The Cellular Location of Adenyl Cyclase in the Pigeon Erythrocyte

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It has been reported by Sutherland, Rall, and Menon (1) that particulate preparations from a number of tissues, including pigeon erythrocytes, are able to synthesize adenosine 3',5'-phosphate (cyclic 3',5'-AMP) when they are incubated in the presence of adenosine triphosphate and Mg++. The name adenyl cyclase was proposed for the enzyme system catalyzing the synthesis of cyclic AMP when they are incubated in the presence of adenosine triphosphate and Mg++. The name adenyl cyclase was proposed for the enzyme system catalyzing the synthesis of cyclic AMP. This enzyme catalyzes the conversion of ATP to cyclic AMP in various biological systems. The enzyme is present in a wide variety of tissues and has been purified from many sources.

The pigeon erythrocyte was chosen for the present investigation because its relatively simple intracellular structure and because it provided a uniformity of cell type not found in more organized tissues such as liver, brain, and muscle, where blood vessels, connective tissue, and a multiplicity of cell types occur.

The work of Rajam and Jackson (2) and of Neville (3) shows that, under certain conditions of homogenization, both the nuclei and the cell membranes can be sedimented by centrifugation at low speed. The centrifugal behavior of a particle is determined by its inherent properties such as size, shape, and density; however, this behavior may be modified by the mechanical or other association of the particle with other cell components during centrifugation. It is probable that the sedimentation behavior of the membranes of pigeon erythrocytes, which were prepared by exposure of cells to hypotonic conditions, is due at least in part to the envelope of the cell collapsing around the heavy nucleus. Whether the simultaneous sedimentation of membranes and nuclei is due to their mechanical association or to the size of the membrane, it has been found that these two components can be separated effectively by utilizing a dispersion procedure which extensively fragments the membranes and yet does little damage to the nuclei.

Disruption of cells by the use of a pestle homogenizer or of units such as the Waring Blender has the basic disadvantage that it is difficult to control the disruptive force applied to the cells, and, furthermore, the contents of the broken cells are subjected to further disruption. For the present investigation a device was constructed that utilizes the principle of the apparatus described by Melner, Lawrence, and French (5). The apparatus allows the stresses which are applied to the cell to be standardized and provides the further advantage that once the cell is disrupted the cell contents are subjected to minimal further stresses.

Experimental Procedure

Adult white pigeons of either sex were obtained from Palmetto Pigeon Plant, Sumter, South Carolina, and rats (albino Wistar strain) were obtained from Albino Farms, Red Bank, New Jersey. All reagents were of analytical grade and were used without further purification. All unbuffered solutions used in the preparation of tissues and for the fractionation procedures were prepared in glass-distilled water and adjusted to pH 6.8 to 7.2 with sodium carbonate.

The preparation of tissues and the fraction procedures were performed at 0–4°C. The densities of sucrose solutions were computed from the data contained in the 40th edition of the Handbook of Chemistry and Physics, and are expressed as specific gravities at 20°C. Centrifugal forces are given as the maximal value attained at the tip of the tube. Adenyl cyclase was assayed by the method of Rall and Sutherland (6). In this report 1 unit of adenyl cyclase activity is defined as the quantity of enzyme that will produce, in 15 minutes at 37°C, 1 × 10^-4 mole of cyclic 3',5'-AMP in an incubation mixture containing 0.04 M Tris, pH 7.4, 2 × 10^-2 M ATP, 3.3 × 10^-3 M MgSO4, 8.7 × 10^-4 M caffeine, 1 × 10^-4 M NaF, and 1 × 10^-4 M Versene.

Cytochrome oxidase was assayed by the method of Cooperstein and Lazarow (7). Phosphate was estimated by the method of Fiske and SubbaRow (8). Sucrose was estimated by the method of Seifter et al. (9), DNA by the method of Burton (10), and protein according to Lowry et al. (11). Bovine serum albumin was used as a standard for the protein determinations and commercial purified DNA as a standard for the DNA determinations.

