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# NUCLEOSIDEDIPHOSPHATASE ACTIVITY IN PLASMA MEMBRANE OF RAT LIVER

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SUMMARY

1. The intracellular distribution of nucleosidediphosphatase activity has been investigated in rat liver with ADP and IDP as substrates and compared with those of reference enzymes.

2. After differential centrifugation in 0.25 M sucrose, the distribution of the enzyme acting on IDP is similar to that of glucose-6-phosphatase; ADPase distribution is comparable to that of 5′-AMPase.

3. Deoxycholate considerably increases nucleosidediphosphatase activity on IDP, but has little effect when ADP is used as the substrate. The detergent does not affect the distribution of ADPase; on the contrary, significant changes of the IDPase distribution are observed if the test is performed with or without deoxycholate.

4. Enzyme distribution curves have been determined after centrifugation in a sucrose gradient of a particle preparation obtained by centrifuging the homogenate at $3.7 \cdot 10^6 \times g \cdot \min$. When granules are layered at the top of the gradient no clear distinction is seen between the enzyme distribution curves. When granules are deposited at the bottom of the gradient, a striking separation of the nucleosidediphosphatase distribution curves can be observed. The behaviour of IDPase is similar to that of glucose-6-phosphatase; ADPase behaves like 5′-AMPase.

5. In purified plasma membrane preparations, ADPase and 5′-AMPase show a specific activity approx. 25 times higher than that found in the homogenate. IDPase is only 5 times purer than in the homogenate. Some properties of the nucleosidediphosphatase associated with the plasma membrane are given.

6. The results are discussed with respect to the presence in rat liver of two nucleosidediphosphatases, one associated with the plasma membrane and the other with endoplasmic-reticulum membranes.

## INTRODUCTION

In our studies of purified rat liver plasma membrane preparations, a significant nucleosidediphosphatase (nucleosidediphosphate phosphohydrolase, EC 3.6.1.6) activity was found to be associated with these preparations. Conflicting results exist in the literature as to the presence of a nucleosidediphosphatase in the plasma membrane. Cytochemical data[1] indicate that an ADPase is associated with this cellular component

and that an enzyme acting on IDP, but not on ADP, is found both in plasma membranes and in cytomembranes. On the other hand, EMMELOT *et al.*[2] reported no or very weak ADPase activity in purified rat liver plasma membrane preparations and EMMELOT AND BENEDETTI[3] list a significant IDPase activity in similar preparations. This situation made it mandatory for us to reexamine the intracellular distribution of nucleosidediphosphatase in this tissue. The work reported here strongly suggests that a nucleosidediphosphatase is effectively associated with the plasma membrane; it can be tested with ADP as a substrate, and is not activated by deoxycholate. In agreement with the results of NOVIKOFF AND HEUS[4] our results show that another nucleosidediphosphatase is present in endoplasmic-reticulum membranes and that this enzyme can be tested with IDP in the presence of deoxycholate.

## METHODS

### *Tissue fractionation*

Experiments have been performed with male and female Wistar rats weighing 200–300 g. The animals were killed by decapitation after 20 h of fasting. Fractionation of the liver homogenate by differential centrifugation was achieved using the technique of DE DUVE *et al.*[5]. A nuclear fraction (N), a 'heavy' mitochondrial fraction (M), a 'light' mitochondrial fraction (L), a microsomal fraction (P), and a soluble fraction (S) were isolated using the fractionation scheme described by these authors. Density-gradient experiments were performed using a method similar to that of BEAUFAY *et al.*[6], except that the tissue was homogenized by 25 up-and-down strokes with a loose-fitting pestle of a Dounce homogenizer (Blaessig Glass, Rochester, N.Y.). Plasma membranes were isolated according to the method of NEVILLE[7] with the modifications introduced by EMMELOT *et al.*[2].

### *Enzyme assays*

Nucleosidediphosphatase assays were performed at 30°. The medium contained 5 mM nucleoside diphosphate, 50 mM Tris buffer (pH 7.5) and 5 mM $CaCl_2$ in a 0.6-ml volume. In the case where IDP was the substrate, 0.03 % deoxycholate was added to the medium. The test was stopped by adding 2.4 ml of 8 % cold trichloroacetic acid, and after filtration in the cold, inorganic phosphate was measured by the method of FISKE AND SUBBAROW[8].

