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Effect of Restricted Food Intake on Growth and Composition of Preweanling Rat Brain.* (29806)

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The weight and chemical composition of the rat brain tends to be less affected by dietary factors than most other organs of the body. Lehr(1) found that restricting food or protein intake of adult rats did not affect total nitrogen, protein, or deoxyribonucleic acid content of the cerebral cortex. Allison(2) noted that the ratio of protein to DNA in rat brain was not altered by the level of protein in the diet as opposed to liver, kidney and muscle tissue. Mandel(3) found that adult rats on protein-free diets for 2 months showed no changes in brain protein or deoxyribonucleic acid levels in spite of considerable loss in body weight. It has been demonstrated(4, 5) that the brains of rats, whose body weights were held constant from birth to 16 days of age, increased in weight by well over 100%. Most other organs of the body gained much less than this and some, such as the liver, actually lost weight. No chemical analyses were done on the brains.

Methods and materials. Litters of 5-day-old Wistar rats were split and half of the newly formed litters were left with lactating dams at all times. The remaining litters were placed with lactating dams for 3 to 6 hours daily, the time being regulated so that their body weight gain was 20-25% of that of the rats that suckled *ad libitum*. All young were weighed daily to insure adequate control of weight gain. Only one preweanling rat died during the experiment. All dams were fed *ad libitum* a nutritionally complete diet containing 30% casein as the protein source. At 20 days of age, each of the young was anesthetized with ether, the skull opened, the olfactory lobes dissected from the brain and the brain removed by severing at the level of the foramen magnum. The brain was immediately rinsed with ice water, blotted and weighed. Brain lipids were extracted(6) by homogenizing in 19 volumes of chloroform-methanol (2-1). The resulting suspension was filtered through a preweighed fine porosity sintered glass filter. The residue on the filter was washed 2 times with 3 ml portions of chloroform-methanol (2-1), after which the residue was dried to constant weight and total non-lipid solids calculated by difference. Total unwashed lipid solid weight was obtained by drying a portion of the chloroform-methanol extract. The remainder of the lipid extract was washed with .2 volumes of .1 M potassium chloride, followed by 2 washings with .2 volumes of chloroform-methanol - .1 M potassium chloride (3-48-47) and a final washing with chloroform-methanol-water (3-48-47). A portion of this washed lipid solution was dried and weighed to obtain the washed lipid weight. The washed lipid extract was used to analyze for cerebrosides(7), cholesterol(8) and phospholipid phosphorus(9). The phospholipid value was obtained by multiplying the phosphorus value by 25. Brains of *ad libitum* fed 5-day-old rats were similarly analyzed to determine the magnitude of the changes in brain growth and composition during the experimental period of 5 to 20 days of age.

Results and discussion. Brain components were calculated as percent of the wet brain weight and appear in Table I. While only the percentages of brain total lipids, cerebrosides and cholesterol were significantly lower than

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TABLE I. Effect of Restricted Food Consumption on Percent Composition of Wet Rat Brain.

Age of rats when sacrificed	5 days	20 days *ad libitum* feeding	20 days restricted feeding
No. of rats	17	24	24
Body wt (g)	12.6* (17)†	49.5 (24)	21.0‡ (24)
Brain analyses			
Wt (mg)	528 (17)	1438 (24)	1167‡ (24)
% Water	88.01 (6)	80.65 (20)	81.31 (20)
% Non-lipid solids	7.70 (6)	11.61 (20)	11.56 (20)
% Unwashed lipid solids	4.29 (6)	7.74 (20)	7.13‡ (20)
% Washed " "	2.91 (6)	5.88 (20)	5.50‡ (20)
% Phospholipids	2.14 (6)	3.98 (20)	3.94 (20)
% Cholesterol	.46 (6)	1.05 (20)	.92‡ (20)
% Cerebrosides	.03 (6)	.52 (20)	.36‡ (20)

* Mean values.
† No. of analyses indicated in parentheses. Sometimes 2 or 3 brains were combined for analysis.
‡ Significantly ($P < .01$) lower than values for 20-day-old *ad libitum* fed rats.

controls when food intake was restricted from 5 to 20 days of age, the absolute amount per brain of each component measured was significantly lower in the restricted food intake group than in the controls.

