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FURTHER TOXICOLOGIC STUDIES OF ACUTE HYDRAZINE TOXICITY IN MICE

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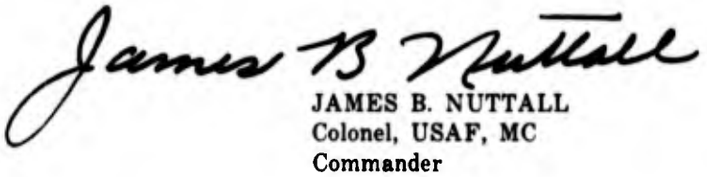
FOREWORD

This report was prepared in the Department of Biochemistry, City of Hope Medical Center, Duarte, Calif., under contract No. AF 41(609)-2949 and task No. 630207. It was monitored by James H. Merritt, Physiological Chemistry Section, USAF School of Aerospace Medicine. The paper was submitted for publication on 28 July 1966. The work was accomplished between 1 July 1965 and 31 March 1966.

The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

The five lots of imidazoleacetic acid used in this experiment were obtained from the California Corporation for Biochemical Research, Los Angeles, Calif.

This report has been reviewed and is approved.



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ABSTRACT

Toxicologic studies of acute hydrazine toxicity in mice were continued. The results indicate that acute toxicity of hydrazine probably is not mediated through a histamine-release mechanism. Various experiments showed that the type of lethal seizure produced by hydrazine probably has no relationship to the sound-induced seizures in susceptible strains of mice.

It was found that sodium phenobarbital had a marked protective effect against hydrazine toxicity when given in subhypnotic amounts. Sodium phenobarbital had an additive protective effect when it was administered together with the previously studied protective mixture (AGKO) containing arginine, glutamate, α-ketoglutarate, and oxalacetate. NaBr also was found to be protective and was found to act additively with either sodium phenobarbital or with the AGKO mixture.

It was found in the course of the above experiments that imidazoleacetic acid, a substance not protecting mice against acute hydrazine toxicity, had interesting analgesic and hypnotic effects in mice. The quantitative aspects of these effects were worked out, and it is suggested that this agent should be explored further as a possibility for human use.

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I. INTRODUCTION

Following our previously published and reported toxicologic studies on acute hydrazine toxicity in mice (4), we began studies of the biochemical basis for hydrazine toxicity (5). Although work along this line is proceeding, it became apparent that numerous avenues could still be explored by further direct toxicologic studies. In the present report the results of these latter studies are presented.

II. GENERAL METHOD

All of the experiments were carried out as in previous reports. Swiss male mice of an inbred Swiss stock, weighing approximately 25 gm., were used in all experiments. Unless otherwise stated, every experiment was performed with a minimum of 10 animals in each experimental group. The mice were starved for approximately 18 hours prior to a single intraperitoneal injection of about 0.1 ml. of a freshly prepared hydrazine solution (pH 7.0). The injection of hydrazine, in most instances, was preceded by 30 minutes by the injection of physiologic saline or by a similar volume of neutral test solution. After the initiation of the experiment the animals were observed continuously for several hours. Final observations were made approximately 24 hours after the injection of hydrazine.

III. INFLUENCE OF SUBSTANCES SHOWING LITTLE PROTECTIVE ACTION AGAINST ACUTE HYDRAZINE TOXICITY

It was of interest to test the influence of histamine, itself, and of an antihistaminic substance on acute hydrazine toxicity, since in some instances small lung hemorrhages were observed in mice which had died from seizures produced by the hydrazine injection. This suggested the possibility that there might be histamine release at the tissue level by hydrazine, although it is well known that mice are extremely resistant to the toxic action of administered histamine. The initial experiments showed that histamine phosphate at a level of 4 mmoles/kg. had no protective action against hydrazine given at a level of 3 mmoles/kg. In the latter preliminary experiments the toxicity of hydrazine was greatly enhanced by histamine pretreatment, histamine-injected animals dying earlier than the controls. The enhancement of the hydrazine toxicity was attributed to the phosphate moiety, and subsequent experiments were performed with histamine dihydrochloride. It is seen from table I that in the latter form histamine gave no protection against hydrazine toxicity. Benadryl, an antihistaminic substance, did not have any protective effect either. The latter two results indicate that probably a histamine-release mechanism is not involved importantly in hydrazine toxicity.

