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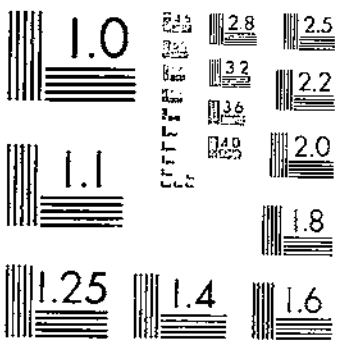

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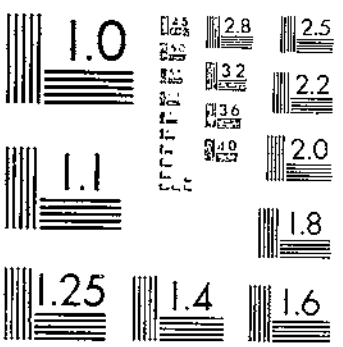

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TOXICITY OF FOOD CONTAINING SELENIUM AS SHOWN BY ITS EFFECT ON THE RAT

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INTRODUCTION

The fact that plants grown in certain areas of the northern Great Plains produce definitely toxic symptoms when eaten by man or animals has been known for some time. The ailment has been called "alkali disease" (*13*)[1], a broad term commonly used in the West for several disorders. As a result of studies undertaken at the South Dakota Agricultural Experiment Station by Franke and others, this problem was brought to the attention of the United States Department of Agriculture, and a cooperative study was subsequently undertaken by the Bureaus of Chemistry and Soils, Animal Industry, Plant Industry, and Home Economics, for the purpose of determining if possible the causative agent.

Franke and associates (*6, 7, 8, 9, 10, 11, 12, 14, 15, 23*) have already published the results of studies in which selenium is definitely indicted as the substance responsible for the conditions observed. Several other reports from the United States Department of Agriculture (Byers (*1*), Byers and Knight (*2*), Horn, Nelson, and Jones

[1] Italic numbers in parentheses refer to Literature Cited, p. 24.

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(*16*) Knight (*20*), and Hurd-Karrer (*17*, *18*, *19*)) deal with the occurrence of selenium in soils and its effect on plants.

The findings described here are the results of the studies made with rats in the nutrition laboratory of the Bureau of Home Economics. These cover the preliminary observations made early in 1931 in which evidence was first obtained that selenium was the factor involved, and also long-time feeding tests designed to show how much of the seleniferous food may be included in the diet of the rat without some evidence of injury. A few studies were also made to determine the amount of selenium stored in the different parts of the body, the rate of storage, and the rate of elimination.

PRELIMINARY STUDIES

MATERIAL AND METHODS

Before definite progress could be made it was necessary to become familiar with the symptoms that develop in the rat when the toxic plant material is included in the diet. K. W. Franke of the South Dakota Agricultural Experiment Station supplied large samples of wheat grown in a district known to produce toxic grain. This grain ground fine was incorporated in the following diet designed to supply all of the nutrients needed for growth and reproduction: Casein, 10 percent; wheat, 65 percent; yeast, 10 percent; butter, 8 percent; cod-liver oil, 2 percent; Osborne-Mendel salt mixture, 4 percent; and salt, 1 percent. Young rats taken at 28 days of age were maintained on this diet until they developed definite pathological symptoms. In each case there was a litter-mate control of the same sex on a diet of the same composition made up with ground wheat from the supply kept for the laboratory stock colony.

TOXIC SYMPTOMS SHOWN BY RATS

During the first 2 weeks the animals on the toxic grain invariably showed a characteristic sharp loss in weight due undoubtedly to the lowered food intake, since most animals exhibit a decided distaste for the toxic food. When hunger eventually compelled the rats to eat the diet they gradually gained in weight, although growth was considerably below normal. At this level of feeding of the particular sample of wheat used (65 percent), definite pathological symptoms appeared after 2 to 3 months. The most pronounced symptom was a generalized edema, and in many animals the abdomen became greatly distended (fig. 1, *A*). Autopsy of such animals revealed a large amount of fluid, sometimes as much as 150 cc, in the abdominal cavity, and in some cases death seemed to be the result of suffocation from the pressure of this excess fluid against the diaphragm. Generally the fluid was free from blood although in several rats there were definite indications of hemorrhage. The livers of these animals usually presented a nodular atrophic appearance (fig. 1, *B*), and very often were shrunk to one-half or one-third the normal size. The lobes were abnormal in size and very much distorted; in some instances several were grown together in a single hard mass and attached to the nearby tissues.

IDENTIFICATION OF SELENIUM AS THE TOXIC AGENT

As soon as the character of the toxic symptoms was established, definite efforts were made to identify the substance that was responsible. The soil in the district from which the toxic plant material came was largely of shale origin, and it was suggested that some of the rarer elements, such as lithium and vanadium, might be involved. Accordingly, salts of these elements, as well as sodium fluoride, were tested by incorporating each one separately in an adequate diet, which was then fed to young rats. Although the amounts used were definitely toxic, as evidenced by the appearance of pathological symptoms in the rats, the effects produced were so dissimilar to those produced by the toxic wheat that these elements were promptly eliminated as suspects.

PLATE 1.—*A*, Rat showing the distended abdomen typical of animals maintained on sublethal quantities of grain containing selenium; *B*, liver of rat shown in *A*.

About this time in conference H. G. Knight, Chief of the Bureau of Chemistry and Soils, called attention to the known toxicity of thallium and selenium, with the suggestion that the latter might very possibly be the toxic agent sought. Acting on this suggestion, tests were made in which selenious acid and sodium selenate were added in minute amounts to the diet of rats. On a diet containing 0.003 percent of selenious acid, the animals developed the characteristic edema and nodular appearance of the liver shown by the rats on 65 percent of toxic wheat, indicating that the symptoms of selenium poisoning were similar to, if not actually identical with, those produced by toxic grain.

While these studies were under way, W. O. Robinson, of the Bureau of Chemistry and Soils, analyzed samples of grain and reported the presence of relatively large amounts of selenium in the wheat. This fact further substantiated the conclusion that the selenium in the food was responsible for its toxicity.

Final confirmation of this assumption was given by feeding tests with specially grown wheat supplied by the Bureau of Plant In-

dustry. The first lot of wheat grown on natural soil to which a selenium salt had been added was tested on rats and reported by Nelson, Hurd-Karrer, and Robinson (*21*). The second lot of grain was grown in nutrient solution in washed quartz sand to which small amounts of sodium selenate had been added. About 300 g of this wheat were available for testing. "Control" grain grown on washed sand without the addition of the selenium salts was also available. Four diets containing skim-milk powder, 15 percent; wheat, 73 percent; yeast, 5 percent; butter, 5 percent; and cod-liver oil, 2 percent, were made up (1) with control wheat grown on sand without selenium; (2) with wheat grown on sand to which sodium selenate was added; (3) with the naturally toxic wheat supplied by Franke; and (4) with normal wheat to which selenious acid was added so that the diet contained 0.003 percent of selenium. Four litter-mate rats were maintained, one on each of these diets, for 5 weeks, when the supply of specially grown wheat became exhausted. All four rats were then killed and autopsied.

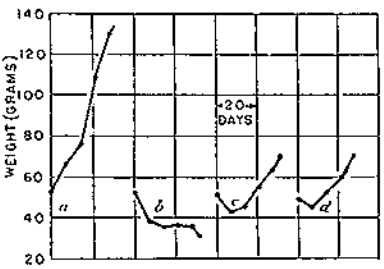

FIGURE 2.—Growth curves of four litter-mate rats fed (*a*) control wheat grown in nutrient solution only, (*b*) artificially selenized wheat grown in nutrient solution to which sodium selenate was added, (*c*) naturally "toxic" wheat containing selenium, and (*d*) a diet containing wheat to which selenious acid was added.

