Experimental Liver Damage Associated with Hematologic Changes in Hogs*

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VARIOUS investigators have reported the occasional association of macrocytic anemia with disorders of the liver (cirrhosis, acute yellow atrophy, etc.[1, 2]). In 1936 a macrocytic anemia in which the blood picture resembled that of Addisonian anemia and of sprue was described by the author and J. A. Pons[3] in the late, or visceral, stage of schistosomiasis mansoni in Puerto Rico. This type of anemia has been correlated with portal cirrhosis of the liver found postmortem in advanced cases of schistosomiasis mansoni,† which latter condition is produced by infestations with a parasitic trematode that lives in the portal system of the liver, bringing about chronic dysentery and liver damage (portal cirrhosis), splenomegaly, anemia, and in some advanced cases, death. From our observation on the study of the blood picture in such cases, we believed that the macrocytic anemia was produced by a deficiency in the specific antianemic factor (Castle) conditioned by chronic liver damage (cirrhosis) and not necessarily by gastric dysfunction. It was suggested that the anemia might be brought about by faulty storage of the hematopoietic principle, or its failure to form in the liver, a deficiency which results in macrocytic anemia.

EXPERIMENTAL STUDIES

Having obtained information from clinical and pathological studies regarding the association of liver damage and macrocytic anemia in man, it was our intention to duplicate experimentally, if possible, the results in animals. Thus, it seemed feasible to produce cirrhosis of the liver by three different agents: 1. Repeated infestations with *Schistosoma mansoni;* 2. repeated infestations with *Fasciola hepatica* (the liver fluke of cattle); 3. administration of variable quantities of carbon tetrachloride. The last method has been shown[4] to produce cirrhosis of the liver in animals, very similar to the Laennec type observed in man. The damage caused by this drug seemed to be limited to the liver, and because cirrhosis of the liver had been successfully produced in rats,

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† Unpublished observations by Dr. E. Koppisch, Department of Pathology, School of Tropical Medicine.

rabbits and dogs by various investigators,[5, 6] it was decided to use hogs in our experiments. It was soon found that methods 1 and 2 of producing liver damage had to be abandoned, as one animal failed to show any evidences of parasitic infestation after receiving several hundred thousands of schistosoma cercariae by the skin and intraperitoneal routes at varying intervals over a period of eight months. The intermediate host (*Limnaea cubensis*) of *Fasciola hepatica,* a fresh water snail, was not available at the time to carry out experimental infection with this parasite.

Material and methods: Five Berkshire pigs (3 males and 2 females) of imported stock, but born in Puerto Rico from the same litter, and of about three months of age, were used. The animals weighed about 10-15 kilos each. The hogs were kept in a large spacious yard, with cement floor and under very clean conditions. Carbon tetrachloride was administered by stomach tube in measured amounts at intervals of from once daily to once every two weeks. The dosage for each animal varied from 1 cc. per kilogram to 7 cc. per kilo daily. The total amount given to all animals varied from 380 to 737 cc. and the drug was given over periods ranging from two to about four months.

The diet consisted of fresh, unspoiled refuse from the University Hospital kitchen, supplemented by raw vegetables and fruits. Milk was given occasionally. Liberal amounts of carbohydrates were supplied. In addition, each animal received daily 10 grms. of a mixture of equal parts of calcium carbonate and calcium chloride, given in the feedings. The purpose of this was to ameliorate through the calcium the acute toxic effects of carbon tetrachloride on the liver.

Blood was drawn from the tail vein (by removing the scab of the previous wound, a free flow of blood was obtained from the distal segment of the tail) at weekly intervals. Two to three cc. were collected in bottles containing 6 mgs. of dry ammonium oxalate and 4 mgs. of potassium oxalate for each cc. of blood.

Erythrocyte and reticulocyte counts, hemoglobin determinations, volume of packed red cells, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, leucocyte counts, differentials and platelet counts were performed weekly. The hemoglobin was determined in grams and percentage by a single Newcomer-Klett instrument with a solid standard. One hundred per cent hemoglobin was considered equivalent to 14.5 grams of hemoglobin per 100 cc. of blood. Smears for differential white cell counts, reticulocytes and a study of red cell morphology were prepared with unoxalated

blood. The usual technique was followed in performing the blood counts and certified apparatus was used.

