AN EXPERIMENTAL STUDY OF THE ADDITIVE AND ANTAGONISTIC ACTIONS OF SODIUM OXALATE, AND SALTS OF MAGNESIUM AND CALCIUM IN THE RABBIT.

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PLATE 95.

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INTRODUCTION

On the basis of the hypothesis that magnesium favors inhibition of the various functions of the nervous system, Meltzer and Auer studied extensively in this laboratory the action of magnesium salts upon various animals. In injecting magnesium sulphate subcutaneously,[1] they found that a certain dose, which varies with the species of animals, is capable of producing profound anesthesia and paralysis from which the animal recovers. For rabbits this dose amounts to about 1.5 gm. of magnesium sulphate ($MgSO_4 + 7\ H_2O$) administered in a molecular solution. Larger doses cause the death of the animal, as a rule, by respiratory paralysis. With an effective but non-fatal dose in subcutaneous injections the development of the depressing, inhibitory effect is gradual and fairly slow. When the maximum is reached, the turn for the recovery sets in soon; there is practically no real plateau to the inhibitory curve. The descending limb of this curve—the recovery—is steeper than the ascending one. When a magnesium salt is injected intramuscularly, the inhibitory as well as the fatal effects set in more promptly and with smaller doses.

In the course of their studies, Meltzer and Auer[2] found that calcium, which is chemically closely related to magnesium, is biologically appar-

[1] Meltzer, S. J., and Auer, J., *Am. Jour. Physiol.*, 1905, xiv, 366.

[2] Meltzer, S. J., and Auer, J., *Am. Jour. Physiol.*, 1908, xxi, 400.

ently the antagonist of the latter. When calcium is injected intravenously, shortly before or immediately after the respiration stops, into an animal which has received a fatal dose of magnesium, the animal will recover in less than a minute, provided, of course, that the circulation is still effective during the calcium injection. This biological antagonism is a remarkable fact and can be made the basis of many problems worth investigating. So far, at least, it is not known that between calcium and magnesium salts which have the same anion a chemical antagonism exists; no precipitation, for instance, occurs *in vitro* when a solution of magnesium chloride is mixed with a solution of calcium chloride. Calcium chloride is nevertheless strikingly antagonistic to magnesium chloride as far as the life of animals and plants is concerned.

What effect would the deprivation of the animal body of some of its calcium have upon the behavior of the animal? There are a number of acids and salts which precipitate calcium compounds *in vitro*. Will the administration of these calcium-precipitating compounds, let us say oxalic acid or oxalates in general, bring out symptoms indicating an increase of magnesium action? By precipitating calcium within the body a certain amount of unantagonized magnesium would be set free. Would this fact become manifest by the appearance of inhibitory and paralytic phenomena? The symptoms of oxalate poisoning do not speak for it; in general they possess rather the opposite character: excitation, tremor, and convulsions. But the amount of magnesium thus set free and the inhibition which it may be capable of exerting, might under these circumstances be too small to play a perceptible part, in the presence of the violent opposite symptoms which are brought out by another exciting factor of the oxalate. Could, however, the depressing component of the calcium-precipitating oxalate be brought out by a simultaneous administration of a subminimal dose of a magnesium salt? This was the problem which we tried to solve experimentally.

While we were at work on this problem, Schütz[3] published a brief preliminary communication in which he says that the susceptibility to magnesium injections could be increased occasionally, but not

[3] Schütz, J., *Wien. klin. Wchnschr.*, 1913, xxvi, 745.

constantly by sodium oxalate. A few months later Starkenstein,[4] with whose work we were not familiar until after we had given a preliminary communication of our results,[5] stated in a preliminary report that he found "like Schütz that the addition of oxalates constantly gave a visible increase of the magnesium narcosis."

We shall describe briefly our experiments bearing upon the problem under discussion and the conclusions to which they point.

EXPERIMENTAL PART.

We experimented exclusively on rabbits. Magnesium sulphate in M solution ($MgSO_4 + 7\ H_2O$) and sodium oxalate in 3 per cent solution[6] were injected separately and practically simultaneously, either into the lumbar muscles on opposite sides of the spine, or subcutaneously into each flank, the injection being usually followed by brief massage. All doses were estimated and are here reported in gm. of the salt per kilo of body weight. Most of the experiments were performed on a series of three animals, two serving as controls and receiving subtoxic doses of either magnesium sulphate or sodium oxalate alone. The experimental animal received the same dose of both salts.

