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Studies on Barbiturates. XXV. The Effect of Vitamin C Level on Barbiturate Depression in Guinea Pigs*

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King, *et al.* (1, 2), found that a large series of organic compounds, notably aromatic ketones and alcohols of the terpene series, as well as barbiturates caused an increased excretion of vitamin C in the urine of rats kept on a diet affording minimal vitamin C synthesis. Since the vitamin C content of the tissues did not deviate from the normal, it was concluded that the increased excretion was due to an increased synthesis. Ritz, Samuels and Addis (3) found an increased urinary excretion of vitamin C in rats fed carvone or salicylates. They concluded that feeding of carvone did not deplete the tissues of ascorbic acid while salicylates did. Gruber, *et al.* (4), found an increased vitamin C excretion in rats when fed sodium diphenyl hydantoinate (Dilantin sodium), a compound chemically related to barbiturates; the increased excretion being due to a loss of vitamin C from the tissues, notably the adrenals and the liver.

These findings suggested the following problems: (*a*) Is there a correlation between the vitamin C level of the diet and the degree of depression produced by barbiturates in animals depending entirely on dietary sources for their vitamin C supply? (*b*) In such animals, or humans, on a diet just barely sufficient in vitamin C, would the oft-repeated administration of certain barbiturates produce scorbutic states of various magnitudes? If a correlation between vitamin C level was found to exist, it was hoped that these studies might throw some light on the more general problem of the mode of action of vitamin C as a detoxifying agent.

EXPERIMENTAL

(*a*) *Effect of Vitamin C Level on Barbiturate Depression.*—For these experiments, healthy guinea pigs, weighing between 250 and 300 Gm., were fed a basal diet completely devoid of vitamin C, but complete in all other respects (5). After the animals ceased to gain weight, they were divided into groups as nearly balanced as possible with respect to weight and general health. One of these groups was used as a negative control and received the basal diet only, while the other groups received 0.25, 0.5, 1.0 and 2.5 mg. of pure vitamin C daily by mouth. It will be recalled that 0.5 mg. of

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vitamin C daily is just sufficient to maintain weight and permit no gain; 1.0 mg. of the vitamin will permit virtually normal growth. Each vitamin group was divided in turn into three groups, one of which served as a control and received daily intraperitoneal injections of 1 cc. of saline, while the other groups were given sodium pentobarbital (Nembutal) and sodium phenobarbital, respectively, daily by the intraperitoneal route.

Since the entire experiment involved a total of 50 animals, the determination of the mean sleeping time was not considered expedient and a system of scoring numerically the degree of depression was devised. If animals showed no deviation from the normal, a score of 0 was given, while the presence of general lassitude was awarded a score of 1. If the animal could not maintain its normal posture and assumed a lateral position, a score of 2 was awarded. Complete abolishment of the righting reflex was scored 5. The ability to locomote also was scored as 1, 2 or 3, depending upon the degree of incoördination observed. Using such a scoring method, the highest possible degree of depression was represented by a total score of 11. At no time did any of the animals lose its corneal reflex from the doses of barbiturates employed. The injections and observations were made daily for a period of 30 days.

Table I.—Effect of Vitamin C Intake on Sodium Pentobarbital[a] Depression in Guinea Pigs

Number of Animals	Daily Intake of Vitamin C (Total Mg.)	Average Degree of Depression at 30 Min.	Average Degree of Depression at 60 Min.	Improvement in Animals During Second 30-Min. Period in Per Cent
4	0.0	9.25	9.9	−1.1
4	0.25	9.9	7.3	26.3
4	0.5	6.6	3.9	40.9
4	1.0	7.0	2.7	61.4
4	2.5	5.5	1.8	67.3

[a] 15 mg./Kg.

Table I shows the results of the experiment with sodium pentobarbital given in doses of 15 mg.[1] At the end of 30 minutes after the time of injection, the animals on the higher vitamin levels were not so depressed as the ones on the lower vitamin intake, but the difference was not a marked one. At the end of 60 minutes, however, the difference was more significant. It will be noticed that the negative controls were actually slightly more depressed at the end of 60 minutes than they were at the end of 30 minutes. In the last column of the table, the degree of recovery during the second 30-minute period is expressed in terms of percentages, *i. e.*,

$$\frac{(\text{av. depression at 30 min.} - \text{av. depression at 60 min.}) \times 100}{\text{av. depression at 30 min.}}$$

From these data it is plain that the lower the vitamin C level, the greater the depression and the more protracted the recovery.

