DIFFERENCE SPECTRA, CATALASE- AND PEROXIDASE ACTIVITIES OF ISOLATED PARENCHYMAL AND NON-PARENCHYMAL CELLS FROM RAT LIVER

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SUMMARY. The reduced minus oxidized difference spectra from isolated parenchymal and non-parenchymal cells from rat liver indicate that the non-parenchymal cells contain a considerable amount of peroxidase. This interpretation is favoured by the more than 30 times higher specific activity of peroxidase (EC 1.11.1.7) in the non-parenchymal cells as compared to the parenchymal cells. The catalase (EC 1.11.1.6) activity in the non-parenchymal cells is 4 times lower than in the parenchymal cells. These results are consistent with an antimicrobial function of the non-parenchymal cells in liver.

The mammalian liver consists primarily of hepatocytes (parenchymal cells) and reticulo endothelial cells (Kupffer cells). As much as 35% of the liver cells are non-parenchymal cells representing 5 to 10% of the liver mass[1]. In an earlier paper[2] we have shown that parenchymal cells contain only L-type pyruvate kinase (EC 2.7.1.40) while the M_2-type was enriched in the non-parenchymal cells. From this distribution pattern we concluded that Kupffer cells do not significantly participate in gluconeogenesis. Histochemical studies have indicated that the main function of the Kupffer cells in the liver might be related to their phagocytic properties[3]. To obtain more information on the function and properties of the non-parenchymal cells, we separated intact parenchymal and non-parenchymal cells from rat liver and determined the difference spectra of these cell types. For the reason that the results were indicative for a large amount of peroxidase in the non-parenchymal cells, we also investigated the distribution of peroxidase and catalase activities between the different cell types. It is concluded that the biochemical data obtained are consistent with phagocytic and antimicrobial functions of the non-parenchymal cells in rat liver.

MATERIALS AND METHODS. Parenchymal cells were isolated from rat liver essentially according to the method of Berry and Friend[4], using collagenase (0.05%) and hyalurinidase (0.10%) as proteolytic enzymes in Hank's balanced salt solution. The cells were purified by differential centrifugation for 2 min at 45 g. The cells were resuspended in Hank's balanced salt solution and recentrifugated. This procedure was repeated 4 times. By centrifugation at this speed the non-parenchymal cells can be removed effectively from the parenchymal cells. The cells obtained, of which at least 90% excluded trypan-blue and were therefore considered to be intact, were not contaminated, as judged by light microscopy.

The non-parenchymal cells were isolated by two different methods both of which were based on a method described by Mills and Zucker-Franklin[5]. The first method was as described earlier[2]. In the second method a total cell suspension, obtained by the Berry and Friend[4] method, was incubated with 0.25% pronase for 1 h[6]. All parenchymal cells were destroyed after 1 h and the non-parenchymal cells were collected by centrifugation for 5 min at 600 g. The cells were resuspended in Hank's balanced salt solution and recentrifugated. This procedure was repeated 4 times. The non-parenchymal cells isolated by both methods did not differ in the assays and spectra mentioned under RESULTS.

Pyruvate kinase was assayed as described earlier[2]. Catalase was determined according to Bergmeyer[7] while peroxidase was measured with 3,3'-diamino benzidine (DAB) as hydrogen donor[8]. Difference spectra were run on a Perkin-Elmer 356 and on an Amino DW-2 spectrophotometer set for split-beam optics.

RESULTS

Fig. 1 shows the reduced minus oxidized spectrum of a homogenate of a typical parenchymal cell preparation. The interpretation of the maxima and minimum is indicated in the figure. The dotted line gives the succinate-reduced (antimycin A present) minus oxidized spectrum which allows the detection of cytochrome b. Fig. 2 gives the same kind of spectra for a homogenate of a typical non-parenchymal cell preparation. It can be seen that this difference spectrum differs markedly from that of the parenchymal cells (Fig. 1). Although the amount of protein present in the experiment of Fig. 2 is half of the amount used in Fig. 1, the peak in the 430 nm region is even higher. This high peak interferes with the determination of the mitochondrial cytochrome spectra. The succinate-reduced minus oxidized spectra show that at least the cytochrome b content of the different cell types is about equal. From these spectra we can conclude that the liver parenchymal cells show a normal cytochrome spectrum as for example is also found with intestinal cells[9], while the non-parenchymal cell spectrum seems to be related to spectra obtained with leucocytes[10]. As the leucocyte spectrum is considered to be the consequence of the high peroxidase content of these cells[11], the distribution of the