We have shown previously, as have others, that administration of pyridoxal enhances the toxicity of hydrazine. The latter results were repeated (table I) with pyridoxal pretreatment at 2 mmoles/kg. All of the experimental animals died within 30 minutes after the administration of hydrazine. The latter experiment, however, does not rule out the possibility that hydrazine may exert its toxic effect by combining with pyridoxal phosphate at the cellular level. Since it is known that aminothiols form thiazolidine derivatives with aldehyde groups, it was thought possible that pretreatment with such substances might protect the aldehyde groups of pyridoxal phosphate in tissues against reaction with administered hydrazine.

TABLE I

Influence of several substances on acute hydrazine toxicity in mice

Substance tested	Dose (mmoles/kg.)	Cumulative deaths (%)			
		30 min.	60 min.	90 min.	24 hr.
None	—	30	70	70	80
Histamine dihydrochloride	4.0	80	90	90	100
Benadryl hydrochloride	0.08	90	90	90	100
Pyridoxal hydrochloride	2.0	100	100	100	100
2-Mercaptoethylamine	0.96	70	90	90	90
L-Cysteine	0.96	0	40	50	50

*All mice received 3 mmoles/kg. of hydrazine.

If hydrazine were reacting with such aldehyde groups in a manner producing toxicity, the aminothiols might afford some protection. 2-Mercaptoethylamine at a tolerated dose level showed no protection against 3 mmoles/kg. of hydrazine. The same molar concentration of L-cysteine may have afforded some protection, and this will be explored in further experiments. It is interesting that in the pyridoxal-treated group all of the animals showed severe tremors within 2 to 3 minutes after the injection of hydrazine. These tremors were distinctly different from the seizures ordinarily produced by hydrazine alone.

Another set of experiments was performed, based on some work done in connection with protecting mice of the DBA strain against sound-induced lethal seizures (1, 7). The highest susceptibility to audiogenic seizures in this strain of mice occurs at approximately 3 weeks of age. When mice of this age were given a single injection of epinephrine (10 to 15 μg.) prior to test procedure, there was a remarkable degree of protection against the seizures, lasting 1 to 4 hours. The administration of glucose (100 mg.) gave some protection. A combination of glucose and epinephrine gave continued protection against seizures for as long as 24 hours. Similar experiments to those above were performed with glucose, epinephrine, and norepinephrine (alone or in combinations) in order to test whether protection against hydrazine toxicity could be achieved. The test substances were given 2 hours before hydrazine was injected at a level of 3 mmoles/kg. It is seen from the results in table II that neither epinephrine, norepinephrine, nor glucose alone gave significant protection, nor did epinephrine and norepinephrine when given with glucose. This suggests there is no similarity in the mechanisms of the audiogenic seizures in DBA mice and the hydrazine-induced seizures in the Swiss mice employed in the present experiments. Another protective agent against audiogenic seizures was 6-n-propylthiouracil. Feeding of this antithyroid substance gave complete protection against audiogenic seizures in mice (6). Experiments were performed in which mice were given this substance at 5 mg./day for 4 days by intraperitoneal injection in a suspension made with asolectin. Control animals were given comparable amounts of asolectin daily for the same period of time. Thirty experimental animals and 15 controls were used in all. At the end of 4 days of treatment all the animals were fasted for 18 hours, and on the 5th day each animal received the standard dose of hydrazine (3 mmoles/kg.). There was no protection, whatsoever, observed in the animals which received the drug.

TABLE II

Effects of norepinephrine and epinephrine with and without glucose on hydrazine toxicity in mice

Substance tested	Cumulative deaths (%)			
	30 min.	60 min.	90 min.	24 hr.
None	30	60	60	60
Epinephrine*	40	70	80	80
Norepinephrine*	10	40	50	80
Glucose†	20	60	60	70
Epinephrine* + glucose†	10	80	80	90
Norepinephrine* + glucose†	10	40	50	90

All mice received 3 mmoles/kg. of hydrazine. There were 10 mice in each group.

*25 μg. of free base per 25 gm. mouse.

†100 mg. of glucose per 25 gm. mouse.