5'-AMPase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5) was assayed at 30° in a medium containing 5 mM 5'-AMP, 5 mM $MgCl_2$ and 50 mM Tris buffer (pH 7.5) in a 0.6-ml volume. The reaction was stopped by the addition of 2.4 ml of 8 % trichloroacetic acid, and inorganic phosphate determinations were made on the filtrate.

Cytochrome oxidase (cytochrome *c*:oxygen oxidoreductase, EC 1.9.3.1), glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) and acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) were measured according to the method of DE DUVE *et al.*[5], NADH–cytochrome *c* reductase (NADH:cytochrome *c* oxidoreductase, EC 1.6.99.3) according to ERNSTER *et al.*[9], proteins by the method of LOWRY *et al.*[10].

Units of enzymatic activity are defined as the amount of enzyme causing the decomposition of 1 μmole of substrate per min under the conditions of the assay. One cytochrome oxidase unit is defined as the amount of enzyme causing a logarithmic

decrease of the concentration of reduced cytochrome *c* by 1 unit per min per 100 ml of incubation mixture[5].

Nucleoside diphosphates, 5'-AMP, glucose 6-phosphate, NADH and cytochrome *c* were obtained from the Sigma Chemical Co.

## RESULTS

### *Distribution after differential centrifugation*

Table I shows the distribution of enzymes after differential centrifugation. The nucleosidediphosphatase activity was measured with IDP or ADP as the substrate, with $Ca^{2+}$ or $Mg^{2+}$ in the incubation medium. Reference enzymes are: cytochrome oxidase for mitochondria, glucose-6-phosphatase for endoplasmic-reticulum membranes, 5'-AMPase for plasma membranes, acid phosphatase for lysosomes. In addition, NADH–cytochrome *c* reductase, an enzyme recovered mainly in microsomes, was also determined.

TABLE I

INTRACELLULAR DISTRIBUTION OF ENZYMES

Absolute values are given in mg/g for proteins and in units/g fresh wt. of liver for enzymes. E, cytoplasmic extract; N, nuclear fraction; M, heavy mitochondrial fraction; L, light mitochondrial fraction; P, microsomal fraction; S, final supernatant.

| *Enzymes* | *Absolute values* | *Percentage values* | | | | | |
|---|---|---|---|---|---|---|---|
| | *E+N* | *N* | *M* | *L* | *P* | *S* | *Recovery* |
| Proteins | 244 | 12.8 | 27.2 | 4.7 | 22.8 | 30.6 | 98.1 |
| Cytochrome oxidase | 32.6 | 9.2 | 83.0 | 8.8 | 2.8 | — | 103.8 |
| Glucose-6-phosphatase | 18.9 | 6.5 | 6.8 | 7.3 | 75.5 | 1.4 | 97.5 |
| NADH–cytochrome *c* reductase | 52.0 | 3.6 | 24.2 | 7.3 | 64.5 | 2.5 | 102.1 |
| Acid phosphatase | 7.24 | 5.4 | 36.4 | 32.4 | 24.8 | 6.6 | 105.6 |
| ADPase + $Mg^{2+}$ | 7.47 | 35.1 | 31.2 | 4.0 | 33.2 | 4.7 | 108.2 |
| ADPase + $Ca^{2+}$ | 8.68 | 31.8 | 37.0 | 4.0 | 28.8 | 3.2 | 104.8 |
| IDPase + $Mg^{2+}$ | 82.1 | 7.4 | 8.5 | 7.2 | 64.5 | 3.9 | 91.5 |
| IDPase + $Ca^{2+}$ | 70.0 | 9.4 | 7.7 | 7.0 | 69.5 | 2.7 | 96.3 |
| 5'-AMPase | 7.45 | 37.5 | 26.5 | 7.1 | 39.6 | 13.2 | 123.9 |

The IDPase distribution resembles that of glucose-6-phosphatase and NADH–cytochrome *c* reductase.

