Table I. Composition of Rations

Ration	Weaning to 125-Lb. Wt.	126-Lb. Weight to Market Wt.
Ground yellow corn	82.20	87.70
Soybean meal (50% protein)	13.00	7.50
Ground limestone	1.00	1.00
Steamed bonemeal	1.00	1.00
Iodized salt	0.50	0.50
Trace minerals[a]	0.05	0.05
Vitamin supplement[b]	0.10	0.10
Vitamin B_{12} supplement[c]	0.05	0.05
Antibiotic supplement[d]	0.10	0.10
Vitamin A and D mix[e]	2.00	2.00
% Digestible protein, calcd.	11.78	9.52
% Lysine, calcd.[f]	0.54	0.40

[a] Adds following to ration in p.p.m.: Mn, 29.6; Fe, 36.5; Cu, 2.5; Co, 0.83; Zn, 42; and K, 3.9.

[b] Contains 2000 mg. riboflavin, 4000 mg. pantothenic acid, 9000 mg. niacin, and 10,000 mg. choline chloride per pound of supplement.

[c] Contains a minimum of 9 mg. vitamin B_{12} per pound.

[d] Contains 10 grams terramycin per pound.

[e] Contains 14 grams vitamin A having 10,000 I.U. per gram; 4 grams vitamin D having 9 I.U. per gram; and 890 grams ground yellow corn.

[f] 0.4% L-Lysine added in addition to these amounts to the rations of half the swine.

crease in enzyme activity occurred. About half as much isocitric dehydrogenase activity was found in the liver as in the heart, but there was no dietary effect on the activity in the liver.

Table II. Feedlot Performance

	Control	+0.4% Lysine
No. swine	40	40
Initial wt., lb.	61.3	61.4
Final wt., lb.	196.1	193.0
Average daily gain, lb.	1.68	1.64
Average daily feed, lb.	5.67	5.58
Feed/lb. gain	3.38	3.40

Table III. Swine Carcass Data

	Control	+0.4% Lysine
No. swine	20	20
Slaughter wt., lb.	200.6	197.9
Back fat, in.	1.56	1.50
Carcass length, in.	28.94	29.15
Dressing, %	71.86	72.31
Ham, %	12.89	13.28
Loin, %	9.66	9.82
Picnic, %	6.19	6.24
Butt, %	4.24	4.48
Total % lean cuts (4)	32.98	33.82
Av. loin area, sq. in.	3.33	3.41

The data obtained for the xanthine oxidase activity in the liver are shown in Figure 2. Average values of 2.58 ± 0.68 and 2.82 ± 0.89 (μl.) of oxygen uptake per mg. of nitrogen per hour were found for the control and lysine-supplemented groups, respectively. Corresponding values of 42.4 ± 12.4 and 44.5 ± 15.8 μl. of oxygen uptake per gram of fresh weight per hour were obtained, respectively. These observations do not demonstrate as great a response to lysine as that of Bothwell and Williams (*3*), who reported that xanthine oxidase activity decreased to about 50% in the livers of rats deprived of lysine. The low lysine dietary group of swine in the present study were not as deprived of lysine as the rats referred to above. The present data suggest that lysine functions in swine in a manner similar to that in rats.

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Literature Cited

(1) Aquirre, A., Wallace, H. D., Combs, G. E., *J. Anim. Sci.* **19,** 1246 (1960).
(2) Baur, L. S., Filer, L. J., Jr., *J. Nutr.* **66,** 545–54 (1958).
(3) Bothwell, J. W., Williams, J. N., Jr., *Proc. Soc. Exptl. Biol. Med.* **85,** 544–7 (1954).
(4) Cahilly, G. M., Kelly, R. F., Brooks, C. C., Davis, J. W., Graham, P. P., *J. Anim. Sci.* **19,** 1232 (1960).
(5) Colowick, S. P., Kaplan, N. O., eds., "Methods in Enzymology," Vol. **1,** pp. 699–704, Academic Press, New York, 1955.
(6) Dhungat, S. B., Sreenivasan, A., *J. Biol. Chem.* **208,** 845–51 (1954).
(7) Siebert, G., Dubuc, J., Warner, R. C., Plaut, G. W. E., *Ibid.*, **226** 965–75 (1957).
(8) Snedecor, G. W., "Statistical Methods," 5th ed., Iowa State University Press, Ames, Iowa, 1956.