Table II shows a similar experiment with soluble phenobarbital administered in doses from 15 to 50 mg. Regardless of the dose of phenobarbital used, it will be noticed that it is almost consistently true that the higher the vitamin C level, the less the depression. Since phenobarbital is slower to take effect and has a longer duration of action than pentobarbital, the time relations are more difficult to correlate with the vitamin level. However, in those cases where extended observations were made, the animals on a high vitamin C level recovered more quickly. Small doses of phenobarbital were used because accumulation was expected, particularly in animals on the lower vitamin levels. However, no observable accumulation occurred at any level up to doses of 50 mg. of phenobarbital.

At the end of the experiment, two of the animals in the phenobarbital series and one in the pentobarbital series which were receiving 0.25 mg. of vitamin C daily, were now given 2.5 mg. of the vitamin daily for 7 days. As the vitamin supply was thus restored, the response to both barbiturates became less severe progressively. For example, the score representing the depression of the animal receiving daily injections of pentobarbital progressively fell from 11 to 5 as the vitamin was deposited in the tissues under the influence of the higher vitamin feeding.

Table II.—Effect of Vitamin C Intake on Sodium Phenobarbital Depression in Guinea Pigs

Number of Animals	Daily Intake Vitamin C[a]	Responses to Varying Doses of Sodium Phenobarbital[b] 15 mg. (7 Days) After 30 Min.	15 mg. (7 Days) After 60 Min.	30 mg. (10 Days) After 30 Min.	30 mg. (10 Days) After 60 Min.	40 mg. (10 Days) After 30 Min.	40 mg. (10 Days) After 60 Min.	50 mg. (4 Days) After 30 Min.	50 mg. (4 Days) After 60 Min.
4	0.0	4.3	4.8	5.3	7.6	[c]	[c]	[c]	[c]
4	0.25	2.6	3.0	3.9	4.3	6.8	6.5	5.1	7.1
4	0.5	2.8	3.6	2.9	2.7	3.7	2.9	6.5	6.7
4	1.0	1.9	2.0	1.2	0.9	1.8	1.9	2.5	2.4
4	2.5	1.1	1.9	0.9	0.6	1.6	2.2	3.0	2.8

[a] Total mg.
[b] Mg. per Kg.
[c] All animals dead.

(*b*) *Effect of Barbiturate on the Vitamin C Level.*—It was thought that the repeated administration of barbiturates might deplete the tissues of vitamin C to such an extent that animals on a bare subsistence vitamin intake would develop a scorbutic state. This did not appear to be the case, for the growth

[1] Throughout this paper, doses of barbiturates are expressed in terms of mg. per Kg.

curves of animals on either barbiturate were substantially the same as those of the saline controls. Furthermore, the vitamin C content of the adrenals and the livers of many of the animals were determined by the indophenol titration method (16). The vitamin content of these tissues was essentially the same in both groups. These results are in agreement with other data in the literature. Therefore, it seems improbable that in humans on a low vitamin C level, the frequent administration of barbiturates would cause an increased vitamin requirement.

There is considerable evidence in the literature that vitamin C may play a role in the detoxification of many types of organic compounds (3, 4, 6, 7, 8, 9, 10). One way in which vitamin C could be of assistance in the detoxification of barbiturates is by the formation of a simple barbiturate-ascorbate complex. To test such a possibility, the vitamin C content of the guinea-pig urine was determined before and after mild acid hydrolysis and was found not to vary. Since mild hydrolysis made no more vitamin available, it was concluded that no such complex was eliminated, at least in a manner analogous to glycuronate conjugation, unless the complex was capable of reacting with the indophenol dye used for titrating the urine. King, *et al.*, observed similar results with albino rats (1, 2).

Because vitamin C is a sugar acid lactone, it is conceivable that the tissues can convert it in part to a glycuronic acid. Since glycuronic acid is widely recognized as a detoxifying agent, it was thought possible that the vitamin detoxified barbiturates through glycuronate complex formation. To test such a hypothesis, the reducing substances of guinea-pig urine of barbiturized animals were determined by the Scales (11) method. The reducing substances present, however, were substantially accounted for in terms of ascorbate itself.

On the basis of the last two findings, any conjugative mechanism for the detoxification of barbiturates must be denied or it must be assumed that the conjugation takes place in the tissues and that the conjugates are so labile as to preclude their presence in the urine.