The general distribution pattern is similar for 5'-AMPase and ADPase (Fig. 1). Both show the highest specific activity in the nuclear fraction; however, the diphosphatase is more abundant than 5'-AMPase in the heavy mitochondrial fraction, whereas the nucleotidase exhibits a larger proportion of soluble activity. This distribution is quite different from that of the reference enzymes: cytochrome *c* oxidase, glucose-6-phosphatase and acid phosphatase.

### *Effect of deoxycholate*

As described by ERNSTER AND JONES[11], deoxycholate increases nucleosidediphosphatase activity on IDP, GDP and UDP, but has little effect when ADP or CDP are used as the substrate; all these activities were determined on the microsomal fraction of rat liver. This led us to investigate the effect of deoxycholate in the reaction

medium on ADPase and IDPase activity found in fractions isolated by differential centrifugation. Our results are recorded in Table II and Fig. 2. Deoxycholate has little effect on ADPase activity, and the distribution patterns of the enzymes are similar whether or not the detergent is present in the reaction medium. IDPase activity, on the contrary, is strikingly decreased if deoxycholate is not added to the test. The microsomal fraction (P) is especially affected in agreement with the results of

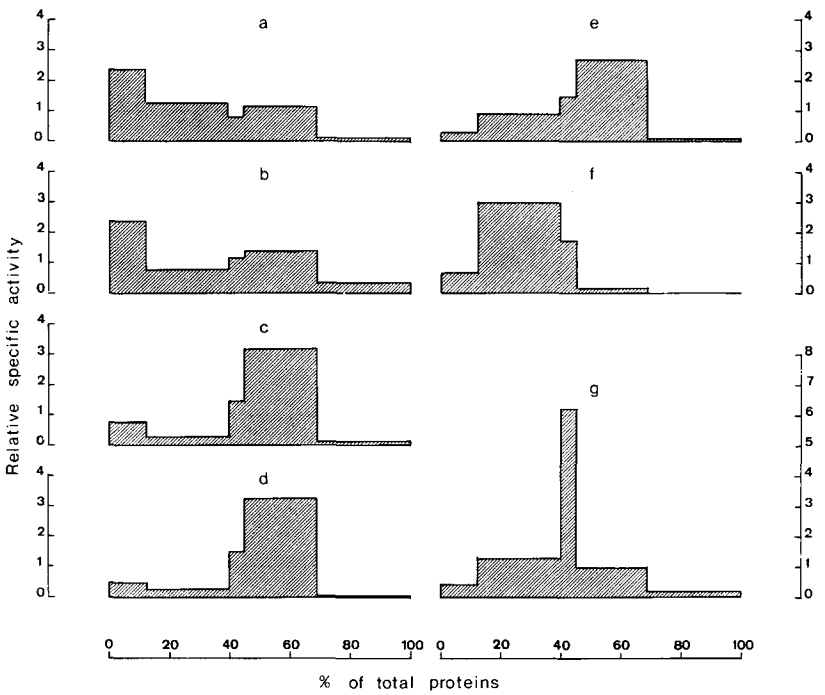



Fig. 1. Distribution patterns of enzymes: (a) ADPase; (b) 5′-AMPase; (c) IDPase; (d) glucose-6-phosphatase; (e) NADH–cytochrome *c* reductase; (f) cytochrome oxidase; (g) acid phosphatase. Ordinate: relative specific activity of fractions (percentage of total activity recovered/percentage of total proteins recovered). Abscissa: relative protein content of fractions (cumulatively from left to right).

TABLE II

EFFECTS OF DEOXYCHOLATE ON NUCLEOSIDEDIPHOSPHATASE ACTIVITY OF SUBCELLULAR FRACTIONS

Determinations were made in both the presence and absence of 0.03% deoxycholate. Values are given in units/g fresh wt. of liver. See legend of Table I for abbreviations.

| *Fraction* | *ADPase* | | *IDPase* | |
|---|---|---|---|---|
| | *— Deoxy-cholate* | *+ Deoxy-cholate* | *— Deoxy-cholate* | *+ Deoxy-cholate* |
| E | 4.63 | 5.25 | 15.6 | 61.3 |
| N | 3.45 | 3.05 | 5.8 | 7.6 |
| M | 2.06 | 2.18 | 3.2 | 5.9 |
| L | 0.29 | 0.28 | 0.7 | 3.1 |
| P | 1.84 | 2.06 | 9.0 | 46.6 |
| S | 0.14 | 0.10 | 2.4 | 1.8 |

