THE DEVELOPMENT IN VITRO OF PARTICLES FROM CYTOPLASM

II. PARTICLES FROM HEMOGLOBIN AND DEOXYRIBONUCLEIC ACID

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Plates 70 to 72

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In the foregoing study it was demonstrated that the incubation of a bacteriologic medium after an addition of soluble materials from certain mammalian cells resulted in the development of characteristic protein particles (1). It was postulated that a polymerization of cytoplasmic proteins resulted from their interaction with trace components of the medium in the presence of an enzyme.

Several compounds were found which in broth enhanced the development of particles from filtered lysates of erythrocytes. Some chemicals could initiate particle formation from such lysates in distilled water. One such substance, deoxyribonucleic acid (DNA), was particularly interesting because it is a normal constituent of mammalian cells. The studies described here were carried out with a system composed of red cell lysate, DNA1, and water.

EXPERIMENTAL

Standardization of Reactants.—Lysates of mammalian erythrocytes were prepared by washing the cells twice with 10 volumes of physiologic saline and then lysing the washed cells with 10 volumes of deionized water. All particulate material was removed by centrifuging the lysed cell preparation at 4500 g for 30 minutes.

The clear lysates were standardized photometrically on the basis of hemoglobin content. A unit of lysate was defined as five times that amount of lysate which, when added to 4 ml. deionized water, gave 55 per cent transmittance in a Coleman photoelectric nephelometer at 540 m/μ. One unit varied between 0.35 and 0.55 ml.

For convenience the DNA was also measured in units. One unit of DNA was defined as, 5 mg. of DNA in 1 ml. deionized water at pH 3.5. A stock solution of 200 ml. (units) of DNA was prepared as routine and the pH adjusted with 1.0 N HCl. The resulting opaque, white DNA solution was stored at 4°C.

Preliminary Observations.—

When DNA and lysate were mixed in optimal proportions in sterile deionized water, a pink turbid solution resulted. If the mixture was incubated at 37°C, the pink color gradually

1 General Biochemicals, Inc., Chagrin Falls, Ohio.
PARTICLE DEVELOPMENT IN VITRO FROM CYTOPLASM. II

changed to brown indicating that the hemoglobin was being oxidized. In addition to the color change, the solution became increasingly granular and a brown sediment gradually formed.

Microscopic examination of the DNA-lysate mixture at various times during incubation revealed the following. The initial pink solution was found to yield only amorphous acidophilic material when stained by Wright's method. Preparations observed under the phase microscope did not reveal the presence of any particulate elements. Particles which could be seen in phase preparations first appeared during the 2nd hour of incubation, but these would not remain intact during fixing and staining. A few smudges of basophilic material were observed in the stained preparations. By 4 hours' incubation true basophilic particles, 1 to 8 microns in diameter (Fig. 1), which could be dried and stained without losing their spherical configuration, were developing at a rapid rate.

Text-Fig. 1. The temporal relationship between the development of visible turbidity, particles, and sediment.

The basophilia of the particles was probably due to the presence of DNA bound to the hemoglobin, because 2 per cent of the DNA originally added to the lysate was found in the particle mass. Text-fig. 1 illustrates the sequential relationship between the development of visible turbidity, sediment, and basophilic particles from DNA and red cell lysates.

The initial turbidity apparently was the result of a non-specific precipitation of protein by DNA. However, this precipitation was not required for the formation of basophilic particles, because it could be removed by centrifugation without affecting the subsequent development of particles. Turbidity was useful though as an indicator of optimal reaction conditions. Table I shows that both DNA and hemoglobin had to be present in proper proportions for a maximum yield of sediment. One unit of DNA reacted optimally with one unit of lysate in 20 ml. of sterile deionized water at a pH of 5.0 and a temperature of 37°C.

Conditions for Particle Development.—The temperature requirements were those previously described (1). When DNA was added to lysates which had been heated to 60°C., or above, particles did not develop on subsequent incubation at 37°C. The optimal pH range for particle formation from DNA in deionized water was 4.8–5.8, which was lower than that required for the
# TABLE I

*Amount of Visible Sediment* Formed from Different Mixtures of DNA and Lysate

<table>
<thead>
<tr>
<th>Units of DNA</th>
<th>Units of Lysate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>0.05</td>
<td>±</td>
</tr>
<tr>
<td>0.1</td>
<td>±</td>
</tr>
<tr>
<td>0.2</td>
<td>±</td>
</tr>
<tr>
<td>0.5</td>
<td>±</td>
</tr>
<tr>
<td>1.0</td>
<td>±</td>
</tr>
</tbody>
</table>

* The total amount of sedimentable material formed after 16 hours' incubation at 37°C.

