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Selenium in Proteins from Toxic Foodstuffs

Edgar Page Painter

SELENIUM IN PROTEINS FROM TOXIC FOODSTUFFS

I. The Removal of Selenium from Toxic Protein Hydrolysates.

II. The Effect of Feeding (1) Toxic Proteins (2) Toxic Protein Hydrolysates and (3) Toxic Protein Hydrolysates from which the Selenium has been Removed.

By

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Introduction

The importance of the selenium problem has been established. Knight (1) in an address before The Association of Official Agricultural Chemists summarized the progress made on the problem, conservatively emphasized its importance, and stressed the need for extensive research. It is a paramount problem not only in South Dakota, but in many other states as well.

Franke (2) reported a new toxicant occurring naturally in certain samples of plant foodstuffs. He also reported (3) that the toxicant was carried in the protein fraction and was not removed by ordinary solvents. In 1932 the problem was brought to the attention of the United States Department of Agriculture and Robinson (4) found selenium in grain samples which Franke had found toxic.

The major portion of work published on this problem has dealt with the effects produced by the toxicant on the animal body. The gross pathology produced by feeding toxic foodstuffs has been described (2,3,6). The albino rat has been used in these investigations, however, similar effects are produced in farm animals (5). Franke and Potter (6) found abnormally low hemoglobin levels in rats fed toxic grain. The effects produced in chickens by toxic grains has been studied by Franke and Tully (8) and Tully and Franke (9). Franke and Moxon (7) studied the action of toxic proteins on enzymatic activity and have reported on the inhibiting effect of toxic proteins on carbon dioxide production during yeast fermentation.

Since it has been shown that toxic foodstuffs producing the so-called alkali disease contain selenium, a series of investigations was started

to determine its chemical nature, and to determine, if possible, whether or not selenium in some compound (or compounds) is the sole toxicant in the toxic foodstuffs. In the first paper, Franke and Painter (10) showed that the selenium in toxic grains was in the protein and was not removed by the solvents for metallic selenium or selenium salts. It was found that when the proteins were hydrolized with acids most of the selenium was present in the hydrolysate but some was in the humin. The selenium in the hydrolysate was in some organic combination. Painter and Franke (11) hydrolized toxic proteins with acids and studied the selenium distribution in the insoluble humin and in the hydrolysate. The proportion of total selenium in the insoluble humin increased when the humin formation was increased. This resulted when concentrated mineral acids were used, and when carbohydrates were added to the hydrolizing solution. It was also shown that the two amino acids, tryptophane and tyrosine, condensing with aldehydes or carbohydrates in some manner to form humin, contained very little selenium when they were isolated, and this selenium was probably due to contamination. Hydrolysis with hydriodic acid resulted in a selenium free hydrolysate. The effect of alkaline hydrolysis has been studied by Franke and Painter (12). It was found that when toxic proteins were hydrolized with alkali in the presence of plumbite, some of the selenium appeared in the lead sulfide formed. A similarity between labile sulfur and "labile selenium" was shown. By combining strong acid hydrolysis and alkaline hydrolysis (12) a selenium free hydrolysate resulted. A possible explanation for this was pointed out from the work of Butz and du Vigneaud (13) who showed that methionine was converted to homocystine by strong acid hydrolysis and that the sulfur in this amino acid, homocystine, was labile like that of cystine. The possibility that selenium replaces sulfur in the amino acids, cystine and methionine, has

been further discussed (11).

The reviews of Bradt and collaborators (14) have shown that organic selenium compounds are very similar to those of sulfur in both preparation and reaction. Hurd-Karrer (15) has shown that the absorption of selenium by plants depends upon the selenium sulfur ratio.

Beath et al (16) reported selenium in poisonous plants, but the selenium in the plants he reported had different properties than the selenium in the toxic foodstuffs reported by Franke (2).

Plan of Experiment

In Part I of this paper, the removal of selenium from the hydrolysate is reported. It has been found (11) that most of the selenium present in toxic proteins is in the hydrolysate, when hydrolysis is carried out by the usual methods.

Since amino acids are the products of protein hydrolysis, methods for the separation of these were used. The method which appeared the most practical for the removal of selenium was perfected so that a selenium free fraction could be bio-assayed by the method used when determining the toxicity of the grains.

It should be emphasized that orthodox procedures were not rigidly followed, and alternations of methods were freely practiced. This appears justified inasmuch as an unidentified compound (or compounds) containing selenium is present in toxic protein hydrolysates. In case a selenium homologue of cystine or methionine were present, it would not have properties exactly like those of the sulfur compounds, but they probably would be similar in some respects. To keep this concept in mind particular emphasis is given the two amino acids cystine and methionine.