Dog blood was drawn from the femoral veins of animals anesthetized with secobarbital. Pigeon blood was drawn from the hearts of pigeons anesthetized with ether. The washed erythrocytes were prepared as previously described (12), with the exception that chilled 0.154 M sodium chloride solution was used instead of Krebs bicarbonate buffer.

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1 The assay procedure previously has been performed at 30°C; therefore, units reported here are two-thirds as large as those reported previously.
Disruption of Pigeon Erythrocytes—The choice of a suspension medium for the disruption and subsequent fractionation of the pigeon erythrocytes was a compromise between several factors. If the cells were disrupted in isotonic sucrose, in the absence of added electrolytes, the nuclei burst and the resulting gel made further fractionation impractical. The bursting of the nuclei could be prevented by disruption of the cells in isotonic electrolyte solutions, but the use of electrolytes resulted in aggregation of the smaller particles and limited the usefulness of subsequent differential centrifugation. The addition of calcium ions to the isotonic sucrose, as suggested by the work of Schneider and Petermann (13), effectively prevented the bursting of the nuclei; however, in the present investigation this procedure had the disadvantage that calcium ions interfered with the assay of adenyl cyclase.

The most satisfactory compromise was to disrupt the cells in 0.9% NaCl solution, separate the particles into nuclei and smaller particulate matter, and then resuspend the smaller particles in sucrose solutions for further fractionation.

Fractionation of Pigeon Erythrocytes into Nuclear and Non-nuclear Fractions: Preparation of "78,000 × g Particles"—Table I is presented to show that the distribution of adenyl cyclase between particulate fractions of disrupted erythrocytes was dependent both on the method of disrupting the cells and on the relatively minor differences in the methods of fractionation.
TABLE I

**Distribution of adenylyl cyclase among particulate fractions of pigeon erythrocytes**

Particles were prepared by one of the following procedures.

**Procedure A (Lysis of Cells with Water)—**Packed cells (2 ml) were lysed by thorough mixing with 8 ml of chilled water. Sodium chloride (0.3 M) was added to give a final added salt concentration of 0.15 M. The lysed cells were centrifuged at 600 X \( g \) for 15 minutes and the resulting supernatant fluid was centrifuged at 78,000 X \( g \) for 1 hour.

**Procedure B (Lysis of Cells with Water and Layering)—**This procedure was similar to Procedure A, except that 20 ml of the cell lysate (plus NaCl) were layered over 10 ml of a solution comprising 0.15 M sodium chloride, 0.001 M Versene, and 20\% (w/v) glycerol.

**Procedure C (Pressure Homogenization and Layering)—**Packed cells (2 ml) were suspended in 18 ml of 0.15 M sodium chloride-0.001 M Versene and dispersed in the pressure homogenizer. The resulting particle suspension was layered over 10 ml of 0.15 M sodium chloride-0.001 M Versene-20\% (w/v) glycerol and centrifuged at 600 X \( g \) for 15 minutes. The entire supernatant was then centrifuged at 78,000 X \( g \) for 1 hour.

All values are corrected for an initial sample of 1 ml of packed cells.

<table>
<thead>
<tr>
<th>Homogenization procedure</th>
<th>Experiment</th>
<th>Adenyl cyclase</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>600 X ( g )</td>
<td>78,000 X ( g )</td>
<td>78,000 X ( g )</td>
</tr>
<tr>
<td>Water lysis (A)</td>
<td>1</td>
<td>400 units x 10^-4</td>
<td>13.0 mg</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>358</td>
<td>0.1</td>
</tr>
<tr>
<td>Water lysis + layering (B)</td>
<td>3</td>
<td>332</td>
<td>17.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>175</td>
<td>12.2</td>
</tr>
<tr>
<td>Pressure homogenizer + layering (C)</td>
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<td>26</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>43</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Both adenylyl cyclase activity and DNA content of the fractions obtained are shown.

