Biochimica et Biophysica Acta, 356 (1974) 207–218

BBA 76676

THE EXCHANGE AND MAXIMAL NET FLUX OF GLUCOSE ACROSS THE HUMAN ERYTHROCYTE

II. THE EFFECT OF TWO SULPHYDRYL ENZYME INHIBITORS, CHLORMERODRIN AND *p*-CHLOROMERCURIBENZENE SULFONIC ACID

HERBERT ZIPPER and RICHARD C. MAWE

Department of Biological Sciences, Hunter College, CUNY, New York City, N.Y. and Special Research Laboratory for Oral Tissue Metabolism, Veterans Administration Hospital, Brooklyn, N.Y. (U.S.A.)

SUMMARY

The exchange and maximal net fluxes of [^{14}C]glucose across the membrane of the human red cell were measured. The effects of *p*-chloromercuribenzene sulfonic acid and chlormerodrin on these two parameters of glucose transport were determined. At low concentrations *p*-chloromercuribenzene sulfonic acid (a non-penetrating organic mercurial) was found to readily inhibit the exchange flux but not the net efflux. At extremely high concentrations of *p*-chloromercuribenzene sulfonic acid the maximal net efflux showed some degree of inhibition. Chlormerodrin (a penetrating organic mercurial) inhibited both the exchange and net fluxes in the same manner. The addition of insulin in certain instances reduced the degree of inhibition caused by the organic mercurials. Insulin had no effect on the amount of either *p*-chloromercuribenzene sulfonic acid or chlormerodrin which bound to the red cell. From the results obtained, it is suggested that there exist glucose-reactive sites on both the outer and inner surfaces of the membrane. The results also suggest a carrier system possessing different sites or molecular arrangements for glucose egress and for glucose entry.

INTRODUCTION

The ability of $HgCl_2$ and organic mercurial derivatives [1] to inhibit glucose transfer across cell membranes and the reversal [2] of this inhibition after the addition of cysteine have focused attention on the role of membrane sulphydryl groups in the penetration of this nonelectrolyte. Two organic mercurials which inhibit glucose transport in human erythrocytes are *p*-chloromercuribenzene sulfonic acid (PCMBS), a substance which does not penetrate the red cell membrane, and chlormerodrin, which may penetrate the red cell membrane under certain conditions. The interaction of these mercurial compounds with human red cells and red-cell mem-

Abbreviation: PCMBS, *p*-chloromercuribenzene sulfonic acid.

brane components has been measured and it has been reported that the inhibition of glucose transport results from the interactions of these compounds with the sulphydryl groups located on the outer surface of the membrane [3, 4]. However, the effect of these compounds on both the exchange and net flux of glucose in the intact cell has not been investigated. We report here on the ability of the penetrating mercurial (chlormerodrin) to inhibit both the net and exchange flux of glucose and the ability of the non-penetrating mercurial (PCMBS) to inhibit the exchange flux but not the net efflux of glucose. The latter is inhibited to a small degree by PCMBS but only at very high concentrations. The reversal of the mercurial inhibitions by insulin in certain instances was observed. The results suggest that there are sites concerned with glucose transport which are present on both the outer and inner membrane surfaces.

MATERIALS AND METHODS

Chemicals

Bovine insulin (24 units/mg), and PCMBS were from Sigma Chemical Co., St. Louis, Mo.; chlormerodrin was a gift from Dr H. L. Friedman of the Lakeside Laboratories, Milwaukee, Wisc.; chlormerodrin labeled with ^{203}Hg, PCMBS labeled with ^{203}Hg and D-[$^{14}C_6$]glucose were supplied by ICN Corp., City of Industry, Calif.; porcine insulin (23 units/mg) was from Mann Research Laboratories, N.Y.C., N.Y.; porcine ^{131}I-labeled insulin was from Abbott Laboratories, Chicago, Ill. All other materials were of reagent grade.

