Ploidy Distribution of Nuclei in Rat Liver Following Hypophysectomy.* (23423)

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It has been reported recently(1,2) that following hypophysectomy there is an increase in the ratio of diploid to tetraploid nuclei in rat liver. Bass *et al.*(1) using adult rats observed a 30% increase in percentage of diploid nuclei in the liver. DiStefano and Diermeier(2) have presented evidence that in young rats there is an arrest of nuclear activity following hypophysectomy. This results in maintenance of polyploid distribution present at time of operation. However, neither of these studies took into consideration the effect of reduced dietary intake on the liver. Because severe dietary restriction has been shown to modify polyploid distribution of parenchymal cells(3), it seemed desirable to compare the effects of reduced food intake on this distribution in adult rat liver with the alterations observed in hypophysectomized animals which invariably reduce their caloric intake.

By evaluation of liver samples taken both at time of hypophysectomy and 21 days later at time of sacrifice it was possible to show that the ploidy shifts previously reported(1) were directly correlated with hypophysectomy, as no significant shifts were observed in pair-fed animals of corresponding age and weight.

Methods. 125-135 g Sprague-Dawley rats obtained from Endocrine Laboratories, Madison, Wis., were employed. They were housed individually in a constant temperature room, fed on canned dog food and allowed free access to water. The animals were divided into 3 groups: (1) a group of normal rats fed *ad libitum;* (2), hypophysectomized animals; and (3), normal rats pair-fed with the hypophysectomized animals. All animals were killed by decapitation at the same time each day. A biopsy sample was taken from the tip of median lobe of the liver of each rat in both control groups, at the beginning of the experimental period, and at the time the animals were sacrificed a more representative liver sample was taken. These specimens were fixed in 10% formalin, dehydrated and prepared according to standard histological procedure. Ten percent homogenates of liver tissue were prepared(4) and the nuclei counted on a certified Spencer Bright Line hemocytometer. Mitotic indices were determined on tissue sectioned at 5 μ. The nuclei were stained by the Feulgen technic and the cytoplasm counterstained with fast green. 10,000 nuclei/animal were counted, and values expressed as the number of dividing nuclei/100 parenchymal nuclei. The ploidy distribution of liver nuclei was determined on hepatic tissue sectioned at 20 μ, stained by the Feulgen technic, and DNA content of 50 to 100 whole nuclei estimated by the method of Swift(5).

Results. Data presented in Table I show certain effects of hypophysectomy and reduced food intake on the young adult rat. It is seen that pair-fed animals when compared with hypophysectomized controls have similar changes in body weight, liver weight and nuclei/gram of liver. Therefore, the reduced food intake in the hypophysectomized rat appears to be the major factor responsible for these changes.

Table II shows polyploid distribution of parenchymal nuclei from livers of these animals. Biopsy samples taken at the beginning of experiment have slightly higher diploid values than those taken at the time of sacrifice. This is probably due in part to the location from which the biopsy sample was taken. There are, however, no significant differences between the initial and final samples of hepatic tissue taken from the *ad libitum* control and pair-fed animals. No biopsy samples were taken from the hypophysectomized animals and it was assumed that initial polyploid

* This investigation was supported by grants from Nat. Cancer Inst., U. S. Public Health Service and Anna Fuller Fund.

TABLE I. Effects of Hypophysectomy and Reduced Food Intake on Adult Rat.

	Ad lib. control (5)*	Pair-fed control (5)	Hypophysectomized (5)
Body wt, g (initial)	132.3 ± 2.4†	127.3 ± 4.9	123.1 ± 4.5
(final)	273.0 ± 8.9	155.2 ± 10.3‡	138.9 ± 5.4‡
Liver wt, g	12.1 ± .6	5.1 ± .6‡	5.6 ± .4‡
Nuclei × 10⁸/g	2.4 ± .2	3.6 ± .2‡	3.9 ± .2‡
" × 10⁸/liver	29.1 ± 2.3	17.9 ± 1.0‡	21.5 ± 2.9§

* No. of animals. † Represents a mean figure with its stand. error. ‡ $p < .01$ when compared to *ad lib.* control. § $.05 < p < .1$.

p = probability from Fisher's Table "t."

distribution was the same in the 3 groups. The samples taken from the latter group at termination of the experiment indicate a significantly higher percentage of diploid nuclei associated with a corresponding reduction in tetraploid nuclei. The changes in number of octaploid nuclei are probably not significant, as the number was relatively small in each group. This differs from observations of Bass, Grisham, and McArdle(1), who reported a higher percentage of octaploids in hypophysectomized rats. The reason for this difference is not apparent. However, since synthetic diets were not employed, it is possible that dietary differences could be responsible.