It was found that when the pigeon erythrocytes were lysed by exposure to hypotonic conditions (Procedure A), the adenylyl cyclase could be sedimented by centrifugation at 600 X \( g \) for 15 minutes. This observation was in agreement with the findings of Sutherland et al. (1). It was also found that treatment of the cell lysate with a pestle homogenizer did not alter significantly the distribution of adenylyl cyclase or DNA. If the lysate of the cell lysate was layered over a denser medium (Procedure B), a significant proportion of the adenylyl cyclase remained suspended after centrifugation of the system at 600 X \( g \) for 15 minutes.

The results differed markedly from those cited above when the pressure homogenizer was used to disrupt the cells and the homogenate was layered over a denser medium before centrifugation. In this case only a small fraction of the adenylyl cyclase activity was sedimented at 600 X \( g \). The “nuclear” fraction contained most of the DNA and, on microscopic examination, was seen to be predominantly typical nuclei, very few appearing to be grossly damaged. The presence of most of the adenylyl cyclase activity in the 78,000 X \( g \) precipitate, which contained no visible nuclei and only a small fraction of the DNA, provided strong evidence that the adenylyl cyclase was not primarily associated with the nuclei. The particles obtained at 78,000 X \( g \) by the above procedure were subjected to further fractionation and they will be referred to in this paper as “78,000 X \( g \) particles.”

An attempt was made to subfractionate these pigeon erythrocyte 78,000 X \( g \) particles by suspending them in 0.25 M sucrose with a pestle homogenizer and subjecting the suspension to conventional differential centrifugation. It was found that the adenylyl cyclase was distributed between the precipitate obtained by centrifugation at 10,000 X \( g \) for 10 minutes (mitochondrial fraction) and that obtained by subsequent centrifugation at 78,000 X \( g \) for 1 hour (microsomal fraction). The results were variable and appeared to depend on the quantity of salt in the original particle preparation and on the efficiency of its suspension in 0.25 M sucrose.

**Density Gradient Fractionation of Pigeon and Dog Erythrocyte 78,000 X \( g \) Particles—**If particles of various densities are suspended uniformly throughout a medium of varying density, and the system is subjected to a gravitational force, the particles will tend to move toward the region of their own density. This technique is discussed in several excellent reviews (14-16). The 78,000 X \( g \) particle preparations, each derived from 2 ml of packed pigeon erythrocytes, were subjected to the density gradient fractionation described under “Experimental Procedure.”

**Materials and Analytical Methods—**The distribution, in a typical experiment, of adenylyl cyclase activity and protein among Fractions 1 to 12 is shown in Fig. 2. The protein estimation showed that the 78,000 X \( g \) particles had been resolved into two components. The lesser component occurred in Fraction 1 and consisted mainly of the material that sedimented during the density gradient centrifugation. It contained no cyclase activity. The major component occurred in the region of Fraction 7, and in this region the distribution of adenylyl cyclase closely followed that of protein. The density of Fraction 7 at 20° was computed to be 1.18.

Washed dog erythrocytes were also subjected to pressure homogenization, layering, and differential centrifugation (Procedure C). A very small precipitate was obtained by centrifugation of the homogenate at 600 X \( g \), whereas the yield of 78,000 X \( g \) particles was very similar to that previously observed with pigeon erythrocyte. Microscopic examination of the 600 X \( g \) precipitate showed that it consisted of unbroken and partially

![Fig. 2. Gradient density fractionation of pigeon erythrocyte 78,000 X \( g \) particles derived from 1 ml of packed cells.](http://www.jbc.org/DownloadedFrom.../by guest on October 29, 2017)
broken erythrocytes. It was suspended in water to complete the rupture of the cells, and the suspension was centrifuged at 78,000 × g in the presence of a small amount of sodium chloride. The resulting precipitate contained only 1.2% of the total sedimentable protein of the original sample. The 78,000 × g particles, which thus comprised 98% of the particulate protein of the erythrocyte, were subjected to the gradient density fractionation. The distribution of protein among Fractions 1 to 12 is shown in Fig. 3.

The quantity of protein present in Fraction 1 was considerably smaller than that obtained from pigeon erythrocytes; however, both the total quantity and the distribution of protein in the major component, which occurred in the region of Fraction 8, were very similar to those found previously for pigeon. This major component comprised 90.5% of the total sedimentable protein of the original sample. The density of Fraction 8 at 20° was computed to be 1.16.

