

Starvation and the Glycogen of the Brain and Vital Organs of the Rhesus Monkey

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ABSTRACT The brain, visceral organs and muscle of the rhesus monkey were examined for their total glycogen contents after starvation periods of from 1 to 4 days. The glycogen concentration of the brain increased significantly during the 4 days, reaching a maximum of 50% above normal levels on day 2 of starvation. In contrast to the glycogen accumulation by the brain, the glycogen reserves of the liver fell precipitously during the first day of deprivation and remained at the new low level of 3 to 5% of the control over the next 3 days. Muscle glycogen remained essentially unaltered over the first 3 days of starvation and decreased by 50% on day 4. The glycogen of the heart, lung, spleen and kidney did not change significantly during the 4 days of starvation. The elevated cerebral glycogen concentrations brought about by 48 hours of starvation were not reversed by 4 days of ad libitum feeding. *J. Nutr.* 104: 1189-1194, 1974.

INDEXING KEY WORDS adult monkey · brain · heart · lung · liver · spleen · kidney · muscle · glycogen · starvation · newborn monkey · rhesus

Tissue glycogen reserves (1) and the effects of starvation on tissues (2) and their constituents (3) have been reviewed. The precautions (4) necessary to insure reliable glycogen analyses of brain tissue have probably been the major contributing factor for the lack of extensive quantitative data on this organ. Kerr and Ghantus (5) found that starvation tended to increase cerebral glycogen concentrations slightly. Elevated brain glycogen concentrations have also been found under circumstances which produce brain damage¹ (6-10). Recently it was shown that learning deficits were caused by nutritional privation (11).

In view of the latter findings, starvation and its effect on the total glycogen of the brain of the rhesus monkey (*Macaca mulatta*) were reexamined. The visceral organs and muscle were also assayed for their glycogen content. It was found that cerebral glycogen concentrations were significantly elevated, 50% above control levels, on day 2 of starvation. The changes in the glycogen stores of the liver and

muscle were similar to those already reported in the literature.

METHODS

Seventy-seven rhesus monkeys were used in this study, 16 newborns and 61 juveniles. The newborn monkeys (0.5 kg) were delivered surgically and maintained as described elsewhere (9, 12, 13). All juveniles (4 to 5 kg) were imported from India and quarantined for 90 days before they were used for experiments. These monkeys were 2 to 4 years old and were fed a commercial stock diet² while they were at NIH. Eleven control monkeys were used and designated in tables 1 and 2 as sustaining 0 hours of starvation. Seven newborn monkey controls were killed at delivery and four juvenile monkeys were taken from

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¹Petersen, R. O., Rivera, A., Jr., Kahn, K., Mueller-Heubach, E. & Myers, R. E. (1970) Alterations of DNA, RNA, glycogen and microsomes of the brains of recovering hypoglycemic monkeys. *Federation Proc.* 29, 835 (abstr. 3333).

²Purina monkey chow, Ralston Purina Co., St. Louis, Mo.

TABLE 1
Effects of starvation on the cerebral glycogen of newborn monkeys

Starvation (hr)	0	12	24	48
	<i>mg glycogen/g brain</i>			
Group				
1. 20% food intake ¹				
Newborn	0.77	1.00	0.85	1.07
	0.53	0.91	1.32	0.68
	0.48	0.63	0.63	0.73
	0.60			
	0.59			
	0.71			
	0.39			
Average 1	0.58	0.85	0.93	0.83
±SE	0.05	0.11	0.20	0.12
P < *		0.05	0.05	0.05

¹ Except for 0 hour controls, monkeys were fed glucose at 35 kcal per day, approximating 20% of normal energy intake. * The t test was made between "0-time" privation and 12, 24 and 48 hours of privation.

the colony without fasting and were killed. These juveniles were used as controls for groups 2 and 3.

