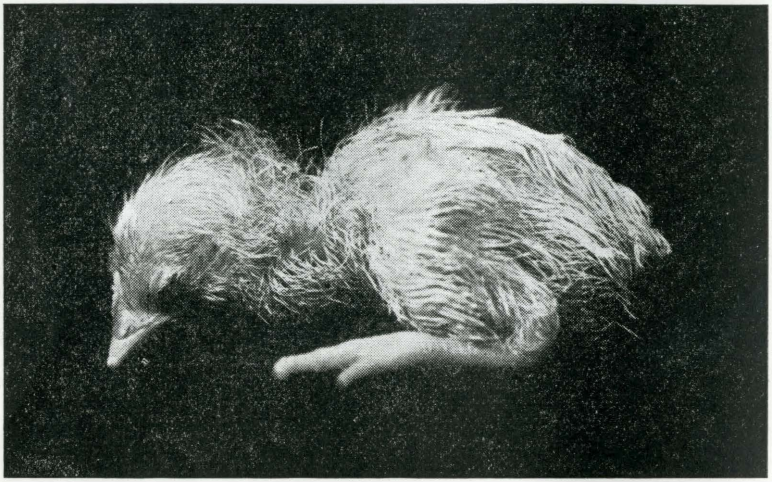


Fig. 10.—Day old chick, hatched from an egg laid by a hen which was fed seleniferous grains. Notice the weak condition and wirey down.

Hatching Experiments. In the spring of 1931, 12 dozen eggs were obtained from a farm in the "alkali disease" area. These eggs were incubated under the usual conditions. Following is a report on the hatchability of these eggs.

Number of eggs set	133
Dead germs 7th day	7
Infertile 7th day	6
Dead embryos 14th day	14
Hatched on 21st day	5

* These studies have been carried out in cooperation with W. C. Tully, formerly head of the Poultry Department and W. E. Poley, present head of the Poultry Department.

Of the five chicks that hatched on the twenty-first day one died on the twenty-second day and one died on the twenty-third day. Eggs which did not hatch were opened on the twenty-third day and the age of the embryos was estimated as follows:

Alive in shell on 23rd day	5
Fully developed to 21st day	34
Fully developed to 20th day	1
Fully developed to 19th day	8
Fully developed to 18th day	26
Fully developed to 16th day	6
Fully developed to 14th day	11
Fully developed to 10th day	2
Fully developed to 7th day	3
Fully developed to 3rd day	3
Hatched on 21st day	5
Indefinite age	2

The five embryos which were alive in the shells and five picked at random from the rest of the group were saved for further examination and the balance were burned to prevent any possibility of spreading disease among the college flock. Careful examination of the ten embryos saved, showed that eight of them were deformed. The results of this experiment were reported by Franke and Tully (21). Figure 11 is a photograph of two of the chicks which were alive in the shell. Figure 12 is a photograph of one of the embryos which was dead in the shell. This chick had only one eye which was centered somewhat over the beak. The upper beak was very short and underdeveloped and the lower beak was flat and thin. There was no evidence of wings and the legs were short, each terminating as a single toe, with no evidence of foot development.

Series 2.—In April, 1931, another lot of twelve dozen eggs was obtained from the same farm as those in the preceding report. Thirty-four control eggs from the Poultry Department were set in the same incubator and given the same treatment throughout the incubation period as those from the affected area. The following table gives a summary of the results:

		Experimental eggs		Control eggs	
		Number	Per cent	Number	Per cent
Total set		141		34	
Infertile		5	3.57	4	11.76
Dead germ		13	9.29	11	32.35
Hatched		16	11.43	19	55.88
Dead Embryos		107	75.71		
Fertility			96.43		88.24
Hatchability on basis of fertile eggs			11.85		63.33
Deaths of chicks which hatched during first twenty four hours	1st day		6		
	2nd day		1		
	3rd day		1		
	4th day		1		
	5th day		1		1
	6th day		1		
Mortality			68.75%		5.26%
Living July 1, 1931 (2 months old)			31.25%		94.74%

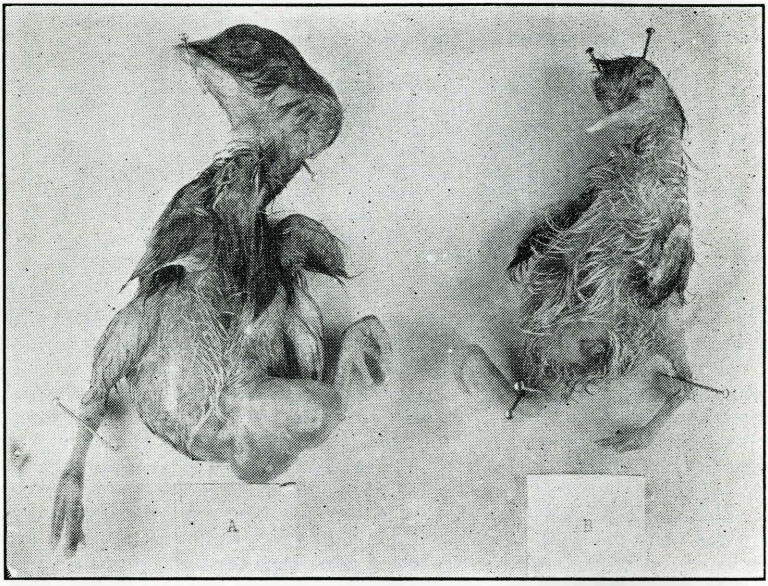


Fig. 11.—Two chicks which were alive (in the shells) on the twenty-third day.

Figure 13 is a photograph of a typical deformed embryo from this series. The hatchability of this second lot of eggs is somewhat higher than that of the first lot. This is probably due to the fact that the hens were getting different feed during the period when the second lot of eggs was produced.

Series 3.—A lot of 57 eggs was obtained from a farmer living in an "alkalied" area in the southern part of the state. He complained that he had always had poor hatches. These eggs were obtained during the latter part of April, 1931, and at that time the hens were obtaining a considerable amount of green feed. This lot of eggs hatched much better than the two preceding lots, but the mortality of the chicks which hatched was very high. The enlargement at the back of the neck as shown in Figure 11A was the most prominent abnormality observed in this series. Figure 14 is a photograph of one of the chicks which hatched in this series. This photograph was taken several days after hatching, and shows how the down failed to become fluffy.

Fig. 12.—A typical deformed chick. Notice the eye, beak and foot deformities and the absence of wings.

Series 4.—A Study of the Effect of "Alkalied" Grain on Growing Chicks and Poultry.—This series was started in the spring of 1931 for the purpose of observing the effects of "alkalied" grain on growing chicks and to raise pullets to the laying age so that eggs could be obtained from hens which had been fed "alkalied" grain continually from the time they were hatched.

Procedure: Four lots of Rhode Island Red Chicks and four lots of Single Comb White Leghorn Chicks were fed the following rations:

	Rhode Island Reds				Single Comb White Leghorn			
	Lot 1 %	Lot2 %	Lot 3 %	Lot 4 %	Lot 5 %	Lot 6 %	Lot 7 %	Lot 8 %
Ground yellow corn "normal"	25			25	25		25	
Ground yellow corn "alkalied"		25	25			25		25
Ground barley "normal"	25		25		25		25	
Ground barley "alkalied"		25		25		25		25
Ground wheat "normal"	15		15	15	15		15	
Ground wheat "alkalied"		15				15		15
Wheat bran	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Wheat flour middlings	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Meat and bone scraps	12	12	12	12	12	12	12	12
Dried buttermilk	5	5	5	5	5	5	5	5
Pearl grits	1	1	1	1	1*	1*	1*	1*
Common salt	1	1	1	1	1	1	1	1
Cod liver oil	1	1	1	1	1	1	1	1
	100	100	100	100	100	100	100	100

*ground Nebraska limestone

Results: Chicks fed a ration containing 65 per cent of toxic grain showed a distinct inhibition in growth, while the chicks fed 25 per cent of toxic grain in their ration showed practically normal growth. The chicks fed 65 per cent of toxic grain also had ruffed feathers and characteristics of nervousness were exhibited when they were about four weeks old. The start of egg production by the pullets was delayed in the groups which received 65 per cent of toxic grain in their ration. The egg production was reduced in the same group. These results have been reported in detail by Tully and Franke (22).

During the latter part of the summer of 1931 Dr. Henry G. Knight, Chief of the Bureau of Chemistry and Soils, notified us that Robinson (23) had found selenium in some of the toxic wheat which we had sent to them. This suggestion resulted in two poultry series in which selenium was fed in the form of sodium selenite added to the normal ration.

Series 5. The Effect of Inorganic Selenium on Laying Hens.—Twenty producing Rhode Island Red Hens were fed a normal ration to which had been added 26 p.p.m.* of selenium as sodium selenite. The hens immediately began to restrict their food consumption and in two weeks they had lost an average of over a pound of weight, which was undoubtedly due mainly to the decrease in food consumption. The egg production of these hens was markedly decreased.

Series 6. The Effect of Inorganic Selenium on Pullets.—Leghorn pullets were fed selenium as sodium selenite at a level of 6.5 p.p.m. in an all mash ration. These birds immediately decreased their food consumption similar to the birds in Series 5, therefore, the selenium content of the ration was decreased to 3.25 p.p.m. Sixteen settings totaling 305 eggs were made from this pen.

* p.p.m. is an abbreviation for parts per million. Example: 25 p.p.m. of selenium in the ration is equivalent to 25 pounds of selenium in a million pounds of the ration.

The average weights and the food consumption decreased noticeably even with a ration containing 3.25 p.p.m. of selenium. Deformed embryos were obtained similar to those resulting from the feeding of 65 per cent of "alkalied" grain.

Series 7. The Effect of Inorganic Selenium on Growing Chicks.—In this series four groups of chicks were placed on rations containing 0, 2, 4, and 8 p.p.m. of selenium as sodium selenite.

Results: The chicks in the 8 p.p.m. group showed a depression in the rate of growth but the 2 and 4 p.p.m. groups showed no significant effects from the selenium in their rations.

Fig. 13.—"Alkalied" chick, eyes and upper beak missing, feet deformed.

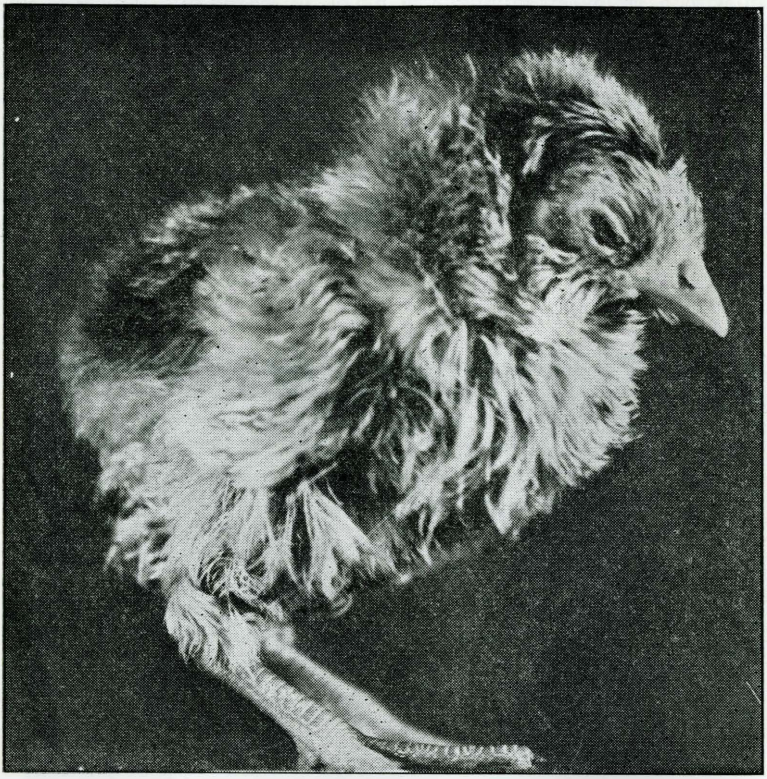


Fig. 14.—"Alkalied" chick from series 3.

Injection of Selenium Salts into the Egg Before Incubation.—The results of Series 6 indicated that selenium was causing the deformities, therefore, to further prove that selenium was the cause selenium salts were injected into the air cell of normal eggs before incubation.

Several settings of eggs were used in the experiment. The eggs were candled and the outlines of the air cells marked on the shell with a pencil. The area over the air cell was swabbed with an iodine solution for the purpose of sterilizing the shell in this area. The selenium solutions were injected by puncturing the shell with a hypodermic needle. After the injection was completed the punctures were closed with discs of thin paper soaked with egg white. In the first settings it was necessary to use various concentrations and amounts of selenium so as to find a suitable dosage. The preliminary results indicated that .1 of a cc. of solution was the most suitable amount for injecting into the air cell of the egg.

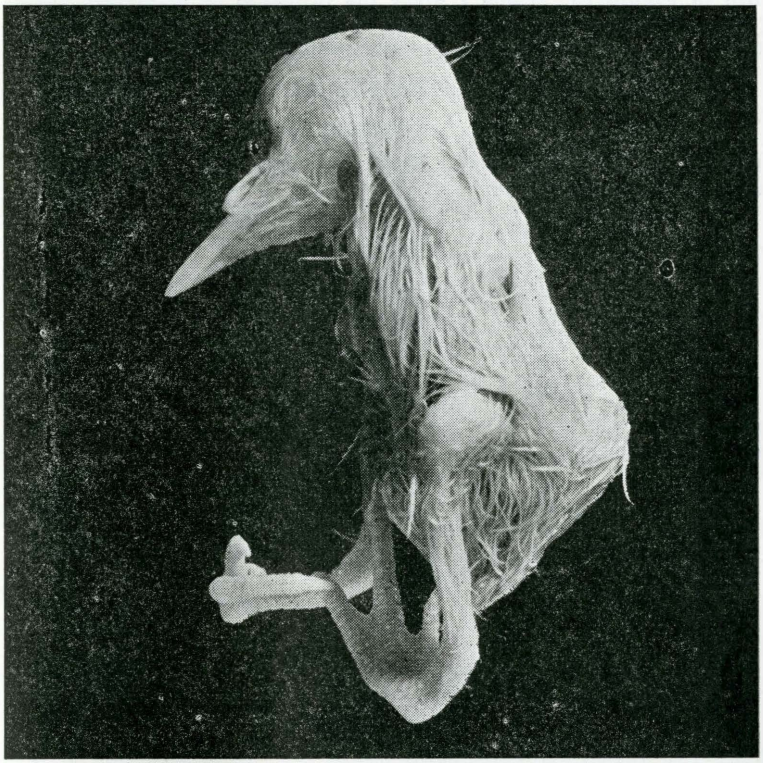


Fig. 15.—Deformed embryo resulting from the injection of .01 p.p.m. of selenium into the air cell of a fertile egg before incubation.

The concentrations of the solutions were varied so as to give from .01 to 1. p.p.m. of selenium when injected into an egg weighing 50 grams. The average weight of the eggs used minus the weight of the shell was very near to 50 grams.

After the eggs had been injected with selenium they were incubated according to the usual procedure.

Results: The results of these injection experiments have been reported by Franke et al (24). Many of the chicks which hatched from these injected eggs showed abnormalities similar to those resulting when toxic grains or selenium salts were fed to laying hens. Selenium in a

concentration as low as .01 p.p.m. (selenite) caused abnormalities as shown in Figure 15. Table 1 gives the incidence of mortality and deformities resulting from the injection of the indicated amounts of selenium in the form of sodium selenite and sodium selenate.

A summary of the results obtained by the injection of selenium ions.

Table 1

p.p.m. Se as Sodium Selenite	Total Embryos Examined	Dead %	Abnormal %	Normal %
0.9	5	60.0	20.0	20.0
0.8	16	12.5	31.0	56.5
0.7	4	50.0	25.0	25.0
0.6	4	50.0	50.0	0
0.5	12	50.0	8.3	41.7
0.1	131	24.4	2.3	73.3
0.02	78	53.8	3.8	42.3
0.01	64	34.3	9.4	56.3
p.p.m. Se as Sodium Selenate				
1.0	21	76.2	19.0	4.8
0.8	15	13.3	86.7	0
0.7	11	27.3	18.2	54.5
0.6	26	7.7	57.7	34.6
0.5	40	17.5	5.0	77.5
0.4	18	22.2	27.8	50.0
0.1	36	11.1	0	88.9

Series 8.—During the spring of 1936 another series of poultry experiments was started for the purpose of answering the following questions:

(1) What is the effect of toxic grain containing different levels of selenium on (A) the weights of laying hens? (B) a. the number, b. size or weight, c. fertility, d. hatchability of the eggs produced? (C) the food consumption of the laying hens?

(2) How long after hens are changed from a normal ration to a ration containing 65 per cent of toxic grain (15 p.p.m. Se) does it take for the toxicity to affect the hatchability of the eggs?

(3) How long does it take for the toxic effects to disappear after the hens are changed from "toxic" back to a normal ration?

(4) Will the feeding of elemental sulphur at a level of 1 per cent in the ration either prevent or alleviate the toxic effects of selenium, or in any way affect the action of selenium?

Procedure: Three pens of individually mated White Leghorn pullets were fed a control ration for six weeks and their normal egg production, fertility, etc. was determined. At the end of this six weeks period pen No. 2 was changed to a toxic ration and pen No. 3 was changed to the toxic ration to which had been added 1 per cent of powdered sulphur Pens 2 and 3 were left on the toxic rations for a period of five weeks and were then changed back to the normal ration for six weeks. Individual records on each egg and incubation records on each hen were kept. The following table gives the composition of the rations used by these three pens:

Percentage Composition of the All-Mash Rations

	Control Pen 1	Toxic Pen 2	Toxic plus Sulphur Pen 3
Ground corn (Toxic #570, 29 p.p.m. Se		25.0	25.0
Ground corn (Normal)	25.0		
Ground barley (Toxic #587, 15.4 p.p.m. Se)		25.0	25.0
Ground barley (Normal)	25.0		
Ground wheat (Toxic #607, 30 p.p.m. Se)		15.0	15.0
Ground wheat (Normal)	15.0		
Wheat bran	7.5	7.5	7.5
Wheat middlings	7.5	7.5	7.5
Meat and bone meal	8.0	8.0	0.0
Alfalfa leaf meal	5.0	5.0	5.0
Buttermilk powder	5.0	5.0	5.0
Salt	1.0	1.0	1.0
Cod liver oil	1.0	1.0	1.0
Selenium content pens 2 and 3	0	(15.15 p.p.m.)	(15.15 p.p.m.)
	100.0	100.0	100.0

Note: One percent of sulphur added to ration of pen No. 3.

Results: The results of this series have been reported by Poley, Moxon, and Franke (25). The laying hens lost weight during the period which they were fed toxic rations and egg size decreased correspondingly. Feeding of the toxic rations had no appreciable effect on the egg production or fertility. There was an appreciable decrease in food consumption during the period when toxic rations were fed. The hatchability decreased to zero when the toxic rations were fed. There were no normal chicks hatched from eggs laid after the seventh day of feeding selenium containing rations. Figure 10 shows a typical chick hatched from pen No. 2 during the first week after changing. Figure 16B shows the same chick at 6 weeks as compared with A a normal chick of the same age from pen No. 1. The apparent toxic effects of selenium on embryo development and hatchability disappeared on the sixth day after restoring the normal ration. No monsters were produced in the control pen while in pens No. 2 and 3 monsters characteristic of selenium poisoning were produced. One per cent elemental sulfur in the ration had no effect upon the action of selenium. The average hatchability in pens No. 2 and 3 decreased rapidly after changing to the toxic ration and was quickly restored to normal after changing back to control ration, as shown in Table 2.

TABLE 2

Types of Embryo Deformities. Percentage of fertile eggs incubated.

