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METABOLIC AND ELECTROLYTE CHANGES PRODUCED BY LITHIUM IONS IN THE ISOLATED RAT DIAPHRAGM*

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Abstract—The effects of lithium ions on glucose metabolism and the tissue content of monovalent cations were studied in rat hemidiaphragms incubated *in vitro*. The entrance of lithium ions into the cell was associated with an increase in the rate of glucose utilization and glycogen synthesis. As lithium was taken up by the tissue, there was a concomitant loss of potassium. The loss of potassium could not account for the stimulation of glucose metabolism produced by lithium and it was concluded that lithium ions *per se* increased glucose uptake and particularly glycogen synthesis. Preincubation of hemidiaphragms in media containing different concentrations of lithium ions resulted in an increase in the rate of glycogen synthesis during a subsequent incubation in the absence of lithium. The stimulation of glycogen formation was directly related to the tissue content of lithium. Lithium ions did not significantly alter the cyclic AMP content of the tissues under conditions at which the rate of glucose metabolism was markedly enhanced.

As a consequence of the effectiveness of lithium salts in the treatment of affective disorders [1–4] considerable interest has developed in the pharmacological actions of this cation. Although lithium has been known to have effects similar to those of sodium in several biological systems, its charge-to-radius ratio tends to ally it to the larger alkaline earth metals, particularly magnesium. It is not surprising then that lithium ions have been shown to affect several enzyme systems that require calcium or magnesium ions for activity. For example, Li^+ has been demonstrated to inhibit hormone-induced activation of adenylate cyclase in several tissues without lowering basal activity of the enzyme [5–9]. Such an action of lithium ions may be the result of a competition with magnesium ions, as Wang *et al.* [9] showed that the inhibitory effect of Li^+ on prostaglandin E_1-stimulated adenylate cyclase activity can be overcome by raising the magnesium concentration in the reaction medium.

The activity of the enzyme system involved in sodium transport, i.e. the Na,K-stimulated ATPase, has been shown to be affected by Li^+. While this ion substituted poorly, if at all, for the sodium ion in promoting phosphorylation of the enzyme [10, 11], it is capable of stimulating the dephosphorylation step of the reaction in a manner similar to that of potassium ions. However, it is less effective than K^+ in this regard [11, 12].

Studies with intact animals [13] and isolated muscle preparations [14–16] have shown that lithium ions have marked effects on carbohydrate metabolism. Recent studies from this laboratory have shown that, in the isolated rat diaphragm, lithium increases glucose utilization and glycogen synthesis in a manner that is additive to that of insulin [17]. The present study is concerned with the mechanism of action of lithium in stimulating carbohydrate metabolism, with particular emphasis on the correlation between changes in tissue electrolytes and alterations in glucose utilization and glycogen synthesis.

METHODS

Male Wistar strain rats weighing between 125 and 150 g were used. The animals were fasted overnight and killed by decapitation. Hemidiaphragms were blotted lightly on filter paper and weighed on a torsion balance.

Incubation. In experiments reported in Tables 1–3, tissues were incubated at 37° in Erlenmeyer flasks with 2 ml of solutions containing 0·040 M HEPES (*N*-2-hydroxy-ethyl piperazine-*N*-2-ethane sulfonic acid) adjusted to pH 7·2 with NaOH, 0·005 M $MgCl_2$ and KCl, NaCl or LiCl as indicated to give a total concentration of 0·110 M. Glucose or U-^{14}C-glucose (*ca* 40,000 c.p.m.) was present at 0·006 M.

In another set of experiments, hemidiaphragms were preincubated for 30 min at 37° in the presence of 0, 2·5, 7·5 and 25 mM LiCl. The media contained 0·040 M HEPES, 0·005 M $MgCl_2$, 0·010 M KCl and NaCl to give a total concentration of monovalent cation chlorides of 0·113 M. The tissues were rinsed briefly in saline and incubated for 5 min in a medium containing HEPES, $MgCl_2$ and KCl as above, 0·107 M NaCl and 12 mM U-^{14}C-glucose (*ca* 80,000 c.p.m.). After three rinsings in saline (5 sec each), the tissues were digested in 30% KOH for determination of radioactive glycogen, as described below. In parallel experiments, the tissue lithium content was determined at the end of the 5-min incubation. The conditions were identical to those above, except that the medium for the 5-min incubation contained nonradioactive glucose and the final rinsings were in 0·28 M sucrose.

