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Abstract

As the first step to analyze the autoimmune disease of red cells the recognition mechanism of macrophage to red cells or erythrophagocytosis has been studied in vitro by using mouse peritoneal macrophage and homologous and cells and the following results were obtained: 1. In Hanks solution, the mouse macrophage hardly phagocytizes living red cells, both homologous and heterologous ones. But in the presence of mouse serum, the macrophage phagocytizes heterologous red cells selectively but does not phagocytize homologous ones. 2. The macrophage actively phagocytized homologous red cells prior to treatment with concanavalin A (Con A) at a concentration as low as 1.95 ltg/ml. 3. Red cell agglutination was clearly recognized in those treated with Con A at 62.5 lag/ml or more, but not at 1.95 ltg/ml. 4. The red cell agglutination by Con A was inhibited with D-glucose, D.mannose and a-methyl glucopyranoside at the concentration as low 1.5 mM, while the phagocytosis was suppressed only at a very high concentration of the sugars, 1, 000 mM. 5. Fragility test of the red cells treated with Con A showed a lower resistance of red cells to hypotonic solution than those treated with Con A at the concentration of 31.25 p.g/ml or more 6. Electron microscope observation revealed no membrane damage of red cells by treating with Con A at a concentration of 1.95 ,ag/ml, where erythrophagocytosis was observed. The membrane damage occur. red by treating with Con A at 31.25 ltg/ml or higher. 7. All the data indicate that the phagocytosis of homologous red cells by macrophage is induced by the adherence of a small amount of Con A, which induces no detectable changes of red cell surface and red cell membrane as revealed by agglutination test, fragility test, electron microscope observation and circular dichroism. On the basis of these observations a possible recognition error to homologous red cells by adsorbing a minute quantity of foreign substances on their surfaces has been discussed.

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STUDIES ON THE CELLULAR RECOGNITION MECHANISM: EFFECT OF CONCANAVALINA ON THE PHAGOCYTOSIS OF HOMOLOGOUS RED CELLS BY PERITONEAL MACROPHAGE

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The mechanism of autoimmunity is unknown but it should be the problem to be solved urgently as the ideal treatment of the autoimmune disease will be made solely on the theoretical basement. The problem seems to lie in the faulty cell recognition mechanism of the patients. Immunological response to foreign materials may be conveniently divided into two types; the one, no macrophage participating and the other, macrophage participating. MILLER and others (1, 2, 3), and COUDKOWICZ and associates (4) showed that a production of antibody for foreign soluble material, globulin, is conducted by the antigen reacting cells or antibody forming cells, while FISHMAN and coworkers (5) and BROWN and associates (6) indicated that the production of antibody for foreign gross particles and corpuscles such as T_2 bacteriophages and blood cells, is triggered by the phagocytic process of the macrophage. Thus, in the case of immune responce to own cells macrophage should participate, *e.g.* in immuno-hemolytic anemia the recognition error to own red cells may occur at the macrophage level. As is generally known, the macrophage does not phagocytize the homologous functioning red cells. The macrophage, however, phagocytizes actively even homologous red cells when they are chemically denatured or dead (7), but it is also true that those denatured or dead cells are of the character of foreign substances and are much less effective in forming antibody specific to functioning cells. Thus, it is very important and interesting to study how the macrophage recognizes materials either self or non-self in its phagocytic process. The mechanism should lie in the function to recognize even a slight difference of the surface of particles.

Concanavalin A (Con A), a plant agglutinin, agglutinates red cells and tumor cells by binding to their surfaces (8, 9). The fact seems to indicate that Con A would alter the cell surface as in general chemical and thermal treatments. But the problem is whether or not functioning

red cells are made susceptible to macrophage phagocytosis by the action of Con A at a lower concentration than that induces changes in the structural conformation of cell surface and cell membrane. The author attempted to observe how macrophage detects and ingests the foreign bodies selectively, distinguishing from the cells and components of its host and how the recognition error occurs in macrophage to its own functioning red cells. The paper presents the fact that the functioning homologous red cells are phagocytized by macrophage when they have been treated with Con A at a very low concentration in which Con A does not cause the detectable changes in red cell membrane and surface as revealed by red cell agglutination test, fragility test, electron microscope observation and circular dichroism (CD).

MATERIALS AND METHODS

Macrophages were obtained from Ehrlich tumor ascites. As already demonstrated by YOKOMURA and SENO (10), SHIMIZU, YOKOMURA and SENO (11) rather intact peritoneal macrophages can be obtained from tumor ascites than those from inflammatory ascites obtained by the intraperitoneal injection of various foreign substances. Adult mice of ddN strain of both sexes weighing from 17 to 25 grams were inoculated with Ehrlich ascites tumor cells and the peritoneal fluid was withdrawn 3 to 4 days after the tumor cell inoculation according to the method of YOKOMURA and SENO (10) and SHIMIZU, YOKOMURA, SENO (11). The harvested peritoneal fluid was diluted 10 times with cold Hanks solution, poured into Petri dish (2 cm in diameter) containing a cover-slide (18×18 mm) and incubated at 37°C for 60 min. After the incubation the cover-slide, on which the macrophages were adhered, was taken out and rinsed gently with freshly prepared warm Hanks solution (27 to 30°C) according to the modified method of RABINOVITCH (12). In this way macrophages obtained settled on the surface of the cover slide. These macrophages on cover-slide were used for the phagocytosis test. During the incubation fibrin clots appeared occasionally and they were removed with a steel needle.

Red cell suspensions were prepared with the blood from retro-orbital sinus of mice, ear vein of rabbit, heart of guinea pig and jugular vein of cow and chick. The blood was diluted with cold glucose-free Hanks solution and immediately centrifuged at a low speed (100×g) for 10 min. The precipitated red cells were washed 4 times with glucose-free Hanks solution and finally resuspended in the same solution adjusting the red cell number to 10^6 red cells per cu mm. The cells were used without post-treatment or after treating with mouse serum, Con A and glutaraldehyde. Besides these, red cell ghosts were used for the observation of the conformational changes of the cell membrane by circular dichroism.

The blood serum was prepared with blood from retro-orbital sinus of mice and rat, and jugular vein of chick. The blood was kept at 37°C for 30 min,

allowed to stand at 4°C for 12 hr, centrifuged at 100×g for 10 min and serum was separated.