Growth curves are shown in figure 2. The animal given the control wheat grew normally and presented no pathological changes at autopsy, while the one fed wheat grown on sand to which the selenium salt had been added showed the liver and other changes typical of selenium poisoning. The other two rats grew at a subnormal rate but otherwise showed no toxic symptoms. The amount of selenium present in these two diets was probably too small to cause development of lesions within a period of 5 weeks, although it did affect growth. The results with these four rats, however, offered incontrovertible evidence that the selenium in the grain was responsible for its toxicity.

FOOD INTAKE IN RELATION TO DEVELOPMENT OF TOXIC SYMPTOMS

At this point the question was raised as to whether the lowered food intake of the rats on the toxic grain had anything to do with the development of symptoms, i. e., whether some of the symptoms were merely starvation effects. To answer this, feeding tests were conducted in which one animal of a pair was given a diet containing 70 percent of the toxic grain and the other 70 percent of the nontoxic laboratory stock diet grain. The food allowance of the rat on the nontoxic grain was restricted in amount to the quantity eaten by its litter mate on the toxic-grain diet, so that both rats of a pair consumed the same quantity of food. Five pairs were fed in this way.

After various lengths of time, all of the rats on the toxic grain died. As this happened, the litter-mate control on the nontoxic grain was killed, and both rats were autopsied at the same time. All five rats on the toxic grain showed the typical symptoms asso-

ciated with selenium poisoning, while the litter mates on the nontoxic grain were normal in every way. Growth curves and postmortem photographs of a typical pair are given in figures 3 and 4.

Particular attention should be given to the appearance of the liver, testes, and fat deposits. The liver of the rat (16687) on the toxic grain was hard, nodular, and shrunken to one-half its normal size. The testes were small and the spleen, not visible in the photograph, was about one-half as large as that of the rat (16688) on the nontoxic grain. Although these two rats ate the same quantity of food, the one given the wheat containing selenium showed little, if any,

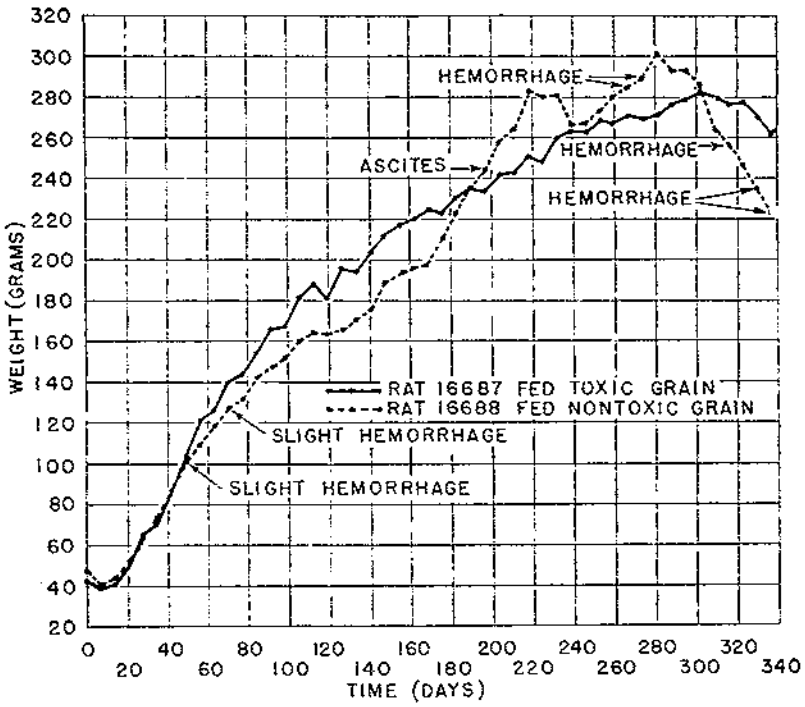

FIGURE 3.—Growth curves of two litter-mate rats fed identical quantities of diets of the same composition except that one was made up with wheat containing selenium and the other with normal wheat free from selenium.

fat deposits, while the one on nontoxic grain, despite the fact that its food intake was restricted, had a good supply of subcutaneous fat. The rat (16687) on the toxic grain showed the distended abdomen characteristic of the animals with marked ascites. Subsequently there was a leakage of serous material from the bladder with an accompanying loss in body weight. This condition was never severe but rather persistent. The material was generally faintly tinged with blood.

These findings ruled out any possible suggestion that the symptoms shown by rats on the toxic grain were in any degree ascribable to chronic starvation.

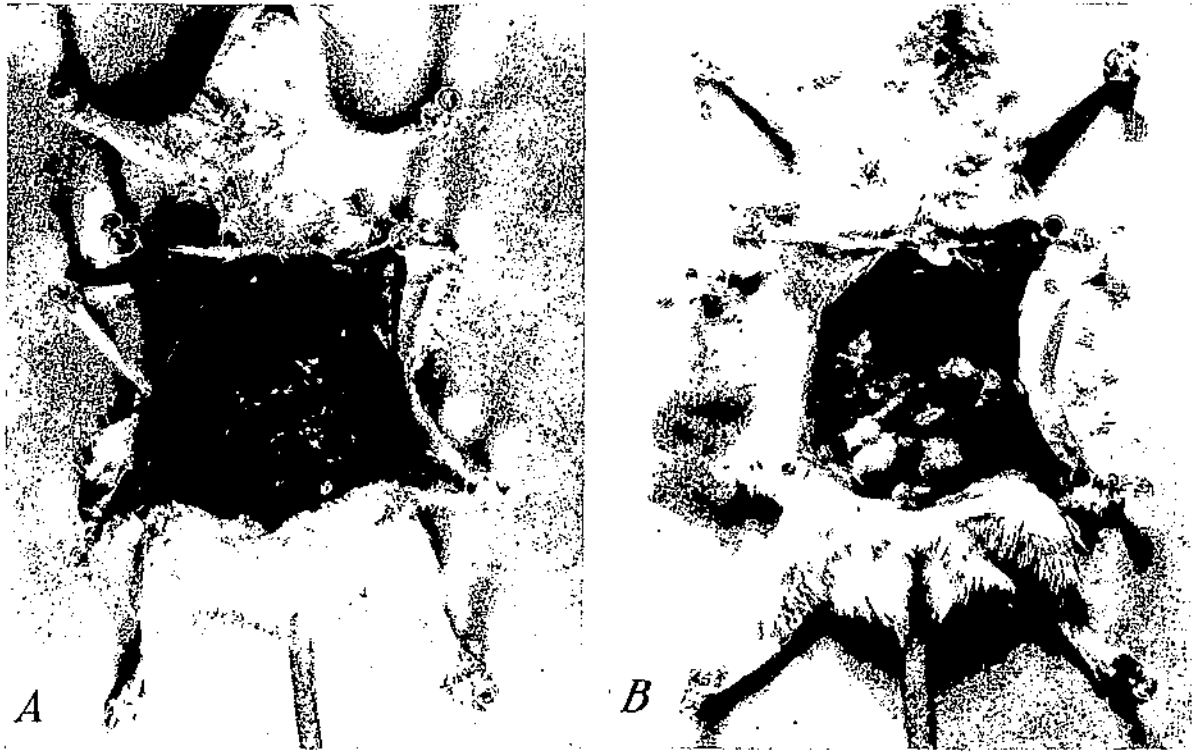

FIGURE 4.—Post-mortem photographs of the rats whose growth curves are shown in figure 3: A, Rat 16687 was given a diet made up with wheat containing selenium; B, rat 16688 had a diet of the same composition except that the wheat was free from selenium. Both rats consumed identical quantities of food.