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Glucose utilization and glycogen synthesis. The initial and final glucose concentrations in the incubation media were determined by the glucose oxidase method after deproteinization with $ZnSO_4$ and $Ba(OH)_2$. Incorporation of ^{14}C from U-^{14}C-glucose was determined by the method of Thomas *et al.* [18] as described previously [17].

Cyclic AMP analysis. The diaphragms were frozen by immersion in liquid nitrogen. Adenosine 3',5'-monophosphate (cyclic AMP) in diaphragms was extracted by powdering the frozen tissue in a mortar cooled in liquid nitrogen containing 3 ml trichloroacetic acid per 100 mg wet weight of tissue. The frozen tissue TCA powder was transferred into a centrifuge tube, liquified by immersion in a boiling water bath for 10 sec, and then sonicated twice for 15 sec. The mixture was centrifuged at 4° and an aliquot of the clear supernatant solution was removed and transferred into a 40-ml glass-stoppered centrifuge tube. If lithium was to be analyzed, suitable dilutions were made from this solution. The TCA was extracted with ether and the ether removed by bubbling nitrogen through the sample. An aliquot was analyzed for cyclic AMP by the protein-binding method of Gilman [19].

Electrolyte determinations. To measure total tissue content of sodium, potassium and lithium, the following procedure was adopted: the diaphragm, after removal from the incubation vial, was rinsed three times with 0·28 M sucrose, blotted on filter paper, and homogenized in 20 vol. of 0·625 N perchloric acid. The homogenate was centrifuged for 15 min at 4° and an aliquot of the supernatant fluid was removed for electrolyte determinations. The cation concentrations in suitably diluted samples were measured by atomic absorption spectrophotometry. Concentrations of sodium, potassium and lithium in the incubation media were also determined.

RESULTS

Previous studies of the action of lithium ions on carbohydrate metabolism in isolated diaphragm have suggested that it is necessary for lithium to be taken up by the tissue in order to exert its metabolic effect [16]. It is, therefore, of interest to correlate metabolic measurements with determinations of lithium content in diaphragm incubated *in vitro*. As lithium has been shown to exchange with sodium and potassium in tissue [20], it is also of importance to measure alterations in concentrations of these ions under our experimental conditions. In parallel experiments, we have studied the effect of lithium on glucose metabolism and electrolyte changes under different experimental conditions.

Effect of lithium on glucose metabolism and tissue electrolytes. Hemidiaphragms were incubated for 30 or 60 min in the presence and absence of 10 mM KCl and glucose uptake and incorporation of glucose into glycogen determined (Table 1). Statistical evaluations of these data are reported in Table 2.

In the control medium in which the only added monovalent ion was sodium, there was a high rate of glucose utilization and glycogen synthesis. Under these conditions, the activity of the sodium pump would be expected to be low. The inclusion of 10 mM potassium in the medium, to stimulate the sodium pump, had only a small effect on glucose metabolism, although there was a tendency for potassium ions to decrease glucose uptake and glycogen synthesis. This action of potassium was statistically significant in the case of glycogen synthesis after 60 min of incubation (Table 2A).

