AN EXPERIMENTAL STUDY ON THE PHAGOCYTOSIS OF ASCITES TUMOR CELLS AGAINST BENZIDINE YELLOW SYSTEM PIGMENTS

SUZUKI, Akira

Kobe, J. Med. Sci. 15, 53-70, June 1969

AN EXPERIMENTAL STUDY ON THE PHAGOCYTOSIS OF ASCITES TUMOR CELLS AGAINST BENZIDINE YELLOW SYSTEM PIGMENTS

Akira SUZUKI

Department of Pathology, Division 2
Kobe University School of Medicine

Indexing Words
ascites tumor cells;
benzidine yellow pigment;
phagocytosis

Akira SUZUKI. *An Experimental Study on the Phagocytosis of Ascites Tumor Cells against Benzidine Yellow System Pigments.* Kobe J. Med. Sci. 15, 53-70, June 1969——The purpose of the present study is to elucidate the biological characteristics of tumor cells from the viewpoint of phagocytosis. The author has discovered a marked phagocytosis of some kind of newly synthetized Benzidine Yellow series pigment by Ehrlich ascites tumor cells under the use of some specific surfectant. Pigment solution was prepared by systematically combining 5 kinds of Benzidine Yellow series pigment with delicate difference in chemical structure with 6 kinds of surfectant as the solvent. States of phagocytosis by Ehrlich ascites tumor, Yoshida sarcoma, and AH-130 ascitic hepatoma were compared and differences were found among them. Even the same kind of tumor cells exhibit different phagocytotic ability through minor difference in chemical structure. Differece in solvent also gave variations in phagocytotic ability.

INTRODUCTION

In Ehrlich's ascites tumor cells it is a rare occurrence that the tubules or vacuoles of smooth surfaced endoplasmic reticulum are adjacent to the cell membrane. Invagination of the cell membrane is also said to be rare (Selby et al.). Phagocytosis or pinocytosis is not usually found under ordinary circumstances in Ehrlich's ascites tumor. The author has found phagocytosis of a newly synthesized organic pigment by Ehrlich's ascites tumor cells in an extremely high percentage. Phagocytosis of the pigments of Benzidine Yellow series by the Ehrlich ascites tumor occurred in an extremely high rate. Concerning the pigments of Benzidine Yellow series, the influence of the changes of chemical structure and the differences in the surfectant used as solvents on the susceptibility to phagocytosis of pigments in the same series was investigated. As the material for this study, Yoshida sarcoma cells in rats and Yoshida's ascites hepatoma cells AH-130 were used in addition to Ehrlich's ascites tumor cells. The results were as follows.

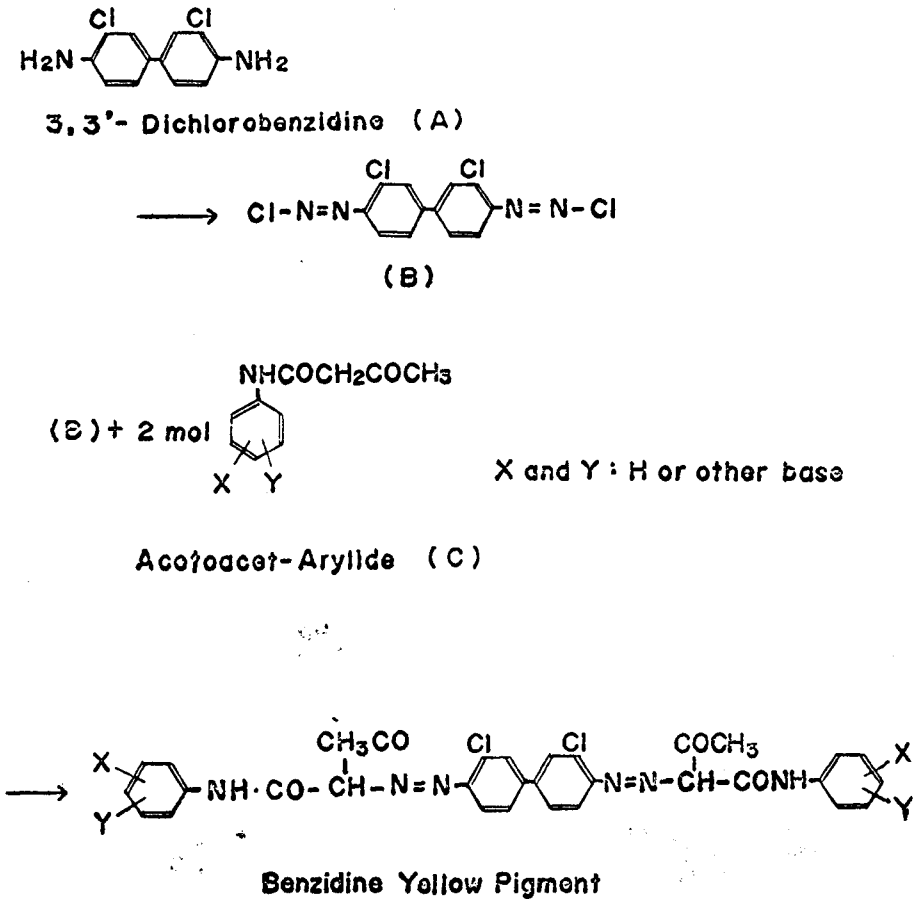
MATERIALS AND METHODS

The pigment of Benzidine Yellow series used was obtained by reaction between tetrazo-3,3'-Dichlorobenzidine and acetoacet-arylide (Chart 1).

By slightly changing the structure of benzene nucleus of acetoacet-arylide,

Received for publication February 21, 1969
Author's Name in Japanese: 鈴木昭

Chart 1. Benzidine Yellow Pigment.



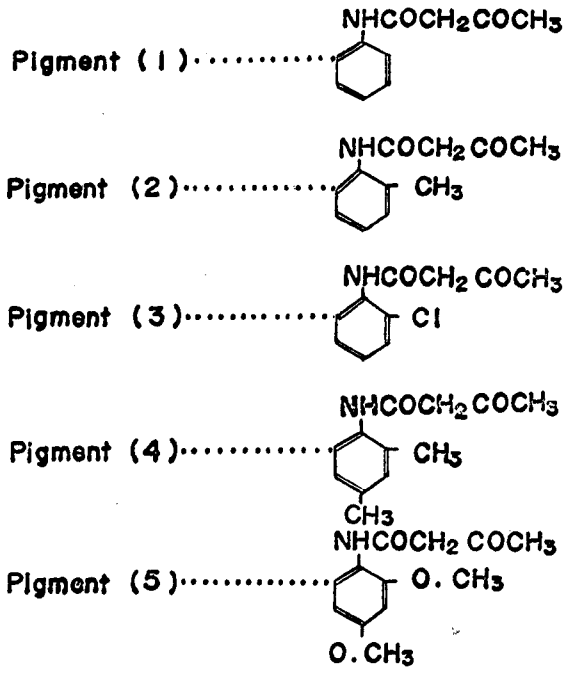
similar pigments of Benzidine Yellow derivativs with slightly different chemical structure may be obtained. Due to the difficulty in synthesis, only the 5 pigments shown in Chart 2 with variations in the structure of benzene nucleus of the acetoacet-arylide were used in the present experiment (Chart 2).