Since space does not permit an introduction to the methods of separating amino acids, what references seem necessary are given when the experimental

results are discussed.

In Part II of this paper the results obtained by feeding the fractions reported in Part I are given. In this part (II) a study was made of the toxicity of the selenium containing and selenium free fractions.

I. THE REMOVAL OF SELENIUM FROM TOXIC PROTEIN HYDROLYSATES.

The source and method of separation of the protein used in this investigation has been described (10). Selenium was determined by the alkaloidal test described by Horn (17) and the procedure described by Robinson et al (18). When a three gram sample gave a negative selenium test, it was considered selenium free. The proteins were hydrolized with sulphuric acid (10), the insoluble humin filtered off and washed, and the acid removed with barium hydroxide. The barium sulfate was centrifuged and washed several times with hot water. The washings were added to the neutral (free from sulfuric acid or barium) hydrolysate. This hydrolysate was used at the starting point of all the separation procedures. The solution was concentrated by vacuum distillation or diluted with distilled water when adjustments in volume were necessary.

Due to the amount of humin formed during the hydrolysis, the tryptophane present in the protein was probably decomposed. The work of Gortner and his collaborators (20) and Holm and Greenback (21) indicate that this amino acid is practically quantitatively decomposed during acid hydrolysis when humin formation is large.

After each separation the approximate quantity of selenium in the fractions was determined.

Extraction methods:

1. Extraction with organic solvents.

Marx (unpublished data) extracted a toxic protein hydrolysate with chloroform, petroleum ether, alcohol and benzene. In no case was selenium

removed, neither was a crystalline compound obtained.

2. Extraction with Butyl Alcohol.

It was found (Potter, unpublished data) that nearly all the selenium passed into the butyl alcohol fraction when a toxic protein hydrolysate was separated by the method described by Dakin (22). After 72 hours of continuous extraction only a trace of selenium remained in the neutral aqueous solution. All the amino acids except the basic and dicarboxylic amino acids and lysine (?), hydroxy glutamic acid (?) and tyrosine (?) are extracted by butyl alcohol. Pirie (23) has shown that methionine is readily extracted by butyl alcohol. That cystine slowly passes into the butyl alcohol fraction has been shown by Zahnd and Clark (24), and Hess and Sullivan (25). They also found that cysteine passes into the butyl alcohol more readily than cystine.

Precipitation methods:

1. Precipitation with phosphotungstic acid:

The well known method for the precipitation of the bases: lysine, arginine and histidine, together with cystine, precipitated less than half the selenium from toxic protein hydrolysates. Both the methods described by Morrow (26) and Vickery and Leavenworth (27) were used. Thimann (28) has shown that proline is partly precipitated by phosphotungstic acid. The solubility of cystine phosphotungstate depends upon the degree of racemization. Hoffman and Gortner (29) and Plimmer and Lowndes (30) found that the phosphotungstate of i-cystine is much more soluble than the phosphotungstate of l-cystine. Undoubtedly there was appreciable racemization of cystine during hydrolysis by the method used.

2. Precipitation with copper:

Town (31) and Brazier (32) use copper carbonate as an amino acid precipitant. Separation of the amino acids is effected by the solubilities of their copper salts in different solvents. The water soluble copper salts

contained from a trace to about half the selenium present in the hydrolysate. Consistent results were not obtained when precipitations were carried out under similar conditions. The amino acid complexes of copper insoluble in water are those of leucine, phenyl alanine, and aspartic acid (31, 32). Vickery and Leavenworth (27) have shown that cystine is 95% precipitated by copper hydroxide in a solution containing cystine and histidine. Meuller (33) prepared the copper salt of methionine using copper carbonate and found that it was almost insoluble in cold water, but moderately soluble in hot water.

Vickery and White (34) use cuprous oxide to precipitate cysteine. Cystine is boiled with sulfuric acid in the presence of tin to reduce it to cysteine. The cuprous mercaptide precipitates quantitatively. By using this procedure on toxic protein hydrolysis, approximately half the selenium appeared in the cuprous mercaptide precipitate. Methionine is not precipitated under the conditions employed (34).

3. Precipitation with silver sulfate and silver oxide:

The silver precipitation of Kossel and Kutscher (35) for the determination of arginine, lysine and histidine is probably the most satisfactory procedure for precipitating these amino acids. By following the procedure described by Vickery and Leavenworth (36) a very small amount of selenium was in the precipitates. A trace of selenium was in the precipitate formed in both neutral and alkaline solutions. However, the amount was so small that it was probably due to a contaminant. These authors have shown (27) that a small amount of cystine is precipitated by silver sulfate in acid solution and that none is precipitated by silver oxide in alkaline solutions.

