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A $Na^+ + K^+$-stimulated adenosine triphosphatase in "microsomal" fractions from rat liver

Recently, numerous studies have revealed the presence of an ATPase in "membranous" structures that is dependent upon $Mg^{2+} + Na^+ + K^+$ for optimal activity and is inhibited by cardiac glycosides. The enzyme system has been found in the red-cell membrane[1] and in the microsomal fractions of heart[2-5], skeletal muscle[6], brain and kidney[2,6]. Although JARNEFELT[8] was unable to observe activity in the liver, EMMELOT AND BOS[9] found enzymic activity but only in the "cell-membrane" fraction of liver.

The present study was designed to investigate whether a "microsomal" fraction from rat liver possessed ATPase activity which could be stimulated by $Na^+ - K^+$ and inhibited by ouabain.

The isolation medium utilized by SKOU[2,3] for heart, brain and kidney tissue was found to be suitable for liver tissue. The solution consisted of 0.25 M sucrose, 5 mM EDTA, 30 mM histidine·HCl, and 50 mM 2-amino-2-methyl-1,3-propanediol (pH 6.8). Just prior to homogenizing the tissue, 0.2% sodium deoxycholate was added to the isolation medium.

Adult male rats were decapitated, bled, the livers removed, homogenized and subjected to centrifugation using a previously described procedure[7]. Fractions were collected at 600 × *g* for 15 min (P6), 10 000 × *g* for 30 min (P10), 20 000 × *g* for 30 min (P20), 80 000 × *g* for 30 min (P80) and 100 000 × *g* for 70 min (P100). The pellets were washed lightly with the isolation medium, suspended in 0.25 M sucrose containing 1 mM EDTA and 30 mM histidine at pH 7.00 as suggested by SKOU[2,6], and then stored for varying periods at −5°.

RNA, DNA, glucose-6-phosphatase (EC 3.1.3.9) and ATPase were determined in each of the subcellular fractions and on an aliquot of the homogenate by procedures indicated in the table legends. Estimation of 5′ nucleotidase (EC 3.1.3.5) activity was performed according to the procedure of HEPPEL AND HILMOE[10]. The activity of the glucose-6-phosphatase did not change during the storage period. Protein was estimated by the biuret procedure[11]. Staining and microscopic examination revealed little or no mitochondria or nuclei in fractions P20, P80 or P100. No whole cells were visible.

From the data in Table I, and previous studies using brain suspensions[7], it would appear that fractions P20, P80 and P100 consist mostly of microsomal material. The highest specific activity for glucose-6-phosphatase as well as the highest total amount was found in fractions P20, P80 and P100. HERS *et al.*[12] observed that glucose-6-phosphatase was confined to the microsomes of the liver cell and hence served as a "microsomal marker". The present results on liver glucose-6-phosphatase are in agreement with these authors and with the data of HULSMANS[13]. The high RNA and low DNA levels lend additional evidence to the microsomal nature of the above subcellular fractions.

The results in Table II indicate the presence of an ATPase which is stimulated by $Na^+ + K^+$ and inhibited by ouabain. The "activity ratio" (B : A) increases with storage of the preparation at −5°. This is due to a greater decrease in the Mg^{2+}-stimulated ATPase than the $Mg^{2+} + Na^+ - K^+$-stimulated enzyme and is similar to results found in heart tissue[2,6]. It is noteworthy that the fractions exhibiting the

TABLE I

RNA, DNA AND GLUCOSE-6-PHOSPHATASE ACTIVITY OF RAT-LIVER SUBCELLULAR FRACTIONS

RNA and DNA were determined by the method of HUTCHINSON *et al.*[14]; glucose-6-phosphatase was determined by the procedure of SWANSON[15] at 37° and pH 6.5. The fractions are the sediments collected at the indicated forces, × 10^{-3} (average *g*) and resuspended in 0.25 M sucrose, 30 mM histidine·HCl, 1 mM EDTA (pH 7.00).