ERNSTER AND JONES[11], while the activity of the nuclear fraction (N) is only slightly increased by the detergent. As illustrated in Fig. 2 significant changes occur in the IDPase distribution depending on whether the test is performed with or without deoyxycholate. The distribution pattern is typically microsomal in its presence, but becomes similar to that of ADPase (and of 5′-AMPase) in its absence.

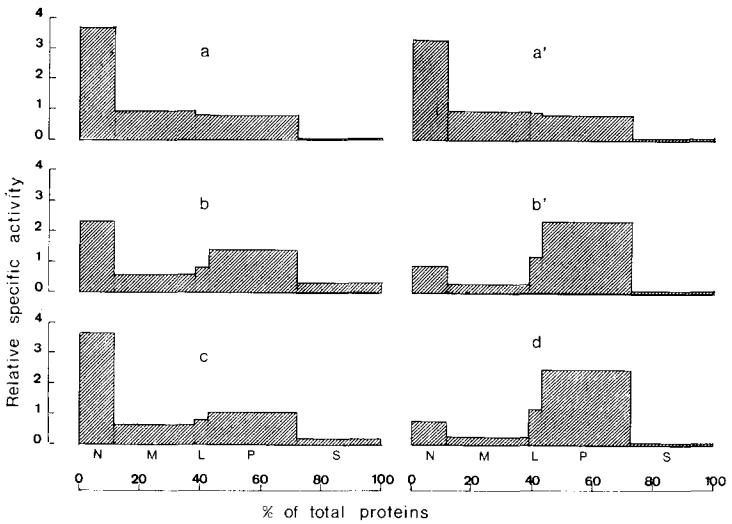



Fig. 2. Effects of deoxycholate on the distribution of nucleosidediphosphatase. (a) ADPase without deoxycholate; (a′) ADPase with deoxycholate; (b) IDPase without deoxycholate; (b′) IDPase with deoxycholate; (c) 5′-AMPase; (d) glucose-6-phosphatase. For an explanation of the graph see Fig. 1.

*Distribution after centrifugation in a sucrose gradient*

The enzyme distributions were established after centrifugation in a sucrose gradient with a density ranging from 1.09 to 1.24. Because 5′-AMPase and ADPase are recovered in a significant amount in each sedimentable fraction after differential centrifugation, in the gradient experiments we used granule preparations obtained by centrifuging the homogenate at $3.7 \cdot 10^6 \times g \cdot$min in rotor No. 40 of the Spinco L-HV centrifuge. These preparations correspond to the sum of N, M, L and P fractions isolated by differential centrifugation and contain 80–90 % of the homogenate activity with respect to the enzymes measured. In one experiment the granules were resuspended in aqueous sucrose (density 1.034) and layered on the top of the gradient; in another experiment, the granules were diluted in aqueous sucrose (density 1.25) and placed at the bottom of the centrifuge tube.

The results are illustrated in Fig. 3. When granules are layered a broad distribution is observed for 5′-AMPase, glucose-6-phosphatase, nucleosidediphosphatase and NADH–cytochrome *c* reductase. It is practically impossible to distinguish among these distribution curves. Acid phosphatase distribution is comparable to that found after centrifugation of mitochondrial fractions in the same gradient[6]. Cytochrome oxidase was not determined in this experiment. When granules are deposited at the bottom of the gradient, one can see a separation of the nucleosidediphosphatase's distribution curves. IDPase exhibits a median density of 1.22, much higher than that which is found when granules are layered. The same phenomenon is observed for glu-

cose-6-phosphatase and NADH–cytochrome *c* reductase, which are characterized by a median density of 1.226 and 1.211, respectively. ADPase resembles 5′-AMPase in showing a distribution pattern similar to that found when granules are layered; median density is about 1.17 for each enzyme. Acid phosphatase distribution is relatively

