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CORRELATION BETWEEN (Na^+–K^+)-ACTIVATED ATPase ACTIVITIES AND THE RATE OF ISOTONIC FLUID TRANSPORT OF GALLBLADDER EPITHELIUM

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SUMMARY

1. Relative and absolute ATPase activities have been determined in rabbit and guinea pig whole gallbladder homogenates.

2. Absolute (Na^+–K^+)-ATPase activities were correlated with the rate of fluid transport in both species. The ratio of equiv Na^+ transported per mole ATP was 1.4 and 2.0 in rabbit and guinea pig, respectively.

3. Variation in fluid transport of individual gallbladders (40–140 μl $H_2O \cdot h^{-1} \cdot cm^{-2}$) could be directly correlated with (Na^+–K^+)-ATPase activity.

4. Stimulation of the (Na^+–K^+)-ATPase system by 10^{-9} M ouabain and 1.5 mM K^+ in rabbit gallbladder also increased the rate of fluid transport.

5. These results indicate that the (Na^+–K^+)-ATPase system plays the primary role in transcellular isotonic fluid transport in gallbladder despite the absence of a secretory potential.

INTRODUCTION

In most epithelial tissues specialized for transcellular transport of NaCl the only actively transported ion is sodium. This ion transport generates an electrical potential gradient which provides the driving force for the movement of Cl^-. The transport of Na^- is linked to the membrane-bound ouabain-sensitive (Na^+–K^+)-ATPase system[1,2] (EC 3.6.1.3) first described by SKOU[3]. Strong evidence exists for a primary function of this enzyme system in transcellular NaCl transport in frog skin[4], toad bladder[5], intestines[6], renal tubules[7], pancreatic electrolyte secretion[8], the formation of aqueous humor[9], and cerebrospinal fluid[10]. Gallbladder epithelium reabsorbs NaCl and water in isotonic proportions[11,12] but it lacks a secretory potential in contrast to other Na^+-transporting tissues[13,14]. Based on this observation a neutral coupled NaCl carrier system for transcellular salt transport has been postulated[13]. In this aspect isotonic fluid reabsorption in gallbladder resembles that of the proximal tubule of rat[15] and rabbit[16] kidney. In the latter tissues no transtubular potential differences can be measured during NaCl reabsorption but despite this, the (Na^+–K^+)-ATPase system in the kidney plays a primary role in Na^+ reabsorption[7,17,18].

For this reason we tried to correlate the rate of fluid transport in gallbladder

with the previously described (Na^+–K^+)-ATPase system[19]. Since DIAMOND[12] described significant lower rates of fluid transport in guinea pig gallbladders than in the rabbit, we used both species.

We also looked for a stimulating effect of low ouabain concentration on the rate of fluid transport since this concentration stimulates (Na^+–K^+)-ATPase activity in rabbit gallbladder homogenates[19].

MATERIALS AND METHODS

Tissue preparation

Albino rabbits of both sexes were anesthetized intravenously with nembutal and exsanguinated by cardiac puncture. Albino guinea pigs were killed by a blow on the head. After opening the abdomen, the neck of the gallbladder was ligated and the bladder carefully removed and suspended in a gassed Ringer solution at 38°. Some bile was withdrawn with a syringe before the bladder was cannulated according to the method of DIAMOND[12]. The rest of the bile was removed by means of a washing procedure described by WHEELER[20]. After removal of bile the bladders were immediately lyophilized at −20° and stored in a dessicator at the same temperature for later enzyme assays.

Water transport measurements

Water fluxes were measured gravimetrically by weighing the bladder every 5 min as described by DIAMOND[12]. The effect of low ouabain and K^+ concentrations on the rate of water transport was measured as follows: The experiment was divided in six 30-min periods. The first period started when the rate of water transport had reached a steady state. After this initial control period the serosal bathing solution was replaced either by normal Ringer (38°, O_2 satd.) for control experiments or by Ringer with 10^{-9} M ouabain and 1.5 mM K^+ (K^+ replaced by Na^+). The bathing solution was also renewed after the third and fifth period. After each experiment the bladder was incised and spread on a moistened filter paper to measure the surface area by planimetry. Gallbladders which had lost more than 30% of their initial activity after 3 h were discarded.