Number days of feeding toxic ration	Pen Number	Hatched	Dead	Alive—not hatched	Malposition	Edema		Twisted		Missing toes	Missing Beak				Protruding eyes	Eyes missing	
						Neck	Head	Neck	Legs		Bud	¼	½	¾		one	both
1-5	2	84.5	11.9	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6-12	2	18.6	64.4	17.0	6.8	59.3	16.9	6.8	18.6	0.0	8.5	0.0	10.2	3.4	8.5	0.0	0.0
13-19	2	2.1	95.7	2.1	17.0	76.6	25.5	19.1	29.8	4.3	0.0	2.1	17.0	6.4	19.1	0.0	4.3
20-26	2	0.0	93.0	7.0	16.3	62.8	34.9	4.6	34.9	0.0	11.6	11.6	18.6	2.3	23.3	2.3	10.7
27-33	2	0.0	75.0	25.0	7.1	17.9	25.0	0.0	46.4	7.1	53.6	17.9	3.6	0.0	32.1	0.0	35.7
34-40*	2	27.5	60.0	12.5	2.5	3.5	22.5	0.0	12.5	7.5	17.5	10.0	7.5	2.5	10.0	10.0	12.5
41-47	2	91.1	8.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1-5	3	80.0	12.7	7.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6-12	3	7.4	77.8	14.8	12.9	46.3	44.4	11.1	25.9	0.0	22.2	0.0	11.1	3.7	11.1	0.0	1.9
13-19	3	0.0	93.3	6.7	2.2	44.4	17.8	22.2	57.8	4.5	64.4	0.0	0.0	4.5	44.5	2.2	15.6
20-26	3	0.0	100.0	0.0	8.3	27.8	19.4	2.8	38.9	2.8	55.6	2.8	11.1	0.0	50.0	11.1	13.9
27-33	3	0.0	93.5	6.5	6.5	19.4	9.7	3.2	29.0	22.6	74.1	0.0	0.0	6.5	35.4	9.7	32.3
34-40*	3	18.8	75.0	6.2	3.1	31.3	28.1	3.1	31.3	6.3	50.0	0.0	3.1	0.0	25.0	6.3	21.9
41-47	3	93.3	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* Normal ration restored on 35th day.

Series 9.—This series was started in the fall of 1936 to determine the approximate amount of "alkalied" grain, containing a known amount of selenium, that could be fed to laying hens without appreciably affecting: 1. hatchability 2. viability of chicks hatched and 3. state of health of chicks and laying hens and also to determine the amount of selenium in the eggs and in the meat when the hens were fed rations containing various amounts of selenium.

Procedure: Four pens of laying hens were fed the following rations:

	Pen 1 (Control)	Pen 2 (2½ p.p.m. Se)	Se mg.	Pen 3 (5 p.p.m. Se)	Se mg.	Pen 4 (10p.pm. Se)	Se mg.
Alkali corn #570 (29.0 p.p.m. Se)		4	(1.16)	8	(2.32)	16	(4.64)
Alkali barley #587 (15.4 p.p.m. Se)		4	(.6)	8	(1.23)	16	(2.46)
Alkali wheat #607 (30.0 p.p.m. Se)		2.43	(.73)	4.85	(1.45)	9.7	(2.90)
Ground corn (Se free)	25	21		17		9	
Ground barley (Se free)	25	21		17		9	
Ground wheat (Se free)	15	12.57		10.15		5.3	
Wheat bran	8	8		8		8	
Wheat middlings	8	8		8		8	
Meat and bone	8	8		8		8	
Alfalfa Leaf Meal	5	5		5		5	
Dried buttermilk	5	5		5		5	
Salt	0.5	0.5		0.5		0.5	
Cod liver oil conc.	0.5	0.5		0.5		0.5	
	100.0	100.0		100.0		100.0	

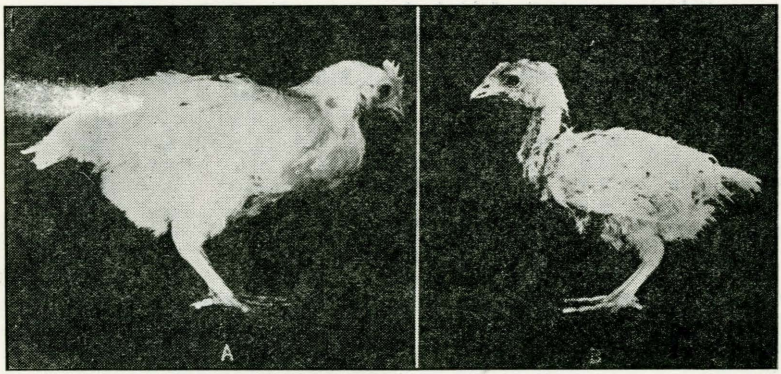


Fig. 16.—A. Control bird 6 weeks old from Pen 1, Series 8
B. "Alkalied" bird 6 weeks old from Pen 2, Series 8.

All birds were individually mated and pedigree hatched. The eggs which did not hatch were opened for study. Complete records were kept of all embryos from the unhatched eggs. The eggs were candled on the seventh and fourteenth days after setting and all infertile eggs were saved for selenium analysis.

With 10 p.p.m. of selenium in the ration the hatchability of normal chicks was decreased nearly to zero after the first week and characteristic embryo deformities occurred. With 5 p.p.m. of selenium in the ration hatchability was not appreciably affected, however, the characteristic wirey down was quite prevalent among the chicks and the mortality rate was rather high. A concentration of 2.5 p.p.m. of selenium in the ration produced no apparent effects on viability of the chicks.

Selenium Content of Eggs from Pens No. 2, 3 and 4.—The eggs were boiled (hard) and the whites and yolks separated out and dried. They were then analyzed for selenium. At the end of the experiment two hens were taken from each of pens No. 2, 3 and 4 for selenium analysis. The hens were dissected and the various organs and tissues were removed and dried for selenium analysis. The distillation method was used for selenium analysis and the results are shown in the tables below:

Table 3

Selenium content of eggs analyzed from pens No. 2, 3, 4

		Average selenium content (dry weight)	
		White	Yolk
Pen 2	2.5 p.p.m. Se in ration	11.3 p.p.m.	3.60 p.p.m.
Pen 3	5.0 p.p.m. Se in ration	19.0 p.p.m.	5.88 p.p.m.
Pen 4	10.0 p.p.m. Se in ration	41.3 p.p.m.	8.42 p.p.m.

Table 4

Selenium content of birds from pens No. 2, 3, 4, Series 9

Parts per million dry weight

Hen No.	p.p.m. Se in Diet	Ovary	Upper Oviduct	Liver	Small Int.	Kidneys	Proventriculos	Light Meat	Dark Meat	Soft-shelled Egg
1081	10	15	30	25	——	15	7.3	——	23	25
1079	10	14	23	9	3	3.6	14.7	8	2.4	55
1024	5	4.6	5	4	4	10	10.5	13.2	6.33	—
1029	5	4.4	11	<2	——	——	——	——	10	—
1059	2.5	6.3	30	trace	0	9.5	2.6	0	——	—
1075	2.5	17	7.3	0	1.25	9	7	4.25	9.7	—

Summary of Experimental Poultry Work.—The cause of low hatchability of eggs from the seleniferous area of South Dakota has been traced to selenium in the feeds eaten by the laying hens. The fertility of the eggs was high but they failed to hatch because of the high incidence of monstrosities. Most of the monsters were weak and died before the twenty-first day and those which did live to the twenty-first day were unable to pip the shell because of weakness or deformities. The most prominent deformity throughout the entire group of monsters was lack of the full sized upper beak which enables the normal chicks to pip the shell. Other abnormalities of importance were missing eyes, missing and deformed feet, missing or small wings, wiry down and edema of the head and neck.

Similar deformities have been produced by feeding hens inorganic selenium in their ration and also by injecting very small amounts of selenium into the air cells of fertile eggs before incubation.

The influence of various amounts of seleniferous grain on egg production, hatchability, growth of young chicks, tolerance limits for normal hatchability, etc. has been determined. The selenium content of eggs and meat from hens fed various amounts of seleniferous grains has also been investigated.

Experiments With Laboratory Animals

Feeding Experiments with Laboratory Animals.—One hundred and twenty-eight series of albino rats, two series of rabbits, and one series of guinea pigs have been fed on various seleniferous diets. About 20 dogs have also been used in these investigations. These feeding experiments will be divided into the following general classes for discussion:

1. Series dealing mainly with symptoms of the "alkali disease" in laboratory animals. This will include physiological and pathological studies.
2. Series dealing mainly with experiments on the feeding of vitamins, minerals, and special diets.
3. Series dealing mainly with nature of the toxic (selenium containing) factor.
4. Series dealing mainly with self-selection of diets by laboratory animals.
5. Series dealing mainly with toxicity of various grains and toxicity of selenium and other less common elements.

Several of the series were conducted for the purpose of determining the toxicity of certain samples of grain and will not be discussed individually but will be discussed collectively in connection with the toxicity of the various grains.

Preliminary Experiments.—At the start it was necessary to find a suitable laboratory animal with which to conduct the feeding experiments with the toxic grain. Albino rats were chosen as the first animals to try and they have proved to be so satisfactory that they have been used for most of the work. The first series was started on December 12, 1929, and was of a preliminary nature.

The rats used in this work were produced in our own rat colony the stock of which is of Wistar Institute origin. Unless otherwise stated in the discussions of the individual series the rats were weaned at 21 days of age and maintained for one week on McCollum's diet No. 1 (26) and then placed on experiment in individual cages at 28 days of age.

Diet (Control Animals)		Diet (Experimental Animals)	
Ground corn (grown in Brookings County*)	70.0%	Ground corn No. 388 (toxic, grown in the affected area,	70.0%
Casein	11.0	Casein	11.0
Sucrose	15.0	Sucrose	15.0
Lard	2.0	Lard	2.0
Calcium carbonate	1.4	Calcium carbonate	1.4
Sodium chloride	.6	Sodium chloride	.6

It will be noted that the above diets are identical except for the area in which the corn was grown. This will be the only difference in the diets in practically all succeeding series.

The selenium content of a sample of this corn No. 388 has been determined at some time within the last two years to be 25.0 p.p.m. This selenium content for the corn would make a selenium concentration of 17.5 p.p.m. in the above diet containing 70 per cent of the corn.

* An effort was made to secure locally grown grains of known history for all control diets and except for one or two series the control grains were grown in Brookings County.

Symptoms of Selenium Poisoning in Rats.—The results of this experiment were very striking. Within a few days those rats getting the diet containing toxic corn decreased their food consumption to about half the amount being consumed by the control rats. A marked decrease in the rate of growth paralleled the decrease in food consumption. The mortality was also very high in the group fed the toxic diet. The first rat died on the thirtieth day of the experiment and on the sixtieth day 13 out of 30 rats on toxic diet were dead. By the one hundredth day all but six of the 30 were dead. None of the rats receiving the control diets died during the course of the experiment which ended on the one hundred thirty fifth day at which time all of those receiving toxic diet had died.

All of the rats were carefully autopsied at death. In all cases the rats which received the toxic diet showed severe pathology of the liver. Livers typical of those encountered in this series are shown in Figure 17. The liver pathology was very uniform in that it involved, in practically all cases, atrophy of the central lobes, hypertrophy of the caudate and lateral lobes, and general cirrhotic condition accompanied by necrosis and hemochromatosis. In general this has been the typical liver pathology in all of the rats on the various seleniferous diets which have been fed. Figure 18 shows photomicrographs of typical normal and "alkalied" livers. During the course of the autopsies on the rats intramuscular hemorrhages around the joints of the leg bones as well as brashness of the bone shafts were noted in a large number of cases. This pathology, like the liver pathology and also pathology of the gastro-intestinal tract in the form of hemorrhages in the mucosa of the stomach and small intestines, has also appeared quite consistently in the feeding experiments where seleniferous diets were used. Edema and ascitic fluid in the abdominal cavity have also been common symptoms.

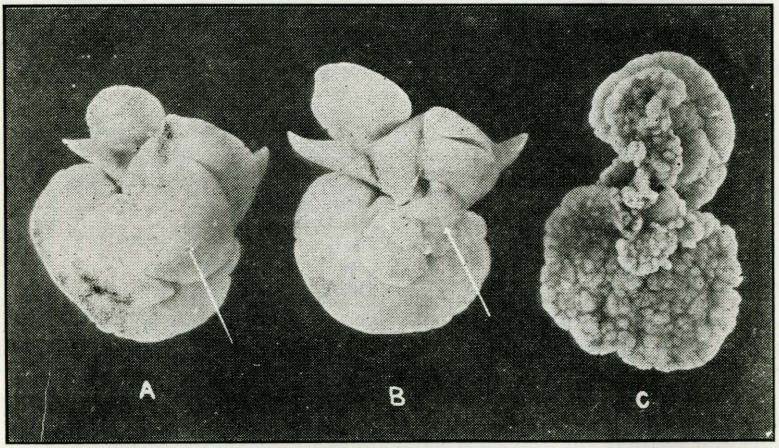


Fig. 17.—A. Normal rat liver
B. Slightly affected rat liver
C. Severely affected rat liver.
Notice the atrophy of the central lobe in B as indicated by arrow. Compare with A.

Series 4.—This series was started April 29, 1930, for the purpose of determining the effect of feeding "alkalied" corn at various levels in the diet. The same corn No. 388 was used in this series as was used in Series 1.

Four groups of rats were used in this series. Diets were made from the same formula as used in Series 1.

		Calculated Se content of diet
Group A	Control 70 per cent normal corn	0.
Group B	Experimental 70 per cent (toxic) corn No. 388	12.25 p.p.m.
Group C	Experimental 46.66 per cent No. 388 corn plus 23.33 per cent normal corn	8.16 p.p.m.
Group D	Experimental 23.33 per cent No. 388 corn plus 46.66 per cent normal corn	4.08 p.p.m.

The main results shown by this series was that the corn when fed as only 23.33 per cent of the diet caused a marked decrease in the growth rate, decrease in food intake and atrophy of the central lobes of the liver.

A sample of supposedly toxic wheat was received from the affected area and Series 9 was set up to determine its toxicity and also to determine whether or not the symptoms in the rats fed toxic wheat would be the same as those produced by the toxic corn.

Series 9.—This series was started on Nov. 6, 1930. Two groups of rats were given the following diets:

Control Group		Experimental Group	
Wheat, control (grown in Brookings County)	70.0%	Wheat (toxic) No. 459	70.0%
Casein	11.0	Casein	11.0
Sucrose	15.0	Sucrose	15.0
Lard	2.0	Lard	2.0
Calcium carbonate	1.4	Calcium carbonate	1.4
Sodium chloride	.6	Sodium chloride	.6

Wheat sample No. 459 has been analyzed and found to contain 25.0 p.p.m. of selenium which would give a selenium content of 17.5 p.p.m. in the above experimental diet.

The results of this series indicated that the wheat was equally as toxic as No. 388 corn. The growth, food consumption, and symptoms were very much the same as in Series 1.

Toxicity is Carried by Different Grains.—Series 1 and Series 9 demonstrated that both corn and wheat carried the toxic factor. A sample of emmer and a sample of barley were obtained from a farm which was known to produce toxic wheat and toxic corn.

Series 11.—The toxicity of the emmer was determined in this series, started on December 3, 1930. It was found to be less toxic than the wheat and corn of the former series. The selenium content of the emmer has since been found to be much less than that of the corn and wheat. The selenium content of the emmer was 15.0 p.p.m. while that of the corn and wheat was 25 p.p.m. With the emmer making up 70 per cent of the diet the selenium content of the diet was therefore 10.5 p.p.m.

Series 12.—The toxicity of the barley No. 455 was determined in this series, started on December 15, 1930. The barley contained 17.0 p.p.m. of selenium and the diet contained 11.9 p.p.m. of selenium. The post-mortems of these rats revealed internal lesions similar to those produced by

toxic corn, wheat and emmer. The toxicity of the barley as measured by growth, food consumption, and lesions was about equal to the toxicity of the emmer in Series 11. The incidence of death of rats fed various toxic grains has been reported by Franke (27).

Toxic and Normal Grains Fed Alternately.—At this point it had been demonstrated that corn, wheat, emmer and barley carried the toxic factor (selenium) when raised on affected farms. Questions were often asked as to what the result of feeding, alternately, toxic and non-toxic feeds would be, so Series 13 was planned to determine the effect of such feeding on rats.

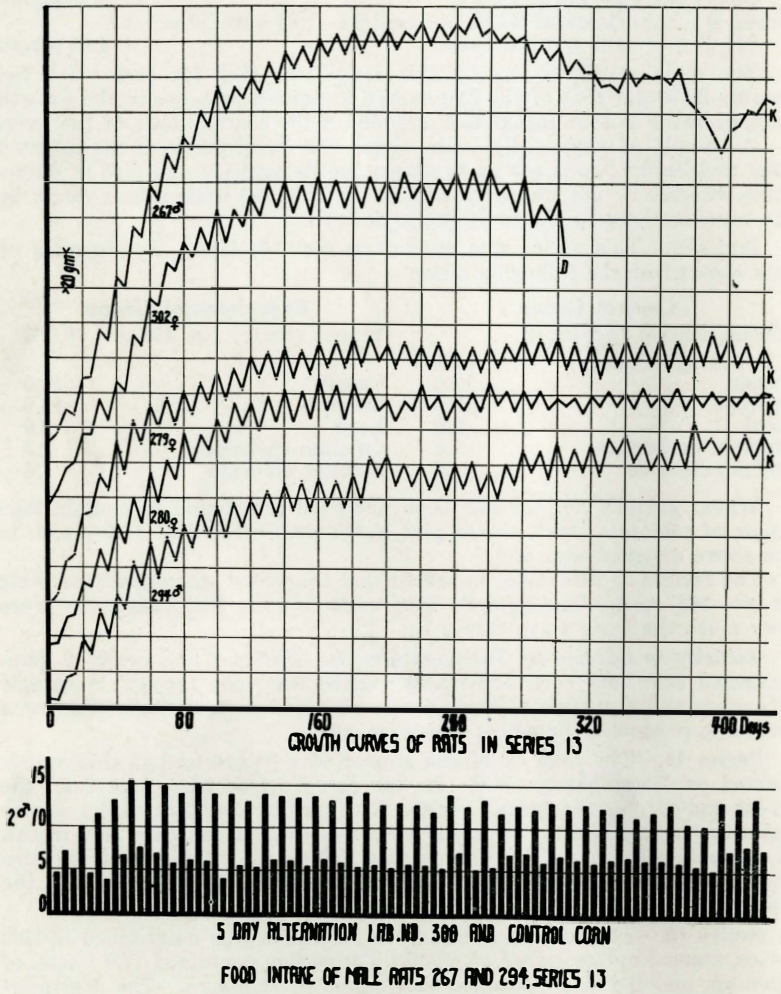


Chart I

Series 13.—This series was started on December 15, 1930. Four groups of five rats each were fed as follows:

Group A Fed 15 days on "alkalied" diet and 15 days control diet, 15 days "alkalied" diet, etc., until the end of the experiment.
Group B Fed 10 days on "alkalied" diet and 10 days control diet, 10 days "alkalied" diet, etc.
Group C Fed five days on "alkalied" diet, then five days control diet, five days "alkalied" diet, etc.
Group D Fed five days control diet, five days "alkalied" diet, five days control diet, etc.

These alternations continued for 420 days at which time all of the rats in Group C were dead, and one was living in each of Groups A and D and four were living in Group B. The diets used in this series were the same as used in Series 1.

Results: The results of this series have been discussed in detail by Franke (28). The alternation of the diets resulted in a rhythmic gain and loss of weight with a net gain on every change to the control diet. (See Chart I). There was likewise a rhythmic increase and decrease in food consumption, as shown in Chart I.

Toxicity of Small Amounts of the "Alkalied" Grains in the Diet.—There was demand for more information regarding the amount of toxic grain that could be fed without injurious effects. The results of Series 4 indicated that No. 388 corn when fed at a level as low as 23.33 per cent of the diet caused a decrease in the rate of growth and showed other toxic effects.

Series 14.—This series was made up of five groups of ten rats each, as follows:

		Selenium Content of Diets
Group A	Control diet 70 per cent control wheat	0
Group B	Experimental Diet No. 1 containing 17.5 per cent toxic wheat (No. 459) 52. 5 per cent control wheat	4.38 p.p.m.
Group C	Experimental Diet No. 1 containing 35 per cent toxic wheat (No. 459) 35 per cent control wheat	8.75 p.p.m.
Group D	Experimental Diet No. 1 containing 52.5 per cent toxic wheat (No. 459) 17.5 per cent control wheat	13.13 p.p.m.
Group E	Experimental Diet No. 1 containing 70 per cent toxic wheat	17.5 p.p.m.

