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Modification of Selenium Distribution in the Rat

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MODIFICATION OF SELENIUM DISTRIBUTION IN THE RAT

By

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

In many semiarid regions plants may take up toxic quantities of selenium. If livestock consume very large amounts of such plants they will ingest enough selenium to produce toxic symptoms. This presents a serious problem to the farmers and ranchers in such areas and is certainly the cause of much financial loss.

Since the discovery by Franke in 1934 (5) that selenium was the cause of the condition then known as "alkali disease", a great deal of work has been done on the problem of selenium poisoning. As a result of this research, several substances have been discovered which give complete or at least partial protection against selenium poisoning. Although protection by certain compounds has been established, very little work has been done which aids in the elucidation of the actual mechanism involved in the protective action.

Recently it has been shown that reduction of selenite inhibition of yeast respiration by arsenite and lactate was due to interference with a concentrating mechanism (3). This study was undertaken in the hope that an investigation of the effect of these substances on selenium distribution in the rat might provide new information as to the mechanism of the protective action.

REVIEW OF LITERATURE

A review of the selenium literature has not been made in recent years but there are a few reviews which cover the literature up to 1949. A thorough discussion of the selenium research which had been done at South Dakota State College up to 1937 was prepared by Moxon (18). In 1943 Moxon and Rhian (22) prepared a general review of the literature up to that date. The latest history and review of the selenium problem was prepared by Trelease and Beath (32), who stated the scope of their book as "Selenium-its geological occurrence and its biological effects in relation to botany, chemistry, agriculture, nutrition and medicine."

The extensive research resulting from the discovery that selenium was the cause of "alkali disease" lead to the disclosure of the protective effect of arsenic against selenium poisoning. Moxon (19) was the first investigator to demonstrate the protective action of arsenic. In the course of his studies on the combined toxicities of selenium, tellurium, arsenic, vanadium, nickel, tungsten, and molybdenum, he found that sodium arsenite in the drinking water would alleviate liver damage in rats fed on seleniferous wheat. In 1939 (21) a more complete report was made on similar work in which rats were again fed on seleniferous wheat with various elements being administered in the drinking water. Molybdenum, fluorine, chromium, vanadium, cadmium, zinc, cobalt and uranium all caused an increase in mortality while sodium tungstate brought about a partial decrease in liver damage and mortality. Sodium arsenite again gave good protection at the level of 5 parts per million of arsenic. When a lower level of arsenic was used, characteristic

liver damage prevailed but some of the other symptoms of selenium poisoning were alleviated.

The demonstration of the selenium-arsenic antagonism in the rat was followed shortly by the demonstration of the same antagonism in various other animals. It has been shown (29) that the typical symptoms of selenium poisoning in the dog could be alleviated by feeding sodium arsenite. Moxon (20) succeeded in demonstrating the protective effect of arsenic against selenium in hogs by placing sodium arsenite in their drinking water. In later studies with organic arsenicals, Wahlstrom and others (34) reported the same result. Moxon and others (26) in studying the antagonism in cattle found that steers on seleniferous range showed improved condition and growth when sodium arsenite was blended with the regular salt mix. When chickens were used as the experimental subjects the protective effect of arsenic was more difficult to demonstrate except in the case of hatchability. Moxon and Wilson (23) were successful in showing that 2.5 parts per million of arsenic in the drinking water of laying hens was sufficient to partially counteract the depressant effect of selenium upon hatchability, but complete protection was not afforded even at the 5 parts per million level of arsenic.

Several forms of arsenic have been shown to be effective in the prevention of symptoms of selenium poisoning. Besides the form of sodium arsenite which has already been mentioned, DuBois and others (4) have shown that sodium arsenate was effective against selenium in the form of seleniferous wheat, sodium selenite or selenium cystine but that the arsenic sulfides As_2S_3 and As_2S_5 were ineffective. Many organic arsenicals have been found to give protection against selenium poisoning

but usually to a lesser extent than do inorganic salts. For instance it has been shown (9) that arsanilic acid and 3-nitro-4-hydroxyphenylarsonic acid will give partial protection against as much as 19 parts per million of selenium fed to rats in the form of seleniferous wheat. Neither compound gave complete protection, however, even when they were included at levels of 86 parts per million and 25.6 parts per million of arsenic, respectively. The effect of these same compounds in hogs (34) was slightly different since 0.02 percent arsanilic acid and 0.005 percent 3-nitro-4-hydroxyphenylarsonic acid gave excellent protection against ingested selenium.

A few other organic arsenicals have been used with varying degrees of success. Moxon and others (24) could obtain only partial protection by feeding neoarsphenamine and sulfarsphenamine to rats receiving selenium in their diets. In some later work Hendrick and Olson (8) fed sodium methyl arsenate and calcium methyl arsonate to rats but were not able to obtain any protection against the symptoms of selenium poisoning.

Despite the numerous papers that have been published pertaining to the antagonism between selenium and arsenic, little is known of the actual mechanism involved. The suggestion has been made (1) that the absorption of selenium is decreased due to the combination of selenium and arsenic in the gastrointestinal tract. Moxon and others (25) were able to show, however, that the protection by arsenic against selenium induced liver damage and death was independent of the route of administration of either substance. Since injected arsenic will give protection against orally administered selenium it seems unlikely that the

protective mechanism could be merely an interference with absorption.

Klug and others (13) have suggested the mechanism might involve an important enzyme system such as succinic dehydrogenase. They were able to demonstrate that the levels of this enzyme in the livers of rats were lowered when sodium selenite was added to the diet. The succinic dehydrogenase levels could be returned to normal, however, by administration of sodium arsenite.

A few facts as to the mechanism of the antagonism have been ascertained by work with rats. Several workers have shown that rats will exhale a volatile compound when injected with selenium, but varying ranges of excretion have been reported. Schultz and Lewis (30) discovered that rats injected with sodium selenite would exhale 17 to 52 percent of the injected dose within eight hours. When Peterson and others (27) injected rats intraperitoneally with sodium selenite they were able to recover 30 to 35 percent of the injected dose as a volatile excretory product. A comparatively low recovery was obtained by McConnell (17) when he injected rats with sodium selenate and was able to trap only 3 to 10 percent of the injected dose at the end of twenty four hours. Under the conditions of his experiment it was found that the selenium content of the liver was the highest of any organ tested. Because of its relatively high selenium concentration and the fact that the liver mince gave off a garlicky odor characteristic of the volatile compound, he suggested that the liver was the production site for the compound. It was not until several years later that a study was made to determine the effect of arsenic upon the excretion of the volatile product. Kamstra and Bonhorst (11) conducted a study using sodium selenite in which they

recovered 7.6 to 23.6 percent of the injected dose as a volatile product. Upon injection of sodium arsenite, however, the excretion of the volatile selenium compound was almost completely inhibited.

Studies on the distribution of selenium in tissues of animals have included few in which the effects of various protective substances were considered. When McConnell (16) injected rats with radioactive sodium selenate, he found that the selenium level in the blood reached its peak in two hours. The liver, kidney and intestinal tract contained the highest concentrations of selenium of the organs tested, with the liver having the highest of the three. In a similar study with mice, Heinrich and Kelsey (7) also used radicisotopes to study the selenium distribution. When they injected sodium selenite they found that one half of the injected dose was in the liver at the end of the first hour.

Peterson and others (28) have also investigated the effect of arsenic on selenium distribution in rats by means of feeding experiments. Sodium arsenate and sodium selenite were administered intragastrically with a blunt hypodermic syringe and, after a period of several days of feeding, an analysis was made of the tissues and excretions. No differences were found in the selenium content of tissues from rats receiving both selenium and arsenic and those receiving only selenium. The excretion of selenium by the kidneys also was not affected by administration of the arsenic. In a similar experiment Klug and others (12) fed rats a diet containing seleniferous corn and then added 5 parts per million of arsenic as sodium arsenite to the drinking water. After a period of 10 to 12 weeks the tissues of the rats were analyzed but again no significant difference could be seen between the selenium content of

the rats receiving both arsenic and selenium and those receiving only selenium.

Bonhorst (2) has contributed some of the most recent information concerning the mechanism of the selenium-arsenic antagonism in a study on the effect of various anions upon yeast respiration. He reported that 10^{-4} M selenite inhibited yeast respiration when glucose or ethyl alcohol was used as substrate. This inhibition was reduced by the presence of arsenite, arsenate, or phosphate. When lactate, acetate or pyruvate was used as substrate it was found that selenite did not inhibit yeast respiration. Recent data (3) show that the decrease in inhibition of respiration in yeast by the arsenic and other protective substances was accompanied by a decrease in selenite uptake. This was essentially due to an interference with a concentrating mechanism since 15 milligrams of wet yeast removed one half of the selenite from 3 milliliters of medium which was 2.5×10^{-5} M in selenite. If such a concentrating mechanism were present in certain tissues of animals, one would expect a rapid rise in selenium content of such tissues after injection of selenite. It should be possible to design a distribution study which would give information concerning the presence of such a concentrating mechanism and the effect on it produced by various protective substances.