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SELENIUM TOXICITY

Effect of Arsenic on Selenium Metabolism in Rats

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Since Moxon (*11*) found that arsenic reduced the toxicity of seleniferous grains, a number of animal studies on the effect of arsenic compounds on selenium metabolism have been reported. Moxon and DuBois (*12*) concluded that the selenium content of livers from rats on a diet containing seleniferous wheat was decreased by sodium arsenite addition to the water. While limited data for swine under similar conditions indicated that arsenic increased the selenium content of the liver and decreased it in the hair and certain other tissues (*10*), results with dogs (*19*), cattle (*14*), and rats (*7, 13, 15*) on chronically toxic diets show no role for arsenic in the alteration of selenium metabolism. Further, Petersen *et al.* (*18*) administered sublethal doses of selenite and selenite plus arsenite to rats by stomach tube and concluded from their results that arsenite did not cause any significant variation in the absorption, excretion, or tissue deposition of the selenium. On the other hand, recent studies with chickens indicate that arsenic in seleniferous diets decreases selenium deposition in eggs (*8*) and increases it in muscle and liver tissues (*1*).

Kamstra and Bonhorst (*5*) first reported an effect for arsenic on the exhalation of selenium. They found that the amount of selenium exhaled by rats injected with sublethal doses of selenite was greatly reduced by arsenite injections. This work was confirmed by Ganther and Baumann (*2*), who also found that arsenite markedly increased excretion of the selenium into the gastrointestinal tract and its level in the kidneys while reducing its level in the blood, liver, and carcass. Palmer and Bonhorst (*16*) in a similar study found that arsenite decreased the level of selenium in the liver, but for periods of up to 3 hours following injection the arsenite increased the selenium in the blood.

The variability in the results of the

Investigations of the effect of arsenic on the metabolism of selenium by rats were made in an effort to explain the protective effect of arsenicals in chronic selenium poisoning. When selenite, selenate, arsenite, and arsenate injections were used, selenium exhalation was markedly reduced by the arsenic. As the level of selenium was decreased, however, its exhalation fell off rapidly and, at levels of the order of those found in chronically toxic diets, became quantitatively unimportant in the excretion of the element. Further, under conditions involving low levels of selenium, no effect of arsenic on selenium exhalation or excretion in the urine or feces could be demonstrated.

experiments cited has been explained, at least in part, by Ganther and Baumann (*2*). Among the factors which they discussed, the effect of the level of selenium administered seems significant in comparing the metabolism of this element in acute and chronic selenosis. The importance of the level of selenium administered is emphasized in the following investigations of the effect of arsenic on selenium metabolism by rats.

Materials

The rats used in the various experiments were all males of the Sprague-Dawley strain. Sodium and potassium selenate were purified as previously described (*4*), and the radioactive selenium used was obtained from Oak Ridge National Laboratories in the form of $H_2Se^{75}O_3$. Wheat containing 20 p.p.m. of selenium on analysis by the Klein method (*6*) was obtained from a seleniferous area. Analytical reagent grade sodium arsenite and sodium arsenate were used, and the arsanilic acid was supplied by Abbott Laboratories (North Chicago, Ill.).

Experimental

Exhalation Studies. Equipment used to collect selenium exhaled by the rats consisted of a rectangular plastic chamber of about 3 liters volume with a small air inlet near the top and a side outlet connected in series with two all-glass absorption bottles. The bottles were fitted with a sintered glass inlet and contained 75 ml. of 1% mercuric oxide in concentrated nitric acid. They were in turn connected to a vacuum source adjusted to draw 400 to 500 ml. of air through the system each minute. All connections were of glass or of tygon tubing. Two such systems were built, and they were alternated in use to ensure that the effect of any treatment was not the result of differences in the systems.