Table 1. Effects of lithium and potassium ions on glucose uptake and glycogen synthesis by rat diaphragm incubated *in vitro**

Incubation Medium	Incubation (Min)	N	Glucose uptake (μmoles g wet wt)	Glycogen synthesis (μmoles C^{14}-glucose incorporated into glycogen g wet wt)
125 mM Na	30	10	5·79 ± 0·55	3·08 ± 0·13
125 mM Na	60	11	11·05 ± 0·49	5·68 ± 0·27
115 mM Na + 10 mM K	30	10	5·76 ± 0·53	2·78 ± 0·18
115 mM Na + 10 mM K	60	11	9·84 ± 0·66	4·13 ± 0·32
100 mM Na + 25 mM Li	30	11	7·26 ± 0·81	4·79 ± 0·31
100 mM Na + 25 mM Li	60	10	16·79 ± 0·74	10·53 ± 0·61
90 mM Na + 25 mM Li + 10 mM K	30	11	6·23 ± 0·33	4·41 ± 0·14
90 mM Na + 25 mM Li + 10 mM K	60	10	13·30 ± 0·69	8·49 ± 0·53

* Diaphragms were incubated at 37° in media of the composition indicated in Methods; gas phase, 100% oxygen.

Lithium at 25 mM clearly stimulated glucose uptake after 60 min of incubation. Significant increases in the incorporation of glucose into glycogen in the presence of lithium were observed after both 30 and 60 min of incubation. The stimulation of glucose uptake by lithium ions tended to be lower when potassium ions were present in the medium, although this effect of potassium was not significant. The effect of lithium on glycogen synthesis was not affected by 10 mM KCl (Table 2B).

Changes in tissue concentrations of monovalent cations during incubation under the same experimental conditions used for the experiments in Table 1 are recorded in Table 3.

In an all-sodium medium, where the sodium pump is operating at a low rate, the tissues lost potassium and gained sodium ions. However, when 10 mM KCl

Table 2. Significance of the effects of potassium and lithium on glucose uptake and glycogen synthesis

Incubation time (min)		Glucose uptake Δ ± S.E.M.	P	Glycogen synthesis Δ ± S.E.M.	P
A Effect of potassium (10 mM)					
30	Na, no K vs Na + K	−0·03 ± 0·76	NS	−0·30 ± 0·23	NS
60	Na, no K vs Na + K	−1·21 ± 0·81	NS	−1·55 ± 0·65	<0·05
30	Li no K vs Li + K	−1·03 ± 0·86	NS	−0·38 ± 0·30	NS
60	Li, no K vs Li + K	−3·49 ± 1·01	<0·005	−2·04 ± 0·81	<0·025
B Effect of lithium (25 mM)					
30	Na, no K vs Li, no K	+1·47 ± 1·00	NS	+1·71 ± 0·35	<0·001
60	Na, no K vs Li, no K	+5·74 ± 0·87	<0·001	+4·85 ± 0·64	<0·001
30	Na + K vs Li + K	+0·47 ± 0·61	NS	+1·63 ± 0·23	<0·001
60	Na + K vs Li + K	+3·46 ± 0·95	<0·005	+4·36 ± 0·60	<0·001

Table 3. Changes in tissue concentrations of monovalent cations during incubation of rat diaphragm *in vitro* in the presence and absence of lithium*

Incubation			Changes in monovalent cations† (μmoles/g wet wt)				Total monovalent cations	Glucose uptake‡	Glucogen synthesis‡
Medium	(min)	N	ΔNa	ΔK	ΔLi	Δ(Na + Li)	(μmoles/g wet wt)	(% of control)	(% of control)
125 mM Na	30	3	+32 ± 7·5	−26 ± 5·2			144 ± 4·8	100	100
125 mM Na	60	4	+47 ± 3·2	−44 ± 2·1			144 ± 3·4	100	100
115 mM Na + 10 mM K	30	8	+21 ± 1·8	−23 ± 1·8			139 ± 2·2	100	90
115 mM Na + 10 mM K	60	5	+22 ± 4·2	−28 ± 2·6			136 ± 3·2	89	73
100 mM Na + 25 mM Li	30	5	+13 ± 1·9	−38 ± 2·6	+26 ± 0·5	+39 ± 2·0	140 ± 3·2	125	156
100 mM Na + 25 mM Li	60	9	+21 ± 2·4	−55 ± 3·2	+34 ± 1·0	+55 ± 2·6	139 ± 2·3	152	185
90 mM Na + 10 mM K + 25 mM Li	30	11	+3 ± 1·4	−22 ± 1·7	+20 ± 0·9	+23 ± 1·7	138 ± 2·6	108	143
90 mM Na + 10 mM K + 25 mM Li	60	7	+5 ± 2·3	−28 ± 2·9	+24 ± 0·6	+29 ± 2·4	135 ± 4·1	120	150

* Conditions of incubations were identical to those of experiments reported in Table 1.