MATERIALS AND METHODS

All experiments were carried out with male albino rats of the Sprague-Dawley strain. In each instance the selenium, which was used for injecting the rats, was in the form of radioactive selenite obtained from the Oak Ridge Laboratories.

Arsenic-Selenium Experiment: All injections in this study were made subcutaneously in the flank. The injections of sodium arsenite preceded the selenite injections by ten minutes and were made in the opposite flank. At varying times after the selenite injections the rats were anesthetized with ether. The carotid artery and the jugular vein were then severed and a blood sample collected in a beaker containing sodium citrate as an anticoagulant. After the blood sample had been collected, 10 milliliters of a 5 percent solution of sodium citrate were injected into the left ventricle of the heart in order to flush the blood from the intact organs. This method gave very good results when pressure was applied to the severed vessels in the neck to prevent leakage. In most instances the kidneys and livers were straw colored when removed.

Homogenates were prepared from the liver, kidney and spleen for study of selenium distribution since these organs have been reported to accumulate the highest levels of selenium (16). The milliliters of water added per gram of organ in preparing the homogenates were: liver, 4; kidney, 1; spleen, 2. The use of varying amounts was prompted by differences in ease of preparing and pipetting the various homogenates as well as differences in activities. Trichloroacetic acid extracts were also prepared from the liver homogenates and blood by mixing 1 milliliter of the sample with 1 milliliter of a 10 percent trichloroacetic acid solution

and then centrifuging. One milliliter portions of the blood, homogenates and trichloroacetic acid extracts were pipetted into planchets and mixed with 0.5 milliliter of a 25 percent solution of mercuric acetate to reduce loss of the selenium by volatilization. The samples were then dried over a hot water bath and counted with a Geiger-Muller counter.

In all the distribution studies in which the samples were dried and counted, there was considerable self-absorption by the biological material. Approximately 65 percent of the activity in the blood and 55 percent of that in the liver was not available for counting because of this absorption. This was not considered to affect the trend of the results since the samples which were compared received identical treatment. In every case the data were reported as obtained except for correction of the activity for background and dead time of the counting tube. No attempt was made to calculate actual distribution by taking into consideration dilutions and absorption since work has already been done using methods which gave results requiring much smaller corrections (7,12,16, 28).

Lactate-Selenium Experiment: In general the procedure for this study was the same as that described under the arsenic-selenium experiment. A few variations which were introduced into the procedure are given below. Rats were fasted twenty-four hours prior to the start of the experiment so that the glycogen levels would be as low as possible. The selenite injections were made ten minutes after magnesium lactate injections and, twenty-five minutes later, the rats killed by a blow to the head. Ether anesthesia was avoided in this experiment since it has been reported (31) to affect lactic acid levels. A blood sample was collected as previously

described but in this instance sodium fluoride was used as an anticoagulant to prevent further glycolysis. The remainder of the procedure for the distribution study involving lactate was carried out as described under the arsenic-selenium section with the exception that only liver homogenates (3 milliliters of water to 1 gram of liver) and blood were used to check the effects of lactate on selenium distribution.

In checking the effect of injected magnesium lactate upon lactic acid levels in the blood, the following procedure was used for determining lactic acid. A blood sample was collected as described and a protein free filtrate of the blood to be used for analysis was prepared according to the Van Slyke and Hawkins modification of the method of Folin and Wu (33). A freshly prepared mixture of eight parts of N/12 sulfuric acid and one part of a 10 percent solution of sodium tungstate was added directly to a sample of 1 milliliter of blood in a centrifuge tube. The coagulated protein was then removed by centrifugation. The quantity of lactic acid in the blood was determined by a slightly modified version of the method described by LePage (14). The principle of the determination is that lactic acid is oxidized to acetaldehyde by heating in concentrated sulfuric acid and then a quantitative color reaction is used for the determination of acetaldehyde. The quantities of all substances described in LePage's procedure were doubled to give added ease in handling. A lactic acid standard solution containing 1 milligram of lactic acid in 5 milliliters was prepared from lithium lactate (6) and then a working stock solution was prepared from this solution each day. Five milliliters of the working stock solution (equivalent to 0.05 milligram of lactic acid) were added to a clean tube and carried through the pro-

cedure with the blood filtrate. In the actual analysis, 1 milliliter of each protein free filtrate was placed in a clean centrifuge tube to which 20 percent copper sulfate had been added. Each tube was then filled to the 10 milliliter mark with water. Approximately 1 gram of calcium hydroxide was added to remove interfering materials and then the tubes were allowed to stand for thirty minutes with frequent shaking. After the thirty minute period the solution was centrifuged and a 1 milliliter portion of the supernatant was transferred to a clean test tube containing two drops of a 4 percent copper sulfate solution. Seven milliliters of concentrated sulfuric acid were added slowly while shaking the tube in an ice bath to prevent further oxidation of acetaldehyde to acetic acid. The volume of seven milliliters of acid was chosen since this was the smallest quantity that could be read conveniently with an Evelyn colorimeter. This quantity is different from that described in LePage's procedure but Stone (15) has stated that a range of 6.15 to 9 volumes of sulfuric acid to one volume of sample will give maximum color. The tubes were placed in boiling water for five minutes and then cooled to below 20°Centigrade. Two drops of a 1.5 percent p-hydroxydiphenyl solution were then dispersed in the sample and the tubes incubated for thirty minutes at 28 to 30°Centigrade. At the end of this time they were placed in boiling water for ninety seconds to dissolve any excess reagent, cooled and transferred to colorimeter tubes. The transmittance was determined at 565 millimicrons on an Evelyn colorimeter, using a reagent blank to zero the instrument. The percent transmittance was converted to optical density and the amount of lactate calculated by the following form:

$$\frac{\text{Density of unknown}}{\text{Density of standard}} \times .005 \times 100 \times 100 = \text{milligrams of lactic acid per 100 milliliters of blood.}$$

RESULTS

Demonstration of the Selenium-Arsenic Antagonism by Single Injections

In work previously mentioned (25) it was shown that injected arsenite would definitely protect against injected selenite when the substances were administered in daily subtoxic doses over an extended period of time. Some work done in this laboratory has indicated that arsenic will protect against a single lethal dose of selenite (10) but since this work was not very conclusive and since the point seemed important to the rest of this problem, an unequivocal demonstration of this phenomenon was attempted.

Forty five rats were divided into three groups of fifteen which were then further subdivided into three groups of five. Each of the large groups received one of three levels of selenium (4, 5 or 6 milligrams of selenium per kilogram of body weight). Then each subgroup of five rats received one of three levels of arsenic in the form of sodium arsenite (0, 3 or 4.5 milligrams of arsenic per kilogram of body weight). The deaths occurring during an observation period of four days were recorded and are shown on Table 1.

The rats which received 6 milligrams of selenium per kilogram and no arsenic all died within twenty eight hours after injection. In the two groups receiving 4 and 5 milligrams of selenium per kilogram and no arsenic, most of the deaths occurred within seventy two hours. Two rats in the 5 milligrams per kilogram group and one in the 4 milligrams per kilogram group, however, did not die until the fourth day. It is evident that perfect protection was obtained under the conditions of this experiment since there was 86 percent mortality among the rats receiving selen-

TABLE 1

Protection against a single lethal injection of sodium selenite by a single injection of sodium arsenite (five rats per group).

Group	Mg. of Se per kilo	Mg. of As per kilo	Deaths at fourth day
1	4	0	4
2	4	3	0
3	4	4.5	0
4	5	0	4
5	5	3	0
6	5	4.5	0
7	6	0	5
8	6	3	0
9	6	4.5	0

ium alone but no deaths among the rats receiving arsenic injections.