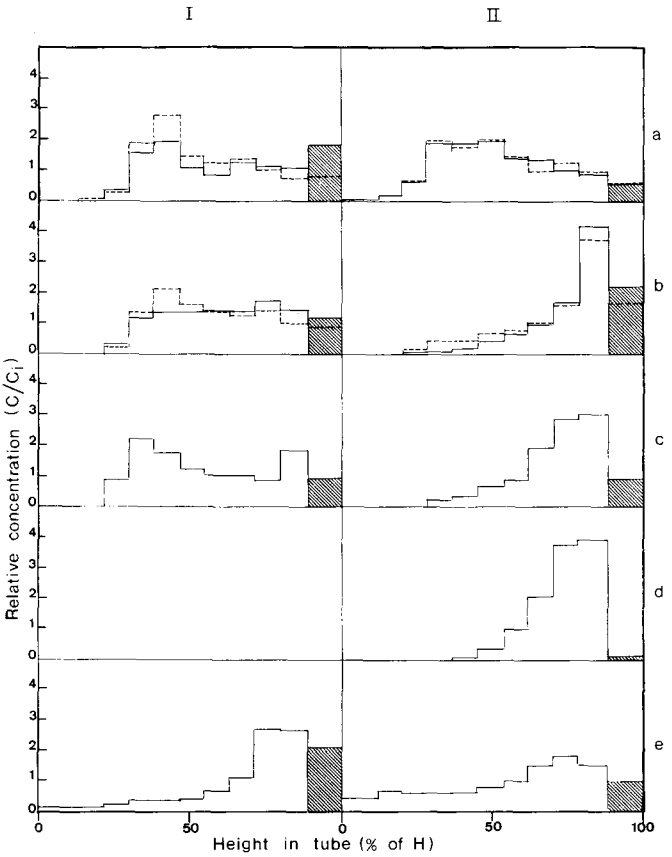



Fig. 3. Distribution of particle-bound enzymes after centrifugation (for 2.5 h at 39000 rev./min in SW 39 head of the Spinco model L-HV preparative ultracentifuge) of a granule fraction of rat liver (see text for explanation) through a 0.776–2.969 molal sucrose gradient in water. (I) The particles were initially diluted in 0.265 molal sucrose and layered at the top of the gradient; (II) the particles were initially diluted in 3.187 molal sucrose and put at the bottom of the tube under the gradient. Abscissa: percentage of the height of the liquid column in tube (H). Ordinate: relative concentration, *i.e.*, ratio of the observed activity (C) to that which would have been found if the enzyme had been homogeneously distributed throughout the whole gradient ($C_i$). Filled blocks (▨) are used for the bottom subfractions to indicate that they include material falling beyond the limits of the gradient. (a) ———, 5′-AMPase; ---------, ADPase. (b) ———, glucose-6-phosphatase; --------, IDPase. (c) NADH–cytochrome *c* reductase. (d) Cytochrome oxidase. (e) Acid phosphatase.

flatter than that observed in the first experiment; however, the difference is not very important. Cytochome oxidase has a median density of 1.21; such a high value has been observed by BEAUFAY *et al.*[6] with mitochondria exposed to a high sucrose concentration.

TABLE III

COMPLEMENTARY DATA ON EXPERIMENTS OF FIG.3

Values given refer to the sum of the activities of all the fractions expressed in percentages of the total activity included in the gradient.

| *Enzyme* | *Experiment* | |
|---|---|---|
| | *I* | *II* |
| 5′-AMPase | 102.0 | 112.0 |
| ADPase | 104.1 | 99.2 |
| Glucose-6-phosphatase | 99.3 | 90.2 |
| IDPase | 108.1 | 112.0 |
| NADH–cytochrome *c* reductase | 87.5 | 103.0 |
| Cytochrome oxidase | — | 83.5 |
| Acid phosphatase | 104.5 | 107.0 |