† For calculating the changes in tissue Na and tissue K, the following initial values were measured: Na, 43 ± 0·7 μmoles/g (N = 8); K, 91 ± 2·6 μmoles/g (N = 8).

‡ Values were calculated from results presented in Table 1.

was present in the medium, the net exchange of tissue potassium for external sodium was much less extensive, especially at 60 min, reflecting a stimulation of the sodium pump by the extracellular potassium. When 25 mM LiCl was substituted for the equivalent amount of NaCl, there was an accumulation of Li in the tissue; in this instance, the rise in sodium was smaller than that observed in the absence of lithium ions in the medium. The loss of tissue potassium was identical, within experimental error, to the increase in sodium plus lithium ions. In a medium containing both Li^+ and K^+, the increase in tissue sodium was much smaller than in the absence of extracellular potassium. The tissues accumulated lithium and lost potassium ions. As seen in the absence of added KCl, the tissue gain in sodium plus lithium was identical to the loss of potassium. In the presence of 10 mM KCl in the incubation medium, the accumulation of lithium ions was significantly less, at both 30 and 60 min ($P < 0.001$), than the increase in lithium measured in the absence of external potassium.

In the last column of Table 3, the relative rates of glucose uptake and glycogen synthesis (obtained from Table 1) are recorded. The correlation between the uptake of lithium ions by the diaphragm and the stimulation of glucose utilization and glycogen synthesis is immediately apparent. The entrance of lithium into the tissue is associated with a loss of potassium, but the changes in tissue potassium do not appear to be related to the metabolic effects of lithium. For example, in the sodium medium containing 10 mM KCl, the loss of potassium is exactly the same after 60 min as in the medium containing both 10 mM KCl and 25 mM LiCl, but the rates of glucose utilization and glycogen synthesis are much higher in the latter case.

Correlation between tissue content of lithium and the rate of glycogen synthesis. Preliminary experiments were carried out to determine the rate of uptake of lithium ions by the diaphragm at 37°. The results showed that there was a very rapid uptake of lithium during the first 5 min undoubtedly due to filling up of the interstitial space. This was followed by a slower rate of net uptake over the next hr. At 30 min, the amount of lithium that had entered the tissue was more than 80 per cent of the value at 60 min (Table 3). In several experiments, the rate of uptake of lithium by diaphragms incubated in Krebs–Ringer bicarbonate medium was measured; the results obtained were essentially the same as in diaphragms incubated in HEPES buffer containing potassium.

For the subsequent experiments, a preincubation of 30 min was selected. At the end of this period, the hemidiaphragms were rinsed and transferred to a medium containing 12 mM U-^{14}C-glucose but no LiCl. Incorporation of isotope into glycogen was determined after a 5-min incubation and, in parallel experiments, tissue lithium content was measured. The experimental details are described in Methods and the results of the experiment are presented in Fig. 1.

There is a steady increase in the rate of glycogen synthesis with the rise in the level of tissue lithium. It is important to note that a relatively small amount of lithium caused a large increase in the rate of glycogen formation. At 1 μmole/g of tissue lithium, the rate of glycogen synthesis is increased about 65 per cent. Significant metabolic effects of lithium can probably be demonstrated at even lower tissue concentrations of this ion.