The work of Holter, Ottesen, and Weber (17) and others indicated that the densities of liver mitochondria and microsomes, as determined by density gradient centrifugation, are in the ranges 1.10 to 1.20 and 1.25 to 1.30, respectively. In the system just described, therefore, one would expect liver microsomes to occur in the pellet at the bottom of the tube, and the mitochondria to be located in the same region as the particles carrying adenyl cyclase (see “Discussion” for densities of microsomes).

**Fractionation of Rat Liver**—Since the density of the particles carrying adenyl cyclase was very similar to that suggested for mitochondria of liver, it seemed desirable to homogenize and fractionate rat liver by the current procedure, even though from previous experiments in which different methods of fractionation had been used it had been reported that mitochondria were not the carriers of adenyl cyclase (1). The distribution of adenyl cyclase in homogenates prepared in a pestle homogenizer and in the pressure homogenizer were compared.

Rats were killed by a blow on the head and their livers were perfused with chilled 0.25 M sucrose. The livers were chopped into small pieces and forced through a fine stainless steel sieve. The sieved tissue could be readily suspended in 0.15 M sodium chloride or 0.25 M sucrose for dispersion in either a pestle homogenizer or the pressure homogenizer.

When the tissue was dispersed in 0.15 M sodium chloride, by use of either homogenizer, all the adenyl cyclase activity could be sedimented by centrifugation of the homogenate at 600 × g for 10 minutes. It was evident that such a salt solution was not a desirable medium for dispersal, since microscopic examination of the 600 × g precipitate disclosed extensive aggregation of small particulate matter. Therefore, the use of sucrose was investigated. The results obtained when the liver tissue was dispersed in 0.25 M sucrose by either the pestle or the pressure homogenizer are shown in Table II.

When the pestle homogenizer was used (Procedure D), the adenyl cyclase activity was distributed among all fractions. The use of the pressure homogenizer (Procedure E) resulted in all the activity being in the 78,000 × g precipitate; however, dissociation of the adenyl cyclase from nuclear material could not be demonstrated in this experiment because of the occurrence of large amounts of DNA in the 78,000 × g precipitate.

The main reason for the fractionation of the rat liver was to investigate the possible association of adenyl cyclase with whole or fragmented mitochondria. Also shown in Table II are the cytochrome oxidase activity and the protein content of the fractions. Whereas the technique used for dispersion of the tissue sample had a marked effect on the distribution of adenyl cyclase, the distribution of cytochrome oxidase was virtually unaffected. With either the pestle or the pressure type of homogenizer, the bulk of the cytochrome oxidase was sedimented by centrifugation at 10,000 × g for 10 minutes. Neither the protein content nor the microscopic appearance of this mitochondrial precipitate was significantly influenced by the type of dispersion procedure used. The complete lack of correlation between the activities of adenyl cyclase and cytochrome oxidase, and the absence of a significant portion of the adenyl cyclase from the typical mito-

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**Table II**

<table>
<thead>
<tr>
<th>Homogenization procedure</th>
<th>Adenyl cyclase</th>
<th>DNA</th>
<th>Cytochrome oxidase</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>600 X g ppt.</td>
<td>10,000 X g ppt.</td>
<td>78,000 X g ppt.</td>
<td>600 X g ppt.</td>
</tr>
<tr>
<td>PESTLE (D) .......</td>
<td>131</td>
<td>67</td>
<td>177</td>
<td>6.47</td>
</tr>
<tr>
<td>PRESSURE (E) .............</td>
<td>0</td>
<td>0</td>
<td>215</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Sieved liver tissue (2 g) was homogenized in 20 ml of 0.25 M sucrose by use of either a pestle homogenizer (Procedure D) or the pressure homogenizer (Procedure E). The resulting homogenate was centrifuged successively at 600 X g for 15 minutes and at 78,000 X g for 1 hour. All results are corrected for an initial sample of 1 g of sieved tissue.
ehondrial fractions, established that adenyl cyclase was not primarily associated with the mitochondria.

