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EFFECTS OF Ca^{2+} AND Mg^{2+} UPON AMINO ACID TRANSPORT IN RAT RENAL CORTEX SLICES

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SUMMARY

1. The roles of Ca^{2+} and Mg^{2+} in the transport of amino acids were examined in rat kidney cortex slices *in vitro*. The absence of either Ca^{2+} or Mg^{2+} from the incubation fluid was associated with increased inulin space and slightly decreased K^+ content of the slices although no significant alterations of total tissue water nor Na^+ content were noted. Decreased net accumulation of glycine, cycloleucine and α-aminoisobutyric acid were found upon removal of either divalent cation from the incubation fluid with no corresponding effects upon efflux from prelabeled tissues. No effects of divalent cations were noted upon lysine transport.

2. It is concluded that Ca^{2+} and Mg^{2+} have critical roles in the transport of amino acids in rat cortical slices which are related to an active pump mechanism.

INTRODUCTION

The interaction of Ca^{2+} and membranes is widespread in biological systems and is important in the maintenance of several membrane fucntions[1]. The Ca^{2+} binding properties of kidney membranes is dependent upon MgATP[2] which is a substrate for $(Na^+ + K^+)$-ATPase[3]. Furthermore Ca^{2+} is bound to membrane phospholipids[4–7] which are necessary for the maintenance of $(Na^+ + K^+)$-ATPase activity[8–10]. On the other hand, under certain conditions Ca^{2+} may inhibit $(Na^+ + K^+)$-ATPase activity of crude membrane fragments prepared from renal cortex[3].

Previous reports have related external medium concentrations of Ca^{2+} to maintenance of transport of amino acids by liver slices[11] and of monosaccharides by kidney cortex slices[12]. The present series of experiments describe the effects of alteration of the medium content of Ca^{2+} and Mg^{2+} upon the distribution of water, Na^+, K^+ and amino acids in rat renal cortical slices.

MATERIALS AND METHODS

Male albino (Simonson) rats weighing 200–225 g were utilized. They received standard rat chow (Purina) and tap water *ad libitum*.

Radioisotopes utilized were α-amino[1-^{14}C]isobutyric acid, 1-aminocyclopentane [1-^{14}C]carboxylic acid, L-[^{14}C]lysine and [*carboxy*-^{14}C]inulin (New England Nuclear).

Nonradioactive amino acids were obtained from Nutritional Biochemical. Purity of all amino acids and of inulin were assured by paper chromatography. All flasks contained 0.25 μC of amino acid or 0.50 μC of inulin per 2 ml of medium. The final concentration of amino acid per flask was 0.065 mM, unless otherwise specified.

Krebs–Ringer bicarbonate buffer contained (mM): Na^+, 145; K^+, 5; Ca^{2+}, 1.25; Mg^{2+}, 1; Cl^-, 140; PO_4^{3-}, 1.17; and HCO_3^-, 10.

Slices, 0.4-mm thick, were obtained from each pole of decapsulated kidneys with a Stadie–Riggs microtome, and the first slice from each pole was discarded. The subsequent two slices were utilized. Slices were preincubated in Krebs–Ringer bicarbonate buffer at 22° for 10 min. After completion of the incubation period, slices were removed from the flasks, quickly rinsed twice in 0.9% NaCl, lightly blotted and weighed. Incubation flasks were equilibrated with O_2–CO_2 (95:5, by vol.).

Total tissue water was determined by drying slices in a vacuum oven at 105° for 18 h and reweighing, the difference between wet and dry weights representing the water content. Extracellular space was determined by [*carboxy*-^{14}C]inulin distribution as described by ROSENBERG *et al.*[13]. Intracellular fluid equals the difference between total tissue water and extracellular space.

Isotopes were extracted from tissues by boiling 10 min in stoppered test tubes after which the tubes were centrifued 100 × *g* for 10 min, and the supernatant fluids were used for the determination of radioactivity. 200-μl samples of media and of supernatant fluid were counted in a Beckman liquid scintillation spectrometer using 10 ml of 20% Bio-Solv 3 (Beckman Co) in Fluoralloy (Beckman Co). The distribution ratios of amino acids were expressed as the ratio counts/min per ml intracellular fluid to counts/min per ml medium.