EFFECT OF DIFFERENT QUANTITIES OF SELENIUM (SELENIOUS ACID) IN THE DIET

After it was established that selenium was responsible for the toxicity of the plant material, the next logical step was to determine how the severity of the symptoms was related to the amount of selenium in the diet. Selenious acid was used in these feeding tests. A solution of known concentration was prepared and used to make up 13 diets in which the concentration of selenium ranged from 4.5 to 6,100 p. p. m. These diets were fed to rats 28 days of age. Although a limited number of animals was placed on each diet, the results (table 1) show a definitely graded effect. All of the animals on the diets containing 24.5 or more p. p. m. of selenium died and the length of time the animals survived was roughly proportional to the amount of selenium present. Those on 600 or more p. p. m. lived only a few days and died in convulsions. The rats on 61 to 310 p. p. m. of selenium also died within a short time, but their deaths were not violent and no gross tissue changes were visible at autopsy. On diets containing 24.5 to 49 p. p. m., the rats lived 30 or more days and showed the ascites and liver changes ascribed to the poisoning effect of selenium.

TABLE 1.—*Behavior of rats fed diets containing different quantities of selenium in the form of selenious acid*

Selenious acid in the diet (percent)	Quantity of selenium in the diet	Rats fed	Survival of rats (days)	Condition of rats at termination of test
	P. p. m.	*Number*		
1	6,100	2	7, 7	Died in convulsions.
0.5	3,100	2	7, 9	Do.
0.1	600	2	7, 8	Do.
0.05	310	2	4, 9	No abnormalities.
0.01	61	2	15, 22	Do.
0.005	49	5	40, 46, 34, 37, 37	Severe ascites, liver lesions, kidneys and spleen affected.
0.003	37	7	100, 60, 38, 57, 32, 102, 42.	Severe ascites, liver lesions, kidneys enlarged, pancreas affected.
0.0015	27	5	186, 117, 114, 41, 102	Ascites, liver lesions, pancreas affected.
0.001	24.5	2	62, 108	Ascites, liver lesions.
0.003	18.4	13	127, 103	Some ascites, slight liver changes, kidneys affected.
0.0030 as Na_2SeO_4	13	2	29	Slight changes in liver.
0.0015	9.1	3		No abnormalities.
0.7 mg selenium per 100 g wheat.	4.5	7	77	Some animals had spotted livers.

[1] 1 killed at end 38th week.
[2] 1 killed at end 14th week.
[3] All killed at end 38th week.
[4] 6 killed at end 18th week.

On diets containing 13 to 18.4 p. p. m., some animals lived and some died, showing that the threshold level of the lethal dose was within these limits. Most of the animals in these groups showed the typical edema and liver atrophy seen in the rats maintained for long periods on the toxic grain. Those on the diet containing 18.4 p. p. m. most nearly resembled the rats on the diet containing 65 percent of toxic grain. Evidently the continuous ingestion of slightly sublethal doses of selenium results in a chronic subacute form of poisoning.

Animals on diets containing 9 p. p. m. or less of selenium derived from selenious acid did not show any discernible retardation of growth or development of pathological symptoms.

EFFECT OF DIFFERENT LEVELS OF WHEAT CONTAINING SELENIUM IN THE DIET

During the course of this investigation the question arose as to the amount of toxic grain or other "selenized" material that could be regularly included in the diet without eventual development of some ill effects. Several hundred pounds of the toxic wheat had been supplied by Franke. Since this was sufficient to feed a rather large colony of rats for some time, it was decided to feed the grain at several different levels to carefully matched lots of rats and compare their behavior with a control group given the same diet made up with nontoxic grain. Inasmuch as Franke's observations on chickens showed that effects of poisoning might not appear until the second generation, plans were made for continuing as many of the young rats as possible on the respective diets on which they were reared.

PROCEDURE

The basal diet used in the preliminary studies was rather costly and required considerable time to prepare. For use in the later studies a less complex diet was therefore devised in which skim-milk powder was substituted for the casein and Osborne and Mendel salt mixture, giving a diet of the following percentage composition: Skim-milk powder, 30; wheat, 58; yeast, 5; butter, 5; and cod-liver oil, 2. Past experience and knowledge of the requirements of the rat justified confidence that this diet would be adequate for growth and reproduction.

Robinson's analyses of the grain showed that it contained about 15 p. p. m. of selenium. Accordingly a diet made up with 58 percent of this grain would contain 8.7 p. p. m. of selenium. The feeding tests with selenious acid had indicated that rats could live at least 38 weeks on a diet containing 9 p. p. m. of selenium. Therefore one diet was made up in which the entire 58 percent of wheat was replaced by toxic grain. The others in the series were made up with a mixture of nontoxic wheat and toxic wheat so that they contained respectively 40, 20, 10, 5, and 2.5 percent of toxic grain, giving concentrations of selenium of 6.0, 3.0, 1.5, 0.75, and 0.38 p. p. m. As before, the nontoxic wheat was from the supply kept for the regular stock colony. One diet containing 58 percent of nontoxic grain was also included in the series. The diets containing the small percentages of toxic grain were included in the series because it had been suggested that selenium, like boron, might have a stimulating effect when ingested in small amounts, although larger amounts were toxic.

Seven lots of rats, each containing two males and six females 4 weeks of age, were then segregated, one for each of the seven diets. Every precaution was taken to make the lots as nearly comparable as possible. The young rats were drawn from two colonies, equal numbers of males and females being taken from each. Fourteen litters in all were used. Two litters of seven males, one from each of the two colonies, were distributed, one of each litter to a lot. In the case of the females three or four animals were selected from each litter, and litter mates were put on alternate levels of the toxic

grain, rather than all of one litter on the low levels and all of another on the high levels. It was felt that this would give data more satisfactory for purposes of comparison.

After the lots of animals were arranged and established on the different diets, they were cared for in the same way as the regular breeding colony, i. e., they were weighed once a week, the pregnant mothers were separated from the group several days before parturition, and the young were weaned at 28 days of age.

The seven original lots were continued 8 months on the respective diets on which they were reared. Because of the rapidly increasing size of the colony, the second-generation animals were kept only 6 months. The first litter reared by each female of the second generation was continued until it was 12 weeks old. All other young of these second-generation animals were killed at 4 weeks of age.

After the first study had been under way for some time the animals on the diet that contained 5 percent of toxic grain seemed to show a superior performance over the control group in number of young born and percentage reared. There was no explanation for this other than the suggestion already made that certain amounts of selenium in the diet might have a stimulating effect. Before accepting this explanation, however, it seemed advisable to carry out further feeding tests to see whether these results were repeated, and a second test was therefore planned.

For this purpose a new basal diet was devised in which the protein content was considerably reduced in quantity and improved in quality over that of the basal diet used in the first study, since attention had been called to the fact that this basal diet contained a relatively high percentage of protein. The percentage composition of this diet was as follows: Skim-milk powder, 15; meat scrap, 5; whole wheat, 68; yeast, 5; butter, 5; and cod-liver oil, 2. Diets containing 2.5, 5, and 10 percent of toxic grain were used as before. The three lots of rats established on these three diets each contained two males and four females. The group on the control diet contained two males and six females. The usual precautions were taken to make the lots as nearly comparable as possible.

Additional tests were also made with high levels of the toxic grain; namely, 50, 60, 65, and 70 percent. The percentage composition of the basal diet in this case was: Skim-milk powder, 13; meat scrap 5; whole wheat, 70; butter, 5; yeast, 5; and cod-liver oil, 2. The five lots of rats fed these four diets and the control diet were very well matched, since every member of each lot was paired with a litter mate of the same sex in each of the other lots.

This study was terminated at the end of 8 months, when the surviving animals were killed and autopsied. The young were killed and autopsied when weaned at 4 weeks of age.

During the course of the feeding tests all animals were observed for the appearance of symptoms characteristic of selenium poisoning, and at the termination of the experiment autopsies were performed. Nearly all of the members of the seven original lots in the first study were examined by C. D. Stein, associate veterinarian, Bureau of Animal Industry. G. T. Creech, veterinarian, of the same Bureau made

histological examinations of the tissues of several animals fed on the 58-percent level of toxic grain and one of the controls on normal grain.

