OBSERVATIONS ON THE PROTECTIVE EFFECT OF LINSEED OIL MEAL AND SOME EXTRACTS AGAINST CHRONIC SELENIUM POISONING IN RATS [1]

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(Received for publication October 27, 1954)

It is generally recognized that protein may minimize the severity of selenium poisoning (Moxon, '37; Smith, '39; Gortner, '40; Smith and Stohlman, '40; Rosenfeld and Beath, '46), although there is less agreement as to the completeness of protection that may result from the various types of supplements investigated (Smith and Stohlman, '40; Moxon, '41). Moxon ('41) found crude casein and linseed oil meal the most effective of several protein supplements tested with rats but whole beef liver and linseed oil meal were the best with dogs. In the present study, continued work with protein supplements with rats has shown linseed oil meal more effective than purified casein in protecting against poisoning by either naturally occurring selenium or sodium selenite. The activity of certain linseed oil meal extracts has also been demonstrated. Evidence is presented which suggests that the active principle of the linseed oil meal probably is not protein in nature, indicating a need for some re-evaluation of protein effects and a consideration of other feed constituents in future studies.

[1] Approved for publication by the Director of the South Dakota Agricultural Experiment Station as paper No. 330 of the Journal Series.

METHODS

Male Sprague-Dawley albino rats were used throughout these experiments. They were housed in individual wire cages and allowed food and water ad libitum. The rats were placed on experiment at a weight of about 70 to 80 gm, the averages for the various groups in each experiment being within a two to 3 gm range. Bi-weekly weights were kept, and the livers were observed for gross damage and weighed at the end of the experiment. The ratio of liver weight per 100 gm of body weight affords a numerical means of expressing the extent of liver damage.

The diets used in these studies all contained salts IV (Phillips and Hart, '35), 2.0% ; animal protein factor,[2] 0.1% ; dried brewers' yeast, 2.0% ; and lard, 3.0%. The remaining 92.9% of the diet was made up of corn, seleniferous corn, and purified casein alone and with or without linseed oil meal or its fractions. The seleniferous corn was used in an amount necessary to give the desired selenium level and non-seleniferous corn was then used to complete the diet. In preparing diets containing Na_2SeO_3, the salt was dissolved in 70% ethanol and sprinkled on the other ingredients. Vitamins A and D were supplied by administration of diluted haliver oil once weekly.

EXPERIMENTAL AND RESULTS

Casein and linseed oil meal diets at various selenium levels

Linseed oil meal or casein was added to the diets at the 21 or 8% level respectively, at which concentrations each supplement furnished the same amount of protein (N $\times$ 6.25). Table 1 presents the data obtained with the two protein supplements with different levels of selenium as seleniferous corn. Without selenium in the diets growth was better with casein than with linseed oil meal. The same was true at 7 p.p.m. of selenium, but the difference was minor and survival and pro-

[2] Nutritional Biochemicals Corporation, Cleveland, Ohio.

tection against liver damage were better on the linseed oil meal diet. At 10 and 13 p.p.m. of selenium, growth was more severely retarded on the casein diet than on the linseed oil meal, and the survival and liver damage data indicated a much greater degree of protection against selenium poisoning by the linseed oil meal. Complete protection, however, was not

TABLE 1

The toxicity of various levels of selenium to rats fed casein and linseed oil meal
(8 rats per group)

PROTEIN SUPPLEMENT	SELENIUM [1] IN DIET	PROTEIN CONTENT OF DIETS	RESULTS OF 42-DAY EXPERIMENT		
			Average gain in weight of survivors [2]	Survival	Liver damage [3]
	p.p.m.	%	*gm*	%	
8% casein [4]	0	15.4	256	100	None (5.0)
	7	15.7	170	88	Moderate (3.8)
	10	15.8	121	38	Severe (2.4)
	13	16.0	...	0	Severe (2.1)
21% linseed oil meal [5]	0	14.2	187	100	None (5.1)
	7	14.5	157	100	Slight (4.7)
	10	14.7	132	100	Slight (4.6)
	13	14.8	80	63	Moderate (3.5)

[1] Added as seleniferous corn.

[2] Initial weights averaged 66 to 69 gm among groups.

[3] The figures in parentheses are ratios of grams of liver weight per 100 gm of body weight at time of death or sacrifice.

[4] Vitamin-free (Nutritional Biochemicals Corporation), 85.4% protein.

[5] "Old process" expeller type (Spencer-Kellogg), 32.2% protein.

obtained on the linseed oil meal diets at any of the three levels of selenium. The data also indicated 10 p.p.m. of selenium to be satisfactory for use in further work.