Intramuscular Injections.

An abbreviated typical protocol follows.

Experiment I.

Rabbit A.—Oct. 2, 1913. Magnesium sulphate alone. Grey female. Weight 1,550 gm.

11.10 Right lumbar muscles: magnesium sulphate M, 4.3 cc. = 0.7 gm. per kilo of body weight.

11.23. Lying down, head up, breathing rapidly.

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[4] Starkenstein, E., *Wien. klin. Wchnschr.*, 1913, xxvi, 1235.

[5] Gates, F. L., and Meltzer, S. J., *Zentralbl. f. Physiol.*, 1913–14, xxvii, 1169. Starkenstein, E., *Zentralbl. f. Physiol.*, 1914, xxviii, 63; *Arch. f. exper. Path. u. Pharm.*, 1914, lxxvii, 45.

[6] Merck's reagent, "Sörensen's oxalate." Impure oxalates are not soluble to 3 per cent.

11.32. Can be placed on side.

11.34. Moves eyes and head when approached.

11.40. When tail is pressed, raises head and turns on belly. Moves head and looks around.

11.57. Hops when tail is pressed.

12.13. Hops around voluntarily, watching other rabbits. Remains well.

Rabbit B.—Oct. 2, 1913. Magnesium sulphate and sodium oxalate. Grey male. Weight 1,720 gm.

11.13. Right lumbar muscles: magnesium sulphate M, 4.8 cc. = 0.7 gm. per kilo. Left lumbar muscles: sodium oxalate 3 per cent, 5.6 cc. = 0.10 gm. per kilo.

11.24. Lying prone, with head on floor. When pushed over on side, lies passive. Respiration full; 17 in ¼ min. No response to pressure on tail; lid reflex good.

11.38. Same position. Lid reflex hardly perceptible.

12.01. Respiration slow and deep; 13 in ¼ min.

12.12. No response to stimuli, no lid reflex.

12.37. Turns over and lies on belly. Head sinks to floor and is raised at intervals. No response to pressure on tail.

1.08. Crouching in a corner. Recovers.

Rabbit C.—Oct. 2, 1913. Sodium oxalate alone. Grey male. Weight 1,480 gm.

11.21. Left lumbar muscles: sodium oxalate 3 per cent, 4.88 cc. = 0.1 gm. per kilo of body weight.

11.28. Sitting up, alert, changes position frequently. Starts suddenly without apparent cause.

11.43. Active, hops around, laps water, licks site of injection.

12.14. Has been behaving normally. Does not remain in one position long. No further effects noted.

The contrast in the behavior of the controls and the experimental animal is striking. The control animals were but little affected: this dose of sodium oxalate produced only trivial symptoms in Rabbit C, and the magnesium animal, Rabbit A, while weak and stupid, was at no time paralyzed or anesthetic. The third rabbit, however, ten minutes after the injections, was deeply anesthetized and remained passive and insensible for an hour. Two points are of particular interest: (1) In spite of the depth and long duration of the narcosis the respiration continued of good volume and rate and the animal was at no time in danger. (2) The animal regained power of voluntary movement before the return of sensibility to painful stimuli. A series of experiments with similar doses is given in Table I.

TABLE I.

Magnesium Sulphate and Sodium Oxalate, Intramuscularly.

No. of experiments.	Dose per kilo of body weight.		Average duration of deep inhibition. Animal relaxed on side.	Died.	Remarks.
	Magnesium sulphate.	Sodium oxalate.			
	gm.	*gm.*	*min.*		
6	0.7	0.10	89	0	
6	0.7	—	10	0	Only two out of six relaxed at all.
4	—	0.10	0	0	Visible effect questionable.

Subcutaneous Injections.

When the injections were made subcutaneously, somewhat larger doses had to be employed. A typical protocol of an experiment follows.

Experiment II.

Rabbit A.—Oct. 9, 1913. Magnesium sulphate alone. Slate colored female. Weight 2,030 gm.

10.16. Left flank, subcutaneously: magnesium sulphate M, 6.5 cc. = 0.8 gm. per kilo. Massage for 20 seconds.

10.31. Lying down, head and ears erect, breathing rapidly.

10.47. When disturbed hops away clumsily.

11.15. Crouching quietly, head up, ears flat on back. Respiration good.

11.50. Raises head to sniff at nearby objects.

12.20. Sitting up, washing paws. Remains well.

Rabbit B.—Oct. 9, 1913. Magnesium sulphate and sodium oxalate. Grey male. Weight 1,755 gm.