To determine whether the chemical form of selenium or of arsenic affects the amount of selenium exhaled, rats averaging about 250 grams and maintained on a diet of Purina Laboratory Chow (Ralston-Purina Co., St. Louis, Mo.) were injected intraperitoneally with sodium selenite or with potassium selenate, both with and without the simultaneous injection of either sodium arsenite or sodium arsenate. The salts were injected at a level of 2 mg. of selenium or of both selenium and arsenic per kg. of body weight. When both selenium and arsenic salts were injected, they were mixed and given in a single dose. Immediately after injection, the rats were placed in the plastic chamber, and the collection of volatile selenium was started. After 6 hours, the rats were removed, and the contents of both absorption flasks were combined and analyzed for selenium by the method of Klein (*6*).

A study of the amount of selenium exhaled by rats on naturally seleniferous diets, and the effect of arsanilic acid in the diet on selenium exhalation, was next made. Rats that had been on Purina Laboratory Chow until they reached about 400 grams in weight were used. They were starved for a 24-hour period, and then one of the rats was allowed to consume a seleniferous diet for 1 hour. The diet contained 10 p.p.m. of selenium and was composed as follows: wheat (nonseleniferous), 30.9%; wheat (seleniferous), 50.0%; casein, 12.0%; salts USP XIV, 2.0%; corn oil, 3.0%; and vitamin B_{12}–starch mix (27.5 μg. of B_{12} per gram), 0.1%. An additional rat was allowed to consume the same diet, to which 0.015% of arsanilic acid had been added, for 1 hour. The amount of feed eaten was determined, and the rats were placed in the equipment used to collect exhaled selenium. After 8 hours, the rats were removed and starved overnight. On the following 3 days, the treatment was repeated for each rat. This extended collection period was necessary to provide enough selenium in the absorption bottles for analysis by the Klein method. Four such paired collections were completed.

In a similar experiment, radioactive selenium as selenite was used. Normal corn (nonseleniferous) replaced the wheat in the diet, and nonradioactive sodium selenite was added at a level of 3 p.p.m. of selenium. To half of this diet, sodium arsenite was added at a level of 15 p.p.m. of arsenic. Rats averaging 70 grams in weight were individually housed on wire and fed Purina Laboratory Chow to a weight of about 90 grams; these were used in the experiment. One rat was placed on the diet containing selenite, another on the diet containing selenite plus arsenite. After 5 days on these diets, feed was restricted to 5 grams per day on two consecutive days. On the following day, the individual rats were allowed 3 grams of feed to which radioactive selenite (63 μc. radioactivity in 1.5 μg. of selenium) had been added. After allowing 1 hour for consumption of the feed, the rats were placed in the breath collection system which was somewhat modified. For experiments in which radioactive selenium was used, a 1- × 60-cm. column with a fritted glass disk at the bottom replaced the first absorption bottle. It contained 15 ml. of the mercuric oxide–nitric acid mixture, and efficiently trapped the selenium, no significant count being recovered in the absorption bottle following it in the system. After about 6 hours, the rats were removed, and the absorption mixture was made to a definite volume and counted using a Geiger-Mueller dip counting tube. Six pairs of rats were treated in this manner, except that two of the pairs were fed in the collection chamber, consuming their feed in about 30 minutes. This change in procedure

Table I. Selenium Exhalation by Rats Following Injection of Selenite or Selenate and Effect of Simultaneous Injections of Arsenite or Arsenate

Av. Wt. of Rats[a] (Grams)	Salt Injected[b]	Se Exhaled in 6 Hours (% Injected)
210	Sodium selenite	22
258	Sodium selenite + sodium arsenite	2
246	Sodium selenite	25
278	Sodium selenite + sodium arsenate	2
300	Potassium selenate	11
286	Potassium selenate + sodium arsenite	2
191	Potassium selenate	16
213	Potassium selenate + sodium arsenate	1

[a] All data are averages for 3 rats.
[b] Salts were injected at a level of 2 mg. of Se or As per kg. of body weight.

seemed to have no effect on the results.

Following the suggestion by Ganther and Baumann (*2*) that the amount of selenium injected appeared to influence the distribution of the element in rats, a study of the relationship between the level of injected selenite and the amount exhaled was made. In addition, the effect of level of injected arsenite was studied. Rats which had been maintained on laboratory chow were used. They were injected intraperitoneally with 1 ml. of a solution containing $Na_2Se^{75}O_3$ (4.3 μc.) plus sufficient carrier sodium selenite to bring the level of selenium to that desired. When arsenite was administered with the selenite, it was added at the desired level and a single injection was made. In all cases, the volume of solution administered was 1 ml. Immediately following injection, the rats were placed in the collection system for from 4 to 6 hours, and the measurement of radioactive selenium collected was made as previously described here.