For these pigments with slightly different structure, the following solvents were used.

(a) Polyoxyethylene laurylether
(b) Polyoxyethylene nonylophenolether
(c) Conjugated material of naphthalene sulfonic acid and formaldehyde
(d) Polyoxyethylene alkylether
(e) Polyoxyetnylene alkylphenolether
(f) Mixture of solvent (e) and solvent (c) ((e) : (c) = 4 : 1)

All these solvents were surfactants. Solvent (c) is the surfectant of anion system, while all the others (a), (b), (d), and (e) were non-ionic surfectants. The above

Chart 2. Benzidine Yellow Derivatives.



mentioned pigments and solvents were combined and each pigment solution was used. In the combination of pigment 1 and solvent a, pigment 1 and solvent b, pigment 1 and solvent c, pigment 2 and solvent a, pigment 2 and solvent b, pigment 2 and solvent c, pigment 3 and solvent a, pigment 3 and solvent b, pigment 3 and solvent c, pigment 4 and solvent a, pigment 4 and solvent b, pigment 4 and solvent c, original solution was consisted of 10% pigment containing material, 2% solvent, and 88% water. This was further diluted to a constant dilution with sterile distilled water upon experiment. In the combination of pigment 1 and solvent d, and pigment 1 and solvent e, the original solution contained 20% of pigment containing material and 6% of solvent. In the combination of pigment 5 and solvent c, pigment 5 and solvent e, and pigment 5 and solvent f, the original solution was consisted of 15% pigment and 4% solvent.

Mice of pure dd strain were used to study the phagocytosis of these pigments by Ehrlich's ascites tumor cells. Ten mice were used for each kind of pigment solution, for a total of 170 mice. Approximately 2 million of Ehrlich's ascites tumor cells were transplanted in the peritoneal cavity of each animal. On the 4th and the 5th day after transplantation, pigment solution was injected intraperitoneally. On the 7th day after transplantation, ascites was taken to study the pagocytotic ability of the tumor cells. Upon the use of pigment 1, 2, 3, and 4, 0.1% solvent and 0.5% pigment were mixed to prepare aqueous emulsion, 0.2cc of which was injected once daily into the peritoneal cavity of the tumor-bearing mice. When pigment 5 was used, 0.1% solvent and 1.5% pigment were mixed and prepared into aqueous emulsion after dilution,

0.2cc of which was injected into the peritoneal cavity of the Ehrlich ascites tumor-bearing mice.

In the experiments using Yoshida ascites hepatoma AH-130 and Yoshida sarcoma, male rats of Gifu strain and Donryu were used, 10 animals for each pigment solution for a total of 340 rats. Pigment solution was diluted with water as in case of Ehrlich ascites tumor, 2cc of which was injected intraperitoneally. Yoshida ascites hepatoma AH-130 or Yoshida sarcoma cells, 10,000,000 in number, was transplanted into the abdominal cavity. On the 3rd and the 4th day after transplantation, pigment emulsion solution was injected. Ascites was obtained on the 5th day after transplantation to study the phagocytotic ability comparatively.

In order to avoid the mixing of saprophytic bacteria, the pigment solution was intraperitoneally injected after sterilizing at 60°C for more than 24 hours.

The study on phogocytotic ability of tumor cells against pigment was conducted by preparing smear preparation of fluid drawn from tne abdominal cavity and staining with May-Giemsa's stain. Some preparations were studied without staining.

For comparative evaluation of the degree of phagocytotic ability, immersion was used to observe tumor cells. Scarcely any variation in size was found in the pigment granules in tumor cells, while a considerable variation was found when they were phagocytized by cells other than tumor cells. When 1-3 pigment granules were found in 1 tumor cells, (+) was used. Presence of 4-8 pigment granules in one tumor cell was expressed by (++), while more than 8 pigment granules in one cell was showh by (+++).

RESULTS

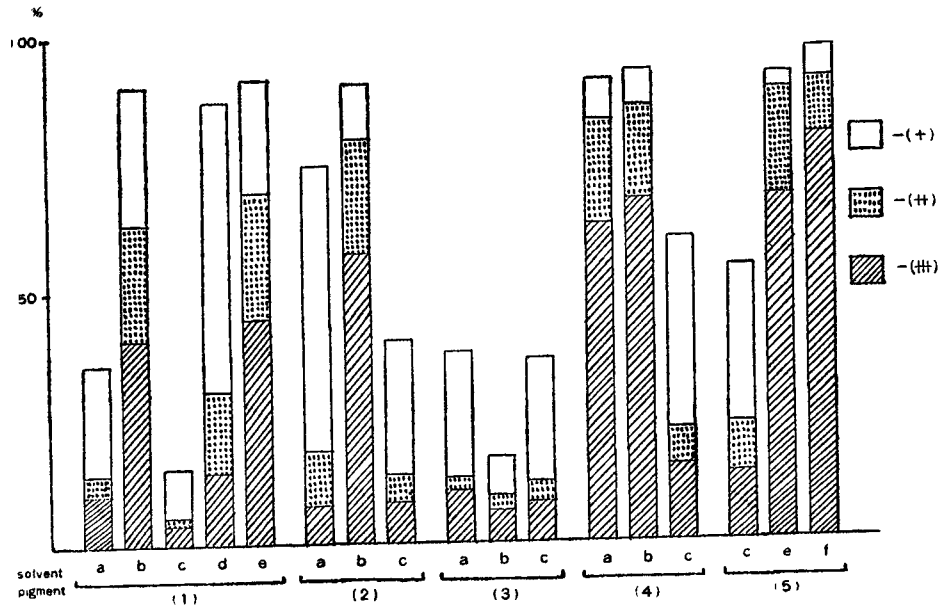
1. The phagocytizing ability of Ehrlich ascites tumor cells.

Pigment administered into the abdominal cavity was phagocytized into the phagocytes in the abdominal cavity in a considerably large amount. Pigment 1, 2, 3, 4, and 5 were phagocytized into the cells as yellow or yellow-brown or brown pigment granules. These pigment granules were quite distinct even after May-Giemsa's stain, to be convenient for the classification of the cells with optical microscopy and evaluation of the phagocytotic ability.