IV. PROTECTIVE EFFECTS OF SODIUM PHENOBARBITAL AND ITS ADDITIVE PROTECTIVE EFFECTS WITH THE AGKO MIXTURE

A continuation of the studies of protection against acute hydrazine toxicity showed in preliminary experiments that sodium phenobarbital alone could give protection against acute hydrazine toxicity. A series of experiments was performed in which groups of mice were pretreated with intraperitoneal injection of AGKO mixture (4 mmoles/kg.), sodium phenobarbital (60 mg./kg.), or a mixture of both prior to the injection of different amounts of hydrazine. The isomolar mixture (AGKO) contained arginine, glutamate, α-ketoglutarate, and oxalacetate (4). The barbiturate and the AGKO mixture were injected separately, since the solutions of the AGKO mixture and the sodium phenobarbital could not be mixed without precipitation of some of the components. Saline-injected animals were used as controls. Each point shown on figure 1 is the result obtained from a group of 10 or more mice. It is seen that both AGKO and phenobarbital in the doses administered gave approximately the same protection against acute hydrazine toxicity. The LD_{50} for the saline-injected controls was approximately 2.6 mmoles/kg. of hydrazine, and that for the groups receiving the AGKO mixture was approximately 4.5 mmoles/kg. In four groups of 10 animals receiving phenobarbital alone and in two of three groups receiving the AGKO mixture alone, there was 100% mortality within 2 hours when 6.5 mmoles/kg. of hydrazine was given. There was a remarkable additive protective effect when the AGKO mixture was given at the same time as the phenobarbital before the administration of the hydrazine. The approximate LD_{50} for this combination was 6.3 mmoles/kg. of hydrazine. None of the animals receiving this mixture died within 120 minutes of the administration of 4 to 5.5 mmoles/kg. of hydrazine.

A subsequent experiment was performed in which ten groups of 10 animals each were employed. A control group received saline alone 30 minutes prior to the administration of 6.5 mmoles/kg. of hydrazine. The remaining nine groups all received 60 mg. of sodium phenobarbital before the administration of hydrazine. One of these groups received no additional injection, while the remaining eight groups received injections of the AGKO mixture containing from 1 to 8 mmoles/kg. of each constituent, the substances being present in the mixture in isomolar amounts. The results in

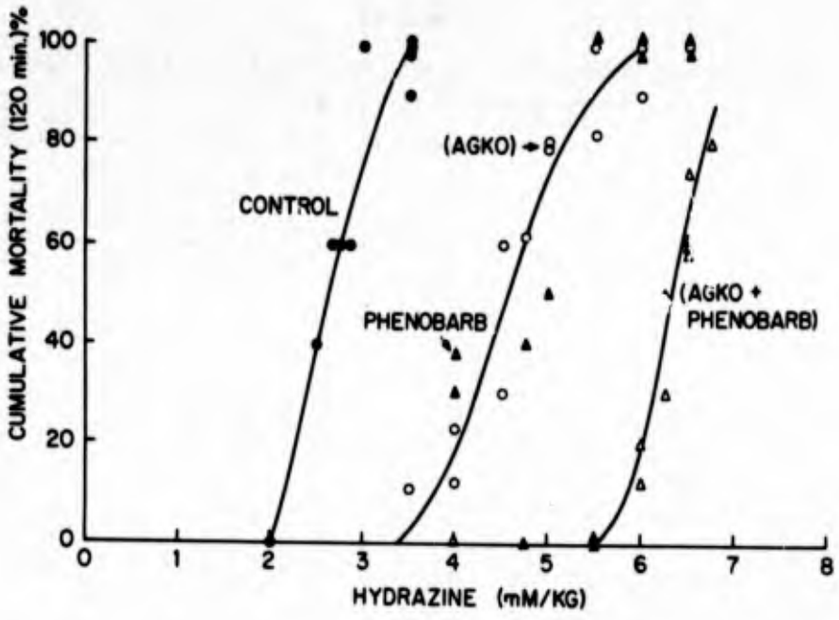

FIGURE 1

The additive effects of the AGKO mixture and sodium phenobarbital in protecting mice against hydrazine toxicity.

figure 2 show the cumulative mortalities in the barbiturate-injected groups as a function of the amount of AGKO received at different times after the injection of hydrazine. The group receiving saline alone is not included in figure 2, 100% mortality being achieved within 15 minutes of the hydrazine injection. It is seen from the results in figure 2 that the optimal doses of the AGKO mixture lie between 3 and 4 mmoles/kg.

Another experiment (fig. 3) was then performed similar to the one for which the results are shown in figure 2 with the exception that all of the animals of eight groups of 10 mice each received 3.5 mmoles/kg. of the AGKO mixture 30 minutes before the injection of 6.5 mmoles/kg. of hydrazine. The control group received no sodium phenobarbital, while the remaining seven groups received from 10 to 70 mg./kg. From the results shown in figures 2 and 3, it can be concluded that the amounts of AGKO and sodium phenobarbital used originally (fig. 1) were approximately the optimal amounts for protection against acute hydrazine toxicity under the conditions of our experiments.