Gastric analysis (histamine) was done in some animals every two weeks.

A control period of 4-5 weeks before administration of the carbon tetrachloride (and during which the above procedures were performed), was observed in all the experimental animals.

Examinations of feces to determine parasitic infestation were carried out at monthly intervals in all animals.

Liver damage was estimated by clinical evidences observed in animals under study, icteric index determinations at weekly intervals and by gross examination of organ at autopsy. No other liver function tests were made.

Blood studies and weight determinations in control animals (Nos. 1 and 2) were continued from five to six months under same conditions as experimental animals, indicating the adequacy of diet and that the amount of blood drawn weekly did not cause anemia.

Autopsy was performed on four of the five animals. Microscopic sections for histologic examinations were made of liver, spleen, pancreas, kidneys, adrenals, lungs, heart, salivary glands and thyroid. Hematoxylin eosin stain was employed.

RESULTS

Clinical evidences of effect of carbon tetrachloride administered: Animals Nos. 4 and 5 lived seventeen and eight weeks, respectively, after ingestion of the first amount of the drug. Both animals received about the same proportion of the drug per kilogram of body weight. Both continued to gain weight until death, but the rate of growths as compared with the control animals was greatly diminished. Hog No. 1 which had been used as control for over five months was, upon the deaths of hogs Nos. 4 and 5, employed as a test animal.

The earliest symptoms of carbon tetrachloride poisoning, noticed about two weeks after administration, were anorexia and weakness, followed later by increased irritability and convulsive movements of the extremities. When death was near, weakness and stiffness of the legs were marked. No signs of acites or jaundice were observed in hogs Nos. 4 and 5, but evidences of jaundice in hog No. 1 were observed two weeks before death; the skin over the abdomen became brick red in color. The sclerae remained unchanged, however, and there were no noticeable changes in the tongue or in the mouth. The urine was scant, brick red in color and had an offensive ammonia odor.

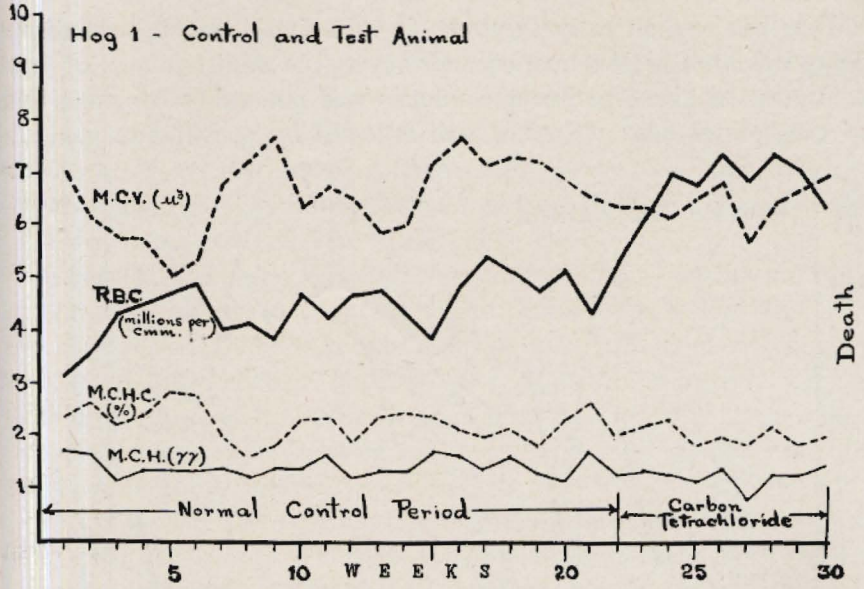

FIGURE 1: Red blood cells, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, before and during administration of carbon tetrachloride.