4. Precipitation with mercury salts:

Three mercury salts, the sulfate, acetate and chloride have been studied. Each appeared to be superior to other amino acid precipitants in

precipitating selenium. Precipitation with Hopkins and Cole's reagent (37) in 5% sulfuric acid resulted in only partial precipitation of the selenium present. It was found that more selenium was in the precipitate when precipitation was carried out in a near neutral solution. However, appreciable amounts of selenium remained in the solution when mercuric sulfate was used. When mercuric acetate was used, more amino acids were precipitated from solution than when using the sulfate or chloride, but some selenium remained in solution. Mercuric chloride has proved to be the best precipitant for the selenium compounds yet found. It would completely precipitate the selenium from a near neutral hydrolysate.

The following procedure was developed which precipitated the selenium so nearly quantitative that present methods for the detection of selenium were not sensitive enough to detect it in a reasonable amount of the soluble hydrolysate. To the hydrolysate from 100 grams of toxic protein in three liters of solution solid barium carbonate is added in excess. Two liters of saturated mercuric chloride are then added while the solution is stirred. A flaky precipitate forms immediately. The solution is allowed to set at room temperature, with frequent stirring, for one hour. The mercury precipitate and undissolved barium carbonate is filtered off using a Buchner funnel and the precipitate washed two or three times with water. The filtrate is practically free from selenium. After the mercury, chlorine, and barium have been removed and the amino acids dried, the alkaloidal test on a three gram sample is negative. A positive test can be obtained on less than 0.1 gram of the toxic protein, so less than 1/30 of the selenium originally present is in this filtrate. The distillation method (18) indicated less than 3 p.p.m. selenium. As in all similar procedures, results are somewhat variable.

Over sixty mercuric chloride precipitations have been made. One

precaution must be considered. The mercuric chloride must be saturated. It should be dissolved by heating, and the solution used after it has cooled down to room temperature.

In general mercury salts precipitate histidine and tryptophane quantitatively, and cystine and tyrosine to a less extent. Pirie (23, 38) and Mueller (33) and du Vigneaud and Myer (39) used mercury salts to precipitate methionine. In his later paper, Pirie (38) describes the combined action of mercuric acetate and phosphotungstic acid to precipitate methionine. In both Pirie's procedures methionine is recovered from a final precipitation in which mercuric chloride is used. He states that both the sulfate and chloride ion have an inhibiting action on the precipitation of methionine in an amino acid solution. In both the older methods for isolating methionine (33, 39) the amino acid was precipitated from a solution neutral to congo red. Mercuric sulfate in 5% sulfuric acid has been used to precipitate cystine (37). Under these conditions Vickery and Leavenworth (27) found 86% of the cystine precipitated. These authors also (36) use Hopkin's reagent to precipitate histidine.

By the method described for removing the selenium from toxic protein hydrolysates, the selenium in the hydrolysate from 100 grams of protein is in the mercuric chloride precipitate which contains approximately 5 grams of amino acids.

Discussion

The results obtained by precipitating selenium from toxic protein hydrolysates using different amino acid precipitants does not restrict the properties of the selenium compound to those identical with any one amino acid. In fact more than one compound of selenium is indicated. It would be very difficult to conceive a single compound partially precipitated by every one of the precipitants used but only completely precipitated by one.

However, it is entirely probable that other precipitants than mercuric chloride would quantitatively precipitate the selenium compounds under very definite conditions. Few conclusions can be made regarding the chemical properties of the selenium compound (or compounds) when only one property -- that of the solubility of several of its derivatives -- has been studied. In this regard the properties are very similar to those of several amino acids. The properties in regard to solubility are unlike those of any of the amino acids except cystine, methionine, histidine and possibly tryptophane. Every amino acid precipitant precipitating these amino acid precipitates some selenium and every hydrolysate fraction containing these amino acids in appreciable quantity contained some selenium. Since the solubilities of these amino acids resemble each other closely, and since little is known of the solubilities of the compounds of methionine, it is difficult to eliminate the known amino acids.

One difficulty has deterred progress toward isolating a selenium containing compound. When the metallic precipitate of amino acids containing a concentration of selenium was decomposed with hydrogen sulfide, a large proportion of the selenium always was in the metallic sulfide formed. This has made several separations of the selenium containing compounds of toxic protein hydrolysates by precipitation very difficult. This was striking when cystine in a toxic hydrolysate was reduced to cysteine. Upon decomposing the cuprous mercaptide with hydrogen sulfide, all of the selenium in the precipitate appeared in the cuprous sulfide formed, which suggests that an unstable selenol existed. The selenides of heavy metals are extremely insoluble compounds.