Analytical methods

Samples labeled with ^{131}I or ^{203}Hg were counted in a Packard Model 2001 Spectrometer well counter system. The amounts of [^{203}Hg]PCMBS and [^{203}Hg]-chlormerodrin which bound to the human red cell were measured under the experimental conditions used to determine flux measurements. All other methods concerned with determining fluxes, osmolarities, and hematocrits are as previously described [5].

Red cells

Blood from healthy donors obtained by venapuncture was collected into a citrated tube (5 mg sodium citrate per ml blood). The blood was passed through three layers of gauze and washed 3 times at 4 °C in glucose–saline solution adjusted to pH 7.3 with phosphate buffer. The glucose concentration was 0.075 M, and the osmolarity was 380–390 mosM. The washed red cells were brought to room temperature (22–23 °C) and an aliquot of red cells was equilibrated with [$^{14}C_6$]glucose as described below.

Flux Measurements

Aliquots of 10–50 μl of the solution(s) containing the agent(s) to be tested were added to 0.5 ml of packed cells (hematocrits between 75 and 80 %) previously equilibrated with 25 μl [$^{14}C_6$]glucose (125 μCi/ml) for 30 min at 22–23 °C. A control sample without the agent was run concurrently under identical conditions. The incubation times of the mercurials and insulin with the glucose-equilibrated red cells

varied from 30 to 60 min and is noted in the appropriate tables. In a typical flux experiment, 10 μl of a red-cell suspension which has been incubated with tracer and experimental agents as described above, is rapidly stirred into a beaker containing 10 ml of saline–phosphate buffer (pH 7.3) with glucose (380–390 mosM) or without glucose (295–310 mosM). Samples (5–6) were removed at 5–7-s intervals (the first sample was usually obtained within 1.5–3 s of the addition of the 10-μl aliquot of red cells) and treated as described by Mawe and Hempling [6].

In the mercurial binding experiments, both 30- and 60-min incubation periods were used. This mimicked those flux experiments with mercurial alone where the inhibitor is present for 30 min prior to analysis and those where mercurial was used in conjunction with insulin (i.e. mercurial present for 60 min prior to analysis).

Theoretical considerations

The exchange flux is either the influx or the efflux of glucose when the concentrations of the sugar inside and outside the red cell are equal during the course of an experiment. The conditions under which the exchange flux were measured were the same as that of a two-compartment closed system discussed by Solomon [7] and Sheppard [8]. The kinetics of the exchange have been described by Mawe and Hempling [6] and Zipper and Mawe [5].

The net flux is a measurement of the change in glucose content of the red cell when there is a difference between the internal and external concentrations of the sugar. The maximal net flux (that which was measured in the experiments reported here) is the net flux under ideal conditions that is, when the concentrations of the sugar on one side of the membrane remains well above the saturation level of the reactive site while on the other side the concentration of the sugar remains as close to zero as possible for the length of the measurement. The kinetics of the maximal net flux have been described by Zipper and Mawe [5].

RESULTS

PCMBS

Glucose-loaded red cells were incubated with different quantities of PCMBS for 30 min at 22–24 °C and net and exchange fluxes of [^{14}C]glucose were determined as previously described [5, 6]. The results (Table I) show the following: (1) At the lowest concentration of PCMBS (0.05 μmoles/ml packed cells), the exchange flux is inhibited by 51 % while the net flux is unaffected. (2) Over the concentration range of 0.05–7.8 μmoles PCMBS/ml packed cells, the percentage inhibition of the exchange flux ranged from a low of 41 % to a high of 73 %. As a result the exchange flux at a PCMBS concentration of 1.9 was reduced to the level of the normal net flux value. (3) By comparison, the net efflux of glucose was not significantly inhibited until the cells were exposed to a PCMBS concentration of 1.4 μmoles/ml packed cells.