For the treatment with Con A the red cell suspension was added to the equal volume of Con A solution. The final concentration of Con A was 1.25 to 2,000 μg/ml (5×10^5 red cells/mm^3) and these suspensions were incubated at room temperature for 30 min using a rotatory incubator (6 cycles/min). For the inhibition test by sugars an equal volume of these Con A-sugar mixtures was added to the red cell-macrophage systems (see below) in place of Con A, and after incubation the red cell agglutination and phagocytosis were checked microscopically. The Con A sugar mixtures were prepared by mixing the solutions of α-methyl glucopyranoside, D-glucose and D-mannose, 0.001 to 1 M in each, with an equal volume of Con A solution, 62.5 μg/ml, and incubated for 30 min at 37°C.

For glutaraldehyde treatment of the red cells, they were fixed with 1.25 %, glutaraldehyde in Millonig's phosphate buffer solution (pH 7.4) at 15°C for 30 min, washed 5 times with glucose-free Hanks solution by repeated centrifugation and allowed to stand overnight at 5°C and used for phagocytosis test.

For observation of phagocytosis, monolayer macrophages on cover-slide were incubated with the red cells, treated and non-treated, at 37°C for 60 min shaking gently (60 cycles/min). The final red cell number in the medium was 1.5×10^4 red cells/mm^3 and the medium volume was of 2-3 ml in each experiment. After the incubation the cover-slide having macrophages was rinsed gently with glucose-free Hanks solution, air-dried, fixed with methanol and stained with Giemsa. Phagocytosis rate was obtained as the ratio of the number of the macrophages adhering red cells to the total number of 1,000 to 1,500 macrophages counted because the red cell adhesion observed by light microscope showed partially ingested features of red cells in the macrophage under the electron microscope.

Electron microscope observation was made on the red cells agglutinated by Con A and the macrophages incubated with red cells treated with Con A. The Con A-treated red cells and those mixed with tumor ascites macrophage were incubated for 60 min at 37°C, centrifuged and the packed cells were fixed with 1.25% glutaraldehyde in Millonig's phosphate buffer solution (pH 7.4), post-fixed with 1 % OsO_4 for 60 min, washed twice with water, dehydrated through alcohol and imbedded in Epon by the conventional method. The thin sections were prepared by microtome, Porter-Blum MT-1, stained with uranil acetate and lead citrate, and observed under electron microscope, Hitachi HU-11A.

For the observation of the conformational changes of red cell membrane the red cell ghosts were used which were prepared by pouring red cells into the hypotonic PBS, 20 mOsM, pH 7.4, according to the method of DODGE (13). These ghosts were treated with Con A at varied concentrations. Loss of α-helix was measured by the depth of the troughs at 200-250 nm of CD spectra. CD spectra were drawn by using the apparatus, Jasco ORD/UV-5 with CD attachment. Samples were prepared as follows; The red cell ghost suspension, 300 μg ghost protein/ml (this concentration corresponds to 5×10^4 intact red cells per mm) in the phosphate buffer, was homogenized into 3 to 7 μ particles with a

tephron homogenizer before recording CD spectra and used immediately, as the ghosts treated with Con A agglutinated to produce remarkable noise in the CD spectrum. The light path of the cuvette was 5 mm. By using the residual suspension the protein contents were estimated by biuret reaction (13) after solubilizing the ghost with 0.5% sodium deoxycholate. Osmotic fragility test was carried out by the method of PARPART (15) by estimating the amount of hemoglobin released from hemolysed red cells.

The reagents used were Con A purchased from SERVA Co. Led., α-methyl glucopyranoside from Sigma Chemical Co. Ltd., Giemsa and OsO_4 from A. Merck. The other reagents were the commercial products of the highest purity.

RESULTS

In Hanks solution, the mouse macrophage phagocytized actively the glutaraldehyde fixed red cells irrespective of their origin but hardly phagocytized unfixed living red cells, both homologous and heterologous ones. The chick red cells were phagocytized by the macophages in all cases tested. In the presence of mouse serum, 10%, however, the macrophages phagocytized heterologous red cells selectively but not homologous ones (Table 1). However, in the presence of heterologous serum, the

TABLE 1 PHAGOCYTOSIS TEST OF MOUSE PERITONEAL MACROPHAGE TO RED CELLS OF VARIOUS ORIGINS, TREATED AND NON-TREATED, IN HANKS SOLUTION WITH OR WITHOUT HOMOLOGOUS SERUM

Origin of red cell	Number of macrophages phagocytizing red cells (%)		
	Fresh red cells in Hanks soln.	Fresh red cells in Hanks soln. containing mouse serum	Fixed cells in Hanks soln.
Mouse	1.5	1.4	97.0
Guinea pig	3.3	42.2	99.1
Cow	2.0	37.0	—
Rat	6.0	62.0	99.0
Rabbit	9.7	97.0	97.5

TABLE 2 PHAGOCYTOSIS OF MOUSE RED CELLS BY MOUSE PERITONEAL MACROPHAGE PHAGOCYTOSIS OF HETEROLOGOUS SERUM TREATED MOUSE RED CELLS BY MOUSE PERITONEAL MACROPHAGE.

Origin of treated serum	Origin of red cells	In Hanks soln. containing treated serum, phagocytized macrophage ratio (%)
Mouse	Mouse	1.4
Rat	Mouse	1.6
Chick	Mouse	5.2
Mouse	Chick	89.9
Chick	Chick	73.3
Hanks only	Chick	43.4

The red cells were mixed with hanks solution contaning 10% serum and the macrophages were added to allow incubation for 60 min at 37°C.

mouse macrophage did not phagocytize the homologous red cells (Table 2), even though the macrophage retains its phagocytic activity even in the heterologous serum as revealed by phagocytosis test with chick red cells. Con A treatment of unfixed red cells made them susceptible to phagocytosis by macrophages.

The unfixed mouse red cells, which never adhered to or phagocytised by mouse macrophage even in the presence of homologous serum, were actively taken up by the macrophages after treating with Con A at a very low concentration, 1.95 μg/ml (5×10^5 red cells/mm^3), where red cell agglutination was not observed (Table 3). Red cell agglutination by