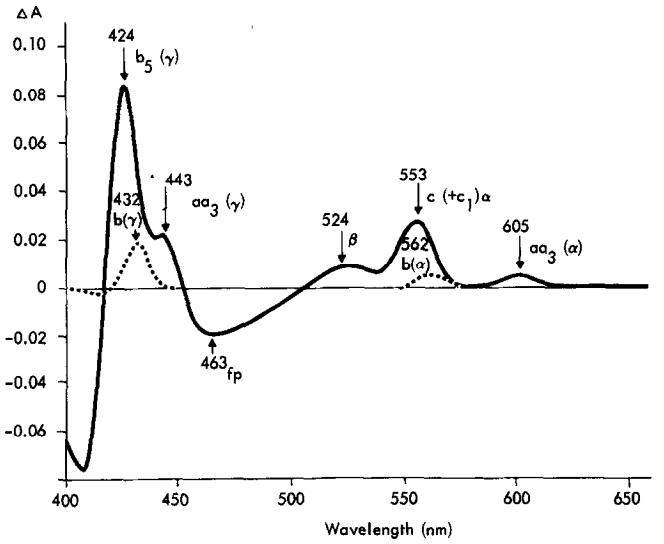

Fig. 1 Difference spectra of the parenchymal cell homogenate. —— reduced (with dithionite) minus oxidized (0.05% H_2O_2). ---- reduced (with succinate (10 mM) + antimycin A (4 µg)) minus oxidized (0.05% H_2O_2). The protein concentration with both spectra was 5.1 mg.

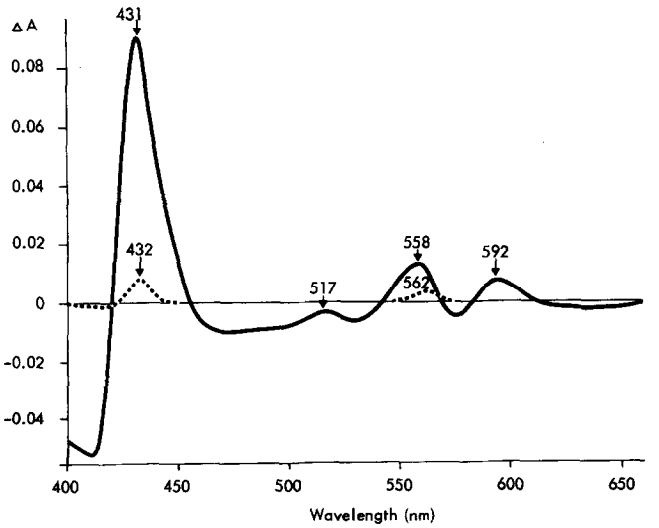

Fig. 2 Difference spectra of the non-parenchymal cell homogenate. —— reduced (with dithionite) minus oxidized (0.05% H_2O_2)). ---- reduced (with succinate (10 mM) + antimycin A (4 µg)) minus oxidized (0.05% H_2O_2). The protein concentration with both spectra was 2.54 mg.

activities of peroxidase and catalase between the different cell types was determined. To ascertain the intactness of the cells pyruvate kinase was used as a marker because this enzyme is solely located in the cytoplasm and does not adhere to membranous structures (unpublished). Table I shows the pyruvate kinase acti-

TABLE I

INTERCELLULAR DISTRIBUTION OF L- AND M_2-TYPE PYRUVATE KINASE

Source of homogenate	activity at 1 mM P-enolpyruvate (nmol min^{-1} mg^{-1} protein) ± SEM (n)	activity at 1 mM P-enolpyruvate + 0.5 mM Fru-1,6-P_2 (nmol min^{-1} mg^{-1} protein) ± SEM (n)
Whole rat liver	18.1 ± 2.0 (10)	124.4 ± 10.5 (10)
Parenchymal cells	11.8 ± 1.1 (8)	134.6 ± 16.3 (8)
Non-parenchymal cells	28.3 ± 4.4 (5)	27.6 ± 5.0 (5)

vities of the liver homogenate, the parenchymal and non-parenchymal cells. In accordance with an earlier report[2], parenchymal cells do only contain the L-type pyruvate kinase while the activity on protein base further supports the intactness of the cells used. From the absence of Fru-1,6-P_2 activation it can be concluded that the non-parenchymal cells contain exclusively M_2-type pyruvate kinase. This is a further evaluation of earlier obtained results[2] in which the non-parenchymal cell fraction also contained a considerable amount of L-type pyruvate kinase probably due to the impurity of the earlier non-parenchymal cell preparations.