If one of the known amino acids contained selenium in one of its usual organic combinations, the selenium would undoubtedly replace a sulfur or oxygen atom (14). The probability that it would replace sulfur has been suggested (11). If this were the case, or if selenium replaces an oxygen,

the properties of this compound would be different from the analogous amino acid. However, it would be expected that the properties of the compounds would be somewhat similar. A compound entirely unlike those of the known protein hydrolysis products is not precluded.

There is evidence that most of the selenium is in a compound very similar to cystine. All cystine and cysteine precipitants used precipitates some selenium, and some selenium is removed from toxic hydrolysates under the same conditions that labile sulfur is split off from cystine or cysteine. The selenium in toxic proteins was extracted by butyl alcohol more readily than cystine. It has also been shown (11) that a compound of selenium is present which decomposes more readily in acid solutions than cystine. Mercuric chloride was found to precipitate probably all the selenium from toxic protein hydrolysates. Cystine cannot be completely precipitated in this manner. The quantitative precipitation of cystine by heavy metals is precluded because 1/6 of the sulfur is oxidized when silver (40) mercury or copper (41, 42) salts are employed and which may occur with other metals. Evidently this did not happen with a selenium compound. It is known that sulfur is more easily oxidized than selenium.

In an earlier paper (11) it was stated that the molar selenium-sulfur ratio was approximately 1:173 in the protein under investigation. Since many of the properties of the selenium containing compounds closely resemble those of the sulfur containing amino acids, the difficulty in separating the two is obvious. This difficulty is further augmented by the unstability of the selenium in its naturally occurring linkages.

The separation of selenium was discontinued at this time to determine the toxicity of the products under investigation.

II. THE EFFECT OF FEEDING (1) TOXIC PROTEINS, (2) TOXIC PROTEIN HYDROLYSATES, AND (3) TOXIC PROTEIN HYDROLYSATES FROM WHICH THE SELENIUM HAS BEEN REMOVED.

Up to the present time only results obtained by feeding toxic grain and the recombined products have been reported (2, 3, 6, 8, 9). It seemed advisable to determine the relative toxicity of the grain, the protein, the protein hydrolysate, and to determine if the removal of selenium would render the protein hydrolysate non-toxic. In an earlier paper (10) it was stated that the only difference known between "normal" and "toxic" grains was the presence of selenium in the latter. Vanadium may contribute to the toxicity of some of the grains since Byers (43) found this metal in one sample, but vanadium is much less toxic than selenium. Present methods of selenium analyses indicate that the most toxic grain samples bio-assayed (2) do not contain over 25 p.p.m. selenium. Some corn samples that had lower selenium contents were just as toxic as wheat samples. Franke and Potter (44) found that 22.3 p.p.m. selenium in a diet added in the form of sodium selenite was not as toxic as many grain samples. Two explanations appear possible: (1) that the selenium in toxic foodstuffs is in a compound which is more toxic than inorganic selenium salts, or (2) there is some toxicant other than selenium in the foodstuffs.

Toxicity has been compared by the incidence of death, restriction of food intake, growth rates, and gross pathology in experimental animals.

Preliminary feeding trials of toxic protein hydrolysates conducted in this laboratory indicated that they were toxic when the protein was hydrolized with hydrochloric or sulfuric acids. In these cases the protein was separated from the bran and starch, hydrolized and recombined with the bran and starch when made up into a diet. There was some residual protein in the bran and starch and some selenium was lost in the humin. In this paper experiments are reported where a direct comparison of toxicity of a hydrolysate and grain was attempted.

Experimental

Although Franke (3) reported that the toxicant in the foodstuffs under investigation was carried in the protein fraction, no direct comparison between the protein and foodstuff, based upon equal nitrogen content, was made. A small amount of protein, soluble in 0.2 N sodium chloride was lost and not fed.

In order to determine the effect on animals of the chemical treatment used to separate the selenium compounds, the following feeding trials (Series 101) were carried out.

Group A was fed a diet containing toxic wheat.
Group B was fed a diet containing toxic protein.
Group C was fed a diet containing the hydrolysate from toxic proteins.
Group D was fed a diet containing the hydrolysate from toxic protein from which the selenium had been removed.

It should be emphasized that in these feeding trials strict series of controls were used. In all feeding trials a fraction was fed from a "normal" wheat (Laboratory No. 648) which had received the same treatment as the experimental. These will be referred to as control groups A, B, C, and D.

