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Guanidine Retention and Calcium Reserve as Antagonistic Factors in Carbon Tetrachloride and Chloroform Poisoning.*

A. S. MINOT AND J. T. CUTLER. (Introduced by Paul D. Lamson.)

From the Department of Pharmacology, Vanderbilt University, School of Medicine.

An earlier note[1] reported the rather empirical observation that dogs on a lean meat diet can be made either highly resistant or susceptible to carbon tetrachloride intoxication by adding or withholding calcium salts for a few days previous to the administration of carbon tetrachloride. Furthermore calcium therapy was found highly effective in the treatment of cases of poisoning.

The intoxication produced in susceptible dogs was described in the earlier note and is characterized by gastro-intestinal irritation, nervous disturbances and convulsions, followed by weakness, depression, and death. There is a retention of bile pigments in the blood and a hypoglycemia which usually becomes extreme before death and which seems in most cases to be the immediate cause of death. The level of total calcium in the blood is usually within normal limits, nevertheless either a lack of or excessive demand for calcium ions seems to be indicated by the relief afforded by the furnishing of extra available calcium. It was at first suggested that a depression of ionized calcium due to combination with bile pigments might account for the apparent calcium lack. This is probably one factor in the need for extra calcium, but further work has indicated that it is probably not the only or even the most important factor.

A similar toxic picture can be produced by the administration of guanidine compounds. Following subcutaneous doses of 250 mgm. of guanidine hydrochloride per kilo body weight, dogs show fibrillary twitching of muscles, tetanic convulsions, gastro-intestinal irritation with bloody vomitus and diarrhea, with usually a period of extreme depression and weakness for some time before death, which occurs 12 to 15 hours after the drug is injected. Four or 5 hours after the guanidine is administered there is a marked fall in blood sugar and the hypoglycemia becomes extreme unless a diet very rich in calcium and carbohydrate has been fed for some time pre-

* This investigation is one of a series of studies being made under the direction of Dr. P. D. Lamson on the pharmacology and toxicology of carbon tetrachloride. The work is being carried on with the support of the International Health Board.

[1] Minot, A. S., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 617.

ceding the experiment. In this intoxication as well as in carbon tetrachloride poisoning, relief is afforded by the prompt intravenous administration of calcium salts.

Further evidence of a more fundamental similarity in the 2 conditions was furnished by the determination of guanidine in the blood of large numbers of animals. A carefully controlled adaptation of the method for guanidine determination described by Major and Weber[2, 3] was used. Guanidine and sugar determinations were made on samples of blood taken before and at intervals after the administration of the toxic drug in the following types of intoxication: I. Following the oral administration of 4 cc. CCl_4 per kilo to dogs on the following diets: (a) on a diet of meat without extra calcium; (b) on a diet of meat with extra calcium; (c) on a diet of bread and milk. II. Following the oral administration of 4 cc. CCl_4 per kilo + 4 cc. alcohol per kilo to dogs on the diets described above. III. Following the inhalation for 1½ hours of chloroform vapor of a concentration sufficient to maintain complete anesthesia. IV. Following the subcutaneous administration of varying doses of guanidine hydrochloride.

From these studies it was found that both carbon tetrachloride and chloroform cause some interference with normal metabolism which results in a tendency for guanidine to accumulate in the blood. Until more is known of the origin and normal disposal of guanidine nothing definite can be concluded regarding the site or nature of this interference. From the similar effects produced by 2 well known liver poisons one is tempted to attribute the disturbance to a failure on the part of the damaged liver to contribute some necessary step in normal metabolism. This point has, however, not yet been proved. It seems probable that precursors of guanidine are abundant in meat since the increase in guanidine following the administration of carbon tetrachloride is largely avoided if the animals are fed a bread and milk diet. There is also, however, an endogenous source of guanidine because in the more severe intoxications produced by giving alcohol with carbon tetrachloride and by chloroform there is an accumulation of guanidine even when meat is not eaten.

Within a few hours after an appreciable increase of guanidine in the blood the typical symptoms described above appear and there is a fall in blood sugar. The hypoglycemia is more extreme and usually proves fatal in the animals on a low calcium diet. The difference in the severity of intoxication in the high and low calcium groups

[2] Major, R. H., and Weber, C. J., *Johns Hop. Hosp. Bull.*, 1927, xl, 87.

[3] Major, R. H., and Weber, C. J., *Arch. Int. Med.*, 1927, xl, 891.

on the meat diet seems to lie in the difference in the severity of the subsequent hypoglycemia rather than in the concentrations of guanidine in the blood. Further evidence that the additional dietary calcium is responsible for the avoidance of dangerously low blood sugar levels was furnished by other experiments in which rapidly falling sugar levels were restored and maintained at a safe level by the administration of calcium salts. This effect on blood sugar is, however, not the only basis for the relief afforded by calcium because when a normal blood sugar level is maintained by the administration of dextrose the nervous symptoms persist unless calcium is also given.

The more severe intoxications produced by carbon tetrachloride given with alcohol, or by chloroform, apparently do not differ qualitatively from that produced by carbon tetrachloride alone. There is a higher and more rapid rise in guanidine in the blood which occurs to some extent even when meat is not eaten. This rise is soon followed by severe hypoglycemia and death. Calcium has the same antagonistic action here as in cases of carbon tetrachloride poisoning, though in these cases the need is more imperative. Mere preliminary feeding of a high calcium diet is usually inadequate to prevent serious symptoms of intoxication. When calcium feeding is supplemented by persistent calcium and dextrose therapy marked temporary relief is afforded and permanent cures can often be effected unless gastro-intestinal hemorrhages become uncontrollable.

Of most importance in establishing a causal relationship between the symptoms seen in any of the cases just described and the observed concentrations of guanidine in the blood are the results obtained from the group of animals receiving guanidine hydrochloride. When similar symptoms were produced quite comparable concentrations of guanidine were found in the blood whether the drug administered to produce the intoxication was carbon tetrachloride, chloroform, or guanidine hydrochloride.

The reason for the protective and curative action of calcium salts reported in our earlier paper[1] is apparently due to the antagonism between calcium and the guanidine which tends to accumulate in the blood. Practical suggestions for the safe use of carbon tetrachloride would, therefore, emphasize both the advisability of avoiding meat, and of furnishing adequate dietary carbohydrate and calcium for several days before and after the administration of the drug. Should cases of intoxication occur calcium and dextrose therapy would be indicated and in our work with dogs has proved very effective.