showers of these degenerated leucocytes which are found in the peripheral blood stream. Experiments are at present in progress to determine the exact nature of this mechanism.

This is a preliminary report.

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The Relation of Calcium to the Toxicity of Carbon Tetrachloride in Dogs.*

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In the treatment of several million cases of hookworm disease with carbon tetrachloride a few deaths have occurred. Some of these were probably due to the taking of alcohol with the drug, and others to mechanical obstruction by ascaris worms. In a very few instances, however, carbon tetrachloride was apparently the direct cause of death. In an attempt to explain these cases extensive studies have been carried out by Lamson and his associates.[1] These authors found that it was practically impossible to produce visible signs of intoxication in dogs by the oral administration of carbon tetrachloride in doses even up to the capacity of the stomach (250 cc.). Despite the general normal condition, pathological examinations and various functional tests showed that even single small doses caused considerable damage to the liver, while typical cirrhosis of the liver could be produced by repeated administration.[2, 3, 4, 5]

The work was transferred in 1925 to the Vanderbilt University Medical School. Continued studies here with various liver function tests gave results quite analogous to those reported in the earlier papers. The death of a great number of experimental animals, however, indicated that some new factor had been introduced in spite of the fact that the same carbon tetrachloride was used. In contrast to the earlier results, doses as low as 4 cc. per kilo body

* 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.

weight (the smallest amount capable of influencing the phenoltetrachlorpthalein test) now proved fatal in practically all cases, while attempts to produce cirrhosis with repeated doses were futile because the subjects all died.

The usual symptoms in the dogs in this laboratory, after receiving 4 cc. of carbon tetrachloride per kilo by mouth, were a loss of appetite and a noticeable lack of energy during the first 20 to 30 hours. Some of the dogs then rapidly became unconscious, or so sluggish that they could be aroused only with great difficulty. Others, and these were more common, showed muscle twitchings, starting as a fine tremor of the toes and lips and increasing in severity over a period of several hours, until nearly the entire musculature was involved in typical tetany. Frequently the diaphragm was also involved, causing spasmodic breathing. Death occurred in either type 40 to 60 hours after the drug was administered.

The similarity of the convulsive symptoms to infantile tetany, as well as the fact of their appearance only after the work was transferred to Nashville, suggested a possible calcium deficiency in the new diet. During the previous experiments the food had been a well balanced ration consisting of meat, corn-meal, and bone meal baked into a cake. Here the diet had been almost exclusively lean beef scraps containing very little bone. There had been, therefore, a very radical decrease in the calcium intake. Nevertheless dogs which did not receive carbon tetrachloride seemed to be well nourished on this new diet. Apparently some condition caused by the carbon tetrachloride was making unusual demands on the organism which could not be met by animals kept for some time on a diet deficient in calcium.

The most consistent change found in dogs after the oral administration of carbon tetrachloride was a marked increase in the concentration of bilirubin in the blood as shown by determination of the icteric index.[6] This constituent, which is normally present only in traces in dog's blood, began to increase about 12 hours after the dose was given, and the more severe signs of intoxication appeared soon after the bilirubinemia reached a high level. Several observations serve to link this change with the possibility of calcium lack. It has been shown that the slowed clotting time of blood in obstructive jaundice can usually be restored to normal by the addition of ionized calcium.[7] Furthermore, Bowler has reported that the lethal intravenous dose of calcium chloride is much higher for jaundiced than for normal dogs.[8] Earlier papers by King and Stewart[9] and King, Bigelow and Pearce[10] show that bile pigments

combine with calcium in the blood. This protects the organism against the toxicity of bile pigments, but at the same time depletes reserve of calcium in the tissues and causes an increased elimination. There may be an actual increase in the total calcium present in the blood, but much of it is combined with bilirubin so that a decreased concentration of *ionized* calcium results. Conditions of tetany were not reported in any of these experimental studies. It seems quite possible, however, that while animals on an adequate ration may have a sufficient reserve of calcium to prevent such acute symptoms, nevertheless a removal of ionized calcium might readily be disastrous to animals kept for some time on a deficient diet.

To test this point calcium chloride was administered intravenously to dogs in the convulsive or unconscious stages of intoxication from carbon tetrachloride. Within 15 to 30 minutes the animals walked around the laboratory in a fairly normal condition. The muscle twitching disappeared temporarily. Relapses into the comatose or convulsive condition occurred frequently, but the response to intravenous calcium injections was always prompt. By repeated medication at intervals of 3 or 4 hours the animals could be brought through the crisis of the intoxication and completely cured. Relapses needing repeated medication recurred as long as the icteric index remained high, usually for 2 or 3 days. Furthermore, the simple addition of calcium salts to the meat diet for a week or more preceding the administration of carbon tetrachloride made the dogs here show the same tolerance for the drug as reported by Lamson.[1] Large doses, 4 to 6 cc. per kilo, which had been consistently fatal, now produced no sign of intoxication, except the usual liver damage as evidenced by liver function tests.

The oral administration of calcium salts has failed to save animals already showing signs of carbon tetrachloride intoxication. Death was delayed somewhat but the absorption of calcium was too slow to be adequate. The oral administration of ammonium chloride as an indirect means of influencing the blood calcium[11, 12] was more effective. When the drug was retained the animals recovered. Persistent vomiting in some cases, however, made it necessary to resort to intravenous calcium therapy.

These results show that (1) carbon tetrachloride is relatively very toxic for dogs that have been on a prolonged low calcium diet; (2) signs of intoxication can be prevented by the continued addition of calcium to the diet; or (3) cured either by the intravenous injection of calcium chloride, or the oral administration of ammonium chloride.

These observations all suggest that the immediate cause of death in these dogs may be attributed to the lack of ionized calcium in the blood, secondary to the bilirubinemia, following the administration of carbon tetrachloride. Furthermore, the possibility that the production of serious symptoms by the lack of dietary calcium might occur as readily in any other jaundiced condition, may be a point of considerable clinical importance.

Further experimental work is being done regarding the total and ionized calcium in the blood following carbon tetrachloride administration and in other jaundiced conditions, and also on the effect of the parathyroid hormone as a therapeutic agent. This work together with the experimental data already accumulated will follow this preliminary note in a later publication.

In man uncomplicated deaths from carbon tetrachloride have been so rare that there is no typical picture of intoxication. It is, however, known that such deaths occur 24 hours or more after the administration of the drug. Convulsions have been reported in some cases of poisoning. The majority of these cases have occurred in children in whom calcium deficiency is a common clinical condition. Whether deaths following anthelmintic treatment can be attributed to a calcium deficiency remains to be investigated, but the foregoing experiments would indicate the advisability of an adequate calcium diet for a considerable period preceding carbon tetrachloride administration, and suggest the possibility of calcium therapy in cases of acute intoxication.

This is a preliminary report.

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