The experiment in figure 4 shows the times of death of mice after a dose of 6.5 mmoles/kg. of hydrazine in a saline-injected control group and in groups of 10 mice each receiving 4 mmoles/kg. of AGKO mixture, 60 mg./kg. of sodium phenobarbital, or a mixture of AGKO and sodium phenobarbital in the above amounts. All of the control mice died within 30 minutes after the injection of hydrazine. The time of death was delayed in both the groups receiving AGKO and sodium phenobarbital alone, the delay in the case of sodium phenobarbital being somewhat greater, the first death occurring 60 minutes after the injection of hydrazine. Here again, the additive effect of AGKO and sodium phenobarbital is demonstrated clearly, only 20% of the animals in the latter group having died at 120 minutes after hydrazine, at which time 80% of the mice receiving AGKO and all of the mice receiving phenobarbital were dead. Even at 24 hours after the hydrazine administration, the animals receiving the dual injection of protective substances showed a decreased mortality, only 70% having died at this time.

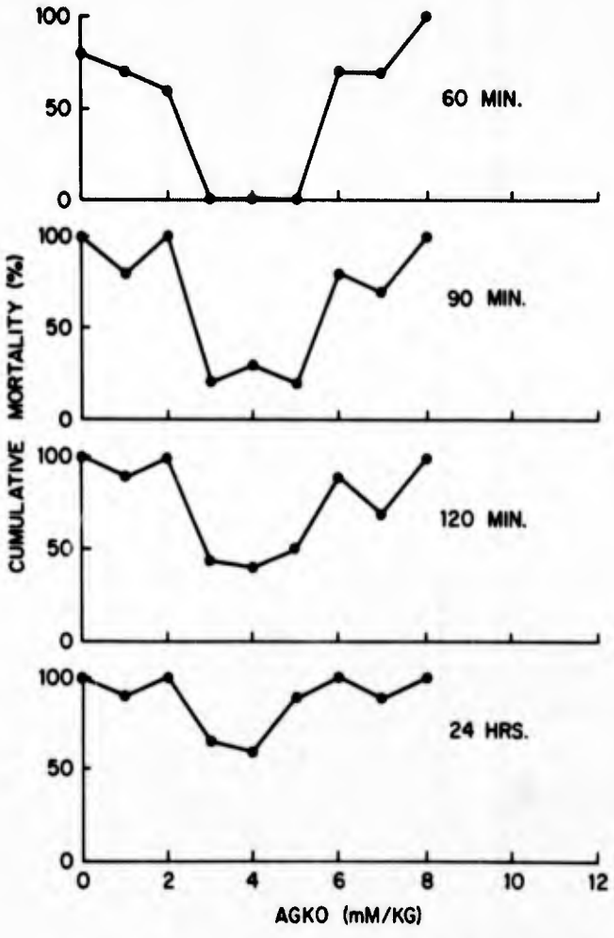

FIGURE 2

The protective effects of different amounts of the AGKO mixture administered together with a constant amount of sodium phenobarbital.

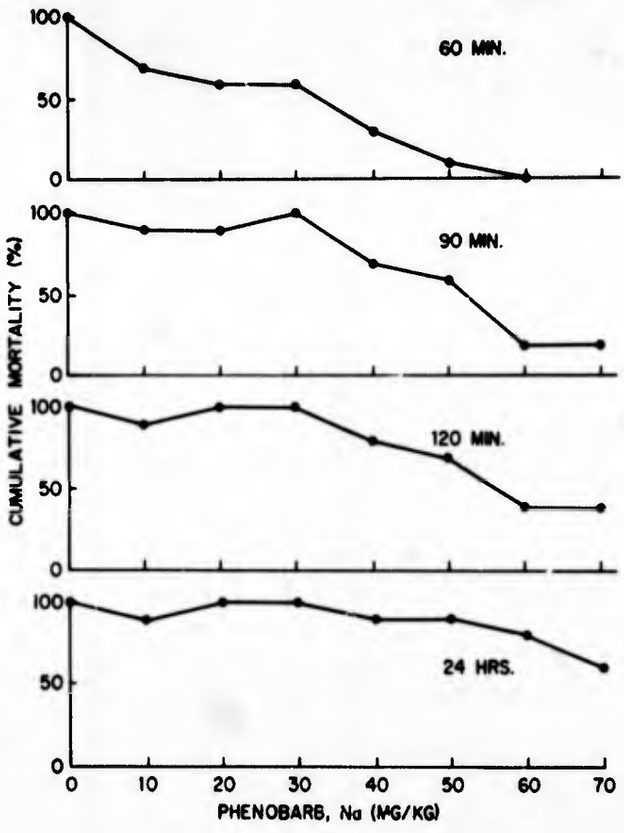

FIGURE 3

The protective effects of different amounts of sodium phenobarbital administered together with a constant amount of the AGKO mixture.

