[Chem. Pharm. Bull. 22(2) 439—445 (1974)]

UDC 547.269.1.09 : 577.352.4

Effect of Sulfur-Containing Compounds on Experimental Diabetes. XI.[1] Effect of Thiol and Disulfide Compounds on Glucose Transfer into Red Blood Cells

Takehisa Chiba

Research Laboratory, Santen Pharmaceutical Co., Ltd.[2]

(Received October 24, 1973)

Studies were made on the influence of thiol and disulfide compounds on glucose uptake into red blood cells (RBC) of normal and alloxan diabetic rats.

1) Incorporation of glutathione (GSH), 2-mercaptopropionylglycine (MPG), oxidized GSH and oxidized MPG into RBC was examined. MPG was found to be incorporated into RBC. When MPG was allowed to react with RBC at 37° for 30 minutes, approximately 30% of MPG in the reaction solution was incorporated into RBC (alloxan diabetic (AD)-RBC). Four to ten per cent of the incorporated MPG existed in the oxidized form.

2) Incorporation of glucose into normal RBC and AD-RBC was examined. Incorporation of glucose into AD-RBC was inhibited.

3) In normal RBC, such compounds as MPG and cystein which were incorporated into RBC increased glucose uptake, while GSH showed a tendency to inhibit incorporation of glucose. Incorporation of glucose was inhibited by oxd GSH, oxd MPG and cystine.

4) Influence of various inhibitors was studied. Incorporation of glucose was inhibited by SH reagents ($HgCl_2$, parachloromercuribenzoate (PCMB), parachloromercuribenzene sulfonate (PCMBS), NEM), but not by insulin. Potassium borohydride increased glucose uptake.

5) Disappearance of glucose from normal RBC and AD-RBC was studied. Fifty per cent of glucose disappeared from normal RBC 9 minutes later and from AD-RBC 19 minutes later. The time required for the disappearance of 50% of glucose from normal RBC and from AD-RBC was shortened to 7 minutes and to 16 minutes respectively by the addition of MPG.

As mentioned above, there is a difference in incorporation of glucose into RBC between normal RBC and AD-RBC. Furthermore, promotion of glucose uptake by MPG and cysteine and inhibition of glucose uptake by oxd MPG and cystine were discussed.

Studies were made of the orientation of a sulfhydryl group which is specifically reactive in transporting compounds into red blood cells (RBC).[3] Particularly, sulfhydryl groups of the cell membrane are known to have significant activities in the transport of Na^+, K^+[4] and sugar.[5]

The authors examined transfer of thiol and disulfide compounds into RBC, using glutathione (GSH), oxidized glutathione (oxd GSH), 2-mercaptopropionylglycine (MPG) and oxidized MPG (oxd MPG)[6] and found that MPG is incorporated into RBC, but GSH, oxd GSH and oxd MPG are not. On the other hand, studies were also made on MPG uptake into RBC of normal and alloxan diabetic (AD) rats, and it was revealed that MPG uptake is increased in AD-RBC.

Many works have been carried out concerning glucose transfer into RBC.[7] In earlier studies, the intermediation of a sulfhydryl group at the cell surface, an enzymatic phospholy-

1) Part X: T. Chiba and S. Yoshikawa, *Yakugaku Zasshi*, **93**, 379 (1973).
2) Location: *Shimoshinjo-cho, Higashiyodogawa-ku, Osaka.*
3) H.S. Jacob and J.H. Jandle, *J. Clin. Invest.*, **41**, 779 (1962); R. Weed and G. Berg, *Fedration Proc.*, **22**, 213 (1963).
4) R.M. Sutherland, A. Rothstein and R.I. Weed, *J. Cell Physiol.*, **69**, 185 (1967).
5) K. Kakinuma, *Metabolism and Disease.*, **8**, 818 (1971).
6) T. Chiba and S. Yoshikawa, *Yakugaku Zasshi*, **89**, 248 (1969).
7) P.G. Lefevre, *Symposia Soc. Exptl. Biol.*, **8**, 118 (1954); W.R. Lieb and W.D. Stein, *Biophys. J.*, **10**, 585 (1970).