Excretion Study. As part of an experiment on the effect of sulfate on selenium excretion reported by Halverson *et al.* (*4*), the effect of arsanilic acid on fecal and urinary excretion of selenium was investigated. Rats averaging about 90 grams in weight were allotted to two groups of eight animals each. They were individually housed in stainless steel metabolism cages. One group was fed a basal corn-type diet (*4*) containing 5 p.p.m. of selenium added as potassium selenate. The other was fed the same diet to which 0.015% of arsanilic acid was added. All rats were given an equal amount of feed each day, the amount given being increased with time and being such that all of it was consumed. Four consecutive 4-day periods of urine and feces collection were used during the experiment. Urine and feces from each animal were collected separately. The individual samples were pooled in a manner that gave two pooled samples per group per period. The pooled samples were analyzed in duplicate for selenium (*6*).

Results

Exhalation Studies. The results in Table I confirm the reports of others (*2, 5*) that arsenite markedly reduces the exhalation of selenium by rats injected with selenite at a level approaching an acutely toxic dose. Indeed, both arsenite and arsenate were effective in this respect when injected in combination with either selenite or selenate. The data also indicate that the rat is more efficient in converting selenite to the volatile form than it is in converting selenate. Whether this means that selenite is an intermediate in the metabolism of selenate needs further study.

The data for the exhalation of selenium where rats were fed diets containing seleniferous wheat are summarized in Table II. Under the conditions used, the percentage of ingested selenium exhaled was very small. This is in agreement with the findings of Ganther and Baumann (*3*) for rats fed selenate. Feeding arsanilic acid caused a slight reduction in the average value; however, this difference was largely the result of the value obtained for one rat in the seleniferous group, and it failed to prove statistically significant. Since the rats were in the collection equipment for only one third of the experimental period, the data do not represent the entire amount exhaled. However, in view of the report of McConnell (*9*), most of the selenium exhalation probably occurred during the collection period.

The data in Table III confirm those in Table II. The percentage of ingested selenium exhaled during the collection period was again very small, and the sodium arsenite apparently had no effect on it. Longer collection periods might have given somewhat greater recoveries, but they would also increase the error should there be any volatilization from the feces.

Table IV shows that as the level of injected selenium is decreased the percentage exhaled falls off rapidly. At the lower levels of injection, it approximates those found in the feeding experiment. Ganther and Baumann (*3*) also found that when either selenite or selenate was injected at low levels only small percentages of the selenium were exhaled. At the highest level of injection, the percentage of selenium exhaled was somewhat higher than that shown in Table I or reported by Kamstra and Bonhorst (*5*). This may indicate a superiority for the isotopic method over the chemical method of determination. The percentage of injected selenium recovered when arsenite was injected

Table II. Exhalation of Selenium by Rats Fed Diets Containing Seleniferous Wheat with and without Arsanilic Acid

Diet	Av. Initial Wt. of Rats[a] (Grams)	Av. Se Ingested (μg.)	Av. Se Exhaled[b] (% Ingested)
Seleniferous	399	396	1.8 (0.8–4.3)
Seleniferous + arsanilic acid	402	427	1.2 (0.8–1.8)

[a] Data are averages for 4 rats.
[b] Selenium exhaled during 8-hour collection period following feeding. Figures in parentheses show range in results. $P = 0.41$ by t test for paired values (*21*).

Table III. Exhalation of Selenium by Rats Fed Diets Containing Sodium Selenite with and without Sodium Arsenite

Diet	Av. Initial Wt. of Rats[a] (Grams)	Av. Se Ingested[b] (μg.)	Av. Se Exhaled[c] (% Ingested)
Seleniferous	139	9.80	0.45 (0.21–0.67)
Seleniferous + sodium arsenite	144	9.95	0.45 (0.16–0.67)

[a] Data are averages for 6 rats.
[b] Selenium ingested during feeding period just prior to breath collection.
[c] Selenium exhaled during 6-hour collection period. Figures in parentheses show ranges in values.