Effect of Arsenic on Selenium Distribution

It was considered desirable to keep the injections in this study at subtoxic levels so that the normal metabolism of the rat would not be disturbed more than necessary, therefore eight rats were injected with 1 milligram of arsenic and 0.68 milligram of selenium per kilogram in a preliminary trial. A second group of eight rats served as controls and were injected with only sodium selenite. The data for this trial are given in Figures 1 to 3. The time-distribution curves for the blood in Figure 1 indicate that the injection of arsenic prior to selenium injection increased the level of selenium in the blood during the first few hours but three hours after injection the selenium levels of the two groups tended to approach common values. The curves for the trichloroacetic acid extracts of the blood indicate that the effect of the arsenic was to decrease the selenium levels in comparison with the controls. Since the counts were so low, however, the differences amounted to only a few counts per minute and consequently were not considered significant. The situation in the liver (Figure 2) was just the opposite from that in the blood since the rats receiving only selenite injections accumulated more selenium than did the arsenic treated group. The trichloroacetic acid extracts of the liver were slightly higher in selenium concentration than were the similar extracts of the blood but differences between the controls and treated animals were still very small. From the data for the spleen and kidney in Figure 3 it can be seen that the amount of selenium accumulated by the spleen was quite low although the injection of arsenic tended to reduce the selenium level after the first hour.

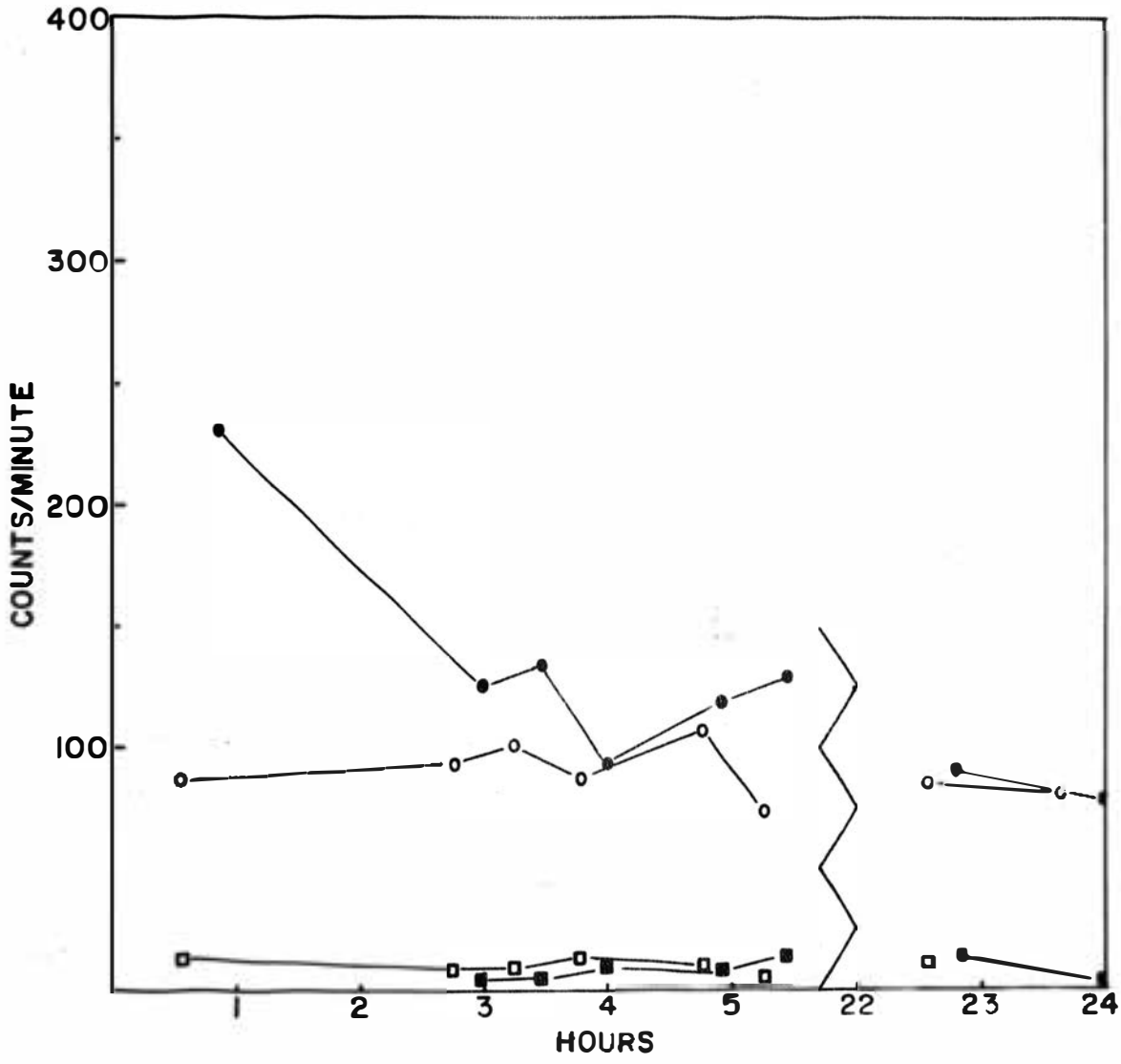

Figure 1. Effect of 1 milligram of arsenic per kilogram upon the activity in the whole blood and trichloroacetic acid extracts from rats receiving 0.68 milligram of selenium per kilogram.

	Sample counted	Injection
○	Blood	Selenium
●	"	Selenium + Arsenic
□	Trichloroacetic acid extract	Selenium
■	" " "	Selenium + Arsenic

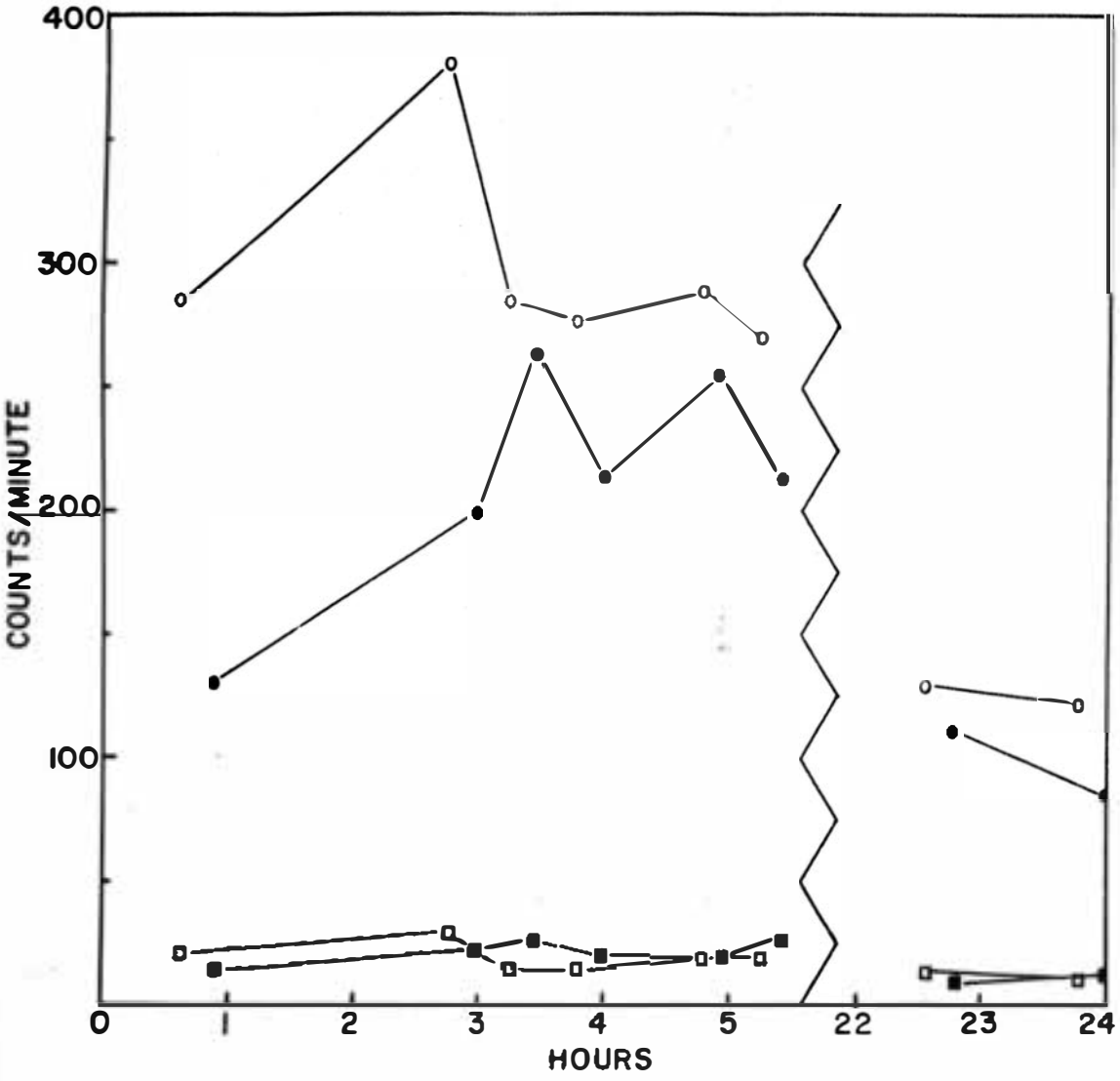

Figure 2. Effect of 1 milligram of arsenic per kilogram upon the activity in homogenates and trichloroacetic acid extracts of livers from rats receiving 0.68 milligram of selenium per kilogram.