Studies were then made with different preparations of linseed oil meal. Hexane-extracted expeller meal and petroleum ether-extracted raw ground flax proved to be as active as untreated expeller meal, thus indicating that the fat fraction was inactive.

Protective effect of linseed oil meal plus casein against naturally occurring and inorganic selenium

Experiments with combinations of casein and linseed oil meal (table 2) show a striking difference in the toxicity of the seleniferous diets containing casein alone and those containing linseed oil meal alone or with various casein levels. The addition of 6 or 12% casein in the presence of linseed oil meal complemented the protective effect of the meal somewhat as evidenced by increased growth and liver size. While this experiment did not include a group receiving casein alone at a level equivalent in protein to that of the linseed oil meal plus 12% of casein, data from an accessory study indicated that 21% of casein does not afford material protection.

The results in table 2 demonstrated that sodium selenite is similar in behavior to naturally occurring selenium, except that it appears somewhat more toxic in the present work. Therefore, inorganic selenium was used in all future work instead of seleniferous corn, thus simplifying the preparation of diets. Because of the satisfactory response obtained with linseed oil meal in the presence of casein, 12% of casein was included routinely in subsequent diets. Such a practice eliminated the possibility of protein deficiency with diets containing linseed oil meal fractions.

Feeding trials with ethanolic extracts of linseed oil meal

Attempts to extract the protective principle from linseed oil meal with acetone failed to remove any activity. Water extraction was unsatisfactory because of the mucilages. Continuous extraction with 95% ethanol in a Soxhlet greatly reduced the activity of the residue although none was observed in the extract. Since the prolonged heating of the solvent during extraction might have destroyed the protective principle, another experiment using cold solutions of absolute and of 50% ethanol was undertaken. Feeding tests with the cold extracts indicated only slight protection with the absolute ethanol preparation, but significant protection with the 50%

TABLE 2

Study of the selenium protective effect of linseed oil meal with diets containing added casein and selenium as toxic corn or sodium selenite

(6 rats per group)

DIET VARIANTS [1]			PROTEIN CONTENT OF DIETS	RESULTS OF 42-DAY EXPERIMENT		
Selenium form	Casein in diet	Linseed oil meal [2] in diet		Average gain in weight of survivors [3]	Survival	Average liver weight per 100 gm body weight
	%	%	%	gm	%	gm
No selenium						
1	12	0	18.7	262	100	5.9
Seleniferous corn (10 p.p.m.)						
2	12	0	19.5	88	33	2.7
3	0	21	16.2	163	100	5.5
4	6	21	20.8	185	83	6.0
5	12	21	25.3	197	100	5.9
Sodium selenite (10 p.p.m.)						
6	12	0	18.7	24	17	3.1
7	0	21	15.3	112	83	5.5
8	6	21	19.9	139	83	5.3
9	12	21	24.5	151	100	5.9

[1] Variants added at expense of corn; casein contained 85.4% protein, linseed oil meal 36.8%, control corn 9.2% and seleniferous corn 11.2%.

[2] Commercial hexane-extracted, unheated product.

[3] Initial weights averaged between 78 to 79 gm among groups.

ethanol extract. A reduced activity of linseed oil meal was also noted following extraction with 50% ethanol. Since recombination of the extracts with their residues produced full activity, it was evident that no material part of the

TABLE 3

Protection against selenium poisoning by hot ethanolic extracts of linseed oil meal[1]
(4 rats per group)

LINSEED OIL MEAL OR FRACTION ADDED TO BASAL DIET[2]		RESULTS OF 28-DAY EXPERIMENT	
Description	Level added	Average gain in weight of survivors[3]	Survival
	%	gm	%
No selenium			
1 (Control basal)	0	188	100
10 p.p.m. selenium as Na_2SeO_3			
2 (Control basal)	0	61	25
3 Linseed oil meal	20.0	153	100
4 50% EtOH extract,[4]			
a. 20% linseed oil meal equivalent	3.4	140	100
b. 40% linseed oil meal equivalent	6.8	167	100
5 50% EtOH residue,[4] 24% linseed oil meal equivalent	20.0	83	50

[1] Commercial hexane-extracted, unheated product.

[2] Basal diet contained 12% casein (18.7% total protein) and addition of linseed oil meal or extracts was at the expense of corn.

[3] Initial weights averaged between 75 to 77 gm among groups.

[4] The linseed oil meal was extracted by heating at 70°C. for two hours with 7 volumes of 50% aqueous ethanol. The extract was filtered off hot by suction and the residue was promptly washed by using about three volumes of fresh solvent at similar temperature. The combined extracts and washings were dried in flat pyrex pans in a 75°C. forced draft oven. The residue was air dried.

protective principle had been destroyed in the extraction procedure.