Efflux of isotope from prelabeled slices (60 min, 37°) was determined after quickly rinsing in 0.9% NaCl twice, blotting lightly and transferring to fresh buffer equilibrated with O_2–CO_2 (95:5, by vol.), each flask of which was sampled at varying time intervals during reincubation. The data was expressed as per cent of the original iso-

TABLE I

WATER, Na^+ AND K^+ CONTENT OF KIDNEY CORTEX SLICES

Total tissue water, inulin space and Na^+ and K^+ content of kidney cortical slices were determined on fresh slices prior to or after incubation at 37° in Krebs–Ringer bicarbonate buffer (KRB) with or without added Ca^{2+} or Mg^{2+}. Each value represents the mean (± 1 S.D.) of five incubations.

Condition	*Total tissue water (% of wet wt.)*	*Inulin space (% of wet wt.)*	*Na^+ content (mequiv/kg intracellular fluid)*	*K^+ content (mequiv/kg intracellular fluid)*
KRB (60 min)	79.5 (± 0.8)	28.7 (± 2.2)	108 (± 4.8)	92 (± 3.1)
KRB *minus* Ca^{2+} (60 min)	80.3 (± 0.4)**	33.5 (± 2.4)***	115 (± 5.1)**	78 (± 5.6)§
KRB *minus* Mg^{2+} (60 min)	80.2 (± 0.5)**	30.3 (± 1.6) **	110 (± 4.9)**	83 (± 5.6)***
KRB (30 min)	80.7 (± 1.9)	25.3 (± 0.6)	112 (± 2.5)	93 (± 3.6)
Fresh cortical slices (not incubated)	79.3 (± 0.6	*	52 (± 3.8)§	140 (± 6.4)§

* Not determined; calculation of intracellular fluid based upon inulin space of 28.7% of wet wt.

** $P < 0.05$.

*** $P < 0.05$.

§ $P > 0.005$.

tope remaining in the tissue at each time interval and was plotted on semi-logarithmic paper. The slopes were calculated by the method of least squares.

Na^+ and K^+ were extracted from tissues with 1.0 M HNO_3 at room temperature for 48 h and aliquots of the extracts were taken for flame photometry using an internal Li^+ standard.

The significance of all determinations was calculated using Student's t test.

RESULTS

Whereas absence of either Ca^{2+} or Mg^{2+} from Krebs–Ringer bicarbonate buffer led to no significant alterations of total tissue water, the absence of Ca^{2+} caused significant elevation of inulin space (Table I). A slight decrease of K^+ content and increase of Na^+ content of slices incubated in the absence of Ca^{2+} were noted whereas lesser effects were noted in the absence of Mg^{2+}.

The absence of either Ca^{2+} or Mg^{2+} was associated with reduced distribution