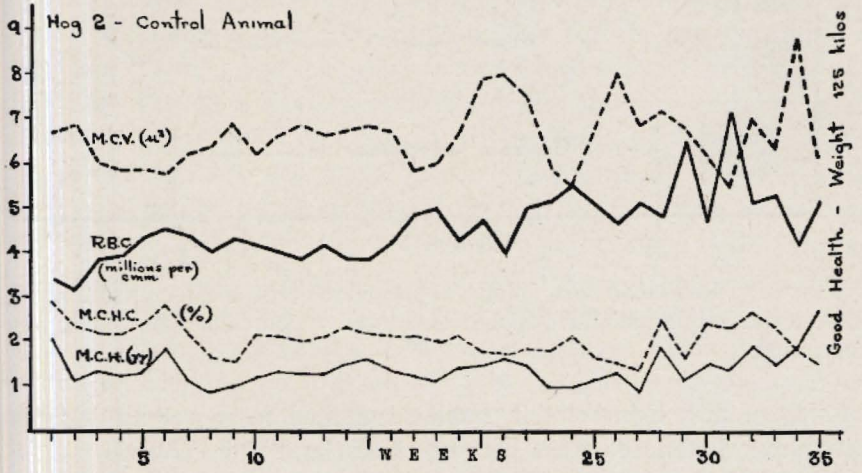

FIGURE 2: Red blood cells, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration.

Diarrhea was an early symptom in all animals, but it soon disappeared as well as the anorexia. However, the daily amount of food consumed by the experimental animals was noticeably less than that of the control hogs. Diarrhea was followed by constipation and the

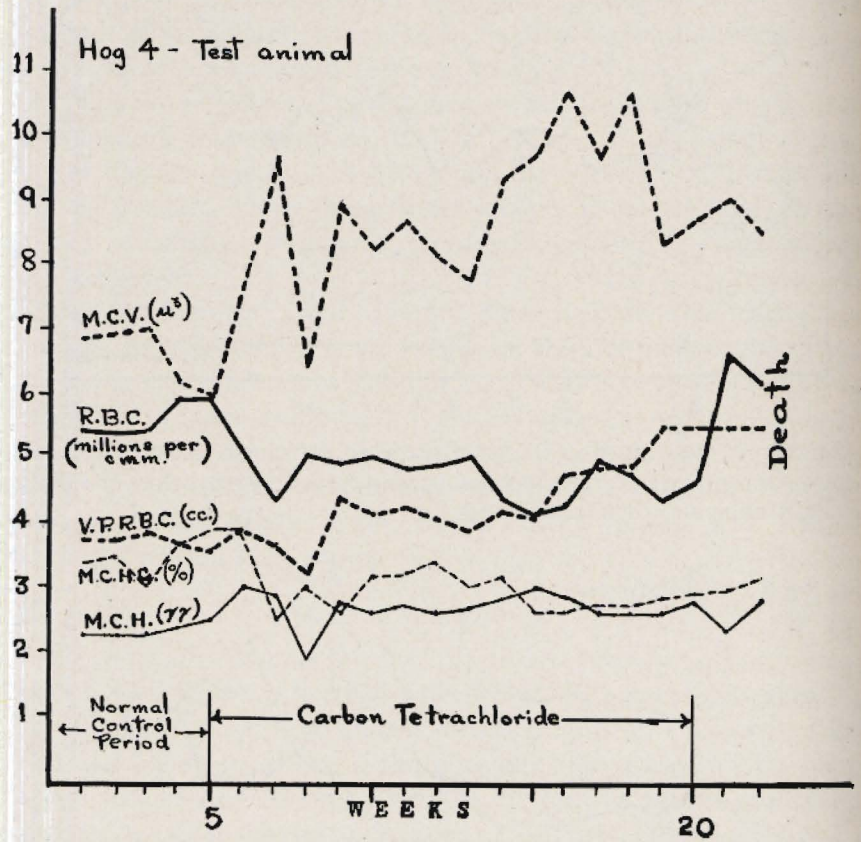

FIGURE 3: Red blood cells, volume of packed red cells, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration.

passing of dry, light-colored, small segmented feces, resembling goat droppings. Later, the feces became chalky and clay colored.