Chart II shows the average growth curves of the animals in this series and indicates that 17.5 per cent of the "alkalied" wheat No. 459 in the diet is slightly toxic while the larger amounts are distinctly toxic.

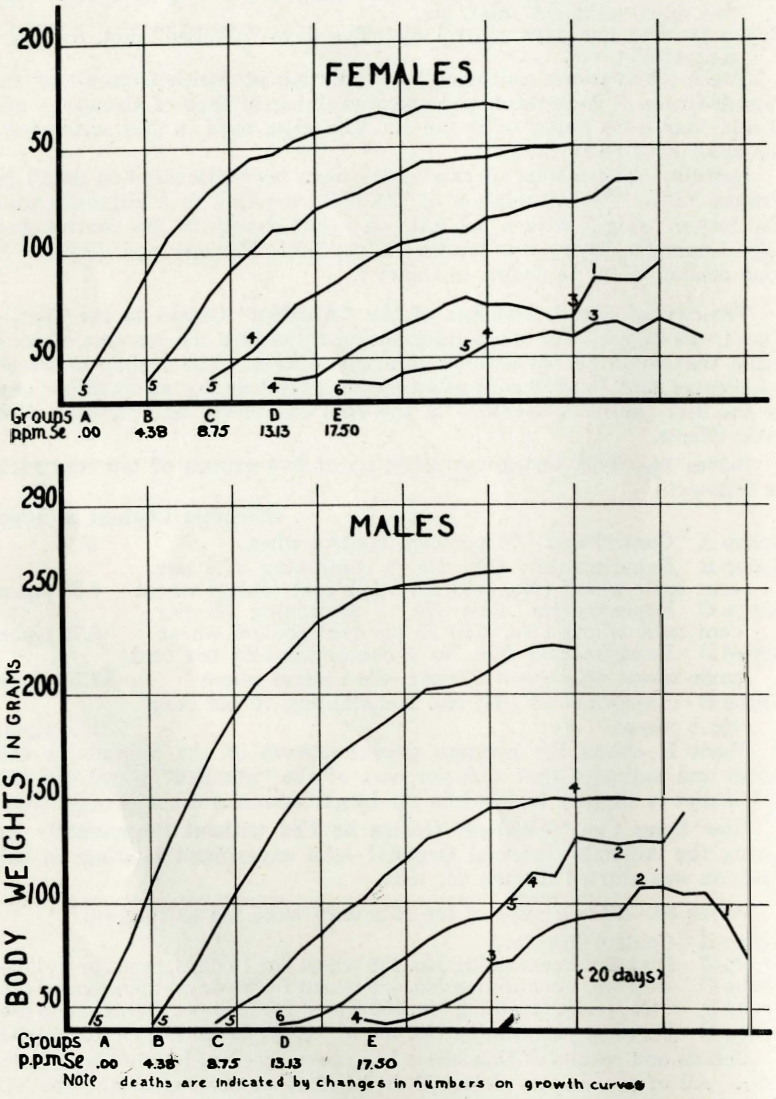
How Long Can "Alkalied" Grains be Fed without Permanently Injuring the Animals' Internal Organs?—An experiment bearing on this question was started August 24, 1931.

Series 30.—Five groups of ten rats each were fed as follows:

Group A Control diet No. 1
Group B Diet No. 1 containing No. 459 wheat for 10 days, then control diet
Group C Diet No. 1 containing No. 459 wheat for 20 days, then control diet
Group D Diet No. 1 containing No. 459 wheat for 30 days, then control diet
Group E Diet No. 1 containing No. 459 wheat for 40 days, then control diet

Details and results of this series have been discussed in full by Franke (29). All of the rats in Group E died before or soon after the fortieth day of experiment, when they were changed back to normal diet. All ex-

cept two of the rats in Group D died by the 180th day. In Group C all except three of the rats died by the 100th day and in Group B one of the rats died during the experiment.



Note deaths are indicated by changes in numbers on growth curves

Chart II

Chart III shows the growth curves of the rats in this series and Figure 19 shows the livers of male litter mates from Groups A, B, C, D which were alive on the 100th day. The atrophy of the central lobes and the other pathology is very evident.

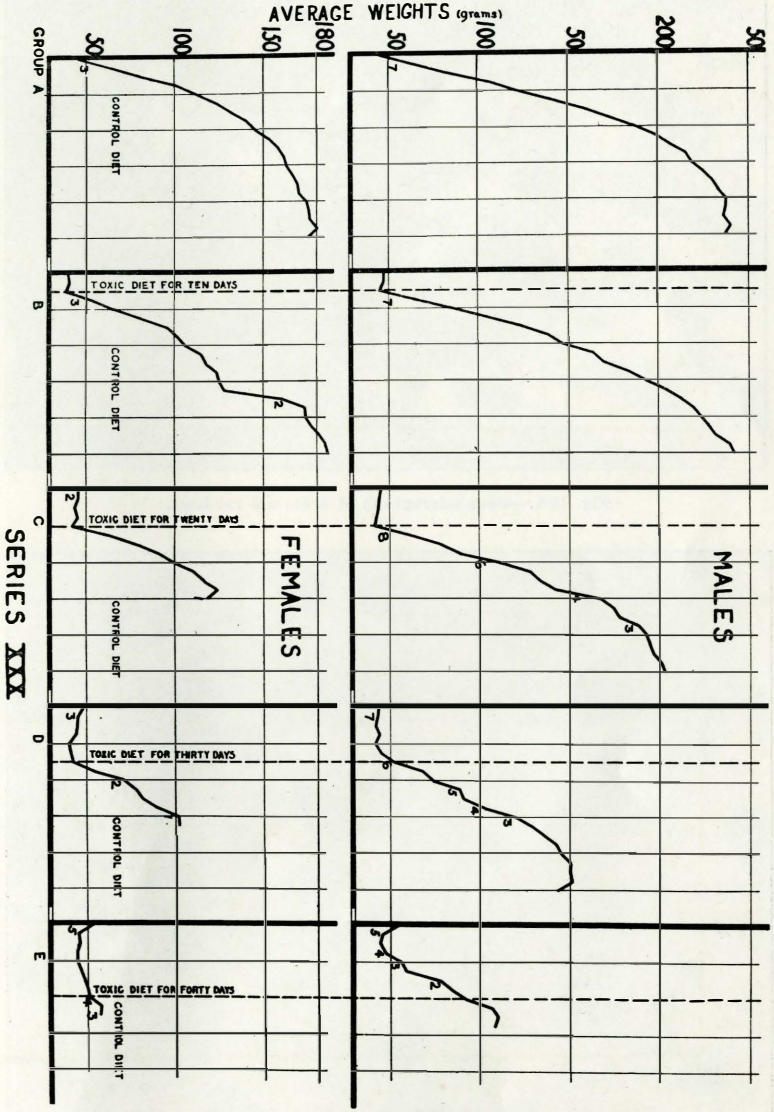


Chart III

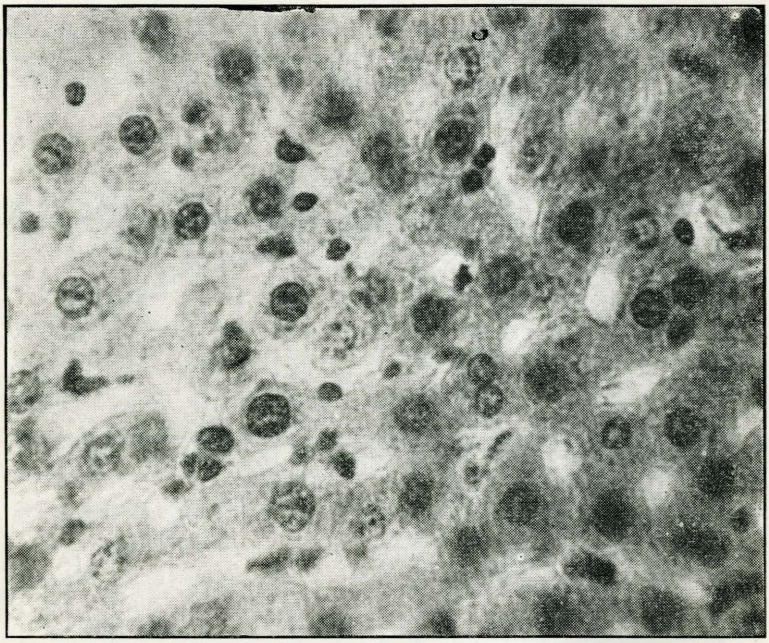


Fig. 18A.—Photomicrograph of a normal rat liver.

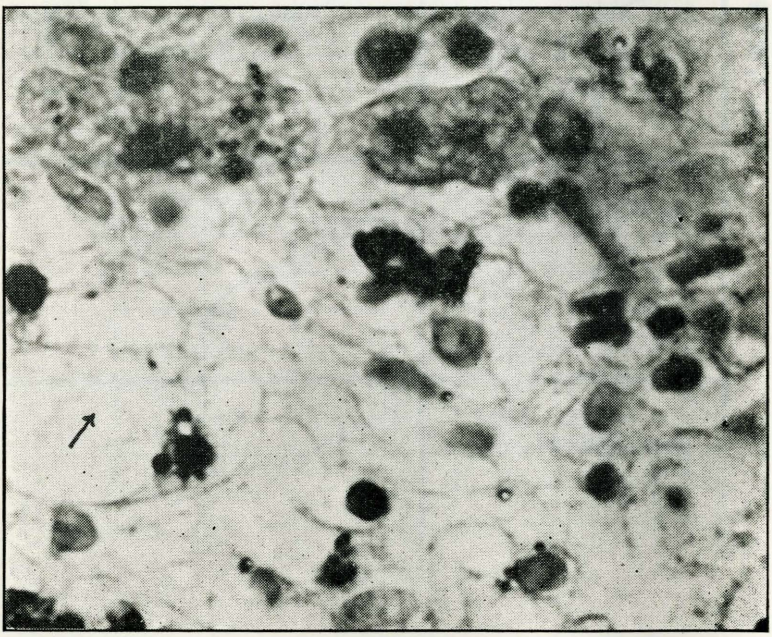


Fig. 18B.—Photomicrograph of an "alkalied" rat liver. Note destruction of cells at point indicted by arrow.

Effect of Selenium Containing Grains and Inorganic Selenium on the Blood Picture of the Albino Rat

"Alkalied" Grain Causes Anemia.—It was observed in some of the first feeding experiments with rats that anemia was a prominent symptom. This symptom has been prominent throughout all of the experimental work.

Series 74.—This series was started on November 18, 1932, for the purpose of developing methods for studying the blood of the albino rat. The preliminary results obtained in this series showed that low red cell counts accompanied by correspondingly low hemoglobin levels and low red cell volumes were common in the rats at death. Red cell counts as low as 450,000 cells per cu. mm. of blood were observed. These preliminary results led to a more thorough study of the hematology in Series 78, 85 and 87.

Series 78.—This series was started on December 28, 1932. A system of restricted controls was used to determine the effect of restricted food intake as well as the effect of "alkalied" grain upon the blood picture and the growth of the animals. Three groups of rats were given the following diets:

Group A Control wheat diet, ad lib
Group B Control wheat diet,restricted
Group C No. 582 wheat diet, ad lib

The restricted controls of Group B were fed the same amount of control wheat diet daily as their litter mates in Group C consumed of No. 582 wheat diet on the previous day. Examinations of the blood of some of the rats in this series were made at the time of their death. Low red cell counts, low hemoglobin levels, and low cell volumes were observed in animals of Group C, while the levels of the same constituents in the blood of animals from Groups A and B appeared to be normal. The animals of Group B made better growth, although they received no more of the control diet than those of Group C consumed of the toxic diet.

Series 85.—This series was started on May 4, 1933. The set up of this series was similar to that of Series 78.

Group A Control wheat diet, ad lib
Group B Control wheat diet, restricted
Group C No. 582 wheat diet, ad lib

In this series an attempt was made to obtain a sample of blood from each rat just before death. The blood was withdrawn from the abdominal aorta by means of a hypodermic needle and syringe, as decribed by Swanson and Smith (30). The details of this series as well as Series 87 have been reported by Franke and Potter (20). Hemoglobin determinations were made on all the samples and cell counts were made on many of the samples. In most cases blood smears were made and the differential leucocyte count determined. It was observed that animals in Group C which died within the first 20 days of the experiment, showed no decline in hemoglobin levels while those which died after the 20th day of the experiment showed extremely low hemoglobin levels in most cases. The hemoglobin levels of the rats in Group B were practically the same as the hemoglobin levels of their litter mates in Group A. The rats in Groups A and B were sacrificed at the time their litter mates in Group C died.

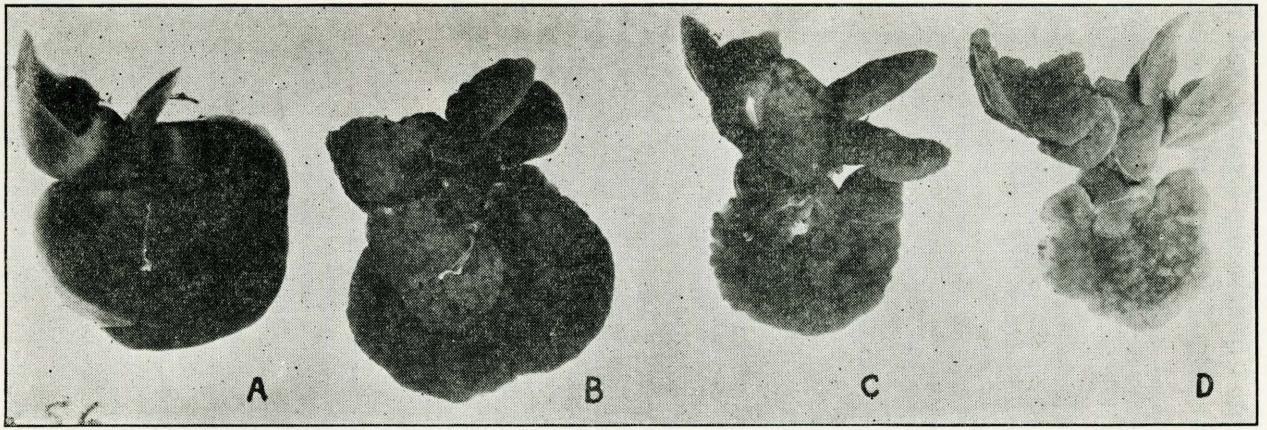


Fig. 19.—Livers from male litter mates in Series 30, Group A, B, C and D.
A. Liver from rat fed control diet.
B. Liver from rat fed "alkalied" diet 10 days, then control diet.
C. Liver from rat fed "alkalied" diet 20 days, then control diet.

Series 87.—In this series started June 30, 1933, blood was obtained by clipping off small portions of the rats' tails. Samples were obtained at three day intervals for hemoglobin determinations and smears for leucocyte counts. As in Series 85 it was observed that the rats which died in the first 20 days of the experiment had high hemoglobin levels while those which died later had very low hemoglobin levels. These low levels ranged from 2 to 10.8 grams of hemoglobin per 100 cc. of blood. The normal hemoglobin levels observed in Groups A and B ranged from 13 to 20 grams of hemoglobin per 100 cc. of blood.

Inorganic Selenium also Causes Anemia.—It has been demonstrated that selenium was present in the toxic grains used in the preceding series on blood studies and it seemed desirable at this time to study the effect of inorganic selenium salts upon the composition of the blood.

Series 97.—This work was started on January 8, 1934. Four groups of nine rats each were fed as follows:

Group A Control wheat diet
Group B Control wheat diet +22.5 p.p.m. Se as Na_2SeO_3
Group C Control wheat diet +33.5 p.p.m. Se as Na_2SeO_3
Group D Control wheat diet +52.0 p.p.m. Se as Na_2SeO_3

The control wheat diet used in this series was made up as follows:

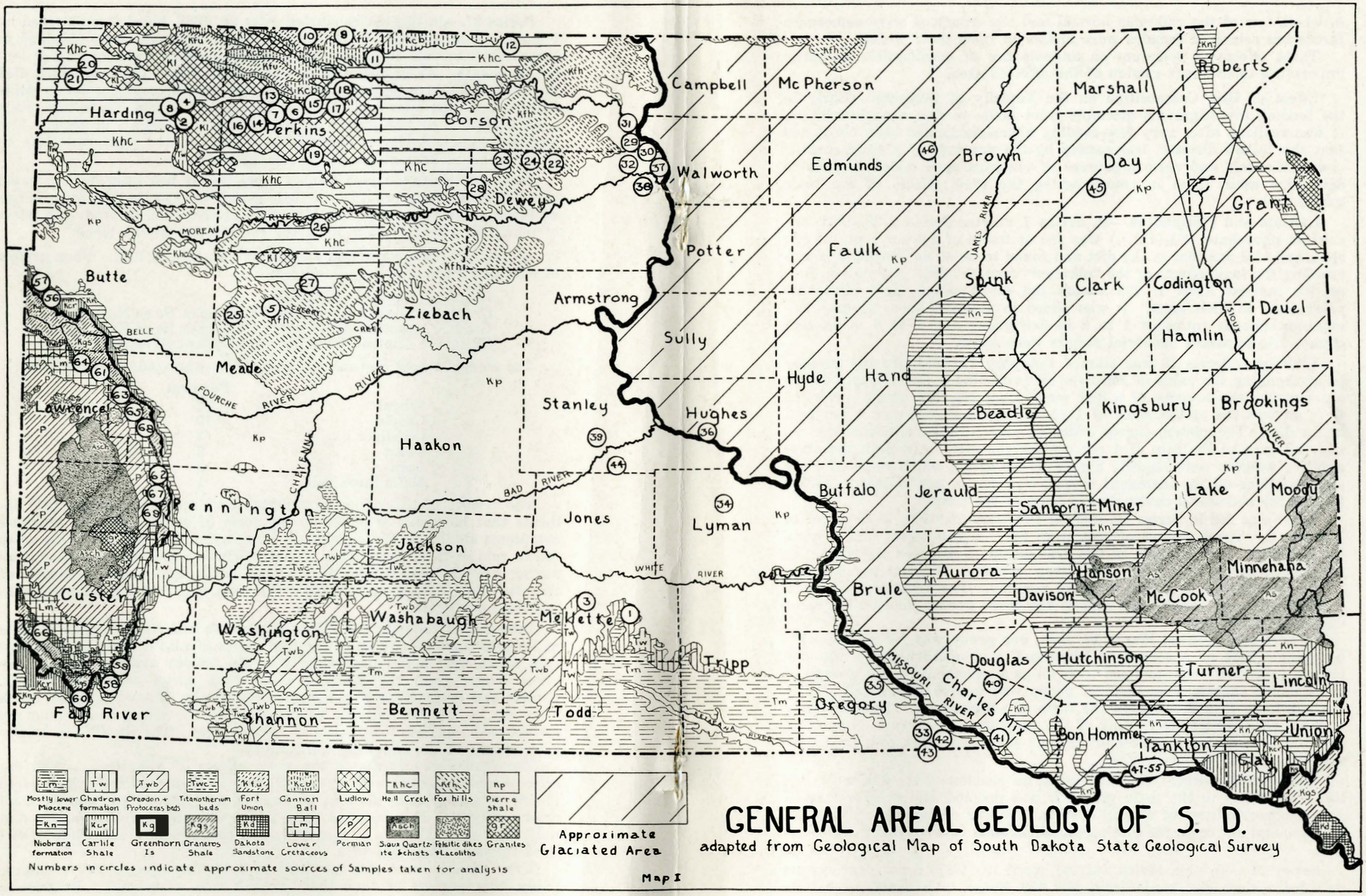
	Per cent
Wheat	82
Casein	10
Cod-liver oil	2
Yeast	2
Lard	3
McCollum's salt	1

The results of this series as reported by Franke and Potter (31) indicate that inorganic selenium in the form of sodium selenite produces symptoms similar to those produced by selenium containing grains when fed to rats in the normal diets. This conclusion was reached after comparing growth, food intake, hemoglobin levels, and gross pathology of rats fed diets containing inorganic selenium and rats fed diets containing the toxic grain.