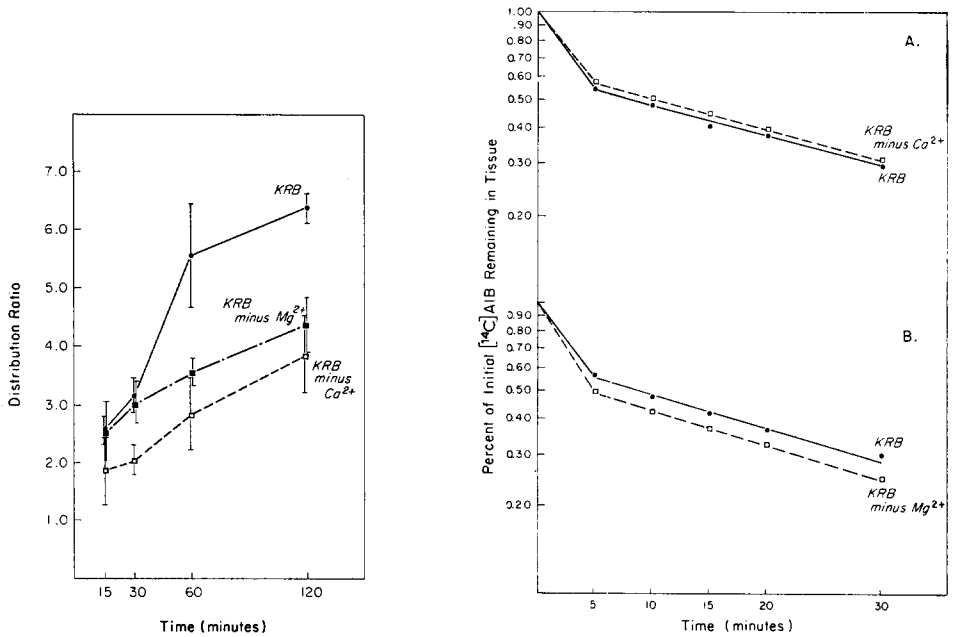

Fig. 1. Effect of removal of Ca^{2+} or Mg^{2+} from Krebs–Ringer bicarbonate buffer (KRB) upon net accumulation of α-aminoisobutyric acid by kidney cortical slices. After preincubation of the slices at 22° for 10 min in buffer containing both Ca^{2+} and Mg^{2+}, the slices are transferred to flasks containing buffer *minus* either Ca^{2+} (□), Mg^{2+} (⊠) or *plus* Ca^{2+} and Mg^{2+} (●) and incubated at 37° in the presence of α-aminoisobutyric acid (0.065 mM). The results are expressed as the distribution ratio of counts/min in the intracellular fluid as compared with the media and represent the mean ± 1 S.D of at least five flasks for each condition at each time period.

Fig. 2. Efflux of α-aminoisobutyric acid (AIB) from kidney cortex slices in Krebs–Ringer bicarbonate buffer (KRB) containing Ca^{2+} and Mg^{2+} (●), *minus* Ca^{2+}, A (□) or *minus* Mg^{2+}, B (□). Slices were preincubated in KRB with Ca^{2+} *plus* Mg^{2+} and containing α-aminoisobutyric acid (0.065 mM) for 60 min at 37°, rinsed twice in 0.9% NaCl, lightly blotted and transferred to flasks containing KRB with or without Ca^{2+} or Mg^{2+} and incubated at 37°. Aliquots of media were removed from each flask at varying intervals. Data was expressed as percent of initial α-aminoisobutyric acid remaining in tissue at each interval. Each point represents the mean of at least three determinations.

ratios of α-aminoisobutyric acid, cycloleucine and of glycine (Table II). The effect was seen as early as 15 min after the slices were placed in the cation-deficient media (Fig. 1.) However, the accumulation of lysine, either under ordinary conditions where the isotope was added to incubation fluid initially at low concentrations or when added under conditions appropriate for the stimulation of exchange diffusion, *i.e.* after preloading with a high concentration of unlabelled lysine, was not impaired by elimination of Ca^{2+} and Mg^{2+} from the incubation fluid (Table III).

TABLE II

EFFECT OF ABSENCE OF Ca^{2+} OR Mg^{2+} ON ACCUMULATION OF α-AMINOISOBUTYRIC ACID, AMINOCYCLOPENTANE 1-CARBOXYLIC ACID AND GLYCINE BY KIDNEY CORTEX SLICES

Concentration of amino acids was 0.065 mM, 0.25 μC of [^{14}C]amino acid in Krebs–Ringer bicarbonate buffer with or without Ca^{2+} or Mg^{2+} (pH 7.4). Slices were incubated at 37° for 60 min. Each value represents the mean (± 1 S.D.) of six incubations.

Condition	*Distribution ratio*	*Significance of difference from control (P)*
α-Amino[1-^{14}C]isobutyric acid		
Control	4.10 ± 0.48	
Minus Ca^{2+}	2.86 ± 0.25	< 0.01
Minus Mg^{2+}	2.65 ± 0.18	< 0.01
1-Aminocyclopentane [1-^{14}C]carboxylic acid		
Control	3.32 ± 0.48	
Minus Ca^{2+}	2.14 ± 0.26	< 0.01
Minus Mg^{2+}	2.20 ± 0.29	< 0.01
[2-^{14}C]Glycine		
Control	5.65 ± 0.59	
Minus Ca^{2+}	3.56 ± 0.66	< 0.01
Minus Mg^{2+}	3.58 ± 0.57	< 0.01

TABLE III

EFFECT OF ABSENCE OF Ca^{2+} AND Mg^{2+} ON EXCHANGE DIFFUSION OF AMINO ACID IN KIDNEY CORTICAL SLICES

Non-radioactive lysine was present at 30 mM. [^{14}C]Lysine (0.2 μC per flask) was added at 0.065 mM Flasks were preincubated at room temperature, 10 min, followed by incubation at 37° in Krebs–Ringer bicarbonate buffer (KRB) with or without Ca^{2+} and Mg^{2+}. In some instances, 30 mM lysine was added for a 60-min preincubation at 37° followed by incubation with [^{14}C]lysine, 0.065 mM, for 10 min. Each value represents the mean (± 1 S.D.) of five incubations.