On the basis of the results as shown in figures 1 through 4, it may be concluded that the combination of the AGKO mixture and sodium phenobarbital can give highly significant protection against acute hydrazine toxicity. It is of great interest that there is a marked potentiation of the protective action of AGKO when used in conjunction with sodium phenobarbital. Small doses of phenobarbital have been used for many years in the treatment of various types of epilepsy. In milder types of epilepsy, this treatment has been efficacious in the control of seizures and when combined with Dilantin has proved better than when either drug was given alone. It can be suggested from the above results that the AGKO mixture or various combinations of its components may be effective in treatment of epilepsy and other convulsive disorders and in increasing the antiepileptic effects of phenobarbital, Dilantin, and other agents which are employed in the treatment of epilepsy. This is particularly attractive because all components of the AGKO mixture are known metabolites in the human body and possess no known toxicity when given in reasonable amounts.

V. PROTECTIVE EFFECTS OF BROMIDE AGAINST ACUTE HYDRAZINE TOXICITY

Since bromides are among the oldest known anticonvulsant agents, it was of interest to test the effects of these substances on acute hydrazine toxicity. Surprisingly, bromides proved to be effective protective agents. The results in table III show that NaBr at 3 mmoles/kg. or higher showed a remarkable protective action against a challenging dose of 3 mmoles/kg. of hydrazine. At the three highest levels tested only one of 30 animals died within 24 hours

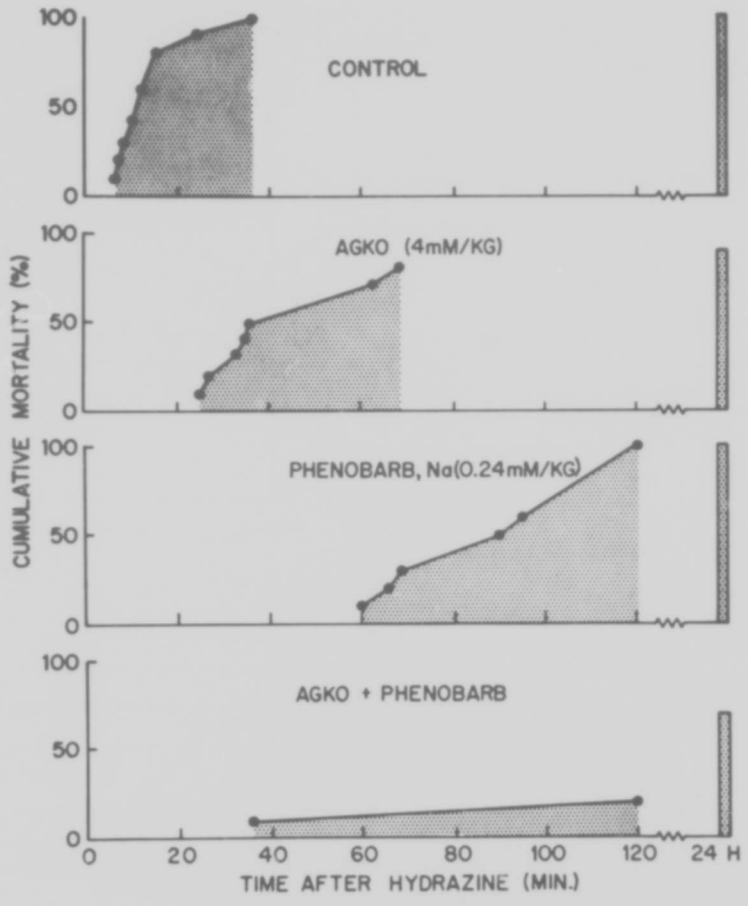

FIGURE 4

The cumulative mortality of mice receiving optimal amounts of the AGKO mixture, sodium phenobarbital, or a combination of the two.

after the administration of hydrazine. Although KBr also was protective, the highest level (8 mmoles/kg.) was toxic by itself, and the general extent of protection at lower levels was somewhat less than that shown by NaBr. This may be attributable to the toxic effects of the K^+ ion itself. The above results show clearly that the bromide ion has a highly significant protective action against hydrazine in our standard test system in mice.

