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Quantitative Distribution of an Esterase Among Cytoplasmic Components of Mouse Liver Cells.*

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It was previously demonstrated by Dounce[1] that nuclei of rat liver cells, isolated by differential centrifugation of cell suspensions treated with citric acid, contained a concentration of esterase which was about 50% of that found in the whole tissue; methyl butyrate was used as the substrate. If the ratio of nuclear to cell volume is taken to be 6:100 for hepatic cells, as approximated by Marshak,[2] then the separated nuclei contain only around 3% of the esterase activity of the cell. It is therefore apparent that a study of the cytoplasmic components of the liver cell is essential to an understanding of the intracellular localization of the esterase. The unusually high concentration of esterase in the liver lends interest to an investigation of this nature since the special accumulation of the enzyme in this tissue can hardly be considered a merely fortuitous circumstance. Just which of the many esterifications and de-esterifications known to take place in the liver are catalyzed by this enzyme still remains to be elucidated. The distinct differences in the nature and properties of liver esterase and lipase have been known for some time.[3]

The quantitation of the activities observed in the various separated fractions as related to the activity of the whole cell has been emphasized in the present investigation since, as Danielli[4] has pointed out, the centrifugal segregation of cellular components may result in an alteration of the enzymatic activities as they exist *in situ* through separation of naturally occurring inhibitors, activators, etc.

Experimental. Livers from 2 or 3 female ZAF1 strain mice were used in each experiment. The portal vein was clamped prior to the removal of the organ, ether anesthesia being used throughout. In the subsequent procedures up to the point of the determination of enzyme activity, care was taken to maintain the tissue as close to 0°C as possible.

The whole livers were put through a tissue press and weighed. The material was transferred to a mortar and ground thoroughly for 4 minutes with the slow addition of 4 volumes of alkalized physiological saline solution (2 cc of 0.1N NaOH per liter of 0.85% NaCl). The diluted tissue sample was further ground in a Potter-Elvehjem homogenizer[5] for about 20 seconds. This ground tissue preparation was assumed to be a 5 times dilution of the original tissue sample.

The differential centrifugation procedure employed was essentially that of Claude[6] with some modifications and additions and is summarized in Fig. 1. The washings indicated in the figure were carried out with volumes of physiological saline solution equal to that of the supernate in each case. The values of (g) were calculated from the center of the centrifuge tube in every instance. The time for the whole separation procedure from the initial removal of the liver was approximately 3 hours. Esterase activity was determined by a manometric method similar to that of Rona and Lasnitzki[7] in a Warburg apparatus. 0.5 cc of the enzyme solution was placed in the sidearm of the reaction flask and 1.5 cc of

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1 Dounce, A. L., *J. Biol. Chem.*, 1943, **147**, 685.