	Sample counted	Injection
O	Liver	Selenium
●	"	Selenium + Arsenic
□	Trichloroacetic acid extract	Selenium
■	" "	Selenium + Arsenic

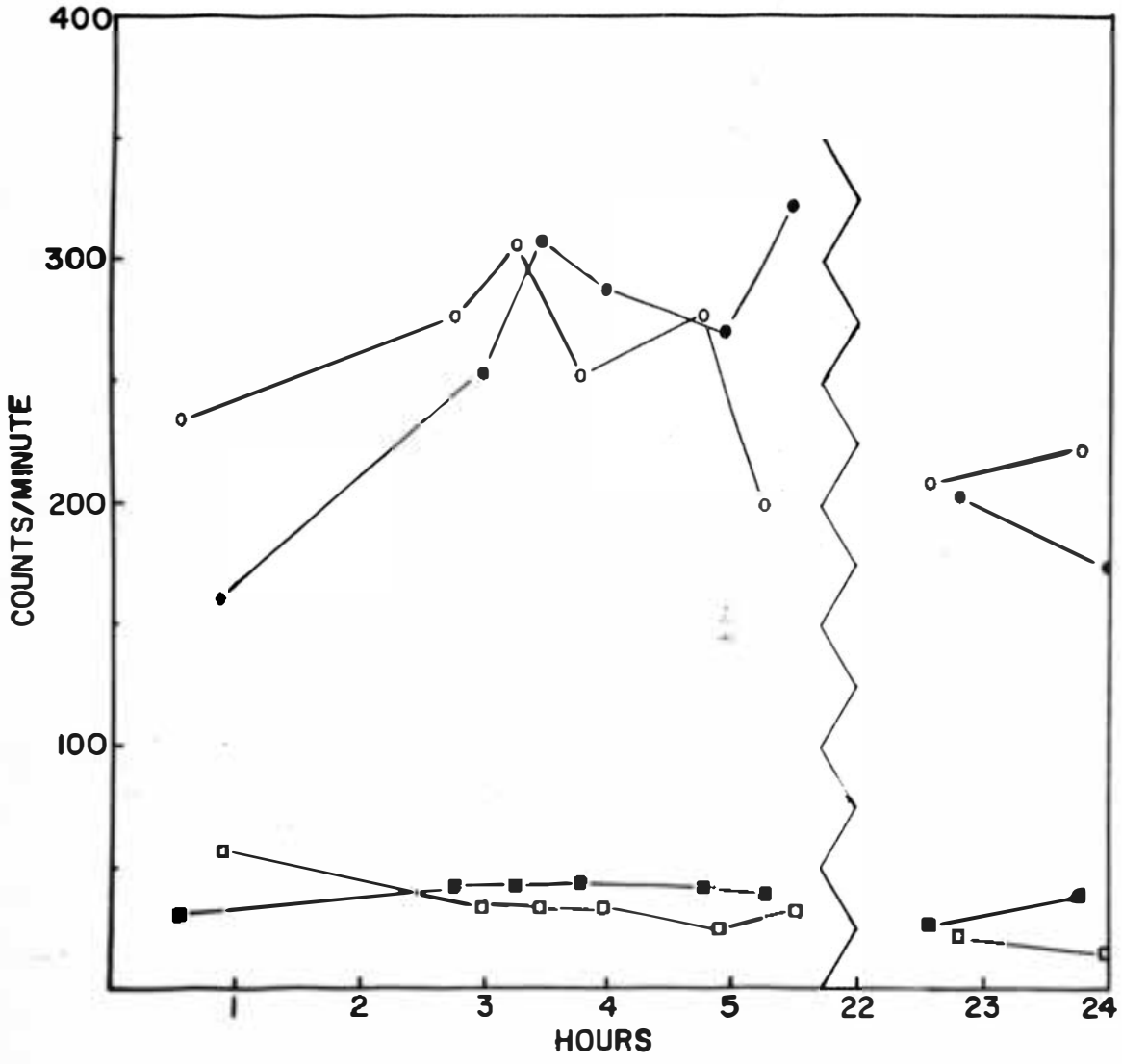

Figure 3. Effect of 1 milligram of arsenic per kilogram upon the activity in the kidneys and spleens of rats receiving 0.68 milligram of selenium per kilogram.

	Sample counted	Injection
O	Kidney	Selenium
●	"	Selenium + Arsenic
■	Spleen	Selenium
□	"	Selenium + Arsenic

The difference between the two groups, however, only amounted to approximately ten counts per minute. Activities obtained for the kidneys indicate that arsenic tends to inhibit selenium accumulation for the first three hours. After this time the selenium accumulation appeared to increase since the kidney levels were higher in rats receiving arsenic. Neither trend could be supported by subsequent experiments.

Since the differencee between the groups of rats treated with selenite and those which had received both arsenite and selenite were not as great as desired, a second trial was designed in which the arsenite injections were increased to 2 milligrams per kilogram. The selenite injections were also increased (1.36 milligrams per kilogram) so that higher activities could be obtained from the various samples. On the basis of the results of the preliminary trial the trichloroacetic acid extracts of the blood were omitted. In most instances the trends in this experiment were the same as those in the preliminary trial. The time-distribution curves for the blood in Figure 4 show that the activities were much higher than in the preliminary trial and that the highest selenium concentration was reached in the first thirty minutes. This compares favorably with work by McConnell (16) in which it was reported that maximum concentration was reached within the first fifteen minutes. The blood of rats injected with arsenic and selenium again attained much higher levels than did those injected with only selenium, the difference being approximately four fold at the end of the first hour. The time-distribution curves for the liver in Figure 5 show that the highest concentration in that organ was reached within the first hour. As in the preliminary trial, the injection of arsenic reduced the level of se-