Fraction	RNA		DNA		Glucose-6-phosphatase	
	μg RNA/mg protein	total (%)	μg DNA/mg protein	total (%)	μmoles P/mg protein/h	total (%)
P6	18.4	8.2	29.5	51.0	1.2	5
P10	13.1	6.4	3.3	6.1	3.5	16
P20	24.2	7.0	5.0	5.6	5.5	15
P80	27.2	9.3	3.9	4.0	8.6	27
P100	80.0	18.5	7.8	5.6	10.7	23
SPN	23.2	51.0	3.1	26.4	0.72	15

$Na^+ + K^+$-stimulated ATPase possessed a very low 5'-nucleotidase activity (about one-fifth of the ATPase activity) under the same conditions of assay as the ATPase. These "microsomal" fractions are in contrast to the liver cell-membrane preparations of EMMELOT AND BOS[9] which were high in 5'-nucleotidase (about one-half the ATPase) and low in glucose-6-phosphatase activity (about 1.6 μmoles P/mg protein/h).

TABLE II

THE $Na^+ + K^+$-STIMULATED ATPASE OF SUBCELLULAR FRACTIONS FROM RAT LIVER

The incubation medium consisted of 3 mM ATP, 3 mM $MgCl_2$, 100 mM NaCl, 20 mM KCl, 30 mM Tris (pH 7.00); generally 0.1 ml enzyme suspension was added after 5 min temperature equilibration at 37° and incubation was continued for 15 min. The reaction was stopped by the addition of 0.1 ml of cold 50% trichloroacetic acid and aliquots (0.4 ml) were assayed for inorganic phosphate released by the method of FISKE AND SUBBAROW[16], using Amidol as reducing agent. The fractions represent the pellets as described in Table I.

Fraction	Days at −5°	μg P/mg protein/h				
		(A) Mg^{2+}	(B) $Mg^{2+} + Na^+ + K^+$	(B):(A)	(B) $Mg^{2+} + Na^+ + K^+$ + ouabain	
P20	5	144	160	1.10		
	9	80	121	1.51		
	12	96	130	1.36	(10^{-4} M)	107
					(10^{-3} M)	77
P80	10	110	146	1.33		
	12	73	113	1.55	(10^{-4} M)	91
P100	12	83	126	1.52	(10^{-4} M)	94

It should be mentioned that freshly prepared liver "microsomal" fractions did not exhibit any apparent stimulation upon addition of $Na^+ + K^+$. However, the cardiac glycoside ouabain still depressed the activity of the enzyme in the presence of $Mg^{2+} + Na^+ + K^+$ but not with Mg^{2+} alone. This indicates that the $Na^+ + K^+$ activity was present but was "masked" by a high basic Mg^{2+}-stimulated ATPase. The high Mg^{2+}-stimulated ATPase activity which tends to obscure the activity of the enzyme in the presence of $Na^+ + K^+$ was first found in heart-muscle fractions

by Skou[2,6]. These results were also obtained in similarly prepared rat, guinea-pig and rabbit-heart "microsomal" fractions[5]. Storage at −5° effects a destruction of the Mg^{2+}-stimulated enzyme thereby revealing the stimulation due to $Na^+ + K^+$, which stimulation can be inhibited by ouabain.

The data presented suggest that the fractions described as having ATPase activity which could be stimulated by the addition of $Na^+ + K^+$ and inhibited by ouabain, consist essentially of endoplasmic reticulum. The possibility of small fragments of cell membrane being associated with the microsomal fraction in these preparations cannot be excluded. The contribution, however, of this "contaminant" to the total ATPase observed in the present study would be very low.

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Effect of oxygen and *N*-ethylmaleimide on the inactivation of ribonuclease by γ-radiation

N-ethylmaleimide is a member of the group of thiol reagents (including iodoacetic acid and phenylmercuric acetate) which are able to sensitize bacteria to the lethal action of ionizing radiation when present during irradiation[1–3]. It was suggested[2,3] that it might react with –SH groups (or possibly –S· free radicals) which result from the radiation-induced breakage of –S–S– bonds, necessary for the functional state of some proteins. Combination of *N*-ethylmaleimide with either of the sulphur