The Ringer solution had the following composition (mM): NaCl, 120; NaH_2CO_3, 25.0; KCl, 7.0; $CaCl_2$, 1.0; $MgCl_2$, 1.0; NaH_2PO_4, 1.2 (pH 7.4 ± 0.1). During the experiment the solution was stirred by a constant stream of oxygen bubbles (95% O_2–5% CO_2 mixtures) saturated with water vapor.

Determinations of extracellular space

The extracellular space was determined with [^{14}C]inulin (Radiochemical Centre, Amersham, England). Bladders were incubated for 1 h in Ringer solution with 0.2% inulin (0.1 μC/ml). After gentle blotting on Ringer-impregnated filter paper the water content was determined and the dry tissue extracted for 48 h in 0.1 M HNO_3. All samples were counted in a Packard liquid β-scintillation counter (Model 314E) using BRAY's[21] scintillation fluid.

ATPase assay

(Na^+–K^+)-stimulated and Mg^{2+}-activated ATPase activities were determined by means of the method previously described[19]. Medium A (complete) gives total

ATPase activity. The average of Media B (no K^+), C (no Na^+), D (10^{-4} M ouabain) and E (no K^+, 10^{-4} M ouabain) gives Mg^{2+}-activated ATPase activity. The difference between both activities gives the (Na^+–K^+)-stimulated ATPase activity. Medium F (1.5 mM K^+, 10^{-9} M ouabain) was used to study the stimulating effect of low K^+ and ouabain concentrations on the (Na^+–K^+)-ATPase system. For enzyme assays the frozen-dried preparations were homogenized in twice distilled water in an all-glass Potter–Elvehjem tissue grinder (about 2.5 mg dry wt./ml). 50 μl of the homogenate were added to 0.75 ml incubation medium. 100-μl samples of these mixtures were incubated for 1 h at 37° and assayed for inorganic phosphate[22]. Enzyme activity is expressed in moles P_i liberated·kg^{-1} dry wt.·h^{-1}.

Calculation of Na^+/ATP ratios

The net Na^+ flux per h was calculated from the water transport rate since the absorbed fluid is isotonic with the luminal content (150 mequiv Na^+ per l). The ratio of Na^+ transported per mole ATP split by (Na^+–K^+)-ATPase was calculated from the formula:

$$\frac{\mu l\ H_2O \cdot 0.150\ \text{equiv}\ Na^+ \cdot h^{-1} \cdot cm^{-2}}{\text{mg dry wt.} \cdot (\text{moles}\ P_i\ \text{liberated} \cdot kg^{-1}\ \text{dry wt.} \cdot h^{-1}) \cdot cm^{-2}}$$

RESULTS

The relative and absolute ATPase activities of rabbit and guinea pig gallbladder homogenates in the various substrate media are shown in Table I. The similarity of these activities in both structures may reflect their identical morphology and physiology.

The inhibition in Medium B (no K^+) was less than in the other media, which agrees

TABLE I

ATPASE ACTIVITIES OF RABBIT AND GUINEA PIG GALLBLADDER HOMOGENATES IN THE VARIOUS SUBSTRATE MEDIA

ATPase activity in Medium A is set at 100 %. Mean values with standard error of the mean (S.E.). Number of determinations in parentheses.

Medium	*ATPase activity* (%)	
	Rabbit	*Guinea pig*
A (complete)	100	100
B (no K^+)	79.1 ± 2.1 (8)	78.0 ± 2.4 (10)
C (no Na^+)	68.4 ± 3.0 (9)	69.1 ± 2.1 (10)
D (10^{-4} M ouabain)	73.9 ± 2.0 (9)	70.2 ± 2.8 (9)
E (no K^+, 10^{-4} M ouabain)	69.0 ± 2.2 (9)	65.5 ± 2.3 (10)
Average Media C, D, E	70.9 ± 2.5	68.5 ± 2.1
	Absolute activity (*moles ATP hydrolyzed·kg^{-1} dry wt.·h^{-1}*)	
Total ATPase	6.39 ± 0.52 (9)	4.88 ± 0.3 (9)
(Na^+–K^+)-ATPase	1.86 ± 0.23 (9)	1.54 ± 0.1 (9)

with the very low K_m value for K^+ activation of the (Na^+–K^+)-ATPase system[19]. The stimulation in this medium is probably due to the small amount of tissue K^+ in the homogenates and for this reason the average inhibition was calculated from the Media C, D and E only. The lower inhibition value of Medium D is due to the antagonizing effect of K^+ on the inhibition of the (Na^+–K^+)-ATPase system by ouabain[23].