Mice transplanted with Ehrlich's ascites tumor cells and later infused with the above mentioned pigment solution survived after sampling of ascites on the 7th day after transplantation. The period of survival was not much different from those of animals with Ehrlich ascites tumor but without administration of pigment solution. No prolongation of life thus occurred upon administration of pigment in mice with Ehrlich's ascites tumor cells. In mice with Ehrlich's ascites tumor cells given pigment solution, the ascites tumor cells were transplanted on the 7th day after the first transplantation to another animal. All cells proved to be transplantable.

In the optic microscope, the phagocytized pigment granules were noted to various extent within the cytoplasm of ascites tumor cells. In extreme instances, a considerably large quantities of phagocytized pigment granules were found within the ascites tumors in collection. Under some circumstances, vacuole-like unstained

Chart 3. Phangocytizing activity of Ehrlich ascites tumor cells.



Hof was formed around the phagocytized pigment granules. Occasionally some of these pigment granules were found in the site probably representing the nucleus of tumor cells, but the presence of pigment granules within the cell nucleus could not be definitely established.

Phagocytizing ability of Ehrlich ascites tumor cells was classified into (+), (++), and (+++) as stated before. Results obtained are shown in Chart 3. In this Chart the average value of the phagocytizing ability of tumor cells in each animal upon each administration is shown. As shown in the Chart, the phagocytizing ability of Ehrlich ascites tumor cells, differed according to the variation of chemical structure. For pigment 3, the phagocytizing ability was low upon the use of any of solvent a, b, and c, 12.4% of 3a, 8.1% of b, and 11.2% of 3c showing (++) and (+++), representing extremely low ratio. In the other pigments 1, 2, 4 and 5, the phagocytizing ability was either extremely low or very high. In other words, in pigment 1, 2, 4 and 5, the phagocytizing ability distinctly varies as the emulsion of pigment solution changes. In pigment 1, 2, 4, and 5, the phagocytizing ability was low when solvent c was used. Upon the use of pigment 1 and solvent c, 5.2% showed phagocytizing ability of (++) (+++), while the corresponding figure was 13,2% in pigment 2 and solvent c, 20.8% in pigment 4 and solvent c, and 21.6% in pigment 5 and solvent c, representing a considerably low values. Even upon the use of the same pigment, in pigments 1, 2, 4 and 5, the ability of phagocytosis was quite high when some solvent was used. Phagocytizing ability of tumor cells of (++) and (+++) was seen in 62% upon the use of pigment 1 and solvent b, 71.6% upon the use of pigment 1 and solvent e, 82.8% in pigment 2 and solvent b, 83.2% in pigment 4 and solvent a, 86.4% in pigment 4

and solvent b 90.4% in pigment 5 and solvent e, and 92.4% in pigment 5 and solvent f. When pigment 1, 2, 4, and 5 were used in combination with solvent b, e, and f, the phagocytizing ability of tumor cells would probably give higher values. Solvent a showed different phagocytizing ability upon the use of pigment 1, 2, and 4. The phagocytizing ability of (++) and (+++) are shown in 83.2% of 4a, giving a very high value. Upon the use of pigment 1 and 2, the ability of phagocytosis is rather low. The phagocytizing ability of (++) and (+++) was obtained in 14% upon the use of pigment 1 and solvent a, while the corresponding value was 18.8% in the use of pigment 2 and solvent a.

In the light of these results, pigment 4 and 5 appear to be most readily phagocytized by Ehrlich ascites tumor cells among 5 pigments of Benzidine Yellow series, with quite similar chemical structure, while pigment 3 is not easily phagocytized. The phagocytizing ability of the phagocytizing cells may differ according to the kind of solvents. When solvent c was used, the phagocytizing ability of the cells was extremely weak, while the use of solvents b, e, and f gave a very high phagocytizing ability of the tumor cells.

2. The phagocytizing ability of Yoshida ascites hepatoma AH-130 cells.

Upon administration of these pigment emulsion in rats with Yoshida ascites hepatoma AH-130 cells intraperitoneally, pigment granules were phagocytized in a very high percentage by phagocytes in the peritoneal cavity, as in the Ehrlich's ascites tumor cells administered into the peritoneal cavity. However, on this occasion, pigment granules were phagocytized by the tumor cells in a very low percentage. The days of survival were approximately the same as those of non-treated rats with ascites hepatoma AH-130, giving no influence to the days of survival.

The administered pigments were demonstrated as yellow or, yellow-brown, and brown particles in the cells as in Ehrlich ascites tumor cells. With May-Giemsa's stain, pigment granules were clearly distinguished.

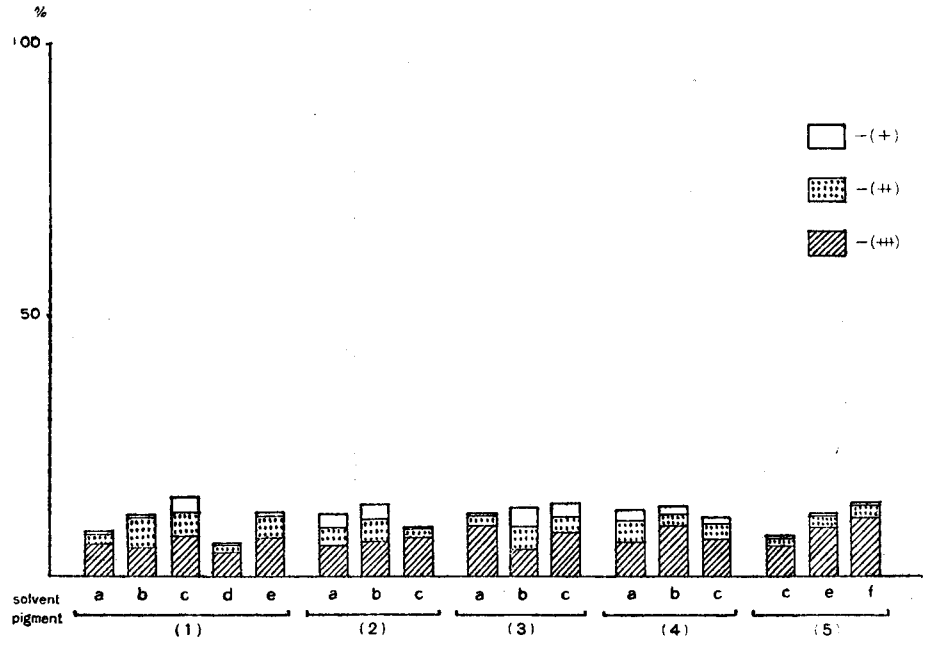
Pigment granules within the tumor cells are found scattered in the cytoplasm. Pigment granules were found in the intercellular space densely contacted with island-forming tumor cells or in the cytoplasm of densely contacted tumor cells in minute quantities.