The protein from toxic wheat (Laboratory No. 582) fed to Group B was prepared by the same procedure as that used in previous investigations.

The following method was used to prepare the hydrolysates for animal feeding. The hydrolysate of proteins has been described (11). The insoluble humin was filtered off and the sulfuric acid removed in the manner already described for preparing the hydrolysates prior to the separation procedures. Care had to be taken to ensure that there was no barium in the hydrolysate. It was found that excess sulfuric acid had to be present before all the barium was removed. The pH of the hydrolysate was acid (between 4-5) but indicators could not be depended upon. Instead

dilute sulfuric acid was added until no more barium sulfate precipitated, then several cc. of very dilute acid were added. The solution was concentrated to a thick syrup by vacuum distillation at not over 60° C. The syrup was removed from the distillation flasks and placed in evaporating dishes. At this time sodium hydroxide was added until the end point of methyl red was reached. The neutral hydrolysates were then placed on a steam bath to remove most of the water, then finally dried in a vacuum oven at 60° C. Some ammonia escaped during drying. The amino acids we then ground in a mortar, and kept in a dessicator until incorporated into diets and fed to Group C.

Preparation of the Selenium Free Hydrolysates

The method of precipitating the selenium from toxic protein hydrolysates by mercuric chloride has been described. A precipitate may form in the clear solution after the mercuric chloride and excess barium have been filtered off if it is allowed to stand, but this may be disregarded. The mercury in the filtrate is removed with hydrogen sulfide and the barium with dilute sulfuric acid. The mercury, barium and sulfate free filtrate is evaporated to near dryness several times in vacuum to remove as much chlorine as possible. The filtrate is then made up to about 1 liter and the remaining chlorine removed by the addition of boiling hot suspensions of silver oxide. This is added until the pH reaches 4. The silver chloride and excess silver oxide is then filtered off and washed several times with hot water. The small amount of silver in the solution is removed as the sulfide by the addition of hydrogen sulfide and the volume of the solution reduced by vacuum distillation to about 500 cc. It is then tested with a few cc. of dilute sulfuric acid to ensure complete removal of barium. If it is free from all toxic metals, dilute sodium hydroxide is added and the pH adjusted to that described for the

hydrolysates. This amino acid fraction is then dried in the manner already described. Each one of these filtrates was tested for the presence of selenium before it was incorporated into diets and fed to Group D. The same procedure was carried out using a hydrolysate of control protein which served as a control group (D).

The usual practice was to hydrolize 300 grams of protein at a time and divide it, taking half for the hydrolysate diet and carrying the other half through the selenium precipitation procedure.

Preparation of the Diets

The diets were prepared so that their nitrogen contents were equal. It has been stated (3, 10) that the toxicant was carried in the protein of toxic foodstuffs so protein nitrogen was used as the basis for the preparation of the diets.

The wheat diets were the same as those used by Franke and Potter (6). The following table gives the diets used in each of the eight groups:

	Series 101 diets			
Toxic and control groups	A	B	C	D
Component in %				
Ground whole wheat	82			
Commercial casein	10	10	10	10
Cod liver oil	2	2	2	2
Dehydrated yeast (Northwestern)	2	2	2	2
McCollum's Salt Mixture No. 185	1	3	2	2
Lard	3	5	5	5
Cornstarch		15	15	15
Commercial sugar		45.2*	43.2+	41.8+
Protein		17.6*		
Protein hydrolysates			20.8+	
Protein hydrolysates after mercuric chloride precipitation				22.2+
Cystine (Eimer and Amend C.P.)			0.1	0.1

* This was not the same for the control and experimental diet because the proteins used did not contain the same percentage of nitrogen.
\+ The different diets varied as the nitrogen content of the hydrolysates. The figure given represents an average figure but not the percentage in every diet. The material was being prepared while the feeding was in progress so the weight of the hydrolysates and sugar varied somewhat depending upon the percentage of nitrogen in the hydrolysates.

The diets were copied after the experimental diet used in this laboratory as much as practicable. Fourteen and five tenths (14.5) percent of the protein (N x 6.25 x 100) in the toxic wheat diet is in the whole wheat added. All other diets, except the control whole wheat diet, contained the same amount of protein nitrogen as this diet. This difference in the nitrogen in the protein and hydrolysates necessitated varying another constituent of the diet. The sugar was the constituent varied.