It next was of interest to see whether NaBr could exert a synergistic effect with the substances previously shown to be protective. Experiments were performed at a high level of hydrazine, 6.5 mmoles/kg. Under these circumstances all of the control animals were dead within a short time (table IV). NaBr alone, at the 8 mmoles/kg. level, did not appear to show significant protection at this dose of hydrazine. The AGKO mixture alone showed some protection. The combined administration of the NaBr and the AGKO mixture appeared to be considerably more effective than either one alone at the earlier time intervals after hydrazine administration. Likewise, NaBr and phenobarbital seemed to show a synergistic effect and appeared to be as protective as the administration of the AGKO mixture with phenobarbital. When all three protective

TABLE III

Influence of NaBr and KBr on acute hydrazine toxicity in mice

Substance tested	Dose (mmoles/kg.)	Cumulative deaths (%)			
		30 min.	60 min.	90 min.	24 hr.
None	—	30	70	70	80
NaBr	1.92	57	57	73	86
	3	0	10	10	30
	4	0	10	10	20
	5	20	20	20	40
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	10	10
KBr	3	0	30	30	40
	4	0	10	10	30
	5	20	30	30	40
	6	0	0	10	40
	7	0	10	10	20

*All mice received 3 mmoles/kg. of hydrazine. There were 10 mice in each group, with the exception of the group receiving the lowest level of NaBr. The latter group had 7 mice.

modalities were employed (i.e., when AGKO, phenobarbital, and NaBr were given together), there appeared to be no additional protective action over that shown when any two of the solutions were given together.

Although the subject needs further elucidation, it already is apparent that bromides may be of great value in the treatment of acute hydrazine toxicity. The mechanism of action of NaBr on the central nervous system is not known. The above experiments possibly may open up new avenues for exploration of the pharmacology of bromides.

VI. ANALGESIC AND HYPNOTIC EFFECT OF IMIDAZOLEACETIC ACID IN MICE

Initial observations

In the course of screening experiments it was found that 10 male mice became unconscious almost immediately when imidazoleacetic acid (IMA), a substance showing no protective effects against hydrazine toxicity, was injected intraperitoneally in 0.1 ml. of a neutral solution at a level of 4 mmoles/kg. Essentially the same results were obtained in a subsequent experiment with 5 male mice (fasted; 25 gm.) which had been given the same dose of IMA. In less than 5 minutes the mice became comatose and lay on one side. Shortly thereafter, they became completely inert. When examined at 1 hour after the injection, the mice were colder than normal to the touch. Momentary arousal could be achieved by the pinching of the tail or by other vigorous handling. One of the mice in this group died during the night, but the other 4 were alert and eating the following morning and behaved normally for several days of observation.

In a further experiment, ten groups of 10 fasted male mice each were given intraperitoneal injections of IMA in 0.1 ml. at the following dose levels: 0.0156, 0.0312, 0.0625,

TABLE IV

Influence of NaBr with and without other protective substances on acute hydrazine toxicity in mice

Substance tested	Dose (mmoles/kg.)	Cumulative deaths (%)			
		30 min.	60 min.	90 min.	24 hr.
None	—	90	100	100	100
NaBr	8	30	100	100	100
AGKO mixture*	4 of each substance	20	50	80	90
AGKO mixture + NaBr	4; 8	0	20	40	90
Phenobarbital	0.24	0	10	60	100
Phenobarbital + NaBr	0.24; 8	0	0	10	60
AGKO + phenobarbital	4; 0.24	0	10	10	70
AGKO + phenobarbital + NaBr	4; 0.24; 8	0	20	20	80

All mice received 6.5 mmoles/kg. of hydrazine. There were 10 mice in each group.

*The isomolar mixture contained arginine, glutamate, α-ketoglutarate, and oxalacetate (4).

0.125, 0.25, 0.50, 1, 2, 3, and 4 mmoles/kg., respectively. At doses through 0.25 mmoles/kg. there were no visible effects in the injected animals. The mice receiving 0.5 mmoles/kg. of IMA became lethargic but did not fall asleep. At the 1 mmole/kg. level all of the animals fell asleep within 30 minutes; 8 of the 10 regained an upright position within 90 minutes, while 2 mice awoke only after 6 hours. The mice receiving 3 and 4 mmoles/kg. slept for from 3 to 6 hours. One of the animals receiving the 4 mmole/kg. dose of IMA died 2 hours after the injection. At all times the sleeping mice could be aroused by sufficiently strong pinching of the tail, but immediately after the cessation of stimulation they lapsed again into a somnolent state.