10.18. Right flank, subcutaneously: magnesium sulphate M, 5.6 = 0.8 gm. per kilo. Left flank, subcutaneously: sodium oxalate 3 per cent, 8.75 cc. = 0.15 gm. per kilo. Both sides massaged for 20 seconds.

10.53. Sitting up naturally.

11.04. Lying with chin on floor. Respiration slower and deep; 21 in ¼ min.

11.13. Placed passively on side without a struggle. Respiration 18 in ¼ min.

11.37. No response to pressing tail. Respiration shallow.

12.50. Trace of lid reflex. No response to pressing tail. Respiration of fair depth; 14 in ¼ min.

2.35. No lid reflex. No response to pressing tail.

3.52. Animal lying as before. No response to stimuli. Breathing entirely abdominal, of fair depth; 14 in ¼ min.

4.28. Does not resist handling. Voluntarily moves head, tail, and legs slightly. Observation discontinued.

Oct. 10, 1913. 9.15. Sitting up in cage. Rather quiet.

Rabbit C.—Oct. 9, 1913. Sodium oxalate alone. Grey female. Weight 1,510 gm.

10.22. Left flank, subcutaneously: sodium oxalate 3 per cent, 7.5 cc. = 0.15 gm. per kilo. Massage for 20 seconds.

10.43. Hops around licking the floor and sniffing at objects.

10.53. Sitting up, behaving normally.

11.14. Hops off actively when approached. No effects noted from injection.

Here again neither the oxalate nor the magnesium alone was effective. Together they produced a profound depression with a period of anesthesia and paralysis lasting more than four hours, followed by a gradual complete recovery.

Table II summarizes experiments with subcutaneous injections.

TABLE II.

Magnesium Sulphate and Sodium Oxalate, Subcutaneously.

No. of experiments.	Dose per kilo of body weight.		Average duration of deep inhibition. Animal relaxed on side.	Died.	Remarks.
	Magnesium sulphate.	Sodium oxalate.			
	gm.	*gm.*	*min.*		
8	0.8	0.15	123 +++	1	In four animals anesthesia extended into the night following. One died next day without recovery.
8	0.8	—	0	0	Practically no effect. Drowsiness in four cases.
8	—	0.15	0	0	No effects observable.

The cited protocols and the two tables illustrate the results obtained in these series of experiments. With the exception of two failures at the beginning, before the proper relation of dosage was determined, the experimental animal in every instance was definitely more deeply affected than the controls. The differences between the various experiments were only of degree, and depended upon the relation of the dose employed and the mode of administration, whether subcutaneous or intramuscular. With proper dose the

contrasts were striking and constant; while the controls were hardly visibly affected, the experimental animals were deeply anesthetized and paralyzed, the character of this inhibition being in general similar to that caused by large effective doses of magnesium alone.

In the following particulars the depression of the animals which received sodium oxalate and magnesium sulphate seemed to differ from that of animals which received magnesium alone. (1) The period of anesthesia and paralysis is a fairly long one, especially after subcutaneous injections, when the state of inhibition may last even 4 hours and longer; whereas after an effective sublethal dose of magnesium alone the entire state of depression is of a comparatively short duration. (2) In animals which receive oxalate and magnesium the deepest stage of anesthesia and paralysis tends to become stationary and is of long duration—the inhibitory curve has a long plateau—and the recovery takes place gradually; whereas with magnesium alone the inhibitory curve has hardly any plateau, and the animal after reaching the acme of anesthesia and paralysis either recovers quite rapidly or the depression leads to death by respiratory paralysis.

The increase of depression following the injection of subminimal doses of sodium oxalate and magnesium sulphate which was definitely established in these experiments cannot be considered simply as a summation of two similar effects. The symptoms brought on by oxalates are entirely dissimilar to those of magnesium inhibition. In our experiments the symptoms which follow the injections of sodium oxalate in toxic doses exhibit the character of excitation; anxiety, restlessness, hypersensitiveness, and tonic and clonic convulsions, which finally lead up to asphyxia and to a fatal termination. In such subminimal doses as we have employed, the toxic symptoms, if there were any, consisted at most in excitation and increased alertness; but there was never any manifest depression. It seems, therefore, that the strikingly depressing effect which the addition of a practically non-toxic dose of sodium oxalate to a subminimal dose of magnesium produces, must be ascribed to the ability of the oxalate to precipitate calcium from the body fluids and thus eliminate an element which biologically is antagonistic to magnesium.