In a previous report [5], an acceleration of the net efflux of glucose in the human red cell treated with insulin was reported. In addition, note was taken of the ability of insulin to reduce the degree of inhibition of the exchange flux obtained with phospholipase C. Consequently, it was decided to conduct experiments in which both the mercurial and insulin were present. The results obtained with PCMBS

TABLE I

THE AVERAGE NET AND EXCHANGE FLUXES OF [U-^{14}C]GLUCOSE IN HUMAN RED CELLS EXPOSED TO DIFFERENT CONCENTRATIONS OF PCMBS

Red cells were incubated with PCMBS for 30 min at 22–24 °C. The PCMBS concentration is expressed as μmoles/ml packed cells. Flux values are expressed as mmoles per l cell water per s. There was no significant difference ($P > 0.10$) between the net efflux values obtained with the three lowest PCMBS concentrations and their respective controls.

PCMBS concn	Net flux ± S.E.			Exchange flux ± S.E.			Exchange/net flux ratio	
	Controls	Treated cells	% inhibition	Controls	Treated cells	% inhibition	Controls	Treated cells
0.05	2.59 ± 0.20	2.41 ± 0.26	7	6.63 ± 0.25	3.27 ± 0.43	51	2.56	1.36
0.35	2.13 ± 0.23	1.95 ± 0.18	8	6.22 ± 0.16	3.70 ± 0.24	41	2.92	1.90
0.7	1.64 ± 0.05	1.44 ± 0.14	12	7.28 ± 0.58	2.87 ± 0.92	61	4.43	1.99
1.4	2.09 ± 0.15	1.71 ± 0.10	18	5.26 ± 0.21	2.82 ± 0.25	46	2.52	1.65
1.9	2.33 ± 0.06	1.81 ± 0.08	22	6.13 ± 0.21	2.16 ± 0.22	65	2.63	1.29
3.8	2.23 ± 0.14	1.53 ± 0.11	31	5.85 ± 0.54	1.56 ± 0.19	73	2.62	1.02
7.8	1.76 ± 0.17	1.18 ± 0.12	37	5.54 ± 0.31	1.78 ± 0.31	68	2.98	1.51

TABLE II

THE AVERAGE NET AND EXCHANGE FLUXES OF [U-^{14}C]GLUCOSE IN HUMAN RED CELLS

Measurements were made on: (1) cells incubated with PCMBS for 30 min; (2) cells incubated with PCMBS for 30 min followed by insulin for 30 min; (3) cells incubated with insulin for 30 min followed by PCMBS for 30 min; (4) cells exposed to PCMBS and insulin simultaneously for 30 min. The incubations took place at 22–24 °C. The concentration of PCMBS was 0.7 μmoles/ml packed red cells. The insulin concentration was adjusted to be at 0.14 μmoles/ml packed red cells. Fluxes are expressed as mmoles per l cell water per s. The values for *P* represent the presence or absence of significant differences between values obtained from the PCMBS-treated cells and the values obtained when insulin is present.

	Net flux±S.E.	% inhibition	*P*	Exchange flux±S.E.	% inhibition	*P*	Exchange/net flux ratio
Control (untreated)	2.25±0.07	—	—	6.10±0.19	—	—	2.71
PCMBS-treated cells	1.76±0.07	22	—	2.11±0.20	65	—	1.20
PCMBS-treated cells exposed to insulin	1.99±0.10	12	>0.10	3.93±0.24	36	<0.01	1.97
Insulin-treated cells exposed to PCMBS	2.36±0.15	0	<0.01	4.04±0.32	34	<0.01	1.71
Cells treated with PCMBS and insulin simultaneously	2.23±0.15	0	<0.05	3.70±0.91	39	<0.05	1.66

in combination with insulin are shown in Table II. At the concentration of PCMBS used, 0.7 μmole/ml packed cells the net flux was inhibited by 22 %. However, if insulin is added to the cell suspension either before PCMBS addition, or together with PCMBS, the PCMBS inhibition of the net flux noted above is prevented. If the cells are exposed to insulin after PCMBS treatment, the inhibition of the net flux is still noted but is slightly reduced. The exchange flux of red cells from the same population exposed only to the same concentration of PCMBS was reduced by 65 %. However, if insulin was present either before, after or during incubation with PCMBS, the degree of inhibition was 34–39 % and represented a recovery of 40–45 %.