# TABLE II

*Effect of Various Compounds on Turbidity and Particle Formation from DNA and Hemoglobin*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Percentage Concentration</th>
<th>Amount of Turbidity and Particles</th>
<th>Typical Particles*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>8.5</td>
<td>4+</td>
<td>+</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>10.0</td>
<td>2+</td>
<td>+</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>10.0</td>
<td>3+</td>
<td>-</td>
</tr>
<tr>
<td>Sodium nitrite</td>
<td>10.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Copper sulfate</td>
<td>10.0</td>
<td>3+</td>
<td>-</td>
</tr>
<tr>
<td>Mercuric chloride</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium cyanide</td>
<td>10.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sodium phosphate</td>
<td>10.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Glucose-one-phosphate</td>
<td>10.0</td>
<td>+</td>
<td>Distorted</td>
</tr>
<tr>
<td>Adenosine-triphosphate</td>
<td>1.0</td>
<td>3+</td>
<td>+</td>
</tr>
<tr>
<td>Ribonuclease†</td>
<td>10.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Deoxyribonuclease‡</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4+</td>
<td>+</td>
</tr>
</tbody>
</table>

* +, present; −, absent.
† Added to DNA in water before addition of lysate:
   Inoculum, 1 unit lysate
   Medium, 20 ml. water
   DNA, 1 unit

Development of particles in bacteriologic media. The optimal pH range for the DNA reaction could be varied somewhat by changing the ionic strength of the water menstruum with sodium chloride.

*Inhibition of Particle Formation.*—It can be seen in Table II that phosphates,
cyanide, and heavy metals inhibited particle formation. Ribonuclease (RNase) also was inhibitory if it was added to DNA in water prior to the addition of lysate. However treatment of the lysate with RNase before the addition of DNA did not prevent particle formation. When DNA was treated with deoxyribonuclease (DNase), the DNA-lysate reaction was also inhibited but to a lesser degree than with RNase. A trace of turbidity did develop after prolonged incubation. Microscopic examination of this turbidity revealed the presence of a few distorted, bizarre particles rather than the usual spheres. Apparently RNase, though unable to depolymerize DNA, was able to interfere with particle formation, whereas DNA, following depolymerization by DNase, still retained some particle-initiating activity.

**Particle Composition.**—Dried red cell lysates were considered as pure hemoglobin, although trace amounts of other substances were undoubtedly present. Dried particles, developed from lysates and DNA in water, contained 88 per cent protein, 12 per cent DNA, and a trace of lipide.

Purified serum globulin and commercially obtained hemoglobin both reacted with DNA to give a trace of visible precipitate in water. However, particles,

### TABLE III

<table>
<thead>
<tr>
<th>Tube</th>
<th>Experimental procedure</th>
<th>Counts in particles of sediment after</th>
<th>Per cent incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st wash</td>
<td>2nd wash</td>
</tr>
<tr>
<td>1</td>
<td>1 unit lysate + BSA $^{131}$, Incubate 37°C - 24 hrs.</td>
<td>650</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>1 unit lysate + BSA $^{131}$ + 1 unit DNA, Incubate 37°C - 24 hrs.</td>
<td>11,231</td>
<td>10,383</td>
</tr>
<tr>
<td>3</td>
<td>1 unit lysate + BSA $^{131}$ + 1 unit DNA, Incubate 0°C - 24 hrs.</td>
<td>786</td>
<td>258</td>
</tr>
<tr>
<td>4</td>
<td>1 unit lysate + 1 unit DNA, Incubate 37°C - 24 hrs. to form particles. Then add BSA $^{131}$ and reincubate.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1 unit lysate + BSA $^{131}$ + 1 unit DNA, Incubate 0°C - 24 hrs. Then incubate 37°C - 24 hrs.</td>
<td>8,651</td>
<td>7,705</td>
</tr>
</tbody>
</table>

Total reaction volume 20 ml. Approximately 40,000 counts BSA $^{131}$ added to each tube. Particles washed with a solution of KI and BSA in saline.

