The Quantitative Estimation of the Changes Induced by Benzydamine in the Mitochondria of Developing Mouse Hepatocytes

Experiments on the effects of benzydamine on developing mouse hepatocytes have been carried out[1]. Ultrastructural changes found during these experiments indicate that benzydamine causes elongation of the major mitochondrial axis and increase of the ratio of the volume of the cristae to the volume of the mitochondria. In this note we present quantitative estimations of these changes in the mitochondria provoked by benzydamine. For this purpose the coefficient η is introduced and its value is calculated for 2 mitochondrial populations, one normal (η_c) and the second from benzydamine treated mice (η_b). The coefficient η is defined using the formula suggested by WEIBEL et al.[2] for volume fractions of cellular components.

The definition of the coefficient η is as follows:

$$\eta = \frac{Vv_c}{Vv_m} \qquad \text{(a)}$$

where Vv_c denotes the volume fraction occupied by cristae and Vv_m denotes the volume fraction occupied by mitochondria. For the calculation of volume fractions the grid containing 10,000 test points per 100 cm², prepared according to WEIBEL et al.[2], is put randomly on 100 electron micrographs (dimensions 18×24 cm) taken from the hepatocytes of the normal and benzydamine treated mice. The control population contains 498 mitochondria. 525 mitochondria from benzydamine treated mice represent the experimental population of the organelles.

The formula [4] from[2] is applied. According to this formula:

$$Vv_c = \frac{P_c}{P_T} \quad \text{and} \quad Vv_m = \frac{P_m}{P_T}$$

After transformation we obtain:

$$\eta = \frac{P_c}{P_m} \qquad \text{(b)}$$

where P_c is equal to the sum of test points lying over all cristae of the control or experimental material and P_m is equal to the sum of all test points lying over the whole area of the corresponding population of the mitochondria. Using this formula we find the value of η_c to be equal to 16.53 and for the η_b the value 25.44 is obtained.

For the study of the behaviour of single mitochondria in the control and experimental population, the coefficient η is calculated for each mitochondrion separately and then for the values of η_c and η_b obtained for the whole populations of mitochondria a statistical analysis is applied. For the calculation of η for each mitochondrion separately, the formula (b) is used, but in this case P_c is equal to the sum of the test points lying over cristae of only one mitochondrion and P_m is equal the sum of test points lying over the area of one mitochondrion. The results of this procedure may be seen in the Figure. This figure shows that the coefficients η are distributed in the following way: the coefficients η_b of the benzydamine treated mitochondria are shifted in the direction of greater values, while the coefficients η_c of the control mitochondria are limited by smaller values. It is interesting to note that the mode assumes different values for control and experimental mitochondria. In the case of control mitochondria this value is equal to 15.6, while for experimental mitochondria the value 24.4 is found. The difference between these 2 values shows that the mitochondria from control and benzydamine treated mice represent 2 statistically different populations.

The data presented in this paper show clearly that benzydamine causes the increase of the volume of internal membranes per mitochondrion, confirming the conclusions drawn previously from ultramorphological study[1]. This does not necessarily mean an increase in the level of activity of mitochondrial enzymes, since augmented volume of internal membranes may concern primarily the structural proteins. The results of other investigations on enzymic induction[3] suggest, however, that it is reasonable to expect an increase in the level of activity of at least some mitochondrial enzymes in benzydamine treated mouse liver[4].
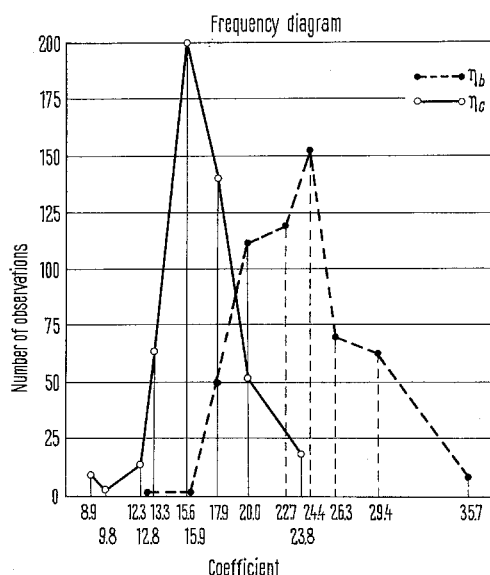
A frequency diagram of the coefficients η_c and η_b. The ordinate gives the number of observations and the abscissa the values of the coefficients. The shift of the values of η_b for mitochondria of benzydamine treated mice in the direction of greater values is clearly seen.

Riassunto. Gli autori hanno studiato l'effetto della Benzidamina sui mitocondri di epatociti di topo dal 3° al 5° giorno di vita. È stato introdotto un coefficiente η per la valutazione quantitativa delle alterazioni. La Benzidamina provoca un aumento del rapporto fra il volume delle «cristae» e il volume dei mitocondri corrispondenti.

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