The Action upon the Motor Nerve Endings.

Among the general effects of magnesium salts their depressing action upon the motor nerve endings stands out prominently. In minimal effective doses these salts reduce and in larger doses they completely abolish the conductivity of the nerve endings. In a series of experiments we have studied directly the combined action of sodium oxalate and magnesium sulphate upon this intermediary link between nerve and muscle. The sciatic nerve was cut under ether, the animal permitted to recover completely, and then the motor reactions of foot and toes to faradic stimulations of the peripheral end of the sciatic nerve were studied under the influence of the salts under discussion. Seventeen experiments were made upon rabbits. In fifteen there were two rabbits to each experiment, one an experimental animal and one a control. The experimental animals received subcutaneous injections of 0.6 to 0.8 gm. of magnesium sulphate in one side and 0.15 to 0.2 gm. of sodium oxalate. The fifteen control animals received injections of 0.6 to 0.8 gm. of magnesium sulphate alone. Two rabbits received injections of 0.15 and 0.2 gm. of sodium oxalate alone. For faradic stimulations a Porter induction coil, armed with one Daniell cell, was used. The cut sciatic nerve was stimulated before and at various intervals after the injection of the salt solutions, and the degree of the reactions to the various strengths of stimuli was noted. The results obtained in the experimental and control animals were compared and brought into relation with the general condition of the respective animals.

In both the sodium oxalate animals stimulation of the sciatic nerve before and at various times after the injection gave prompt reactions; strong tetanic flexion of the foot and abduction of the toes.

In eight of the magnesium controls stimulation of the sciatic nerve gave normal responses at the various periods after the injection. In the seven other controls there were slight degrees of reduction in the response to the stimulations; the reaction was less prompt, the extent of the contractions was lessened, or the distance of the secondary coil, in order to be effective, had to be shortened.

Of the experimental animals, in thirteen the conductivity of the peripheral nerve endings was definitely more deeply affected than in

their controls. In some cases the conductivity was so depressed that at the time when the general narcosis was at its height no response could be obtained from the stimulation of the sciatic nerve even with a 40 mm. coil distance. In two of the experimental animals the reduction in the response to stimulation of the sciatic nerve was not greater than that of their controls, although the general signs of anesthesia in the experimental animal were quite deep.

The depressing effect upon the motor nerve endings never outlasted the central effects, while there were cases in which the loss of sensation still continued after the motility seemed to be normal again.

The Antagonistic Action of Calcium.

Calcium, as stated in the introduction, is biologically antagonistic to magnesium, and our present experimental results led us to the conclusion that the increase of the depressive action of subminimal doses of magnesium by the addition of a subtoxic dose of sodium oxalate was due to the calcium-precipitating property of this salt. On the other hand, we found that the anesthesia and paralysis produced by a combination of subminimal doses of the two salts was of much longer duration than the same condition produced by an effective dose of magnesium sulphate alone. The question presented itself: Would calcium cause a recovery from the profound long-lasting state of depression caused by the combined action of the two salts, and especially would the recovery be as prompt and as rapid as in cases of magnesium anesthesia? We made a large number of experiments, but our results may be presented in the following single sentence: The antagonistic action of calcium is just as striking and prompt in the prolonged anesthesia brought about by the combination of oxalate and magnesium as it is in the anesthesia produced by magnesium alone. The following protocol is typical for all experiments in this series, and the photographs (Figs. 1 and 2) taken of this experiment are a good illustration of the results.

Experiment III.

Rabbit I.—Mar. 9, 1914. Magnesium sulphate alone. Grey and white male. Weight 1,860 gm.

1.58. Right back, subcutaneously: magnesium sulphate M, 5.9 cc. = 0.8 gm. per kilo. Massage for 1 min.

2.16. Sits quietly in corner of box, or lies down.

2.23. Crouching on forepaws, head and ears up. Respiration fair volume, slow; 19 in ¼ min.

2.32. Lying at full length, head and ears up. Backs up into sitting posture; rather heavy and quiet.

2.47. Crouching quietly in corner of box. Respiration full volume; 14 in ¼ min.

3.07. Photographed (Fig. 1).

3.16. Photographed (Fig. 2).

3.45. Behaving normally and has shown no further effects. Remains well.

Rabbit II.—Mar. 9, 1914. Magnesium sulphate and sodium oxalate. Black and white female. Weight 1,540 gm.