In order to minimize the possibility that an impurity in the IMA was responsible for the effects observed, samples from five different synthetic batches of IMA, meeting the usual chromatographic and analytic requirements for purity, were tested. Groups of 5 mice, each maintained in different cages, were given a single intraperitoneal injection at a dose level of 3 mmoles/kg. The results are shown in table V. All of the lots of IMA tested were effective. The minimal sleeping time of any of the mice was over 2 hours, while 7 of the animals slept at least 7 hours, the longest period of observation. It is not possible to determine whether or not the differences observed between different lots of IMA are significant or whether they are attributable to variability in responsivity of the mice to the substance or to lack of precision of the testing end points. The following morning all 25 mice appeared to be normal in every respect. Autopsies performed on one animal chosen at random from each cage showed the brain and all internal organs to be normal. There was a question as to whether or not the livers and kidneys appeared slightly hyperemic by comparison with normal controls.

Quantitative estimates of analgesic and hypnotic effects in mice

Analgesic effect. In all of these experiments the IMA employed was from lot No. 50107. The analgesic effectiveness was measured in mice by a modification of a commonly

TABLE V

Hypnotic effectiveness of five lots of imidazoleacetic acid

Lot No.	Time to fall asleep* (min.)		Sleeping time† (min.)
	Av.	Range	
30367	24	19-27	247, 283, 297, 347, >420
40026	30	13-51	145, 170, 177, 300, 309
50107	28	22-30	131, 151, 193, 227, 370
590882	11	9-20	259, 280, >420, >420, >420
670981	21	8-28	227, 233, >420, >420, >420

All male mice, approximately 25 gm. in weight and fasted for 18 hours, received an intraperitoneal injection of the compound in 0.1 ml. of solution at pH 7.0. The dose level was 3 mmoles/kg.

*The time from injection until the mouse lay on its side with eyes closed.

†The end of sleep was considered to be the time at which the mouse assumed an upright sitting or crouching position.

used "hot plate" method (2). A thermostatically controlled hot plate was set at 55° C. A bottomless restraining cylinder, 5 inches in height, was made from a tin can. Mice were placed on the hot plate in the cylinder and were observed. The reaction time was measured with a stop watch from the moment the feet of the mouse touched the hot plate. The end point employed was either the licking of the front feet or the climbing or jumping out of the cylinder. All other behavioral signs were disregarded. If the response time required was less than 30 seconds, the result was called negative; if it was 30 seconds or greater, it was considered positive. The animals were not allowed to remain on the hot plate longer than 35 seconds. Ten cages of mice were used, each containing 8 to 10 fasted male mice averaging 25 gm. in body weight. The reaction times of the mice in each cage were measured, and then the animals were injected with 0.1 ml. of neutral solution containing IMA. The control reaction times averaged 10 seconds, the range of values being between 1 and 15 seconds. The reaction times were then remeasured at 30 and 120 minutes after the injection. In those instances in which positive results were obtained, the results at the period giving the maximal number of positive responses were employed for the points shown in figure 5. No analgesic effects were noted by the above procedure at

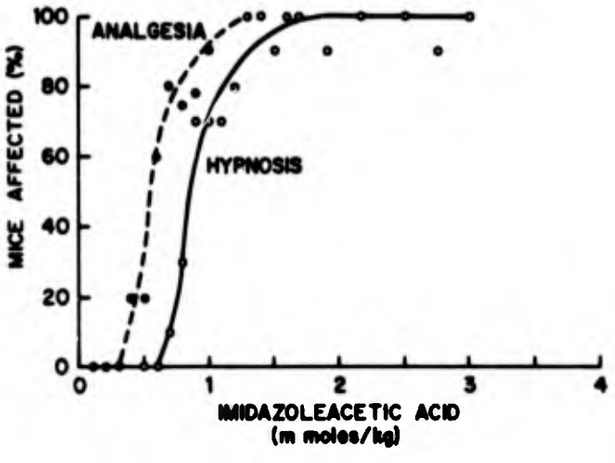

FIGURE 5

Analgesic and hypnotic effects of different doses of imidazoleacetic acid.

the 0.1 to 0.3 mmole/kg. level, while 90% of the mice receiving 1 mmole/kg. gave a positive result.