RESULTS

Although the data cannot be presented in detail, enough are given to show the relative toxicity of the different diets as judged by the effect on growth, reproduction, and development of gross pathological lesions in the animals. In comparing results for the first- and second-generation animals of the first study it must be kept in mind that the animals of the first generation were continued until they were 9 months old, while those of the second generation were kept only 6 months.

GROWTH

Average weight curves for the first- and second-generation males of the first study and for those of the third generation that were continued for 12 weeks are given in figure 5. Weight curves for the females are not included, because after the tenth or eleventh week increased and variable weights due to pregnancy made them of little value for purposes of comparison. Up to that time the females showed the same relative trends as the males.

The animals in the first study on the diet containing 58 percent of toxic grain gradually lost weight after the sixteenth to eighteenth week and appeared decidedly stunted. Most of these animals were thin and emaciated, and at autopsy little subcutaneous fat was found. The animals on the 40-percent level showed slight but definite retardation of growth. This was more pronounced in the second generation than in the first.

The animals of the first and third generations on 2.5, 5.0, 10.0, and 20.0 percent of the toxic grain grew at practically the same rate as the controls on 58 percent of normal grain. The curves indicate that the second-generation animals in the 2.5-, 5.0-, and 10.0-percent groups weighed more at 4 weeks of age and grew faster than the animals in the control group. This seeming superiority, however, can be attributed only indirectly to the effect of the selenium.

As stated earlier in this report, the seven lots of the first generation were made up with animals from two different colonies. The young from one of them invariably weighed more when weaned than the young from the other. The females from this latter colony showed the effects of the seleniferous food more strongly than the females from the first colony in that more of them failed to produce young. This accounts entirely for the higher starting point of the growth curve for the second-generation animals in the 10-percent group, since it consisted entirely of young of mothers that produced the heavier offspring. The starting points for the weight curves of the 2.5- and 5-percent groups are between those of the control and 10-percent groups because they contained a preponderance of the larger rats.

Average growth curves for the males in the second study on the higher levels of toxic grain are given in figure 6. Growth curves for

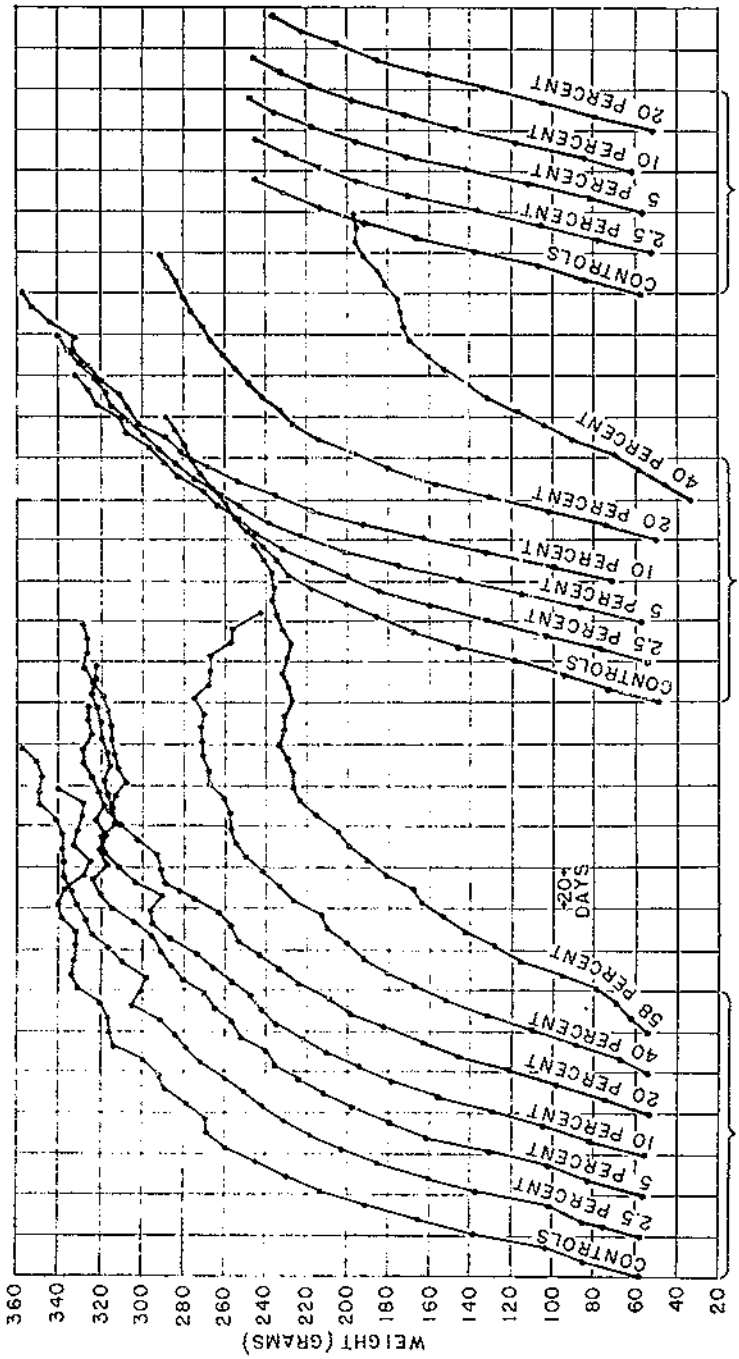

FIGURE 5.—Growth curves of first (A), second (B), and third (C) generation of male rats used in the first study and fed various percentages of wheat containing selenium incorporated in an adequate diet.

the groups of animals on the low levels of toxic grain are not given, since they paralleled closely the one for the control group on nontoxic grain.

The animals on 60, 65, and 70 percent of toxic grain showed the characteristic initial loss in weight due to decreased food intake.

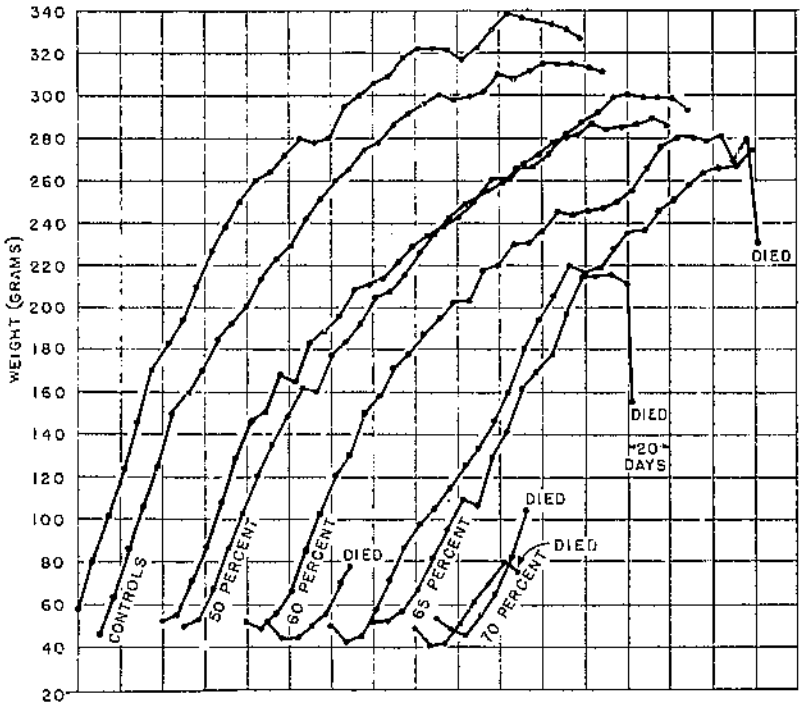

FIGURE 6.—Growth curves of rats fed diets containing 50, 60, 65, and 70 percent of wheat containing selenium, and of control rats fed 70 percent of wheat containing no selenium.