lation, was regarded as an essential step in the passage of glucose and glycerol across the human red cell membrane.[8] Later, whether glucose is bound by absorption with a specific site of the membrane or is transported by Carriers in the membrane was discussed.[9] The first stage of the former non-carrier-mediated transport was presumed to be the special association of substrate with the active sites of absorption at the cell surface,[10] and identification of glucose-binding component on the RBC membrane was made.[9] Glucose transport into RBC is inhibited by SH-reagents[11] and the inhibition mechanism differs with inhibitors.[12]

In this investigation, the authors studied the effects of thiol and disulfide compounds on glucose transport, using RBC of normal and alloxan diabetic rats and report the results.

Experimental

1) **Materials**——Male 120—150 g Wistar rats were used for all experiments. Alloxan diabetic rats with blood sugar levels of higher than 200 mg/dl which was produced by intraperitoneal administration of 200 mg/kg of alloxan were used. Blood was collected in test tubes containing heparin and was centrifuged at 4°, 3000×*g*, for 10 minutes, after which white blood cells and buffy coat were removed with suction by an aspirator. RBC was washed twice with phosphate-NaCl solution (a mixture of 1 part of 0.15M phosphoric acid buffer of pH 7.4 and 9 parts of 0.145M NaCl).

2) **Compounds used**——Ninety-eight per cent purity of 2-mercaptopropionylglycine (MPG) and 98% purity of oxidized MPG (oxd MPG) were synthesized by the authors; GSH, oxd GSH, cysteine (CySH), cystine, parachloromercuribenzoate (PCMB), parachloromercuribenzene sulfonate (PCMBS), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), special grade reagents, were provided by Nakarai Chemicals, Ltd.; EDTA and triethanolamine, both special grade reagents, were provided by Wako Pure Chemicals Co., Ltd.

3) **Methods**——a) Transport of Thiol and Disulfide Compounds: 1.0 ml of 70% Haematocrit value RBC (a mixture of 7 parts of RBC and 3 parts of phosphate buffer) was allowed to react with a mixture of 0.75 ml of phosphate-NaCl solution and 0.25 ml of a thiol or disulfide compound or a SH reagent at 37°.

b) Transport of Glucose: 1.0 ml of 70% Haematocrit value RBC was allowed to react with a mixture of 1.0 ml of phosphate-NaCl solution and 1.0 ml of 15 μM glucose at 37°. Uptake of the compounds in to RBC was measured by Morita, *et al.*'s method[13] described below.

After a certain time, the reaction solution was cooled with ice and was centrifuged, and glucose, SH and S-S compounds in the supernatant were assayed. As much supernatant as possible was removed from RBC, and the RBC and a small amount of the remaining supernatant were stirred homogeneously by using a thin glass rod. Glucose, SH and S-S compounds in a certain amount of the RBC were assayed, and at the same time, the hematoclit value was determined by using the remaining RBC. The blood level CR can be obtained by the following equation:

$$CR = \frac{100}{H}\left\{CSR - CS\left(1 - \frac{H}{100}\right)\right\}$$

where CS is the concentration of the supernatant, CSR is the concentration of the mixture of RBC and a small amount of supernatant and H is the hematoclit value in percent.

4) Determination of MPG and oxd MPG——Determination of MPG: To 0.5 ml of the supernatant or the RBC solution, 4 ml of water and 1 ml of 30% trichloroacetic acid (TCA) were added and the mixture was centrifuged. Three ml of the resulting supernatant was passed through a column (1.0×2.5 cm) of Dowex 50 W resin (100—200 mesh, H type) and the resin was washed 5—6 times with small portions of water. 0.5 ml of the initial eluate was discarded and the following 5 ml was collected for use as amino group-free MPG and S-S (AGF-SH, S-S), and the SH amount was determined by Ellman's method.[14] A two ml aliquot was mixed with 0.1 ml of 0.01M DTNB-ethanol solution (DTNB reagent) and 1 ml of 1.5M triethanolamine (TEA) buffer of pH 8.80, and colorimetry was performed at 412 mμ.