The phagocytizing ability of Yoshida ascites hepatoma AH-130 cells against pigments of Benzidine Yellow series was very low as compared with that of Ehrlich's ascites tumor. Very low values were obtained upon the use of pigment 1, 2, 3, 4, and 5. The phagocytizing ability of tumor cells of (++) to (+++) was seen at the highest, 13.6% (pigment 5 and solvent f) and 12% (pigment 1 and solvent c). The other combinations gave even lower values. In the results obtained within the range of the authors' experiment, Yoshida ascites hepatoma AH-130 cells scarcely phagocytize pigments of Benzidine Yellow series. Chart 4 summarizes these experimental results. Average values of phagocytizing ability are shown in this Chart.

3. The phagocytizing ability of Yoshida sarcoma cells.

Intraperitoneal administration of these pigments in rats with Yoshida sarcoma

Chart 4. Phagocytizing activity of AH-130 ascites tumor cells.



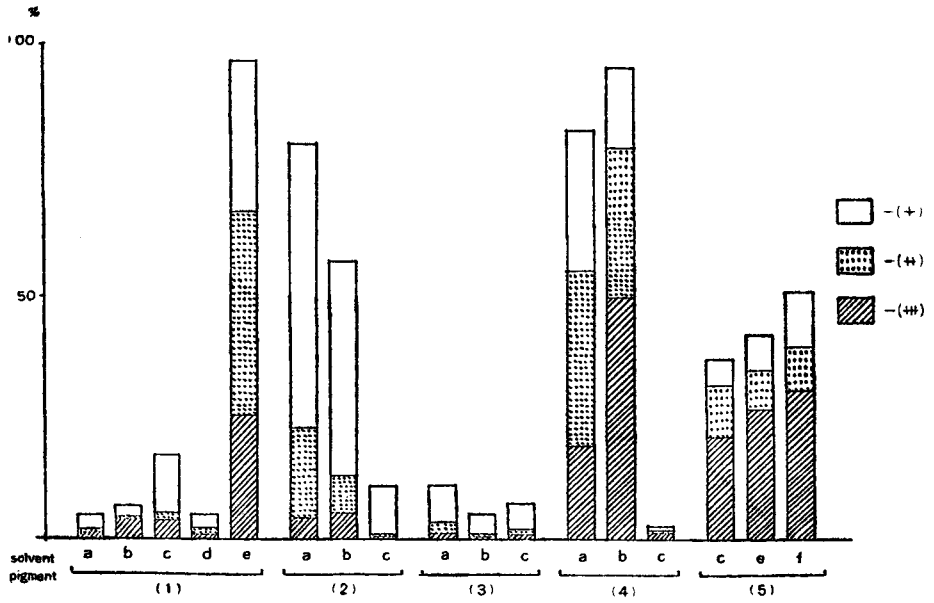
resulted in phagocytosis of a large amount of pigment granules by the phagocytes in the peritoneal cavity as in Ehrlich ascites tumor cells or AH-130 rats. These pigment granules were yellow, yellow-brown, or brown as in the other experiments. After Giemsa stain, distinct pigment granules were noted within the cytoplasm.

The period of survival of rats with Yoshida sarcoma given pigment granules were not much different from rats without administration of pigments. There was scarcely any difference on the days of survival.

In the optical microscopic observation, the administered pigment granules phagocytized by the tumor cells appeared to be fine or coarse granules scattered within the sarcoma cells. Pigment granules were also found within cells undergoing mitosis.

Upon comparis on between Yoshida sarcoma and Yoshida ascites hepatoma AH-130, a considerably large amount of pigment granules are found phagocytized in the Yoshida sarcoma cells. As a whole, the phagocytizing ability was somewhat lower than that of Ehrlich ascites tumor cells (Chart 5). The phagocytizing ability shown in the Table represents the average value in each group of administration. In Yoshida sarcoma cells, the phagocytizing ability was extremely low upon administration of pigment 3, regardless of the solvent. The phagocytizing ability of (++) and (+++) was seen in 3.6% in the combination of pigment 3 and solvent a, in 1.0% in the combination of pigment 3 and solvent b: and in 2.2% upon the use of pigment 3 and solvent c. In the other pigments 1, 2, 4, and 5, variation in solvents gave difference in phagocytizing ability. In pigment 1, 2, and 4, the use of solvent c gave low phagocytizing ability. Only pigment 5 gave slightly higher phagocytizing ability despite the

Chart 5. Phagocytizing activity of Yoshida sarcoma ascites tumor cells.



use of solvent c, (++) and (+++) being seen in 31.2%.

High phagocytizing ability of Yoshida sarcoma was seen in the combined use of pigment 1 and solvent e, pigment 4 and solvent a, and pigment 4 and solvent b. The highest frequency of phagocytosis of (++) and (+++) in pigment 5 was 39% at the combination of pigment 5 and solvent f, and thus lower values were obtained. Phagocytizing ability was very low in pigment 2. Only 22.4% showed phagocytizing ability of (++) and (+++) at the combination of pigment 2 and solvent a. At the high rate of phagocytizing ability in combination of pigment 1 and solvent e, pigment 4 and solvent b, pigment 4 and solvent a, phagocytizing ability of (++) to (+++) was seen 66%, 79.6%, and 54.6%, respectively.

Although the phagocytizing ability of Yoshida sarcoma cells failed to show specific difference depending on the solvent, upon the use of pigment 1, intense phagocytizing ability was seen only at the combined use with solvent e. In pigment 4, the rate of phagocytizing ability was quite low in the combined use with solvent c, and a considerably high phagocytizing ability was seen only when solvent b and a were used. These experimental results are summarized in Fig. 6.

DISCUSSION

When a substance goes through the cell membrane, the mechanism of so-called phagocytosis or pinocytosis and that of transmembranosis are said to be present, besides Novikoff's interpretation of almighty pinocytosis. The choice of the mechanism through which the substance enters the cell is said to depend upon the size of the

Chart 6.