The rate of growth of all of the rats on these higher levels of toxic grain was considerably less than that of the control group.

REPRODUCTION

The breeding records of the different groups were very interesting, albeit somewhat difficult to explain. The pertinent data for the females in the first study are tabulated in table 2. No record is given for the females of the third generation since only a scattered few of these bore young before they were killed at the age of 12 weeks. The data on reproductive performance of all lots in the second study are given in table 3.

TABLE 2.—*Effect on reproduction in the first and second generations of test rats used in the first study of various levels of wheat containing selenium incorporated in an adequate diet*

Toxic wheat in diet		Generation and number of females		Females having young		Total young	Total litters	Average young per litter	Average young per bearing female	Young reared	
		First generation	Second generation								
Percent	*P. p. m.*			*Number*	*Percent*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
0	0	6	.....	6	100	70	13	5.4	11.7	30	42.9
2.5	.38	6	.....	5	83.3	72	13	5.5	14.4	23	31.9
5.0	.75	6	.....	6	100	90	18	5.0	15.0	75	83.3
10.0	1.5	6	.....	5	83.3	42	10	4.2	8.4	15	35.7
20.0	3.0	6	.....	3	50.0	38	7	5.4	12.7	19	50.0
40.0	6.0	6	.....	4	66.7	30	8	3.8	7.5	5	16.7
58.0	8.7	6	.....	2	33.3	7	2	3.5	3.5	0	0
0	0	.....	11	10	90.9	134	19	7.1	13.4	46	34.3
2.5	.38	.....	9	6	66.7	63	10	6.3	10.5	31	49.2
5.0	.75	.....	41	29	70.7	431	52	8.3	15.0	325	75.6
10.0	1.5	.....	7	7	100	100	11	9.1	14.3	72	72.0
20.0	3.0	.....	7	5	71.4	61	9	6.8	12.2	25	41.0
40.0	6.0	.....	2	2	100	17	3	5.7	8.5	0	0
58.0	8.7	.....	0	.....	.....	.....	.....	.....	.....	.....	.....

TABLE 3.—*Effect on reproduction in the test rats used in the second study of various levels of wheat containing selenium incorporated in an adequate diet*

Toxic wheat in diet		Females	Females having young		Total young	Total litters	Average young per litter	Average young per bearing female	Young reared	
Percent	*P. p. m.*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
0	0	6	6	100	120	22	5.5	20	57	47.5
2.5	.38	4	4	100	90	19	5.1	21	52	54.2
5.0	.75	4	3	75	27	6	4.5	9	13	48.1
10.0	1.50	4	4	100	74	14	5.3	18.5	46	62.2
0	0	3	3	100	81	12	6.8	27	64	79.0
50.0	7.5	3	1	33.3	1	1	1.0	1	0	0
60.0	9.0	4	0	0	0	0	.....	.....	.....	.....
65.0	9.9	3	0	0	0	0	.....	.....	.....	.....
70.0	10.5	3	0	0	0	0	.....	.....	.....	.....

In each of the seven original lots used in the first study there were six females; therefore the values for the number of females bearing young, total number of young born, total number of litters born, and percentage of young reared in the case of the different lots may be compared. All of these values were highest for the group on 5 percent of toxic grain. On the levels above this there was a gradual decrease in values paralleling the increase in toxic grain, with the exception of the values for the 20-percent group. At the 58-percent level only seven young were born and none reared.

In the second generation the different groups on the various levels of toxic grain did not contain the same number of females. Therefore only the values for average number of young per litter, average number of young per bearing female, and percentage of young reared are valid for purposes of comparison. These values for the lots on 5 and 10 percent of toxic grain, especially for percentage of young reared, were higher than the ones for the control group. In the groups on 2.5 and 20 percent of toxic grain the average number of young per litter was slightly less than in the control group, although

in each case the percentage of young reared was slightly greater than that of the control group. In the 40-percent lot there were fewer young per litter and decidely fewer young per bearing female than in the control group. This lot did not rear any young.

In contrast to the results described for the 5-percent lot in the first study, the corresponding group in the second study gave values for average number of young per litter and average number of young per bearing female that are significantly less than those for the control group. The value for the percentage of young reared was practically the same as that of the control group.

Although the 10-percent group reared a higher percentage of young than did the control group, the difference is not so great as it was in the second-generation animals of the first study. These findings do not lend support to the suggestion that small quantities of selenium in the diet might stimulate biologic processes.

One female of the 50-percent lot gave birth to one young that died. None of the other females on the higher levels produced young.

TOXIC SYMPTOMS

The results of the examination of the rats for toxic symptoms are summarized in tables 4 and 5. Data on third-generation animals of the first study are not given, since they were killed at either 4 or 12 weeks of age and, with one exception, showed no abnormalities. In grouping the animals according to severity of symptoms, "slight" has been used to indicate the condition of the rats showing merely a spotted liver. This may or may not have been an abnormal condition. "Moderate" indicates definite but not extensive liver changes, while "severe" denotes atrophy of the liver and very marked ascites.

TABLE 4.—*Degree of toxic symptoms shown by the first and second generations of rats used in the first study and fed various levels of wheat containing selenium incorporated in an adequate diet*

Toxic wheat in diet	Generation and number of rats fed		Rats dying before end of test period		Rats showing indicated type of toxic symptoms				Remarks
	First generation	Second generation	Number	Survival period (days)	No symptoms	Slight	Moderate	Severe	
Percent					*Number*	*Number*	*Number*	*Number*	
0	8		1	65	8				1 rat died from effects of parturition.
2.5	8		1	177	7		1		Do.
5.0	8		0		8				
10.0	8		0		8				
20.0	8		0		6	2			
40.0	8		3	225, 215, 226	2	3	2	1	
58.0	8		1	221		1	5	1	1 rat was not autopsied.
0		30	0		20				Do.
2.5		22	0		21	1			
5.0		75	2	117, 63	62	9			2 rats may have died of pneumonia. They showed no signs of selenium poisoning. 4 rats were not autopsied.
10.0		15	0		13	2			
20.0		19	0		18	1			
40.0		5	1	36			2	2	1 rat may have died of pneumonia. It did not show signs of selenium poisoning.
58.0		0							

TABLE 5.—*Degree of toxic symptoms shown by rats used in the second study and fed various levels of wheat containing selenium incorporated in an adequate diet*

Toxic wheat in diet	Rats fed	Rats dying before end of test period		Rats showing indicated type of toxic symptoms			
		Number	Survival period (days)	No symptoms	Slight	Moderate	Severe
Percent	*Number*			*Number*	*Number*	*Number*	*Number*
0	8	0		8			
2.5	6	0		6			
5.0	6	0		6			
10.0	6	0		6			
0	5	0		5			
50.0	5	0				1	4
60.0	6	3	39, 167, 203		0		5
65.0	5	3	63, 122, 201				5
70.0	5	5	43, 48, 68, 208, 216				4

[1] Died after 39 days. [2] 1 rat was not autopsied.

In the first study practically all of the animals on the diet containing 58 percent of toxic grain developed moderate or severe symptoms. As a matter of fact, diets containing 58 percent or more of this particular grain never failed to produce the characteristic ascites and liver changes, although the time of appearance of external evidence of the ascitic condition varied considerably with different animals. No attempt was made to determine the exact length of time required for the development of tissue changes due to selenium poisoning. Histological examination of the livers of these rats showed the presence of small hemorrhages, leucocyte and round-cell infiltration, and a general or uniform cloudiness of the liver cells, while the liver of the rat on 58 percent of nontoxic grain showed none of these abnormalities. Significant lesions were not present in any of the groups below the 40-percent level.