The icteric index was soon elevated in animals receiving carbon tetrachloride. A rise of 50 units was observed as early as the second week after ingestion of the drug. This increase was maintained around 50 units until death. During control period the icteric index did not go over 2 units in all the animals.

As it seemed that either too large or too frequent amounts of carbon

tetrachloride were responsible for the absence at autopsy of chronic liver damage (cirrhosis), it was decided to administer smaller doses per kilo at less frequent intervals to hog No. 1. This procedure, and the fact that this animal was about ten months old and weighed 75 kilos, apparently had no protective effect, as death occurred on the eighth week after administration of the drug, and no cirrhosis of the liver was found postmortem.

In general it may be said that increased individual susceptibility to carbon tetrachloride in the three experimental animals was not a significant feature. While it is true that one animal lived about twice as long as the other two, the manifestations of poisoning were very much alike in all. Animal No. 3 died early during the period of standardization, congestion and edema of the lungs, with mild, acute bronchitis being observed at autopsy.

Effects on the blood: Moderate degree of variability was observed in the red cell counts, owing to the differences of what constitutes a normal red count in hogs. In some animals the erythrocyte determinations during the period of standardization or control fluctuated between four and five millions per c.mm., while in others the range was from four to six millions. No marked anemia was observed during administration of carbon tetrachloride. In hog No. 5 there was a decided trend toward reduction of red cells, while in Nos. 1 and 4 there occurred a rise, more definite and sustained in the former. This increase in red cells is considered a true polycythemia in hog No. 1, as there occurred a rapid increase which continued until the death of the animal. The rise in the red cells was about 95 per cent greater as compared with the counts during the normal period. In hog No. 4 the gain in red cells was observed almost one week before death and was only slightly higher than normal values. However, it occurred after a previous loss of about two million cells per c.mm. Had greater and more consistent reduction occurred in the erythrocytes, it would have served as a guide to give smaller and less frequent doses of carbon tetrachloride and thereby, perhaps, prolong the life of the animals.

Macrocytosis of the red cells accompanied by slight hyperchromia was observed in hogs Nos. 4 and 5. The elevation in mean corpuscular volume occurred early after administration of carbon tetrachloride and was continued until death. This is the most important and significant morphologic change observed. In both animals the increase varied from 70 to over 100 cubic microns, which is well above the normal limits for all hogs (control period). In animal No. 1 there was no important

change in the mean corpuscular volume during administration of the drug after a short rise.

The short, initial rise in the mean cell hemoglobin in Hog No. 5, which paralleled the elevation in mean cell volume, decreased, and rose again slightly before death. In No. 4, after a slight and early rise in

