Previous studies (1–3) have shown that the metabolism of carbohydrates and ketone bodies in infants and children has distinct peculiarities which are characteristic of these age groups. The two most important differences between the young and the adult human being are (a) the ease with which hypoglycemia develops in infants and children when carbohydrates are withheld from the diet (1) and (b) the pronounced disposition to ketosis at certain ages. Studies (3) made on ketosis showed that under the influence of a ketogenic diet with a ratio of 2.5:1 ketonuria remains minimal in infants up to the age of 7 or 8 months. From then on, ketonuria develops with increasing ease during the first 4 years of postnatal life, reaches a broad peak at from 4 to 8 years, and decreases during prepuberty, at which time it equals values obtained in adults, values which are still much higher than those found in infants.

To understand these findings, one should know more about the effect of age and fasting on the content of glycogen in liver and muscle. The reports of systematic studies thus far made in this field rarely include determinations in animal or human infants, although the period of infancy is obviously of the greatest interest to the problem.

Because Collip and his collaborators (4) described a disposition to ketosis in rats in regard to age that was similar to that which was found (3) in human beings, rats were chosen for the experimental animals.

Results of the experiments now reported show, however, some
differences between rats and human beings in regard to the influence of fasting on the blood sugar level (Fig. 1). In rats 8 to 11 days old and 10 to 12 weeks old, hypoglycemia does not develop after 1 and 2 days of fasting, as it does in human infants and children. Blood sugar values obtained in these rats were not lower after 1 and 2 days of fasting than normal fasting values in full grown animals, whereas in human infants and children, under similar conditions, the values would be around 50 to 60 mg. per 100 cc. of blood.

**Methods**

A total of 119 white rats was divided into three age groups: 8 to 11 days, 10 to 12 weeks, and 2 to 3 years. These animals
were fed the Sherman Diet B as modified by Smith and Bing (5), supplemented with yeast and wheat germ. The first blood sugar and glycogen values were determined 3 hours after the food tray had been removed from the cage or after nursing rats had been separated from their mothers for 3 hours. Determinations of liver glycogen values were also made on twenty-one puppies, 6 days old, in the same manner. During the period of actual fasting, the animals had access to water in their cages.

The animals were bled to death by cutting through the large abdominal or femoral vessels. The content of sugar in the blood obtained from the bleeding animals was determined according to the method of Folin (6).

The content of glycogen was determined by the method used by Pflüger (7), and the range of error was ±0.15 gm. per 100 gm. of fresh tissue. The tissues of the rats and puppies were immersed immediately after death in a 60 per cent solution of KOH. The liver and muscle tissues of from three to six baby rats were pooled together in order to obtain sufficient material.

The water content of the tissue was measured by weighing the tissues before and after incubation for 24 hours at 100°. Here the range of error was ±0.1 gm. per 100 gm. of fresh tissue.

Results

Effect of Age

Before the influence of fasting was studied, data on the influence of age alone on the content of glycogen in liver and muscle were obtained.

It was found that the liver of a week-old rat contains from 3 to 4 times less glycogen than that of a full grown animal (Fig. 2). The values rise within the first 6 weeks and decrease slightly and very slowly from the 6th week on; the average value at 6 weeks was 5.5 gm. per 100 gm. of fresh liver. In view of the results of Deuel and his collaborators (8) on diurnal changes in liver glycogen, it should be mentioned that all rats were killed between 10 and 11 a.m. Deuel (9) found the maximum level of liver glycogen at 39 to 40 days of age. This result agrees satisfactorily with our findings.

Is the low content of glycogen in the liver of the nursing rat,
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together with its increase during the first 6 weeks of life, connected with the change in nutrition which takes place during that time? The experimental results do not support this possibility. The rats were weaned when they were 3 weeks old, and they had no access during the nursing period to any food other than their mothers’ milk, because as soon as the baby rats were 2 weeks old the mother rat was fed outside the cage.

![Graph showing liver and muscle glycogen levels in rats of different ages.](http://www.jbc.org/)

**Fig. 2.** Values for liver glycogen and muscle glycogen in rats of different ages.

The most pronounced increase in content of glycogen in the liver takes place, however, during the nursing period in rats. The curve rises far less steeply after the rats have been weaned. The cause for this increase within the first 6 weeks of postnatal life must, consequently, be exclusively endogenous. Seckel and Kato (10) give further support to this assumption. They found
that in rats no definite diurnal cycle of liver function is demonstrable up to the age of 13 days. The increase in liver glycogen with age is consequently not the only rather impressive change in the function of the liver cells.

Determination of content of glycogen in muscle tissues of rats produced different results (Fig. 2). No distinct and positive difference in glycogen content of muscle tissue of baby rats was found as compared with the two older age groups. Owing to the small amounts of tissue obtainable in animals 1 and 2 weeks old, the range of error may, however, be larger in this age group. Muscle glycogen values for other animals, moreover, such as dogs, rabbits, and guinea pigs, also do not show any differences in corresponding age groups. It should be concluded, therefore, that muscle tissue, in contradistinction to liver tissue, contains like amounts of glycogen at all ages.