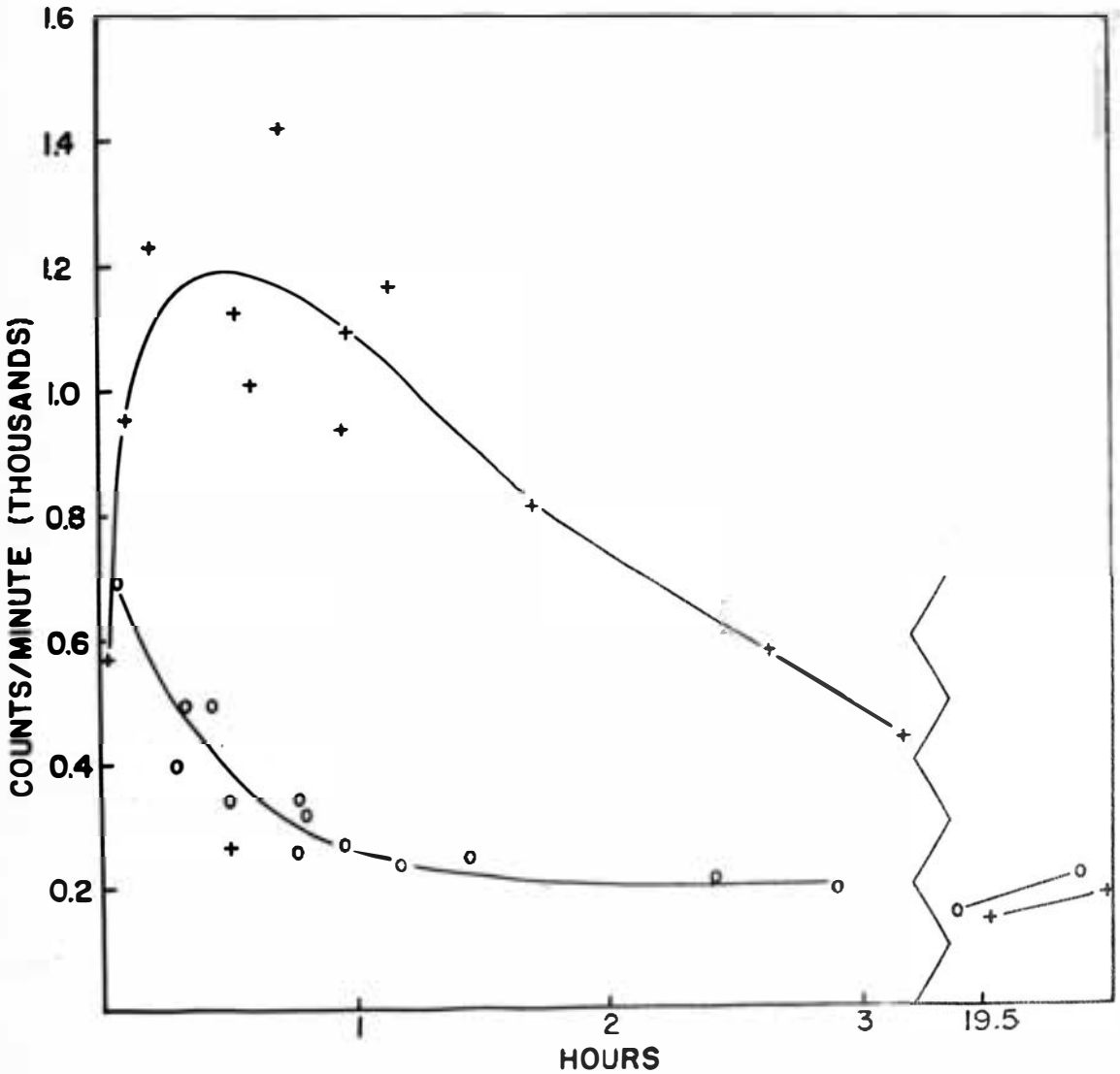

Figure 4. Effect of 2 milligrams of arsenic per kilogram upon the activity in the blood of rats receiving 1.36 milligrams of selenium per kilogram.

O Activity after selenium injections

\+ Activity after selenium + arsenic injections

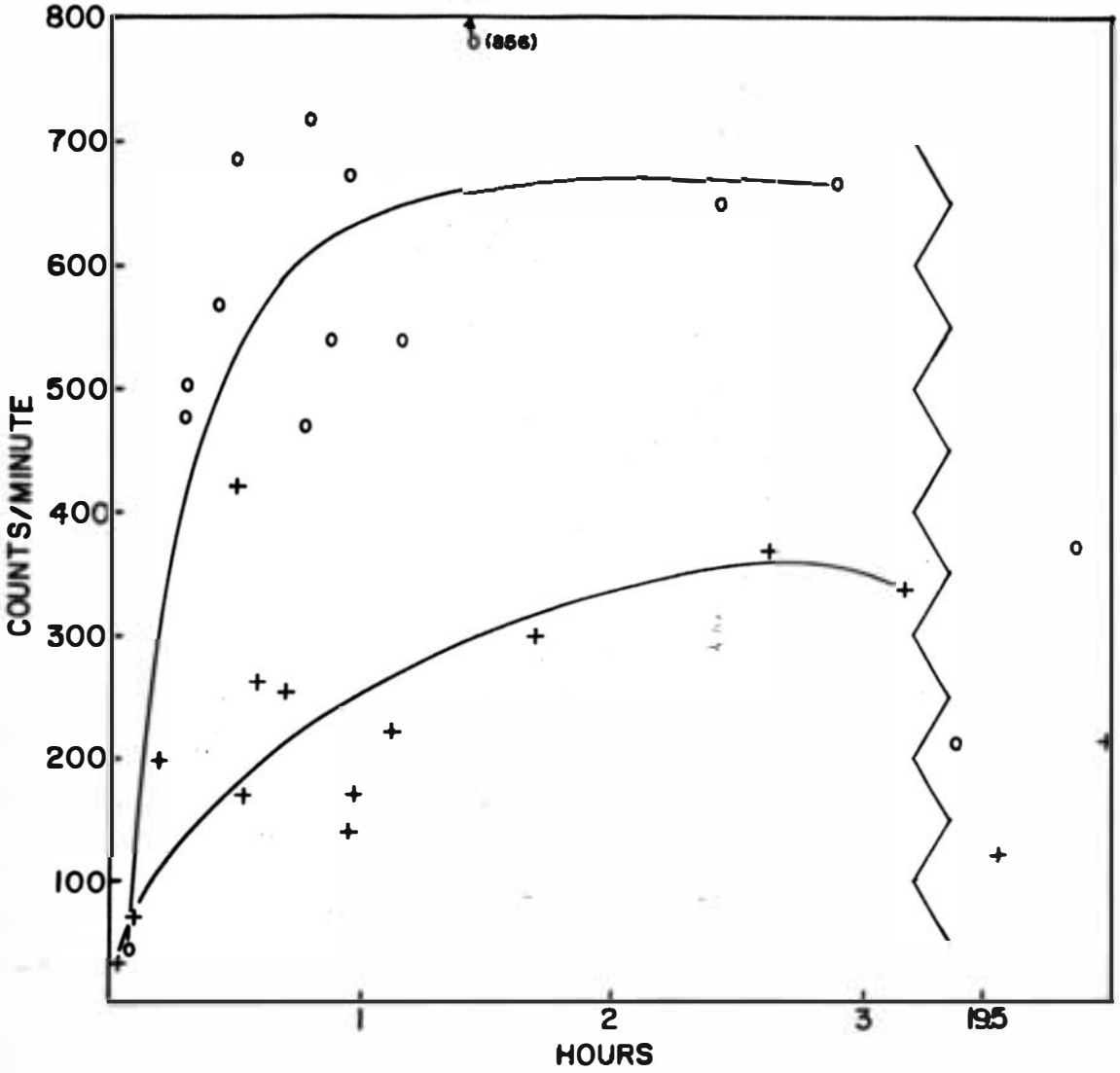

Figure 5. Effect of 2 milligrams of arsenic per kilogram upon the activity in the livers of rats receiving 1.36 milligrams of selenium per kilogram.

O Activity after selenium injections

\+ Activity after selenium + arsenic injections

lenium in the liver, the difference between the control and arsenic treated rats being approximately two fold at the end of the first hour. The kidney values (Figure 6) were rather high again but the individual values varied so widely, both in the controls and in the arsenic treated group that no apparent differences could be detected between the two groups. In the same figure the trichloroacetic acid extracts of the liver closely approximate the low activities obtained in the preliminary trial and from all appearances the administration of arsenic did not influence the selenium distribution in these extracts. At this level of arsenic a difference was noted in the activity of the spleens from the two groups (Figure 7). The general trend of the curves was very similar to that in the blood with the exception that the peak concentration was much lower. At the end of the first hour the spleens of rats receiving both selenium and arsenic had accumulated about twice the amount of selenium as those of the controls. The higher values due to the arsenic decreased very rapidly, however, and at the end of one and one-half hours the activity of the arsenic treated group appeared to fall below that of the controls.

A third trial was also made to determine whether or not increasing the arsenic level to 3 milligrams per kilogram would have any further effect upon selenium distribution. In this study only the blood, liver and kidneys were used to trace the distribution. The data for this experiment are shown in Figures 8 to 10 and will not be discussed in detail since the trends were the same as in the previous trial. It should be noted, however, that the differences demonstrated in previous trials were increased by the higher arsenic level.