		Ehrlich (%)			Yoshida (%)			AH-130 (%)		
		⁜	⁜	+	⁜	⁜	+	⁜	⁜	+
1	a	10.2	3.8	20.8	1.0	0.8	2.8	6.0	2.0	0.4
	b	40.6	21.4	28.4	3.8	0.4	2.2	4.8	6.0	0.8
	c	3.6	1.6	9.6	3.8	1.4	12.0	7.6	4.4	2.8
	d	15.0	16.8	57.8	1.0	1.4	2.6	4.4	1.4	0.2
	e	46.2	25.4	23.2	25.2	40.8	30.2	7.2	4.0	0.8
2	a	8.0	10.8	56.6	4.4	18.0	57.6	5.6	3.4	2.8
	b	58.2	24.6	12.8	5.4	7.6	43.0	6.2	4.4	2.8
	c	8.0	5.2	28.6	0.2	0.8	9.6	7.0	1.6	0.2
3	a	10.4	2.0	26.8	1.4	2.2	7.4	9.6	2.0	0.4
	b	6.2	1.9	7.0	0.4	0.6	4.2	5.0	4.2	3.4
	c	7.8	3.4	26.0	1.2	1.0	5.4	8.2	3.0	2.6
4	a	63.6	19.6	9.0	19.2	35.4	28.4	6.6	4.0	1.6
	b	69.6	16.8	7.6	49.2	30.4	15.8	9.6	2.0	1.8
	c	14.2	6.6	38.6	1.8	0.2	1.0	7.6	2.4	1.4
	d	11.8	9.8	32.6	21.2	10.0	5.4	6.0	1.4	0.2
5	e	69.0	21.4	3.6	26.2	8.0	7.4	9.2	2.4	0.4
	f	81.0	11.4	5.2	30.2	8.8	11.2	11.2	2.4	0.4

particle. According to Miller, blood pigment is the minimum borderline of the former, while Colloid iron is the maximum borderline of the latter according to Moore. The borderline of the size of the particle is approximately 50Å, in other words. When the particle is larger than this, the mechanism of phagocytosis or pinocytosis is generally assumed, while the mechanism of trans-membranosis is employed when a particle is smaller than this. In case of ferritin the phenomenon is somewhat different and exceptional, the size being approximately as great as 100Å. In phagocytic cells, in general, pseudopod formation is said to be observed when the size of the particle is extremely large. Under such circumstances, the cell actively takes in the particle so that the influences of the electric charge of the cell surface and electric charge of the particle is said to be relatively small. However, in the phagocytotic phenomenon in which no pseudopod formation is noted under optical microscope dealing with a relatively small particle, the influence of static electricity between phagocytic cells and phagocytized particles is said to be great. According to Sanders et al, polystylene latex with diameter of 2200Å is absorbed without pseudopod for-

mation. According to Overman, uptake of a particle with diameter of 2640A by fibroblast of cultured chicken fetus through pinocytosis takes place while the role of pseudopod is not clear. The size of pigment particle used in the present experiment is usually between 200Å–1500Å. In the present experiment, consequently, the mechanism of phagocytosis or pinocytosis should be considered and the influence of electric discharge is also taken into consideration in view of the size of the particle.

The pigments of Benzidine Yellow series used in the present experiment made some difference in the behavier taken up by Ehrlich ascites tumor and Yoshida sarcoma cells only on the basis of different radicals attached to the benzene nucleus. The reason for this is not yet understood. This might be due to either the difference in the viscoelasticity of adsorption to the cell membrane or the different chemical change for each of the 5 pigments itself at the cell membrane causing some differnce in the energy metabolism at the surface of the cell membrane. Some other complex factors may also participate but the situation is not yet clear at present. While Low's report of "adsorption but no phagocytosis" is available, Suter said the contact between phagocytes and phagocytized material is the first requirement, the adhesibility being most important. According to Lucke, the ready phagocytability of bacteria treated with antibody is due to the increase in adhesibility of the bacterial surface. In the mechanism of phagocytosis, Fenn demonstrated the phagocytotic phenomenon through the surface tension while a cell is phagocytizing a particle. According to Mudd, Ponder, as well as Berry and Spies who developed their theories, phagocytosis is facilitated under the following conditions.

1) Increase of the value of particle-suspending fluid surface energy
2) Decrease of particle-phagocyte interface energy
3) Decrease of phagocyte-suspending fluid surface energy
4) Mixture of these

According to Tamura, the surface tension fell as the energy source is consumed and metabolism become low. Such difference among the phagocytizing ability of 5 pigments used in the present experiment might be due to the influence on energy changes in the surface tension upon adherance of some radical attached to the benzene nucleus of the Benzidine Yellow series to the cell membrane. Ponder demonstrated that the fat solubility of the pigment influenced the passage through the lipid layer of the cell membrane. He and Parpart et al showed a model of fine structure of the red cell membrane, demonstrating the mixed presence of protein and lipid portions in the red cell membrane, representing an overlap of water-and non-water layer. Dourmashkin et al conducted an electron microscopical observation of liver cells of chickens and mouse sarcoma cells after treatment with saponin solution, demonstrating the composition of cell membrane on the basis of the mixture of protein and lipid. This consists of stakes of lipid surrounded by fiber bundles probably representing protein. Although separation of cell membrane alone has been attempted by various methods, sufficiently clean cell membrane itself has never been isolated todate. Most of the results are based on red blood cells. Although it is not clear to what extent the structure of cell membrane of tumor cells indicates the difference from other kinds of cells, the mixed presence of proteinous and lipid nature cannot be denied. According to Terayama, the lipo protein membrane of cancer cells is

considerably different from normal ones, exhibiting a high permeability against substances with high molecular weight. This may be due to the intense pinocytosis with a high probability. According to Ludford, the cell membrane of cancer cells has a larger permeability to fat soluble pigments than that of 8 normal cell. Lucke pointed out the low permeability of cell membrane of cancer cells against high alcohol. In view of the results of Ludford, Lucke and others, cell membrane of cancer cells contains more abundant fat than that of the normal cells. According to Terayama, the use of surfectant removes cholesterol promptly from the cell membrane. The solvents used in the present experiment were all surfectants, with the property of moving towards the surface rather than the inside of the solution upon being dissolved in a liquid. Surfectants are the substance exhibiting surface activity through being adsorbed on the border surface between liquid and liquid, liquid and gas, or solid and liquid. Thus the surfectants reduce the surface tension and border tension of the solution, facilitating the mutual adsorption at the border surface. In case of tumor cells, it is tempting to suggest that the mixture of surface active agents in the pigment emulsion might indicate the ready adsorption of pigment particles on the cell membrane of tumor cells or the border surface between tumor cells and pigment emulsion. As is clear from the results of the author's experiment, the phagocytotic activity of ascitic tumor cells definitely differs according to the kinds of surfectants. In Ehrlich ascites tumor cells upon the use of solvent C, the phagocytotic ability rather tended to decrease. Similar tendency was also noted in Yoshida sarcoma cells. Solvent C, therefore, does not appear to be quite effective in the biological mechanism of phagocytosis of tumor cells.