DISCUSSION

The data presented in this paper show that there is a definite correlation between the vitamin C intake and the response of guinea pigs, animals unable to synthesize the vitamin, to pentobarbital and to phenobarbital. At present, the explanation for this correlation, however, is a difficult if not an impossible task, for the specific mechanism of action of vitamin C and the modification of metabolic processes by this vitamin are unknown. Although the vitamin is capable of undergoing reversible oxidation and reduction, it has never been definitely identified with any specific enzyme system in animal tissues. The work on rats referred to above, showing that the feeding of barbiturates causes an apparent increase in the vitamin C synthesis by the tissues, makes it permissible to assume that this vitamin plays some role in the detoxification of barbiturates. Our finding on guinea pigs would lend support to this assumption if an exhaustion of the animal's vitamin depot would accompany oft-repeated administration of barbiturates. However this was not the case. Since the vitamin was not depleted from the tissues, it can be inferred that the frequent administration of barbiturates to humans is not likely to produce scorbutic states. This is further suggested by the findings of Wright, *et al.* (12), that the daily administration of 180 mg. (total dose) of phenobarbital to humans produced no change in the vitamin C concentration of whole blood, plasma or urine. The urine of our barbiturate-treated animals contained no abnormal amount of reducing substances such as sugars or glycuronates.

Koppanyi, *et al.* (15), have stated that animals in a good physiological condition can destroy more barbiturates than when they are in a state of "ill health." In observing individual animals used during this experiment it was apparent that the animals appearing to be less vigorous and in ill health were more severely depressed by the barbiturates and that the depression lasted appreciably longer. Indicative of such an empirical interpretation, Hjort, *et al.* (13), found a correlation between the mean sleeping time of mice, under the influence of a synthetic ureide, and the completeness of the diet. The nature of the deficiencies of their diets was not stated, but some were assumed to be deficient in protein, vitamin B complex and perhaps calcium.

Until the specific action of vitamin C is better understood, one is not justified in assigning a definite role to vitamin C in the detoxification of barbiturates; one must be content with stating that the general physiological state of animals is an important factor in modifying the action of hypnotics.

When this paper was completed, our attention was called to a note by Keuter, Richards and Klatt (14) on the action of barbital, pentobarbital and pentothal in vitamin C-depleted guinea pigs. These authors stated that at different levels of C avitaminosis the sleeping time from pentobarbital was markedly prolonged while that from barbital or pentothal was little affected. These authors suggest that general metabolic disturbances and changes in the liver following the C avitaminosis are responsible for the prolongation of pentobarbital narcosis. They suggest that the liver is particularly instrumental in the destruction of the short-acting barbiturates, but not of the long-acting group, pentothal being a notable exception. Thus two groups of investigators using slightly different methods of approach have found that lack of vitamin C leads to a prolongation of sleeping time in pentobarbital narcosis. Here unfortunately the agreement stops because the above investigators report that a long-acting barbiturate, barbital, is not appreciably influenced by lack of vitamin C, whereas we found that another long-acting barbiturate, phenobarbital, behaves like a short-acting barbiturate under identical conditions.

Moreover, there is some reason to question the validity of the conclusion of the above investigators because the main difference that we found between the action of barbiturates at a high and at a low vitamin C level is not so much concerned with the prolongation of sleeping time as with the magnitude of the initial depression. We are inclined to believe that a given hypnotic has a greater initial effect in animals at a lower physiological level than in animals at a higher physiological level. These initial differences were very readily observed in the individual animals.

SUMMARY

1. There is a correlation between the vitamin C level and the response produced by phenobarbital and by pentobarbital in guinea pigs. The higher the vitamin level, the less the depression produced by the barbiturates.

2. The frequent administration of pentobarbital and phenobarbital to guinea pigs did not cause a depletion of vitamin C from the tissues.

3. It is suggested that the most likely cause for the effect of vitamin C level on barbiturate depression is the altered general metabolism produced by the lack of this vitamin rather than an effect produced in major degree by direct conjugation.

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Marihuana Investigations. IV. A Study of Marihuana Toxicity on Goldfish Applied to Hemp Breeding*

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INTRODUCTION

It has been shown in previous articles (1, 2, 3) of this series that individual plants or varieties of hemp, *Cannabis sativa*, react differently to the chemical Beam tests and that the resins vary in amount and reaction to the Beam tests with hemp of different varieties, different ages, or hemp grown under different climatic conditions. The results presented suggested a possibility of obtaining by plant breeding methods a variety of hemp that might contain a low amount of the resin. It was not proved in the previous articles that the resins in the different lots of hemp were different in their physiological activity as measured upon animals.

Although significant progress has been recently made in elucidating the chemistry of marihuana resins, the material is of such complex nature that it may require consider-

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