gains between the groups receiving different varieties but no significant differences between the gains of the groups fed the same variety from different dates of planting. To test the effect of location of planting, Forkedeer oats were planted at the same date but one sample was planted at Perkins, Okla., on Vanoss loam and the other at Lake Carl Blackwell, Okla., on a combination of Port loam and Port clay loam. Similar yields were obtained at both locations. There was no significant difference between the weight gains of the groups which were fed these samples.

In the other two experiments presented in Table III, the nutritive values of two samples of Cimarron oats are compared. One sample was planted in September, the usual time in this area for planting this winter variety, and the other sample was planted in March, which is the usual date for planting spring oats. No significant differences in weight gains were found.

Discussion

Substantial differences in the growth-promoting values existed among the 16 varieties of oats subjected to evaluation in these studies. As the diets contained adequate quantities of required nutrients other than protein, these differences are attributed to differences in the protein of the samples. These differences are apparently characteristic of the varieties, for the samples were grown under conditions as similar as possible, and the findings were in general similar to samples from different plantings.

Samples of oats planted at different dates or locations caused similar weight gains in rats. Although this was not an extensive study, it suggests that these environmental factors are of lesser importance in determining the nutritive value of oat protein than are those of genetic origin.

Cimarron, Forkedeer, and Winter Fulghum Selection (6570), three winter varieties, consistently produced good growth results when fed to rats. There is no indication that the nutritive values of these varieties are significantly better than those of most other winter varieties. However, two winter varieties, Woodward Composite Selection (4829) and DeSoto, consistently caused poor growth results. It is possible that the differences would be of importance when oats were to be used in feeds for nonruminant farm animals and poultry.

There was a trend toward less weight gain in the groups fed the spring varieties of oats than in the groups fed winter varieties. The spring varieties were studied in only two experiments and the same samples of oats were used in both experiments—not a satisfactory test to determine nutritive value. However, one spring variety, Andrew, caused poor rates of growth in three experiments and probably is not as good a protein source as the better winter varieties.

As protein is the limiting factor in these diets, the differences in the growth rates suggest that the poorer oat varieties supply less of one or more of the essential amino acids to the rats. Mitchell and Smuts (*9*) found that lysine was the most limiting amino acid in oats for the growth of rats. Unpublished data from the author's laboratory indicate that methionine and threonine are the next most limiting amino acids in oats. Microbiological assay for lysine and methionine showed that there was no correlation between the levels of these amino acids in the oat samples and the weight gains of groups that received them. If the differences between the varieties are not due to the levels of the growth-limiting amino acids, they may be due to differences in the availability of the essential amino acids to the rat.

It will be important to determine the cause of the differences in the nutritive values of the oat varieties. If these differences are the result of poor availability of a single amino acid, the importance of these results will depend on what other sources of protein will be fed with the oats. If all of the amino acids of the poor oat varieties are less available to the animal, it will be an important consideration whenever oats are used as a protein source for nonruminants.

Acknowledgment

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SELENIUM POISONING

Modification of Selenite Metabolism by Arsenite

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THE FEEDING of various arsenic compounds has shown considerable promise as a means for the control of selenium poisoning in livestock. The protective action of arsenic has been demonstrated by feeding experiments with rats (*3*), hogs (*11*), and cattle (*10*). Organic arsenicals are about as effective as inorganic salts (*8*).

Little is known of the mechanism involved in the antagonism between selenium and arsenic, but a few facts have been ascertained by work with rats and yeast. Analyses of rats fed selenium and arsenic for several weeks revealed no differences in selenium distribution caused by administration of enough arsenic to prevent the appearance of symptoms of selenium posioning (*6*).

In yeast, the study of selenite inhibition of respiration has been broadened to include the effect of several ions and substrates (*2*). The inhibition is reduced by addition of arsenite, arsenate, or

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Partial control of selenium poisoning in livestock has been effected by feeding various arsenic compounds. As an extension of this work, a study of the mechanism of the selenium-arsenic antagonism has been initiated. Arsenic compounds prevent selenite uptake by respiring yeast cells. Injected arsenite apparently blocks transport of selenite from the blood to the liver in the intact rat. Selenite uptake by kidney and spleen appears to be unaffected by arsenite, indicating that arsenite lowers selenite toxicity by its blocking action, and that the efficacy of arsenical feeds in the prevention of selenium poisoning is related to the level of arsenite maintained in the blood stream.

phosphate to the media or when lactate, acetate, or pyruvate are used as substrates.

Under the conditions of the previous experiments (*2*), any treatment that decreases inhibition is accompanied by a decrease in selenite uptake by the yeast cell. This is essentially an interference with a concentrating mechanism as, when aerated for 80 minutes at 31° C., 15 mg. of wet yeast removed half of the selenite from 3.0 ml. of suspending media which was $2.5 \times 10^{-5}M$ in selenite. This gives a final concentration of $2.5 \times 10^{-3}M$ inside the cells, which is about the same as that previously reported to be toxic to yeast in a phosphate buffer.