In the experiments shown in Fig. 1, we determined the concentration of lithium in the medium at the end of the 5-min incubation. This represents the ion that has been lost by the tissue. In diaphragms preincubated for 30 min with 25 mM LiCl, the lithium concentrations in the medium (2 ml) varied between 0·4 and 0·5 mM. Experiments were then done to determine whether a concentration of 0·5 mM LiCl in the medium would influence the rate of glycogen synthesis during a 5-min incubation as in Fig. 1; this was found not to be the case. In six paired experiments, the rate in the absence of added LiCl was 0·29 ± 0·03 μmoles/g/5 min; with 0·5 mM LiCl, the rate was 0·29 ± 0·02.

Possible role of cyclic AMP in the metabolic effects

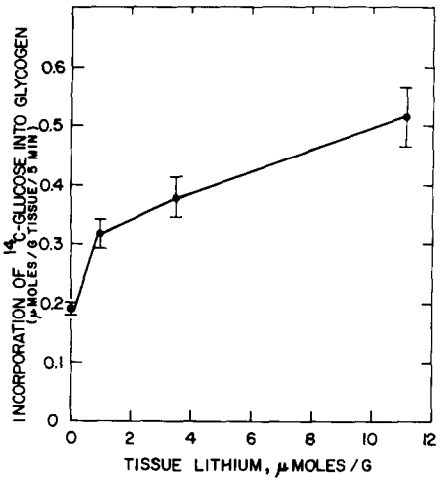

Fig. 1. Relation of rate of glycogen synthesis to lithium present in tissue. Diaphragms were preincubated at 37° for 30 min in media containing different concentrations of LiCl (gas phase, 100% oxygen). The tissues were then incubated for 5 min at 37° in the presence of U-^{14}C-glucose in an Li-free medium and the incorporation of radioactive glucose into glycogen was determined. The tissue content of Li was measured in parallel experiments at the end of the 5-min incubation. The medium concentrations and final tissue contents of Li (in parentheses) were as follows: 2·5 mM (0·97 ± 0·07 μmole/g), 7·5 mM (3·79 ± 0·30 μmole/g), 25 mM (11·09 ± 0·28 μmole/g) (N = 5–8). For details of experiment, see Methods.

of lithium ions. Lithium ions have been reported to increase the ratio of the I-form to the D-form of glycogen synthase in diaphragm [17, 21]. Since the I-form of the enzyme is converted to the D-form by a protein kinase dependent for activity on cyclic AMP [22], it was of interest to determine whether the stimulation of glycogen synthesis by lithium ions was related to a decrease in the concentration of cyclic AMP in the cell. We, therefore, measured the tissue content of this nucleotide in diaphragms which had been treated exactly like those used for the experiments reported in Fig. 1. This consisted of preincubation for 30 min in the presence and absence of 25 mM LiCl followed by a 5-min incubation in a medium not containing LiCl. In ten paired experiments, the tissue cyclic AMP content was 0·67 ± 0·10 nmole/g in tissues not exposed to lithium and 0·57 ± 0·06 nmole/g in tissues preincubated in the medium containing 25 mM LiCl. The lithium ion had no significant effect on the cyclic AMP content of the diaphragm under conditions at which glycogen synthesis was markedly stimulated.

DISCUSSION

The most significant finding in this report is that lithium ions exert their effects on glucose metabolism in muscle after being taken up by the cell. In the experiments in which diaphragms were incubated with 25 mM LiCl (Table 3), the total tissue lithium content reached values that indicated that a large increase in the intracellular concentration of lithium must have occurred. Using values for total H_2O of 0·80 ml/g and extracellular H_2O of cut diaphragm of 0·21 ml/g [23] we have made the following calculation. In diaphragms incubated in a medium containing 25 mM LiCl, the lithium in the extracellular volume would be 5·3 μmoles/g. It is clear that in diaphragms incubated for 30 min or longer the total tissue lithium far exceeds this value, indicating that the lithium ion is present intracellularly. In fact, it can be calculated that under all conditions of the experiments reported in Table 3, the concentration of lithium in the intracellular water exceeds that in the extracellular phase. In the absence of added external potassium, the lithium concentration in the cellular water after 60 min of incubation reaches a calculated value of 49 mM, which is more than double the concentration in the medium. The accumulation of lithium ions observed here is in agreement with results with other tissues, demonstrating that lithium ions can be transported into the cell [24]. It is generally accepted that this process involves interaction of Li^+ with the potassium site of the Na,K-stimulated membranal ATPase [24, 25].