Unlike PCMB which has also been shown to inhibit glucose transport in red cells, PCMBS does not penetrate the red-cell membrane. The lack of penetration by PCMBS has been attributed to the substitution of the sulfonic acid group for the carbonyl group in PCMB and the resulting increase in the hydrophylic character of the molecule [9].

Depending on the atom involved in binding and the orientation of the PCMBS molecule with the surface, a molecule of PCMBS may occupy an area from 20–65 $Å^2$ on the surface of the red cell. At a PCMBS concentration of 0.05 μmole/ml packed cells, where the exchange flux is inhibited while the net is unaffected (Table I), a maximum of 0.4–1.3 % of the surface area of the red cell* can be covered by PCMBS. Where the exchange flux is lowered to the level of the normal net flux and inhibition of the net flux first appears (0.7 μmole PCMBS/ml packed cells), approx. 6–18 % of the cell surface can be covered by PCMBS. At those concentrations where the maximum inhibition of both fluxes is obtained, it can be shown that upwards of 97 % of the cell surface would be covered by PCMBS. It should be noted that even under conditions where the entire cell may be covered with PCMBS, the net efflux is never fully inhibited. The binding of PCMBS to human red cells has an approximate linear relationship to the amount PCMBS added (Table III). As a result, it can be de-

TABLE III

THE BINDING OF [^{203}Hg] PCMBS TO HUMAN RED CELLS

Red cells were incubated with labeled PCMBS for 30 min at 22–24 °C.

Expt No.	μmoles PCMBS added per ml packed cells	μmoles PCMBS bound per ml packed cells	Molecules PCMBS $\times 10^8$ per cell	Molecules PCMBS per 10^3 $Å^2$ surface area
1	0.35	0.25	0.15	1
	0.35	0.25	0.15	
2	1.89	1.05	0.63	4.5
3*	1.89	1.59	0.96	6.8
	1.89	1.56	0.94	
4	3.77	2.45	1.48	10
	3.77	2.43	1.46	
5*	3.77	2.88	2.07	15

* In these experiments the cells were incubated with PCMBS for 60 min. The hematocrits in the different experiments are as follows: No. 1, 84 %; 2, 78 %; 3, 80 %; 4, 84 %; 5, 70 %.

* The value of 145 $\times$ 10^8 $Å^2$ for the surface area of the human red cell was used for these calculations [10].

monstrated that at the point where the net flux is unaffected, but where the exchange flux is brought to the level of the normal net flux, as few as 1–2 molecules of PCMBS per 10^3 Å^2 of the cell surface need be bound. Assuming that each molecule of PCMBS is bound to a single site, each of which is concerned with glucose flux, a maximum of $1.5 \cdot 10^6$–$3.0 \cdot 10^6$ molecules of PCMBS per cell would be required to lower the exchange flux to the level of the normal net flux. Since it can be demonstrated that more PCMBS may be bound to the surface even after the exchange flux has been brought to the level of the normal net flux, the results suggest that the above figure probably represents the maximum number of sites actually involved with glucose transport on the cell surface. This figure for an upper limit in the number of glucose transport sites at the outer surface is in general agreement with the value of $1 \cdot 10^6$–$1.4 \cdot 10^6$ suggested by Van Steveninck et al. [3]. Depending on the conformation assumed by PCMBS with a membrane sulphydryl group, a maximum of from 0.4 to 1.2 % of the total cell surface area of the red cell would contain these reactive sites. These figures are essentially in agreement with that of Widdas [11] who calculated a value of 1 %.