The question naturally arose whether the glycogen content of the livers of baby rats and puppies could be raised above its physiologically low level by administration of dextrose. Because in clinical practice this is a frequent procedure, and is done mainly by parenteral injection, 2 cc. daily of a 10 per cent solution of dextrose were injected subcutaneously into each of twenty-four rats and 16 cc. daily into each of sixteen puppies, when they were 2 days old. When the rats were 8 days old and the puppies 6 days old, they were killed, from 2 to 24 hours after the last injection of dextrose. Seven rats 8 days old and five puppies 6 days old were used as controls. During the period when dextrose was administered, some of the animals were fasted, as shown in Fig. 3.

It may be seen in Fig. 3 that the content of glycogen in the livers of baby rats and puppies increases after administration of dextrose, the maximum content being reached in from 4 to 6 hours. The values remain elevated in the rats for from 12 to 16 hours and in the puppies for about 20 hours after the last injection.

The maximum value for liver glycogen in nursing rats, as well as in puppies, after the administration of dextrose, corresponds exactly to the values obtained in healthy full grown animals. For full grown rats this value is 5 gm. (Fig. 2) and for adult dogs 4.5 gm. per 100 gm. of fresh tissue. The liver of the nursing

1 Heymann, W., unpublished data.
rat and of the puppy seems to be unable to convert into glycogen a larger amount of parenterally administered dextrose, and its capacity to accumulate glycogen is certainly very much smaller than that of older animals.

It is not unlikely that similar conditions exist in human beings, as Burghard (11) found very low liver glycogen values in healthy newborn infants (2 to 3 gm. per 100 gm. of liver), whereas his values for healthy adults were between 6 and 8 gm. per 100 gm. of liver.

The bearing of these experimental investigations on clinical problems which pediatricians meet daily is obvious and will be discussed in another paper. It should be pointed out here, however, that the amount of dextrose injected into the nursing rats and puppies was about 10 times the amount of glycogen stored.
in the liver of the animals. This difference would be found to be smaller in clinical practice.

**Effect of Fasting**

Observations discussed in previous communications (1-3) suggested the possibility that fasting might not deplete the glycogen depots in the liver of infants so fast or so easily as in children or adults. Experimental support for this hypothesis was sought by observing the influence of fasting for 1 and 2 days on the content of glycogen and water in the liver and muscle of rats 8 to 11 days old, 10 to 12 weeks old, and 2 to 3 years old. The results are shown in Fig. 4.

It can be seen that the content of glycogen in the liver decreases under the influence of fasting at an equal rate in animals of all three age groups, in contradiction of the hypothesis suggested. There is no basis for the assumption that it might be retained more tenaciously in the liver of baby rats. Within from 6 to 10 hours it reaches the lowest possible hunger level, regardless of the age of the animals. Nor is there any distinct age difference in the reaction of the content of glycogen in muscle to hunger. The values are lower to start with than those for liver, and the differences before and during fasting are smaller, because there is no complete disappearance of glycogen from the muscles. The correctness of the few high values obtained in determining the glycogen content of the muscle of baby animals has already been questioned; these values, consequently, are not considered in this discussion.

Glycogen is supposedly one of the important factors responsible for the water content of tissues. MacKay and Bergman (12) found that in the liver of rats an average of 3.8 gm. of water was stored with each gm. of glycogen. They concluded that their results with rabbits (13) did "not oppose the frequently quoted statement that with every gm. of glycogen 3 gm. of water are stored." In the fasting rat, however, Greisheimer and Goldsworthy (14) found that the relationship was less marked.

Determination of water content was made at the same time as determination of glycogen content, about one-half the amount of liver or muscle being used for each. The experimental results recorded in Fig. 4 show that under normal conditions the liver
of the baby rat, with a glycogen content averaging 3 times less than that of older animals, has a higher water content than has the liver of older animals, and that the liver of rats 10 to 12 weeks old contains less water than that of rats 8 to 11 days old, in spite of its being able to store 2 and 3 times as much glycogen. Furthermore, fasting deprives the liver of its glycogen stores without correspondingly lessening its water content, with the possible exception of the rats in the 2 to 3 year age group. There is, then, no parallelism whatever between the content of glycogen and

Fig. 4. Glycogen and water content in liver and muscle before and during 1 and 2 days of fasting in rats of three age groups.
that of water in the liver of rats. The very high water content in muscle tissue of baby rats also cannot be explained on the basis of corresponding differences in glycogen content. Some other explanation than glycogen storage, consequently, is responsible for the high water content in the tissues of baby animals. The importance of glycogen as one of the chief water retainers certainly diminishes, in view of these results.