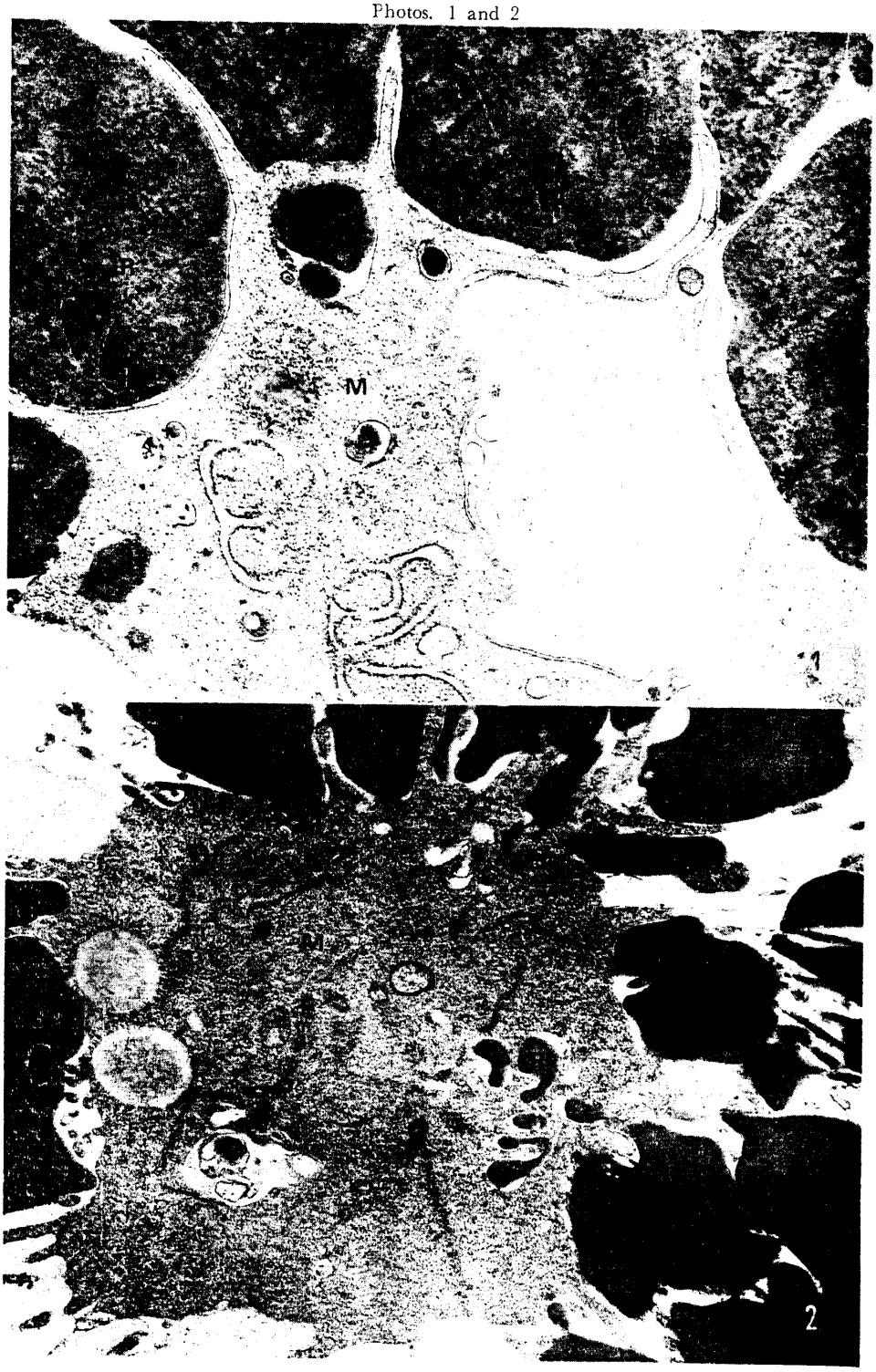
TABLE 3 EFFECT OF CON A ON THE ERYTHROPHAGOCYTOSIS BY HOMOLOGOUS MACROPHAGE AND RED CELL AGGLUTINATION

Con A concentration (μg/ml)	Reaction of macrophage		
	Red cell adherence and partial ingestion	Complete red cell ingestion	Red cell agglutination
0.0	1.5	−	−
0.12	3.0	−	−
0.24	5.0	−	−
0.48	10.86	−	−
0.97	46.0	−	−
1.95	94.0	−	−
3.90	94.0	±	−
7.81	99.0	±	−
15.62	99.0	±	−
31.25	98.0	+	±
62.5	99.0	++	+
125.0	98.0	+++	++

The macrophaces phagocytize actively the homologous red cells treated with Con A even at a low concentration where it does not induce red cell agglutination.

Con A occurred slightly at 31.25 μg/ml (5×10^5 red cells/mm^3) and became marked at higher Con A concentrations (Table 3). Adherence of the red cells to macrophage was also increased with the increase in the Con A concentration (Photos. 1, 2). The complete ingestion of red cells by macrophage was frequently encountered in those treated with Con A at 31.25μg/ml (5×10^5 red cells/mm^3) and became more marked with the increase in Con A concentration for red cell treatment (Table 3 Photos. 3, 4, 5). Red cell ingestion by macrophage was hardly met with in red cells treated with Con A at lower concentration than 31.25 μg/ml (5×10^5 red cells/mm^3). In these experiments the red cells treated with Con A were not washed but the cell suspension was diluted to 1/50 with glucose-free Hanks solution and this red cell suspension was added to macrophages on

Photos. 1 and 2



cover-slide. Therefore, the incubation media contained Con A, 1.5μg/ml (5×10^5 red cells/mm^3) at the highest, but in this medium no intact homologous red cells adhered to the macrophage. These results show that there is no relation between Con A concentrations for red cell agglutination and for red cell phagocytosis by macrophage.

Conformational change of red cell ghost protein by Con A was observed first at an extremly high concentration of Con A, 1, 000 to 2, 000 μg/ml (5×10^5 red cells/mm^3) as the loss or reduction in α-helix content of the membrane protein. As shown in Fig. 1 curve G, the CD spectra of red cell ghosts in Hanks solution showed a typical α-helix feature with two minima in the ultraviolet region, at about 210 and 224 nm. These