Although cold 50% ethanol extraction appeared to remove much of the protective principle, the procedure used to obtain it was lengthy and laborious. In the search for a simpler procedure, the meal was extracted with 50% ethanol at 70°C.

by soaking and then washing and the extract was fed at two different levels (20 and 40% linseed oil meal equivalent). The hot ethanol removed 17% of the dry matter from the linseed oil meal, and left essentially all of the mucilage in the residue. The data in table 3 indicate that practically all of the protective factor was removed by the hot 50% ethanol since the extract, equivalent to 20% linseed oil meal, was essentially as active as linseed oil meal itself. Further, the higher extract level (40% linseed oil meal equivalent) gave protection superior to that of 20% of linseed oil meal, although the growth response was still not equal that of the non-seleniferous controls. The poor response obtained with the residue fraction further indicated a nearly complete removal of the protective principle.

TABLE 4

Failure of lead acetate to precipitate the active principle

(4 rats per group)

DIET DESCRIPTION	AVERAGE GAIN IN WEIGHT AT 28 DAYS [1]
	gm
No selenium (control basal)	178
10 p.p.m. Se as Na_2SeO_3	
Seleniferous basal	72
20% linseed oil meal	154
7.5% lead non-precipitable fraction [2] (80% linseed oil meal equivalent)	151
1.5% lead precipitable fraction [2] (80% linseed oil meal equivalent)	93

[1] Average initial weights 78 gm for each group.

[2] Fractions prepared by treating a water extract of dried hot 50% ethanol extract with excess lead acetate. Hydrogen sulfide was used to remove lead from precipitate and from filtrate. Upon drying at 70°C. the non-precipitable and precipitable fractions contained 13 and 9 p.p.m. lead, respectively.

A subsequent experiment has shown that the ash of the active ethanolic extract is not responsible for its protective effect. Further, it was found that water removed the protective principle from the dried ethanolic extract and then an excess of lead acetate did not remove the activity from water solution (table 4).

DISCUSSION

In these studies linseed oil meal has been found markedly superior to casein in protecting against selenium poisoning. In view of this and of the results with 50% ethanol extracts, it appears that some fraction of the meal other than protein was responsible for the protective effect. The failure of lead to precipitate the extracted active principle from solution supports this view. Whereas the effect of protein on selenium poisoning has received considerable attention, these findings point to a need for the consideration of other food constituents in the search for practical control measures, as well as for a reconsideration of protein effects.

At present there is no explanation of the mechanism by which linseed oil meal prevents selenium poisoning. Halverson and Hendrick ('54) have demonstrated that the anti-vitamin B_6 principle (Kratzer and Williams, '48) is not involved. Linseed oil meal does not reduce deposition of selenium in the liver (Olson and Halverson, '54), and extraction of the active principle with 50% ethanol leaves the mucilage with the inactive residue. Therefore, prevention of selenium absorption by the mucilage, a mechanism suggested for the protective effect of beet pectin (Rosenfeld and Beath, '47), does not seem likely. Identification of the active principle of linseed oil meal and the determination of its mode of action may be helpful in the understanding of the metabolic effects of selenium.

The effectiveness of linseed oil meal in preventing selenium poisoning in farm animals has not been adequately determined. However, in studying the effect of protein content of the diet with sheep fed seleniferous extracts of *Atriplex canescens*, Rosenfeld and Beath ('46) used medium and high protein diets containing 16 and 25% of linseed oil meal and found both protective when compared to a low protein diet without linseed oil meal. Although other protein supplements were included in their diets, it is possible that the protection they observed was largely due to the linseed oil meal. Unpublished studies at this laboratory indicate that this protein supplement is effective against selenium poisoning in hogs and chickens. Since it appears to supply a protective principle in addition to protein, linseed oil meal should probably be considered the supplement of choice in seleniferous areas.

SUMMARY

In rat studies the protective effect of linseed oil meal against selenium poisoning has been demonstrated under several conditions. The inclusion of 6 or 12% casein in the diets did not alter or mask the protective effect of the meal and meals prepared by different methods were all found active. The protective principle was effective against inorganic (selenite) selenium as well as against the form occurring in toxic corn.

The protective principle has been extracted from linseed oil meal with hot 50% aqueous alcohol. It is water-soluble, and an excess of lead did not precipitate it from solution. The ash of active extract was not protective.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Archer-Daniels-Midland Co. for preparing and furnishing the unheated, hexane-extracted linseed oil meal, and to Robert M. Pengra for technical assistance.

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