TABLE II

CORRELATION BETWEEN (Na^+–K^+)-ATPASE ACTIVITIES AND Na^+ TRANSPORT IN RABBIT AND GUINEA PIG GALLBLADDER WALL

Mean values with S.E. Number of determinations in parentheses.

	$\mu l\ H_2O \cdot h^{-1} \cdot cm^{-2}$	$mg\ dry\ wt. \cdot cm^{-2}$	(Na^+–K^+)-ATPase (moles ATP hydrolyzed · kg^{-1} dry wt. · h^{-1})	Na^+/ATP
Rabbit	89.3 ± 5.5 (30)	5.2 ± 0.4 (12)	1.86 ± 0.23 (9)	1.4 ± 0.3
Guinea pig	77.1 ± 5.6 (9)	3.7 ± 0.5 (10)	1.54 ± 0.09 (9)	2.0 ± 0.3

The quantitative correlation between the (Na^+–K^+)-ATPase activities and the net Na^+ fluxes, calculated from the rate of fluid transport is shown in Table II. The (Na^+–K^+)-ATPase activities in the gallbladder of both species were estimated in whole bladder homogenates including the serosal muscle layer. Therefore, the ratios Na^+ transported per mole ATP are somewhat underestimated. It was possible with rabbit gallbladders to prepare a muscle-free preparation which was impossible with the guinea pig gallbladder. The (Na^+–K^+)-ATPase activity in a muscle-free preparation was 2.3 ± 0.3 moles P_i liberated · kg^{-1} dry wt. · h^{-1} as we reported previously[19], while the (Na^+–K^+)-ATPase activity of the serosal muscle layer was now estimated on 0.4 ± 0.2 mole P_i liberated · kg^{-1} dry wt. · h^{-1}. However it was impossible to estimate the dry weight ratio for the mucosal epithelial and serosal muscle layer, but it is reasonable to assume that this ratio will be about 1:1 since the extracellular space in rabbit gallbladder wall is estimated at 64 ± 4% (n = 11). With this assumption the (Na^+–K^+)-ATPase activity of 2.3 moles P_i liberated · kg^{-1} dry wt. · h^{-1} must be halved giving a Na^+/ATP ratio of 2.0 instead of 1.4 based on the (Na^+–K^+)-ATPase activity of the whole gallbladder wall.

Our values for the rate of fluid transport in rabbit gallbladders, 89 ± 30 (S.D.) $\mu l \cdot h^{-1} \cdot cm^{-2}$ is the same as that found by MACHEN AND DIAMOND[24], *i.e.* 84 ± 24. For guinea pig a value of 77 ± 17 (S.D.) $\mu l \cdot h^{-1} \cdot cm^{-2}$ was found, a value which is not signifiantly lower than those of the rabbit, but higher than the value reported by DIAMOND[12] (25 $\mu l \cdot h^{-1} \cdot cm^{-2}$).

In individual gallbladders, however, the rate of fluid transport varied from 40 to 140 $\mu l \cdot h^{-1} \cdot cm^{-2}$. Therefore, we estimated fluid transport as well as (Na^+–K^+)-ATPase activity in 7 guinea pig and 11 rabbit gallbladders, including 3 rabbit gallbladders treated with 10^{-6} M ouabain, a concentration which slightly inhibits (Na^+–K^+)-ATPase activity. This is shown in Fig. 1. In a range of 1.59–4.55 μequiv $Na^+ \cdot h^{-1} \cdot mg^{-1}$ dry wt. the amount of ATP splitted by the (Na^+–K^+)-ATPase system in 1 h correlates well with the net Na^+ flux per h per mg dry weight in both species of gallbladder.

The K^+ activation curve of (Na^+–K^+)-ATPase of rabbit gallbladder showed a maximum between 1 and 2 mequiv K^+ while ouabain concentrations of 10^{-9} M stimulated the (Na^+–K^+)-ATPase system slightly[19]. Both effects together stimulated the transport enzyme system by 25.8 % (Table III).