8) P.G. Lefevre, *J. Gen. Physiol.*, **31**, 505 (1948).
9) P.G. Lefevre, K.I. Habich, H.S. Hess and M.R. Hudson, *Science*, **143**, 955 (1964); W.R. Lieb and W.D. Stein, *J. Theor. Biol.*, **30**, 219 (1971).
10) W.D. Stein, "The Movement of Molecules Across Cell Membranes", Vol. 162 Academic Press., A.B. Pardee., Science, New York, N.Y., 1968, p. 632.
11) R. Weed, J. Eber and A. Rothstein., *J. Gen. Physiol.*, **45**, 395 (1962).
12) A.C. Dawson and W.F. Widdas, *J. Physiol.*, **168**, 644 (1963); A.K. Sen and W.F. Widdas, *J. Physiol.*, **160**, 392 (1962).
13) M. Morita and T. Minesita, *Vitamins* (Japan), **33**, 67 (1966); M. Shindo, K. Okamoto and J. Totsu, *Chem. Pharm. Bull.* (Tokyo), **15**, 295 (1967).
14) G.L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).

Determination of oxd MPG: Oxd MPG was reduced to MPG by Ellman's method[15] and the oxd MPG content was calculated from the difference between MPG contents before and after the reduction. Ten ml of 7.3% ethylenediaminetetraacetate solution (pH 9.00), 10 ml of 1.3M NaOH and 10 ml of 15% KBH_4 were mixed, and the mixture was used as the reduction solution. To 2 ml of AGF-SH and S-S aliquots, 2 ml of the reduction solution was added and it was allowed to stand at room temperature for 50 minutes. While cooling with ice, 1 ml of 4N HCl was added. After 20 minutes, 0.1 ml of DTNB reagent and 2 ml of TEA buffer were added and colorimetry was performed at 412 mμ. The oxd MPG content was calculated by the following equation.

$$\text{oxd MPG} = \frac{1}{2} \times \left(\begin{matrix} \text{MPG amount} \\ \text{after reduction} \end{matrix} - \begin{matrix} \text{MPG amount} \\ \text{before reduction} \end{matrix} \right)$$

5) **Determination of Glucose**——Determination was performed by the enzymatic method.

Result

Uptake of MPG into RBC

One ml of 1.0 μM MPG was allowed to react with RBC and the MPG level in the reaction solution and the incorporated MPG level in RBC were determined at fixed intervals of time. After 10 to 15 minutes, 30% of MPG was incorporated into RBC and after 30 minutes, MPG in RBC gradually increased. Approximately 4% of MPG incorporated into RBC was found to exist in the form of oxd MPG. About 40% of MPG was incorporated into AD-RBC, which was a greater amount as compared with normal RBC. About 7—10% of oxd MPG was found in AD-RBC. Our study proved that little was incorporated into RBC from the reaction solu tion[16]and this evidence suggests that MPG is oxidized after it is incorporated into RBC (Fig. 1). Incorporation of MPG in the presence of glucose was examined. As shown in Table I, MPG uptake was increased in the presence of glucose in both normal RBC and AD-RBC.

Table I. Incorporation of 2-Mercaptopropionylglycine (MPG) into Red Blood Cells (RBC) in the Presence or Absence of Glucose

MPG concn.	Group: Normal-rat rBC μM/ml		AD-rat rBC μM/ml	
	−Glucose	+Glucose	−Glucose	+Glucose
0.3 μM/ml	0.0364±0.0012	0.0636±0.0028	0.0697±0.0025	0.0787±0.0034
0.6 μM/ml	0.0751±0.0048	0.1262±0.0043	0.1248±0.0068	0.1421±0.0058
1.2 μM/ml	0.1323±0.0036	0.1773±0.0083	0.1840±0.0052	0.2317±0.0076

0.3 μM, 0.6 μM and 1.2 μM of MPG were allowed to react with a mixture of 1.0 ml of 70% RBC and 0.75 ml of phosphate-NaCl solution (pH 7.4) respectively at 37° for 5 minutes in the presence or absence of 20 μM glucose and MPG amount in RBC was determined.