From the results obtained, it is possible to speculate that singly or in combination PCMBS may, (1) inhibit the release of glucose from the glucose carrier to the outside medium; (2) inhibit the uptake of medium glucose by free carrier, or (3) the carrier may interact directly with PCMBS and as a result the movement of the carrier is reduced and/or the concentration of usable carrier is reduced. Since Cases 1 and 2 require the inhibition of both fluxes to the same degree, it is suggested that PCMBS acts by interfering with the uptake of medium glucose by the carrier for passage through the cell membrane to the cell interior. As less medium glucose enters the cell, a situation analogous to that of the experimental maximal net efflux condition begins to prevail, i.e. carrier-bound [^{14}C]glucose is brought to the cell exterior released, and carriers primarily glucose-free return to the cell interior. The results show that the flux approaches a rate consistent with that of the normal net flux indicating that the system may be behaving as if glucose were absent from the external medium. At high PCMBS concentrations practically the entire red-cell surface may be bound. Under these conditions non-specific alterations in the membrane could result in inhibition of the net flux, an inhibition which never exceeds 40 % even under these conditions of PCMBS excess.

Chlormerodrin

Glucose-loaded red cells were similarly treated with different concentrations of chlormerodrin. The cells were incubated with this inhibitor both at 22–24 and 4 °C. The incubation time was 30 min. Chlormerodrin is capable of slowly penetrating the red-cell membrane. Approx. 40-50 % of the added chlormerodrin will penetrate the red-cell membrane at 22–24 °C over a period of 30–60 min while at 4 °C the rate of chlormerodrin penetration is reduced by about 80 % [3]. The glucose-loaded cells incubated with chlormerodrin at 4 °C were brought to 22–24 °C prior to the determination of the flux. The flux data obtained from cells treated with chlormerodrin are shown in Table IV. The results show that at 22–24 °C, chlormerodrin inhibits both the net and exchange flux equally well and that the degree of inhibition in both cases increases with increased chlormerodrin levels. At the highest chlormerodrin concentration, both the net and the exchange flux were inhibited by 75–80 %. The

TABLE IV

THE AVERAGE NET AND EXCHANGE FLUXES OF [U-^{14}C]GLUCOSE IN HUMAN RED CELLS EXPOSED TO DIFFERENT CONCENTRATIONS OF CHLORMERODRIN

Red cells were incubated with chlormerodrin for 30 min at 22–24 and at 4 °C. Chlormerodrin concentrations are expressed as μmoles per ml packed cells. Flux values are expressed as mmoles per l cell water per s. The values for the net and exchange fluxes obtained from chlormerodrin-treated cells were all significantly different from their respective controls.

			Net flux ± S.E.			Exchange flux ± S.E.			Exchange/net flux ratio	
Incubation temperature	Initial chlormerodrin concn	Final chlormerodrin concn*	Controls	Treated cells	% inhibition	Controls	Treated cells	% inhibition	Controls	Treated cells
22–24 °C	0.14	0.08	2.62 ± 0.20	2.19 ± 0.15	16	6.13 ± 0.43	5.02 ± 0.26	18	2.34	2.29
	0.27	0.15	2.48 ± 0.17	1.56 ± 0.08	37	5.19 ± 0.33	3.48 ± 0.22	33	2.28	1.98
	0.55	0.30	2.19 ± 0.10	1.08 ± 0.08	51	5.84 ± 0.27	2.52 ± 0.24	57	2.67	2.33
	1.1	0.59	2.31 ± 0.13	0.57 ± 0.38	75	5.79 ± 0.39	1.57 ± 0.30	73	2.51	2.75
	2.7	1.5	2.03 ± 0.10	0.51 ± 0.06	75	4.94 ± 0.70	0.99 ± 0.20	80	2.43	1.94
4 °C	0.14	0.12	2.54 ± 0.12	1.31 ± 0.05	49	5.90 ± 0.22	3.98 ± 0.25	33	2.32	3.04
	0.54	0.46	2.88 ± 0.16	1.47 ± 0.15	49	5.78 ± 0.22	2.35 ± 0.21	59	2.01	1.60

* These values represent the concentration of chlormerodrin at the cell surface or in the medium after incubation for 30 min.