Because it was possible that this increase in percentage of diploid nuclei might be correlated with an increase in percentage of diploid binucleate cells, the percentage of binucleate cells was determined. The results of this experiment are given in Table III. Although a slight but significant increase in percentage of binucleate cells occurred in the livers of hypophysectomized rats, this increase was not sufficient to account for the increased percentage of diploid nuclei shown in Table II. Counts made on stained slides confirmed Harrison's observation(6) that cell suspensions must be employed for this procedure.

To evaluate the possible effect of changes in mitotic activity as an explanation for these ploidy differences between control and hypophysectomized animals, mitotic indices were done on representative samples from each rat liver. These observations are given in Table IV. Mitotic indices were not performed on the biopsied liver specimens because it had been observed in other animals that the percent of dividing cells in such samples was considerably lower than that in more representative samples of liver. Using the biopsy samples taken from the edge of the lobe, a mitotic index of ⅓ to ½ that of a more representative liver sample was obtained. Therefore, mitotic indices were determined on a separate group of animals weighing 133.7 g ± 5.84 g, and an index of .27 ± .025 was obtained. It therefore, may be assumed that the mitotic index in all 3 groups had fallen during the experiments but to no greater extent in one group than another. It cannot be assumed, however, that the rate of fall in each group was the same. The observations of DiStefano and Diermeier(2) would suggest that rate of fall in the hypophysectomized animals is more rapid.

Discussion. The data here presented indicate that the increased percentage of diploid nuclei observed in the liver of the hypophysectomized rat is independent of growth in-

TABLE II. Distribution of Polyploid Nuclei in Parenchymal Cells of Hypophysectomized, Pair-Fed Control and *Ad Libitum* Fed Control Rats.

Class	*Ad lib.* control (5) Biopsy	*Ad lib.* control (5) Final	Pair-fed control (5) Biopsy	Pair-fed control (5) Final	Hypophysectomized (5) Final
	%				
Diploid	23.3 ± .7*	19.8 ± 2.4	24.0 ± 3.2	19.2 ± 3.2	35.4 ± .97
Tetraploid	72.6 ± 2.6	74.2 ± 1.7	74.0 ± 3.5	77.4 ± 2.7	66.0 ± 1.3
Octaploid	4.0 ± 1.7	6.0 ± 1.6	2.0	3.4 ± .9	2.0 ± 1.0

* Represents a mean figure with its stand. error.

TABLE III. Binucleate Cells in Rat Liver. Comparison of data obtained by 2 methods to show that crowding of nuclei seen in hypophysectomized rats and their pair-fed controls invalidates data obtained from 5 μ tissue sections.

Tissue preparation	*Ad lib.* control (5)	Pair-fed control (5)	Hypophysectomized (5)
	%		
Stained on slides	9.92 ± .7*	17.5 ± .6	16.96 ± .2
Cell suspension	18.90 ± 1.0	20.8 ± .8	23.1 ± .6
		p >.1	p <.01

* Represents a mean figure with its stand. error.

TABLE IV. Mitotic Index Determined on Rat Liver of 5 Rats/Group.

	Ad lib. control	Pair-fed control	Hypophysectomized
	.0	.025	.100
	.018	.020	.010
	.092	.010	.020
	.0	.0	.010
	.020	.0	.010
Mean	.026	.011	.030

hibition of the animal as indicated by normal polyploid distribution observed in pair-fed animals at the end of the 21-day experimental period. Although there is an equivalent reduction in mitotic index in each of the 3 groups of rats during this period, it is probable that this reduction in the mitotic index occurs earlier in the hypophysectomized animal as indicated by the work of DiStefano and Diermeier(2).

It has been reasoned(2) that similar ploidy changes in the liver in young rats are merely a reflection of arrest of liver growth following hypophysectomy. However, the data on older rats reported here cannot be explained in this manner, because the percentage of diploid nuclei is higher in the liver of the hypophysectomized animal than in the control animals of comparable age and weight. Removal of the hypophysis from animals in which the characteristic adult ploidy distribution pattern is established results in a return to a ploidy distribution seen in immature animals. This would suggest that there is a direct relationship between hypophyseal function and the maintenance of normal polyploid distribution of liver nuclei. The particular hypophyseal hormone involved is probably somatotropin as it has been shown that this hormone when given to hypophysectomized rats(2) or mice with congenital absence of the hypophysis(7) will restore the polyploid distribution to normal values. Although the mechanism responsible for this effect is not apparent from the data presented, the data suggest that hypophysectomy creates an imbalance between the rate of DNA synthesis and the rate of mitosis.

Summary. 1. Percentage of diploid nuclei observed in livers of young adult hypophysectomized rats is greater than that seen in control animals of comparable age and weight. 2. In the hypophysectomized rat there is a small but significant increase in percentage of binucleate liver cells. 3. Total reduction in mitotic index in 125-135 g rats is no greater in 21 days hypophysectomized rats than in the unoperated animals; however, rate of change may be more rapid in operated animals.

Technical assistance of Miss Catherine Cheatham is gratefully acknowledged.

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Received May 29, 1957. P.S.E.B.M., 1957, v96.