Among 6 kinds of surfectants used in the present experiments, only solution C is an anionic surfectant, while the others were nonionic surfectants. Although a hasty conclusion should not be drawn until experiments are conducted using other kinds of anionic surfectants, anionic surfectant seems to be of no value against the phagocytotic ability of the tumor cells within the range of this experiment under the current assumption. While nonionic surfectant does not dissociate into ions in aqueous solution, anionic surfectant ionizes in aqueous solution and the surface active portion becomes anionic. According to Hampton, Gosselin, Harford, Parks, Clark and others, cell surface membrane has negative electric charge and particles with positive discharge such as ThO_2 are readily adsorbed, while particles with negative discharge such as Hg S are only sparingly phagocytized. Brandt suggested that such surface phenomena were decisive in phagocytosis or pinocytosis. Ponder also pointed out the static electrical effect of the protein on the surface of cell membrane against electric charge of the pigment. According to Ambrose et al, cell membrane of the cancer cells has a high negative electric discharge. Terayama is of the opinion that even normal cells which are present as single cells exhibit considerably high negative electric discharge as in cancer cells. Analysis from this viewpoint revealed the surface activity of nonionic surfectant without dissociation to ions, while the portion with surface activity becomes anion in anionic surfectant resulting in repulsion at the cell membrane of cancer cells. This is probably the reason for the low rate at which phagocytosis by tumor cells occurred when anionic surfectant was used as solvent.

In the author's experiment, only 3 kinds of cells, Ehrlich ascites tumor, Yoshida

ascitic hepatoma AH-130 and Yoshida sarcoma were used as ascites tumor cells. The difference in phagocytosis of tumor cells was studied concerning pigments of Benzidine Yellow series. Each kind of tumor cells has its own characteristics as tumor cells. Especially in Yoshida ascites hepatoma, marked phagocytosis was almost never found. This could be explained by proper biological and cytochemical characteristics of each kind of tumor cells as one phase of the multiplicity of tumor cells. However, details of difference between these tumor cells are not clear at the present stage.

CONCLUSION

1) Five kinds of pigments of Benzidine Yellow series and 6 kinds of surfectants used in the present experiment revealed low phagocytotic ability by ascities hepatoma AH-130.

2) Ehrlich ascitic tumor cells and Yoshida sarcoma cells revealed higher phagocytotic ability against compound with attachment of CH_3 or $O\text{-}CH_3$ group to the benzene nucleus of the Acetoacet-Arylide, especially 2 CH_3 groups than 1 CH_3 group.

3) In Ehrlich ascites tumor, scarcely any difference is found in the phagocytability between compounds with attachment of 2 CH_3 groups to the bezene nucleus of acetoacet arylide and those with 2 $O\text{-}CH_3$ groups, while the compound with 2 CH_3 groups exhibited higher rate of phagocytability in Yoshida sarcoma.

4) Compounds with attachment of Cl group to the benzene ring of acetoacet-arylide are not readily phagocytized by any of the ascites tumor cells used.

5) The use of anionic surfectant as solvent is not suitable for the purpose of elevating the phagocytotic ability as far as the present experiments are concerned.

6) Among pigment of Benzidine Yellow series, the highest rate of phagocytability was seen in acetoacet-arylide with attachment of 2 CH_3 group to the benzene ring at the present stage.

7) From the viewpoint of phagocytosis, each of the three tumor cells, Ehrlich ascites tumor, Yoshida ascites hepatoma AH-130 and Yoshida sarcoma has its own characteristics.

REFERENCES

1. AMBROSE J. E. et al.
Nature. 1956. 177. 576
Differences between the Electrical Charge carried by Normal and Homologous Tumor Cells.
2. BERRY L. J. and SPIES T. D.
Medicine. 1949. 28. 239
Phagocytosis.
3. BRANDT P. W. and PAPPAS G. D.
J. B. B. C., 1960. 8. 675/687
An electron microscopic study of pinocytosis in ameba. 1. The surface attachment phase.
4. CLARK S. L.

J. B. B. C., 1959. 5. 41/50
The ingestion of protein and colloidal materials by columnar absorptive cells of the small intestine in suckling rats and mice.

5. DOURMASHKIN R. R. et al.
Nature. 1962. 194. 1116
Electron microscopic observations on Rous sarcoma virus and cell membranes.

6. GOSSELIN R. E.
J. Gen. Physiol. 1956. 39. 625/649
The uptake of radio colloids by macrophages in vitro.
A kinetic analysis with radio active colloidal gold.

7. HAMPTON J. C.
Acta anat., 1958. 32. 262/291
An electron microscope study of the hepatic uptake and excretion of submicroscopic particles injected into the blood stream and into the bile duct.

8. HARFORD C. G., HAMILTON A. and PARKER E.
J. B. B. C., 1957. 3. 749/756
Electron microscopy of Hela cells after the ingestion of colloidal gold.

9. LOW F. N. and SAMPAIO M. M.
Anat. Rec., 1957. 127. 51/63
The pulmonary alveolar epithelium as an entodermal derivative.

10. LUCKE B., MCCUTCHEON M., STRUMIA M. and MUDD S.
J. Exp. Med., 1927. 49. 797
On the Mechanism of Opsonin and Bacteriotropin Action II.
Correlation between changes in Bacterial Surface Properties and in Phagocytosis caused by Normal and Immune Sera.

11. LUCKE B. and A. K. PARPART
Cancer Res. 1954. 14. 75/80
Osmotic Property and Permeability of Cancer Cells.

12. LUDFORD R. J.
Clarendon Press: Oxford, 1951. 373/418.
Chapter IX. Pathological Aspects of Cytology.
In: G. H. Bourne (ed.), Cytology and Cell Physiology.

13. MILLER F.
J. B. B. C. 1960. 8. 689/718.
Hemoglobin absorption by the cell of the proximal convoluted tubule in mouse kidney.

14. MOORE R. D., HUNAW V. R. SCHOENBERG M. D.
J. Ultrastr. Res. 1961. 5. 244/256
The transport and distribution of colloidal iron and its relation to the ultrastructure of the cell.

15. MUDD S., MCCUTCHEON M. and LUCKE B.
Physiol. Rev., 1934. 14. 210
Phagocytosis.

16. NOVIKOFF A. B., BEAUFAY H. and de DUVE C.
J. B. B. C., 1956 2 Suppl. 179/184
Electron microscopy of lysosomerich fractions from rat liver.

17. NOVIKOFF A. B.
The Cell, 1961. Vol. 2. 423/488
Lysosomes and related particles.

18. NOVIKOFF A. B.

Biol. Bull., 1959. 117, 385.
Lysosomes and physiology and pathology of cells.
19. OVERMAN J. R.
Proc. Soc. Exp. Biol. and Med., 1961. 107. 895/900
Ingestion of latex particles by chicken fibroblasts in tissue culture.
20. PARKS H. F. and CHIQUOINE A. D.
Anat. Rec., 1956. 124, 343.
On the mode of formation of cytoplasmic inclusions of phagocytosed colloidal particles by Kupffer cells.
21. PARPART A. K. and BALLENTINE R. In: BARRON, E. S. GUZMAN (ed.)
New York: Academic Press. 1952. 135.
Trends in Physiology and Biochemistry.
22. PONDER E.
The Cell, 1961. Vol. 2, 1/84
The cell membrane and its properties.
23. SANDERS E. and ASHWORTH C. T.
Exp. cell Res., 1961. 22. 137/145
A study of particulate intestinal absorption and hepatocellular uptake. Use of polystylene latex particles.
24. SELBY C. C. and GREY C. E.
Lichtenberg, S., Friend, C., Moore, A. E., and Biesele, J. J. Cancer Res., 1954. 14. 790/794.
Submicroscopic Cytoplasmic Particles Occasionally Found in the Ehrlich Mouse Ascites Tumor.
25. SUTER E.
Bact. Rev., 1956. 20. 94.
Interaction between Phagocytes and Pathogenic Micro-organism.
26. TAMURA H.
Osaka Univ. Med. 1959. 11 (5). 1221.
About the transition of leucocyte movement.
27. TERAYAMA H.
Saibo Kagaku Shimpozium. 1963. 13. 1/9
Structure of Animal Cell Surface.