For this reason we studied the effect of 1.5 mequiv K^+ and 10^{-9} mole ouabain on the rate of fluid transport. The results are shown in Fig. 2. Stimulation of fluid transport occurred between 30 and 90 min after application. The stimulation in these periods is compared with the stimulatory effect on the (Na^+–K^+)-ATPase activity in Table III.

Ouabain at concentrations higher than 10^{-6} M produced a contraction of the

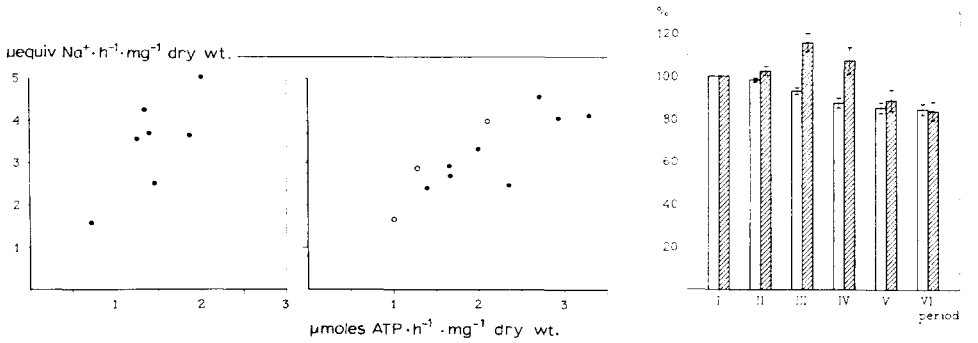

Fig. 1. Correlation between (Na^+–K^+)-ATPase activity and Na^+ transport in individual whole gallbladders of guinea pig (left) and rabbit (right). For three rabbit gallbladders the transport rate is measured after 1-h treatment with 10^{-6} M ouabain and the (Na^+–K^+)-ATPase determined in the presence of the same ouabain concentration (O).

Fig. 2. Effect of low K^+ and low ouabain concentrations on fluid transport in rabbit gallbladder. The open bars represent the control values in normal Ringer solution (n = 10). The shaded bars represent the effect of 1.5 mM K^+ and 10^{-9} M ouabain concentrations (n = 9). Each period is 30 min. The control period is set at 100 %. After this first period the Ringer solution was changed. Mean values with S.E. on top of the bars.

TABLE III

EFFECT OF LOW K^+ AND LOW OUABAIN CONCENTRATIONS ON (Na^+–K^+)-ATPASE ACTIVITY AND FLUID TRANSPORT IN RABBIT GALLBLADDER EPITHELIUM

The (Na^+–K^+)-ATPase activity in Medium A is set at 100 %. The fluid transport in Periods III and IV are expressed as percentages of Period I. Mean values with S.E. Number of determinations in parentheses.

Medium	*(Na^+–K^+)-ATPase* (%)	*Fluid transport*	
		Period III	*Period IV*
A (5 mM K^+, no ouabain)	100	93.1 ± 1.7 (10)	87.4 ± 2.3 (10)
F (1.5 mM K^+, 10^{-9} M ouabain)	125.8 ± 3.5 (11)	115.9 ± 4.2 (9)	107.2 ± 6.1 (9)
	Stimulation (%)		
	25.8 ± 3.5 $P < 0.001$	22.8 ± 4.5 $P < 0.001$	19.8 ± 6.5 $P < 0.01$

smooth muscle fibers which resulted in a decrease in the luminal volume. Gravimetrical measurements became unreliable for this reason and a complete dose-response curve for ouabain could not be established. However, inhibition of fluid transport by high concentration of ouabain has been described by DIETSCHY[25] and MARTIN AND DIAMOND[26]. We also found complete inhibition of fluid transport with 10^{-3} M ouabain within 0.5 h. Similar biphasic effects of ouabain, stimulation by low and inhibition by high concentrations, have been found in toad bladder[5], chicken[17] and dog kidney[18] and in rabbit pancreas[8].