As lithium enters the cell, the net exchange is predominantly with potassium. It is also apparent that lithium ions do not inhibit the sodium pump in the incubated diaphragms. The loss of cellular potassium when the pump was operating, due to the presence of external potassium ions, was the same in the absence and presence of LiCl in the medium (Table 3). Furthermore, there was only a very small gain in tissue sodium when both K^+ and Li^+ were present extracellularly. These findings are consistent with the observations of Tobin *et al.* [11] that the lithium ion does not inhibit and may actually increase the activity of a transport ATPase preparation from brain in the presence of both sodium and potassium ions. Baker [26] also found that lithium ions did not inhibit inorganic phosphate production in spider crab nerve when both sodium and lithium ions were present in equimolar concentrations.

In the experiments reported in Tables 1 and 3, LiCl was substituted for NaCl so that when LiCl was added, the sodium concentration in the incubation medium was decreased. However, changes in external sodium ions of the magnitude used here do not significantly affect glucose metabolism in the diaphragm. In previous studies [16] we measured the rate of glucose uptake in diaphragms incubated in media containing different ratios of sodium to potassium in the incubation medium. There were no significant changes in glucose uptake in these experiments, except that glucose uptake was higher in a medium devoid of potassium.

The mechanism by which intracellular lithium ions stimulate glucose metabolism is unknown. It appears to be the presence of the ion itself in the cell that is responsible for the metabolic alterations observed. Neither the loss of intracellular potassium nor the changes in tissue sodium can account for the enhancement of glucose utilization and glycogen synthesis caused by lithium. In the experiments in which hemidiaphragms were incubated in the presence of 10 mM KCl, there was the same loss of tissue potassium in the presence as in the absence of lithium ions, both after 30 and 60 min of incubation. Yet, glucose metabolism was stimulated only in the tissues incubated with LiCl (Table 3). In a similar manner, the changes in tissue sodium show no precise correlation with the metabolic changes observed. Increases in

the tissue content of either sodium or lithium are associated with an enhancement of glucose metabolism. However, in the presence of the lithium ion, the net gain in tissue sodium is decreased, despite the fact that it is under these conditions that the greatest stimulation of glucose metabolism is observed. In this connection it should be noted that large changes in glucose metabolism can be produced by lithium ions under conditions at which the gain in tissue sodium is minimal (Table 3).

Our results give no support for the view that changes in tissue concentrations of cyclic AMP mediate the effects of lithium ions on glucose uptake and glycogen synthesis. However, it is conceivable that concentrations of cyclic AMP at particular sites in the cell could change in the presence of lithium, without a measurable alteration in the total tissue content of this nucleotide.

It is most likely that lithium influences glucose metabolism at several different sites. Lithium ions have been shown, for example, to increase the rate of transport of 3-*O*-methylglucose into muscle [27]. It can also increase the conversion of the D-form of glycogen synthase to the I-form of the enzyme [17, 21], and has been observed to decrease the cellular concentration of glucose 6-phosphate [17]. The effects of lithium ions on other enzymes involved in glucose metabolism, particularly those catalyzing the steps in the pathway glucose to glycogen, need to be investigated.

Studies of the action of lithium ions on muscle metabolism may be of help for our understanding of the role that the sodium ion plays in the regulation of carbohydrate metabolism in this tissue. The results of Bihler and Sawh [28] are particularly important in this connection. These authors demonstrated that the rate of penetration of 3-*O*-methylglucose into the intact diaphragm preparation was markedly stimulated in a potassium-free medium. The rate of transport of this sugar became progressively elevated in experiments in which the tissues were preincubated in the absence of potassium for increasing periods of time. There was an excellent correlation between the rise in the rate of sugar transport and the increase in the cellular content of sodium and the concomitant loss of potassium. Our own experiments also indicate that the rate of carbohydrate metabolism is elevated in muscle under conditions at which the sodium pump is inhibited and the tissues gain sodium. In an all-sodium medium, the rates of glucose uptake and glycogen synthesis tended to be higher than when 10 mM KCl was also present (Tables 1 and 2A). The decrease in the rate of glycogen synthesis seen after addition of KCl to the medium was statistically significant after 60 min of incubation. In this tissue, then, sodium and lithium ions have similar effects on glucose metabolism, but lithium is much more potent than sodium in this regard.