The average nitrogen content for the hydrolysates was approximately 11.2%, and for the hydrolysates resulting from mercuric chloride precipitate 10.4%. It is realized that this is somewhat low. However, the weight of the protein hydrolysate increases over that of the protein. Some of the ammonia nitrogen was lost, and the hydrolysates were contaminated with small amounts of salt. The proteins which were hydrolized had low nitrogen contents. The nitrogen content of the toxic protein was 13.0%, and of the control protein 13.8%. These figures seem to be very low, indicating that a pure protein was not used. Mitchell and Hamilton (19) give 17.66 and 17.49 percent respectively for gliadin and glutenin, the chief proteins in wheat gluten. The proteins were purified by the method of Blish and Sandstedt (45). Both were taken from wheats of high protein content, 17.68% for the toxic (582) wheat, and 17.5% for the "normal" (648) wheat. The factor 6.25 was used to convert nitrogen to protein although Dill (46) states that the factor 5.7 should be used when determining the protein in wheat gluten. It appears that the method of Blish and Sandstedt did not separate all the protein from the starch. Further purification by dispersing the protein in dilute alkali did not seem advisable because of the alteration of the properties (partial hydrolysis) resulting from dispersion in alkalies. It has already been shown (12) that the selenium in toxic proteins is unstable in alkaline solution.

Albino rats of Wistar origin were the experimental animals used. They had been weaned on the 21st day and maintained on McCollum's Diet I for seven days as described by Burr and Burr (47). They were then divided into eight groups so that litter mates and the same number of males and females were in each group, and placed in individual cages described by Franke and Franke (48). The average weight of the eight groups did not vary 0.5 grams from the mean. The rats were weighed every five days and the food consumption recorded every two days.

Individual growth curves for the eight groups of Series 101 are shown on Chart I.

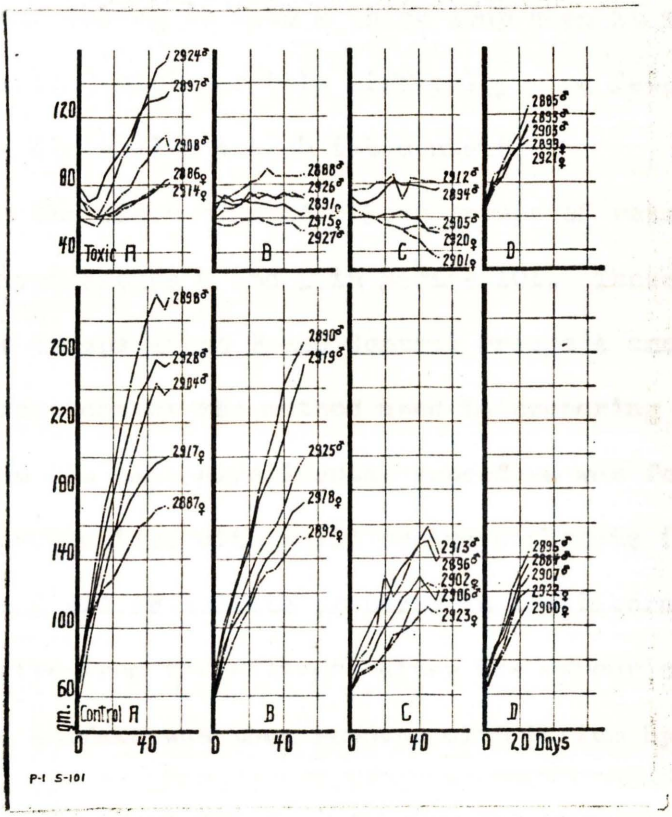

Chart I

Individual growth curves for Series 101.

Toxic groups.		Control groups.
A	Whole Wheat	A
B	Protein	B
C	Hydrolysate	C
D	Hydrolysate after $HgCl_2$ precipitation.	D

Due to the results obtained in the toxic and control Groups C and D it seemed advisable to duplicate these four groups. The toxic hydrolysate which had the selenium removed appeared to be non-toxic; however, it was felt that a period of 25 days was not long enough to warrant conclusions. Furthermore, better growth was obtained in control Group D which had part of the amino acids removed by mercuric chloride precipitation, than in control Group C. Accurate food consumptions were not obtained during the first twenty days of Series 101 on the groups receiving amino acid diets. This difficulty was overcome by adding water to the diet and making it into a thick soup when it was fed. Rose (personal communication) obviates this difficulty when feeding amino acid diets by using diets with a high fat content.

Series 112 was therefore planned. Four groups of rats were fed diets similar to those given Groups C and D in Series 101. These groups are designated as Toxic Groups A and B and Control Groups A and B (Series 112). The following alternations of the method used in preparing the amino acids were made, otherwise the same experimental procedure was followed. The hydrolysates were neutralized with solid sodium carbonate instead of sodium hydroxide, and silver sulfate prepared in the laboratory was used to remove the chlorine from the filtrate after the mercuric chloride precipitation. The sulfate was then removed with barium hydroxide following the precautions already mentioned.