LEGENDS

Fig. 1. Light microscope photograph of phagocytizing Ehrlich ascites tumor cells injected with 2b.
Fig. 2. Light microscope photograph of phagocytizing Ehrlich ascites tumor cells injected with 4a.
Fig. 3. Light microscope photograph of phagocytizing Ehrlich ascites tumor cells injected with 5e.
Fig. 4. Light microscope photograph of phagocytizing Ehrlich ascites tumor cells injected with 1b.
Fig. 5. Light microscope photograph of phagocytizing AH-130 ascites tumor cells injected with 2b.
Fig. 6. Light microscope photograph of phagocytizing AH-130 ascites tumor cells injected with 1b.
Fig. 7. Light microscope photograph of phagocytizing Yoshida sarcoma ascites tumor cells injected with 4a.
Fig. 8. Light microscope photograph of phagocytizing Yoshida sarcoma ascites tumor cells injected with 1e.

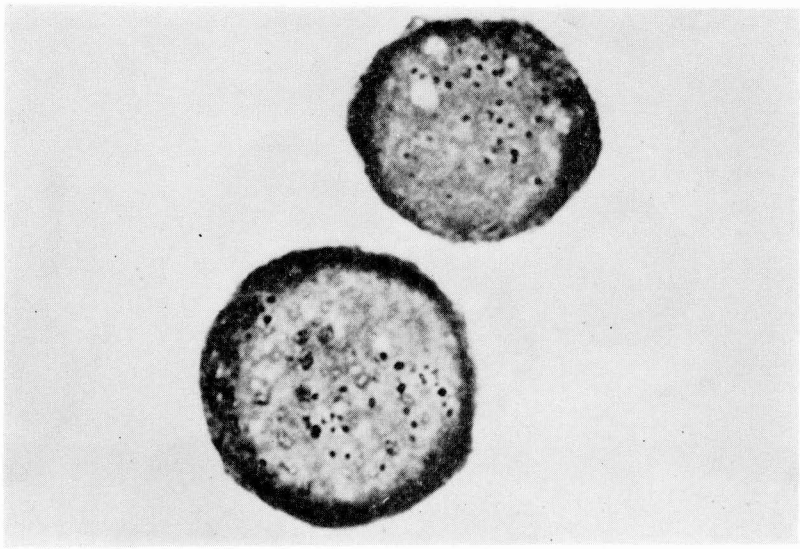


Fig. 1.

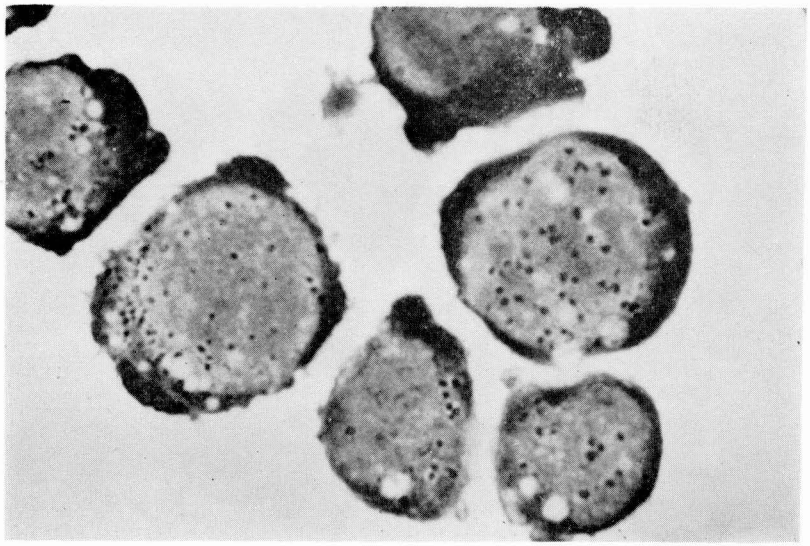


Fig. 2.

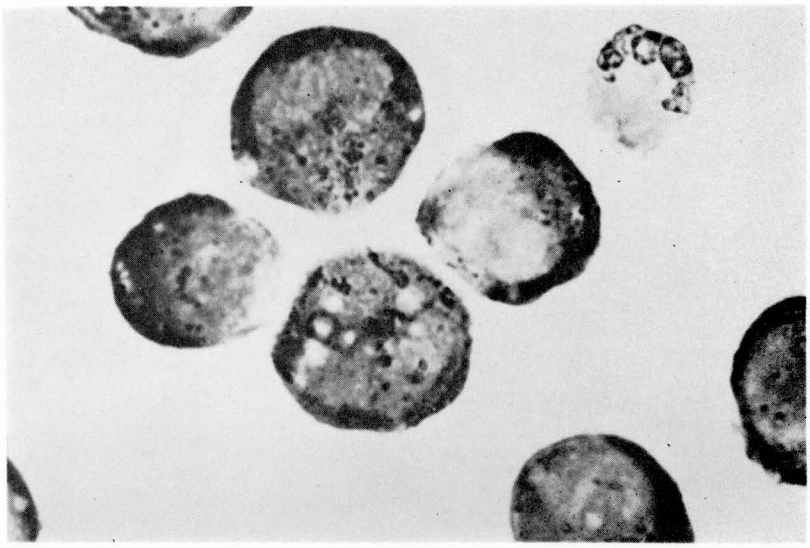


Fig. 3.