Table II shows the peroxidase and catalase activities in the different cell preparations. It can be seen that the non-parenchymal cells contain 30 times more peroxidase activity than the parenchymal cells, and that on the other hand catalase activity is more concentrated in the parenchymal cells. The reason for the relatively low catalase activity in the liver homogenate as compared to activity in the parenchymal cell homogenate cannot be explained at the moment but might be related to the results of

TABLE II

INTERCELLULAR DISTRIBUTION OF PEROXIDASE AND CATALASE

Source of homogenate	peroxidase (μmol min^{-1} mg^{-1}) $\pm$ SEM (n)	catalase (μmol min^{-1} mg^{-1}) $\pm$ SEM (n)
Whole rat liver	7.0 $\pm$ 0.5 (4)	618 $\pm$ 92 (4)
Parenchymal cells	2.6 $\pm$ 0.3 (5)	1472 $\pm$ 315 (5)
Non-parenchymal cells	80.8 $\pm$ 12.7 (5)	368 $\pm$ 38 (5)

Fujiwara et al.[12] who observed the same phenomenon with the enzyme collagenase.

DISCUSSION

The results clearly demonstrate the heterogeneity of liver tissue. Conclusions drawn from work with total liver homogenates must be taken with caution when the different cellular contributions are not taken into account. The high specific activities obtained with the parenchymal cell homogenates, together with the trypan blue exclusion, clearly illustrate the usefulness of the reported cell preparation for cellular distribution studies. A quality test for the non-parenchymal cells is more difficult to assess although also for these cells the trypan blue exclusion is indicative for intactness.

From the distribution of the L- and M_2-type pyruvate kinases we can conclude that, at least in adult liver, the M_2-type is solely located in the non-parenchymal cells. Of course this does not exclude an occurrence of this type in parenchymal cells under special conditions (fetal or regenerating liver cells[13]). However, under all conditions the M_2-type is the only type present in non-parenchymal cells which excludes a significant contribution of these cell types to gluconeogenesis[2].

From histochemical studies it was concluded earlier that the Kupffer cells from rat liver contain peroxidase-like activity whereas the parenchymal cells were hardly positively stained[14,15]. The present paper shows that peroxidase is about 30 times more active in the homogenate of non-parenchymal cells as compared to

the homogenate of parenchymal cells. For reason that this enzyme is involved in antimicrobial systems[16], the present data, together with the lower catalase activity, support an antimicrobial function of the non-parenchymal cells in liver.

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REFERENCES

1. Weibel, E.R., Stäubli, W., Gnägi, H.R. and Hess, F.A. (1969) J. Cell. Biol. 42, 68-91.
2. Van Berkel, Th.J.C., Koster, J.F. and Hülsmann, W.C. (1972) Biochim. Biophys. Acta 276, 425-429.
3. Melly, M.A., Duke, L.J. and Koenig, M.G. (1972) J. Reticuloendothel. Soc. 12, 1-15.
4. Berry, M.N. and Friend, D.S. (1969) J. Cell. Biol. 43, 506-520.
5. Mills, D.M. and Zucker-Franklin, D. (1969) Am. J. Pathol. 54, 147-166.
6. Berg, T. and Boman, D. (1973) Biochim. Biophys. Acta 321, 585-596.
7. Bergmeyer, H.U. (1955) Bioch. Z. 327 , 255-260.
8. Herzog, V. and Fahimi, H.D. (1973) Anal. Biochem. 55, 554-562.
9. De Jonge, H.R. and Hülsmann, W.C. (1973) Eur. J. Biochem. 32, 356-364.
10. Evans, W.H. (1962) Fed. Proc. 21, 45
11. Kirschner, R.H., Getz, G.S. and Evans, A.E. (1972) Enzyme 13, 56-76.
12. Fujiwara, K., Sakai, T., Oda, T. and Igarashi, S. (1973) Biochem. Biophys. Res. Commun. 54, 531-537.
13. Bonney, R.J., Walker, P.R. and Potter, V.R. (1973) Biochem. J. 136, 947-954.
14. Fahimi, H.D. (1970) J. Cell. Biol. 47, 247-262.
15. Wisse, E. (1974) J. Ultrastruct. Res. 46, 393-426.
16. Klebanoff, S.J. (1974) J. Biol. Chem. 249, 3724-3728.