* The higher per cent with fewer counts than tube 2 is caused by label decay and counting with a different scintillation detector.
such as developed from fresh lysates, could not be detected in these precipitates. Apparently some factor present in fresh lysates was required for particle development in addition to protein and DNA. The possibility that the unknown substance was an enzyme was considered for several reasons. Particle-forming activity was greatest at 37°C, but was destroyed at 60°C. A definite pH range was required as was a suitable incubation period. The active substance in fresh lysates could be destroyed in vivo by ionizing irradiation. (Lysates prepared from the blood of animals given near lethal doses of x-irradiation 48 hours previous to bleeding did not form particles, whereas direct irradiation of lysates with doses up to 10,000 r did not affect particle-forming ability.)

\[
\text{Text-Fig. 2. The effect of BSA concentration on the percentage of available BSA incorporated into particles. Additions of BSA were to 20 ml. water with 1 unit each of lysate and DNA.}
\]

\text{The Incorporation of Heterologous Substances into Particles.}—In some of the preliminary studies of particle formation in bacteriologic media it was suspected that polypeptides and polysaccharides of the medium were sometimes trapped within or on the developing particles.

In order to test this possibility bovine serum albumin (BSA), labelled with \text{I}^{131} \text{(2)}, was added to lysate in water just before the addition of DNA. The mixture was incubated at 37°C. to determine whether particles forming under such conditions would contain the radioactive label. The results of this experiment, together with control findings, are presented in Table III.

Approximately 25 per cent of the labelled BSA was firmly incorporated into particles forming at 37°C. In tubes held at 0 degrees no particles formed and nothing which exhibited radioactivity could be centrifuged out of solution. The addition of labelled BSA to tubes containing fully developed particles did not result in incorporation of the label.

The effect of BSA concentration on the amount of BSA incorporated was determined in a second experiment Text-fig. 2. Because the per cent incorpora-
tion increased only slightly with a relatively large increase in BSA concentration, it appeared that the BSA was bound in a non-selective fashion. The actual amount of BSA incorporated was purely a function of the BSA concentration in the reaction medium; this would be the case if an aliquot of the medium were trapped within the particle structure.

Substances which have been incorporated into hemoglobin particles by adding them to lysates and DNA under suitable conditions are listed in Table IV.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Assay method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine serum albumin</td>
<td>Detection of $^{131}$I label. Stimulation of antibodies to BSA in mice and rabbits</td>
</tr>
<tr>
<td>Human gamma globulin</td>
<td>Detection of $^{131}$I label. Stimulation of antibodies to HGG in rabbits</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>Sensitization of rabbits</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Detection of Cr$^{51}$ label</td>
</tr>
<tr>
<td>Catalase</td>
<td>Titration with peroxide</td>
</tr>
<tr>
<td>Urease</td>
<td>Titration with urea</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>Antibacterial activity</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>Antibacterial activity</td>
</tr>
<tr>
<td>Glycogen</td>
<td>Best's carmine stain</td>
</tr>
<tr>
<td>Zymozan</td>
<td>Stimulation of heat-stable bactericidins for <em>Escherichia coli</em> in mice*</td>
</tr>
<tr>
<td>Forssman antigen</td>
<td>Stimulation of C.F. antibody</td>
</tr>
<tr>
<td>Rabbit anti-BSA gamma globulin</td>
<td>Direct titration with BSA $^{131}$</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>Detection of hemagglutinins</td>
</tr>
<tr>
<td>Heat killed <em>Pseudomonas</em> organisms</td>
<td>Complement-fixation with specific antiserum. Microscopic observation.</td>
</tr>
</tbody>
</table>

* While this is not specific for zymozan, it is included because of the reported relationship between zymozan and bactericidal activity for *E. coli* (3).

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IV. The criteria used to indicate the presence of these materials in particles are included.

Photometrically standardized preparations of lysates from several species of normal animals were examined for ability to incorporate labelled BSA into particles with DNA. The amount of BSA incorporated into particles varied between 15 and 40 per cent of the total added to the reaction mixture. Most values were in the 20 to 30 per cent range. The results with any one sample of lysate were reproducible within 1 per cent. Different lysate preparations from bleedings of a single normal animal on different days showed a variance within 5 per cent.

Since BSA could be incorporated into the particles from lysate, it was important to determine whether BSA was bound in addition to or in place of hemog-
globin. This problem was resolved by employing lysate preparations made from chromium (Cr⁴⁺)-labelled sheep red cells. The chromium label was known to be bound entirely to the globin portion of the red cells (4). Washed particles, which developed from 1 unit of the chromium-labelled lysate and 1 unit of DNA, contained 78 per cent of the label originally present in the lysate. The addition of BSA (0.3 mg./ml.) to a similar reaction mixture resulted in particles containing only 57 per cent of the chromium label. This indicated that some of the hemoglobin of the particles was displaced by the BSA.

