THE RESISTANCE OF PUPS TO LATE CHLOROFORM POISONING IN ITS RELATION TO LIVER GLYCOGEN.*

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In a former paper it was shown that the various hemorrhagic diseases of the new-born, known as Buhl's disease, Winckel's disease, and melena neonatorum, are probably all expressions of an asphyxial process. This conclusion was based on the following points: (1) the similarity of the anatomical and clinical findings to those known to be induced by lack of oxygen, viz., cyanosis, edema, fat infiltration, hemorrhages, etc.; (2) the experimental production of the various disease pictures by subjecting pregnant animals to the influence of chloroform, a drug which is known to suppress oxidations; (3) the production likewise of similar conditions by direct asphyxiation of the fetuses through ligation of blood vessels to the uterus.

Most of the experiments were made with guinea pigs, but a few dogs were used also.

While the paper was in press, an article by Whipple appeared, in which he showed that in experiments upon four litters of pups he was unable to produce a central necrosis of the liver lobules during the first three weeks of life by the administration of chloroform for a period of approximately two hours. A necrosis of the central portion of the liver lobule is a nearly constant result of a two hour chloroform anesthesia of an adult dog. A portion of the work incorporated in Whipple's article had already been included in one of his former articles. We had, therefore, in our earlier article made note of the absence of findings in Whipple's experiments and had made the suggestion that perhaps a high content of liver glycogen had been concerned in protecting the pups which he used.

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However, since in our experiments no central liver necrosis was found, although the livers became very fatty, no claim was made that the changes found justified the diagnosis of a true chloroform poisoning, since the presence of a central necrosis has been regarded by some as characteristic of the picture. We felt that perhaps they might be expressions merely of an interference with the fetal blood supply induced by changes in the placenta, of the kind which had already been described by Whipple. Nevertheless, it has seemed desirable to investigate the point further, because from a theoretical standpoint it would be of interest to determine the nature of the relative insusceptibility of pups to late chloroform poisoning. Accordingly, we have carried out experiments with this point in view.

We have been able to corroborate the main conclusion of Whipple that pups are relatively insusceptible to the production of liver necrosis by chloroform. But we feel that the evidence is practically conclusive that this greater resistance is dependent chiefly, if not entirely, on the high glycogen content of the livers of normal, well nourished pups. Our evidence, briefly summarized, is as follows: 1. Pups were made to show the typical liver necrosis, if given chloroform, after adopting measures to deplete glycogen, either by (a) starvation or (b) phlorhizinization and starvation. 2. In adult animals it is well known that increasing the glycogen content of the liver by feeding of carbohydrates serves to protect against poisons which, like chloroform, produce fatty changes in the liver; e. g., phosphorus, arsenic, etc. 3. An examination of the liver of a pup twenty-four hours old showed that as much as 9.07 per cent. of its total weight was glycogen.

It is interesting to compare this amount with the amounts found by Schöndorff in a series of eight adult dogs in which an attempt was made to raise the glycogen content to a maximum by feeding for several days with a large quantity of carbohydrate. The results represent the percentages of the total weights of the livers, and are as follows: 4.3, 7.6, 18.7, 17.1, 16.4, 9.9, 7.3, and 15.

Thus it is seen that our finding in the pup's liver of 9.07 per cent. is greater than or equal to the amounts found in half the adult dogs in which an attempt had been made to crowd the liver with glycogen. In other words, this value of 9.07 per cent. may be considered as indicating a liver that is well filled with glycogen.