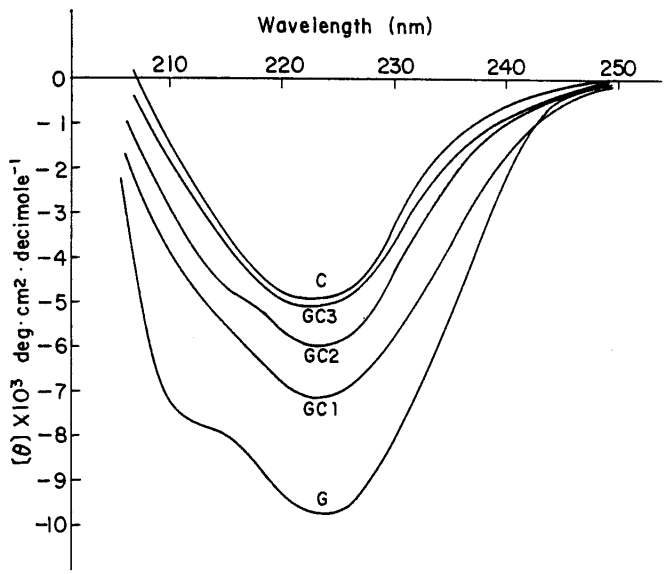
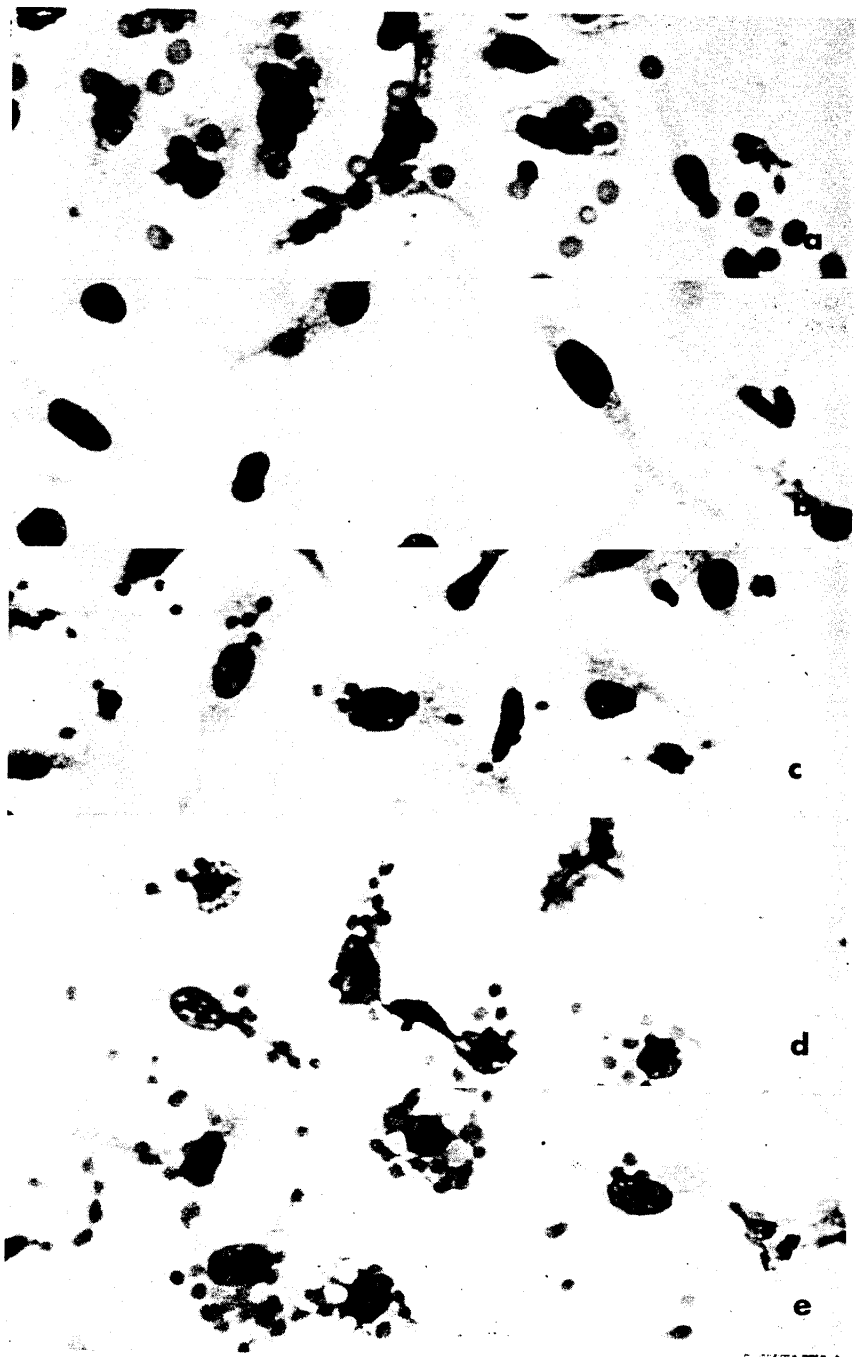


Fig. 1. Effect of Con A on the CD spectra of red cell membrane.
G; native red cell ghosts in 20 mOsM PBS pH 7.4
GC1; Con A-treated red cell ghost: ghosts 300 μg/ml, were incubated with Con A, 500 μg/ml for 30 min 25°C, in PBS pH 7.4 The curve also indicates the average of Con A and native ghosts, suggesting no conformational change of ghosts.
GC2; Con A treated ghosts; incubated with Con A, 1000 μg/ml in PBS pH 7.4
GC3; Con A-treated ghosts; incubated with Con A, 2000 μg/ml in PBS pH 7.4

Photos. 1, 2 Electron microscope picture of mouse red cells adhered to the surface of a mouse peritoneal macrophage. Some parts of red cells are taken by macrophage. The red cells were pretreated with Con A, 31.25 μg/ml (5×10^5 red cells/mm^3) for 60 min. and then incubated with macrophages at 37°C for 60 min. M: macrophage, R: red cell
×40,000: Photo. 1 ×20,000: Photo. 2

Photo. 3



two minima arise from and uniquely characterize the α-helical structure of the polypeptide chain (16). The CD spectrum of the ghosts (300 μg protein/ml) from red cells treated with Con A at the concentration of 500 μg/ml (300 μg protein/ml) (Fig. 1 GC1) gave a curve running just the middle of those drawn with Con A in Hanks solution (Fig. 1, C) and native red cell ghosts (Fig. 1, G). The CD spectrum given by red cells treated with Con A at 2,000 μg/ml (300 μg ghosts protein/ml) (Fig. 1, GC3) was practically identical with that of Con A. The CD spectrum of Con A-D-glucose complex gave the identical curve with that of Con A