The appearance of pronounced injury in one animal of the first generation on the 2.5-percent level cannot be explained unless one assumes that certain animals are definitely more sensitive than others to this type of poisoning. Substantiation of this fact would mean that this susceptible group would have to be taken into consideration in determining the maximum amount of this grain that could be included in the diet of rats with safety.

In the second study one of the animals on the diet containing 60 percent of toxic grain died at the end of 38 days, one on 65 percent after 201 days, and the two on 70 percent at the end of 43 and 48 days, respectively. With the exception of one, all of the animals on the higher levels showed severe lesions, while none of those on the lower levels showed any signs of poisoning.

FOOD CONSUMPTION

The food-consumption records of five litter-mate male rats in the second study, one each on the 50-, 60-, 65-, and 70-percent toxic-grain diets, and one on 70-percent nontoxic-grain diet were analyzed in terms of grams of food eaten per rat per week and of grams eaten per 100 g of body weight per week. These values are expressed graphically in figure 7, and the number of grams of selenium in-

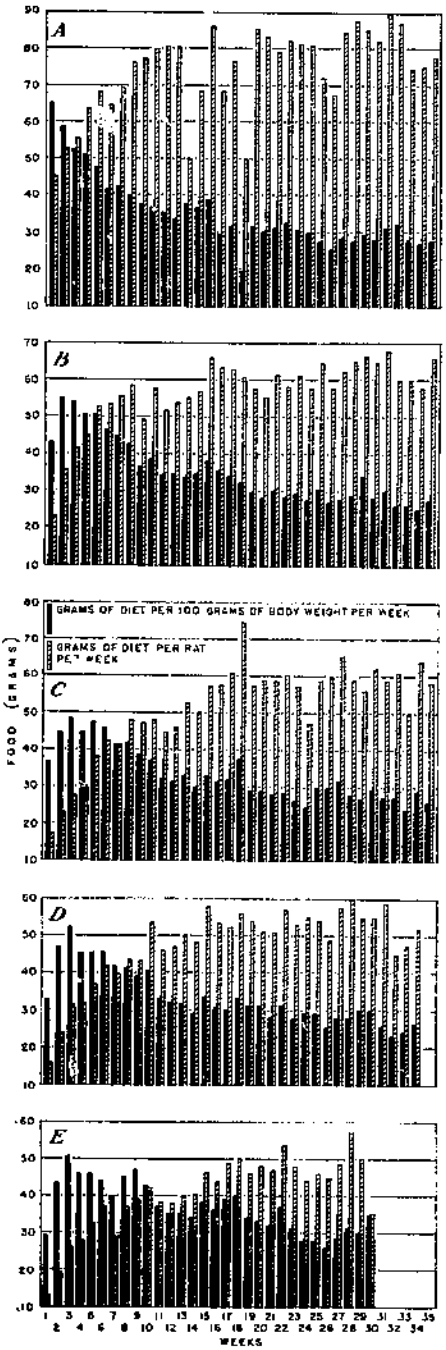

FIGURE 7.—Food intake of rats used in the second study and fed various levels of grain containing selenium incorporated in an adequate diet: *A*, Rat 16290, control on nontoxic wheat; *B*, rat 16291, 50 percent of toxic grain, or 0.000253 g of selenium per 100 g of body weight per week; *C*, rat 16292, 60 percent of toxic grain, or 0.000289 g of selenium per 100 g of body weight per week; *D*, rat 16293, 65 percent of toxic grain, or 0.000312 g of selenium per 100 g of body weight per week; *E*, rat 16294, 70 percent of toxic grain, or 0.000372 g of selenium per 100 g of body weight per week.

gested per 100 g of body weight per week is given in the legend. The total food intake of the rats on the toxic grain was considerably less than that of the control group. The number of grams eaten per 100 g of body weight, however, is about the same for all five animals, with the possible exception of a slightly higher value for the rat on the 70-percent diet. A possible explanation of this may be the fact that the records for this group were not so accurate as those for the other groups, since the rats disliked the diet and scattered it.

The rats on the diet containing 50 percent of toxic wheat ate a quantity of food that supplied 0.00025 g of selenium per 100 g of body weight per week. All of the rats in this group showed definite evidence of poisoning as judged by lower body weight, failure to reproduce, and the characteristic liver changes at autopsy, indicating that the continuous ingestion of very small quantities of selenium will cause tissue damage.

CONCLUSIONS

The results of the first study, in which wheat containing selenium was fed in graded amounts to groups of rats in some cases as long as 8 months, gave convicing evidence that food containing certain amounts of selenium in the diet of the rat may have a profound effect on growth and reproduction. On a diet containing 8.7 p. p. m. of selenium, growth was decidedly stunted. Very few young were born and none reared. The animals on the diet containing 6.0 p. p. m. grew at a subnormal rate and their ability to produce and rear young was seriously impaired. On the diet containing 3.0 p. p. m. of selenium the rats grew normally, but reproduction and ability to rear young were somewhat below normal.

Animals on diets containing 1.5 p. p. m. or less of selenium grew normally. The females of the first generation on the diet containing 0.75 p. p. m. and those of the second generation on 0.75 and 1.5 p. p. m. showed an apparent superiority over the control group in number of young born and percentage reared. However, no definite conclusions could be drawn from these results as to whether this superiority was real or only apparent.

These results compared with those obtained in the preliminary studies with selenious acid indicated that on the basis of selenium content the wheat containing selenium was relatively more toxic than selenious acid.

In the second study the reproductive performance of the group on the diet containing 0.75 p. p. m. of selenium was not superior to that of the control group. The group on the diet containing 1.5 p. p. m. raised a slightly higher percentage of young, but the difference over the value for the control group was not so great as in the case of the second-generation animals of the first study. These findings therefore did not confirm the suggestion that very small amounts of selenium in the diet might have a stimulating effect.

Analysis of the food-intake records of a limited number of animals showed that the daily ingestion of enough seleniferous wheat to supply as little as 0.00025 g of selenium per 100 g of body weight per week resulted in retarded growth, failure to reproduce, and the development of the characteristic symptoms of selenium poisoning.

STORAGE AND EXCRETION OF SELENIUM

In the foregoing studies the livers of the rats showed the most pronounced effects of selenium injury, and therefore it became of interest to know whether this was due to excessive accumulation of selenium in this organ. The results of analyses made by Robinson on a few carcasses of rats fed seleniferous wheat indicated that selenium was stored to a considerable extent in the liver and to a slight extent in the muscles and bones. At the suggestion of H. G. Byers, of the Bureau of Chemistry and Soils, balance experiments were undertaken to determine the rate of excretion of selenium in relation to intake and also the amount stored in the body. Later these investigations were extended to include studies to determine the rate of storage of selenium in the different parts of the body and also the rate of elimination when the animal was returned to a diet containing no selenium. George Bannstark of the Bureau of Chemistry and Soils cared for the animals and made the necessary selenium analyses. Since the report given here was first submitted the results of somewhat similar studies made at Johns Hopkins University have been published (*4, 5*).

PROPORTION OF INGESTED SELENIUM STORED

METHODS

Eleven rats ranging in age from 33 to 105 days were given a diet of the following percentage composition: Wheat containing selenium, 60; normal wheat, 8; meat scrap, 5; skim-milk powder, 15; yeast, 5; cod-liver oil, 2; and butter, 5. The wheat was from the lot used in the studies already reported and contained 15 p. p. m. of selenium. The diet therefore contained approximately 9 p. p. m. Previous experience (pp. 7 and 15) indicated that this was sufficient to produce toxic symptoms but not enough to cause death before the expiration of at least 6 months.