Table II indicates the absolute amounts of components incorporated into the brains between 5 and 20 days of age. These data were obtained by subtracting the absolute amounts of components in the 5-day-old rat brains from the absolute amounts of components contained in the 20-day-old control and restricted food intake groups. In making these calculations, it was assumed that the brain composition of the 20-day-old rats was the same at 5 days of age as the rats actually sacrificed at that age. The data in Table II demonstrate the marked slowing of incorporation of components into the brains during the 15-day period of restricted food intake.

TABLE II. Absolute Amounts of Constitutents Incorporated into Brains Between 5 and 20 Days of Age.

Treatment	Rats fed *ad libitum*	Rats on restricted intake	
			(%)
Brain wt gain, mg	910*	639	(70.2)†
Water, mg	695	484	(69.6)
Non-lipid solids, mg	126	94	(74.6)
Unwashed lipid solids, mg	89	61	(68.5)
Phospholipids, mg	45.9	34.7	(75.6)
Cholesterol, mg	12.7	8.3	(65.3)
Cerebrosides, mg	7.4	4.0	(54.0)

* Mean values.
† Incorporation expressed as % observed in *ad libitum* fed rats.

The brain cerebroside content was affected to a greater extent by the restricted food intake than any other brain component measured. Its level increases at a particularly rapid rate during the preweanling period of mice and rats corresponding with the onset of brain myelination(10,11). Its lower level in the brains of rats on restricted food intake suggests that myelination may be retarded in these animals. Donaldson(12) found that restricting the food intake of older rats, from 30 until 51 days of age, did not interfere with brain myelination when checked by histochemical procedures.

Summary. Food intake of Wistar rats was restricted from 5 until 20 days of age such that body weight gain was approximately 20% of the *ad libitum* fed control rats. The incorporation of nonlipid solids, cholesterol, phospholipids and cerebrosides into the brain was reduced by restricting food intake.

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Liver Cell Damage Produced by Portacaval Shunts.* (29807)

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Increasing evidence has accumulated that portacaval shunts for relief of portal hypertension may produce undesirable side effects such as portasystemic encephalopathy(1), demyelinating disease of the spinal cord(2), hyperbilirubinemia, possibly owing to hemolytic anemia(3) and diabetes mellitus(4). Hepatic siderosis and even generalized hemochromatosis(5) have followed construction of portacaval shunts. Cirrhosis is not necessary for the accumulation of hepatic iron, since increased hepatic hemosiderin has been produced in normal dogs(6) and in rats(7) by end-to-side portacaval shunts alone. Untoward side effects are said to result simply from the diversion of the portal flow from the liver. To investigate further the integrity of the liver cells after shunts, portacaval anastamoses were constructed in rats by microsurgical techniques after which autoradiographic studies of DNA synthesis and fine structural studies were performed.

Materials and methods. The experimental animals consisted of 24 Sprague-Dawley rats, with an average initial body weight of 400 g. All were fed Rockland Farms complete rat diet. They were divided into groups of 8 rats each: Group I. End-to-side portacaval shunts. Group II. Side-to-side portacaval shunts. Group III. Sham operations, with mobilization of the portal vein and vena cava, but without construction of a shunt.

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Two rats from each group were sacrificed 30, 60, 90 and 120 days later. Two hours before sacrifice each animal was given 0.5 microcurie of tritiated thymidine intraperitoneally. A small sample of liver tissue was immediately fixed in buffered osmic acid, and embedded in epon. One micron and 0.1 micron thick sections were cut with a Porter-Blum microtome. The former were stained with toluidine blue for examination under the light microscope, while the latter were stained with lead hydroxide, and examined with a Hitachi HS-7 electron microscope. Portions of the liver were also fixed in 10% neutral buffered formalin after which paraffin sections were stained with hematoxylin and eosin, and subjected to Perl's reaction for iron. Paraffin sections were also coated with Kodak NTB-3 nuclear track emulsion for autoradiography. These were exposed for 16 days, developed in Kodak D-19 solution and stained with Harris hematoxylin.

Results. After end-to-side shunts, the animals lost up to 20% of their initial weight by the end of experiment. The liver weight/body weight ratio remained about the same in all animals. Rats subjected to side-to-side shunts and to sham operations gained up to 25% of initial weight by the end of experiment. The livers after both types of shunts and after sham operations appeared normal in sections stained with hematoxylin and eosin. Small amounts of iron accumulated in the centrolobular zones of animals with end-to-side shunts after 90 days. Autoradiographs had about 3 to 4 times as many labeled hepatocytes in rats subjected to end-to-side shunts