DISCUSSION

One cannot help being impressed by how little it matters that the glycogen content of the liver is low throughout the nursing period.

The fact that ketosis cannot be produced in young human infants and the great ease with which it can be produced in children (3) contradict every expectation so far as the content of glycogen in liver is concerned. One would expect the effects to be reversed, on the basis of the low values for liver glycogen found in nursing human and animal infants and the high values in children and older animals. The newer knowledge of the dominant importance of hormonal regulation of the metabolism of ketone bodies, as affected by the pituitary (15) and suprarenal (16) glands, finds support in these results.

The ease with which hypoglycemia develops in infants subjected to carbohydrate starvation also cannot be explained by the physiologically low glycogen values in liver tissue, because in baby rats hypoglycemia does not develop, in spite of the low content of glycogen in the liver. In children, on the other hand, fasting hypoglycemia does develop, in spite of high glycogen values. As fasting deprives the liver of its glycogen depots in animals of all ages with the same ease, it must be assumed, furthermore, that other factors than glycogen reserves in the liver are responsible for the development of fasting hypoglycemia in the young human being. These factors, in all probability, would have to be sought in age-conditioned differences in hormonal regulation.

Another aspect of these results is concerned with the vulnerability of the liver. The lower the glycogen deposition in the liver, the more is the liver parenchyma supposed to be subject to injury. This opinion is based on experimental as well as
clinical experience. It might be correct, if comparisons were made within one age group; that is to say, if the influence of a liver poison is compared in animals and human beings of the same age that have or have not been subjected to fasting. Without some such qualification, however, the foregoing assumption is wrong. In experiments not reported in this paper, baby rats with their low hepatic glycogen values were found to be distinctly more resistant to the effects of carbon tetrachloride than older animals with their high liver glycogen values. In spite of daily inhalation of carbon tetrachloride, continued to complete narcosis, baby rats kept on gaining weight normally for from 10 to 12 days. This result agrees with the daily experience of pediatricians who encounter clearly recognizable organic diseases of the liver in infants far less frequently than in older children. One may consequently conclude that the low content of glycogen in the liver of the human infant does not decrease the resistance of the liver to injury.

The physiologically low content of glycogen in the liver of animal and human infants, together with the knowledge that a few hours of fasting in rats are sufficient to reduce it to a minimum, certainly makes one more liberal toward the indication for enteral or parenteral administration of carbohydrates in infancy. It has been shown that the parenteral administration of dextrose increases the content of glycogen in the liver of rats for not longer than from 12 to 20 hours. And there is no reason to assume that the situation is essentially different in human infants.

The low glycogen deposition in the liver of infants, moreover, offers a better explanation of the high carbohydrate requirement of infants than has heretofore been proposed. Also, the fact that the liver is larger in infancy than at any other age could be explained by the teleological reasoning that the low glycogen content of this organ has to be compensated by a greater amount of tissue.

As far as the relationship between carbohydrate metabolism and water is concerned, it is improbable that sugar once converted into glycogen retains water solely on account of the hydrophilic properties of glycogen. The lack of parallelism between glycogen content and water content in liver and muscle under
normal conditions as well as under the influence of fasting speaks strongly against such an obviously too simple explanation.

SUMMARY

Rats of three age groups (8 to 11 days, 10 to 12 weeks, and 2 to 3 years) were fasted for 2 days. In none of these animals did hypoglycemia develop. This result differs from that observed in studies made on human infants and children.

The liver glycogen value for rats 8 to 11 days old is one-third that for rats in the two older groups. During the first 6 weeks of postnatal life this value increases steadily, the average value for the animals 6 weeks old being 5.5 gm. per 100 gm. of fresh liver, which is only slightly higher than the average value for the full grown animals (4.6 gm.).

The increase in content of glycogen in the liver of baby rats takes place mainly during the nursing period. The causes for the low content and its rise during infancy are consequently endogenous in nature.

The muscle glycogen values in baby rats are certainly not lower than those in the two older groups, if they differ at all.

Fasting for 1 and 2 days diminishes the content of glycogen in liver and muscle of rats in all three age groups with the same ease. This result does not support the hypothesis that the liver retains its glycogen deposits with greater tenacity during infancy than during later periods of life.

In spite of having the lowest content of glycogen, the livers of baby rats show the highest water content of all three age groups. The diminution in liver glycogen produced by fasting is not accompanied by a corresponding loss in liver water. The important rôle which glycogen is supposed to play as a water binder is not evident from these results.

Baby rats tolerate carbon tetrachloride better than older rats. The low content of glycogen, accordingly, does not make the liver more vulnerable.

The pronounced disposition to ketosis at certain ages, referred to in a previous publication (3), can in no way be explained by the age-conditioned differences in liver glycogen values found in these experiments.
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