Five of the rats were housed singly, one cage contained two animals and a third, four. The cages used were 9 inches in diameter by 8 inches in height. For the small animals the flooring was made of one-fourth-inch mesh screening, but for the larger animals it was made of one-half-inch mesh. Jars containing food and water were kept in the cages, care being taken to arrange the food jars so that scattering was reduced to a minimum. The animals were weighed at the end of each week and records were kept of body weight, food intake, volume of urine, and weight of fecal matter.

The method of separating the feces from urine was similar to that described by Drinker, Thompson, and Marsh (*3*). Each cage was supported directly over a large funnel about 10 inches in diameter, the shortened stem of which rested an inch above a pear-shaped bulb in a small funnel about 2 inches in diameter. Six small projections held this bulb about one-fourth inch from the sides of the funnel which was supported in the mouth of a small graduate by a rubber stopper. The graduate was protected by a cylinder of galvanized iron 8 inches in diameter and 9 inches high. This arrangement permitted the urine to flow down the sides of the large funnel onto the bulb, where it was directed into the graduate by the

funnel. Small amounts of thymol and mineral oil were placed in the graduate to inhibit bacterial decomposition and subsequent loss of selenium by volatilization. A filter paper was placed under the graduate to absorb any urine which might be spilled. The feces falling through the large funnel to the bulb were deflected onto the filter paper at the bottom of the galvanized cylinder.

When the material was collected for analysis, the feces were removed from the filter paper first and then the inside of the cage and the sides of the funnel were washed with water which was added to the urine sample. These collections were made at intervals of 2 or 3 days. After each collection was completed, the soiled cage and other apparatus was replaced with clean material.

The samples of feces and urine could not always be analyzed immediately, and therefore were stored to minimize loss of selenium through oxidation and volatilization. The feces were kept in tightly stoppered bottles in the refrigerator. The urine was put in tightly stoppered flasks containing small quantities of nitric acid and bromine.

During the early part of the work the urine and feces samples analyzed included the amount collected during a week, but later in the study collections made during 2 weeks were used.

At the end of the experimental period, the rats were killed and their bodies prepared for analysis. The stomach and intestinal tract with contents were removed and discarded. This introduced a slight error in that no record was obtained of the selenium in these tissues and in the undigested food. With the exception of the first few animals, the body was divided into three parts for purposes of analysis: (1) Organs of the thoracic and abdominal cavities; (2) skin and hair, feet, tail, and head; and (3) the remainder of the carcass, mostly muscle and bone. In the preliminary tests an attempt was made to separate the muscles and bones, but this process proved too tedious and was therefore not continued. If the analysis could not be done on the same day the rats were killed, the carcasses were stored in the freezing compartment of a refrigerator. Usually the time between death and dissection was not more than 2 days.

The selenium analyses were made according to the method developed by Robinson and associates (*22*). The material taken for analysis was first decomposed, as far as possible, by digestion with 30-percent hydrogen peroxide and concentrated nitric acid on a steam bath. The urine was first evaporated to small volume after addition of the nitric acid, but before the hydrogen peroxide was added. When digestion was completed, bromine and 40-percent hydrobromic acid were added, and after being heated, the mixture was distilled in an all-glass still until only a small quantity of liquid remained in the distilling flask. The bromine in the distillate was then reduced by passing in a stream of sulphur dioxide. After addition of about one-fourth gram of hydroxylamine hydrochloride, the mixture was placed on a steam bath. If only a small amount of organic matter remained undestroyed and the amount of selenium present was more than a trace, the red precipitate of selenium was easily detected. This precipitate was then filtered off and dissolved in hydrobromic acid to which a small amount of bromine had been

added. A solution of gum arabic was added, and sulphur dioxide passed through, after which a small amount of hydroxylamine hydrochloride was added. The quantity of selenium present was estimated by comparing the color of the resulting suspension with one prepared in the same way with a known quantity of selenium.

RESULTS

The results obtained are summarized in table 6. The quantity of selenium ingested was calculated from the food intake. Inasmuch as there was always some scattering of food, these values may be high, although the food-intake figure was adjusted to take care of spillage whenever this could be estimated.

TABLE 6.—*Storage and excretion of selenium by rats fed a diet containing 9 p. p. m. of selenium derived from wheat*

Rat number and sex	Age when put on diet containing selenium	Length of time on diet	Weight at termination of test	Total quantity of selenium ingested	Selenium excreted			Selenium stored in the body				Autopsy findings	
					Urine	Feces	Total	Internal organs	Muscle and bone	Hide, feet, tail, and head	Total	Degree of severity of liver lesions	Degree of ascites
	Days	*Weeks*	*G*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*		
16676, female	105	6	140	2 1	0.86	0.49	1.35	0.17	0.20	0.10	0.47		
16674, male	91	8	226	5 0	1.26	.75	2.01	.23	.50	.26	.93		
16679, male	97	9	277	5 8	2.84	1.51	4.35	.20	.18	.05	.43		
16146, male	88	11	253	[1]	[2] 2.28	1.95	4.23	.08	.45	.13	.65	+ +	
16148, male	89	11	268	[1]	[2] 2.26	1.79	4.05	.15	.50	.23	.88	Trace	
16150, female	105	11	153	9 0	4.24	4.26	8.50	.06	.25	.08	.39	+	
16152, female	105	11	157					.07	.09	.03	.19		
16673, male	33	11	190					.23	.50	[3]	.73	+ +	
16675, female	33	11	124	17 8	3.61	5.85	8.89	.07	.32	.05	.41	+ +	
16678, male	33	11	240					.06	.25	.01	.31	+ + +	+ +
16681, female	33	11	128					.08	.28	.13	.49	+	

[1] Some lost. [2] Analysis lost. [3] Includes 0.09 mg from fluid in abdominal cavity.

In some of the first analyses, magnesium nitrate was used in the preliminary charring. Unfortunately, this caused appreciable loss of selenium, making the values low for the quantity recovered in the first 4 weeks for rat 16146, the first 3 weeks for rats 16148 and 16150, and the first week for rat 16673. Losses in a few other cases have been noted in the table.

With the exception of one case, the quantity of selenium excreted in the urine was greater than that excreted by way of the feces. The total amount recovered in the body tissues of the different animals varied considerably, although it represented only a small fraction of that ingested and in no instance exceeded 1 mg. The animals kept on the diet for 11 weeks did not show greater storage than those maintained for shorter periods, indicating that the storage of selenium in the animal body is not cumulative.

RATE OF STORAGE OF SELENIUM

Five litter-mate male rats and four litter-mate females were placed at 4 weeks of age on a diet containing 50 percent of selenized wheat

from the lot used throughout these studies. This particular diet contained therefore approximately 7.5 p. p. m. of selenium. After 2 weeks on the diet one male rat was killed and the body analyzed for selenium by the method already described. At the end of 4 weeks another male and a female were similarly treated. Thereafter this procedure was repeated at intervals of 4 weeks until all of the animals were disposed of. The initial and final weights of the animals and the total quantity of selenium found in the bodies are shown in table 7. The female that was to have been killed at the end of 16 weeks died before the expiration of that time and therefore no analysis was available.

TABLE 7.—*Storage of selenium by rats placed at 4 weeks of age on a diet containing 7.5 p. p. m. selenium derived from wheat*

Period on the diet (weeks)	Males				Females			
	Rat number	Weight at beginning	Weight at end	Total selenium in body	Rat number	Weight at beginning	Weight at end	Total selenium in body
		G	*G*	*Mg*		*G*	*G*	*Mg*
2	16542	49	50	0.02				
4	16543	48	104	.35	16548	47	70	0.20
8	16545	46	170	.65	16550	42	117	.40
12	16544	47	208	.51	16549	45	136	.21
16	16546	46	222	.75	[1] 16551			

[1] Died before end of 16-week period.

The data indicate that maximum storage of selenium is probably reached at some time between the fourth to eighth week, or rather soon after the animal is given the selenized food. Although in each case the quantity recovered from the female was less than that from the corresponding male, the cases are too few to warrant the conclusion that females store less than males. The values for quantity of selenium stored are of the same order of magnitude as those obtained in the balance study (table 6).