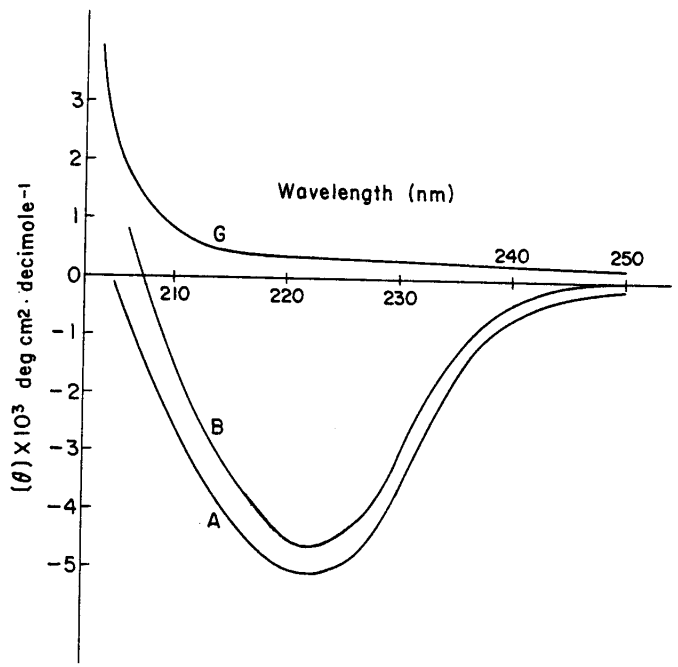
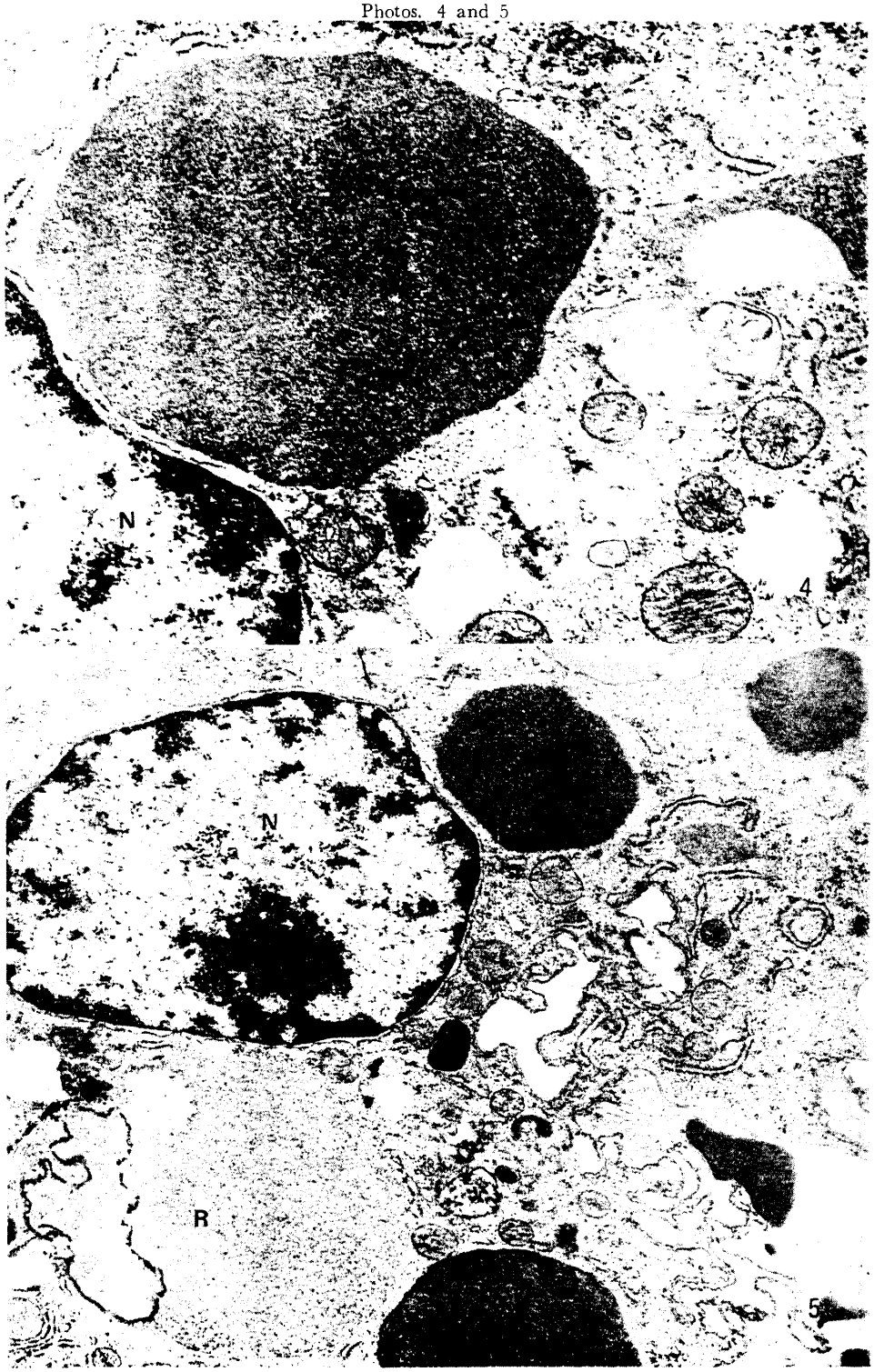


Fig. 2. CD spectra of Con A (A), glucose (G) and Con A-glucose mixture (B) in 20 mOsM phosphate buffer, pH 7.4

Con A-glucose mixture was prepared by mixing 1 ml of Con A 2000 μg glucose 1 M at an equal volume and CD spectrum was obtained with the diluted solution, 50 times.

Photo. 3 Light microscope picture of mouse macrophages ingesting mouse red cells treated with Con A. a, phagocytosis of glutaraldehyde fixed cells b, macrophages given unfixed red cells without pretreatment c, macrophages incubated with the treated cells 1.95 μg/ml (5×10^5 red cells/mm^3) d, Con A-treated red cells 31.25 μg/ml (5×10^5 red cells/mm^3) e, macrophages incubated with the Con A-treated red cells 62.5 μg/ml (5×10^5 red cells/mm^3) The red cell ingestion by macrophage was observed in those treated with Con A at 31.25 (5×10^5 red cells/mm^3) or more and became marked with the increase in Con A concentration. $\times$ 1200

Photos. 4 and 5



(Fig. 2). The results were similar to those of MOLLIE *et al.* (17). Of these CD spectra the helical loss of red cell ghosts by Con A treatment was calculated at the trough, 222 to 224 nm. No helical loss was observed on the red cell ghosts treated with Con A at a concentration less than 500 μg/ml (300 μg protein/ml) (Table 4). In this conception the CD spectrum of

TABLE 4 THE LOSS OF α-HELIX CONTENTS IN THE RED CELL GHOSTS PROTEIN TREATED WITH CON A AS REVEALED BY THE OBSERVATION OF CD SPECTRA.

Con A concentration (μg/ml per 300 μg/ml ghosts protein)	Helix content (%)	Loss of helix (%)
0.0	24	—
62.5	24	00
500.0	24	00
1000.0	19	20
2000.0	11.5	52
Glutaraldehyde fixation (1.25%)	22	8.3

Helical contents were calculated with the depth of troughs at 222–224 nm of cd spectral curves. Method: Refer to the text.