Individual growth curves for these groups which extended to the 70th day are shown in Chart II.

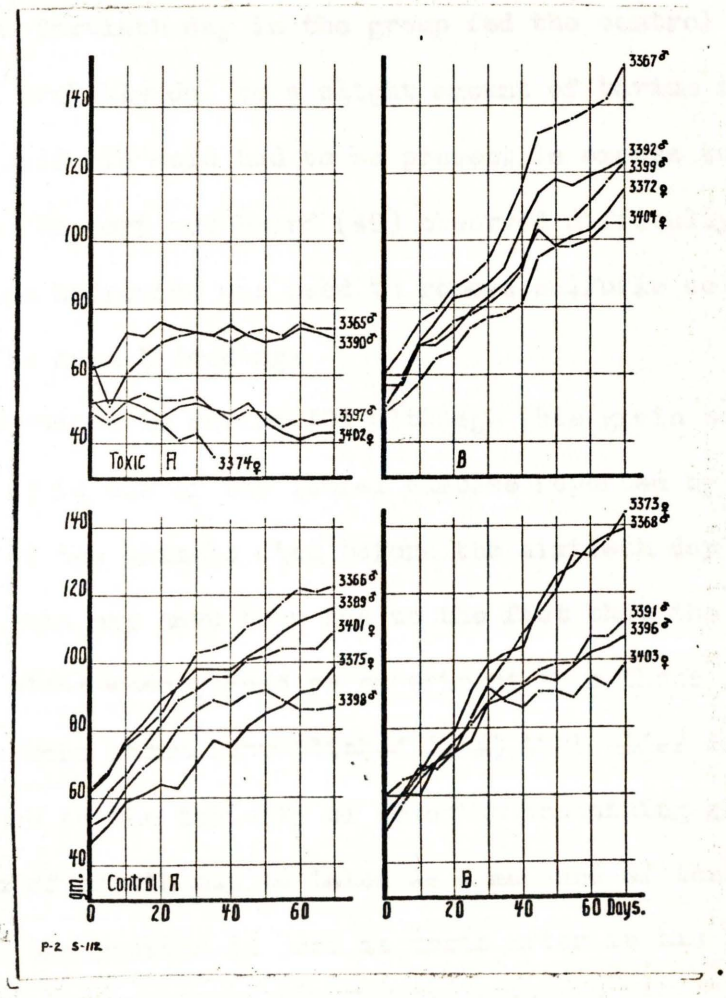

Chart II

Individual growth curves for Series 112.

Toxic groups. Control groups.

A Hydrolysate A
B Hydrolysate after $HgCl_2$ precipitation. B

All animals in both series were killed at the end of the experimental period and autopsies made. In some cases hemoglobin determinations were made following the method already described by Franke and Potter (6).

Discussion

The three control groups A, B, and C of Series 101 show clearly the nutritional values of synthetic and natural diets. The poorer growth rate

using the protein and hydrolysate diet is clearly shown. The depression of growth after the fortieth day in the group fed the control hydrolysate diet (Group C) was probably due to a slight amount of barium in the diet. It was found that sulfuric acid had to be present in excess to completely remove the barium. Rapport and Beard (49) observed difficulty with barium toxicity when barium hydroxide was used to remove sulfuric acid from hydrolysates used in animal feeding.

There were no deaths in Series 101 although this grain sample (Laboratory No. 582) is one of the lethal samples reported by Franke (2) in which over 70% of the animals died before the sixtieth day of experimentation. This may have been due to the fact that the animals averaged 2-3 days older when placed on experiment than those reported by Franke. It has been shown (unpublished data) that older animals have a greater resistance to the toxicity of selenium containing grain.

If depression of growth may be taken as a measure of toxicity it is quite evident that the protein is just as toxic after it has been separated from the wheat, dispersed in acetic acid and re-precipitated. The results here indicate that the protein diet is moretoxic; however, it should be observed that the growth of the control group B is not as good as that in the control group A. Other feeding trials using toxic proteins indicate that the toxicity is not reduced by the method employed in its separation.

It is difficult to state whether or not the hydrolysate diet (Group C) is as toxic as the whole wheat and protein diets. Growth was profoundly suppressed, but the control group also showed marked growth depression when compared with the groups receiving control wheat and control protein diets. The results suggest that the toxicity of the hydrolysate, due to the toxicant in the original grain, had been reduced.