The remaining 66 monkeys were divided into four groups. Group 1 contained nine newborn monkeys who were fed orally 35 kcal/day in the form of glucose (20% of their normal food intake). Group 2 contained 18 juvenile monkeys which were fed 90 kcal/day (20% of their normal food intake) (14) of stock diet.² This particular food intake regimen was selected because it had been used in some of our previous investigations (9, 10, 12). Group 3 contained 17 juvenile monkeys. These monkeys were starved (water ad libitum) for 4 days. Group 4 contained 22 juvenile monkeys. This group was used to study the recovery of the tissue glycogen stores from the effects of starvation. These animals were starved (water ad libitum) for 2 days (the time of maximal cerebral glycogen response), then they were fed a commercial stock diet² ad libitum for 4 days.

Three or more animals from each group were killed at daily intervals by excising their organs under anesthesia (35 mg pentobarbital/kg) as previously described (9, 10, 12). The cerebrum was removed first and frozen instantly, at the temperature of liquid nitrogen, in a mixture of isohexane: isopentane 1:3. The heart, lung, liver, spleen, kidney and adductor muscle of the leg were then excised and frozen in the

same mixture. The tissues were stored at -80°.

The entire cerebral hemisphere (9) was used for the glycogen assay as described by Kerr (4). A modification (12) of the Good et al. method (15) was used to assay the other tissues for their glycogen content. All tissues were assayed in triplicate.

RESULTS

Normal tissue glycogen levels. The normal glycogen concentrations in milligrams/gram wet weight (mean ± SE) for the brain (0.61 ± 0.06), heart (5.2 ± 2.6), and liver (115 ± 39) of the juvenile monkeys were similar to those found for the newborns (9, 12). The lung (0.96 ± 0.05) and muscle (9.2 ± 0.5) glycogen levels of the juvenile were a third that of the newborn (12), whereas the glycogen levels of the spleen (1.7 ± 0.02) and kidney (0.80 ± 0.08) were slightly lower than those of the newborn (12).

Effects of starvation on cerebral glycogen. The data in table 1 illustrate the tendency of the cerebral glycogen of the starved newborn monkey to increase above the control. In five out of nine newborn monkeys deprived of nutrients the cerebral glycogen was greater than 0.77 mg/g, the highest cerebral glycogen concentration found in the brain of a control monkey. The mean cerebral glycogen level of newborn monkeys killed at delivery (0 starvation) was significantly lower than that of newborns killed after 12, 24 or 48 hours of

TABLE 2
Effects of starvation on the cerebral glycogen of juvenile monkeys

Starvation (hr)	0	12	24	36	48	72	96	120
<i>mg glycogen/g brain</i>								
Group								
2. 20% food intake								
Juvenile	0.75		0.68	0.78	1.04	0.69		0.60
	0.50		0.89	0.89	0.92	0.92		1.05
	0.52		0.56	0.67	0.77	0.98		1.05
	0.69		0.72					0.85
								0.93
Average 2	0.62		0.71	0.78	0.91	0.86		0.90
±SE	0.06		0.07	0.06	0.08	0.09		0.08
P < **			NS	NS	0.05	NS		0.05
3. Starved	0.75*		0.69		0.96	1.01	0.74	
	0.50*		1.02		0.89	0.74	0.89	
	0.52*		0.70		1.33	0.90	0.81	
	0.69*				1.12			
					0.51			
					0.90			
					0.94			
					1.20			
Average 3	0.62		0.80		0.98	0.88	0.81	
±SE	0.06		0.11		0.09	0.08	0.04	
P < **			NS		0.05	NS	NS	
Total average	0.62		0.75	0.78	0.96	0.87	0.81	0.90
±SE	0.06		0.06	0.06	0.07	0.05	0.04	0.08
P < **			NS	NS	0.02	0.02	NS	0.05

* The same set of animals was used for the zero-time point of both group 2 and group 3 juveniles. However, they were included only once in the calculation of the total average. ** The *t* test was made between "0-time" privation and 24, 36, 48, 72, 96 and 120 hours of privation.

starvation (fed glucose at 20% of normal energy intake).