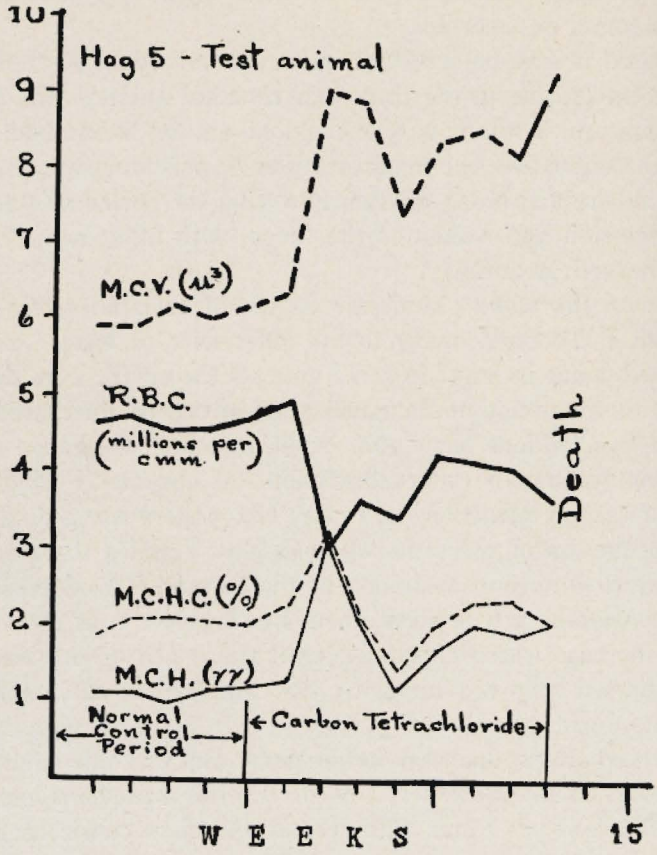

FIGURE 4: Red blood cells, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, before and during administration of carbon tetrachloride.

mean cell hemoglobin, it remained unchanged until death. The mean cell hemoglobin concentration followed closely the mean cell hemoglobin in hog No. 5 and was not altered in hog No. 4. Hog No. 1 showed no change in mean cell hemoglobin and mean cell hemoglobin concentration. The macrocytosis observed was of the round cell type, not oval. Anisocytosis, poikilocytosis, nucleated red cells, were occa-

sionally found in experimental as well as in control animals, and had no apparent relation with the total number of red cells. In fact, when the erythrocyte count in hog No. 1 reached eight millions per c.mm. during administration of carbon tetrachloride, marked anisocytosis and poikilocytosis were observed in stained smears, as well as numerous normoblasts, microcytes and spherical macrocytes and occasional megaloblasts.

White cell counts, differentials and platelet counts are not yet reported in detail in the present work, because of minimal variations obtained in experimental and control animals. The leucocytes fluctuated between 10 and 20 thousands per c.mm. during normal or control periods. In hog No. 4 there was a definite decrease in the total leucocytes three weeks before death, accompanied by a neutrophilia. The decrease was about 50 per cent compared with values obtained during control period. While in hog No. 1, no marked change was observed in the number of white cells, an increase of 40 per cent before death was observed in hog No. 5. The results are too variable to warrant any conclusion. Changes in differential counts were not considered significant.

The platelet counts ranged from 180,000 to 400,000 during the control periods in all animals, but the largest number of counts fell between two and two hundred and fifty thousands. A definite decrease to 100,000 occurred in all hogs two to three weeks before death.

No change in reticulocyte counts was observed at any time. All animals showed a maximum of 2 per cent. Examination of feces during normal and experimental periods were negative for parasite ova and cysts.

Gastric analysis was performed in animals Nos. 1, 2, 4 and 5 at two week intervals. Hogs Nos. 1, 4 and 5 had no fasting free hydrochloric acid. Hog No. 2 showed free acid. However, after hystamine administration free hydrochloric acid was recovered in all animals both during the period of standardization and during the administration of the drug.

Figures Nos. 1, 3 and 4 show the changes in total red cell count, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration during periods of control or standardization and during administration of carbon tetrachloride in experimental animals (hogs Nos. 1, 4 and 5). Similar data is shown in normal animals (Nos. 1 and 2) (Figures 1 and 2). Hog No. 1 was employed first as control for twenty-two weeks and later was used as a test animal. The volume of packed red cells is shown for hog No. 4 in Figure 3.

Postmortem findings: These studies were made by Drs. E. Koppisch and C. Krakower of the Department of Pathology of the School of Tropical Medicine.

Hog No. 3 which died early in the experiment, from no known cause, showed pulmonary congestion and edema with acute bronchitis. In hog No. 1 the subcutaneous fat was intensely yellow and the intestinal mucosa appeared grossly hemorrhagic and contained numerous blood clots. Some amber colored fluid was found in the abdominal cavity. There were irregularly scattered areas of necrosis in the mucosa of the stomach. Unfortunately microscopic sections of stomach and intestines are not available.