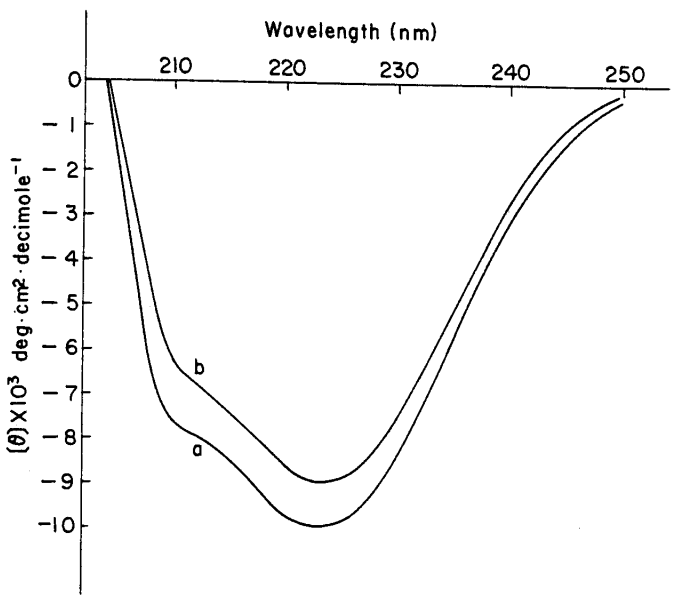


Fig. 3. Circular dichroism spectra of red cell ghosts, native (a) and fixed (b) in 20 mOsM PBS pH 7.4

Fixation was made with 1.25% glutaraldehyde for 30 min. For the refer to the text.

Photo. 4, 5 Flectron microscope pictures of the macrophages ingested red cells

The macrophages were incubated for 60 min at 37°C with the Con A treated red cells, 31.25 μg/10^5 red cells pear mm^3). The some ingested red cells lower their electron densty. M: macrophage R: ingested red cell N: macrophage nucleus × 40,000

glutaraldehyde-fixed red cell ghost was observed, which was phagocytized actively by macrophage. Helical content of the red cell ghosts fixed with glutaraldehyde was 22 % and the helical loss was calculated as 8.3 %. The results were similar to those of LENARD and SINGER (18). This loss was less than that observed after Con A treatment at the concentration of 1,000 μg/ml. This fact shows that the conformational change of the red cell membrane by Con A is not directly concerned with the phagocytosis (Table 4, Figs. 1, 3).

Fragility test revealed that a marked acceleration of hemolysis occurred in the red cells treated with Con A at the concentration of 62.5 μg/ml (5×10^5 red cells/mm^3), but no acceleration on those treated with Con A of 1.95 μg/ml, where the cells were susceptible to the macrophage phagocytosis (Fig. 4). These facts also indicate that the conformational change of the red cell membrane treated with Con A will not be related to the phagocytosis.

The phagocytosis was checked by light microscope and the adherence of red cell or rosette formation surrounding macrophage was taken as phagocytosis,

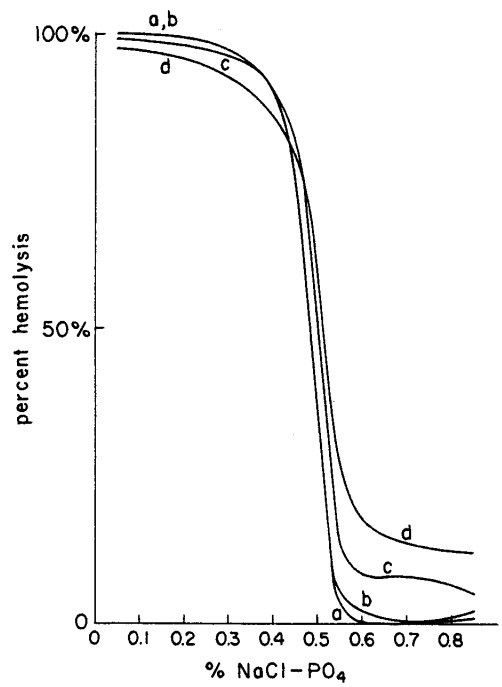
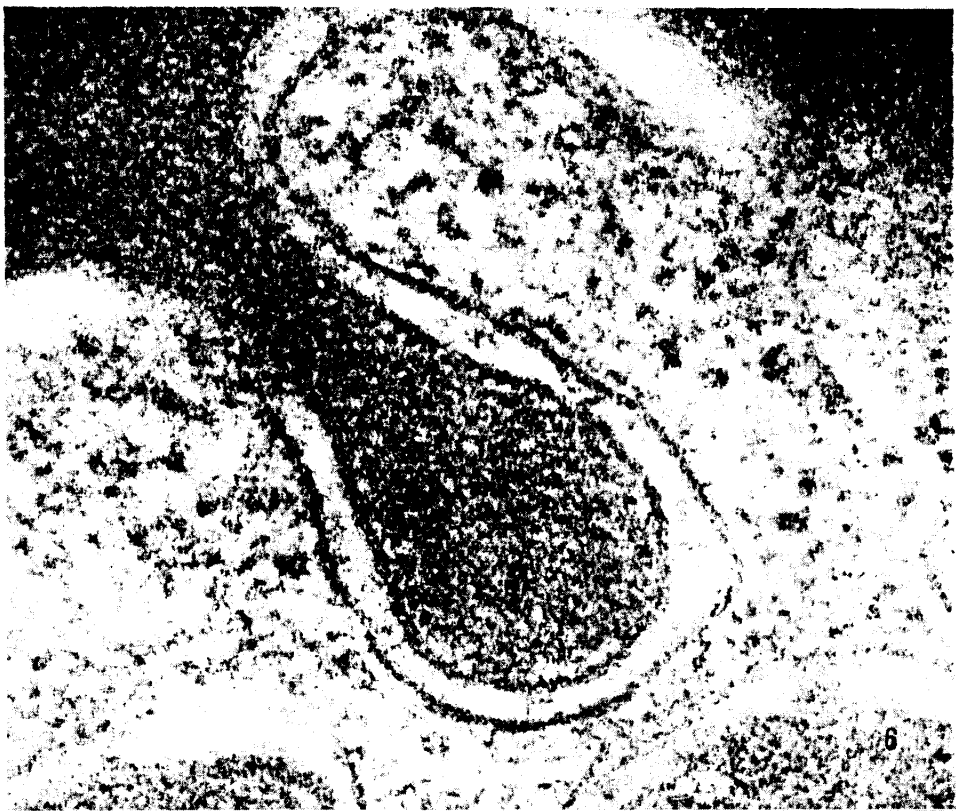


Fig. 4. The effect of Con A on the osmotic fragility of mouse red cells
a: Con A treated with 1.95 μg/ml (5×10^5 red cells/mm^3)
b: no treatment
c: Con A treated with 31.25 μg/ml (5×10^5 red cells/mm^3)
d: Con A treated with 62.5 μg/ml (5×10^5 red cells/mm^3) The method refer to the text.