2.01. Right back, subcutaneously: magnesium sulphate M, 4.9 cc. = 0.8 gm. per kilo.

2.03. Left back, subcutaneously: sodium oxalate 3 per cent, 7.7 cc. = 0.15 gm. per kilo. Massage both sides for 1 min.

2.10. Has defecated. Respiration rapid and rather deep. Restless, changes position often.

2.13. Hind legs dragged a little in walking.

2.21. Crouching, head up, ears back, breathing rapidly; 68 in ¼ min.

2.35. Lying full length, eyes half closed, ears back, chin on floor. Flanks relaxed and bulging. Respiration 50 in ¼ min.

2.49. Lying partly on side, relaxed, head flat on floor. Mere trace of lid reflex. Moves head slightly when tail is touched.

3.05. Placed passively on back, feet in air. Remains there relaxed.

3.07. Photographed with controls (Fig. 1).

3.15. Same condition. Given 8 cc. calcium chloride 0.125 M through left ear vein. Respiration deepens during injection, and before it is completed animal turns over and sits up.

3.16. Photograph taken within 1 minute of injection (Fig. 2).

3.45. Crouching quietly. Hair erect. Hops off actively when disturbed. Then sits up with head and ears up. Remains well.

Rabbit III.—Mar. 9, 1914. Sodium oxalate alone. White female. Weight 1,620 gm.

2.06. Left back, subcutaneously: sodium oxalate 3 per cent, 8.1 cc. = 0.15 gm. per kilo. Massage for 1 min.

2.11. Hind legs dragged a little at times. Rather restless.

2.30. Sitting up or hopping around naturally. Head and ears up. Not restless or anxious. Respiration 42 in ¼ min.

2.48. Behaving normally. Sitting up, quiet. Respiration 35 in ¼ min.

3.07. Photographed (Fig. 1).

3.16. Photographed (Fig. 2).

3.45. Has shown no further effects.

Figs. 1 and 2 illustrate, in the first place, the anesthesia and paralysis produced by the combination of subminimal doses of magnesium and oxalate. They show, further, in a striking way, the antagonistic action of intravenous injection of calcium; it is in all respects similar to the action of calcium in anesthesia by magnesium alone. The respiration becomes deeper and more rapid immediately after beginning the injection, and the return of muscle tone and motor activity can be felt under the hand. Within a minute after the beginning of the injection, often indeed before all of the 8 or 10 cc. of solution is given, the animal draws up its legs, raises its head, turns over and scrambles into a sitting posture, and becomes alert and inquisitive. After an interval the rabbit may gradually sink back into narcosis, and can be restored again by calcium. Occasionally, if too much magnesium and oxalate have been given, a third injection may still be needed and given with success. However, under such circumstances, repeated injections of calcium might finally prove fatal to the animal.

The experiments, showing the depressing effect of magnesium and the antagonistic action of calcium to this depression, are, as we had occasion to learn, frequently demonstrated in many European Universities in lectures on pharmacology or physiology. When magnesium alone is used, the period of the greatest depression is of short duration and the demonstration may either be unconvincing, when the animal is not yet sufficiently narcotized, or it may be a failure, when the calcium injected is administered too late. The anesthesia and paralysis brought about by a combination of sodium oxalate and magnesium sulphate is, as we have seen above, of comparatively long duration. It is therefore a more appropriate method for purposes of demonstration. The animal may receive its double injection 40 to 50 minutes before the time set for the demonstration. If the proper doses are given and the proper procedure is followed out, there is no danger that the animal will not be in deep anesthesia, or that it will die too soon, before the antagonistic effect of the calcium can be shown.

SUMMARY.

The foregoing experiments establish firmly the following facts.

Subcutaneous or intramuscular injections of sodium oxalate in subtoxic doses, when administered to an animal which received a subminimal dose of magnesium sulphate, produce profound anesthesia and paralysis of long duration, although the usual effects of sodium oxalate alone are of a stimulating character. This fact is, in general, in harmony with the results reported by Starkenstein who, however, seems to have used the combination of the two salts in one solution; namely, that of magnesium oxalate.

The combined injections of subminimal doses of sodium oxalate and magnesium sulphate produce a strong reduction, or even, at times, a complete abolition of the conductivity of the motor nerve endings.