2 Marshak, A., *J. Gen. Physiol.*, 1941, **25**, 575.

3 Sobotka, H., and Glick, D., *J. Biol. Chem.*, 1934, **105**, 199.

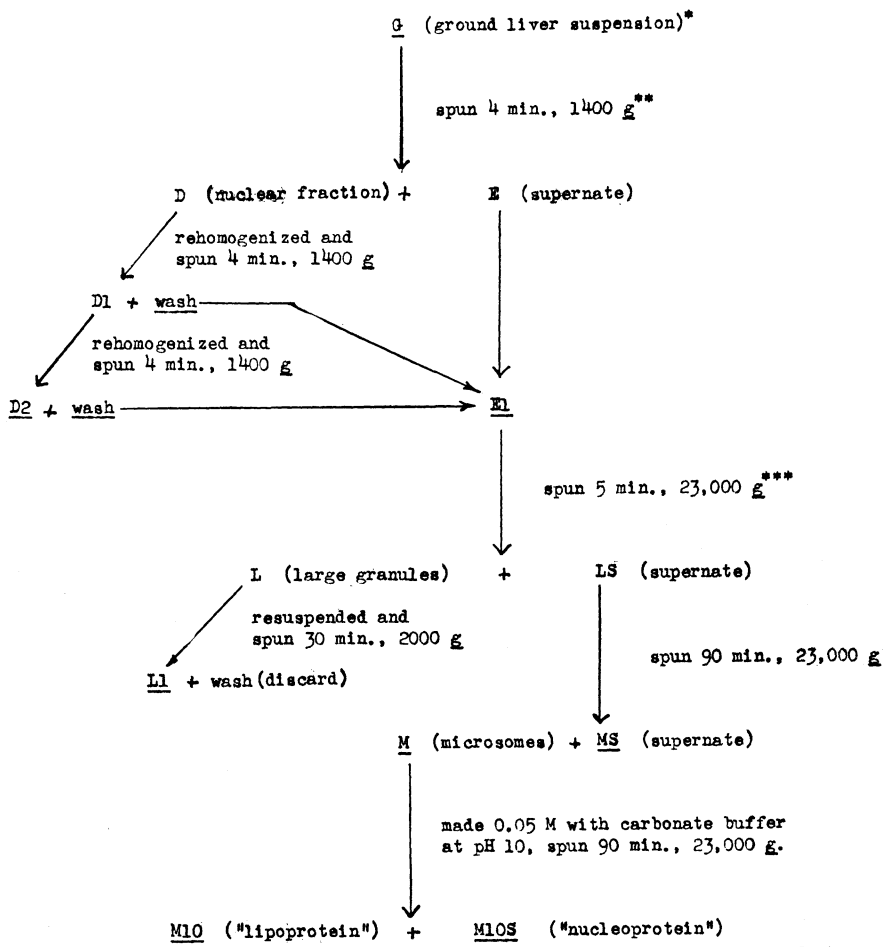
4 Danielli, J. F., *Nature*, 1946, **157**, 755.

5 Potter, V. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1936, **114**, 495.

6 Claude, A., *J. Exp. Med.*, 1946, **84**, 51.

7 Rona, P., and Lasnitzki, A., *Biochem. Z.*, 1924, **152**, 504.

FIGURE I. FRACTIONATION OF LIVER CELL SUSPENSION BY DIFFERENTIAL CENTRIFUGATION



* Ground 4 min. in mortar with 4 volumes of alkalized physiological saline solution, then ground for 20 seconds in Potter-Elvehjem homogenizer.
** Servall angle centrifuge operated in refrigerator.
*** Multi-speed attachment to International centrifuge operated in cold room at —15°C.

bicarbonate-Ringer solution was introduced into the main chamber of the vessel. 0.022 cc of methyl butyrate was pipetted into the main chamber; the final concentration of 1% of the substrate in the complete reaction mixture thus obtained gives the optimum rate of hydrolysis without excess-substrate inhibition. The control vessel contained 0.5 cc of bicarbonate-Ringer solution in the sidearm in place of the enzyme solution. A 5% CO_2, 95% N_2 gas mixture was passed through the system before the contents were mixed. Nitrogen determinations were conducted by the Pregl micro-Kjeldahl method.

Results. The results are shown in Tables I and II. The unit of enzyme activity was chosen as the amount of enzyme which liberates 1 μl of CO_2 per hour at pH 7.4, 37.5°C and at the optimum substrate concentration. In the initial separation of the D and E fractions the cytoplasm was shown to contain most of the esterase of the cell. The N content is about 2.5 times, the enzyme activity per g of wet liver tissue is almost 5 times,

TABLE I.
Esterase Distribution Among Fractions of Mouse Liver Cell.