Influence of Seleniferous Feeds on Growth and Reproduction of Rats at Various Ages.—In some of the first series of rats it was observed that rats which were a few days older than the 28 day average usually survived longer than the younger ones when given seleniferous diets. Franke and Potter (32) have reported the results of several series which were set up for the purpose of determining the effect of selenium on growth and reproduction of rats of various ages. The results are briefly summarized in the following paragraphs.

The resistance of rats to the toxic effects of seleniferous diets increases rapidly between the ages of 21 and 42 days. Animals which were placed on seleniferous diets at 21 days of age lost weight and were all dead in 20 days, while most of those which were fed a selenium free diet until they were 42 days of age and then changed to a seleniferous diet lived for more than 200 days.

Even though some rats survived on seleniferous diets for comparatively long periods of time their growth was subnormal. Matings in which both rats were selenized were completely infertile while matings



GENERAL AREAL GEOLOGY OF S. D.

adapted from Geological Map of South Dakota State Geological Survey

Map I

in which one of the rats was normal and one selenized were sometimes fertile but selenized females were unable to raise their young.

These effects of selenium on animals are of considerable economic importance to livestock raisers of the affected area.

Effect of Diet Composition on the Toxicity of Selenium.—Early in the feeding investigations attempts were made to find a counteractant. It was realized after very few feeding experiments had been conducted that the "alkali disease" was caused by the presence of a toxic constituent. Minerals and various sources of vitamins were added to diets to determine their value in counteracting the toxic effects of the toxic grains.

Calcium and Phosphorus.—In Series 7, started January 29, 1931, tricalcium phosphate ($Ca_3(PO_4)_2$) was fed at levels of 2.8 per cent, 5.6 per cent, and 11.2 per cent in the diet and found to be of no benefit in counteracting the toxic effects of the "alkalied" corn No. 388. Animals in Series 21, started April 1, 1931, were fed mono-sodium acid phosphate (NaH_2PO_4) in their diets. It was added in amounts so as to give calcium-phosphorus ratios of 1 to 2 in one group and 1 to 6 in another group. No apparent beneficial results were noted.

Vitamins.—Series 18 was started February 11, 1931, for the purpose of determining the value of cod liver oil (vitamins A and D) as a counteractant for the toxicity of a diet made from No. 459 wheat. The cod liver oil was fed at the rates of 1 per cent, 2 per cent, and 4 per cent of the diet. The growth curves indicated very little benefit from the cod liver oil even at the rate of 4 per cent of the diet. Although the average live weights were slightly higher in the group which received 4 per cent cod liver oil the increased weight was due in most cases to ascitic fluid in the abdominal cavity or to edema of the tissues.

Yeast was fed in Series 19, started February 26, 1931, as a source of Vitamin B. Three groups of rats were fed No. 459 wheat diet as used for Series 9. The animals in the first group were given no yeast. In the second group they were each given .4 grams of yeast per day and in the third group .8 grams of yeast per day. The yeast was fed as a supplement. At autopsy the rats of the three groups showed the same degree of pathology.

In Series 20, started March 12, 1931, one group was fed supplements of orange juice as a source of Vitamin C and another group was fed orange juice, dry yeast and cod liver oil as supplements. The growth of the animals especially in the last group was improved probably due to the non-toxic food received in the form of supplements. The pathology was not changed by the feeding of the supplements. Figure 20 shows a liver from a rat fed cod liver oil, orange juice and yeast compared with the normal liver in Figure 17A.

Cystine and Sulfur.—Since selenium, an element of the sulfur family, had been found in the toxic grains it was deemed desirable to investigate the possibilities of adding cystine, a sulfur containing amino acid, or elemental sulfur to the diet as counteractants of the toxicity of selenium.

Series 55.—In this series, started April 19, 1932, 5 per cent cystine was fed with a diet made from No. 459 wheat. No beneficial results were noted and there was some evidence of toxicity from the cystine.

Schneider (33) fed cystine at levels of .2 per cent, .4 per cent, and .6 per cent in diets containing 35 p.p.m. of inorganic selenium and noted no alleviating effects.

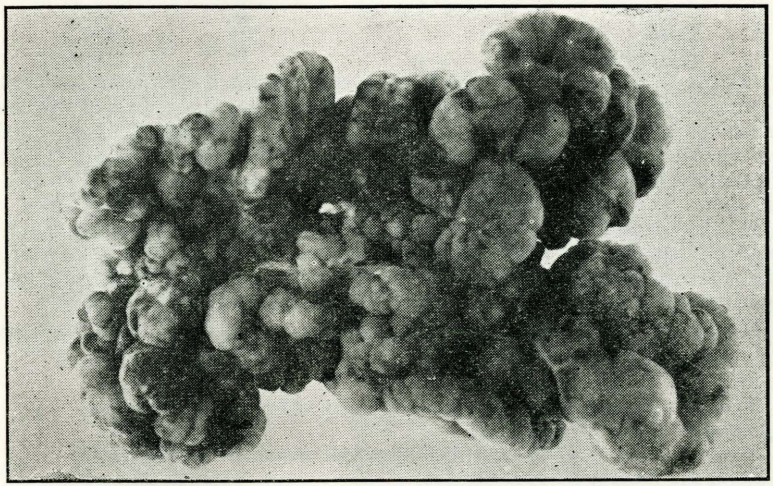


Fig. 20.—Liver of rat fed yeast, cod liver oil, and orange juice as supplements along with a toxic diet.

Series 76.—Sulfur was fed at a level of .5 per cent in this series, started April 18, 1936. Three groups of rats were fed as follows:

Group A Control wheat diet
Group B Control wheat diet +.5 sulphur
Group C Control wheat diet +.5 sulphur +25 p.p.m. Se as Na_2SeO_4

In Group C there was no evidence of beneficial effects from the feeding of the sulfur and in Group B there was some evidence of toxic effects from the .5 per cent sulfur. In poultry experiments just completed in cooperation with the Poultry Department it was found that 1 per cent sulfur in the ration intensified the toxic effects of "alkalied" grain.

Proteins.—Investigations on the influence of the protein content of the diet on the toxicity of selenium were started June 2, 1934, as Series 100. In this series four groups of rats were fed as follows:

Group A High protein diet
Group B Low protein diet
Group C High protein diet +37.5 p.p.m. Se as Na_2SeO_3
Group D Low protein diet +37.5 p.p.m. Se as Na_2SeO_3

The high and low protein diets containing the same number of calories per gram were made up as follows:

Constituents	High protein Per cent	Low protein Per cent
Casein	55	10
Cornstarch	10	25
Sugar	15	45
Cod liver oil	2	2
Yeast	5	5
Lard	10	10
McCollum's salt	3	3

On the thirteenth day of the feeding experiment it was observed that the rats in Groups B and D were failing. Therefore .3 per cent of cystine was added to the diets of these groups. The controls (Group B) responded well but the rats in Group D continued to die. The rats in Group A (high protein diet) made slightly better gains than those in Group B (low protein diet). The rats in Group C (high protein diet + selenium) made a fairly good growth while those in Group D (low protein diet + selenium) were all dead by the fifteenth day. The results obtained in this series were used in planning Series 104 in which the effects of high, low and optimum protein diets on the toxicity of added selenium were studied.

Series 104.—This work was started on August 23, 1934, and consisted of seven groups of rats as follows:

Group A High protein diet.
Group B High protein diet +37.5 p.p.m. selenium as Na_2SeO_3.
Group C Low protein diet.
Group D Low protein diet +37.5 p.p.m. selenium as Na_2SeO_3.
Group E Optimum protein diet.
Group F Optimum protein diet +37.5 p.p.m. selenium as Na_2SeO_3.
Group G Control wheat diet +37.5 p.p.m. selenium as Na_2SeO_3.

The diets in the first six groups were made as follows:

Diet	High Protein	Optimum Protein	Low Protein
Casein	55	20	10
Sucrose	15	36	42
Cornstarch	10	24	28
Cod liver oil	2	2	2
Yeast	5	5	5
Lard	10	10	10
McCollum's salt	3	3	3
Cystine	0	0	.2

The control wheat diet was made as in Series 87.

Results: Averages of the final weights of rats in each group are given in the following table:

	High	High +Se	Optimum	Optimum +Se	Low	Low +Se	Control Wheat +Se
Avg. 5 males	302	223	307	178	322	101	71
Avg. 5 females	198	138	204	108	181	74	47
Avg. all 10	250	180.5	255.5	143	251.5	87.5	59

The results shown in this table indicate that a high protein diet affords more protection against selenium than a low protein diet.

Fat in the Diet.—A series was started on November 6, 1934 for the purpose of determining the influences of the fat content of the diet on the toxicity of selenium to the animal.

Series 111.—Four groups of rats were fed as follows:

Group A High fat diet
Group B High fat diet + 37.5 p.p.m. Se as Na_2SeO_4
Group C Low fat diet
Group D Low fat diet + 37.5 p.p.m. Se as Na_2SeO_4

The diets were made as follows:

Diets	High fat	Low fat
Casein	25	25
McCollum's salt	3	3
Cod liver oil	2	2
Yeast	2	2
Cornstarch	16	26
Cane sugar	22	38
Lard	30	4

Final weights of the rats in Group C were slightly higher than those in Group A while final weights of rats in Group B were slightly higher than those in Group D. All of the rats in Groups B and D were dead before the end of the experiment which ran for 67 days. It was, therefore, concluded that the percentage of fat in the diet had very little influence on the toxicity of the selenium to the animal.

Feeding Experiments Conducted for the Purpose of Determining the Nature of the Toxicant

At the beginning of our experimental feeding work we did not know that selenium was present in the toxic grains and many of the first series of rats were conducted for the purpose of determining the nature of the toxic factor.

Not Caused by Bacteria.—Series 2, started January 16, 1930, was designed to eliminate the possibility that the "alkali disease" was caused by a micro organism. The control and toxic (No. 388 corn) diets were made up the same as the diets used in Series 1. The diets were then heated (autoclaved) for 30 minutes at 15 pounds pressure and a temperature of 120 degrees C. It was found that this treatment did not decrease the toxicity of the "alkalied" corn diet.

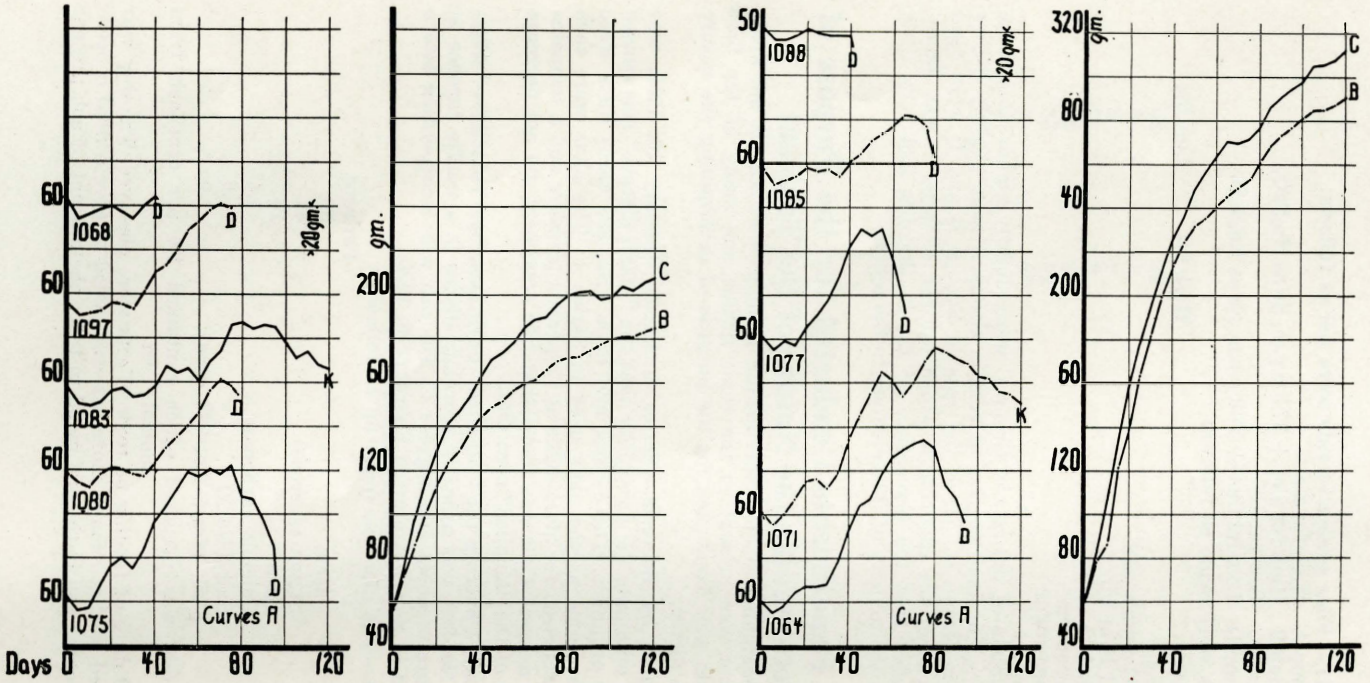
Ashing and Charring the "Alkalied" Corn Destroys Toxicity.—"Alkalied" corn, No. 388, was ashed in porcelain dishes in a muffle furnace at a temperature of about 700 degrees C. The ash was incorporated into a diet and fed in Series 3, started February 6, 1930.

Series 3.—The diet was made up as follows:

	Per cent
Casein	30
Sugar (sucrose)	47
Lard	20
Calcium carbonate	1.4
Salt (NaCl)	.6
Ash (from No. 388 corn)	1.0

A control diet was prepared which contained 1.0 per cent ash from control corn.

After 20 days of feeding no toxic effects were observed. The diet was then changed so that enough more ash was added to give a final concentration of (corn) ash in the diet equal to twice the amount of ash present



SERIES - 36

Chart IV

in the corn of the diet used in Series 1. On the fortieth day still no toxic effects were observed so the composition of the diet was changed again and the ash was replaced by 70 per cent of charred corn. This also proved to be non-toxic, and on the seventieth day the diet was modified so as to contain 70 per cent of untreated "alkalied" corn. Immediately there was a decrease in weight accompanied by other characteristic symptoms. At the time this series was conducted selenium was not known to be the cause of the toxicity and an organic toxicant was suspected. However, at the present time it is known that the toxicity is due to selenium which is volatile to the extent that it is partially driven off by charring or ashing.

It had thus been shown that charring and ashing temperatures had removed much of the toxicity from the "alkalied" grains while heating in moist heat at 120 degrees C. and 15 pounds pressure (autoclaving) had not reduced the toxicity.

Dextrinizing (Toasting) Decreases Toxicity.—In Series 33, started on September 11, 1931, rats were fed diets made from wheat which had been heated at a temperature of 155-165 degrees C. for three hours. This is sometimes called dextrinizing (toasting) because the starches of the grain are supposedly converted into dextrines at such temperatures.

Series 33.—Four groups of rats were fed as follows:

Group A Control wheat diet (wheat autoclaved for one hour at 15 pounds pressure and 120 degrees C.)
Group B No. 459 wheat diet (wheat given no treatment)
Group C No. 459 wheat diet (wheat autoclaved for one hour at 15 pounds pressure and 129 degrees C.)
Group D No. 459 wheat diet (wheat dextrinized)

The autoclaving had very little effect upon the toxicity of the "alkalied" wheat while the dextrinizing decreased the toxicity of the "alkalied" wheat.

Dry Heat Removes Selenium From Grains.—Recently, samples of corn (No. 663) and wheat (No. 607) were ashed, charred and dextrinized, the resulting products analyzed and the loss of selenium by these treatments calculated as follows:

Treatment	Loss of Selenium	
	Corn (No. 663) Per cent	Wheat (No. 607) Per cent
Ashed at a temperature of 700 degrees C.	98.85	98.90
Charred at a temperature of 265 degrees C.	76.90	89.40
Dextrinized at a temperature of 160 degrees C.	27.30	23.76

Toxic Factor Found to be Localized in the Protein Fractions of the Grains.—The next logical step was to determine whether or not the toxic factor was concentrated in any fraction (fat, protein, or starch) of the corn. During the fall and winter of 1930-31 preliminary work was started which led to the preparation of the materials fed in Series 25, started May 12, 1931.

Series 25.—The residue remaining after the toxic corn had been extracted with petroleum ether (to remove the fat) was still apparently as toxic as the untreated corn, thus demonstrating that the toxic factor was not localized in the fat. A second lot of corn was treated with petroleum ether, distilled water, and 5 per cent potassium sulfate solution. The distilled water and the 5 per cent salt solution should have extracted the albumins and the globulins from the corn. The residue was still toxic.

This was as would be expected since the albumin and globulins make up a rather small percentage of the total proteins of corn (34). A third lot of corn was extracted with petroleum ether, 5 per cent potassium sulfate solution, 70 per cent alcohol and .2 per cent sodium hydroxide. This treatment if carried to completion should have the effect of removing practically all the protein from the residue. The residue when fed was only slightly toxic as compared with the residues from the first two treatments which led us to the conclusion that the toxic factor was located in the protein fraction of the corn. More recent studies show that the slight toxicity of the residue after the extraction in the last procedure was due to incomplete extraction of the proteins.

The results of Series 25 indicated that the toxic factor of corn was located in the protein fraction.

Series 36.—This series, started October 7, 1931, had as its objective the localizing of the toxic factor of wheat. The protein was separated from the bran and starch by the following procedure:

Finely-ground wheat was dampened with enough water to form a pasty ball. This was allowed to soak in a small amount of water for 30 minutes. It was then washed with small quantities of water to remove the bran and starch. Sodium hydroxide was added to the bran and starch portions to make a .2 per cent sodium hydroxide solution. This solution was then centrifuged and the bran and starch washed twice with distilled water and then dried. After the bran and starch was separated from the .2 per cent sodium hydroxide solution it was used to disperse the gluten. The mixture was stirred for four hours and the suspension was then centrifuged in a Sharpless Super Centrifuge. A small amount of bran and starch which was recovered by the centrifuge was washed and added to the bran and starch previously separated. The protein was precipitated from the sodium hydroxide solution by adding dilute hydrochloric acid until the isoelectric point of the protein was reached. It was washed twice with distilled water and dried. Both control wheat and wheat No. 459 were treated in this manner. Diets were made by combining bran and starch from toxic wheat No. 459 with the protein from the control wheat, and bran and starch from control wheat with protein from toxic wheat No. 459.

Group A Diet made from No. 459 protein + control bran and starch
Group B Control protein + No. 459 bran and starch
Group C Control protein recombined with control bran and starch

The results of this series were reported in detail by Franke (35). As indicated by chart IV the protein from wheat No. 459 carried practically all of the toxic factor while the bran and starch carried a very small amount of the toxic factor. An examination of the livers from the animals in Group B indicated that the bran and starch caused very little pathology.

It had been shown in Series 25 that the toxicity of toxic corn No. 388 was located in the protein fraction. This was confirmed in Series 39, started October 28, 1931.

Series 39.—In this series three groups of rats were fed as follows:

Group A Diet made from No. 504 corn
Group B Diet made from No. 504 corn protein + cornstarch
Group C Diet made from No. 504 corn residue (starch and hull) + casein

The protein was separated from the corn by the following procedure:

One thousand grams of corn was ground to pass a .25 mm. seive. It was then extracted by stirring it into seven liters of .2 per cent sodium hydroxide where it was allowed to stand for five hours. It was then centrifuged and the residue washed with distilled water centrifuged and dried. Sodium hydroxide extractions were neutralized with HCl so as to precipitate the protein. The precipitate (protein) was washed with distilled water, centrifuged, and dried.

The growth curves and pathology confirmed the results of Series 25.

Series 44.—This work, started December 13, 1931, was similar to Series 39 except that a more toxic corn (No. 523) was used and the diets were prepared much like the wheat series (Series 34). The proteins were extracted from the corn by the same procedure as used in Series 39.

Group A Diet made from control corn residue + No. 523 corn protein
Group B Diet made from No.523 corn residue + control corn protein

Results of this series were similar to those of Series 39 except that they were more striking because of the greater toxicity of corn No. 523 which contained 33.6 p.p.m. of selenium. A more detailed report on the occurrence of selenium in the protein fraction of grains has been published by Franke (35).