The most important changes were limited to the liver. All animals dying from the effects of the drug showed similar changes. Grossly, the livers were of normal consistency, somewhat pale. Microscopically marked central hemorrhagic necrosis with marked vacuolization of the peripheral cells and lobules with round cell infiltration was found. Bile stasis and bile-stained Kupffer cells were noted also. Hog No. 4 showed slight accentuation of the lobular connective tissue, and in the central lobular areas there was considerate increase of fibrous tissue with increased round cell infiltration, including polymorphonuclears. Marked vascular congestion with tubular degeneration was present in the kidneys. Moderate, vascular congestion was found in the lungs and spleen. The adrenals showed hemorrhagic necrosis. The heart, pancreas, thyroid and salivary glands were negative. The anatomical diagnosis for all animals was central necrosis of the liver, fatty infiltration and bile stasis of liver; hemorrhagic necrosis of adrenals and fatty kidneys.

DISCUSSION

The experiments described above have shown that carbon tetrachloride produces extensive liver necrosis in hogs. This confirms the works of others[6, 7]. Though the amount, frequency and length of administration of the drug was quite variable for each animal, similar postmortem findings were observed in all. No chronic liver damage (cirrhosis) was obtained, as it is believed that each animal died too soon after administration of the drug before reaching the stage where chronic liver damage or cirrhosis was produced. The fact that no significant anemia was evident during the course of administration of the drug, deprived us of the best guide to avoid and to delay subsequent sublethal doses of carbon tetrachloride.

The most significant findings regarding the peripheral blood picture

in the experimental animals was a rapid and substantial rise in the mean corpuscular volume. Polycythemia occurred in hogs No. 1 and No. 4 during carbon tetrachloride administration. There was an increase in the total number of red cells as compared with the counts obtained during the five-week control or normal period in the same animal before ingestion of the drug; for in a control animal (No. 2) higher levels for red cells than those obtained in hogs Nos. 1 and 4 were observed when the animal was eight months old. Polycythemia was coincident with periods of diarrhea and vomiting, and may be explained in terms of hemoconcentration through the loss of fluids.

The slight fall in red cells correlated with macrocytosis occurring in hogs Nos. 4 and 5 soon after the administration of carbon tetrachloride may be the result of early liver damage. However, the hematopoietic organs were soon able to compensate for this loss in the presence of further damage to the liver. It is assumed that marked macrocytic hyperchromic anemia did not develop because of the early death of the animals. As a rule, macrocytosis was proportional to the fall in the red cell count.

Although marked macrocytic anemia is lacking in the study, the changes observed in the peripheral red cell morphology, such as macrocytosis of the spherical type resembled closely those described by the author[3] in the late stage of schistosomiasis mansoni in man.

SUMMARY

1. A series of blood examinations were done in five hogs (three experimental and two control animals) before and after ingestion of repeated and variable amounts of carbon tetrachloride. Similar studies were performed in one animal before and after repeated negative attempts at infestation with the cercaria of the trematode, *Schistosoma mansoni,* had been made.

2. Enumeration of erythrocytes, leukocytes, platelets, differential white cell counts and reticulocytes were performed. Estimation of hemoglobin and determination of the volume of packed red cells were made also. Mean corpuscular volume, corpuscular hemoglobin and corpuscular concentration were determined.

3. Clinical observations, gastric analysis and determination of icteric index were performed previous to and during the course of administration of carbon tetrachloride.

4. Postmortem studies of animals dying from carbon tetrachloride poisoning are included.

CONCLUSIONS

1. Changes in the peripheral blood of hogs in which hepatic damage was produced by the administration of carbon tetrachloride have been described. It was intended to produce chronic hepatic damage (cirrhosis), but it is believed that the animals died too soon to effect such changes.

2. Macrocytosis (spherical) of the red cells and polycythemia were the most important changes in the blood observed during the course of carbon tetrachloride poisoning. A slight decrease in number of red cells in some animals was found, also. Macrocytosis was similar to that described by the author in advanced schistosomiasis mansoni in man. It is suggested that changes in red cell morphology and polycythemia are the result of liver damage and hemoconcentration respectively. The latter phenomenon was coincident with diarrhea and vomiting. It is assumed that macrocytosis occurred as a consequence of altered metabolism in the liver of the antianemic principle.

3. Central necrosis of liver with fatty changes and bile stasis was observed postmortem in all animals dying from the effects of carbon tetrachloride.

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