DISCUSSION

The ratio of cation transport to hydrolysis of ATP is constant in a great variety of tissues and calculations of this ratio based on the simultaneously measured (Na^+–K^+)-ATPase activity give values close to 3 (ref. 2). Our calculated values of 2.0 and 1.4 for guinea pig and rabbit gallbladder, respectively, are somewhat lower but still in good agreement with these values. A possible explanation of the relative low values may arise by considering the special type of fluid transport in this tissue. Na^+ is pumped into the intercellular space creating hypertonicity and water follows passively to restore isotonicity along the length of the relatively long intercellular channel[27,28]. During this equilibration some Na^+ may diffuse back into the cell decreasing the net Na^+ transport. For a similar isotonic fluid transport in dog pancreas RIDDERSTAP AND BONTING[8] found a ratio of 1.9. A much higher ratio of 4.0 can be calculated from the O_2 consumption of an *in vitro* transporting rabbit gallbladder measured by MARTIN AND DIAMOND[26]. These authors, however, used for their calculation of Na^+ transported per mole O_2 that part of the O_2 consumption that was inhibited by substituting Cl^- for SO_4^{2-} in the lumen of the bladder. This change in anion inhibits fluid transport but it is unknown whether complete inhibition of the Na^+ pumps in the lateral membranes occurs. For this reason the ratio of 4.0 may represent an overestimation. If we use from their data the amount of O_2 consumption inhibited by ouabain, a Na^+/ATP ratio of 0.8 can be calculated which is closer to our value 1.4 for the whole bladder. Moreover, our measurements show a good correlation between fluid transport and enzyme activity even in individual bladders differing markedly in the rate of fluid transport.

The existence of a neutral coupled NaCl pump, a carrier which transports simultaneously Na^+ and Cl^- as postulated by DIAMOND[11,26] and WHITLOCK AND WHEELER[29] is supported by the following: Simultaneous transport of Na^+ and Cl^- against their electrochemical activity gradients, absence of a short-circuit current or potential difference associated with salt transport, absence of a potential difference expected for an independent Na^+ pump in solutions of nontransported anions and absence of a potential difference expected for an independent Cl^- pump in solutions of nontransported cations. However, the observed biphasic effect of ouabain on fluid transport, the constant ratio for Na^+ transported per mole ATP on the base of (Na^+–K^+)-ATPase activity, the fact that substitution of Cl^- for SO_4^{2-} has no effect on (Na^+–K^+)-ATPase activity[19], and the observation that every monovalent anion can replace Cl^- (ref. 14), but no cation can replace Na^+ (ref. 11) strongly suggest a primary role for the (Na^+–K^+)-ATPase system in fluid transport through the gallbladder wall. This would require an electrogenic coupling of Cl^- with the Na^+ flux

as in other epithelia. Therefore, the Na^+ pump itself has to be electrogenic in nature. The absence of a secretory potential is puzzling but one possible explanation has recently been offered by KEYNES[30]. In a mathematical model for isotonic fluid transport the location of the solute pump at the closed end of the intercellular channel is necessary for isotonicity at the open end[28]. Under these circumstances the pump potential would be attenuated towards the end of the channel in analogy with the electrotonic spread of potential along an axon[30]. This explanation is supported by the greatly increased surface area of the lateral cell membranes by fingerlike invaginations, which make the distance over which the potential can be attenuated many times longer. Even if the Na^+ pumps are located along the whole length of the lateral cell membranes as suggested by the histochemical localization of ATPase in gallbladder[31], the pump potential can be short-circuited by a high Cl^- conductance of these membranes.

An other possible explanation arises from the work of BOULPAEP[32] on single isolated proximal tubules of Necturus kidney. In this tissue there is evidence for low-resistance, extracellular pathways between cells lining the tubule.

The electrical resistance of rat proximal tubule is very low, about 5 $\Omega \cdot cm^2$ (ref. 33), and has been explained as a result of the extracellular shunt pathway[34]. The resistance of rabbit gallbladder epithelium is also unusually low, about 20 $\Omega \cdot cm^2$ (ref. 35; our unpublished observations) and the presence of an extracellular shunt in this tissue could also explain the absence of a secretory potential as in the rat proximal tubule.

Although these phenomena may explain the absence of a secretory potential in gallbladder no definite conclusions can be reached at the moment but the absence of a secretory potential does not prove the existence of a neutral coupled NaCl transport mechanism. Our measurements favor an electrogenic coupling of Cl^- with Na^+, but an internal shunt in the equivalent electric circuit of gallbladder epithelium has yet to be demonstrated.