exchange/net flux ratios for the chlormerodrin-treated cells reflect the almost identical degree of inhibition of both fluxes, that is the ratio remains relatively constant and closer to an average value of 2.3. This data is in marked contrast to the exchange/net flux values obtained from PCMBS-treated cells (Table I) and suggest that the inhibitory action of chlormerodrin may be of a different nature than PCMBS. The values under final chlormerodrin concn in Table IV have been corrected for the passage of chlormerodrin at 24 °C through the cell. They represent the final chlormerodrin concentration which is in an equilibrium distribution in the cell water and the medium after 30 min incubation at 24 °C. At 4 °C the initial concentration of chlormerodrin remains the experimental one through the course of the experiment. When the results are compared, keeping in mind the corrected values, it appears that at the high concentration of chlormerodrin the exchange and net fluxes are inhibited to a greater extent at 24 than at 4 °C. These data indicate that sites may exist on the inner surface. In further support of this point it should be noted that at 22–24 °C, at a chlormerodrin concentration of 0.55 μmole/ml packed cells (Tables IV and VII) ($30 \cdot 10^6$ molecules chlormerodrin per cell) the exchange flux is inhibited by 57 % and the net flux by 60 %. If all the chlormerodrin were bound to the surface, between 5 and 10 % of the red-cell surface would be involved. Unlike the results obtained with PCMBS, the net flux is considerably inhibited. With PCMBS, $30 \cdot 10^6$ molecules bound per cell are required for a 27 % inhibition of the net glucose flux while with chlormerodrin, an equivalent inhibition is observed with $6 \cdot 10^6$ molecules bound per cell.

The possibility arises that glucose could potentiate the inhibitions obtained with PCMBS and chlormerodrin in a manner similar to that reported for dinitrofluorobenzene [12]. Such a possibility does not appear to be present in the experiments reported here. It should be noted that the mercurials are added to glucose-equilibrated cells and as such, interactions between membrane and mercurial occur solely in the presence of glucose. What is actually being measured is a movement of glucose under two precise experimental conditions and it is under those conditions that differences in the manner of the inhibition are obtained.

As was done previously, a group of experiments were run to test the effect of insulin on chlormerodrin inhibition of glucose flux. The glucose-loaded red cells were incubated with the mercurial (30 min) and insulin (30 min) at 4° and at 22–24 °C. The flux measurements were carried out at 22–24 °C.

4 °C. In Table V, it can be seen that the net flux is inhibited by slightly less than 50 %, and whether insulin is added before or after the addition of chlormerodrin, the level of inhibition remains essentially the same as with chlormerodrin alone. On the other hand the exchange flux shows considerable recovery in the presence of insulin. When chlormerodrin-treated cells are exposed to insulin there is a recovery of 50 % and when cells are first treated with insulin and then exposed to chlormerodrin, the recovery is over 60 %.

24 °C. Table VI shows that while the exchange flux is inhibited by chlormerodrin to almost the same degree (56 %) as in the experiments at 4 °C, there is no recovery from the inhibition in the presence of insulin. The net flux inhibited by 48 % by chlormerodrin and this is not significantly altered by prior addition of insulin. However, the degree of inhibition is decreased by 50 % when insulin is added to red cells after exposure to chlormerodrin.

TABLE V

THE AVERAGE NET AND EXCHANGE FLUXES OF [U-^{14}C]GLUCOSE IN HUMAN RED CELLS

Red cells were exposed to: (1) chlormerodrin only; (2) chlormerodrin followed by insulin; and (3) insulin followed by chlormerodrin. The incubation time for each agent was 30 min and the incubation temperature was 4 °C. Fluxes are expressed as mmoles per l cell water per s. The values for *P* represent the presence or absence of significant differences between values obtained from the chlormerodrin-treated cells and the values obtained when insulin is present.