Condition	*Distribution ratio*	*Significance of difference from control (P)*
KRB *plus* [^{14}C]lysine (30 min)	3.69 (± 0.28)	
KRB *plus* [^{14}C]lysine, *minus* Ca^{2+}, *minus* Mg^{2+} (30 min)	3.77 (± 0.29)	> 0.05
KRB (60 min) followed by KRB *plus* [^{14}C]lysine (10 min)	4.05 (± 0.51)	
KRB *plus* lysine (30 mM, 60 min) followed by KRB *plus* [^{14}C]lysine (10 min)	8.34 (± 1.14)	
KRB *plus* lysine (30 mM, 60 min) followed by KRB *minus* Ca^{2+}, *minus* Mg^{2+} + [^{14}C]lysine (10 min)	8.53 (± 1.90)	> 0.05

The efflux of α-aminoisobutyric acid from slices which were preloaded for 60 min with isotope and subsequently incubated in modified Krebs–Ringer bicarbonate buffer was not effected by elimination of either Ca^{2+} ($P > 0.2$) or Mg^{2+} ($P > 0.2$) from incubation fluid (Fig. 2).

The effect of absence of Ca^{2+} or Mg^{2+} on net accumulation of α-aminoisobutyric acid was seen under conditions of either high or low concentrations of α-aminoisobutyric acid in the incubation fluid (Fig. 3). In all instances, the effects of absence of Ca^{2+} or Mg^{2+} was to reduce v_{max} whereas K_m was not altered.

Whereas the effect of ouabain upon net accumulation of amino acid was greater than that of absence of either Ca^{2+} or Mg^{2+} alone, the combination of ouabain as wel