If such a concentrating mechanism were operative in any tissue or tissues of the animal body, that tissue should show a rapid rise in selenium following injection of selenite and should be more susceptible than other tissues to selenium toxicity. One of the chief symptoms of selenium toxicity is necrosis of the liver, and McConnell (*7*) has reported that concentration of selenium in the liver rapidly reaches a high value.

Moxon, Paynter, and Halverson (*9*) found that injected arsenite would protect rats against death from injected selenite when both salts were administered in small repeated doses. According to more recent work (*4*), arsenic will protect against single lethal doses of selenite. Arsenite will prevent the exhalation of volatile selenium compounds by selenite-injected rats (*5*). There is, then, reason for considerable interest in any effect of arsenite on the distribution of injected selenite.

Experimental

Male albino rats of the Sprague-Dawley strain were maintained on laboratory rations until they reached a weight of 150 to 200 grams. Selenite injections were made subcutaneously in the flank about 10 minutes after sodium arsenite had been injected in the opposite flank. Sodium selenite was used for preliminary studies and radioactive potassium selenite, obtained from the Oak Ridge national laboratories, was used for distribution studies. In the tracer studies, rats were anesthetized with ether, and an incision—sufficient to sever both the carotid artery and jugular vein—was made in the side of the neck. After blood had been collected in a citrated beaker, 10 ml. of a 5% citrate solution were injected into the left ventricle to facilitate drainage of blood from the body tissues.

Trichloroacetic acid extracts were prepared by mixing equal volumes of homogenate and 10% acid solution. The counts for all the tissues were uniformly low and appeared to have little significance; only the values for the liver extracts are reported.

Organs were removed from the animal and homogenized in the following amounts of water for each gram of organ: liver, 4 ml.; kidney, 1 ml.; and spleen, 2 ml. The variation in dilution of the homogenates was prompted by differences in ease of handling and in activity observed in preliminary experiments. One-milliliter aliquots of blood and tissue homogenates were pipetted into planchets and mixed immediately with 0.5 ml. of 25% mercuric acetate to reduce loss of selenium through volatilization. The samples were then dried and counted. The counts are reported as obtained, as it was felt that any effort to calculate actual distribution would yield unreliable and misleading figures, and would add nothing to information in the literature. The primary interest was in differences produced by arsenite injection, and the data are presented for the purpose of demonstrating these differences.

Results

Preliminary experiments indicated that 2 mg. of arsenic per kg. protected against a lethal dose of selenite (5 mg.), and that any combination of arsenite and selenite giving a total dose above 11 mg. of the elements per kg. was lethal (usually in 1 or 2 hours as against survival of 6 to 24 hours with doses of selenite well above the lethal level). In no case did animals survive if arsenic injection was delayed more than an hour after that of selenite.

Within the rather narrow limits indicated by these results, an experiment was set up in an effort to determine the toxicity of combinations of selenite and arsenite (Table I).

It was originally planned that work with selenium-75 would be done with levels of arsenite and selenite well below those known to cause toxic symptoms. However, the effects of arsenite fell off rapidly as the injection was decreased below 2 mg. of arsenic per kg. of body weight. For this reason, and to give fairly high counting levels, the experiments reported here were carried out with injections of 1.36 mg. of selenium and 2 mg. of arsenic per kilogram. A few animals received 3 mg. of arsenic per kg., but the results differ so slightly from those reported here that further work at this level was abandoned.

The only large differences obtained were those in blood and liver. The time-distribution curves in Figure 1 were obtained by plotting time of sacrifice of an animal against counts per minute for 1 ml. of blood. Selenite was injected at zero time. The time-distribution curves for the activity found in the liver homogenates of the same rats appear in Figure 2. The arsenite injections retarded the passage of selenium from the blood into the liver for several hours after the injection.

The kidney homogenates and the trichloroacetic acid extracts from the liver show little variation between treated and control animals (Figure 3). Figure 4 shows the values for the spleen homogenates; these follow somewhat the same pattern as is shown by the blood, but the values are much lower.

Discussion

Essentially, all that is established is the fact that arsenite definitely delays the entry of selenite into the liver. It would probably be of interest to determine whether or not this delay and the accompanying high blood-selenium levels are associated with increased urinary excretion. Arsenite prevents exhalation of volatile selenium by selenite-injected rats (*5*); hence it is reasonable to expect an increase in excretion by other routes.

While the evidence is still meager, this work supports the idea that the mechanism of selenite-arsenite antagonism is essentially the same in the rat as it is in yeast. There is also some evidence that acute toxicity of selenite is associated with the same mechanism which is responsible for the production of volatile selenium compounds.

The best explanation of observations that have been made on the action of selenite in the rat would appear to require an assumption that selenite can act in several ways and at different locations.