Another series of importance in connection with feeding experiments on the nature of toxicants is Series 89, started August 23, 1933. This series was undertaken in an attempt to show whether or not acid hydrolysis would destroy the toxic factor in the protein from wheat. The hydrolysis breaks the proteins down to amino acids, the building stones of the protein molecules.

Series 89.—Four groups of rats were fed as follows:

Group A Normal bran starch + crude gluten (No. 582)
Group B Normal bran starch + No. 582 gluten hydrolysate (HCl)
Group C No. 582 bran starch + No. 582 crude gluten
Group D No. 582 bran starch + No. 582 gluten hydrolysate (HCl)

Wheat No. 582 contains 30 p.p.m. Se

The gluten was hydrolyzed with HCl, and the excess HCl was removed by vacuum distillation as much as possible and the remainder was neutralized with NaOH. The excess sodium chloride was removed by electrodialysis.

The hydrolysis of the crude gluten with HCl destroyed much of the toxicity and the hydrolysis of the normal gluten evidently destroyed some of its growth-promoting factors as indicated by a lower growth rate in Group B than in Group A. The lower growth rate in Group B than in Group A and the higher growth rate in Group D than in Group C would further indicate that much of the toxicity of the No. 582 gluten was destroyed by the hydrolysis.

More recent work has shown that the acid insoluble humin, formed during hydrolysis of toxic gluten, contains rather high concentrations of selenium which accounts for the loss of toxicity and selenium in the hydrolysate. Hydrolysis by sulfuric acid does not cause as much humin formation and does not destroy the toxicity as much as hydrolysis by hydrochloric acid.

Animals Will Avoid Selenium Containing Feeds if Given a Choice.—Many of the stockmen of the seleniferous areas are of the opinion that range animals have the ability to recognize the seleniferous vegetation and will avoid it if non-toxic vegetation is available. Experiments carried out in 1925 by the Animal Husbandry Department at South Dakota State College* showed that hogs had the ability to recognize and avoid seleniferous corn. Normal and seleniferous corn was placed in small piles in a pen and the hogs were turned in. In several trials the hogs always refused the piles of toxic corn and ate the selenium-free corn.

Several experiments in this department have shown that rats also have the ability to recognize seleniferous feeds (Franke and Potter (36)). A preliminary experiment was started in May, 1932.

Series 59.—In this series two groups of rats were given free choice of two diets exactly the same except that one was made from normal wheat and the other was made from seleniferous wheat. In 30 day periods the rats ate less than 7 per cent as much of the toxic wheat as they did of the selenium free diet.

Series 113.—This series was set up in November, 1934.

Group A Five rats were placed in a cage with five feed cups containing control wheat diet to which had been added 0., 7., 15., 30., and 60. p.p.m. of Se as Na_2SeO_3. Each feed cup was made up daily to about one-half the amount that the rats would eat daily. At intervals the least toxic diet was removed from the cage, thus forcing the rats to eat more of the toxic diets.

Group B Five rats were placed in a cage and given four feed cups containing the following diets: 1. control wheat; 2. 75 per cent control wheat + 25 per cent toxic wheat No. 582; 3. 50 per cent control wheat + 50 per cent toxic wheat No. 582; 4. 100 per cent toxic wheat No. 582.

The food intake in each group was recorded daily for each diet. After 10 days the control diets were removed from both groups and after 15 days the next least toxic diet was removed. In every case the rats would eat the least toxic diet in their cage before eating any of the other diets.

Possibly Land Should Be Used for Grazing.—The fact that animals are able to recognize seleniferous feeds, especially after they have had a little experience with the seleniferous feeds, indicates that the affected areas could possibly be used for grazing if the animals were given enough free range so that they would not have to graze the highly toxic areas. Grazing experiments are being outlined to test out such a plan of range land management.

A Statistical Study of the Toxicity and Selenium Content of Seleniferous Diets.—During the spring and summer of 1936 Franke and Painter (37), made a statistical study of the feeding records from five years of experimentation. The study revealed a high degree of correlation between toxicity and the selenium content of the various grains. It also showed that selenium from various sources (inorganic salts, corn, wheat, barley and emmer) varied in toxicity. Assuming that selenium

*Verbal communication from Prof. J. W. Wilson, Head of the Animal Husbandry Department and Director of the South Dakota Experiment Station.

was the only toxicant present in the grains the relative toxicity of selenium in the different diets was: Wheat >corn >barley >selenate >selenite.

Diets containing more than 10 p.p.m. of selenium (as it occurs in grains) caused a pronounced restriction of food consumption and the gain per gram of diet was less for the seleniferous diets than for the control diets. Diets containing selenium in amounts as low as 5 p.p.m. prevented normal growth. The toxicity of seleniferous diets was found to be more closely correlated with the selenium content of the diet than with the quantity of selenium consumed per day. This would indicate that the plane of nutrition of an animal has a direct relationship to the toxic effects of selenium.

Toxicity and Selenium Content of Milled Products of Wheat.—A sample of No. 459 toxic wheat was milled for us by Dr. C. H. Bailey, Division of Agricultural Biochemistry, University of Minnesota.

Series 27.—This work, started June 10, 1931, was a series for testing the toxicity of the various fractions. All of the milled products were toxic which showed that the toxicity was not localized in any particular part of the wheat kernel.

Selenium content of the wheat and milled products.

Wheat No. 459	24.0 p.p.m.
Patent flour	37.5 p.p.m.
Clear flour	18.7 p.p.m.
Shorts	26.2 p.p.m.
Bran	26.2 p.p.m.

The results of Series 27 were confirmed in a later series (Series 119.)

Horn, Nelson and Jones (38) have reported that the "toxic principle" is quite uniformly distributed in the milled fractions of wheat. A statistical study of the toxicity of various seleniferous diets (37) has shown that the selenium contained in the bran, flour and shorts is equally toxic.

Selenium in the Corn Kernel.—The germs were removed from a large number of kernels of toxic corn by splitting the kernels and picking the germs out. Care was taken to make a complete separation of the germ and the remainder of the kernels which consisted mainly of hull and starch. Selenium analysis showed that the germs contained 30 p.p.m. of selenium and the remainder of the kernels contained 18 p.p.m. of selenium.

Toxicity of Inorganic Selenium Compounds and Other Inorganic Compounds.— Although it has been quite definitely proven that selenium is the cause of the "alkali disease" in South Dakota and "blind staggers" in Wyoming the possibility remains that certain other elements might contribute to the cause of unexplained livestock disturbances of the Great Plains.

Byers (39) reported the presence of selenium, arsenic, vanadium, and chromium in a soil from the selenium area of South Dakota. Beath, et al (4, 6) reported the presence of molybdenum in Wyoming soils in quantities great enough to produce toxic vegetation. Tellurium has also been found in the soils and in a few native plants of Wyoming but it has not been found in crops grown on dry farms or irrigated lands in that state (6).

The toxicity of selenium has been extensively investigated in this laboratory. Several series of rats have been fed on various amounts of different inorganic selenium compounds. The toxicities of sodium selenite and sodium selenate are of somewhat the same order as the toxicity of the selenium in seleniferous grains (27, 37). Schneider (33) and Munsell and Kennedy (39) have also studied the effects of inorganic selenium on the rat.

Experiments on the toxicity of selenium, tellurium, and vanadium to enzyme activity (40) showed that the toxicity of the salts Na_2TeO_3, Na_2SeO_4, Na_2HAsO_3, $NaVO_3$, and Na_2SeO_3 increased in the order named. In studies on the toxicity of selenium, vanadium, and arsenic to the developing chick embryo (24) selenium was found to be the most toxic and also the only one of the elements which would cause monstrosities.

Franke and Moxon (41) reported on the toxicity of selenium, tellurium, arsenic, and vanadium, when injected intraperitoneally into the albino rat. The compounds in their decreasing order of toxicity were sodium tellurite, sodium selenite, sodium metavanadate, sodium arsenite, sodium selenate, sodium arsenate, sodium tellurate. Molybdenum as ammonium molybdate was found to be non toxic up to 160.0 mg. of molybdenum per kg. of body weight.

Two series of experiments on rats, Series 125 and Series 128, have been conducted to determine the relative toxicities of orally ingested arsenic, selenium, tellurium, vanadium, and molybdenum. (Franke and Moxon, unpublished data.) The elements were fed at the rate of 25 p.p.m. in Series 125 and 50 p.p.m. in Series 128. Hemoglobin determinations were made on the blood of the animals at frequent intervals.

It was found that of the elements investigated selenium was the only one which tended to cause anemia. The decreasing order of toxicity as measured by growth, food consumption and pathology was selenium, vanadium, tellurium, arsenic, and molybdenum. Arsenic and molybdenum were both relatively non-toxic even when fed at a level of 50 p.p.m. in the diet. The rats which received tellurium in their diet showed considerable loss of hair especially from the sides of their bodies. The garlicky odor of methyl telluride as described by Franke and Moxon (41) was noticed in the breath of the rats within a day after they were given the diets containing tellurium. Likewise the rats which were given selenium containing diets had the odor of methyl selenide in their breath within a day.

Toxic Effects of Orally Ingested Selenium on Dogs.—A number of dogs have been fed on diets containing inorganic selenium and seleniferous corn diets (Moxon and Franke, unpublished data). They were fed a diet containing a large amount of corn. The formula is as follows:

	Per cent
Corn (yellow)	72
Casein	18
Lard	5
Salt (NaCl)	1
Calcium Carbonate ($CaCO_3$)	1
Calcium Phosphate (Ca_3PO_4)	1

The above mixture was ground, mixed and cooked for one-half hour with live steam. After it had cooled 2 per cent of cod liver oil was added and it was remixed.

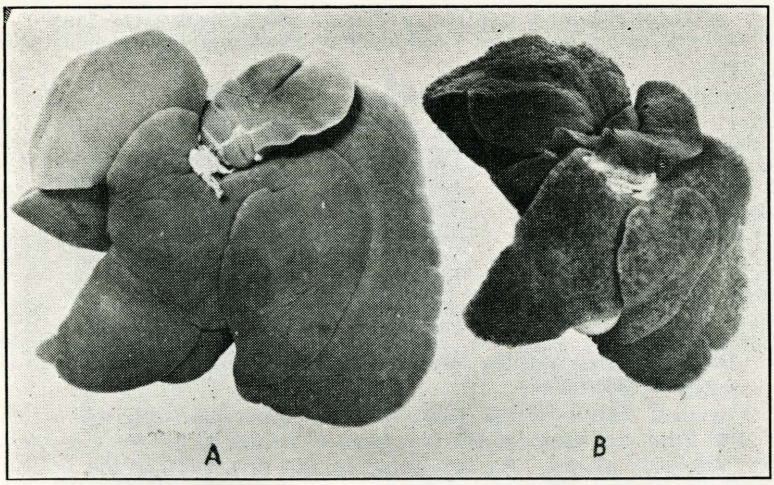


Fig. 21.—A. Normal dog liver. B. Liver from dog fed toxic corn No. 570.

The dogs given inorganic selenium salts were fed the above diet to which 20 p.p.m. of selenium had been added in the form of sodium selenite. Those which were fed the seleniferous corn were given the above diet in which the 72 per cent of normal corn had been replaced by 72 per cent of "toxic" corn No. 570 which contained 29 p.p.m. of selenium. This gave a selenium concentration of 20 p.p.m. in the diet.

At weekly intervals blood samples were drawn from the femoral artery of each dog. Determinations made on the blood included red and white cell counts, hemoglobin, cell volume, non-protein nitrogen, calcium, phosphorus and chlorides. At autopsy a careful inspection was made for pathological changes and tissues and organs were saved for the determination of their selenium content.

The following conclusions can be drawn from the feeding experiments with dogs:

Seleniferous diets cause an inhibition of growth and a decrease in food consumption. The decrease in food consumption is much more pronounced when inorganic salts of selenium are fed than when seleniferous grains are fed.

The changes in the composition of the blood were confined mainly to the calcium and phosphorus levels. In some cases the phosphorus levels were reduced to almost half of the normal levels while the calcium levels showed a slight decrease.

The dogs became dull-eyed and sluggish soon after they were placed on the seleniferous diets. Some of them reached a stage wherein the nervous system appeared to be involved. They would wander about aimlessly much the same as animals with "blind staggers" (4, 6, 7). Definite pathology of the liver was observed as shown in Figure 21.

The selenium determinations on the tissues and organs indicate that selenium is stored in practically every part of the body.

Average Selenium Content of Tissues and Organs of 10 Dogs
All determinations except on blood made on dried material and calculated as such.

Material	Average	High	Low
Liver	31.1 p.p.m.	67.0 p.p.m.	12.0 p.p.m.
Kidneys	28.4 p.p.m.	66.6 p.p.m.	6.0 p.p.m.
Heart	5.0 p.p.m.	16.6 p.p.m.	trace
Spleen	4.95 p.p.m.	23.3 p.p.m.	0.0 p.p.m.
Lungs	3.50 p.p.m.	7.0 p.p.m.	0.0 p.p.m.
Intestines	2.20 p.p.m.	10.0 p.p.m.	0.0 p.p.m.
Brain	2.00 p.p.m.	10.0 p.p.m.	0.0 p.p.m.
Pancreas	7.40 p.p.m.	24.0 p.p.m.	0.0 p.p.m.
Blood cells (wet weight)	.28 p.p.m.	.40 p.p.m.	0.0 p.p.m.
Meat	2.53 p.p.m.	10.0 p.p.m.	0.0 p.p.m.
Bones	.86 p.p.m.	2.45 p.p.m.	0.0 p.p.m.

Rib bones from one dog were analyzed and found to contain 22.6 p.p.m. of selenium.

Chemical Nature of the Toxic Factor.—When the work was started in 1929 little was known about the chemical nature of the toxic factor of the "alkalied" grains. As mentioned in the first part of the bulletin, (page 8) Bushey did some preliminary work on the chemistry of the toxic factor but left no record of his work when he died.

Some of the early feeding experiments (Series 3) showed that ashing or charring the grains decreased their toxicity. This suggested that the toxic factor was probably organic in nature since most inorganic poisons are not destroyed by the ordinary ashing or charring temperatures. Selenium, however, is partially driven off from the grain at temperatures as low as 160 degrees C. The next important development showed that the toxic factor was linked up with the protein of the grains which further suggested that it was organic.

The toxic effects of the "alkalied" grains on animals did resemble somewhat the toxic effects of arsenic and other inorganic poisons. This fact possibly played a part in the original suggestion that selenium might be involved. After selenium was found in the grains the problem of determining the chemical nature of the toxic factor became still more complex because of the small amounts of selenium present and the relatively inaccurate methods of analysis for selenium. Analysis of the proteins from toxic grains showed that the selenium followed the toxicity.

Hydrolysis of the proteins from toxic wheat with hydrochloric acid destroyed some of the toxicity as shown by Series 89. Subsequent analysis of the acid insoluble humin formed during the hydrolysis revealed that it contained selenium which partly explains the decrease in the toxicity of the acid soluble hydrolysis products. When the proteins were hydrolyzed with sulfuric acid there was more humin formed and likewise more of the toxicity was destroyed.

The probable nature of the selenium in the protein has been investigated by Franke and Painter (42). They concluded that little or no selenium was present in the toxic protein as metallic selenium or as an inorganic salt. The fact that most of the selenium remained in combination with some organic compound of the products of hydrolysis suggests that it might be in some unidentified amino acid. The possibility that selenium might replace the sulfur in cystine or methionine was early

considered. Complete removal of the selenium from toxic protein hydrolysates left them non-toxic to rats (43).

The probability that plants may metabolize selenium in a manner similar to sulfur has been investigated (44, 45). Sulfur-selenium ratios have been determined on different parts of various plants and also in proteins from certain cereals. Although there appears to be a close relationship between sulfur and selenium in many cases, selenium evidently does not take a part analogous to sulfur in the physiology of the plants. The sulfur-selenium ratios in "toxic" gluten from wheat was in the order of 1:150. The separation of a selenium containing fraction of the protein hydrolysis, from sulfur containing fraction, is difficult; especially, since sulfur and selenium compounds are very similar in their properties and reactions. Further evidence that selenium is associated with cystine has been presented by Jones, et al (46). The selenium analog of cystine has been prepared by Fredga (47) and by Gordon (48). To date, however, nothing has been published on the toxicity of selen-cystine.

Selenium

The Element.— Selenium was discovered in 1817 by John Jacob Berzelius and Gotlieb Gahn during the course of their examination of a sulfuric acid plant at Gripshalm, Sweden. It was observed in the sediment of the acid plant. In their examination of the element they noted that it gave off a peculiar odor much like that of tellurium. The new element was named selenium which comes from the Greek word "selenos," the moon. Tellurium was named from the Greek word meaning earth. Of the known elements selenium ranks about fiftieth in abundance (49).

Selenium is a member of the oxygen-sulfur family which also includes tellurium. Selenium closely resembles sulfur in its properties and exists in at least three allotropic forms.

(1) Amorphous selenium formed as a finely divided brick-red precipitate when a solution containing the selenous ion SeO_3 is reduced with sulfur dioxide.

(2) Vitreous selenium formed when amorphous selenium is heated to 217 degrees C. and quickly cooled. It is a black, brittle, glassy mass and has no definite melting point, but softens at 100 degrees C. and is completely molten at 250 degrees C.

(3) Metallic selenium obtained by heating vitreous selenium at a temperature of 210 degrees C. for a period of time. The mass finally melts, the temperature rises to 217 degrees C. and metallic selenium solidifies in a granular, crystalline form. Metallic Se is the only form of selenium which will carry electricity and finds use in photoelectric cells.

Aside from its occurrence in soils selenium is found associated with sulfur in many native sulfur deposits (50). The sulfur from the Hipari Islands north of Sicily, the northernmost island of the Hawaiian group and from Japan contains selenium even to the extent that it shows a red-brown coloration.

The sulfur deposits from southern United States are apparently free from selenium (51). Selenium is found in some of the sulfide ores especially certain iron pyrites from Greece, Spain, Germany and the Scandinavian peninsula. Whether or not it exists as a selenide or in some

other form in the pyrite ores has not been determined. The analyses of a number of iron concretions from the Pierre and Niobrara geological formations for selenium, in this department, has shown that the selenium was independent of the pyrite but was associated with iron oxides. The chemical reactions of selenium and its compounds are similar to those of sulfur and sulfur compounds. Commercial uses of selenium are limited mainly to the glass, rubber and electrical industries.

Methods of Analysis for Selenium used for Determinations Reported in This Bulletin

Procedure.*—For vegetation and animal tissues a three-gram sample is used unless it is known that selenium content of the material is above 30 p.p.m., then size of sample should be reduced. For soils and geological materials a 10-gram sample is used. Digest in usual Kjeldahl manner in digestion-distillation flask (Figure 22) until clear. (For grains and vegetative samples this requires approximately one hour. Many tissue samples require as long as two to three hours for digestion and use much more acid than grain samples do.) During digestion a receiving flask containing Br_2:HBr mixture (1:10) is placed on the condenser at beginning and Br_2 added from time to time so that there is always an excess in the flask.

After digestion is completed, allow contents of flask to cool, then add enough water to dilute the acid contents of flask to about 40 to 50 per cent (usually three-fourths to equally as much water as quantity of acid used in digestion). This dilution is necessary even though there is a large quantity of water in the receiving flask to prevent fuming due to heat formation when contents of the receiving flask are added to the digestion flask.

Allow flasks to cool after dilution then add the contents of the receiving flask to the distillation flask. Also add 20. to 75. cc. of HBr to the distillation flask, unless it is thought the selenium content of the sample is low and there is a large excess of Br_2 in the receiving flask. Replace receiving flasks, with just enough water to cover the opening of the delivery tube on the condenser. Distill contents of flask. At the very outset of this distillation a few grams of Br_2 should pass over into the receiving flask. If this does not happen add enough of the Br_2:HBr (1:10) solution to cause Br_2 to pass into receiving flask. If Se content of sample is low distill 50 to 75 cc. of solution into receiving flask or until contents of distillation flask become clear.