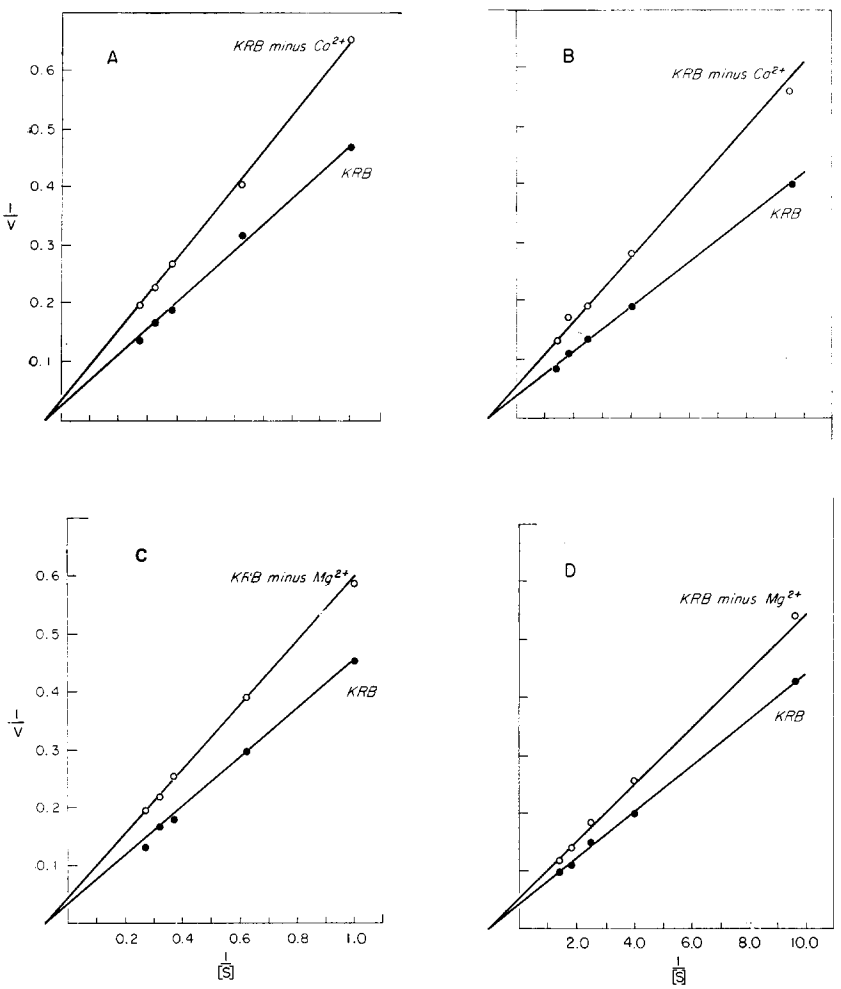

Fig. 3. Lineweaver–Burk plot of reciprocal of velocity of uptake of α-aminoisobutyric acid by kidney cortex slices *vs.* reciprocal of substrate concentration of α-aminoisobutyric acid. Slices were incubated in varying concentrations of α-aminoisobutyric acid in Krebs–Ringer bicarbonate buffer (KRB) with Ca^{2+} and Mg^{2+} (●), *minus* Ca^{2+}, A and B (○) or *minus* Mg^{2+}, C and D (○). [*S*] in mM, *v* in mmoles/l per 30 min, 37°.

as deletion of either medium divalent cation resulted in an augmented reduction of α-aminoisobutyric acid accumulation in each instance (Table IV).

TABLE IV

EFFECTS OF OUABAIN AND ABSENCE OF Ca^{2+} OR Mg^{2+} ON ACCUMULATION OF α-AMINOISOBUTYRIC ACID BY KIDNEY CORTEX SLICES

Ouabain ($8 \cdot 10^{-4}$ M) was added to Krebs–Ringer bicarbonate buffer (KRB) with or without added Ca^{2+} or Mg^{2+}. Incubation conditions were as described in Table II. Distribution ratios are expressed as mean (± 1 S.D.) of at least five incubations.

Condition	*Distribution ratio*	*Significance of difference from control (P)*
KRB	4.34 (± 0.70)	
KRB *plus* ouabain	2.09 (± 0.06)	< 0.005
KRB *minus* Ca^{2+}	3.01 (± 0.26)	< 0.01
KRB *plus* ouabain, *minus* Ca^{2+}	1.54 (± 0.10)	< 0.0005
KRB *minus* Mg^{2+}	3.07 (± 0.46)	< 0.05
KRB *plus* ouabain, *minus* Mg^{2+}	2.02 (± 0.15)	< 0.0005

DISCUSSION

It has been previously demonstrated that Ca^{2+} has profound effects on membrane integrity and permeability although chemical specificity of Ca^{2+} binding to membranes seems to vary with the experimental model[4–7,14,15]. The results obtained here suggest that incubation of rat renal cortical slices in Ca^{2+}-free media is associated with an alteration in the distribution of water as well as a significant decrease of K^+ concentration in intracellular fluid although Na^+ concentration was not altered. Acceleration of the leak of solutes from kidney cells by treatment with EDTA in addition to the absence of Ca^{2+} from the medium has been demonstrated by the work of KLEINZELLER *et al.*[16]. Under those circumstances, steady-state water content of the tissue was not increased. The Na^+ pump was not affected by the lack of Ca^{2+} although electrochemical potential decreased[16]. It is noteworthy that LASSITER *et al.*[17] have reported that Ca^{2+} did not affect the osmotic permeability of the proximal tubule to water, while the permeability of the distal tubule to water was decreased by Ca^{2+}.

The transport of some amino acids is dependent upon the presence of Ca^{2+} in the incubation fluid. Whereas net accumulation is decreased in the absence of medium Ca^{2+}, efflux of α-aminoisobutyric acid from prelabeled slices is not altered thus suggesting an effect upon a pump mechanism rather than acceleration of leak. The relationships of these observations to alterations in cation content as well as to energy metabolism are uncertain. The stimulating effect of Ca^{2+} on O_2 consumption has been found in the renal medullar[18] and in the small intestine[19]. Ca^{2+} also stimulates gluconeogenesis in isolated rat renal tubules[20]. Studies of active sugar transport in kidney cortex by KLEINZELLER *et al.*[12] demonstrated similar effects of Ca^{2+} on both Na^+-dependent and Na^+-independent systems leading to his postulation of an effect of Ca^{2+} on the source of metabolic energy for transport. Other Ca^{2+}-dependent transport systems include stimulation of *p*-aminohippuric acid accumulation in kidney tubules[21] and transport of α-aminoisobutyric acid by rat liver slices[11]. On the other hand, lack of

medium Ca^{2+} or Mg^{2+} had no effects on glucose metabolism or transport by fat cells[22].