An intravenous injection of calcium salts brings on a recovery from the profound and prolonged effects of the combined action of sodium oxalate and magnesium sulphate, which is as prompt as is observed in experiments in which effective doses of magnesium alone were given. This fact is the more noteworthy, since depressions of long duration produced by prolonged continuous injections of magnesium solutions alone do not respond very promptly and effectively to calcium injections.

As will be recalled, the starting point for our investigation was the hypothesis that substances which are capable of precipitating calcium —a biological antagonist of magnesium—ought to be capable of increasing the depressive effect of magnesium. Our experiments proved that this assumption was correct. This would seem, therefore, to justify the interpretation that the augmenting action of sodium oxalate has its cause in the ability of the latter to precipitate calcium and thus increase within the body the amount of unantagonized magnesium. However, we wish to state expressly that this view is, for the present, still no more than a hypothesis and does not exclude other possible interpretations of our facts. As we pointed out it speaks against this hypothesis that oxalates do not produce phenomena of depression; the toxic symptoms produced by oxalates exhibit distinctly signs of increased and not of decreased irritability.

EXPLANATION OF PLATE 95.

FIG. 1. Rabbit III. Sodium oxalate 3 per cent, 0.15 gm. per kilo. Alert, ears erect. (Caught by instantaneous exposure.) Rabbit II. Sodium oxalate 3 per cent, 0.15 gm. per kilo. Magnesium sulphate M, 0.8 gm. per kilo. Deeply anesthetized and quite relaxed. Rabbit I. Magnesium sulphate M, 0.8 gm. per kilo. Crouches quietly as placed. Ears back.

FIG. 2. Rabbits III and I as before. Rabbit II within a minute has received 8 cc. of calcium chloride 0.125 M into the marginal ear vein (note clip). Alert and sensitive; right paw blurred from movement.

III II I

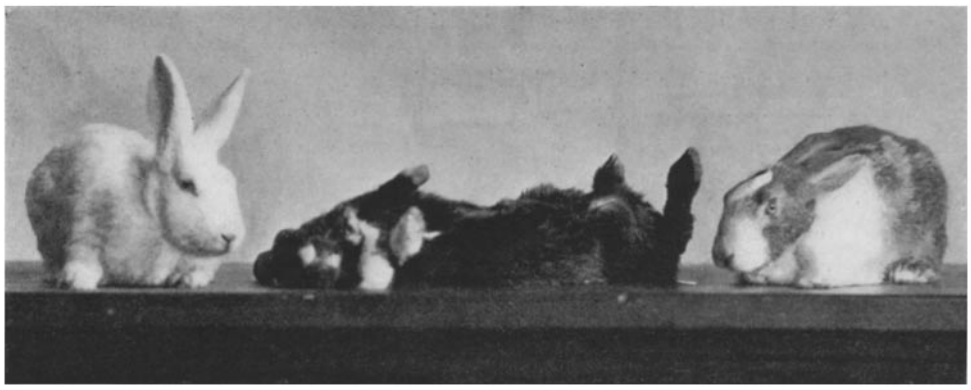

FIG. 1.

III II I

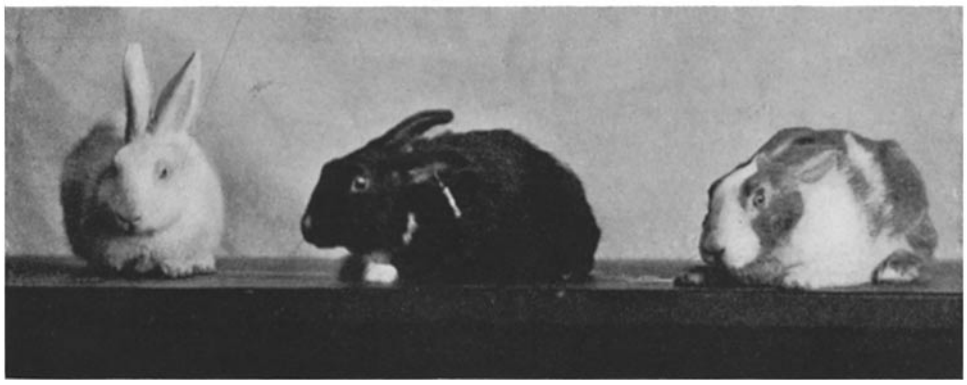

FIG. 2.

(Gates and Meltzer: Action of Sodium Oxalate, Magnesium, and Calcium Salts.)