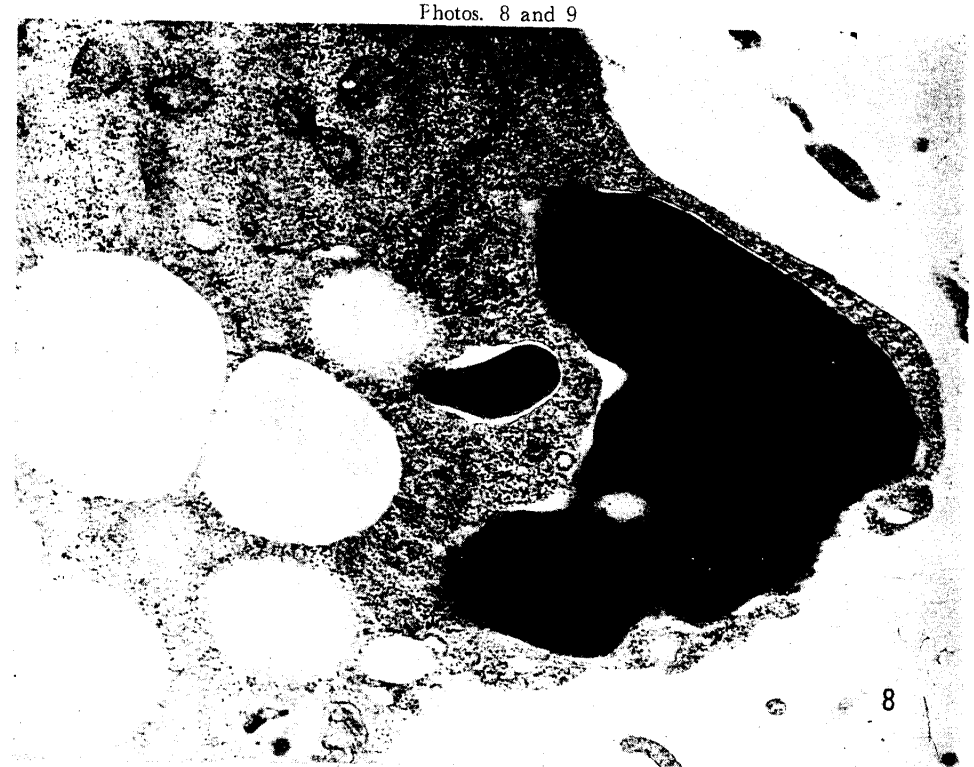
Photo. 6 An enlarged picture of the cytoplasmic surface of macrophage (M) to where a red cell (R) is adhered. Amorphous substance, probably Con A, can be seen in intercellular space (I). A picture taken from the sample treated similarly as in Photo. 1. ×280,000

Photo. 7 Demolition of the mouse red cell membrane after treating with Con A. a, intact red cell membrane without Con A treatment, b, treated with Con A 1.95 μg/ml (5×10^5 red cells/mm^3), c, treated with Con A 31.25 μg/ml d, treated with Con A 1,000 μg/ml (5×10^5 red cells/mm^3). By treating of the mouse red cells with Con A at the concentrations 1.95 μg/ml or more the demolition of the membrane (arrows) can be seen. ×280000

Photos. 6 and 7



Photos. 8 and 9



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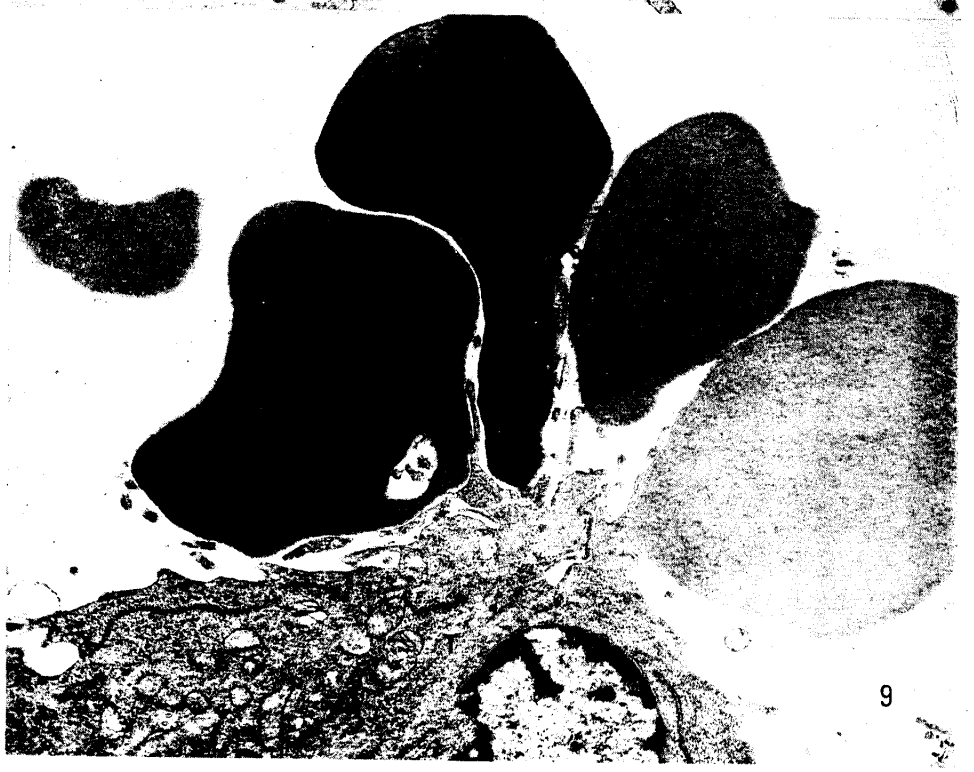

9

as the adherence is the first step of phagocytosis (19, 20, 21). Electron microscope observation revealed that in the red cells adhered to macrophage or forming rosetts surrounding the cell, some part of their cytoplasm was taken into the macrophage (Photos. 1, 2). Some ambiguous fibrillar structures were observed between the macrophage surface and adhered red cell membrane (Photo. 6). The membrane structure of red cells treated with Con A at 1.95 μg/ml (5×10^5 red cells/mm^3) remained intact (Photo. 7, b) but by treating with Con A at 1,000 μg/ml (5×10^5 red cells/mm^3), where the helical loss was observable by CD spectra, a marked distortion of the membrane structure was observed (Photo. 7, d). The red cells treated with Con A at the concentration of 31.25 μg/ml (5×10^5 red cells/mm^3) showed a slight irregularity in the membrane struc-

TABLE 5 THE EFFECT OF SUGARS ON THE PHAGOCYTOSIS OF CON A TREATED-RED CELLS AND RED CELL AGGLUTINATION BY CON A.