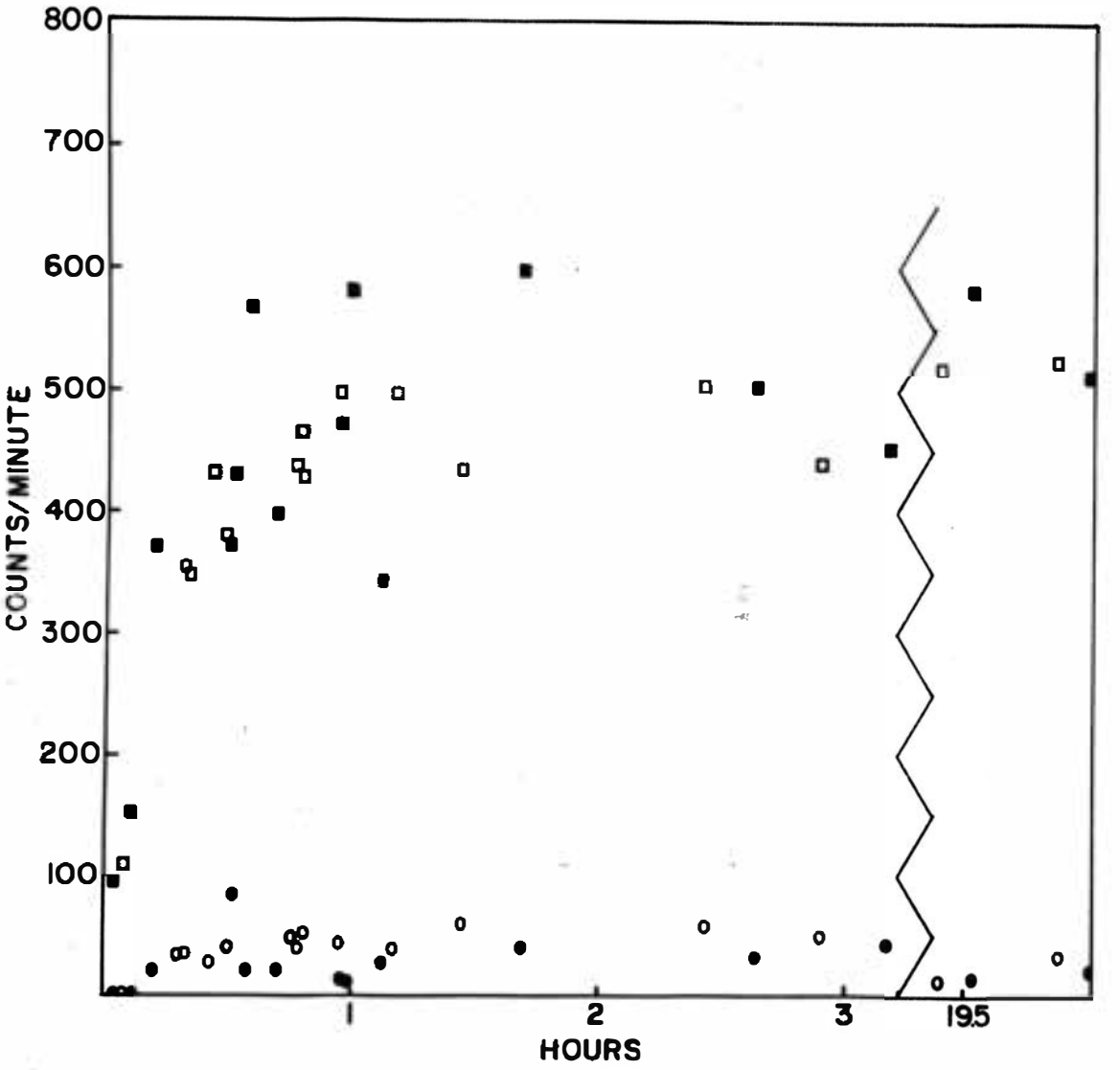

Figure 6. Effect of 2 milligrams of arsenic per kilogram upon the activity in the kidneys and liver trichloroacetic acid extracts from rats receiving 1.36 milligrams of selenium per kilogram.

	Sample counted	Injection
□	Kidney	Selenium
■	"	Selenium + Arsenic
0	Trichloroacetic acid extracts	Selenium
●	" "	Selenium + Arsenic

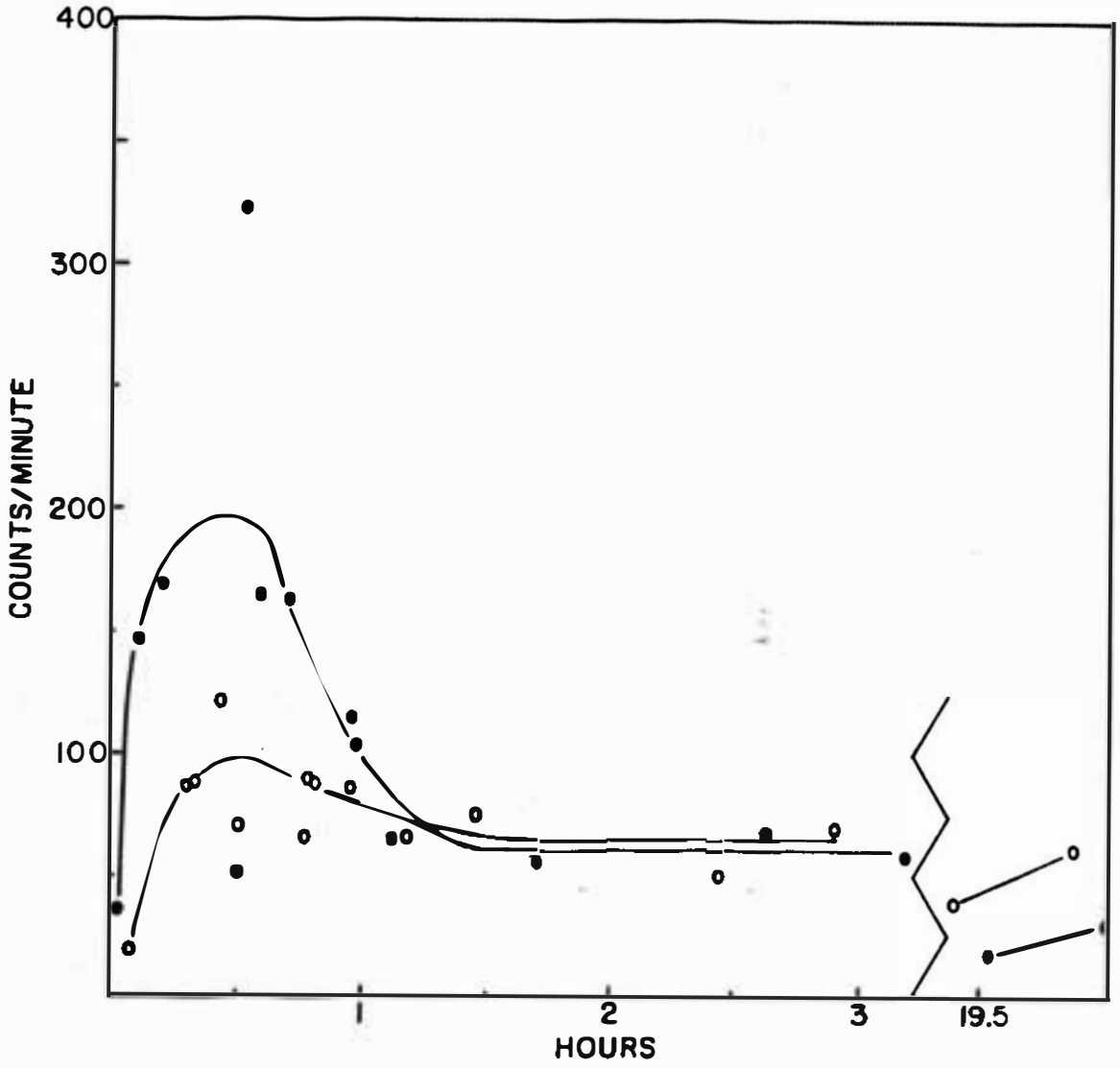

Figure 7. Effect of 2 milligrams of arsenic per kilogram upon the activity in the spleens from rats receiving 1.36 milligrams of selenium per kilogram.