If a large amount of Se is present a great excess of Br_2 must be used in the distilling flask and distillation should continue until contents of distillation flask are clear—even though 200 or more cc's. are collected in the receiving flask. When distillation is complete discard residue in distillation flask. Filter contents of receiving flask into a suitable flask large enough to hold the filtrate and approximately 25 cc. of water used to wash the distillation flask and the filter paper. The precipitate which

* This method is essentially that of Robinson, Dudley, Williams and Byers ('52) with a few variations in details.

is collected on the filter paper is unidentified as to composition. It is useless and is discarded. The filtrate is reduced with SO_2 until clear and excess SO_2 passed in for a few seconds. One-fourth to one-half grams of hydroxylamine hydrochloride is added and the flasks are allowed to heat on the steam bath for one hour to affect complete precipitation of the selenium. After the flasks are removed from the steam bath they are allowed to set for at least two hours, and longer periods are permissible up to 48 hours.

There is a danger of some loss of selenium if solution is heated too long on steam bath or allowed to set too long after removal from steam bath. After the "setting period" the selenium is filtered out on an asbestos mat, which has been washed with Br_2:HBr mixture, in a Gooch crucible. If no selenium filtered through, and this danger is slight, the filtrate is saved for recovery of the unused HBr and the filtering flask rinsed with water. If any selenium filters through, refilter. The selenium is then dissolved and allowed to filter through the mat with 5 cc.

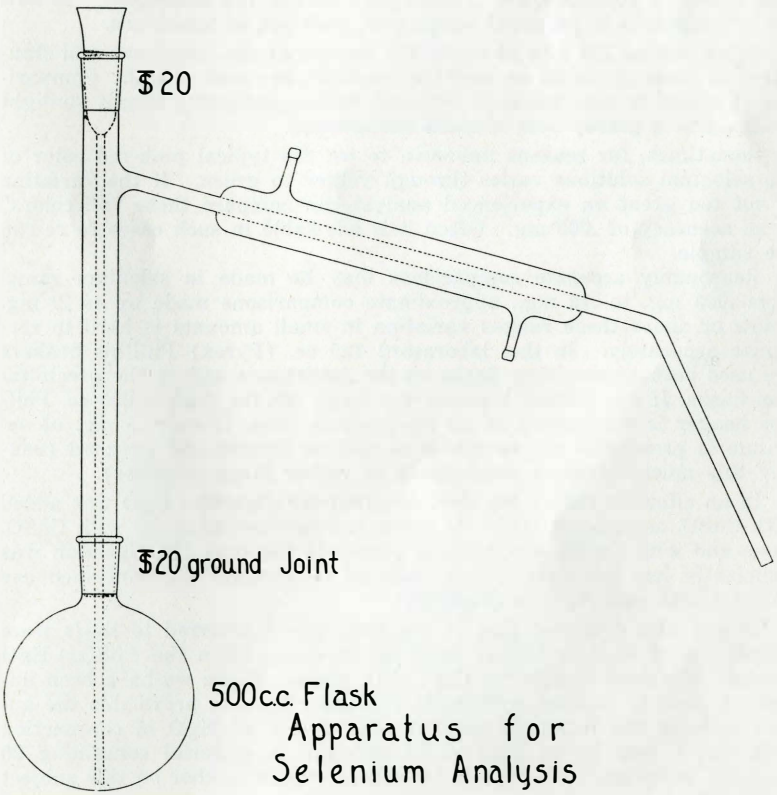


Fig. 22.

of the Br_2:HBr (1:10) mixture. Suction is used to filter the selenium but the Br_2:HBr solution is allowed to drip through for at least five minutes before suction is applied to affect complete washing of the mat.

Approximately 10 to 12 cc. of water, or less, are used to wash the mat free from Br_2:HBr solution. The selenium solution in the flask is now poured and washed into a test tube graduated at 25 cc. One cc. of 0.33 per cent agar-agar solution is added and mixed with the Br_2:HBr solution. The total volume now should not exceed 23 cc. The solution is reduced with SO_2 until clear and an excess of SO_2 is added. One cc. of 10 per cent hydroxylamine hydrochloride solution is added. The test tubes are placed in a water bath at 80 degrees C. and maintained at that temperature for one hour. They are then removed from the bath and allowed to set at least two hours before they are compared.

For purposes of comparison standard selenium solutions are prepared from a solution of selenium in Br_2:HBr (1:10) standardized so 1.0 cc.= .1 mg. Se. To the necessary amount of standard selenium solution is added 5 cc. of Br_2:HBr (1:10) solution and 1 cc. of 0.33 per cent agar and the volume is made up equal to that of the unknowns with water. The standard solutions are treated just as are the unknowns. A new set of standards is prepared along with each set of unknowns.

After setting for 2 to 24 hours the volume of the unknowns and standards is made up to 25 cc. and the solutions are well mixed. Comparison of colors is then made in reflected light—preferably bright sunlight (but not in a glare) over a black background.

Sometimes, for reasons unknown to us, the typical pink-red color of the selenium solutions varies through yellow to green. If the variation is not too great an experienced analyst can compare these "off-colors" to an accuracy of .005 mg. Often it is advisable in such cases to re-run the sample.

Reasonably accurate comparisons may be made in selenium range from .005 mg. to .10 mg., approximate comparisons made up to .2 mg. Below or above these ranges variation in small amounts is hard to recognize accurately. In this laboratory 125 cc. (Pyrex) Phillips beakers are used both as receiving flasks on the condensers and as the precipitation flasks. If the volume becomes too large for the flask a 250 cc. Phillips beaker is substituted at an appropriate time. If over .5 mg. of selenium is present in the sample it should be filtered and weighed (usually this much selenium precipitates in rather large particles).

In an effort to reduce the time required for digestion HgO was added with $CuSO_4$ as catalyst (53). In a few comparative analyses with $CuSO_4$ alone and with $CuSO_4$ and HgO as catalysts the time for digestion was sufficiently less with the double catalyst to warrant the continued use of the $CuSO_4$ and HgO as catalysts.

It was also observed that in the tests above referred to there were indications of slightly higher selenium recovery when the $CuSO_4$+HgO catalyst was used in place of the $CuSO_4$ alone. While we have been unable, to date, to conduct sufficiently extensive tests to prove this the author believes the increased recovery due to use of HgO in conjunction with $CuSO_4$ may be as high as 10 per cent in material containing 20 p.p.m. of selenium. We hope to be able to report further on this subject in the future.

A few analyses made to illustrate the value of the bromine trap during digestion also illustrate the increased recovery when HgO + $CuSO_4$ replaces $CuSO_4$ alone as catalyst. (These digestions were performed on a regular Kjeldahl digestion unit with no attempt to catch selenium which might escape during the digestion process. Otherwise the distillation and estimation of selenium was performed in the usual manner.)

The following table shows the results obtained:

Sample No.	Wt.	Digested on Kjeldahl Unit Digestion Time Clear	Finished	Catalyst	p.p.m. Se
607	4 grams	32 min.	55 min.	$CuSO_4$, 1 gram	6
607	4 grams	15 min.	25 min.	$CuSO_4$ + HgO 1 gram each	17½
587	4 grams	38 min.	55 min.	$CuSO_4$	0
587	4 grams	15 min.	25 min.	$CuSO_4$ + HgO	11
570	4 grams	33 min.	55 min.	$CuSO_4$	1¼
570	4 grams	15 min.	25 min.	$CuSO_4$ + HgO	12½

Selenium content of the above samples by the regular method of analysis is as follows: 607 — 32 p.p.m.; 587 — 15 p.p.m.; 570 — 25 p.p.m.

On several occasions the wet oxidation method of Williams and Lakin (54) for selenium analysis has been used, but we have been unable to find any particular advantage in its use. Our experience has been that we can not obtain higher results with this method than with the digestion-distillation procedure outlined above, and that there is but a slight saving of time by the use of the wet oxidation method. We have not been able to digest samples by the wet method in less than one hour and the average time is approximately 90 minutes, no less than for the Kjeldahl method. Therefore the only saving in time is the time required for cooling before and after dilution of the digests in the Kjeldahl method.

The Geological Distribution of Selenium

The selenium which is found in the soils of South Dakota and other parts of the Great Plains area is also a component of the geological formations from which the soils were formed. At this point it would seem desirable to give a brief discussion of the geology of the area along with the discussion on the distribution of selenium. According to the present knowledge of the distribution of selenium in this area it first appeared in the permian series of formations which were deposited in the latter part of the paleozoic or the first part of the mesozoic era of geologic time.

Geologic time has been roughly divided into the following five eras:

Era	Began About
Cenozoic or present age (Age of mammals)	60 million years ago
Mesozoic or Age of reptiles	185 million years ago
Paleozoic	550 million years ago
Proterozoic	1450 million years ago
Archeozoic	3000 million years ago

The diagram on page 69 indicates the era during which the various formations were laid down and also indicates which formations are selenium bearers. It will be noted that most of the selenium bearers were deposited during the Mesozoic era which is fairly recent when geologic time as a whole is considered. It will also be noted that most of the seleniferous deposits are of the Cretaceous periods during which time the present Great Plains area was covered by a shallow sea. In general this shallow sea occupied an area which extended from the Gulf of Mexico on the south to the Mackenzie river on the north and from somewhere in the vicinity of the South Dakota-Minnesota, Iowa-Nebraska boundaries on the east to somewhere near the Wyoming-Idaho boundary on the west. In fact it covered most of what is now the Great Plains, the Rocky Mountain belt and the Mackenzie River Basin. During the time occupied by the Jurassic, Triassic and Permian periods just preceding the Cretaceous period, smaller areas were covered by shallow seas in some of which selenium bearing formations were deposited and which were of course later covered over by the Cretaceous formations. Information on the selenium content of shales from other continents of the world would indicate that the Mesozoic and Paleozoic were possibly periods of universal selenium deposition. Minomi (55) found the following values:

European Paleozoic shales	average 36 samples	1.1 p.p.m. Se
Japanese Paleozoic shales	average 14 samples	24 p.p.m. Se
Japanese Mesozoic shales	average 10 samples	.4 p.p.m. Se

Primary Source of the Selenium Which Found its Way into the Geological Formations.—During the periods in which the selenium was deposited a range of mountains, older than the present Rocky Mountains, occupied a part of the area now called the Cordilleran Geanticline to the west of the sea (6). Active volcanos existed in this range of mountains and since it has recently been shown that selenium is present in the lava and gases from active volcanos in Hawaii (56) and in lava and gasses from volcanos in the Valley of the Ten Thousand Smokes (57) it is

thought that it was also present in the volcanos of the Mesozoic era. In fact, with the information on hand there is no other logical explanation of its source.

The material which made up the formations deposited in the bottom of the sea was originally a part of these same mountains. It was transported eastward by the tides and therefore the deposits in the western part of the old sea bed are much thicker and of coarser material than the formations in the eastern part of the area. The Cretaceous formations in western Wyoming exceed 20,000 feet in thickness and contain many coarse particles, while in eastern South Dakota the formations are less than 1,000 feet in thickness and are made up of very fine claylike particles.

The selenium may have been a constituent of magmas which intruded into and upon the present material before it was transported by the sea and deposited, or it may have been conveyed by winds with the gasses and volcanic ash from active volcanos and deposited in the sea where it would immediately be bound to certain soluble iron compounds and deposited in the bottom of the sea along with the sedimentary materials which were being moved from west to east by the tides. The former theory has been favored by Beath et al (58). According to Byers (56) the latter theory is supported by the fact that ferric hydroxide absorbs and precipitates selenium from very dilute solutions (51, 56, 59). This reaction is undoubtedly the reason for the absence of selenium from sea water. Strock (51) has reported the presence of .004 mg of selenium per liter of sea water from Helgoland. However, Byers* has been unable to detect selenium in any sea water except where contamination could be traced to an industrial plant such as a sulfuric acid plant situated nearby on the seacoast or on a nearby river leading into the sea. Furthermore, Byers has stated that he has found considerable selenium in the muds taken from the bottom of different parts of different oceans and seas.

The hypothesis that selenium was carried over the old sea by winds and deposited in the water is given further support according to Byers (56) by the fact that the zones of the Niobrara and Pierre formations which are high in selenium content also contain considerable bentonite which is presumed to be derived from volcanic ash.

Selenium Content of South Dakota Geological Formations.—During the past winter a number of samples representing important geological formations which outcrop in this state have been analyzed for selenium‡. Map No. I is more or less self explanatory through its legends. It should be mentioned, however, that the small figures in the circles correspond to the same numbers in the diagram on page 69 and the table on pages

* Verbal communication

‡ The author wishes to express his thanks to E. P. Rothrock, State Geologist, for making available, through the state Geological Survey, a large number of the samples; to Dr. W. V. Searight, Professor of Geology at the University of South Dakota and K. M. Sandals, formerly geologist with the State Planning Board and at present Water Utilization Specialist with the Resettlement Administration, for their assistance in sampling, identifying, and cataloging the geological material; to Professor O. A. Beath, Research Chemist and Dr. S. H. Knight, State Geologist of the University of Wyoming and Dr. H. G. Byers of the Bureau of Chemistry and Soils, U.S.D.A. for helpful information concerning the geology of the Great Plains area. Special thanks are also due Mr. Sandals for his valuable assistance in correlating and presenting the results.

72, 73 and mark the approximate location of the source of the samples. It will also be noted on the map that the formations have been more or less covered over by glacial material in all of the section east of the Missouri river. There are, however, areas along the east side of the river which have not been glaciated and small areas along the west side of the river which have been glaciated. There are also small outcroppings of Pierre and Niobrara formations in various parts of the east river country as indicated by the samples 45 and 46, and 47 to 55.

Geology of the area in the Black Hills and immediately surrounding the Black Hills is unique in that practically all of the sedimentary geological formations and especially those of the Cretaceous period, occur almost in concentric rings around the area. An examination of this Black Hills area on the map and on the columnar section of South Dakota shown in Col. 1 in the diagram on page 69 will show that the core of the Black Hills is of igenous rock (granite), which has been moved up to this level by an upwarping of the formations and a subsequent erosion of the formations above. This has left the sedimentary formations standing at an angle with the uppermost formations on the outside and the lower formations next to the core. The outcroppings of the various formations around the Black Hills furnished several of the samples which were analyzed and afford an opportunity for further studies on the distribution of selenium, especially in the sedimentary formations and the soils derived therefrom.

There has been some controversy whether the Laramie group, which is composed of the Hell Creek, Cannonball, Ludlow and Fort Union formations of the northwestern part of the state, belongs to the Cretaceous or to the Tertiary system. The South Dakota State Geological Survey considers that the first three formations, which are spoken of collectively as the Lance formation, belong to the Cretaceous period. The Fox Hills formation, which is directly between the Laramie group and the Pierre below, is definitely Cretaceous and the White River formations which cover a considerable area in the south central part of the western half of the state are Tertiary.

At the close of Cretaceous time or at some early part of Tertiary time the Pierre formation which is now exposed in a relatively large area of western South Dakota was covered over by the Fox Hills formation and the Laramie group of formations. Then before the White River formations were deposited the Laramie and Fox Hills formations were eroded away so that the White River formations of the southern part of western South Dakota rest directly upon Pierre shale. The White River formations are quite readily eroded and have exposed considerable Pierre shale which they formerly covered.

An examination of the selenium content of samples analyzed as shown in the Table on pages 72, 73 indicates that the important selenium bearers are the Pierre and Niobrara formations with very little selenium in the samples from the other formations analyzed. It will be noted also, that the coal and lignite shale samples (10, 16, 19, 22, and 25) contained selenium, while No. 18, a sample of pyrite and coal from the Ludlow formations, was selenium-free. The low selenium content of the Tertiary samples Nos. 1 to 8 and especially those from the White River formations is in agreement with the results reported by Byers (60).

	AGE / AREA			1 SOUTH DAKOTA	2 S.W. SO. DAK. N.W. NEB. E. WYO.	3 MONTANA	4 WYOMING	5 EASTERN COLO. WESTERN KAN.	6 WESTERN COLORADO
CENOZOIC	QUATERNARY			GLACIAL DRIFT ALLUVIAL DEPOSITS & LOESS		GLACIAL DRIFT		LOE SS	
CENOZOIC	TERTIARY	PLIOCENE		LITTLE WHITE RIVER BEDS -SANDS-	WHITE RIVER Low in Se			OGALLALA	
		MIOCENE		NEBRASKA BEDS SHEEPCREEK BEDS ARIKAREE 2					
		OLIGOCENE		WHITE RIVER GROUP BRULE 3 CHADRON 4-5					
CENOZOIC? MESOZOIC?	TERTIARY? CRETACEOUS?	EOCENE?		FORT UNION 1-10			FERRIS Se		
			LANCE	LUDLOW 8 CANNON BALL 11,20-21 HELL CREEK	11-18		MEDICINE BOW Se		
MESOZOIC	CRETACEOUS	UPPER CRETACEOUS	MONTANA GROUP	FOX HILLS 22-28	FOX HILLS Se	BEAR PAW Se			
				29 to 43 PIERRE ••	PIERRE Se	JUDITH RIVER Se	LEWIS Se	BEECHER ISLAND	MESAVERDE
					" Se B	CLAGGET Se		UNDIFFERENTIATED	
					" Se C	EAGLE	MESAVERDE Se	SALT GRASS	
					" Se D			LAKE CREEK Se	
					" Se E	TELEGRAPH CREEK	STEELE Se	WESKAN Se	
					" Se F			SHARON SPRING Se	
			COLORADO GROUP	46-54 NIOBRARA	NIOBRARA Se		NIOBRARA Se	SMOKY HILL Se	
								FORT HAYES Se	
			BENTON GROUP	CARLILE 55,56	CARLILE Se	COLORADO Se	BENTON FRONTIER Se	BLUE HILL Se FAIRPORT	
				GREENHORN 57	GREHORN Se		MOWRY Se	PFEIFER JETMORE HARTLAND LINCOLN	MANCOS Se
				GRANEROS 58	GRANEROS Se		THERMOPOLIS Se	GRANEROS	
				DAKOTA 59	DAKOTA FUSON LAKOTA		DAKOTA Se	DAKOTA	DAKOTA
		LOWER CRETACEOUS		FUSON LAKOTA 60-65		KOOTENAI			
		CRETACEOUS JURASSIC		MORRISON 66	MORRISON	MORRISON	MORRISON Se		
	JURASSIC	UPPER JURASSIC		UNKPAPA			SUNDANCE n. Se		
				SUNDANCE					
	TRIASSIC			SPEARFISH			CHUGWATER Se		
PALEOZOIC	CARBONIFEROUS	PERMIAN		MINNEKAHTA OPECHE			DINWOODY Se PHOSPHORIA		
		PENNSYLVANIAN		MINNELUSA			TENSLEEP		
		MISSISSIPPIAN		PAHASAPA ENGLEWOOD					
	ORDOVICIAN	UPPER ORDO		WHITEWOOD					
	CAMBRIAN	SARATOGAN		DEADWOOD					
PRE-CAMBRIAN									

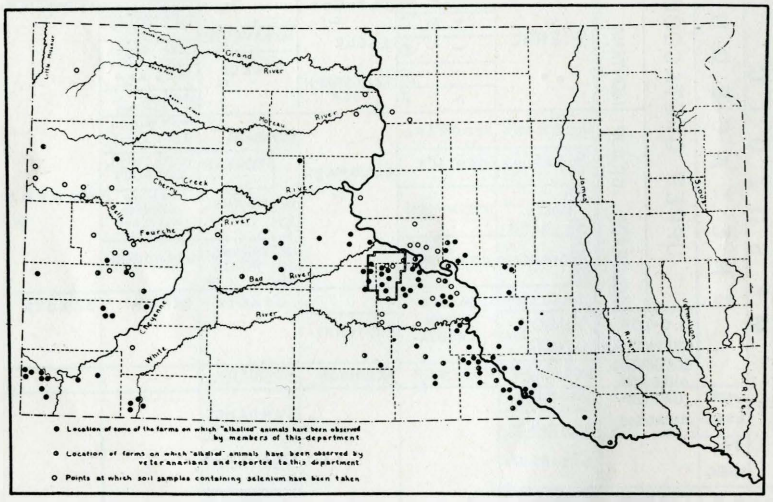
Chart V

No Attempt Has Been Made To Show Relative Depth Of Formations

Se—Selenium Found **Figures in Column One Refer to Samples Analyzed**

Compiled from: Sandals columnar section of South Dakota-Water Resources, Vol. 1, S. Dak. State Planning Board 1937
Beath, Eppson, and Gilbert, Wyoming Agri. Expt. Station Bull. No. 206, 1935
Byers, U.S.D.A. Tech. Bull. No. 530, 1936 S. Dak. State Geological Survey Map, 1932

Samples from the Laramie group (Samples No. 9 to 22) were all negative for selenium except the coal and lignite samples. The lack of selenium in the Laramie group and also in the soils of the same area agree very well with the geographical occurrence of selenium shown on Map II. The Fox Hills formation which is of a more or less sandy nature appears to be relatively selenium-free also. The samples of Pierre shale (Samples No. 29 to 46) as would be expected from all previous work, were all positive in varying amounts except one. As has been reported by Byers (2) our results would also indicate that various zones of the Pierre contain selenium in a variety of concentrations. Work is under way at the present time on the analysis of uncontaminated samples of Pierre from a well core which should give added information on the selenium content of the various zones of the Pierre. The Niobrara (Samples No. 47 to 55) which was taken from an outcropping near Yankton, shows a comparatively high but varying content.