5 A number of analyses made by Demant (Ueber den Glycogengehalt der Leber neugeborener Hunde, Ztschr. f. physiol. Chem., 1887, xi, 142) on the livers of pups show even higher amounts than the one obtained by us. Thus the livers of three pups, aged 1 hour, 3½ hours, and 3 hours, respectively, showed, by Brücke's method, percentage values of glycogen of 11.389, 9.527, and 8.443, re-
Roger\textsuperscript{6} long ago recognized the detoxicating action of the liver against strychnin. Since then it has been learned that the liver exercises a similar action against many other poisonous substances, and that this protective property of the liver is chiefly dependent on glycogen. Rosenfeld\textsuperscript{7} has shown that animals fed upon carbohydrates are in general less susceptible to all those drugs which produce fat accumulation in the liver. We have found that generous feeding of sugar to adult dogs renders them much less susceptible to the late poisonous manifestations of chloroform. For a number of years it has been the custom of many of the surgeons in England, where chloroform is extensively used as a surgical anesthetic, to insure in their patients a good supply of glycogen by generous feeding of carbohydrates.\textsuperscript{8} Recently, Opie and Alford\textsuperscript{9} have shown that in mice the feeding of carbohydrates exerts a decidedly protective action against the development of liver necrosis by chloroform. Furthermore, Rosenbaum\textsuperscript{10} has shown that after a protracted chloroform narcosis the liver is actually poor in glycogen.

The mechanism of this protective action of glycogen is not clear. Is it a property of unchanged glycogen itself, or is it rather a property of glucose? It is, of course, well known that in the condition of so called acidosis of the type which occurs during fasting, diabetes, and some other conditions, the feeding of alcohol, sugars, and other substances possessing alcohol groups, is accompanied by a marked diminution in the output of the acetone bodies in the urine. Chloroform poisoning is one of those conditions. The question of how sugars and other substances accomplish a diminution of this respectively. During the twelve days after birth the amount of liver glycogen was found gradually to diminish. It is interesting to note, therefore, that the diminution of resistance of pups against chloroform poisoning, which occurs with increasing age, parallels a diminution in the amount of liver glycogen. Mendel and Leavenworth (Chemical Studies on Growth.—III. The Occurrence of Glycogen in the Embryo Pig, \textit{Am. Jour. Physiol.}, 1907-08, xx, 117) speak of high values for pups as being in contrast to those for other fetal tissues; and they seriously question the truth of the statement that in general fetal tissue is rich in glycogen.

\textsuperscript{6} Roger, G. H., \textit{Action du foie sur la strychnine}, \textit{Arch. de physiol. norm. et path.}, 1892, iv, 24.
\textsuperscript{7} Rosenfeld, G., \textit{Fettbildung}, \textit{Ergebn. d. Physiol.}, 1903, ii, pt. 1, 50.
\textsuperscript{8} Beddard, A. P., \textit{A Suggestion for Treatment in Delayed Chloroform Poisoning}, \textit{Lancet}, 1908, i, 782.
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A particular type of acidosis has been investigated by Woodyatt. On the basis of the so-called Cannizarro reaction, which has been extensively studied by Ciamician and Silber, he suggests that sugars and other so-called antiketogenic substances undergo oxidation with a simultaneous reduction of ketones and keto-acids. But even if this does explain how antiketogenesis is effected, it does not explain the protective action against liver necrosis. We cannot seriously consider the necrosis to be dependent upon an acidosis in the ordinary sense; for even in fatal cases of diabetes with high grade acidosis, there is no extensive liver necrosis. On the other hand, it is possible that the protective part played by glycogen against chloroform necrosis is more of a physical nature, accomplished perhaps by altering the permeability of the cell or certain parts of the cell to the chloroform. In this connection, it has been shown by Bechhold and Ziegler that glucose, alcohol, and glycerin retard the diffusion of some substances into gels. The protective action ascribed by Whipple to the fetal blood islands found in the livers of these young pups has no evidence to support it, except that blood islands are present in the livers of the insusceptible pups; and, moreover, we have found limited areas of necrosis in livers which still contain some of these blood islands, as shown by the presence of occasional giant-cell and small collections here and there of mononuclear leucocytes. We shall discuss other means of inhibiting the production of this necrosis in another article, now in preparation, in which evidence will be brought to show that chloroform liver necrosis is produced by hydrochloric acid, which is formed as a dissociation product of chloroform, according to the equation:

$$\text{CHCl}_3 \rightarrow \text{CCl}_2 \rightarrow + \text{HCl}.$$ 

A number of experimental difficulties were encountered in carrying out the present work. It was found, for example, that it was almost impossible to maintain an even depth of narcosis in the very

young pups; for when the chloroform was given by inhalation they
frequently held their breath for periods of from forty seconds to
over a minute. Indeed, in one experiment one of these periods of
apnea was found to be as long as two minutes and twenty seconds,
during which time the pup lay still and appeared to be deeply anesthe-
tized, but in reality it was entirely conscious and reacted vigorously
to stimulation by pinching of the skin, etc. To obviate this error
regular artificial breathing was usually maintained by alternate com-
pression and relaxation of the thorax. Because of a scarcity of ma-
terial, the risk of killing pups prematurely by giving the chloroform
by injection was not undertaken. Another difficulty was to avoid
death from exposure, since in order to shut off the carbohydrate sup-
ply, the pups had to be kept away from their mothers. Still another
difficulty was in properly gauging the amount of phlorhizin, so that
a two hour chloroform anesthesia would not result in the death of
the pup during the next few hours. There was no uniformity in
tolerance to the phlorhizin, so that this procedure was largely guess-
work.

The chloroform used was Mallinckrodt's, bearing the label "Puri-
fied for anesthesia." Usually an anesthesia was maintained for two
hours, but sometimes for a longer period. After an interval of two
days the pups were killed with chloroform and examined. Sections
of the liver and other viscera were stained with hematoxylin and
eosin, and also with special fat stains, such as Sudan III. After the
pups were taken from their mothers they were kept wrapped in
cotton, and artificial heat was supplied so that the temperature of
the surrounding air in the box was about 30° C. Water was given
daily by subcutaneous injection of 20 to 30 cubic centimeters of 0.85
sodium chloride solution. The phlorhizin was injected subcuta-
neously, sometimes dissolved in warm 1 per cent. sodium carbonate
solution, and at other times suspended in olive oil, according to
Coolen's method. Typical protocols follow.

**STARVATION PRIOR TO ADMINISTRATION OF CHLOROFORM.**

*Experiment 5.—Mar. 10.* Three healthy fox-terrier pups were received into
the laboratory, all of the same litter, and five days old. Their respective weights
were: A, 237 gm.; B, 275 gm.; C, 350 gm.

*Mar. 11.* After twenty-four hours of starvation A was given chloroform
for two hours, and C for three hours.
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Mar. 13. All three pups had lost markedly in weight. The respective weights now were: A, 192 gm.; B, 217 gm.; C, 285 gm. Pup B was given chloroform for two hours. Pups A and C were killed with sudden overwhelming doses of chloroform and autopsied. A shows a moderate amount of fat in the liver with virtual absence of subcutaneous and omental fat. Microscopically the liver shows very marked changes. About the central veins are areas involving roughly one-quarter to one-third of the lobule, in which there is marked necrosis, as indicated by fragmentation of nuclei, hyaline changes in the cells, and deep staining of the necrotic part with eosin. Both here and at the periphery of the lobule the cells are very fatty. Pup C has an excessively fatty liver. There are no important changes in the other organs. Microscopically the liver changes are about the same in kind and degree as those in pup A.

Mar. 14. Pup B was again given chloroform for one hour. At the close of the anesthesia it was cold and in very poor condition; respiration was shallow and infrequent. It was kept all night in an incubator at 35°C.

Mar. 15. Pup B was found in the morning to be in much better condition than in the evening before. Killed with chloroform and examined. Weight, 210 gm. There is marked emaciation with an entire absence of omental and subcutaneous fat; the liver is light brown in color. It is everywhere studded with pinhead-sized areas which are pale and opaque and apparently surround the central veins. Microscopically the liver is found to present marked necrosis and to be intensely fatty. The areas of necrosis are central and involve from one-third to one-half of the liver lobules; they are filled with hyaline areas from which the nuclei have disappeared.