Table IV. Effect of Level of Selenite and of Arsenite Injection on Exhalation of Selenium by Rats

Weight of Rat (Grams)	Selenite Injected (Mg. of Se/Kg. Body Wt.)	Arsenite Injected (Mg. of As/Kg. Body Wt.)	Collection Period (Hr.)	Se Exhaled (% Injected)
		Effect of Selenite Level		
162	1.9	0.0	5.5	32.8
174	1.9	0.0	6.0	35.6
246	1.9	0.0	6.0	28.2
258	1.9	0.0	6.0	20.9
200	1.5	0.0	6.0	20.2
262	1.5	0.0	6.0	25.2
203	1.2	0.0	6.0	11.5
263	1.2	0.0	6.0	14.6
219	0.9	0.0	6.0	9.1
259	0.9	0.0	4.5	9.1
217	0.4	0.0	6.0	1.9
269	0.4	0.0	4.5	2.8
203	0.020	0.0	6.0	0.5
213	0.019	0.0	6.0	0.5
245	0.016	0.0	6.0	1.1
		Effect of Arsenite Level		
160	1.9	3.6	5.5	0.9
172	1.9	3.6	6.0	1.1
226	1.2	3.6	6.0	1.5
226	1.2	1.9	6.0	2.4
222	1.2	0.9	6.0	2.5
111	1.2	0.4	6.0	3.6
136	1.2	0.2	6.0	6.1

Table V. Excretion of Selenium in Urine and Feces of Rats on Diets Containing Selenate with and without Arsanilic Acid

Diet	Period (Days)	Av. Final Wt. of Rats (Grams)	Se Ingested per Rat (μg.)	Feces Excreted per Rat (Grams)	Urine Excreted per Rat (Grams)	Se Content (P.P.M.)		Se Excreted (% Ingested)		Se Not Accounted For (% Ingested)
						Feces	Urine	Feces	Urine	
Basal + 5 p.p.m. Se	1– 4	96	120	3.6	16.1	3.3	2.7	10	36	54
	5– 8	114	155	2.9	19.9	5.4	3.6	10	47	43
	9–12	131	195	4.5	40.6	5.5	2.4	13	48	39
	13–16	147	205	4.4	35.0	6.7	3.7	14	63	23
	All	147	675	15.4	111.6	5.3	3.1	12	51	37
Basal + 5 p.p.m. Se + 0.015% arsanilic acid	1– 4	98	120	4.1	7.8	2.5	5.4	8	35	57
	5– 8	114	155	3.6	11.1	5.4	6.1	12	43	44
	9–12	131	195	4.6	19.3	5.3	4.9	12	49	39
	13–16	146	205	4.6	23.1	4.9	5.5	11	68	21
	All	146	675	16.9	61.3	4.5	5.4	11	49	40

was smaller than that reported by Ganther and Baumann (*2*), which may indicate that the arsenite is more efficient when administered simultaneously with the selenium than when administered in a separate dose prior to injecting the selenite. The effect of the injected arsenite appears to decrease gradually as its level of injection is decreased.

Excretion Study. The data in Table V indicate that the feeding of arsanilic acid had no effect on the selenium content of the feces, or on the percentage of ingested selenium excreted in them. Urine volume was apparently reduced by the arsenical, but the selenium content of the urine was increased, and the amount of selenium excreted in the urine was not altered.

In view of the data in Tables II and III, probably only a very small part of the ingested selenium was excreted in the breath in this experiment, and the selenium not accounted for would be almost entirely that deposited in the body tissues. Based on this assumption, it would appear that the arsenical had no effect on the total amount of selenium deposited there. The arsenical may have altered the selenium content of individual tissues, and this matter needs clarification.

Discussion

That arsenic alters the metabolism of selenium by rats when the dosage of selenium approaches an acutely toxic level has been well documented here and elsewhere (*2, 5*). These same studies and those of others (*9, 17, 20*) have shown the exhalation of selenium to be an important route of excretion when a high level is administered. However, in the studies reported here, the importance of exhalation as a route of excretion diminished markedly as the level administered was decreased. On diets containing 10 p.p.m. of selenium from wheat or 3 p.p.m. of selenium as selenite, and at low levels of injection, the volatilization of the element apparently played a very minor role in its elimination from the rat body. Further, no effect of arsenic on the exhalation of selenium by rats fed seleniferous diets could be demonstrated.