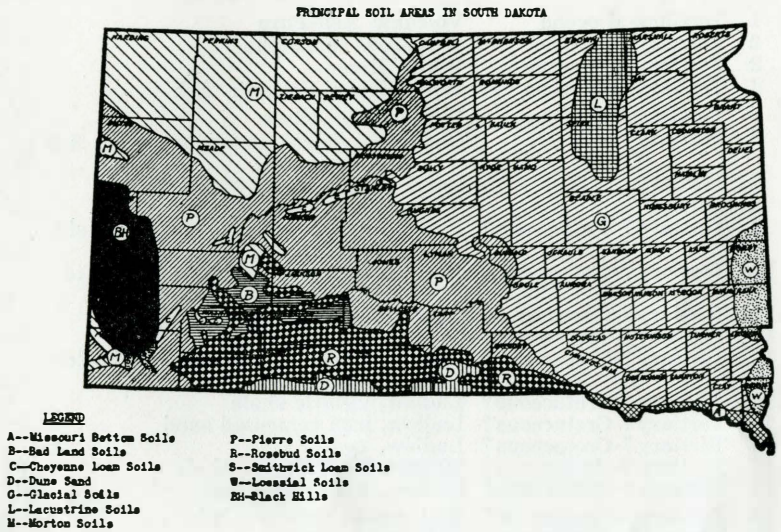


Map II

Several iron concretions were obtained from an outcropping of the Niobrara formation near Laramie, Wyoming, through the courtesy of Professor O. A. Beath of the University of Wyoming. A composite sample of these concretions contained 100 p.p.m. of selenium while a sample of the Niobrara free from the concretions and taken at the same point contained 10 p.p.m. of selenium.

Not enough samples were obtained of formations below the Niobrara to draw many definite conclusions, but as will be noticed in the diagram on page 69 the members of the Benton group have a reputation of being seleniferous in other areas while the Dakota group is probably relatively selenium-free. Although we found no selenium in the two samples of

Morrison analyzed, Beath has reported that it is in general seleniferous but has a relatively low selenium content. Samples of formations older than the Morrison were not analyzed but Beath (58, 61) has just recently reported selenium in formations of Triassic and Permian ages.



Map III*

Map III shows the principal soil areas of South Dakota. A careful comparison of this map will reveal that the soil areas follow very closely certain of the geological formations. The Morton soils in the northwest part of the state include soils derived from the Fort Union, Ludlow, Cannonball, Hell Creek and Fox Hills formations. The Pierre soils are those derived from the Pierre, Niobrara, Carlile, Greenhorn and Graneros formations and the Rosebud and Badlands soils are those derived from the lower Miocene and the White River formations. Most of the toxic areas are included in the areas covered by the Pierre soils, while limited areas of lower toxicity are found on Morton, Rosebud and Badlands soils.

*Made available through the courtesy of Prof. J. G. Hutton, Dept. of Agronomy, South Dakota State College.

Geological Samples and Their Selenium Content

Sample No.	Period	Formation and Description	Se Content p.p.m.
1	Tertiary-Miocene	Volcanic Ash-Pure	0.00
2	Tertiary-Miocene	Arikaree, sandstone	0.00
3	Tertiary-Oligocene	White River	0.00
4	Tertiary-Oligocene	White River-Volcanic Ash	0.00
5	Tertiary-Oligocene	White River, shale	0.00
6	Tertiary	Chadron, coarse sandstone and fine conglomerate	0.00
7	Tertiary	Basal tertiary, clay and shale	1.30
8	Tertiary	White River clay	0.00
9	Tertiary?-Cretaceous?	Fort Union, plant impressions in silicious matrix	0.00
10	Tertiary?-Cretaceous?	Fort Union, coal, lignite black to brown, woody	.50
11	Tertiary?-Cretaceous?	Cannonball, limestone	.50
12	Tertiary?-Cretaceous?	Cannonball, sandstone	0.00
13	Tertiary?-Cretaceous?	Ludlow, ironstone concretion considerable pyrite	0.00
14	Tertiary?-Cretaceous?	Ludlow, lignitic shale	.75
15	Tertiary?-Cretaceous?	Ludlow, iron cemented sand	0.00
16	Tertiary?-Cretaceous?	Ludlow, coal	.50
17	Tertiary?-Cretaceous?	Ludlow, fossiliferous shale	0.00
18	Tertiary?-Cretaceous?	Ludlow, pyrite and coal	0.00
19	Tertiary?-Cretaceous?	Hell creek, lignitic shale	1.00
20	Tertiary?-Cretaceous?	Hell creek, shale	0.00
21	Tertiary?-Cretaceous?	Hell creek, reptile bones	0.00
22	Tertiary?-Cretaceous?	Hell creek, lignite coal, woody	0.00
23	Cretaceous	Fox Hills, fossiliferous sandstone	0.00
24	Cretaceous	Fox Hills, lime concretion, shell fossils	1.50
25	Cretaceous	Fox Hills, coal	1.30
26	Cretaceous	Fox Hills, sandstone, iron stained	0.00
27	Cretaceous	Fox Hills, sand	0.00
28	Cretaceous	Fox Hills, near base transition stone	0.00
29	Cretaceous	Pierre, clay ironstone concretion	0.00
30	Cretaceous	Pierre, shale	1.50
31	Cretaceous	Pierre, clay, ironstone, limonitic	2.20
32	Cretaceous	Pierre, iron concretions	4.00
33	Cretaceous	Pierre, manganese zone	3.00
34	Cretaceous	Pierre, manganese zone	trace
35	Cretaceous	Pierre, manganese zone	5.00
36	Cretaceous	Pierre, shale upper Wheeler formation	.50
37	Cretaceous	Pierre, blue gray shale	3.00
38	Cretaceous	Pierre, iron stained clay, Baculite	1.50
39	Cretaceous	Pierre, shale, verendry zone	.50
40	Cretaceous	Pierre, bentonite, upper chalk zone	24.00
41	Cretaceous	Pierre, shale, fish scale zone	40.00
42	Cretaceous	Pierre, shale, zone below Mn zone and above fish scales	1.30
43	Cretaceous	Pierre, shale manganese zone	2.00
44	Cretaceous	Pierre, shale	29.00
45	Cretaceous	Pierre, shale	.50

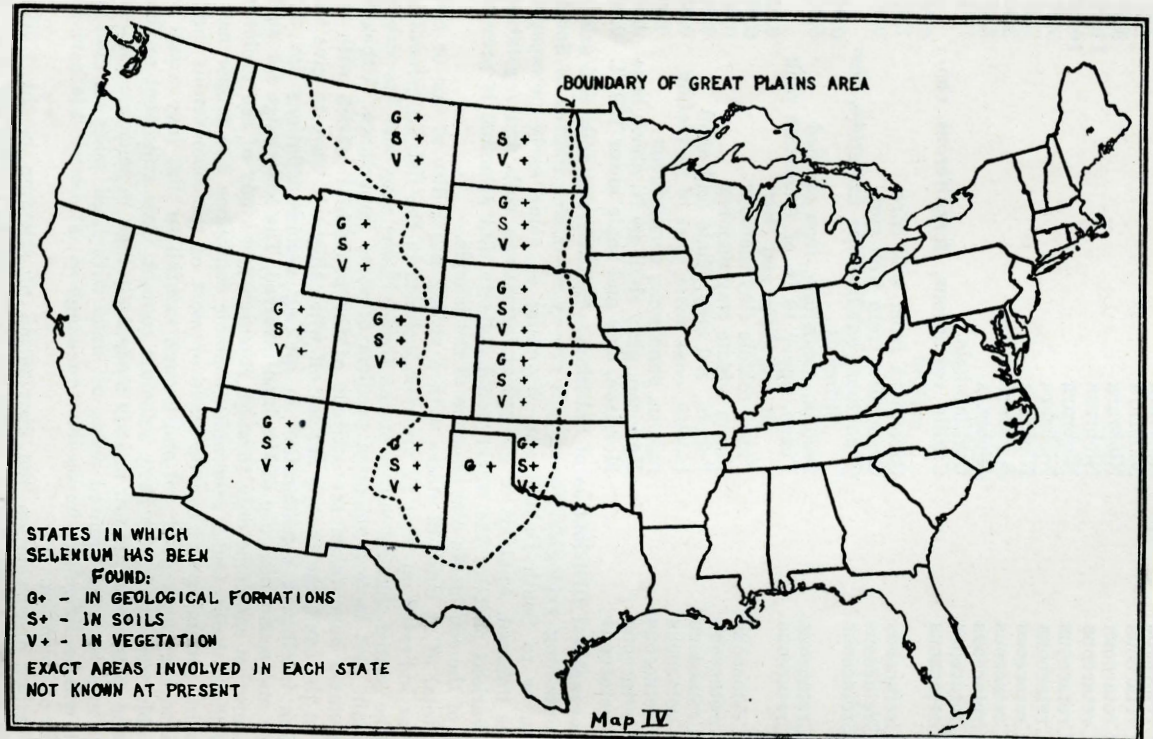
46	Cretaceous	Pierre, shale	.50
47	Cretaceous	Niobrara	3.00
48	Cretaceous	Niobrara	7.00
49	Cretaceous	Niobrara	9.00
50	Cretaceous	Niobrara	11.00
51	Cretaceous	Niobrara	16.00
52	Cretaceous	Niobrara	5.00
53	Cretaceous	Niobrara	3.00
54	Cretaceous	Niobrara	2.00
55	Cretaceous	Niobrara	2.50
56	Cretaceous	Carlile shale	1.00
57	Cretaceous	Carlile calcareous, fossiliferous concretion	0.00
58	Cretaceous	Greenhorn from well core	1.00
59	Cretaceous	Greenhorn from well core	0.00
60	Cretaceous	Granerous, agrilloceous limestone, fossiliferous	0.00
61	Cretaceous	Dakota, sandstone, iron stained	0.00
62	Cretaceous	Fuson, upper 10 ft. of formation pink to red shale and clay	0.00
63	Cretaceous	Fuson middle clay member	0.00
64	Cretaceous	Fuson middle clay member	0.00
65	Cretaceous	Lakota, red clay, shale and sand	0.00
66	Cretaceous	Lakota, coal lower part of formation	0.00
67	Cretaceous	Lakota, sandstone, ferruginous	0.00
68	Cretaceous	Morrison, gray shale 60 ft. above base	0.00
69	Cretaceous	Morrison, clay and shale lower 120 ft.	0.00

Geographical Distribution of Selenium.—Seleniferous soils and selenium containing geological formations are by no means limited to South Dakota or to South Dakota and Wyoming as some early newspaper articles inferred. Analysis of geological formations, soils and vegetation from various parts of the world would indicate that selenium is present on all of the continents and on some of the islands.

A total of at least 12 states with a strong possibility of one or two more as indicated on Map IV are now included in the list. In general it could be stated that all states of the Great Plains area and the Rocky Mountain Belt have a selenium problem of some importance. Although some states have taken the attitude of the proverbial ostrich with its head in the sand, the time is not far off when they will have to give the problem intelligent consideration in making recommendations for the proper and economical use of the land involved. The knowledge for such consideration can come only through research and study of the problem.

Without doubt, under favorable economic conditions and proper management, profitable use can be made of most of the seleniferous lands provided they have good soil and enough rainfall so that they would be desirable were it not for their selenium content. This statement applies to those soils which are low or only moderately high in selenium content. There are, however, limited areas of lands with high selenium content which will be difficult or impossible to manage so as to avoid the harmful effects of selenium.

Further discussion on the distribution of selenium in the United States will not be given here. Those readers desiring further information regarding the areas involved should consult the United States Department of Agriculture bulletins by Byers (2, 60) and the Wyoming Experiment Station bulletins by Beath and associates (4, 6).



REFERENCES: U.S.D.A. TECH. BULLETINS #482 and #530; WYO. AGRI. EXPT. STA. BULLETINS #200 and #206; THE FUTURE OF THE GREAT PLAINS, THE REPORT OF THE GREAT PLAINS COMMITTEE

The geographical distribution of selenium in South Dakota as shown by Maps I and II is in general limited to the part of the state west of the Missouri river. Furthermore, the problem is more serious as a whole in the southern half of the west river country. It will also be noted on Maps I and II that little trouble has been reported from the areas covered by the White River formations which are relatively selenium-free. In general these formations cover Todd and Bennett, and parts of Shannon, Washington, Pennington, Jackson, Washabaugh, Mellette, Tripp and Gregory counties. As would be suspected from the selenium content of the formations given in the Table on pages 72, 73, most of the cases of "alkali disease" shown on Map II are on soils derived from Pierre and Niobrara formations. It was early observed (13, 14) that the "alkali disease" was caused by vegetation grown on soils derived from Pierre shales.

The Selenium Content of Soils and Absorption of Selenium by Plants

During the past four years the selenium content of a large number of soil and vegetation samples from various parts of the state has been determined in this laboratory. The Wyoming Experiment Station has likewise made an intensive study of the selenium content of soils and vegetation in that state and has recently published an excellent article (62) on this phase of the selenium problem. The Bureau of Chemistry and Soils of the United States Department of Agriculture has, under the direction of Dr. H. G. Byers, studied the selenium content of materials from the entire Great Plains area, including intensive studies in certain parts of South Dakota and some of the other states.

From the available data resulting from the above investigations and other specific investigations certain conclusions have been arrived at regarding the selenium content of the soils and its absorption by plants.

The relation of the selenium content of the soil to the selenium content of the parent material is in most cases directly proportional, and since the selenium content of the geological formations is in general not uniform one cannot expect the selenium content of the soils to be uniform. For example, soils derived from Pierre shales are in general all seleniferous. However, certain areas produce vegetation which is highly toxic while other areas produce vegetation which is relatively non-toxic. It has been our observation, however, that land of uniform topography, especially rather large areas of level land, will have a tendency to be rather uniform in selenium content. This can be explained by the fact that the soil was probably all formed from the same stratum of parent material.

We have very little data to show that any certain stratum of a geological formation will be of uniform selenium content but we do have ample data to show that different strata of the same formation will show enormous variations in selenium content. This variation is known to occur in the Pierre formations and studies are underway at the present time to determine these variations on uncontaminated samples from a well core.

On soils of rolling topography derived from the Pierre shale which have probably, in most cases, been derived from two or more strata of the Pierre, a great variation in selenium content is usually found.

As a general statement it can be said that soils will contain selenium if formed from geological formations containing selenium and that the vegetation grown upon such soils will contain selenium. However, it cannot be said that the vegetation grown upon such soils will contain enough selenium to be toxic.

This vegetation may contain only traces of selenium and, therefore, it cannot be assumed that all seleniferous soils will produce toxic vegetation. Furthermore, it must be remembered that just because a soil is underlain by a seleniferous shale is only an indication that the soil will produce toxic vegetation. The only accurate way of determining whether or not an area is toxic is to analyze the vegetation.

The amount of selenium taken up by plants depends upon several factors, such as:

1. The total amount of selenium in the soil.
2. The distribution of selenium in the soil horizons.
3. The chemical form of the selenium in the soil.
4. The chemical compositions of the soil aside from the selenium.
5. The kind of plants.
6. The plant population (the other kinds of plants present).
7. The stage of growth of plants when examined.
8. The part of the plant analyzed (seeds, leaves, stems, roots, etc.)

These factors will be discussed briefly in the following paragraphs.

In the examination of the soil in a given area the first factor mentioned above can be used only as a sort of guide. If the soil is selenium free it will of course produce crops which are selenium free. The sub soil must of course be selenium free also because of the ability of certain deep rooted plants to bring selenium up from the lower horizons. There is also a general relationship between the total selenium content of the soil and of the vegetation especially of the highly seleniferous plants, but there are also other factors which are undoubtedly of more importance than the total selenium content of the soil, especially where crop plants are concerned. Nevertheless, soils containing more than .5 p.p.m. of selenium it should be suspected of being producers of toxic crops.

The distribution of selenium in the soil profile is of importance and must be considered in an inspection of a toxic area. The selenium may be uniformly distributed in the soil profile in which case the analysis of a surface sample would have been sufficient, or the selenium content may increase or decrease with depth in the profile. In the case of an increase in the selenium content with an increase in depth of the profile the analysis of the surface soil would be misleading because many plants absorb selenium from the subsoil as well as from the surface soil.

It has been observed that if in two areas of about equal selenium content in the surface soil the selenium content increases with depth of the profile in one and decreases with depth of the profile in the other the former area will have a reputation of being much more toxic than the latter.

The chemical form of the selenium in the soil is probably the most important factor in the absorption of selenium by a definite crop or kind

of plant. At the present time there are indications that selenium exists in the soil in two general types of compounds, organic and inorganic. The organic form includes most of the selenium which is readily available to crop plants. The inorganic group includes iron selenide, basic ferric selenite and elemental selenium as relatively insoluble forms and calcium selenate as a more soluble form. Selenium in an organic form or as calcium selenate is more readily available to plants because of the greater solubility than selenium in the elemental form or in the form of one of the iron compounds. Iron selenide and basic iron selenite are both practically insoluble in neutral or alkaline solutions such as would be present in the soils of the Great Plains area and the selenium of these compounds is practically non-available to most crop plants of importance but it apparently is available in these forms to certain "converter" plants which contaminate the soil about them with selenium in an organic form readily available to crop plants.

Beath, Eppson and Gilbert (62) have measured the availability of elemental selenium to crop plants (wheat) and to "converter" plants **(Astragalus bisulcatus and A. pectinatus.)** To a selenium-free soil they added finely powdered elemental selenium at a rate of 25 parts per million, and an excess of selenium-free partially decomposed horse manure. The entire mass was kept moist for several months and then wheat was sown. When the wheat was about 8 inches high it was cut and analyzed for selenium. It contained 6.3 p.p.m. of selenium. To another lot of the same soil 25 p.p.m. of elemental selenium was added. The above **Astragali** were planted and in three months a composite sample contained 1,150 p.p.m. of selenium.

The above experiment demonstrated the ability of the "converter" plants to take the insoluble selenium from the soil. Upon decaying these **Astragali** plants will leave the selenium in a form which is readily available to wheat and other crop plants. Beath (62) has demonstrated this by growing wheat plants on seleniferous Morrison shale, and on seleniferous Morrison shale to which had been added some finely ground "converter" plants such as those produced in the preceding experiment. Young wheat plants grown on the shale alone showed a selenium content of 2 p.p.m. and young wheat plants grown on the shale plus the ground "converter" plants contained 227 p.p.m. of selenium.

The absorption of selenium by young corn plants grown on seleniferous Pierre clay soils and Pierre shale has been investigated in this department. Top soil from a seleniferous field and Pierre shale from a nearby outcropping were used. Corn was planted on the samples of the above in pots. It was kept well watered and was cut, dried and analyzed when about 3 weeks old. The results were as follows:

Analyses were made on air dry materials.

Pierre clay soil containing 1.5 p.p.m. of selenium—corn 14.7 p.p.m. selenium.