Sorts and concentration of sugars (mM)		Number of macrophage phagocytized red cells (%)	Red cell agglutination
Mannose	1.0	99.0	+
	1.5	90.0	−
	125.0	63.1	−
	250.0	20.5	−
	500.0	10.3	−
	1000.0	8.9	−
Glucose	1.0	99.0	+
	1.5	89.1	−
	125.0	69.3	−
	250.0	45.9	−
	500.0	23.8	−
	1000.0	8.8	−
*α-MG	1.0	99.0	+
	1.5	95.9	−
	125.0	97.8	−
	250.0	63.7	−
	500.0	7.3	−
	1000.0	3.2	−
no Con A treatment		1.5	−

*α-MG ; α-methyl glucopyranoside

The mouse red cells were previously incubated with Con A, 62.5μg/ml and sugars of varied concentrations for 30 min at room tenperature and reincubated with mouse peritoneal macrophages for 60 min at 37°C.

Photo. 8, 9 Adhesion (Photo. 9) and ingestion (Photo. 8) of the Con A-treated red cells by Ehrlich ascites tumor cell Con A 31.25 μg/ml (5×10^5 red cells/mm^3). Method see text R : red cell, T : Ehrhich ascites tumor cell $\times$ 20,000

ture (Photos. 7, c). The Con A-treated red cells also adhered to tumor cell and finally were ingested by the latter (Photos. 8, 9).

As just described, the phagocytosis of macrophge is triggered by adherence with red cells. Therefore, the phagocytosis of red cells may be the mechanism similar to that of red cell agglutination being mediated by the active groups of Con A surface. Observations revealed that the Con A-induced red cell agglutination was suppressed by D-glucose, D-mannose and α-metyl glucopiranoside (Table 5), suggesting that the binding of Con A to red cell surface was closely related to the above sugar moieties on the red cell surface, but these sugars do not inhibit the red cell phagocytosis by macrophage mediated by Con A at the lower concentration where red cells agglutination was inhibited. That is, the red cells incubated with Con A, 62.5 μg/ml (5×10^5 red cells/mm^3), and the sugars at the concentration of 0.15 mM did not agglutinate in glucose-free Hanks solution, while the suppression of phagocytosis required a large amount of sugars (Table 5). This result suggests that the active group of Con A concerned with red cell agglutination is different from that concerned with the red cell adherence to macrophage surface.

DISCUSSION

As is demonstrated in the present experiment, the mouse macrophage does not phagocytize the intact living red cells in Hanks solution both of homologous and heterogous ones, except chick red cells. But in the presence of the homologous serum they recognized the heterologous ones and phagocytized them selectively. This should be due to the serum factors like opsonin which will adhere to the heterologous red cell surface selectively. Namely, the macrophage recognizes homologous red cells by their intact surface. But the macrophage does not adhere or does not ingest homologous red cells even after treating with heterologous serum (Table 2). This suggests that the changed surface of heterologous red cells being treated with mouse serum should have some groups having the affinity to the mouse macrophage surface, but the homologous red cells cannot have the affinity to homologous macrophage even after treating with heterologous serum factor.

The Con A absorption of red cells to their surfaces seems to alter the homologous red cell surface to have the affinity to mouse macrophage surface. Experiment revealed that the red cells treated with Con A at an extremely low concentration, which does not induce the conformational change of the membrane protein, show the affinity to the macrophage

surface. Such a slight change on the cell surface or the absorption of some "affinity substance" might be related to the error in recognition of macrophage. The macrophage phagocytized actively the homologous red cells when they were affected by glutaraldehyde, tannic acid, saponin, heat and others (7, 12, 22, 23, 24, 25, 26, 27). The principle of recognition error will be the same as in the red cells treated with Con A, but these drastic treatments should be distinguished from the latter. Con A gives the macrophage affinity to red cells at the extremely lower concentration than that it induces the conformational change of the membrane proteins and red cell agglutination. Recently MARTIN *et al.* (28) reported that a tumor-specific immune response was enhanced by pretreatment of tumor cells with Con A. The experiment did not show the mechanism of enhanced immunogenicity of the tumor cells by Con A treatment. However, tumor specific immune response is a kind of autoimmune reaction or very similar one.

Red cell adherence to macrophage due to Con A was hardly inhibited by the sugars such as mannose, glucose, and α-methyl glucopyranoside which inhibit markedly the red cell agglutination by Con A. This fact seems to suggest that some groups other than those concerned with the red cell agglutination will be related to the erythrophagocytosis of macrophage. Thus, the present data indicate the possibility that recognition error at cell level may be induced by the absorption of some "affinity substance" to the cell surface in a minute quantity, which does not induce the conformational change or demolition of red cell membrane.

Of course the "affinity substance" related to the recognition error in autoimmune disease will be completely different from Con A in nature, but a minute quantity of some toxic substance introduced into the body or some abnormal catabolic substances produced in the body may act as the trigger of the recognition error or the autoimmune disease when they are absorbed on the cell surface.

SUMMARY

As the first step to analyze the autoimmune disease of red cells the recognition mechanism of macrophage to red cells or erythrophagocytosis has been studied *in vitro* by using mouse peritoneal macrophage and homologous and cells and the following results were obtained :

1. In Hanks solution, the mouse macrophage hardly phagocytizes living red cells, both homologous and heterologous ones. But in the presence of mouse serum, the macrophage phagocytizes heterologous red

cells selectively but does not phagocytize homologous ones.

2. The macrophage actively phagocytized homologous red cells prior to treatment with concanavalin A (Con A) at a concentration as low as 1.95 μg/ml.

3. Red cell agglutination was clearly recognized in those treated with Con A at 62.5 μg/ml or more, but not at 1.95 μg/ml.

4. The red cell agglutination by Con A was inhibited with D-glucose, D-mannose and α-methyl glucopyranoside at the concentration as low 1.5 mM, while the phagocytosis was suppressed only at a very high concentration of the sugars, 1,000 mM.

5. Fragility test of the red cells treated with Con A showed a lower resistance of red cells to hypotonic solution than those treated with Con A at the concentration of 31.25 μg/ml or more

6. Electron microscope observation revealed no membrane damage of red cells by treating with Con A at a concentration of 1.95 μg/ml, where erythrophagocytosis was observed. The membrane damage occurred by treating with Con A at 31.25 μg/ml or higher.

7. All the data indicate that the phagocytosis of homologous red cells by macrophage is induced by the adherence of a small amount of Con A, which induces no detectable changes of red cell surface and red cell membrane as revealed by agglutination test, fragility test, electron microscope observation and circular dichroism. On the basis of these observations a possible recognition error to homologous red cells by adsorbing a minute quantity of foreign substances on their surfaces has been discussed.

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