Incorporation of Glucose into RBC

Many works have been performed on glucose transport.[7] The authors studied incorporation of glucose, using normal RBC and AD-RBC. Glucose uptake increased with the lapse of time, but incorporation was inhibited in AD-RBC judging from the slower speed of glucose uptake into AD-RBC than into normal RBC (Fig. 2).

Effect of Thiol and Disulfide Compounds on Glucose Transport

Glucose transport into RBC in the presence of MPG, GSH, CySH and their oxidized forms was studied. As shown in Fig. 3, MPG and CySH increase glucose uptake in normal RBC, *i.e.* glucose uptake was increased in the presence of such compounds as MPG and CySH which are incorporated into RBC. Higher concentrations of GSH, which is not incorporated into

15) G.L. Ellman and H. Cysko, *J. Lab. Clin. Med.*, **70**, 518 (1967).
16) T. Chiba and S. Yoshikawa, *Yakugaku Zasshi*, **93**, 379 (1973); M. Horiuchi, T. Chiba, N. Toshioka and S. Okumura, *ibid.*, **93**, 679 (1973).

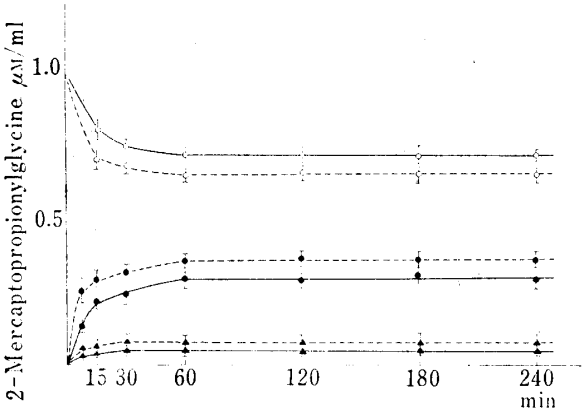

Fig. 1. Incorporation of 2-Mercaptopropionylglycine (MPG) into Red Blood Cells (RBC)

0.25 ml of 1.0μM MPG was allowed to react with a mixture of 1.0 ml of 70% RBC and 0.75 ml of a phosphate-NaCl solution (pH 7.4) at 37° and MPG amount was determined. Oxidized MPG amount in RBC was also determined.
○—○: MPG in medium of normal rat RBC
○---○: MPG in medium of alloxan diabetic rat RBC
●—●: MPG in RBC of normal rat
●---●: MPG in RBC of alloxan diabetic rat
▲—▲: oxidized MPG in RBC of normal rat
▲---▲: oxidized MPG in RBC of alloxan diabetic rat

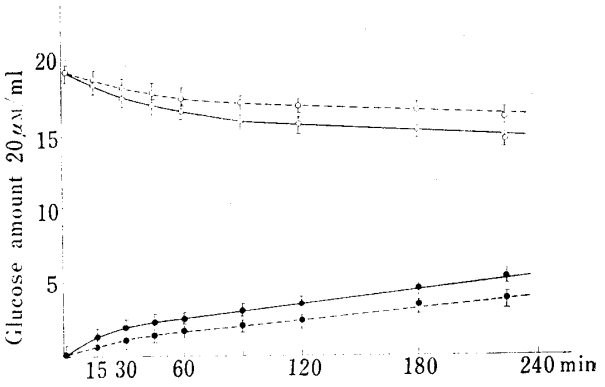

Fig. 2. Incorporation of Glucose into Red Blood Cells

1.0 ml of 20 μM Glucose was allowed to react with a mixture of 1.0 ml of 70% RBC and 1.0 ml of a phosphate-NaCl solution (pH 7.4) at 37° and glucose amount in the reaction solution and in RBC were determined.
○—○: glucose in medium of normal rat RBC
○---○: glucose in medium of alloxan diabetic rat RBC
●—●: glucose in RBC of normal rat
●---●: glucose in RBC of alloxan diabetic rat

RBC, showed a tendency to inhibit incorporation of glucose into RBC. In AD-RBC, GSH also increased glucose uptake (Fig. 3).