*Activity of purified plasma membrane preparations*

In the centrifugation experiments, ADPase was found to have a behaviour similar to that of 5′-AMPase, an enzyme associated with the plasma membrane. Therefore, experiments were performed on purified plasma membrane preparations. The method we used to obtain these preparations is that of NEVILLE[7] with the modifications introduced by EMMELOT *et al.*[2]. The technique consists of isolating from an homogenate (H), by low-speed centrifugation, a pellet enriched in fragments of plasma membranes and a supernatant (S). This pellet is fractionated by centrifugation in a discontinuous sucrose gradient into two subfractions: Mb (plasma membrane fraction) and Sm. Table IV records the results of a typical experiment in which the nucleosidediphosphatase activity and that of reference enzymes have been measured in the homogenate and in the isolated fractions. More than 20% of the ADPase and 5′-AMPase is recovered in fractions obtained after centrifugation in the discontinuous sucrose gradient (Mb+Sm). The other enzymes are found in much smaller proportions; values of recovery allow us to exclude the possibility of this phenomenon being due chiefly to a

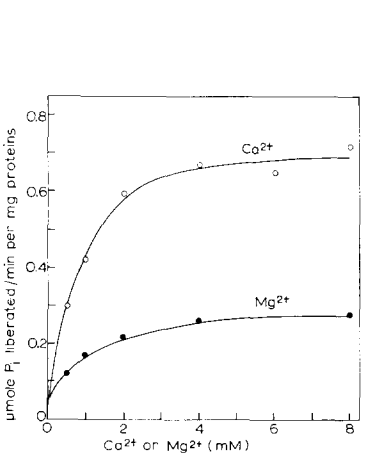
TABLE IV

ENZYMIC ACTIVITY OF FRACTIONS RECOVERED IN PREPARATION OF PURIFIED PLASMA MEMBRANES

Values are given in percentage (%) of the homogenate activity and in units/mg protein (specific activity) for the fractions and in units/mg protein for the homogenate. The amount of protein found in the homogenate was 225 mg/g fresh wt. of liver. For abbrevations see text.

| *Enzyme* | *Homogenate spec. act.* | *S* | | *Mb* | | *Sm* | | *Recovery* (%) |
|---|---|---|---|---|---|---|---|---|
| | | % | *Spec. act.* | % | *Spec. act.* | % | *Spec. act.* | |
| IDPase | 0.202 | 98.0 | 0.206 | 0.8 | 0.98 | 4.7 | 0.272 | 103.5 |
| ADPase | 0.031 | 79.0 | 0.025 | 4.2 | 0.790 | 28.4 | 0.248 | 111.6 |
| 5′-AMPase | 0.033 | 71.5 | 0.024 | 3.9 | 0.790 | 22.2 | 0.206 | 97.6 |
| Glucose-6-phosphatase | 0.122 | 91.5 | 0.119 | 0.06 | 0.043 | 1.7 | 0.060 | 93.3 |
| NADH–cytochrome *c* reductase | 0.227 | 99.5 | 0.282 | 0.03 | 0.054 | 1.0 | 0.075 | 100.5 |
| Acid phosphatase | 0.038 | 96.5 | 0.038 | 0.1 | 0.027 | 0.6 | 0.007 | 97.2 |
| Cytochrome oxidase | 0.178 | 90.5 | 0.375 | 0.01 | 0.012 | 0.4 | 0.014 | 90.9 |
| Proteins | | 96.0 | | 0.16 | | 3.5 | | 99.7 |

selective inactivation of these enzymes in fractions Mb and Sm. In the purified plasma membrane fraction (Mb), ADPase and 5'-AMPase show a specific activity approx. 25 times higher than that found in the homogenate. In this fraction, IDPase is only 5 times purer than in the homogenate, and the other enzymes exhibit a specific activity lower than that associated with the homogenate.



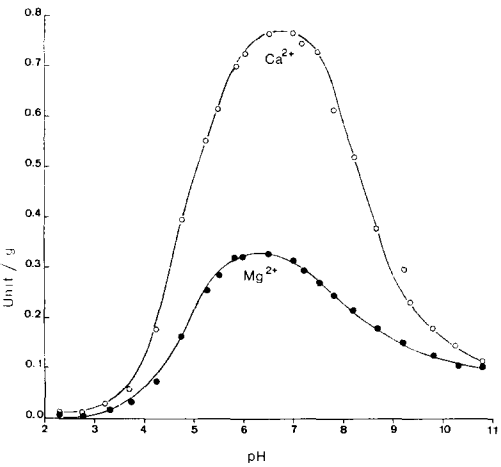




Fig. 4. Effect of $Ca^{2+}$ and $Mg^{2+}$ on the ADPase activity of a plasma membrane preparation. Only liberated inorganic phosphate was measured.