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REFERENCES

1. J. F. J. Cade, *Med. J. Aust.* **36,** 349 (1949).
2. F. K. Goodwin and M. H. Ebert, in *Lithium, Its Role in Psychiatric Research and Treatment* (Eds. S. Gershon and B. Shopsin), pp. 237–252. Plenum, New York (1973).
3. J. Mendels, in *Lithium, Its Role in Psychiatric Research and Treatment* (Eds. S. Gershon and B. Shopsin), pp. 253–268. Plenum, New York (1973).
4. M. Schou, in *Lithium, Its Role in Psychiatric Research and Treatment* (Eds. S. Gershon and B. Shopsin), pp. 269–294. Plenum, New York (1973).
5. T. Dousa and O. Hechter, *Lancet* **1,** 834 (1970).
6. T. Dousa and O. Hechter, *Life Sci.* **9,** 765 (1970).
7. L. Birnbaumer, P. L. Pöhl and M. Rodbell, *J. biol. Chem.* **244,** 3468 (1969).
8. J. Wolff, S. C. Berens and A. B. Jones, *Biochem. biophys. Res. Commun.* **35,** 462 (1969).
9. Y-C. Wang, G. N. Pandey, J. Mendels and A. Frazer, *Biochem. Pharmac.* **23,** 845 (1974).
10. J. C. Skou and C. Hilberg, *Biochim. biophys. Acta* **185,** 198 (1969).
11. T. Tobin, T. Akera, C. S. Han and T. M. Brody, *Molec. Pharmac.* **10,** 501 (1974).
12. G. Hegyvary and R. L. Post, *J. biol. Chem.* **246,** 5234 (1971).
13. P. Plenge, E. T. Mellerup and O. J. Rafaelsen, *J. psychiat. Res.* **8,** 29 (1970).
14. G. Bhattacharya, *Biochem. J.* **79,** 369 (1961).
15. T. Clausen, *Biochim. biophys. Acta* **150,** 66 (1968).
16. E. S. Haugaard, E. Serlick and N. Haugaard, *Biochem. Pharmac.* **22,** 1023 (1973).
17. E. S. Haugaard, R. A. Mickel and N. Haugaard, *Biochem. Pharmac.* **23,** 1675 (1974).
18. J. A. Thomas, K. K. Schlender and J. Larner, *Analyt. Biochem.* **25,** 486 (1968).
19. A. Gilman, *Proc. natn. Acad. Sci. U.S.A.* **67,** 305 (1970).
20. J. Woodward, C. P. Bianchi and S. D. Erulkar, *J. Neurochem.* **16,** 289 (1969).
21. R. S. Horn, O. Wâlaas and E. Walaas, *Biochim. biophys. Acta* **313,** 296 (1973).
22. L. C. Shen, C. Villar-Palasi and J. Larner, *Physiol. Chem. Physics* **2,** 536 (1970).
23. C. P. Bianchi and T. C. Bolton, *J. Pharmac. exp. Ther.* **157,** 388 (1967).
24. H. H. Wespi, *Pflügers Arch. ges. Physiol.* **306,** 262 (1969).
25. M. Maizels, *J. Physiol., Lond.* **195,** 657 (1968).
26. P. F. Baker, *J. Physiol., Lond.* **180,** 383 (1965).
27. P. J. Kohn and T. Clausen, *Biochim. biophys. Acta* **255,** 798 (1972).
28. I. Bihler and P. C. Sawh, *Biochim. biophys. Acta* **225,** 56 (1971).