	Net flux±S.E.	% inhibition	*P*	Exchange flux±S.E.	% inhibition	*P*	Exchange/net flux ratio
Control (untreated cells)	2.70±0.16	—	—	5.67±0.29	—	—	2.10
Chlormerodrin-treated cells	1.37±0.16	49	—	2.21±0.19	61	—	1.61
Chlormerodrin-treated cells exposed to insulin	1.52±0.17	44	>0.10	3.91±0.45	31	<0.01	2.57
Insulin-treated cells exposed to chlormerodrin	1.52±0.14	44	>0.10	4.37±0.56	23	<0.01	2.88

TABLE VI

THE AVERAGE NET AND EXCHANGE FLUXES OF [U-^{14}C]GLUCOSE IN HUMAN RED CELLS

Red cells were exposed to: (1) chlormerodrin only; (2) chlormerodrin followed by insulin; and (3) insulin followed by chlormerodrin. The incubation time for each agent was 30 min and the incubation temperature was 22–24 °C. Fluxes are expressed as mmoles per l cell water per s. The values for *P* represent the presence or absence of significant differences between values obtained from the chlormerodrin-treated cells and the values obtained when insulin is present.

	Net flux±S.E.	% inhibition	*P*	Exchange flux±S.E.	% inhibition	*P*	Exchange/net flux ratio
Control (untreated cells)	2.28±0.10	—	—	6.29±0.21	—	—	2.76
Chlormerodrin-treated cells	1.18±0.12	48	—	2.79±0.32	56	—	2.36
Chlormerodrin-treated cells exposed to insulin	1.70±0.13	25	>0.05	3.14±0.46	50	>0.10	1.85
Insulin-treated cells exposed to chlormerodrin	1.23±0.17	46	>0.10	3.19±0.75	49	>0.10	2.59

TABLE VII

The binding of [^{203}Hg] to human red cells.

Expt No.	Temp. (°C)	μmoles chlormerodrin added per ml packed red cells	μmoles chlormerodrin bound per ml packed red cells	Molecules chlormerodrin $\times 10^8$ per cell	Chlormerodrin molecules per 10^3 Å^2 surface area
1	22–24	0.136	0.094	0.056	0.4
		0.136	0.093	0.056	
2	22–24	0.54	0.50	0.3	2
3	22–24	1.09	0.96	0.58	4
		1.09	0.97	0.58	
4	22–24	1.09	0.97	0.69	5
A	4	0.55	0.27	0.19	1.4
		0.55	0.27	0.19	
B*	4	0.55	0.45	0.31	2.2
		0.55	0.43	0.31	

* In this experiment the cells were incubated with chlormerodrin for 60 min. In all the other experiments the incubation time with chlormerodrin was 30 min. The hematocrits in the different experiments are as follows: No. 1, 80 %; 2, 78 %; 3, 80 %; 4, 70 %; A, 74 %; B, 74 %.

In summary, the results obtained from cells treated with the chlormerodrin–insulin combinations at 4 °C and at 24 °C indicate the following: (1) At 4 °C, regardless of the order of incubation with insulin, the inhibition of the net efflux of glucose is unaltered. The inhibition of the exchange flux is considerably decreased. (2) At 22–24 °C, regardless of the order of incubation with insulin, the degree of inhibition of the exchange flux by chlormerodrin is unaltered. The degree of inhibition of the net flux is unaltered in the case where insulin is added first, while in the case where the insulin is added last, the degree of inhibition of the net flux is considerably lessened.

A diagramatic representation of glucose transport is shown in Fig. 1. The presence of a carrier (designated X) either mobile or of the sort proposed by Lieb and Stein [13], i.e. an aligomeric protein composed of high- and low-affinity sites is assumed. Diagram A represents glucose transport under the experimental conditions for