Fig. 5. Effect of pH on ADPase activity of a plasma membrane preparation. The reaction mixture contained a 50-mM buffer. The buffers used were glycine–HCl from pH 2.3 to 3.25; acetic acid–sodium acetate from pH 3.75 to 5.85; sodium cacodylate–HCl from pH 6.0 to 7.5; Tris buffer from pH 7.5 to 9.2; and glycine–NaOH from pH 8.2 to 10.8.

Some properties of the nucleosidediphosphatase activity found in the plasma membrane preparations were investigated. The reaction is linearly proportional to the amount of preparation and the time of incubation. The amount of phosphate liberated is significantly higher than that of the AMP recovered, as measured by the method of ADAM[12]. This is probably due to the preparation's 5'-nucleotidase activity. $Mg^{2+}$ and

TABLE V

NUCLEOSIDEDIPHOSPHATASE ACTIVITY OF PLASMA MEMBRANE PREPARATION ON DIFFERENT NUCLEOSIDE DIPHOSPHATES

The enzyme assays are carried out at 30° in a medium containing 5 mM nucleoside diphosphate, 50 mM Tris buffer (pH 7.5), with and without 5 mM $MgCl_2$ or $CaCl_2$. Activities are given in units per mg protein.

| *Substrate* | *No addition* | *5 mM $MgCl_2$* | *5 mM $CaCl_2$* |
|---|---|---|---|
| ADP | 0.071 | 0.338 | 0.815 |
| UDP | 0.086 | 0.500 | 1.060 |
| CDP | 0.030 | 0.228 | 0.520 |
| IDP | 0.142 | 0.630 | 1.100 |
| GDP | 0.112 | 0.533 | 0.940 |

$Ca^{2+}$ stimulate the reaction, but its rate in the presence of $Ca^{2+}$ is much higher than that observed in the presence of $Mg^{2+}$. Fig. 4 illustrates the effect of varying concentrations of $Mg^{2+}$ and $Ca^{2+}$; a maximal activity is obtained at approximately a 5-mM cation concentration. The enzyme activity is double when serum albumin is added to the reaction medium at a concentration of 2.4 mg/ml. The optimum pH of the reaction is between 6.5 and 7.0 (Fig. 5). Table V allows us to compare the plasma membrane nucleosidediphosphatase activity with different nucleoside diphosphates at equal concentrations (5 mM). In every case, $Ca^{2+}$ is a more effective activator than $Mg^{2+}$. As indicated in Table VI, deoxycholate does not increase the activity; on the contrary, a slight inhibition is observed with GDP and CDP as a substrate.

TABLE VI

EFFECT OF DEOXYCHOLATE ON NUCLEOSIDEDIPHOSPHATASE ACTIVITY OF PLASMA MEMBRANE PREPARATION

The enzyme assays are carried out at 30° in a medium containing 5 mM nucleoside diphosphate, 50 mM Tris buffer (pH 7.5), 5 mM $CaCl_2$, with and without 0.03% deoxycholate. Activities are given in units/mg protein.

| *Substrate* | *— Deoxycholate* | *+ Deoxycholate (0.03%)* |
|---|---|---|
| ADP | 0.625 | 0.600 |
| IDP | 0.800 | 0.795 |
| UDP | 0.680 | 0.691 |
| GDP | 0.732 | 0.670 |
| CDP | 0.334 | 0.252 |

## DISCUSSION

Distributions exhibited by the rat liver nucleosidediphosphatase activity in centrifugation experiments are different depending on whether ADP or IDP is used as substrate. Compared with reference enzymes, it is seen that the distribution of ADPase resembles that of 5'-AMPase and that the distribution of IDPase, tested in the presence of deoxycholate, is similar to that of glucose-6-phosphatase.