**Text-Fig. 3** The incorporation of BSA into particles of hemoglobin and DNA.

*The Rate of Incorporation of BSA into Particles.*—Text-fg. 3 illustrates the relationship between the amount of BSA incorporated and the period of incubation. The time during which BSA was incorporated most rapidly corresponded to the time during which most of the particles were being formed (See Text-fg. 1). The concentration of BSA affected only the rate at which BSA was incorporated and did not determine the final percentage of BSA incorporated.

*Effect of Antibody on Particle Formation.*—Purified rabbit gamma globulin could be incorporated into particles formed from sheep lysate. However, if the gamma globulin was prepared from high titer anti-sheep erythrocyte serum, it prevented particle development from sheep lysate. The anti-sheep erythrocyte globulin could be incorporated into particles from lysates of other species antigenically unrelated to sheep. The incorporation of labelled BSA into particles from sheep lysates was also blocked by the presence of anti-sheep erythrocyte globulin.

The evidence presented thus far indicates that spherical particles develop at 37°C. and pH 4.8-5.8 through the interaction of hemoglobin and DNA with the aid of some specific component of fresh red cell lysates. During the incubation period, heterologous substances of relatively large molecular size can be

² Obtained from Mr. Roy Weinrach, Department of Medicine, University of Chicago.
incorporated into the particles. The final composition of the particles depends on the amount and kinds of substances (proteins, polypeptides, polysaccharides) present in the reaction medium. For convenience, the letters PIP have been used to designate the particles formed from erythrocyte lysates. The first P stands for "protein, peptide, or polysaccharide"; I stands for "incorporating"; and the second P for "particle."

Having examined in a general way the conditions required for the development of particles from red cell lysates and DNA, we wished to learn something of the structure of the particles and whether or not structure could be controlled in the laboratory. The following experiments were designed toward this end.

**Text-Fig. 4.** The rate of release of BSA from hemoglobin-DNA particles (PIP) digested with trypsin at 37°C.

Factors Affecting PIP Size.—Particle diameter depended primarily on the pH of the reaction mixture, although ionic strength was also important. Fig. 3 shows the relationship between pH and PIP size as determined in deionized water. Indirectly, changes in total protein concentration of the reaction mixture could also affect particle diameter by altering pH and ionic strength. At concentrations above 2 mg./ml. extra protein tended to compete non-specifically for DNA and thus alter particle morphology. It was interesting that the very largest particles developed exclusively within a narrow pH range, and that only these large particles exhibited osmotic properties.

The Intraparticle Distribution of Incorporated BSA.—Some insight into particle structure was gained from the results of three experiments with BSA-PIP.

In the first experiment it was assumed that homogeneous particles would be digested by trypsin at a uniform rate, whereas non-homogeneous structures would probably be digested in a random or irregular manner. Accordingly, suspensions of PIP, containing different con-
centrations of BSA labelled with $^{35}$S, were incubated with sufficient trypsin (1:110) to digest the protein present. Text-fig. 4 shows the rate of breakdown of each preparation as determined by the per cent of total counts released from the particles.

The rate of release of BSA $^{35}$S was the same during the 1st hour for all preparations. Thereafter the rates varied with the concentration of BSA per PIP.

The curves suggest that the particles were similar near the surface but varied internally according to BSA content. Approximately 10 per cent of the bound BSA seemed to be only loosely attached on the surface of the particles, because that amount could be freed by incubation in an alkaline medium without trypsin.

### TABLE V

**Absorption of Anti-BSA Antibody by BSA-PIP**

<table>
<thead>
<tr>
<th>Units* Anti-BSA antibody</th>
<th>Absorbed with†</th>
<th>Per cent antigen bound by antibody (0.08 γ-BSA $^{35}$S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1 γ-BSA nitrogen</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>0.05 ml PIP suspension (containing 10.6 γ-BSA nitrogen)</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Supernatant from 0.5 ml PIP suspension incubated in NRS-borate buffer at pH 8.2, 30 min., 37°C.</td>
<td>15</td>
</tr>
</tbody>
</table>

* Units as determined by the ammonium sulfate precipitation method of Farr (5).
† Absorption carried out in 1/10 normal rabbit serum borate buffer at pH 8.2 for 30 minutes.