O Activity after selenium injection

● Activity after selenium + arsenic injection

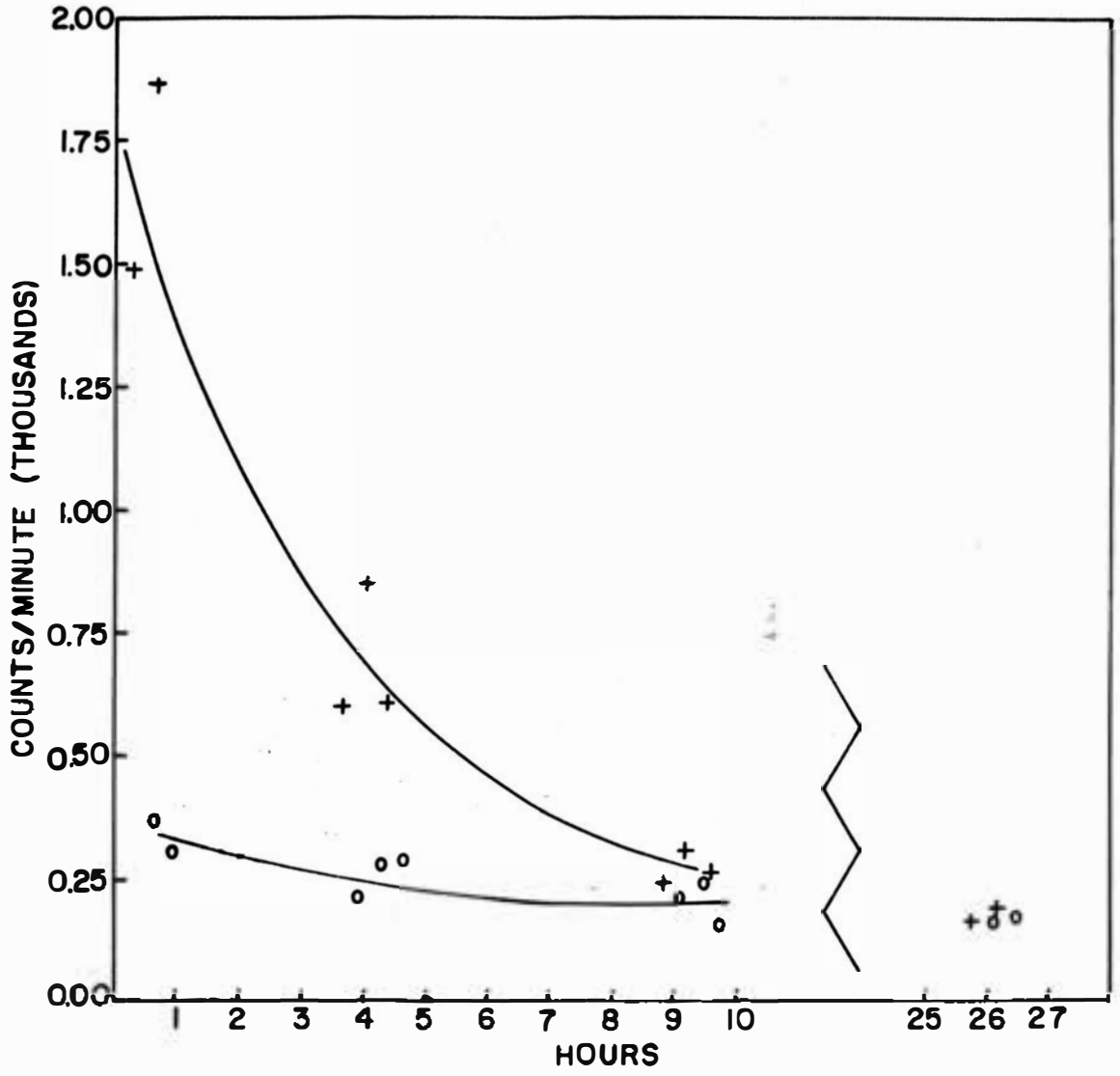

Figure 8. Effect of 3 milligrams of arsenic per kilogram upon the activity in the blood from rats receiving 1.36 milligrams of selenium per kilogram.

0 Activity after selenium injection

\+ Activity after selenium + arsenic injection

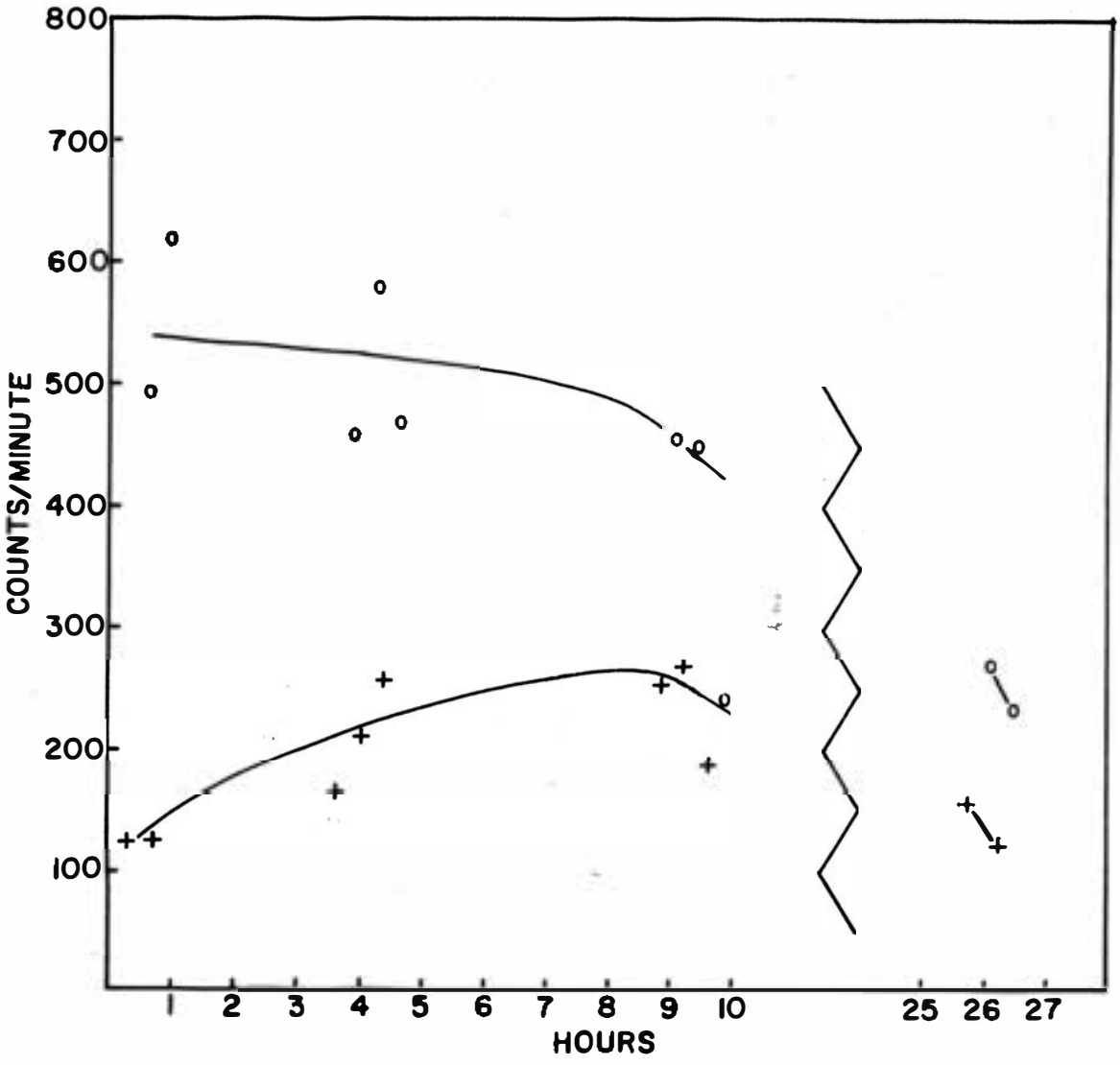

Figure 9. Effect of 3 milligrams of arsenic per kilogram upon the activity in the livers from rats receiving 1.36 milligrams of selenium per kilogram.