Glucose transport was inhibited in the presence of disulfide compounds. The inhibition was observed in both normal RBC and AD-RBC. Particularly, cystine intensely inhibited glucose transport into normal RBC and oxd GSH in AD-RBC (Fig. 4).

Effects of Various Inhibitors on Glucose Transport

It was found that MPG uptake is increased when RBC is treated with SH reagents.[16]

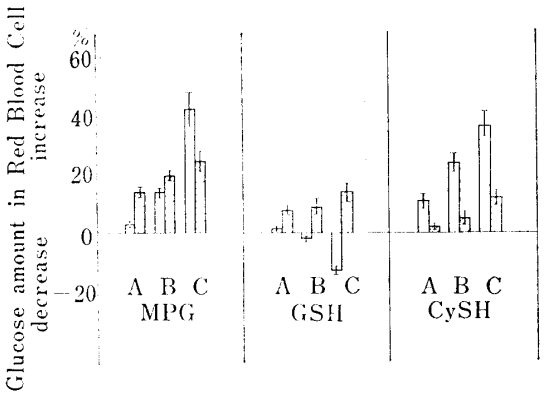

Fig. 3. Effect of Thiol Compounds on the Uptake of Glucose

20 μM glucose was allowed to react with 10^{-3}M, 10^{-4}M and 10^{-5}M thiol compounds (MPG, GSH, CySH) respectively at 37° for 60 minutes and glucose amount in RBC was determined. The glucose uptake in the absense of SH compounds is taken as 100 percent.
□: glucose amount in RBC of normal rat
▨: glucose amount in RBC of alloxan diabetic rat
MPG: 2-mercaptopropionylglycine
GSH: glutathione
CySH: cysteine
A: 10^{-5}M/ml thiol compound
B: 10^{-4}M/ml thiol compound
C: 10^{-3}M/ml thiol compound

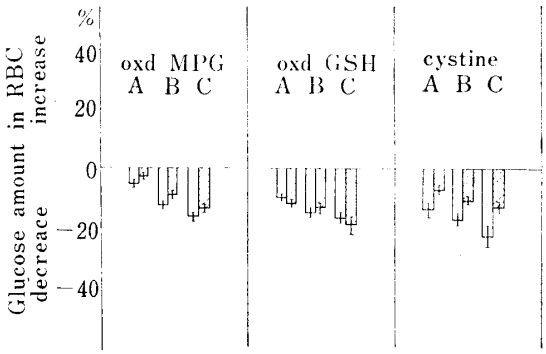

Fig. 4. Effect of Disulfide Compounds on the Uptake of Glucose

20 μM glucose was allowed to react with 10^{-3}M, 10^{-4}M and 10^{-5}M disulfide compounds (oxd MPG, oxd GSH, cystine) respectively at 37° for 60 minutes and glucose amount in RBC was determined. The glucose uptake in the basense of disulfide compounds is taken as 100 percent.
□: glucose amount in RBC of normal rat
▨: glucose amount in RBC of alloxan diabetic rat
A: 10^{-5}M/ml disulfide compound
B: 10^{-4}M/ml disulfide compound
C: 10^{-3}M/ml disulfide compound

Therefore, glucose uptake in the presence of SH reagents was investigated. As shown in Table II, SH reagents decreased glucose uptake. Insulin had no effect. Sodium borohydride increased it. These results agree with the report by Kahlenberg, *et al.*[17]

Table II. Effect of Various Inhibitors on Glucose Incorporation

Addition (10^{-4}M/ml)	Glucose uptake (% of control)	Addition (10^{-4}M/ml)	Glucose uptake (% of control)
None	100	N-Ethylmaleimide	78
$HgCl_2$	64	Sodium iodoacetate	82
PCMB	67	Insulin 0.2 U/ml	102
PCMBS	73	Potassium borohydride	123

Glucose amount incorporated into RBC in the presence of various inhibitors was determined. After RBC and inhibitors were washed with phosphate-NaCl solution and were allowed to react with 20 μM glucose for 30 minutes. Then, glucose amount in RBC was determined.