A second experiment with BSA-PIP further substantiated the hypothesis that the incorporated BSA was primarily "inside" of the particles. Anti-BSA serum was quantitatively absorbed with BSA-PIP and the amount of absorption compared with that obtained with soluble BSA. Table V shows the results of this experiment. A suspension of particles containing 10 γ of BSA, absorbed the same amount of anti-BSA antibody as did 1 γ of soluble BSA. In other words, 10 per cent of the PIP-BSA was available for reaction at the surfaces of the particles. Since absorption was carried out at pH 8.2, most of the PIP-BSA was probably released from the particles during incubation. The control also suggests this, but regardless of whether free or attached only 10 per cent of the BSA, added as PIP, was available for reaction.

In the third experiment particles containing different concentrations of BSA were dehydrated, imbedded in paraffin, and cut into sections approximately 5 microns thick. Fig. 4 shows the change in particle morphology associated with the incorporation of BSA. Particles without added BSA stained uniformly
Throughout. Those developed in the presence of 0.1 mg./ml. BSA exhibited unstained areas within their structures. Particles from a 1 mg./ml. BSA preparation were smaller (presumably because of a pH shift toward the isoelectric point of BSA) and appeared to consist of a single ring of stained material around a clear vacuole.

If one assumes that the unstained areas represent the location of the incorporated BSA, it is apparent why most of the BSA would not be available for surface reaction.

<table>
<thead>
<tr>
<th>Total antibody per unit* of PIP</th>
<th>Surface reacting antibody</th>
<th>Freshly prepared PIP</th>
<th>After 48 hrs. at 4°C.</th>
<th>After 45 min. at pH 8.2</th>
<th>After 2nd incubation, 45 min. pH 8.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>33 units</td>
<td></td>
<td>2.5</td>
<td>0.33</td>
<td>0.029</td>
<td>0.030</td>
</tr>
</tbody>
</table>

* 1 unit of PIP = 0.001 ml packed particles, 25 minutes, 1500 R.P.M.

**The Intraparticle Distribution of Incorporated Anti-BSA Globulin.**—A second series of experiments was carried out to confirm the findings obtained with BSA-PIP.

In this case ammonium sulfate was added to high titer anti-BSA rabbit serum to a concentration of 37 per cent to precipitate the gamma globulin fraction. The precipitate was washed and then dialyzed against tap water for 24 hours. A solution of the gamma globulin fraction contained 10,000 units of anti-BSA antibody per ml. The purified anti-BSA globulin was then incorporated into particles using a rabbit lysate. The particles were thoroughly washed in water and standardized in suspension on a unit basis so that the final preparation contained 33 units of antibody per unit of PIP. The total amount of surface antibody and that which could be removed from the particles can be seen in Table VI.

Although 1 unit of PIP contained 33 units of antibody, only 2.5 units (roughly 10 per cent) were available for reaction with antigen. Essentially all of the surface antibody could be removed by washing and incubation at pH 8.2.

**DISCUSSION**

The studies presented describe a simple reproducible system wherein macromolecules in solution can be brought together to form uniform particles of a definite composition and structure. Although this type of molecular orientation is easily carried out with non-biologic systems employing various polymers (plastics), it has been virtually impossible with biologic material. The work of Schmitt and his associates (6) with the collagen system, in which all molecular

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3 One unit of antibody precipitates 33 per cent of an $^{131}$-labelled BSA antigen at the 0.08 $\gamma$-level as determined by the ammonium sulfate technique of Farr (5).
orientation is linear and results in fiber formation, and the work of Fraenkel-Conrat and Williams (7), with reconstituted tobacco mosaic virus, represent two systems in which purified biologic molecules have been reassembled into oriented structures in vitro.

As reported previously (1), particle-forming ability followed the hemoglobin moiety when lysates were fractionated in the ultracentrifuge. This was also true for rat hemoglobin when crystallized from fresh lysates at zero degrees, and was further confirmed by preparing particles from Cr51-labelled erythrocytes.

Single molecules of hemoglobin are approximately 50 Å in diameter and not visible as individuals with the electron microscope. That portion of lysate which was most active in particle development, when separated by means of ultracentrifugation, consisted entirely of uniform globules of 200 Å diameter (1). Apparently, the individual molecules of hemoglobin were arranged in some precise way, and not free as separate entities. Whether this was a naturally occurring arrangement or some artifact of osmic acid fixation is not known. However, similar globules of a few hundred angstroms’ diameter can be seen in electron micrographs of ultrathin sections of erythrocytes (8). A globular orientation of hemoglobin at the ultramicroscopic level could be important for particle development. Conceivably, such a spherical unit, resembling in miniature the particles formed in vitro, could be the die on which the larger particles were cast.