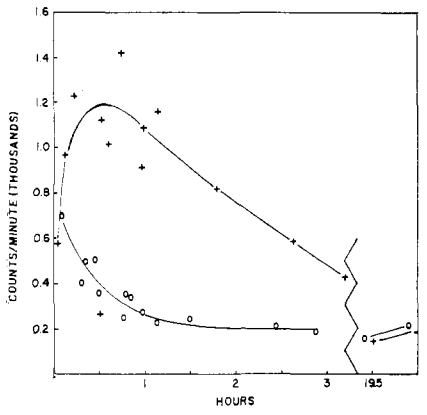

Figure 1. Time distribution of selenium in whole blood after injection of selenite and selenite plus arsenite

+ Values for rats injected with selenite plus arsenite

○ Values for rats injected with selenite

Each symbol represents the activity and time of sacrifice for one rat. Selenium-75 injected at zero time

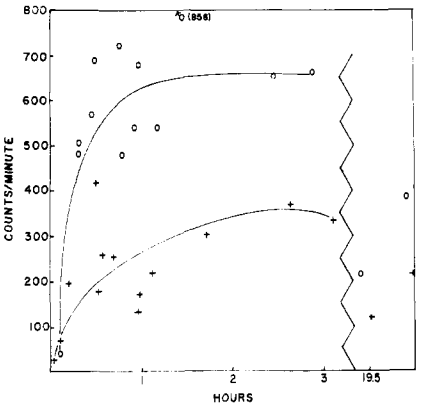

Figure 2. Time distribution of selenium in the liver after injection of selenite and selenite plus arsenite

+ Values for rats injected with selenite plus arsenite

○ Values for rats injected with only selenite

Each symbol represents the activity and time of sacrifice for one rat. Selenium-75 injected at zero time

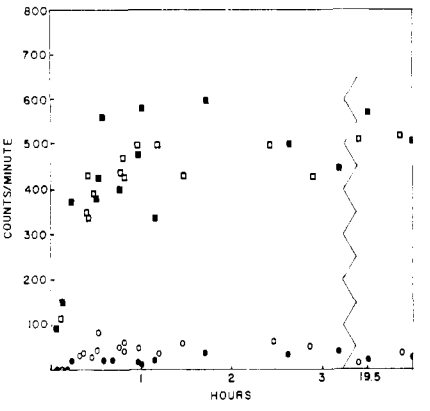

Figure 3. Time distribution of selenium in the kidney and in trichloroacetic acid extracts of the liver after injection of selenite and selenite plus arsenite

■ Activity in kidney of rats injected with selenite plus arsenite

□ Activity in kidney of rats injected with selenite only

● Activity in trichloroacetic acid extract of livers of rats injected with selenite plus arsenite

○ Activity in trichloroacetic acid extract of livers of rats injected with selenite only

The unique reaction of selenite would then be a rapid irreversible one antagonized by arsenic, involved in the formation of volatile selenium and probably initiated at the membrane of liver cells. Another reaction, toxic at a higher level of injection, although not necessarily at a higher concentration at the reaction site, would be a rapid reversible one in which its toxicity and that of arsenite are additive. This could be the reaction that Barron and Kalnitsky (*1*) found to be reversible by dithiols. A third reaction would be a rather slow, nonspecific, complex formation with proteins. This third reaction would account in some measure for the report by Klug, Lampson, and Moxon (*6*) that arsenite had no effect on distribution of selenium in rats that were protected from liver necrosis in a long-term experiment.

If these assumptions are reasonably accurate, the requirements for the blocking of acute selenite toxicity can be postulated rather simply. Enough arsenite must be present to block the first reaction without there being enough arsenite and selenite present to make the second lethal. If these conditions are met for an hour or two, the third reaction and excretion will render the selenite incapable of taking part in the unique reaction which leads to selenium exhalation and death.

Table I. Mortality in Rats Produced by Injecting Indicated Amounts of Selenium as Selenite and Arsenic as Arsenite per Kilogram of Body Weight

(Five rats in each group)

Se, Mg./Kg.	As, Mg./Kg. 0	3	4.5
4	4	0	0
5	4	0	0
6	5	0	0

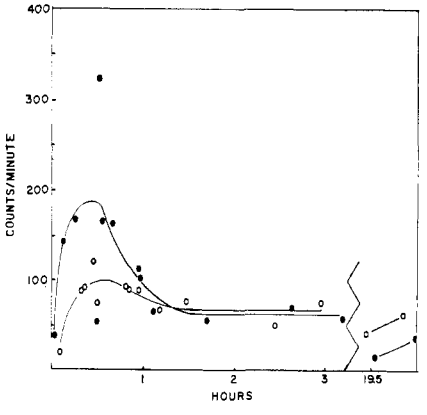

Figure 4. Time distribution of selenium in the spleen after injection with selenite and selenite plus arsenite

● Values for rats injected with selenite plus arsenite

○ Values for rats injected with selenite only

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Alcoholic Fermentation of Blackstrap Molasses—Correction

On page 610 [J. AGR. FOOD CHEM. **5,** 610 (1957)], the author credit should be Walter Borzani, Universidade de São Paulo, São Paulo, Brasil. On page 612, the title, "Continuous Fermentation," should read, "Molasses Fermentation."

WALTER BORZANI