Ganther and Baumann (*2*) reported that when levels of selenium of 1.5 or 2.0 mg. per kg. of body weight were injected, arsenic markedly increased selenium excreted in the feces, decreasing that deposited in the carcass, blood, and liver. Petersen *et al.* (*18*), however, have reported that arsenic has no effect on exhalation, urinary or fecal excretion, or on the deposition of selenium in tissues when 1.4 mg. of sodium selenite per kg. of body weight was administered by stomach tube. Klug *et al.* (*7*) have also reported that arsenic does not alter the deposition of selenium in the tissues of rats fed a diet containing 10.3 p.p.m. of selenium from corn. The data reported here show no effect for the arsenic on excretion of selenium in the breath, urine, or feces of rats fed low levels of selenium. In these various experiments, differences are found in the diets used, the levels of arsenic administered, the age or size of the rats, the route of administration of the selenium and arsenic, and the chemical forms of the elements used. However, the level of selenium administered appears to be the most important consideration in explaining the differences in the findings discussed above, and the amount of an arsenical used to counteract chronic selenium poisoning does not markedly alter excretion in the feces, urine, or breath. Unpublished data from this laboratory indicating that arsenicals do reduce the selenium content of livers of rats on chronically toxic diets, and the reports of other workers (*1, 8, 10, 12*), indicate a need for further study of the effect of arsenic on the deposition of selenium in various tissues at low levels of intake of the element.

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Literature Cited

(1) Carlson, C. W., Guss, P. L., Olson, O. E., *Poultry Sci.* **41,** 1987 (1962).
(2) Ganther, H. E., Baumann, C. A., *J. Nutr.* **77,** 210 (1962).
(3) *Ibid.*, p. 408.
(4) Halverson, A. W., Guss, P. L., Olson, O. E., *J. Nutr.* **77,** 459 (1962).
(5) Kamstra, L. D., Bonhorst, C. W., *Proc. S. Dakota Acad. Sci.* **32,** 72 (1953).
(6) Klein, A. K., *J. Assoc. Offic. Agr. Chemists* **24,** 363 (1941).
(7) Klug, H. L., Lampson, G. P., Moxon, A. L., *Proc. S. Dakota Acad. Sci.* **29,** 57 (1950).
(8) Krista, L. M., Carlson, C. W., Olson, O. E., *Poultry Sci.* **40,** 1365 (1961).
(9) McConnell, K. P., *J. Biol. Chem.* **145,** 55 (1942).
(10) Moxon, A. L., *Proc. S. Dakota Acad. Sci.* **21,** 34 (1941).
(11) Moxon, A. L., *Science* **88,** 81 (1938).
(12) Moxon, A. L., DuBois, K. P., *J. Nutr.* **18,** 447 (1939).
(13) Moxon, A. L., Paynter, C. R., Halverson, A. W., *J. Pharmacol. Exptl. Therap.* **84,** 115 (1945).
(14) Moxon, A. L., Rhian, M., Anderson, H. D., Olson, O. E., *J. Animal Sci.* **3,** 299 (1944).
(15) Olson, O. E., Halverson, A. W., *Proc. S. Dakota Acad. Sci.* **33,** 90 (1954).
(16) Palmer, I. S., Bonhorst, C. W., J. AGR. FOOD CHEM. **5,** 928 (1957).
(17) Petersen, D. F., Klug, H. L., Harshfield, R. D., *Proc. S. Dakota Acad. Sci.* **30,** 73 (1951).
(18) Petersen, D. F., Klug, H. L., Harshfield, R. D., Moxon, A. L., *Ibid.*, **29,** 123 (1950).
(19) Rhian, M., Moxon, A. L., *J. Pharmacol. Exptl. Therap.* **78,** 249 (1943).
(20) Schultz, J., Lewis, H. B., *J. Biol. Chem.* **133,** 199 (1940).
(21) Snedecor, G. W., "Statistical Methods," 4th ed., p. 43, Iowa State College Press, 1946.

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