Separation of "Cell Membranes" from Rat Liver—It was considered that the procedure based on pressure homogenization of tissues and density gradient centrifugation of the non-nuclear fraction of the homogenate provided an approach to the problem of the isolation of cell membranes. An attempt was made to apply these procedures to rat liver tissue. A preliminary experiment showed that if Ca++ was included in the dispersal medium, the non-nuclear fraction contained very little DNA, an indication that the Ca++ could protect the nuclei from rupture during pressure homogenization. The presence of Ca++ precluded the accurate evaluation of the distribution of adenyl cyclase; however, if the particles obtained from the density gradient fractionation were washed with a solution of Versene, the adenyl cyclase could be located.

Sieved liver tissue (2 g) was suspended in 20 ml of 0.25 M sucrose-0.002 M Ca++ and dispersed in the pressure homogenizer. The homogenate was centrifuged at 10,000 × g for 10 minutes to remove the nuclei and mitochondria. The supernatant fluid was centrifuged at 78,000 × g for 1 hour, and the resulting precipitate was subjected to the standard density gradient fractionation.

The distribution of adenyl cyclase activity and protein is shown in Fig. 4. It was found that the quantity of protein in the fractions was much greater than when a comparable sample of erythrocyte particles was subjected to the same procedure, and that the adenyl cyclase was distributed throughout a larger density range. Nevertheless, a concentration of both adenyl cyclase and protein occurred in the region of Fraction 4, most of the activity occurring over the density range 1.18 to 1.21. It was significant that the activity was not concentrated in Fraction 1, where the rough microsomes would be expected to occur (see "Discussion").

Electron Microscopy—It has been shown in the preceding sections that non-nuclear particles obtained from dog, pigeon, and rat tissues can be fractionated by density gradient centrifugation to yield a major component, of characteristic density, with which the adenyl cyclase activity is associated. The material representing the protein peak of this component was subjected to electron microscopy. The samples were suspended in 0.001 M Tris buffer and fixed with 1.5% osmium tetroxide. They were embedded in methyl butyl methacrylate (1:9) and sectioned on a Porter-Blum microtome. The sections were stained with lead hydroxide and examined in an RCA electron microscope (model RCA EMU-2D). It was found that tissues homogenized at 1500 pounds per square inch of pressure yielded samples containing particles that were too small for reliable description. The preparation of samples for electron microscopy required the use of a homogenization pressure that would disrupt a reasonable proportion of the cells of the sample without fragmenting the membranes so extensively that they lost their characteristic appearance. This situation was achieved in the cases of dog erythrocytes and rat liver, but not pigeon erythrocytes. It appeared that once the pigeon erythrocytes were ruptured, the membranes were so fragmented that electron micrographs provided little information. The conditions used are described in the legend accompanying Fig. 5. The behavior of the particles during fractionation was the same as previously described, with the exception that a larger proportion of unbroken cells occurred in the 600 × g precipitates.

**DISCUSSION**

It was considered that the sedimentation of cell membrane fragments by low speed centrifugation was a consequence either of their association with the dense nuclei or of the relatively large size of the fragments which resulted from the dispersion of cells by conventional means of homogenization. The association of adenyl cyclase with the cell membranes of pigeon erythrocytes was suggested when it was observed that the use of the pressure homogenizer, instead of conventional procedures, completely altered the centrifugal behavior of the particles carrying the adenyl cyclase, and yet caused no visible damage to the nuclei. After pressure homogenization, the adenyl cyclase could only be sedimented by high speed centrifugation. The hypothesis concerning the importance of membrane size was supported by the observation that the membrane "ghosts" of dog erythrocytes prepared by lysing the cells with water could be sedimented by relatively low centrifugal forces, whereas passage of the cells through the pressure homogenizer resulted in the cell membrane being fragmented into particles which required high centrifugal forces for their sedimentation. It was concluded that adenyl cyclase was not primarily associated with the erythrocyte nucleus; indeed, when pressure homogenization was used, the bulk of the enzyme activity occurred in a fraction devoid of visible nuclei and substantially free of DNA.