Disappearance of Glucose from Normal RBC and AD-RBC

Twenty μM glucose was allowed to react with RBC at 37° for 30 minutes, and was mixed with phosphate-NaCl solution. Glucose amount which disappeared from RBC was measured. Fifty per cent of glucose disappeared from normal RBC 9 minutes later and from AD-RBC 19 minutes later. Thus glucose quickly disappears from normal RBC.

The time required for the disappearance of 50% of glucose from normal RBC and AD-RBC was shortened to 7 minutes and 16 minutes respectively by the addition of MPG.

MPG increased glucose uptake into RBC (Fig. 3) and promoted disappearance of glucose from RBC. It is not clear whether MPG transport into RBC increases glucose metabolism or accelerates glucose out put. This result suggests that in AD-RBC, membraneous SH groups have been inactivated and the activity is recovered by SH compounds transferring through the RBC membrane (Fig. 5).

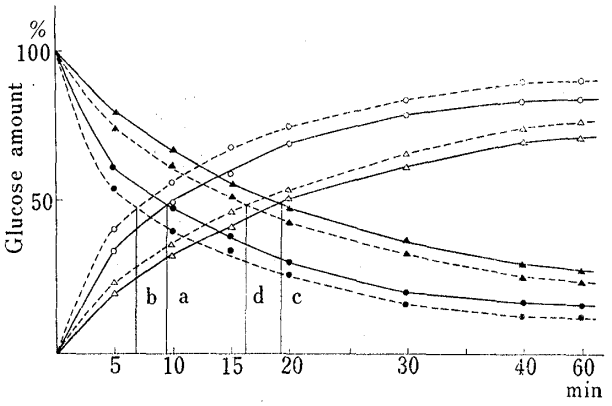

Fig. 5. Disappearance of Glucose from Red Blood Cells

After 20 μM glucose was allowed to react with RBC at 37° for 30 minutes, it was centrifuged and the RBC was transfered to a phosphate-NaCl solution. Glucose amount which disappeared from RBC was determined.

○—○: glucose amount of medium in absence of MPG (normal rat RBC)
○---○: glucose amount of medium in presence of MPG (normal rat RBC)
●—●: glucose amount of RBC in absence of MPG (normal rat RBC)
●---●: glucose amount of RBC in presence of MPG (normal rat RBC)
△—△: glucose amount of medium in absence of MPG (alloxan diabetic rat RBC)
△---△: glucose amount of medium in presence of MPG (alloxan diabetic rat RBC)
▲—▲: glucose amount of RBC in absence of MPG (alloxan diabetic rat RBC)
▲---▲: glucose amount of RBC in presence of MPG (alloxan diabetic rat RBC)
a: cross point of normal rat (absence of MPG)
b: cross point of normal rat (presence of MPG)
c: cross point of alloxan diabetic rat (absence of MPG)
d: cross point of alloxan diabetic rat (presence of MPG)

K_m and V_{max} for MPG and Glucose Transport into RBC

It is known that in case of free diffusion a linear relationship exists between $[S]$ and $[V]$, and that the carrier mediated transport follows Linewear–Burk's equation.[18] Sen, *et al.*

17) A. Kahlenberg, B. Urman and D. Dolansky, *Biochemistry*, **10**, 3154 (1971); R.I. Weed, J. Eber and A. Rothstein, *J. Gen. Phys.*, **45**, 395 (1962).
18) R.W. Hendler, "Protein Biosynthesis and Membrane Biochemistry," John Wiley & Sons, Inc., New york, 1968.