Particle formation seemed to depend in part on a simple ionic binding, probably of the globin portion of hemoglobin to DNA. However because the spherical configuration occurred only when freshly prepared cellular extracts were used, it appeared that particle structure also depended on the presence of some third component not found in commercial hemoglobin preparations. The critical temperature requirements for particle development suggested that the particle-forming component of fresh lysates was enzymic in nature. Whether or not the unknown component is an enzyme remains to be determined, but it does appear to be bound to the hemoglobin moiety.

The fact that a variety of compounds may elicit particles suggests that cells may be stimulated to develop certain types of cytoplasmic structures in response to the presence of an array of agents. There are at least two examples in the literature. One is the in vivo and in vitro formation of Heinz bodies on red cells in response to the presence of phenylhydrazine (9). Another is the formation of basophilic granules within leucocytes in the presence of phenolic substances (10). Still other studies suggest that some inclusions like the Negri body are formed by cells in response to the presence of an appropriate virus (11, 12). In these instances, as in our experiments with extracts of different tissues (1), globins other than hemoglobin, were probably the reacting proteins.

Those investigators interested in biologic membranes might find the large
particles described here worthy of further study. Salts and amino acids apparently pass through freely, whereas larger molecules do not. Once again pH seems important, because as particle size varies, so apparently does porosity. It is interesting to speculate on the possible relationship between the large particles and erythrocyte ghosts which they closely resemble (Fig. 2).

Of immediate practical value are the possible technical uses of the hemoglobin particles. Since particle composition and size can be controlled, it is possible to construct particles having fairly well defined properties. In this way soluble enzymes, antibiotics, antigens, and the like may be made particulate and thus manipulated in ways not possible previously (13).

**SUMMARY**

Spherical particles, 1 to 10 microns in diameter, resulted from the incubation at 37°C. of distilled water lysates of erythrocytes with deoxyribonucleic acid (DNA). The particles consisted of 88 per cent hemoglobin and 12 per cent DNA (dry weight basis).

An unknown factor, presumably an enzyme, present only in fresh red cell lysates, was required for particle development. Particle size was a function of the pH of the reaction mixture. The pH range was 4.8-5.8.

It was possible to trap extraneous proteins and polysaccharides in pockets within the hemoglobin particles during their development to the exclusion of some of the hemoglobin. The amount of any one substance so trapped was proportional to its concentration in the reaction mixture.

Some practical applications of the particles, as a means for making particulate various soluble substances (enzymes, antigens, antibiotics), are suggested.

The author wishes to express his appreciation to Dr. C. Phillip Miller of the University of Chicago for permitting some of these studies to be carried out in his laboratory. Gratitude is also due Dr. Richard Farr of the University of Pittsburgh, and Dr. David Talmage and Mrs. Gloria Freter of the University of Chicago for their assistance in carrying out the isotope studies.

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**BIBLIOGRAPHY**

EXPLANATION OF PLATES

PLATE 70

Fig. 1. Typical basophilic particles from DNA and hemoglobin. × 500.

Fig. 2. Photomicrograph of wet preparation of PIP showing large particle. Phase contrast. × 1500.
(Nelson: Particle development in vitro from cytoplasm. II)
FIG. 3. The relationship between PIP size and the pH of the reaction mixture. × 500. The pH was varied by using HCl (0.1 to 1.0 N) and NaOH (0.1 to 1.0 N). The particles were allowed to dry on glass slides, fixed with methanol, and stained with 1 per cent safranine, aqueous.

FIG. 3 a. pH 6.0.
FIG. 3 b. pH 5.8.
FIG. 3 c. pH 5.4.
FIG. 3 d. pH 5.1.
FIG. 3 e. pH 4.8.
FIG. 3 f. pH 4.4.
(Nelson: Particle development in vitro from cytoplasm. II)
PLATE 72

FIG. 4. Hematoxylin-eosin stained sections (5 microns) of paraffin imbedded PIP with and without incorporated BSA.
FIG. 4 a. No added BSA.
FIG. 4 b. Developed in presence of 0.1 mg./ml. BSA.
FIG. 4 c. Developed in presence of 1 mg./ml. BSA.
(Nelson: Particle development in vitro from cytoplasm. II)