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REFERENCES

1 J. C. SKOU, *Physiol. Rev.*, 45 (1965) 596.
2 S. L. BONTING, in E. E. BITTAR, *Membrane Metabolism and Ion Transport*, Vol. 1, Wiley–Interscience, London, 1970, p. 286.
3 J. C. SKOU, *Biochim. Biophys. Acta*, 23 (1957) 394.
4 S. L. BONTING AND L. L. CARAVAGGIO, *Arch. Biochem. Biophys.*, 101 (1963) 37.
5 T. K. MCCLANE, *J. Pharmacol. Exptl. Therap.*, 148 (1965) 106.
6 S. G. SCHULTZ AND R. ZALUSKY, *J. Gen. Physiol.*, 47 (1964) 567.
7 A. J. KATZ AND F. H. EPSTEIN, *J. Clin. Invest.*, 46 (1967) 1999.
8 A. S. RIDDERSTAP AND S. L. BONTING, *Am. J. Physiol.*, 217 (1969) 1721.
9 S. L. BONTING AND B. BECKER, *Invest. Ophthal. mol.*, 3 (1964) 523.
10 T. S. VATES, S. L. BONTING AND W. W. OPPELT, *Am. J. Physiol.*, 206 (1964) 1165.
11 J. M. DIAMOND, *J. Physiol. London*, 161 (1962) 442.
12 J. M. DIAMOND, *J. Gen. Physiol.*, 48 (1964) 1.
13 J. M. DIAMOND, *J. Physiol. London*, 161 (1962) 474.
14 R. T. WHITLOCK AND H. O. WHEELER, *Am. J. Physiol.*, 213 (1967) 1199.

15 E. FROMTER AND U. HEGEL, *European J. Physiol.*, 291 (1966) 107.
16 M. B. BURG, in K. THURAU AND H. JAHRMARKER, *Renal Transport and Diuretics*, Springer, Berlin, 1969, p. 109.
17 R. F. PALMER AND B. R. NECHAY, *J. Pharmacol. Exptl. Therap.*, 146 (1964) 92.
18 V. E. NACHMOD AND M. WALSER, *Mol. Pharmacol.*, 2 (1966) 22.
19 C. H. VAN OS AND J. F. G. SLEGERS, *European J. Physiol.*, 319 (1970) 49.
20 H. O. WHEELER, *Am. J. Physiol.*, 205 (1963) 427.
21 G. BRAY, *Anal. Biochem.*, 1 (1960) 279.
22 H. H. TAUSSKY AND E. SHORR, *J. Biol. Chem.*, 202 (1953) 675.
23 S. L. BONTING, N. M. HAWKINS AND M. R. CANADY, *Biochem. Pharmacol.*, 13 (1964) 13.
24 T. E. MACHEN AND J. M. DIAMOND, *J. Membrane Biol.*, 1 (1969) 194.
25 J. M. DIETSCHY, *Gastroenterology*, 47 (1964) 395.
26 D. W. MARTIN AND J. M. DIAMOND, *J. Gen. Physiol.*, 50 (1966) 295.
27 J. M. D. TORMEY AND J. M. DIAMOND, *J. Gen. Physiol.*, 50 (1967) 2031.
28 J. M. DIAMOND AND W. H. BOSSERT, *J. Gen. Physiol.*, 50 (1967) 2061.
29 R. T. WHITLOCK AND H. O. WHEELER, *J. Clin. Invest.*, 43 (1964) 2249.
30 R. D. KEYNES, *Quart. Rev. Biophys.*, 2 (1969) 177.
31 G. J. KAYE, H. O. WHEELER, R. T. WHITLOCK AND N. LANE, *J. Cell. Biol.*, 30 (1966) 237.
32 E. L. BOUPAEP, in F. KRUCK, *Transport und Funktion intracellulärer Elektrolyte*, Urban und Schwarzenberg, Münich, 1967, p. 98.
33 U. HEGEL, E. FROMTER AND T. WICK, *European J. Physiol.*, 294 (1967) 274.
34 G. GIEBISCH, *Nephron*, 6 (1969) 260.
35 E. M. WRIGHT AND J. M. DIAMOND, *Biochim. Biophys. Acta*, 163 (1968) 57.