Pierre shale (zone not determined) 29 p.p.m. of selenium—corn 28.0 p.p.m. selenium.

Pierre clay soil (from experimental plot at Dixon, S. D.) 4 p.p.m. of selenium—corn 20 p.p.m. selenium.

Shales of the same selenium content as the above soils usually produce corn and wheat with little more than a trace of selenium. The distribution of selenium between the organic and inorganic fraction of

the Pierre clay from the experimental plot has been determined. The determinations are being made on the other two samples but have not been completed at this time. The separation of the organic fraction of the soil from the inorganic fraction was roughly as follows: (63)

Two hundred grams of the soil was leached with dilute (3 per cent) hydrochloric acid until all calcium was removed. The residue was then extracted with four 800 cc. portions of 4 per cent ammonium hydroxide solution. The results were as follows:

Fraction	Per cent of Original Soil	p.p.m. Se	Per cent of Total Se
Solids from acid extract	28.2	0.6	3.4
Solids from ammonia extract (humus)	10.7	19.4	42.1
Residue after extractions	60.0	4.5	54.5

It is interesting to note that the organic fraction (fraction extracted by ammonia hydroxide) contained 40% of the total selenium of the soil even though it made up only a little more than 10% of the original sample of soil. Any selenium which might be in the soil in the form of calcium selenate or as sodium or potassium selenite would be removed by the first (acid) extraction. This first extraction might also remove any readily soluble organic selenium compounds. Most of the organic selenium would, however, be expected to remain in the humus fraction of the soil. The selenium remaining in the residue after the soil was extracted with acid and ammonia is probably in the form of some insoluble iron compound and possibly a small amount might be present as elemental selenium.

The chemical composition of the soil as a whole would be expected to play an important part in the absorption of selenium by plants. Byers (60) has reported that the results of analyses of plants and soils indicate that on soils of equal selenium content the higher the sulfur-selenium ratio the lower the selenium content of the plants will be. Field experiments in which sulfur was added to the soil for two successive years has failed to show any significant decrease in the selenium content of wheat or corn with an increase in sulfur-selenium ratio of the soils (59).

Different kinds of plants show great difference in their abilities to take up selenium from a soil. The so-called "converter" or "indicator" plants as first reported by the Wyoming Experiment Station (64) have the ability to take up extremely large quantities of selenium from soil or raw shale from which ordinary crop plants would take up only traces of selenium. These "converter" plants are the plants which, in general, make up the type of vegetation which causes "blind staggers" in livestock.

The greatest number of the plants which have the unique ability to convert selenium which is not available to crop plants to a form which is available to crop plants belong to the **Astragali, Stanleya, Oonopis, Atriplex, Mentzelia, Xylorhiza** and Aster genera. There are of course wide variations in the ability of the different species in these genera to absorb selenium from a given source. Beath et al. (4, 62) has indicated that the following species are of importance in Wyoming.

Scientific Name	Common Name
1. Astragalus bisulcatus	Two grooved milk vetch
2. Astragalus pectinatus	Narrow leaved milk vetch
3. Astragalus recemosus	
4. Aster commutatis	
5: Stanleya bipinnati	
6. Oonopsis condensata	
7. Atriplex Nuttallii	Nuttall's salt bush
8. Atriplex canescens	
9. Mentzelia decapatela	
10. Xylorhiza Parryi	Woody aster

Some of these species occur in South Dakota, especially in the southwestern counties and have been listed by Over (65) in his check list of the flora of South Dakota. Over also mentions other species of the genera listed above as of importance in the state. Many samples of certain species of plants from the selenium areas of South Dakota have been analyzed for selenium and found to be highly seleniferous. The Aster **multiflorus**, white aster, is quite common in the seleniferous areas and has been found to contain high concentrations of selenium in many cases. The gumweed **(Grindelia squarrosa)** has been found to contain as high as 800 p.p.m. of selenium. A large part of the area involved in the selenium problem is or should be used for grazing rather than for crop raising. Therefore, the selenium content of the grasses and other vegetation of importance for grazing is really of more significance than the selenium content of crop plants in the Great Plains area and the Rocky Mountain Belt. The relationship of these highly seleniferous plants to the selenium problem and livestock losses in this state has not been determined, but plans are being made for an early study of these phases of the selenium problem.

Many hundreds of samples of cereals and forage crops have been analyzed for selenium in this laboratory in connection with the "alkali disease" project. The toxicity of many samples of wheat, barley and corn has also been determined by a bio-assay method and the relation of the selenium content to the toxicity as shown by the bio-assay methods (rat feeding) has been correlated (37).

The selenium content of the most toxic samples of corn, wheat and barley which we have investigated has been in the range of from 25 to 30. p.p.m. Significant differences have been shown in the ability of different grasses to take up selenium. For example, western wheat grass has in most every case contained a higher concentration of selenium than Buffalo grass growing beside it. There is great need for controlled experimental work to determine the selenium absorbing abilities of different grass plants so that recommendations can be made regarding the kind of grass to plant in seleniferous areas where reseeding is being done.

Does Alfalfa Absorb Selenium?—Alfalfa is a crop of considerable importance in the seleniferous area. There has been some disagreement as to its ability to absorb selenium. Samples of alfalfa from various parts of South Dakota have been analyzed in the laboratory and all contained low to moderate amounts of selenium. None of the samples contained more than 10 p.p.m. of selenium. Beath, Eppson and

Gilbert (6) have stated that a large number of samples of alfalfa grown on selenium bearing raw shales were analyzed and the results showed from zero to only a few parts per million of selenium. On the other hand Byers (60) found 200 p.p.m. of selenium in a sample of alfalfa growing on an irrigated field in Dawes County, Nebraska. He also reports samples of alfalfa growing on unirrigated fields containing 25 p.p.m. of selenium. Available data would indicate that except for the instance reported by Byers, alfalfa does not take up more selenium than most of the grasses.

The plant population or plant associations are of importance since it has been shown that grasses and other plants which are normally low in selenium will usually be of high selenium content when grown in close proximity to a "converter" plant. Thus, when taking vegetation samples for selenium analysis it is very important to note the species of plants growing nearby.

The stage of growth of plants at the time they are sampled for analysis is a factor which must be considered. Beath, Eppson and Gilbert (62) have found great variation in the selenium content of seleniferous plants at various stages of growth. He has also shown that there is a wide variation in the selenium content of various parts (roots, stems, leaves and fruits) of the same plant.

Failure of Sulphur to Prevent the Absorption of Selenium by Plants Under Field Conditions

The work of Hurd-Karrer (44, 45, 66) demonstrates that the addition of sulfur to cultures and soils would inhibit the absorption of added selenium, in the form of sodium selenate, by plants. The results of her work offered some hope to the solution of the problem and the application of sulfur to toxic soils was advocated as a means of preventing the production of toxic vegetation. For several reasons the results of her work are not applicable under conditions which exist in the toxic areas.

In the first place the soils of the seleniferous areas are in general already saturated with sulfur in the form of gypsum. Therefore, it seemed rather inconceivable that the application of more sulfur to such soils would be of any particular value in inhibiting the absorption of selenium by vegetation grown thereon. Sulfur was applied to experimental plots in Gregory County, South Dakota. The field in which these plots were located has produced toxic vegetation for the last 24 years.

In 1934 sulfur in the powdered form was applied to one-fifth acre plots in quantities of 300, 900, and 1500 pounds per acre and gypsum was applied to similar adjoining plots at the ratio of 800 and 1500 pounds per acre. Corn was planted on the plots and in spite of the adverse weather condition sufficient corn was harvested for analyses and experimental feeding work.

In the spring of 1935 the sulfur and gypsum applications were repeated on the same plots and wheat was planted. Again, in spite of the weather conditions, sufficient quantities of wheat were produced for experimental work. The results of these two years of sulfur and gypsum

applications to a seleniferous soil failed to show that sulfur would inhibit the absorption of selenium by corn or wheat (Franke and Painter, 59).

With the available facts concerning the forms of selenium in the soil and the absorption of selenium by plants the reason for the failure of sulfur to inhibit the absorption of selenium by plants in the toxic area is quite obvious.

Beath, Eppson and Gilbert (62) have recently demonstrated that the application of sulfur in various forms increases the rate of absorption of selenium from a selenium-free soil to which ground seleniferous plants have been added.

Results obtained in this department during the last year have shown that a large percentage of the selenium in the soils on the experimental plots to which sulfur was added is of an organic nature. Furthermore, it is very probable that it is this organic form of selenium which is available to crop plants.

Selenium in Irrigated Areas.—The advisability of irrigation in seleniferous areas has been discussed at meetings of various groups within the last three years. As yet we cannot say that there is no danger in irrigating such areas but we can say that the danger is apparently not as great as it might be expected to be.

An examination of the Belle Fourche Irrigation was made in 1934 (2). It was found that the drainage water from the irrigation was carrying away the selenium as it became available for plant use and assuming that good drainage would be maintained and that no highly seleniferous (converter) plants would be introduced, it was concluded that no probable danger exists at the present or would exist in the future in the Belle Fourche project.

In all projects which the Reclamation Service is approving at the present time the highly seleniferous soils are usually automatically eliminated because of their heavy, tight, poor draining character.

In areas heavily infested with seleniferous (converter) plants irrigation is liable to be dangerous because of the tendency for the flooding to distribute the available selenium from these plants over the entire area. A certain irrigation project*, in Wyoming was abandoned because of the great increase in the toxicity of the vegetation after irrigation.

Rainfall and the Absorption of Selenium by Plants.—This is a contraversial point with very little scientific evidence on either side. Some of the residents of the affected areas state that they have more trouble in dry years while others state that their losses are greater during years of most rainfall. A great majority are, however, uncertain as to whether wet or dry years are the worst. Several veterinarians of the area have expressed the opinion that livestock losses are greater during wet years than during dry years.

Byers (60) has presented data which indicates that there is a small decrease in the selenium content of vegetation with an increase in rainfall. Franke and Painter (59) on the other hand have presented data

* Verbal communication—Prof. O. A. Beath, Research chemist, U. of Wyo.

which indicates that crop plants will absorb less selenium during extremely dry years than during seasons of near normal rainfall.

In pastures and ranges heavily infested with highly seleniferous (converter) plants there is a tendency for the selenium made available by these plants to be spread over more of the soil and thus increase the quantity of seleniferous grasses and plants in wet years.

Economic Importance of the Selenium Problem

In certain sections the selenium problem has proven a serious handicap to agricultural development.

At the close of the Civil War the westward movement of population started. Settlers were led farther and farther west by a series of wet years and good harvests and were given homesteads of 160 acres. The settlement of western South Dakota started about 1890 and was very active from the period 1902 to 1908. Even though the land should have been left as grazing land they were required to put it under plow. They were encouraged to go into diversified farming in land which was best fitted for grazing. In the seleniferous areas the homesteaders who set themselves up to raise livestock immediately had large losses of their breeding stock, especially if they had brought well bred animals from farther east. In many cases they had to give up livestock raising and began to raise grain for the market and in many cases they found it necessary to either buy feed from outside the area for their work animals or use tractors. In some areas the selenium problem was directly responsible for the tractor-grain system of farming.

Further difficulty was soon encountered when the farmers were ready to sell their grains from affected areas. The local elevators bought the grains at a discount which sometimes ran as high as 50 per cent. The grains were, of course, shipped out and we have no information as to just how much of a loss they have caused to outside feeders who bought the grains. Hay buyers refused to buy hay from certain areas because of its reported "toxicity". These buyers tried to avoid handling the grains and hay from toxic areas because it had the effect of destroying confidence in their business.

Livestock raisers who have the unfortunate experience of having their stock become "alkalied" not only lose the normal gains on their stock but are often forced to sell them at a discount on the market because of their diseased condition. Hog raisers have found it practically impossible to raise hogs in "toxic" areas. Many farmers have moved into the areas with pure bred hogs and have lost most of them in the first year.

The Resettlement Administration purchased about 100,000 acres of land within the area outlined on Map II. This land was purchased under the marginal land purchase program mainly because of its selenium content and its reputation as a toxic area.

Public Health and the Selenium Problem

We have had reports of people being "alkalied" in the seleniferous areas. Since the problems of public health are outside the scope of the work of this department no detailed study of this phase of the problem was undertaken until the United States Public Health Service started their work last year.

During the spring of 1936 Dr. M. I. Smith, pharmacologist from the United States Public Health Service and Dr. K. W. Franke, as consultant for the Public Health Service, made a study of the relation of the selenium problem to the public health of certain seleniferous areas in South Dakota, Wyoming and Nebraska. The results of their survey have been reported (15). They found that from 127 specimens of urines representing subjects from 90 families in the seleniferous area 8 per cent were selenium-free and 92 per cent contained selenium in amounts ranging from 2 to 133 micrograms of selenium per 100 cc.

Summary and Conclusions

The "alkali disease" is a livestock disturbance of the Great Plains and Rocky Mountain regions. The first written report of this disease was made by an army surgeon in 1856. During the period from 1890 to 1910 the disease was reported in Kansas, Nebraska, South Dakota and Wyoming.

The disturbance was called "alkali disease" because early settlers thought it was caused by the alkali (high salt) waters which are common in the affected areas. Later ergot was suspected as being the causal agent. In 1929 investigation of the disease was started which led to cooperative work with the U. S. Department of Agriculture. The cooperative work resulted in the discovery of selenium in toxic wheat and the soil upon which it was grown.

Selenium poisoning in livestock is of two general types, chronic and acute. The chronic type predominates in South Dakota and is known as the "alkali disease." The acute type is known as "blind staggers" and is quite common in Wyoming.

The general symptoms of the "alkali disease" are:

1. Dullness and lack of vitality
2. Emaciation and rough coat
3. Atrophy of the heart (dish-rag heart)
4. Atrophy and cirrhosis of the liver
5. Anemia
6. Erosion of the long bones, especially the joints, which causes stiffness
7. Loss of the long hair from the mane and tail of horses and from the switch of cattle. Loss of hair from the body of hogs
8. Soreness and sloughing of the hoofs

Selenium is carried in milk of selenized animals to the extent that suckling young are often affected.

In poultry the disease is most apparent in chicks hatched from eggs laid by selenized hens. Many of the eggs fail to hatch because of the high incidence of monstrosities (deformities). The most common deformities are: missing or short upper beaks, missing eyes, edema of the head and neck, and wirey down. The chicks which do hatch are usually weak and have a wirey down.

These deformities can be produced by feeding inorganic selenium or toxic grains or by injecting small amounts of selenium salts into the air cell of the egg. The tolerance levels of selenium for poultry have been investigated.

The toxicity of a large number of different grains has been determined in feeding experiments with albino rats. The symptoms of selenium poisoning in rats involve atrophy and cirrhosis of the liver, hemorrhages in the mucosa of the stomach and small intestines, brashness of the leg bones and intra-muscular hemorrhages around the joints of the leg bones, edema, ascitic fluid in the abdominal cavity, anemia, roughness of the coat, and a general loss of vitality.

Diets containing a small amount of seleniferous grain will produce a slight inhibition in the rate of growth, while diets made up of larger amounts of the seleniferous grain will produce a distinct inhibition in growth, decrease in food consumption, and characteristic pathology of selenium poisoning.

Anemia can be produced experimentally in rats by feeding selenium in the form of seleniferous grains or inorganic selenium (selenate or selenite).

It has been found that ashing or charring of the "alkalied" grains destroys part of the toxicity by driving off part of the selenium. This same effect is produced to a lesser degree by dextrinizing (toasting) grains at a temperature of 155-165 degrees C. In controlled experiments it was found that ashing at a temperature of 700 degrees C. destroyed about 98 per cent of the selenium. Charring at a temperature of 265 degrees C. destroyed from 75 to 90 per cent of the selenium and dexterinizing at a temperature of 160 degrees C. destroyed about 25 per cent of the selenium.

Early in the investigation it was found that the toxic factor was located in the protein fraction of the toxic grains. The protein was separated from the bran and starch of toxic wheat. Rats which were given this protein exhibited the characteristic symptoms of the disease while the rats which were fed the bran and starch showed none of the symptoms.

It has been demonstrated that rats have the ability to recognize seleniferous feeds, and when given a choice of feeds varying in toxicity they will select the one with the lowest selenium content. This fact substantiates the testimony of many farmers and ranchers in the seleniferous areas who are of the opinion that range animals will avoid the vegetation in certain areas because of its toxicity. This observation also brings out the possibilities that seleniferous lands could be used for grazing if the animals were allowed enough free range. It seems likely, however, that slight growth depression might occur which would not be sufficient to be obvious but would make grazing unprofitable in certain toxic areas.

A statistical study of toxicity and selenium content of a large number of seleniferous diets has revealed a high degree of correlation between the toxicity and the selenium content of various grains. The relative toxicity of selenium from different sources was wheat > corn > barley > selenate > selenite. The statistical study also showed that diets containing more than 10 p.p.m. of selenium caused a restriction of growth and food consumption and diets containing selenium in amounts as low as 5 p.p.m. prevented normal growth.

A comparison of the toxicity of inorganic selenium compounds and other inorganic compounds has shown that in the forms investigated selenium is more toxic than arsenic, vanadium, tellurium, or molybdenum. It was also observed that selenium was the only one of these elements which caused anemia at the levels investigated.

Dogs fed seleniferous diets showed an inhibition of growth and a decrease in food consumption. Analysis of tissues and organs from dogs fed the seleniferous diets showed that the highest concentrations of selenium were deposited in the liver, kidneys, heart and spleen, with smaller amounts in practically every part of the body.

The geological distribution of selenium in South Dakota has been investigated. The results of this investigation and other investigations would indicate that selenium, probably of volcanic origin, was deposited along with the sedimentary formations in the shallow sea which covered the Great Plains area and the Rocky Mountain belt during most of the Mesozoic era of geologic time. All of the formations laid down during this time contained more or less selenium. In South Dakota the Pierre and Niobrara formations contain higher concentrations of selenium than the other formations of this era. Furthermore, the incidence of cases of "alkali disease" is much higher in areas where the Pierre and Niobrara formations outcrop than in areas where other formations outcrop. The soils derived from these seleniferous formations are all very heavy clay soils and are often referred to as gumbo soils.

Recent investigations carried out by State Experiment Stations and the Bureau of Chemistry and Soils, U. S. Department of Agriculture, have shown that South Dakota and Wyoming are by no means the only states having a selenium problem. The other states which are probably involved are North Dakota, Nebraska, Kansas, Oklahoma, Texas, New Mexico, Arizona, Utah, Colorado, and Montana. This widespread prevalence of selenium in the Great Plains and Rocky Mountains areas intensifies the seriousness of the selenium problem.

The selenium content of vegetation grown upon seleniferous soils depends upon several factors, some of which are: total selenium content of the soil, distribution of selenium in the different horizons of the soil, the chemical forms of selenium in the soil, the kind of vegetation taking into consideration the plant associations, stage of growth and part of the plant analyzed.

Certain species of plants have the ability of absorbing selenium from forms which are apparently non-available to crop and grass plants. These plants have been called converter plants. Upon decaying these converter plants, among which are several species of **Astragali, Aster, Stanleya,** and **Oonopsis,** leave the selenium which they have absorbed in a form which is available to ordinary crop and grass plants. In many areas of South Dakota it is quite evident that converter plants are playing an important role in the selenium problem. Areas which have a reputation of producting toxic vegetation are at the present time infested with converter plants.

Greenhouse investigations on the selenium problem indicated that the application of sulfur to seleniferous soils might prevent the absorp-

tion of selenium by the plants grown thereon. Sulfur in the form of the element and in the form of gypsum was applied to plots in a field which had produced toxic vegetation for the last 24 years and the results indicated that sulfur would not inhibit the absorption of selenium by plants under field conditions.

Experimental evidence would indicate that plants absorb more selenium during wet years than they do during dry years.

The selenium problem has been of considerable economic importance in the development of certain sections of the state. In certain areas the toxicity of certain crops and grasses has played an important part in the trend toward the tractor-grain system of farming. Many livestock raisers have experienced enormous losses because of the "alkali disease."

Investigations are under way to determine the influence of the selenium upon the health of the people residing in the seleniferous areas.

The analytical method used for the determination of selenium in various materials is given in detail.

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