Table 2 summarizes the data obtained on the effects of starvation on the cerebral glycogen of these juvenile monkeys. Two-thirds of the juveniles fed 20% of their dietary requirements or completely starved had cerebral glycogen concentrations greater than the control animals. Since the cerebral glycogen levels of the juvenile monkeys in the starved and 20% fed group were similar the data of these two groups were combined and expressed as the total average at the bottom of table 2. There was a gradual but significant (16) increase in the total average glycogen content of these brains, which peaked at 50% (0.96 ± 0.07 mg/g) above the normal control levels (0.62 ± 0.06 mg/g) after 2 days of nutritional privation.

Effects of starvation on hepatic glycogen. The glycogen stores of the livers reflected the level of privation of groups 1 thru 3.

The starvation of the juvenile monkey (group 3), as has been reported many times in the past, caused a rapid depletion (78%) of the liver glycogen stores from 115 ± 39 mg/g to 9 ± 4 mg/g in 24 hours (fig. 1). The hepatic stores for the subsequent 3 days of starvation remained at 3 to 5% of their normal levels. The liver glycogen of the animals given 20% of their nutritional needs, on the other hand, decreased by 53% from 102 ± 18 mg/g to 48 ± 10 mg/g in 24 hours. At no time during the experiment did the glycogen stores of this group drop below 25% of control levels. Nevertheless, both groups were combined and were averaged together due to the similarity of the cerebral glycogen responses. The total average hepatic glycogen of both groups combined was maximally and significantly depleted 48 hours after the onset of food deprivation when the livers contained an average of 23 ± 15 mg glycogen/gram of tissue compared to

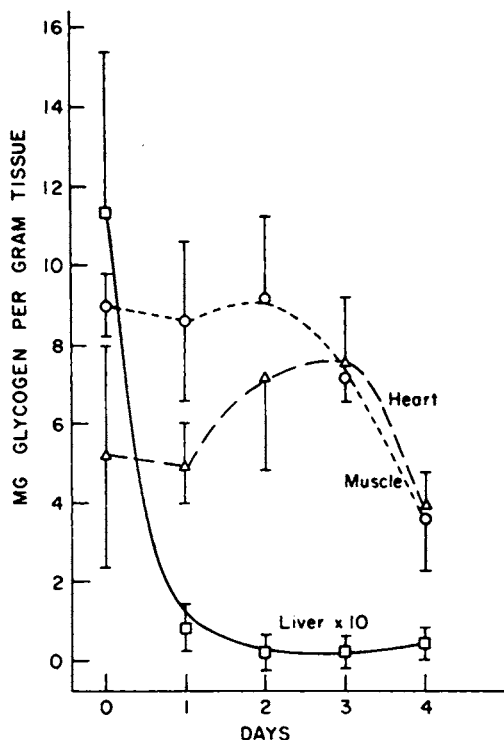


Fig. 1 The effects of starvation on the glycogen of the heart (Δ), liver (\square) and muscle (\circ) of the juvenile monkey. The vertical lines represent the \pm SE of each of the means. Four monkeys were used for the controls and three monkeys were killed on days, 1, 2, 3, and 4.

the control which had 102 ± 18 mg glycogen/gram ($P < 0.001$).

Effects of starvation of muscle and other organs. In contrast to the livers the glycogen stores of the muscles, kidneys, lungs and spleens of the monkeys deprived of 80% of their nutritional needs for 4 days did not change.

The muscle stores of the starved monkeys also remained relatively unchanged during the first 3 days without food. However, on day 4 the muscle glycogen stores were depleted by 50% (fig. 1). The cardiac glycogen stores tended to increase slightly (fig. 1) during the first 3 days of starvation. By day 4 of starvation the cardiac glycogen level was depressed slightly but was not significantly different ($P > 0.1$) from that of the normal control. The lung, spleen and kidney glycogen levels were not affected by the lack of food for 4 days.