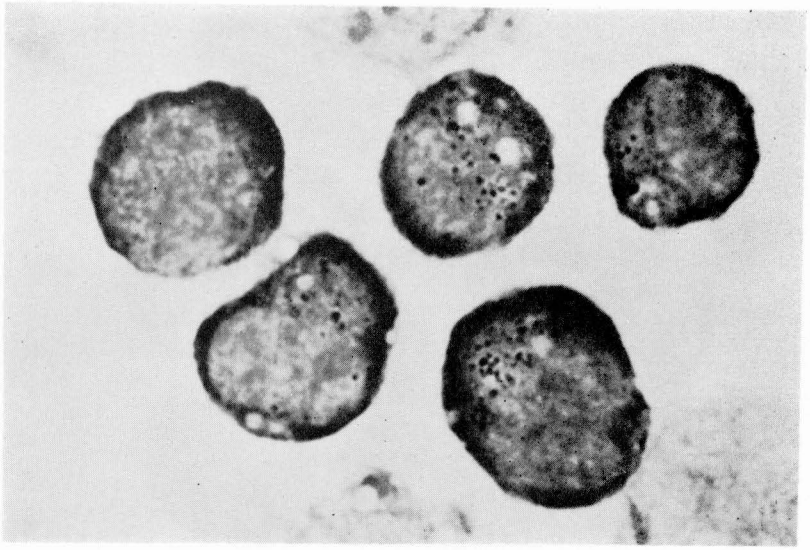


Fig. 4.

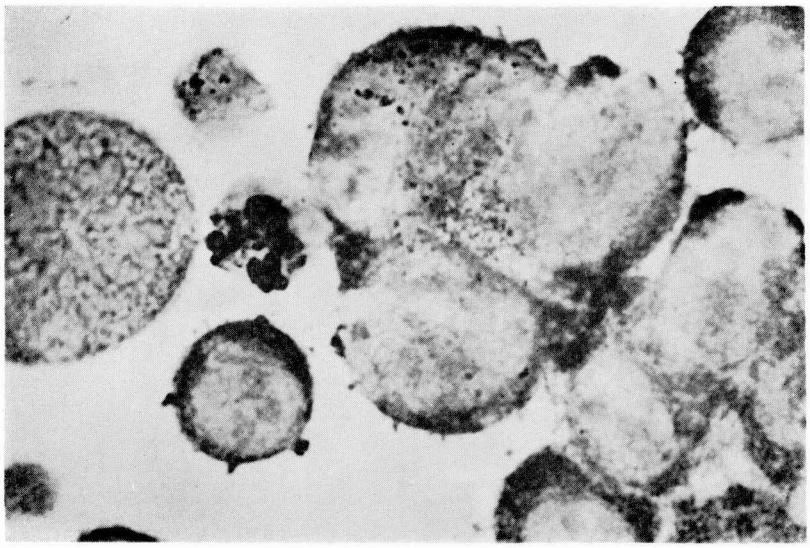


Fig. 5.

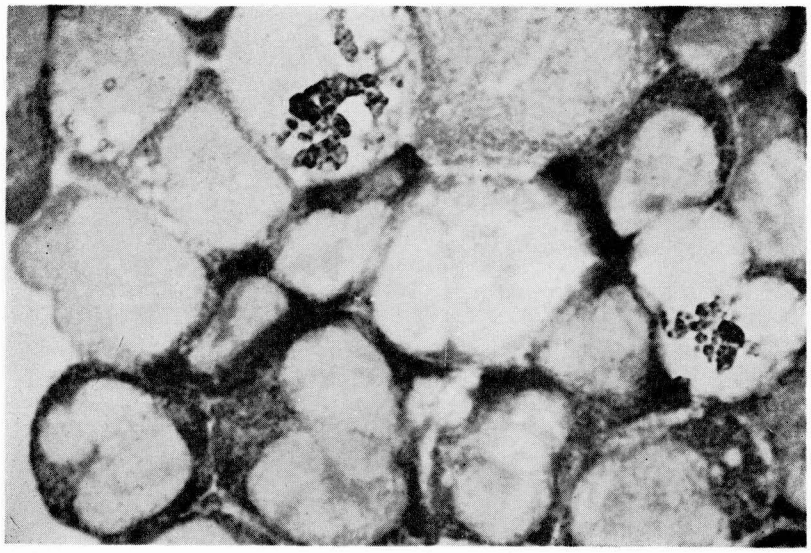


Fig. 6.

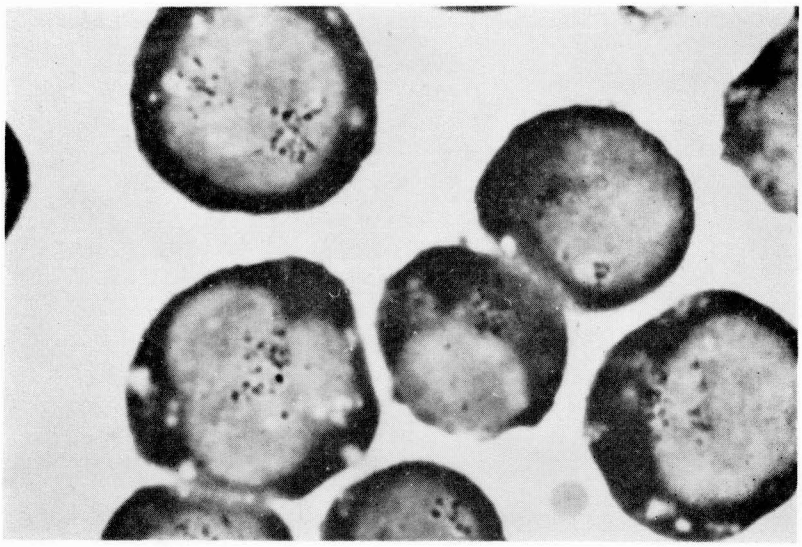


Fig. 7.

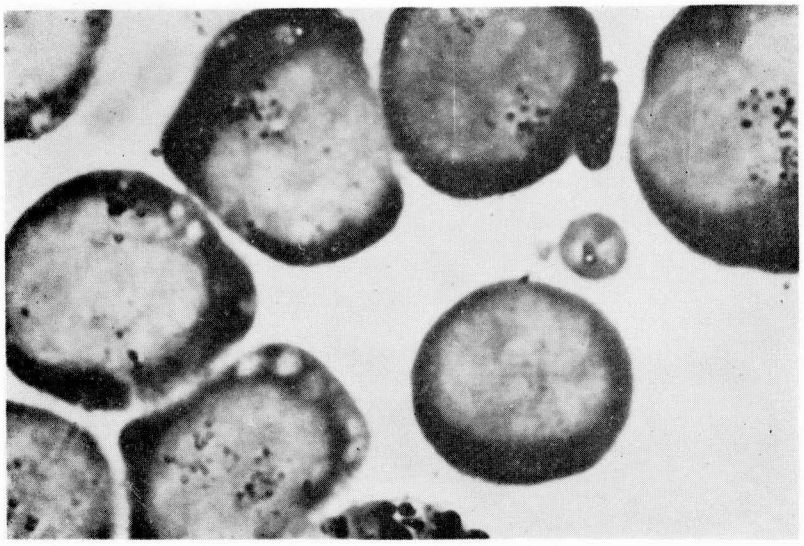


Fig. 8.