Exp.	1			2			3		
Fraction	N yield, % of G	Enz. conc., units/μg N	Enz. yield, % of G	N yield, % of G	Enz. conc., units/μg N	Enz. yield, % of G	N yield, % of G	Enz. conc., units/μg N	Enz. yield, % of G
G	100	16.3	100	100	13.8	100	100	10.4	100
D2	29	7.6	14	27	9.4	19	26	6.8	17
E1	64	20.2	80	67	13.5	65	70	12.6	85
L1	11	23.2	15	10	23.1	17	12	17.5	20
M				10	52.9	39	12	47.0	55
MS				42	3.8	12	46	3.3	15
M10							6	55.0	34
M10S							6	19.0	10

Note: N content of E and G were found to be 2.2% and 3.8%, respectively, based on wet weight of liver tissue.

TABLE II.
Summation of Nitrogen and Enzyme contents of Various Fractions.

	Nitrogen						Enzyme					
	D2 + E1		L1 + M + MS		M10 + M10S		D2 + E1		L1 + M + MS		M10 + M10S	
Exp.	Found	Theory	Found	Theory	Found	Theory	Found	Theory	Found	Theory	Found	Theory
1	93	100					92	100				
2	94	100	62	67			84	100	68	65		
3	96	100	70	70	12	12	102	100	90	85	44	55

and the enzyme activity per μg N is around 2 times as great in the E fraction as in the D2. From the study of the N and enzyme distribution among the particulate fractions of the cytoplasm, Table I, it is clear that, although the greatest part of the N was confined to the supernate, MS, from the microsome separation, the largest portion of the enzyme was contained in the microsome fraction, M.

It has been shown that the separation of the M fraction into "lipoprotein" (M10) and "nucleoprotein" (M10S) fractions can be effected by treatment with a carbonate buffer at pH 10.[8] The separation of the M into M10 and M10S fractions resulted in an equal distribution of N, however, the enzyme was concentrated in the M10 portion. These results were confirmed in duplicate experiments.

Examination of the data reveals that the separation of the various particulate fractions does not result in a noticeable loss of enzyme activity. This is shown by totaling the N values and enzyme activities found in the various sub-fractions and comparing the sums with the theoretically expected values. For example, the sum of the N values found in the L1, M and MS fractions should approximate the observed E value. These comparisons are summarized in Table II. The fact that the summation of enzyme activities approximated the theoretical values indicates that naturally occurring activators or inhibitors of the esterase in the mouse liver cell, if present, were not separated from the enzyme by the fractionation procedures.

It is possible that the presence of considerable activity in the L1 fraction and some activity in the D2 fraction may be derived from contamination of these fractions with microsomes. Electron microscopic examinations of the L1 fraction in other comparable experiments appear to lend weight to this possibility. The presence of some intact cells is another possible source of the activity in the D2 fraction. The esterase activity found in our nuclear fraction (D2) therefore cannot be compared with Dounce's[1] values for isolated hepatic nuclei.

The isolation of pure nuclei with 1% citric acid, essentially the method of Marshak,[2] was carried out in one experiment, but the enzyme activity found was negligible. The esterase concentration in serum was also determined and was found to be less than 1% of that in the liver tissue. Hence no appreciable activity could be ascribed to contamination with serum.

Summary. The esterase in the cytoplasm of mouse liver cells was found to be localized predominantly in the microsome fraction which contained an average of 47% of the enzyme in the whole tissue and 63% of that in the entire cytoplasm. Other cell fractions, the nuclear material, the large cytoplasmic granules, and the supernate from the microsome separation contained an average of 17%, 17%, and 14%, respectively, of the enzyme in the whole tissue. Enzyme activity per μg of N was greatest in the microsomes which contained a concentration that was 4.2 times that of the whole tissue. In the other particulate fractions, the nuclear material, the large cytoplasmic granules, and the supernate from the microsome separation, the enzyme concentration was found to be 0.6, 1.6, and 0.3 times, respectively, that of the whole tissue. The "lipoprotein" fraction of the microsomes displayed about 3 times the enzyme concentration found in the "nucleoprotein" fraction. Summation of the enzyme content of the substituent fractions indicated no appreciable loss during the separation procedures.

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[8] Barnum, C. P., and Huseby, R. A., unpublished data.