O Activity after selenium injection

\+ Activity after selenium + arsenic injection

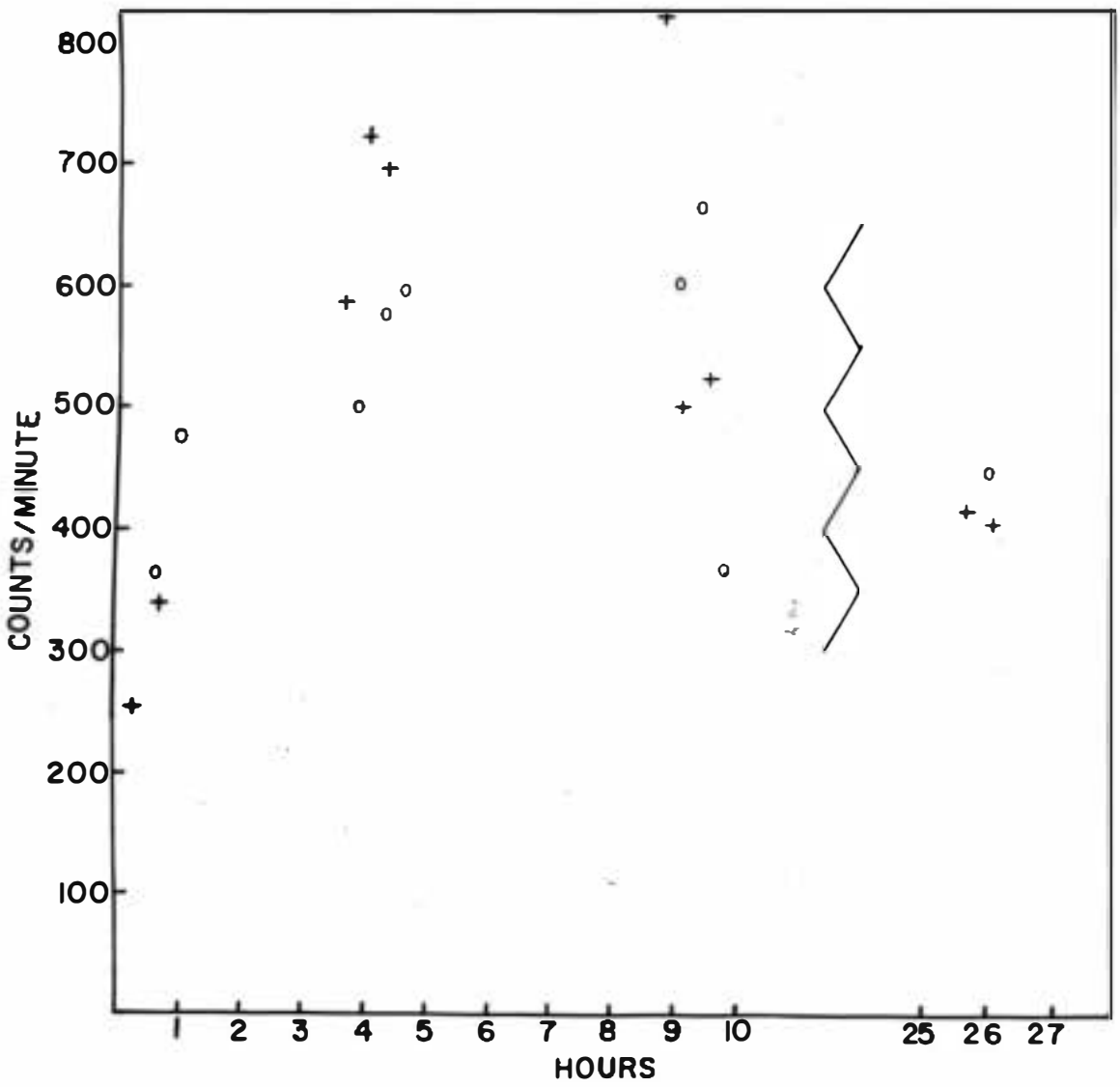

Figure 10. Effect of 3 milligrams of arsenic per kilogram upon the activity in the kidneys from rats receiving 1.36 milligrams of selenium per kilogram.

O Activity after selenium injection

\+ Activity after selenium + arsenic injection

Influence of Lactate on Selenite Distribution

On the basis of the yeast work previously mentioned (2), it was decided to study what effect lactate might have upon selenium distribution. In the course of the study it appeared necessary to establish that the lactate level in the blood of the rats could be significantly varied by subcutaneous lactate injection. Therefore, a study was designed in which five untreated rats were used as controls for normal lactate values and ten other rats were injected with ten milliliters of 5 percent magnesium lactate per kilogram of body weight. Ten minutes after injection with lactate the rats were sacrificed and their blood analyzed for lactic acid by the method previously described. From the data recorded in Table 2 it is evident that magnesium lactate injections caused significant increases in blood lactate levels. Lactate levels in the blood of the control rats were about one third as high as those in the blood of the rats which had been injected with magnesium lactate. The average value in milligrams per 100 milliliters for the controls was 12.6 while the treated rats had an average of 35.2.

For the study concerning the effect of lactate upon selenium distribution, nine rats were injected with 10 milliliters of 5 percent magnesium lactate per kilogram of body weight and nine control rats were injected with selenite only. Twenty five minutes after the selenite injections the rats were sacrificed and treated according to the procedure described under the previous section. In this case activities were determined in the blood and liver only.

Table 3 indicates that the effect of lactate upon the selenium distribution was not as evident as that of arsenic. The effect on the

TABLE 2

The effect of injection of magnesium lactate upon the lactate level of the blood.

Rat No.	Weight	Lactate injection	Mg. of lactate per 100 ml.
1	320	none	13.2
2	240	"	11.9
3	310	"	10.2
4	300	"	15.1
5	270	"	12.6
6	300	10 ml. of 5% Mg lactate per kilo	35.3
7	290	"	39.8
8	295	"	30.8
9	295	"	41.6
10	265	"	37.8
11	330	"	46.2
12	270	"	38.3
13	290	"	37.3
14	290	"	36.8
15	330	"	37.7

TABLE 3

Effect of lactate upon selenium distribution

Activity of Blood counts/min.		Activity of Liver counts/min.		Blood:Liver Ratio	
Se Injected	Se-Lactate Injected	Se Injected	Se-Lactate Injected	Se Injected	Se-Lactate Injected
176	286	181	204	.97	1.40
188	310	210	112	.90	2.77
182	238	238	175	.76	1.36
283	200	212	189	1.34	1.06
230	252	236	187	.97	1.35
247	253	135	168	1.83	1.51
277	309	213	138	1.30	2.24
235	321	218	132	1.08	1.76
191	288	143	155	1.34	1.86
Mean					
223**	273**	198*	168*	1.17**	1.7**

* Difference not significant

** Difference significant at the 2 percent level

selenium level of the blood appeared to be the same as when arsenic was used although the differences were not quite as large. There was more activity in the blood of the rats which were injected with both selenium and lactate than in the blood of the selenium controls, the differences being significant at the 2 percent level. Comparison of the mean liver activities of the control and treated groups indicates that lactate reduced selenium accumulation in the liver. However, statistical analysis revealed that individual variations were so great that the difference between the means was not significant. The ratios between the activities in the blood and liver of each rat were calculated and also appear in Table 3. The ratios for the rats receiving lactate and selenite were considerably higher than those for the controls. The differences between the two groups were significant at the 2 percent level.

DISCUSSION

It is evident from the results that a single injection of sodium arsenite will protect against a lethal injection of sodium selenite and will cause reduced selenium levels in the livers as well as correspondingly high levels in the blood. It appears that the most important point established by the arsenic studies was that arsenite would definitely decrease the accumulation of selenium by the liver. Combining this information with the facts already mentioned, that arsenic prevented the exhalation of a volatile selenium product (11) supposedly produced in the liver (17), it appears that the selenium-arsenic antagonism is essentially the same in the rat as in yeast.

A reasonable explanation of the observations then might be that the toxic reaction involving selenium is antagonized by arsenic, involved in the production of a volatile excretory product and perhaps initiated at the membrane of liver cells. It must be admitted, however, that information is still too meager to state that the antagonism prevents entry into the cell rather than just preventing a toxic reaction after the selenium has entered.

The effect of arsenic upon selenium accumulation by the other organs was not so noticeable. The effect on the selenium concentration in the spleen appeared to be the same as in the blood. The arsenic caused increased activity for the first one and one-fourth hour. Soon after this time the spleen activities returned to the values of the controls and in some cases even dropped to lower values. The attempt to demonstrate a relationship between the administration of arsenic and the

selenium concentration in the trichloroacetic acid extracts and kidneys was unsuccessful. The trichloroacetic acid extracts of the blood and livers were both very low and any differences present were only of the magnitude of a few counts per minute and therefore were not significant. Activities in the kidneys were quite high and it is possible that if any differences were present, they were masked by the activity of unremoved urine. An explanation of this nature seems probable since the individual kidney values varied more widely than in the case of the other samples.

Since it had been shown that lactate produced the same effect on yeast in a selenite medium as did arsenite, it was expected that the effects in rats might also be similar. The results show that in the blood this was true since the selenium levels for rats receiving lactate are higher than for the controls. From the results with arsenic it would be expected that the lactate injections would lower the selenium content of the liver. While a comparison of the mean activities for the control and treated groups bears this out, variations among individuals were so great that the differences were not statistically significant. The ratio of the activity in the blood to that in the liver was calculated for each not as a measure of the effect of lactate on selenium accumulation by the liver. Since the group of rats receiving lactate had much higher ratios than did the controls, it may be said that the administration of lactate does significantly raise the selenium level in the blood without causing the liver values to increase proportionately.

A point of interest which was not considered in this study was the effect of arsenic and lactic acid upon selenium excretion. Since there is a reduction in selenium accumulation by the liver as well as a decrease

in exhalation of the volatile compound, one would expect the excretion in other routes to be increased. Peterson, and others (28) have done some work on this aspect of the problem and have reported that arsenic does not affect selenium excretion. It should be noted, however, that these studies were long term feeding experiments which also gave negative results as to the effect of arsenic on selenium distribution in the various organs. It appears that a study using single injections might also contribute new information as to the effect of arsenic upon selenium excretion by route of the kidney.

SUMMARY

A study was made of the modification by arsenite injections of the selenium distribution and the lethal effects normally resulting from single selenite injections. It was found that a single arsenic injection would give protection against a lethal selenite injection. The arsenite injections were also found to modify selenium distribution in the blood, liver and spleen. A possible mechanism for the protective action is discussed.

The effect of lactate on distribution was also studied but a modification of selenium distribution was noted only in the blood. Further studies are suggested which might reveal additional information on the protective action shown by these two substances.

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