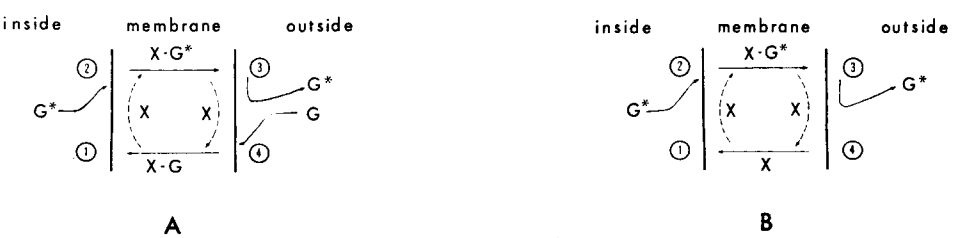

Fig. 1. Diagram A represents experimental conditions to obtain exchange flux measurements and Diagram B represents experimental conditions to obtain maximal net efflux measurements. Non-radioactive glucose is represented by G, [^{14}C]glucose by G*. The carrier entity is designated X and the numbers 1–4 represent the possible sites of PCMBS and chlormerodrin inhibition.

exchange flux, and Diagram B represents glucose transport under the experimental conditions for maximal net efflux. Four possible sites (1–4) of inhibition action are considered and it is suggested that:

(1) PCMBS inhibition of the exchange flux occurs at Site 4 (Diagram A); (2) PCMBS inhibition of the net efflux (occurring at very high PCMBS concentrations) reflects disruption of complexed carrier and also non-complexed carrier sites 3 and 4 (Diagram B); (3) chlormerodrin inhibition of the exchange flux at both 4 and 24 °C consists primarily of disruption of complexed carrier, i.e. Sites 2 and 3 (Diagram A), and in addition at 4 °C chlormerodrin may act at Site 4 (Diagram A) in a manner akin to PCMBS (note relief of this inhibition by insulin); and (4) chlormerodrin inhibition of the net efflux at both 4 and 24 °C reflects disruption of complexed carrier i.e. Sites 2 and 3, and non-complexed carrier, i.e. Sites 1 and 4 (Diagram B). The results as depicted suggest a carrier system possessing different sites for glucose eggress and for glucose entry. It is of course, impossible to know if such sites represent different carrier entities or merely reflect differences in molecular organization of carrier protein. We would suggest here, however, that the behaviour of the glucose flux in the presence of insulin and the differential effects obtained with PCMBS and chlormerodrin suggest that the carrier cannot be considered as a single uniform entity but rather as a multifaceted protein containing numerous sites of variable affinity as perhaps best demonstrated by the model proposed by Lieb and Stein [13].

ACKNOWLEDGEMENT

The authors wish to thank Professor P. G. LeFevre and Professor H. G. Hempling for their helpful suggestions and for critically evaluating the manuscript. The authors wish to acknowledge the technical assistance of Mrs Alice Steele and Mr Julius Levine and the able assistance of Mrs Esther Cinman during the preparation of the manuscript. We also wish to thank Dr P. Person for his very helpful encouragement in carrying out this investigation.

This research was supported by Grant No. GB 7921 from the National Science Foundation.

REFERENCES

1 LeFevre, P. G. (1948) J. Gen. Physiol. 31, 505–527
2 LeFevre, P. G. (1954) Symp. Soc. Exp. Biol. 8, 118–136
3 Van Steveninck, J., Weed, R. I. and Rothstein, A. (1965) J. Gen. Physiol. 48, 617–632
4 Weed, R., Ebor, J. and Rothstein, A. (1962) J. Gen. Physiol. 45, 395–408
5 Zipper, H. and Mawe, R. C. (1972) Biochim. Biophys. Acta 282, 311–325
6 Mawe, R. C. and Hempling, H. B. (1965) J. Cell Comp. Physiol. 66, 95–104
7 Solomon, A. K. (1949) J. Clin. Invest. 28, 1297–1307
8 Sheppard, C. W. (1962) Basic Principles of the Tracer Method, pp. 10–24, Wiley, New York
9 Velick, S. F. (1953) J. Biol. Chem. 203, 563–575
10 Westerman, M. P., Pierce, L. E. and Jensen, W. N. (1961) J. Lab. Clin. Med. 57, 819–824
11 Widdas, W. F. (1954) J. Physiol. 125, 163–180
12 Krupka, R. M. (1971) Biochemistry 10, 1143–1148
13 Lieb, W. R. and Stein, W. D. (1970) Biophys. J. 10, 585–598