RATE OF ELIMINATION OF STORED SELENIUM

Five male rats (four litter mates) and five litter-mate females 4 weeks of age were given the diet containing 50 percent of selenized grain and continued on that diet for 13 weeks. At the end of this time one male and one female were killed and their bodies divided into three parts as described, and analyzed for selenium. The remaining eight animals were then given a diet of the same composition as formerly, but with the wheat component replaced by normal grain containing no selenium. After 2 weeks on this selenium-free diet, a male and a female were killed and their bodies analyzed for selenium. At 2-week intervals this procedure was repeated until all of the animals had been killed and analyzed.

The weights of the animals at the time they were returned to the selenium-free diet, the weights when they were killed, and the quantities of selenium recovered from the carcasses are shown in table 8. It was necessary to have the analyses on the last pair made in another laboratory. No selenium was found in the body of the fe-

male and only 0.08 mg in the muscles and bones of the male. These results seemed rather out of line with the others, and this should be taken into consideration before definite conclusions are drawn from them.

TABLE 8.—*Rate of elimination of selenium by rats given a selenium-free diet after being maintained from 4 to 17 weeks of age on a diet containing 7.5 p. p. m. selenium derived from wheat*

Period on the selenium-free diet (weeks)	Males							Females						
	Rat number	Weight when put on selenium-free diet	Weight when killed for analysis	Selenium stored				Rat number	Weight when put on selenium-free diet	Weight when killed for analysis	Selenium stored			
				Organs	Muscle and bone	Hide, feet, tail, and head	Total				Organs	Muscle and bone	Hide, feet, tail, and head	Total
		G	*G*	*Mg*	*Mg*	*Mg*	*Mg*		*G*	*G*	*Mg*	*Mg*	*Mg*	*Mg*
0	16510	----	195	0.07	0.18	0.13		16524	----	140	0.07	0.25	0.17	0.49
2	16520	223	255	.01	.3	.1	.41	16523	138	156	.01	.25	.08	.34
4	16517	207	253	.01	.4	.1	.51	16520	146	166	.00	.08	.05	.13
6	16518	238	271	.01	.25	.15	.41	16522	155	180	.02	.05	.02	.09
12[1]	16521	207	384	0	0.08	0	.08	16525	132	175	0	0	0	0

[1] Selenium analyses made in another laboratory. See text.

The results with the males would indicate that selenium stored in the body tissues is retained for a considerable time, at least, after the animal ceases to ingest selenium. The results with the females seem to indicate that stored selenium may be gradually eliminated after the animal ceases to ingest the element. Whether these findings are true for the two sexes cannot be stated from the few data available.

Further work along this line is needed. The question at issue is of importance to those concerned with the marketing of meat from animals that may have at one time or another had access to plant material containing selenium.

PERMANENCE OF INJURIES DUE TO SELENIUM POISONING

The results of the studies on the storage of selenium in the body indicated conclusively that the amount of selenium retained in the tissues was small compared to the total quantity ingested. On the other hand, the results of the feeding tests with various levels of wheat as well as selenious acid showed that the severity of lesions due to selenium poisoning is directly proportional to the quantity of selenium in the diet. It seemed, therefore, that although selenium was directly responsible for the tissue changes, its continued presence was not essential to the persistence of these changes. In other words, the question was raised: Are the characteristic lesions of selenium injury also permanent? The animals (table 8) used in the feeding test to determine the rate of excretion of stored selenium gave evidence on this point. The notations with regard to appearance of external symptoms of poisoning in these animals during the feeding tests and the findings at autopsy are shown in table 9.

TABLE 9.—*Conditions during feeding and at autopsy of animals given a selenium-free diet after being maintained from 3 to 17 weeks of age on a diet containing 7.5 p. p. m. of selenium derived from wheat*

Rat number and sex	Condition when put on selenium-free diet	Period on selenium-free diet	Condition during selenium-free feeding	Autopsy findings
		Weeks		
16519, male	No edema	0	No edema	Liver lesions characteristic of selenium poisoning.
16524, female	do	0	do	Do.
16520, male	do	2	do	Liver lesions characteristic of selenium poisoning. Small amount fluid in body cavities. Blood in thoracic cavity.
16523, female	do	2	do	Liver lesions characteristic of selenium poisoning.
16517, male	do	4	do	No report.
16526, female	do	4	do	Liver lesions characteristic of selenium poisoning.
16518, male	do	6	Slight edema	Liver lesions characteristic of selenium poisoning; 4 g liquid in abdominal cavity.
16522, female	do	6	do	Liver lesions characteristic of selenium poisoning; 7 g fluid in abdominal cavity. Bloody fluid in intestine.
16521, male	Ascites present	12½	Marked ascites	No report.
16525, female	No edema	12½	do	Do.

Animals 16519 and 16524 may legitimately be considered as controls, since they were killed and autopsied at the end of the 13-week period, when the group was transferred from the diet containing 7.5 p. p. m. of selenium to the selenium-free diet. Both of these rats showed the liver lesions characteristic of selenium poisoning.

Autopsy reports for five of the eight remaining animals maintained on the selenium-free diet for various lengths of time reveal the presence of characteristic liver lesions. One of the three animals for which no autopsy report was made continued to show increasingly severe symptoms of ascites after being on the selenium-free diet for as long as 12½ weeks.

This evidence would indicate that lesions once formed may remain as permanent injuries after ingestion of selenium is discontinued.

SUMMARY AND CONCLUSIONS

Grains and plant materials that produce the erroneously called "alkali disease" in large animals also produce toxic symptoms in rats. The most prominent of these are generalized edema and liver injury. In advanced cases the liver presents an atrophic nodular appearance and oftentimes is grown together in a single hard mass. Large amounts of a straw-colored fluid collect in the abdominal cavity. These symptoms are identical with those produced in rats by feeding small amounts of selenious acid or selenium salts. The toxic symptoms noted cannot be accounted for on the basis of lowered food intake.

Selenium added to the diet of rats in the form of selenious acid is toxic in proportion to its concentration in the diet. The threshold level of the lethal dose was between 13 and 18.4 p. p. m. The condition of animals on the diet containing 18.4 p. p. m. of selenium most nearly resembled the animals on the toxic wheat diet that contained 9.8 p. p. m.

Wheat containing selenium incorporated in the diet of rats has a detrimental effect on growth and reproduction in direct proportion to the amount of selenium supplied. On a diet containing 8.7 p. p. m., growth was stunted, very few young were born, and none reared. After a period of several months, all animals showed severe toxic symptoms. On the diet containing 6.0 p. p. m., weight was considerably below normal and the number of young born and the percentage reared were less than normal. In the second generation no young were reared. The diet containing 3.0 p. p. m. had a slight effect on reproduction, although growth was apparently normal. When the diet contained 1.5 p. p. m. or less there was no detectable effect on growth or reproduction. The ingestion of toxic wheat supplying as little as 0.00025 g of selenium per 100 g of body weight per week led to development of toxic symptoms.

There is considerable variation in susceptibility of individual rats to selenium injury.

Storage of selenium in the body of the rat is not cumulative. The total quantity stored did not in any case exceed 1 mg. Maximum storage occurred within a few weeks after the animal was given the diet containing selenium. In the case of the males, selenium stored in the body was not entirely eliminated when the selenium regimen was discontinued. Whether this is true of females as well cannot be affirmed from the data obtained in this study.

Effects of selenium poisoning in rats persisted after the animals were returned to a normal diet and even though most of the selenium taken up by the affected tissues had been eliminated. This fact emphasizes the seriousness of selenium poisoning and suggests the need for protective measures designed to eliminate this peril which may endanger the health of human beings as well as of animals.

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