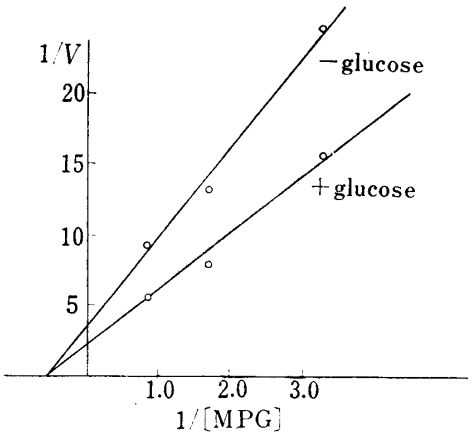

Fig. 6. Incorporation of 2-Mercaptopropionylglycine into Red Blood Cells in the Presence or Absence of Glucose

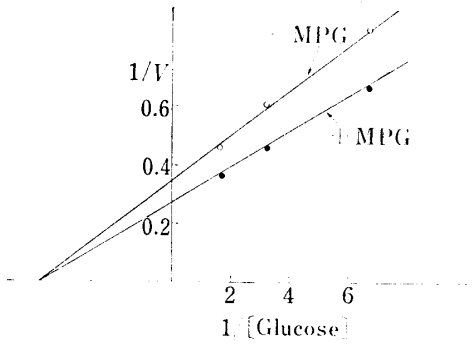

Fig. 7. Incorporation of Glucose into Red Blood Cells in the Presence or Absence of 2-Mercaptopropionylglycine

	Glucose (20 μM)	V_{max}	K_s (μM)
Normal rat RBC	−	0.28	1.68
	+	0.44	1.77
Alloxan diabetic rat RBC	−	0.56	2.39
	+	0.67	2.17

	MPG (1.0 μM)	V_{max}	K_s(mM)
Normal rat RBC	−	3.03	2.25
	+	3.70	2.20
Alloxan diabetic rat RBC	−	3.08	8.21
	+	4.44	8.33

reported that K_m depends on temperature, and the K_m for glucose transport in human RBC is 4.0±0.24 mM at 37°.[19]

MPG uptake in the presence and absence of glucose was investigated.

V_{max} became higher in the presence of glucose, but K_m did not. V_{max} was higher in AD-RBC than in normal RBC (Fig. 6).

Glucose transport in the presence and absence of MPG was also studied (Fig. 7).

In the presence of MPG, V_{max} increased but K_m remained same, and K_m was higher in AD-RBC than in normal RBC.

Discussion

The mechanism of transfer of compounds across the membrane has been reported by Schanker, *et al.*, who demonstrated that non-ionic molecules of many organic weak electrolytes are transferred through the cell lipidic membrane into cells by free diffusion.[20] On the other hand, modification of membraneous protein and lipid results in alteration of permeability to which ion transfer is susceptible. Weed, *et al.*[21] found that inhibition of the membrane by PCMB or PCMBS which have inhibitory activities on SH groups causes alteration of ion transfer which induces loss of K^+ from RBC. The authors reported that thiol and disulfide compounds promote glucose incorporation into rat diaphragm.[22] These evidences suggest that SH groups of the membrane have significant effects on compound transport.[5]

Transfer of thiol and disulfide compounds into RBC was investigated from various aspects.[16] Approximately 30% of MPG and CySH was transported into RBC 30 minutes after reaction (Fig. 1), but GSH was not. Oxd MPG equivalent to 5% of incorporated MPG was found to exist in RBC, and this suggest that MPG was oxidized in RBC. MPG is not bound with the cell membrane, but is incorporated into intracellular cyto plasma. Since

19) A.K. Sen and W.F. Widdas, *J. Physiol.*, **160**, 392 (1962).
20) L.S. Schanker, *Pharmacol. Rev.*, **14**, 501 (1962).
21) R.M. Sutherland, A. Rothestein and R.I. Weed, *J. Cell. Physiol.*, **69**, 185 (1967); P.J. Garraham and A.F. Rega, *J. Physiol.*, **193**, 459 (1967).
22) T. Chiba, *Yakugaku Zasshi*, **89**, 248 (1969); T. Chiba, *ibid.*, **89**, 572 (1969).