The alkaloidal test indicated that the selenium content of the toxic diets fed to groups A, B, C (Series 101) and toxic group A (Series 112) were the same. The method described by Robinson et al (18) gives approximately 100 p.p.m. of selenium for the toxic protein and 80 p.p.m. of selenium in the toxic hydrolysate. If these figures are correct, there is more selenium in the toxic protein diet, but the difference is hardly significant.

In both series better growth resulted in the control groups with the diet containing the hydrolysate after part of the amino acids had been removed by mercuric chloride precipitation than in the groups whose diet contained the untreated hydrolysate. The obvious question is: Did the mercuric chloride precipitation remove some growth depressant or toxicant present in protein hydrolysates? Or were the results due to the removal of something rendering the diet more palatable? The change in color of the hydrolysate after the mercuric chloride precipitation indicated that much of the soluble humin had been removed. Undoubtedly the gustatory effect of protein hydrolysates in diets is a contributing factor in the failure of animals to respond favorably to these diets.

The necropsy of the animals revealed the usual symptoms produced by toxic foodstuffs. The severe pathological disturbances (2) were not as acute in the animals receiving a toxic protein hydrolysate as in the animals receiving the toxic wheat and toxic protein diets. The external appearance of the animals in toxic group C (Series 101) and toxic group A (Series 112) was typical of that generally produced by toxic foodstuffs. The animals on toxic diets were very thin and in general appeared emaciated. All groups receiving control foodstuffs appeared normal except that those receiving the diets containing amino acids were much smaller. This was also true of the animals receiving toxic protein hydrolysates from which

the selenium had been removed. They did not exhibit the typical pathological disturbances observed in the other group. All animals in Series 112 had distended caecums which contained very dark fluid material.

Several hemoglobin determinations did not reveal any abnormally low levels in rats fed toxic protein hydrolysates which Franke and Potter (6) observed in rats fed toxic grain. Only a few samples of blood were taken when the animals were killed and these were not from moribund animals. The two lowest levels obtained in Series 112 were rats No. 3397 and No. 3402, which had hemoglobin levels of 14 and 12.6 grams per 100 cc blood, respectively. The animals in the control group and protein hydrolysate with selenium removed had normal levels.

The voluntary restriction of food consumption observed (2) in animals fed toxic grain diets was observed in the animals fed toxic protein hydrolysate diets. In Series 112 there is a direct correlation between growth and food consumption. The available records of Series 101 indicate that the growth rates and food consumption were in direct proportion. The average food consumption in grams per day for the toxic groups A and B (Series 112) is 3.32 and 6.33 and for the control groups A and B is 5.49 and 6.39. It is evident that the restriction of food consumption is due to the toxicant present in the whole wheat, protein and hydrolysate diets.

Both Series 101 and 112 prove without question that the removal of selenium by the method described removes the toxicant present in toxic protein hydrolysates. This is clearly shown by comparing toxic groups C and D of Series 101 and toxic groups A and B of Series 112. The growth rate, external appearance of the animals, symptoms observed at autopsy and response to the diets all indicate that the toxicant was removed by the mercuric chloride precipitation so completely that the amount left was

innocuous. Either selenium in some compound (or compounds) is the sole toxicant or other toxicants, if present, are likewise precipitated by mercuric chloride. The results obtained do not prove that selenium is the sole toxicant present in toxic foodstuffs but since no toxicant was removed from either toxic proteins or their hydrolysates by organic solvents, nearly all known plant toxicants are eliminated. Observations feeding selenium salts (Franke and Potter (44)) indicated that their action on the animal body is very similar to that of toxic grains, although the symptoms produced were not exactly or consistently the same. If selenium is the sole toxicant in toxic foodstuffs, it is in a much more toxic form than in any of the known inorganic salts of selenium. This difference in the toxic selenium compounds might explain the difference noted when feeding toxic grains and selenium salts.

CONCLUSIONS

A method has been devised whereby the selenium can be removed from toxic protein hydrolysates. The effect of chemical treatment of the proteins from toxic wheat has been studied by animal feeding. Growth curves of animals fed diets containing whole grain, protein, protein hydrolysate and protein hydrolysate after mercuric chloride precipitation are shown.

From the results obtained by the separation trials and feeding series, the following conclusions are made:

1. Most amino acid precipitants remove part of the selenium from toxic protein hydrolysates.

2. The sulfuric acid hydrolysates of toxic proteins are toxic.

3. Mercuric chloride precipitation of toxic protein hydrolysates (under the proper conditions) precipitates the selenium compound (or compounds) so nearly completely that the filtrate is innoxious when fed to albino rats.

4. Mercuric chloride precipitation removes something from sulfuric acid hydrolysates which directly or indirectly inhibits growth.

5. The codein sulfate test has again been found to be a reliable negative test.

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