PHLORHIZIN PRIOR TO ANESTHESIA.

Experiment II.—May 3. Two healthy mongrel pups, two days old, designated as A and B. The respective weights were: A, 390 gm.; B, 370 gm. An injection of 0.05 gm. of phlorhizin was given subcutaneously to A at 9.30 a.m., and repeated at 5 p.m.

May 4. A similar injection of phlorhizin was given again to A at 9.30 a.m. From 1 to 3.30 p.m. both pups were given chloroform. A (phlorhizin pup) took the anesthetic badly and required artificial respiration a number of times. At the close of the anesthesia it was cold and apparently almost dead; both pups were kept in an incubator at about 30°C all night. We have repeatedly noticed that phlorhizinized animals are much more easily killed with chloroform than are others, a fact which has also been observed by Sansum and Woodyatt in this laboratory. The latter, who made metabolism studies of narcotized diabetic dogs, noted also a great depression of all urinary secretion following the chloroform narcosis.

May 5. B was lively, but A (phlorhizin pup) had shallow respirations and moved only when aroused. At 8.30 p.m. A was moribund; therefore both were killed with chloroform and immediately autopsied. A’s (phlorhizin pup) liver was very fatty, B’s only moderately so. Microscopically A showed well marked central necrosis involving about one-third of the lobule. B’s liver had only a slight amount of necrosis. Both livers contained a few bone marrow cells.

DETERMINATION OF GLYCOGEN.

May 29. Bulldog pup born last night in the laboratory. At 10.30 A.M., it was taken from its mother. At 2.25 P.M. it was killed by decapitation. The liver was immediately removed and ground in a meat grinder. To the hash, which weighed 0.709 gm., were added 10 c.c. of hot 60 per cent. KOH, and the whole was then placed on a steam bath. This and subsequent operations were carried out in accordance with Pfüger's method. After hydrolysis, 0.95 gm. of glucose, which is equivalent to 0.07 per cent. in 9.709 gm. of liver, was found by titration. The unhydrolyzed glycogen was also determined by means of the polariscope. By this method a value of 0.39 per cent. was obtained, which is seen to be in close agreement with that found by titration after hydrolysis.

GLUCOSE AND CHLOROFORM.

Mar. 27. Two small adult dogs, designated as A and B. At 10 A.M. A received 15 gm. of glucose in water by stomach tube. From 11 A.M. to 1 P.M. both dogs were given chloroform. At 1 P.M., while still unconscious, A received 25 gm. of glucose in water by stomach tube.

Mar. 28. A was given 50 gm. of glucose by stomach tube.

Mar. 29. Both dogs were killed with chloroform.

Autopsy.—A's (glucose dog) liver shows a slight amount of infiltrated fat. Microscopically there is no necrosis. B's liver is intensely fatty, and microscopically there is well marked necrosis. The amount of subcutaneous and omental fat was about equal in the two dogs.

This experiment was repeated on two other sets of dogs, and similar results were obtained.

SUMMARY AND CONCLUSIONS.

The relative difficulty with which the characteristic central lobular liver necrosis can be produced in young pups after chloroform administration is in some way referable to the high glycogen contents of their livers. Evidence for this conclusion lies in the following facts:

1. Pups can readily be made to show the central liver necrosis which is found in chloroform poisoning in adults, if, prior to the administration of chloroform, they have been starved or starved and made diabetic by phlorhizin.

2. A single quantitative experiment showed that the liver of a normal, well nourished pup, twenty-four hours old, contained as much as 0.07 per cent. of glycogen.

3. The feeding of carbohydrates to adult animals lessens their susceptibility to the production of liver necrosis by chloroform.