The additive effects upon α-aminoisobutyric acid transport of the absence of either medium Ca^{2+} or Mg^{2+} in the presence of ouabain suggests a more complicated relationship that that which directly invokes the ouabain-inhibitable system. The significance of the lack of an effect upon lysine transport by the reduction of Ca^{2+} and Mg^{2+} content of media is not clear. However, previous work by SEGAL *et al.*[23] and SCHWARTZMAN *et al.*[24] suggests that transport characteristics of dibasic amino acids differ from those of other amino acids.

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REFERENCES

1 J. F. MANERY, *Federation Proc.*, 25 (1966) 1804.
2 R. F. PALMER AND V. A. POSEY, *J. Gen. Physiol.*, 55 (1970) 89.
3 F. H. EPSTEIN AND R. WHITTAM *Biochem. J.* 99 (1966) 232.
4 E. ROJAS AND J. M. TOBIAS *Biochim. Biophys. Acta* 94 (1965) 394.
5 D. W. DEAMER AND D. G. CORNWELL *Biochim. Biophys. Acta*, 116 (1966) 555.
6 D. PAPAHADJOPOULOS, *Biochim. Biophys. Acta*, 163 (1968) 240.
7 H. HAUSER, D. CHAPMEN AND R. M. C. DAWSON, *Biochim. Biophys. Acta*, 183 (1969) 320.
8 B. ROELOFSEN, H. BAADENHUYSEN AND L. L. M. VAN DEENEN, *Nature*, 212 (1966) 1379.
9 L. J. FENSTER AND J. H. COPENHAVER, *Biochim. Biophys. Acta*, 137 (1967) 406.
10 A. MALILA AND E. J. MASORO, *Lipids*, 5 (1970) 658.
11 J. K. TEWS AND A. E. HARPER, *Biochim. Biophys. Acta*, 183 (1969) 601.
12 A. KLEINZELLER, D. A. AUSIELLO, J. A. ALEINENDARES AND A. H. DAVIS, *Biochim. Biophys. Acta*, 211 (1970) 293.
13 L. ROSENBERG, S. DOWNING AND S. SEGAL, *Am. J. Physiol.*, 202 (1962) 800.
14 L. WEISS AND E. MAYHEW, *J. Cell Physiol.*, 69 (1967) 127.
15 K. Y. CHANG AND C. W. CARR, *Biochim. Biophys. Acta*, 157 (1968) 127.
16 A. KLEINZELLER, A. KNOTKOVÁ AND J. NEDVIKOVÁ, *J. Gen. Physiol.*, 51 (1968) 326.
17 W. E. LASSITER, A. FRICK, G. RUMRICH AND K. G. ULLRICH, *Arch. Ges. Physiol.*, 285 (1965) 90.
18 S. W. JAIRALA, L. GOLDMAN, A. VIERYRA, A. P. GARCIA AND M. L. ROSIA, *Biochim. Biophys. Acta*, 183 (1969) 137.
19 J. D. BENTLEY, *J. Endocrinol.*, 21 (1960) 161.
20 N. NAGATA AND H. RASMUSSEN, *Biochim. Biophys. Acta*, 215 (1970) 1.
21 J. V. TAGGART, L. SILVERMAN AND E. M. TRAYNER, *Am. J. Physiol.*, 173 (1953) 345.
22 J. LETARTE AND A. E. RENOLD, *Biochim. Biophys. Acta*, 183 (1969) 350.
23 S. SEGAL, L. SCHWARTZMAN, A. BLAIR AND D. BERTOLI, *Biochim. Biophys. Acta*, 135 (1967) 127.
24 L. SCHWARTZMAN, A. BLAIR AND S. SEGAL, *Biochim. Biophys. Acta*, 135 (1967) 136.