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Biochemical Changes Occurring in Mouse Liver Cells with Ageing.* (25253)

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A number of workers have investigated biochemical changes occurring in tissues as animals age, but little information is available concerning such changes in the subcellular fractions of tissues. An opportunity arose in this laboratory, in connection with other studies, to measure nitrogen and phosphorus contents of large and small granules and cytoplasmic supernatant in liver cells from mice ranging in age from 1½ to 21 months. The data obtained demonstrate that as animals age changes occur in relative amounts of these constituents in all 3 subcellular fractions examined.

Method. Inbred virgin female mice of the C3H/Sp strain, reared on laboratory chow and water *ad lib* in air conditioned animal rooms, were employed. They were sacrificed by exsanguination via jugular vein and the livers removed without prior perfusion. Large granule, small granule, and cytoplasmic supernatant fractions were obtained by differential centrifugation employing a sucrose medium, essentially as described earlier(1). Nuclear counts were made(2) on tissue suspensions prior to preparation of the cell fractions. Nitrogen(3) and phosphorus(4) were determined on each fraction prepared, and were calculated on a per nucleus basis.

Results are shown in Table I. There was an increase with age in nitrogen content of all cell fractions examined. Increases were seen in the small granule and cytoplasmic supernatant fractions by 6-7 months, and in the large granule fractions by 10-11 months of age. Similar changes were seen in phosphorus contents of these fractions, but they generally were of smaller magnitude. In the oldest animals studied chemical compositions of the fractions had altered, as evidenced by lowered P/N ratios in all 3 fractions. There are probably no significant differences in numbers of binucleate cells present in livers of animals studied regardless of age(5). It therefore seems safe to conclude from the nitrogen data that, as the animal ages, the amounts of large and small granules and cytoplasmic supernatant increase within the cell. This appears to confirm the report of Franzini *et al.*(6) that with age there is a gradual increase of cytoplasm-nucleus ratio in livers of mice.

There were fewer cells/g of liver tissue, as indicated by decreasing numbers of nuclei, with increasing age but this appears to be largely compensated for by an increase in liver mass (Table I). There is probably less mitotic activity in livers of the older than in those of younger animals(5), which might suggest a decreasing vigor of the cells. However, total content of the 3 fractions examined in liver cells increased with age. That this process may be simply a form of compensatory hypertrophy is suggested by the observations of Weinbach and Garbus(7), who reported a lower absolute level of substrate oxidation by liver mitochondria from aged

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TABLE I. The Effect of Age on the P and N Contents of Mouse Liver Cell Fractions.

Cell fraction		1½-2 mo	6-7 mo	10-11 mo	18-21 mo
		(12)*	(12)*	(9)*	(7)*
Mitochondria	Nitrogen†	27.7 ± 1.1	29.9 ± 1.4	41.6 ± 1.5	45.4 ± 1.5
	Phosphorus†	3.30 ± .44	3.26 ± .13	4.73 ± .21	4.98 ± .13
	P/N ratio‡	11.9 ± .17	11.0 ± .16	11.4 ± .40	11.0 ± .10
Microsomes	Nitrogen	15.3 ± .6	19.8 ± .6	17.8 ± .7	19.9 ± 1.0
	Phosphorus	2.6 ± .08	3.36 ± .1	2.88 ± .12	3.07 ± .2
	P/N ratio	17.0 ± .3	17.0 ± .3	16.2 ± .2	15.4 ± .3
Supernatant	Nitrogen	34.3 ± 1.1	40.8 ± 1.0	45.9 ± 1.5	47.7 ± 1.6
	Phosphorus	2.95 ± .07	3.62 ± .09	3.82 ± .15	3.42 ± .3
	P/N ratio	8.6 ± .1	8.9 ± .1	8.2 ± .1	7.2 ± .5
Nuclei§		281 ±10.	236 ± 8.7	206 ± 5.7	211 ±11.
Liver wt (g)		1.04 ± .03	1.13 ± .04	1.21 ± .04	1.29 ± .03

* Figures in parentheses are No. of animals employed.
† Values are $\mu g \times 10^6$/nucleus. Means and stand. errors are given.
‡ Ratios are μg P/μg N $\times 10^2$. Means and stand. errors of means are given.
§ Values are No. of nuclei/g moist tissue $\times 10^{-6}$. Means and stand. errors are shown.

rats, without any evidence of uncoupled phosphorylation. Their data indicate that with mitochondria from old animals, oxidation proceeded at about ⅔ the rate seen with mitochondria from younger animals. It is interesting that in the present experiments with mice, the large granule nitrogen increased almost inversely proportional to this decrease in oxidative capacity, indicating that perhaps the old animal has successfully compensated for the deteriorating metabolic activity of mitochondria by forming more mitochondria within each cell.

Whether replacement mitochondria have the same metabolic potential as those in younger animals, and whether or not such compensating actions result in a maintenance of normal vigor is not indicated by these experiments. The altered P/N ratios observed would tend to raise a question in this regard.

Summary. Nitrogen and phosphorus contents of large and small granules and cytoplasmic supernatant from livers of mice 1½-2, 6-7, 10-11, and 18-21 months of age were determined and expressed on a per nucleus basis. There were increased nitrogen contents, and relatively smaller increases in phosphorus contents in each of these fractions in older animals. The results, viewed in the light of observations of Weinbach and Garbus (7), suggest that as an animal ages, its liver cells lose metabolic capacity, and, also, that an attempt is made to restore at least some aspects of it by forming more of the active sites within the cell. Cell fractions examined in older animals differed chemically from those in younger animals, however, as indicated by decreased P/N ratios.

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