Hypnotic effect. When IMA was tested at the higher dose levels, it was noted that there always was a period during which there was impairment in the righting reflex before sleep. It was possible to detect this effect either by direct observation of the animals or by turning them over and noting whether or not they returned to a normal position. It was decided to

employ the loss of the righting reflex as an indicator of the onset of the hypnotic effect of IMA. Eighteen groups of 10 mice each were tested at dose levels of IMA ranging from 0.5 to 3 mmoles/kg. The percentages of the animals in which a hypnotic effect was produced at the various doses are shown in figure 5. It is interesting that a dose of IMA (0.6 mmole/kg.) which was 60% effective in the analgesic test was below the level producing a hypnotic effect. The results in figure 6 show that a relationship exists between the average time after injection of IMA required for loss of the righting reflex and the dose of IMA employed. No such relationship was found between the dose of IMA and the total sleeping time. All of the mice which fell asleep in this series of experiments slept for at least 2 hours.

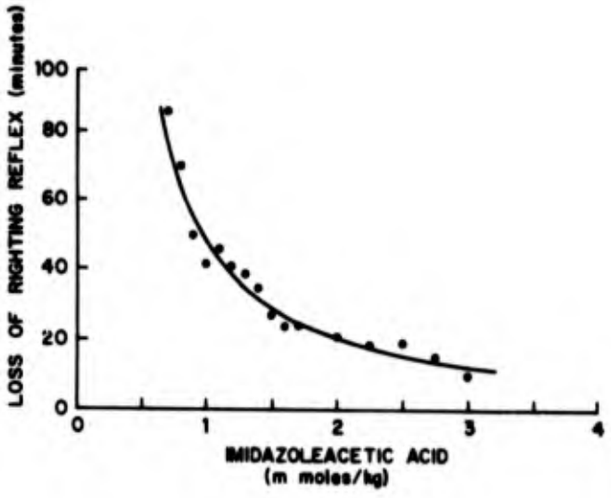

FIGURE 6

Average times required for loss of righting reflex as a function of dose of imidazoleactic acid.

Specificity of effect of imidazoleacetic acid

It was of interest to determine whether other compounds with the imidazole ring would have effects in mice comparable to those observed with IMA. Negative results were obtained with imidazolepropionic acid (dihydrourocanic acid) in experiments with four groups of 5 mice each injected with 1, 2, 3, or 4 mmoles/kg. Experiments in 40 mice with histidine at 4 mmoles/kg. also were negative. The following substances tested at 3 mmoles/kg. in the indicated number of mice also did not produce any of the effects noted with IMA at this level: imidazole, 10; 4-hydroxymethylimidazole, 10; 1-methylimidazole-4-acetic acid, 7; imidazolepyruvic acid, 10; imidazoleacrylic acid, 10. Therefore, it appears that considerable structural specificity must be associated with the biologic effects of imidazoleacetic acid in mice.

Previous biologic work with imidazoleacetic acid

The only previous study that has come to our attention that deals with the biologic activity of IMA was one in which it was found that this substance had an inhibitory action on the stretch receptor neuron of the crayfish, *Pacifastacus leniusculus (Dana)* (3). There appeared to be great specificity in the action of IMA on the stretch receptor neuron, since it was found that the following substances gave negative results: imidazolepropionic acid; 1-methylimidazole-5-acetic acid; histidine; 4-hydroxymethylimidazole; histamine; N-acetyl-L-histidine; histidinol; L-ergothionine; and L-2-thiolhistidine.

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13. ABSTRACT

Toxicologic studies of acute hydrazine toxicity in mice were continued. The results indicate that acute toxicity of hydrazine probably is not mediated through a histamine-release mechanism. Various experiments showed that the type of lethal seizure produced by hydrazine probably has no relationship to the sound-induced seizures in susceptible strains of mice.

It was found that sodium phenobarbital had a marked protective effect against hydrazine toxicity when given in subhypnotic amounts. Sodium phenobarbital had an additive protective effect when it was administered together with the previously studied protective mixture (AGKO) containing arginine, glutamate, α-ketoglutarate, and oxalacetate. NaBr also was found to be protective and was found to act additively with either sodium phenobarbital or with the AGKO mixture.

It was found in the course of the above experiments that imidazoleacetic acid, a substance not protecting mice against acute hydrazine toxicity, had interesting analgesic and hypnotic effects in mice. The quantitative aspects of these effects were worked out, and it is suggested that this agent should be explored further as a possibility for human use.

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