The recovery of cerebral and hepatic glycogen concentrations from starvation. The 22 monkeys in group 4 were fed after 2 days of starvation (the time of maximal cerebral glycogen response) to ascertain the reversibility of the glycogen response. As expected the hepatic glycogen stores recovered rapidly after the onset of feeding. Within 24 hours the hepatic glycogen levels were restored from 23 ± 15 mg/g at the end of 2 days of starvation to 136 ± 11 mg/g ($P < 0.001$).

TABLE 3

Cerebral glycogen of juvenile monkeys recovering from 48 hours of starvation

Recovery from 48 hours of starvation (hours)	0*	6	24	48	72	96
	<i>mg glycogen/g brain</i>					
Group						
4. Starved for 2 days then fed for 4 days	0.96	0.68	0.84	1.06	0.88	0.56
	0.89	0.68	0.76	0.75	0.76	0.84
	1.33	0.70	0.91	1.17	0.39	
	0.90	0.66	0.60			
	0.94	0.65	0.78			
	1.20	0.73	0.99			
	1.12		0.78			
	0.51		1.15			
Average	0.98	0.68	0.85	0.99	0.68	0.70
\pm SE	0.09	0.01	0.06	0.13	0.15	0.14
$P < **$		0.02	NS	NS	NS	NS

* Monkeys were starved for 2 days. ** The t test was made between "0-time" and 6, 24, 48, 72, and 96 hours of recovery from starvation.

The cerebral glycogen levels (table 3) responded somewhat more slowly to the onset of feeding. Although after 6 hours the cerebral glycogen levels were near normal (0.68 ± 0.01 mg/g, control = 0.62 ± 0.06 mg/g), they returned to the elevated state for 2 days and were not normal after 4 days of feeding.

DISCUSSION

The brains of newborn and juvenile rhesus monkeys have responded in a similar manner to many stimuli. Prolongation of "energy privation" has produced neurological and neuropathological changes in monkeys of all ages: fetuses (17), newborns (13) and adults (18, 19). Anoxic episodes of the same order of magnitude have caused similar morphological and chemical changes in both newborn and juvenile monkeys (9, 10, 13, 18). Starvation seems to affect the glycogen levels of the brains of newborn and juvenile monkeys in the same manner (tables 1 and 2). More than half of the newborn monkeys with limited food intake exhibited elevated cerebral glycogen levels. In addition the cerebral glycogen levels of the juveniles were significantly increased by the privation of nutrients. Starvation of monkeys has resulted in cerebral glycogen accumulation of the same order of magnitude as observed with physical and radiation damage to the brain (6-8). It has produced half the cerebral glycogen response found in monkeys recovering from asphyxia (9), circulatory stasis (10) and hypoglycemia.¹

Starvation of several days' duration has not caused any marked variations in the blood glucose levels (20). Consequently, the alteration in the cerebral glycogen level could not have been initiated by changes in the blood glucose concentrations. These changes in the cerebral glycogen were also not caused by the pentobarbital (21) which was given to all the animals including the controls. These changes observed in the cerebral glycogen, however, might have been induced by the metabolic state of the liver (22). The large variations observed in the liver glycogen concentrations made it difficult to determine the extent of depletion which might have induced the accumulation of cerebral glycogen. Nevertheless, the data

suggest that the elevated cerebral glycogen levels which coincide with low hepatic glycogen concentrations might also be causally related. The cerebral glycogen response to starvation could also have been initiated by the effects of starvation on other visceral organs.

The level of cerebral glycogen has been considered to be inversely related to neuronal activity (23). The decrease in neuronal activity (9, 24) could have led to the nonutilization of glucose³ (23) and the concomitant or subsequent increased glycogen accumulation in the brain tissue observed herein. On the other hand, the increased cerebral glycogen might have been the result of changes in the level or activity of various enzymes (3). Nevertheless, it is conceivable that such a decrease in neuronal activity might result in learning deficits (11).

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