Biochemical and morphological studies[13–16] indicate that glucose-6-phosphatase is located in the membranes of the endoplasmic reticulum. Our results suggest that IDPase has the same cellular localization, and this finding agrees with the results of NOVIKOFF AND HEUS[4] and those of AMAR-COSTESEC *et al.*[13]. ADPase seems to be linked to the same cellular component as 5'-AMPase. In the liver, 5'-AMPase, tested at pH 7.5, has been described by several authors as a constituent of plasma membrane[2,17,18]. This strongly suggests that ADPase is found in rat liver plasma membrane. In fact, the specific activity of the enzyme is much higher in plasma membrane preparations than in the homogenate.

EMMELOT *et al.*[2] reported no or very weak ADPase activity in plasma membranes. Their results do not agree with the work reported here or, as pointed out by these authors, with cytochemical data indicating the presence of a nucleosidediphosphatase

in plasma membranes. The reason for these conflicting results is not clear, but it is perhaps due to differences in the rats used in the experiments, as suggested by EMMELOT *et al.*[2]. In fact, the ADPase activity found by these authors in the rat liver microsomal fraction is 4–5 times lower, on a protein basis, than the one we found in the same fraction. The total ADPase activity of the homogenate is not given in their publication. It is interesting to compare our results with morphological observations, which indicate the presence of a nucleosidediphosphatase activity in the plasma membrane. REID[1] recently summarized the morphological results and concluded that ADPase existed only in plasma membranes, while IDPase existed both in plasma membranes and in endoplasmic-reticulum membranes. Our results agree with these morphological observations. However, we think that the IDPase and the ADPase activities of the plasma membrane are due to the same enzyme, so that IDPase associated with the cytomembrane is different from IDPase present in the plasma membrane. There should be a nucleosidediphosphatase, of low specificity, in the plasma membrane which is able to hydrolyse IDP, as well as ADP, and a nucleosidediphosphatase in the endoplasmic reticulum much more active with IDP than with ADP. The latter enzyme would correspond to the microsomal IDPase purified by NOVIKOFF AND HEUS[4] and more recently by YAMAZAKI AND HAYAISHI[19]. According to these authors, the enzyme hydrolyses IDP, GDP and UDP but is quasi-inactive with ADP. Moreover, $Mg^{2+}$ is a more potent activator of the reaction than $Ca^{2+}$. A distinction between the IDPase of the plasma membrane and that of the cytomembrane appears when comparing the effects of deoxycholate on the enzymatic activity and on the distribution after differential centrifugation. The changes observed may be explained by admitting that deoxycholate increases the activity of nucleosidediphosphatase found in the cytomembranes and has no effect on the enzyme associated with the plasma membrane. When tested with IDP, nucleosidediphosphatase distribution reflects that of the cytomembrane if deoxycholate is present, and is significantly affected by the distribution of the plasma membrane enzyme if the detergent is absent. When nucleoside diphosphatase is tested with ADP, no changes are observed because the cytomembrane enzyme, which is the only one sensitive to deoxycholate, is practically unable to split ADP.

One may question whether or not the enzyme acting on ADP is an adenylate kinase, because the ATPase present in the plasma membrane is able to split the ATP formed by the reaction. Previous results obtained by NOVIKOFF *et al.*[20] make this assumption improbable. According to these authors, rat liver adenylate kinase is mainly found in mitochondrial and soluble fractions of the homogenate after differential centrifugation; only a small percentage of the enzyme is recovered in the nuclear and microsomal fractions. Moreover, the activity is stimulated better by $Mg^{2+}$ than by $Ca^{2+}$.

Some properties of the ADPase associated with plasma membrane preparations are comparable to those of potato apyrase (ATP diphosphohydrolase, EC 3.6.1.5)[21,22]: *e.g.*, it is preferentially activated by $Ca^{2+}$; it is an enzyme of low specificity; its optimum pH of activity is at 6.5; it is activated by serum albumin. It would be interesting to see if an apyrase-like enzyme is present in the plasma membrane by comparing ATPase and ADPase activities in purified preparations. This enzyme would be different from the $Mg^{2+}$-ATPase described by EMMELOT AND BOS[23], since the preparations used by these authors seem to exhibit only a weak ADPase activity.

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