MPG uptake increases with increasing MPG concentration, it is considered not to be carrier-mediated diffusion.[18] When GSH is oxidized while suspended in a solution containing neither RBC nor glucose, supply of NADPH from HMP cycle is decreased. In the presence of glucose, even if GSH is oxidized to oxd GSH, the same GSH level is maintained by NADPH and glutathione reductase which are supplied from HMP cycle, and this fact means that presence of glucose has effects on metabolic system in RBC.[23] MPG uptake was further increased in the presence of glucose (Table I). Approximately 10% increase in MPG amount was observed in AD-RBC when compared with normal RBC. It suggests that structural change occurs at the membrane surface. Besides, MPG transfer through the membrane was increased by SH reagents.[16]

Sugar is believed to be transported into RBC by facilitated diffusion and the process is generally referred to as carrier-mediated transport.[24]

Glucose uptake is inhibited by various inhibitors. PCMB, $HgCl_2$, *etc.* are non-competitive inhibitors[25] and phloretin is a competitive one.[26] Glucose uptake was inhibited in AD-RBC as Fig. 2 shows, and by $HgCl_2$, PCMB, *etc.* (Table II).

In the investigation on the effects of thiol and disulfide compounds on glucose uptake, MPG and CySH promoted glucose transfer into RBC, while GSH inhibited it. All the SH compounds promoted glucose transfer into AD-RBC (Fig. 3). Oxd thiol and disulfide compounds inhibited glucose transport into both normal RBC and AD-RBC (Fig. 4).

Thus, presence of MPG and CySH promotes glucose transfer into normal RBC and AD-RBC, and their oxidized forms inhibit it. The same tendency was observed regarding the disappearance of glucose from RBC (Fig. 5).

Evans, *et al.*[27] found that glucose uptake into RBC is increased by treatment of RBC with potassium borohydride, and consider that facilitated diffusion is promoted by the formation of imine bonds with lysyl residues of a protein present in the cell membrane.[28]

On the contrary, Kahlnberg, *et al.* have reported that proteolytic digestion of RBC membranes results in a 3- to 5-fold increase in glucose uptake, while phospholipase digestion of them results in a loss of glucose uptake.[29] They attributed it to the exposure of masked glucose binding sites owing to proteolytic enzymes.

SH compounds is considered to increase glucose uptake by the following mechanisms. 1) Formation of thio-glucose which results from the thioglucoside bond of glucose with SH compounds.[30] 2) Imine bond between lysyl groups of membranous protein and glucose promoted by reductive activity of SH compounds,[28] or 3) Activation of glycolysic enzymes in RBC and of enzymes or carriers in the cell membrane.[8,17]

Thiol compounds which pass through cell membranes activate glucose uptake in intracellular cytoplasma or in the cells but disulfide compounds which do not pass through inhibit glucose uptake on membrane surfaces. It can therefore be presumed that membranous thiol groups are concerned in glucose uptake. This suggests that in diabetic state membranous thiol groups is decreases and accordingly glucose utilization decreases.

Acknowledgement We wish to express our thanks to Mr. Shokyu Mita, president of Santen Pharmaceutical Co., Ltd., for the opportunity of publishing this report and to Dr. Itaru Mita, Executive Vice-President and Dr. Shigeo Okumura, Director, for the technical direction and assistance.

23) S.K. Srivastava and E. Beutter, *J. Biol. Chem.*, **244**, 9 (1969).
24) H. Passow, "The Red Blood Cells," ed. by C. Bishop, Surgenor, Acad. Press., New York, 1961, p. 71; I.M. Glynn, *Progr. Biophys. Biophys. Chem.*, **8**, 242 (1957).
25) A.C. Dawsor and W.F. Widdas, *J. Physiol.*, **168**, 644 (1963).
26) A.K. Sen and W.F. Widdas, *J. Physiol.*, **160**, 404 (1962); P.G. Lefevre, *Science*, **130**, 104 (1959).
27) D.R. Evans, B.C. White and R.K. Brown, *Biochem. Biophys. Res. Comm.*, **28**, 699 (1967).
28) R.G. Langdon and J.R. Sloan, *Proc. N. A. S.*, **57**, 401 (1967).
29) A. Kahlenberg, D. Dolansky and R. Rohrlick, *J. Biol. Chem.*, **247**, 4572 (1972).
30) P.G. Lefevre, *Pharm. Rev.*, **13**, 39 (1961).