The particles carrying the adenyl cyclase were characterized further by density gradient centrifugation. The enzyme was associated with a component that was very similar in both density and yield per unit of volume of packed cells to that derived under the same conditions from dog erythrocytes. In the case of the dog erythrocytes this component comprised over 96% of the total particulate protein of the cell, and it was concluded that it consisted predominantly of fragments derived from the cell membrane. Strong evidence was thus obtained that the equivalent fraction from pigeon erythrocytes was also derived from the cell membrane.

The peak density of this membranous fraction from pigeon erythrocytes was computed to be 1.18. This value was in accord with the observations of Neville (3) and Emmelot and Bos (18).

All electron microscopy was supervised by Dr. Melvin D. Schoenberg in the Department of Pathology. The authors are grateful for his kind cooperation and interpretation of results.
FIG. 5. A, Membranes from dog erythrocytes. Homogenization pressure was 1000 pounds per square inch. X32,400. B, Membranes from rat liver. Homogenization pressure was 750 pounds per square inch. X32,400.
on the centrifugal behavior, in a multilayered system, of cell membranes from rat liver.

When rat liver was studied, it was found that pressure homogenization did not significantly affect the mitochondrial fractions obtained with regard to yield, microscopic appearance, or cytochrome oxidase activity, and that the mitochondrial fractions contained only a small portion of the adenyl cyclase activity. This not only demonstrated the lack of association of adenyl cyclase with mitochondria, but also showed that pressure homogenization did not cause significant fragmentation of these particles. In a single experiment an attempt was made to apply the techniques developed for the above work to the separation of a membrane component from rat liver. Although an accurate evaluation of the distribution of adenyl cyclase was not attempted in this experiment, the results of the density gradient fractionation showed a marked similarity to those previously obtained when pigeon erythrocytes were used. The adenyl cyclase activity was distributed over a greater density range, however, and most of the activity occurred over the density range of 1.18 and 1.21. It was of interest that only a small portion of the adenyl cyclase occurred in Fraction 1, where the rough microsomes would be expected to occur.

The electron micrographs of the major density gradient peak from dog erythrocytes and rat liver served to characterize further the cellular fraction with which the adenyl cyclase was associated. The micrographs showed characteristic structures similar to the cell membranes of liver prepared by Neville (3).

The work of Palade (19) and others has emphasized the complexity of the subcellular structure, particularly with regard to existence of systems of membranes within the cell known as the endoplasmic reticulum. Certain studies have shown that microsomes derived from the endoplasmic reticulum have a high density, but only recently has it become clear (20, 21) that whereas rough microsomes have a high density, the smooth microsomes have a low density. Some appear to have densities similar to those found for cell membranes. The authors have not yet found any careful studies of the possible presence of smooth microsomes in pigeon erythrocytes.

Fouts has studied the distribution of drug-metabolizing enzymes in microsomes and finds them localized in the smooth microsomes (22). He has stated that classical nuclear fractions contain only 10 to 15% of the drug-metabolizing enzymes, whereas classical smooth microsomes contain about 80% of the activity. This distribution is the reverse of the distribution of adenyl cyclase when homogenates are fractionated by centrifugation by usual homogenization techniques. Thus it seems probable that in liver, at least, adenyl cyclase is associated primarily with the cell membrane rather than with the smooth endoplasmic reticulum. An absolute conclusion is not justified yet because of the possibility that the drug-metabolizing enzymes may be localized in specific portions of the smooth endoplasmic reticulum which tend to form microsomes.

It is attractive to speculate that adenyl cyclase forms part of a hormone-responsive system built into the cell membrane. If this were the case, it would be unnecessary for hormones such as the catecholamines and glucose to enter the cell. They may exert their action by influencing the production of cyclic 3',5'-AMP at the membrane; the nucleotide then, in turn, would influence the metabolism within the interior of the cell. In addition to the foregoing, since adenyl cyclase is distributed widely in animal tissues, this enzyme may prove of value as a marker of the cell membrane.

### Summary

1. Homogenates of pigeon erythrocytes were prepared by use of pressure and were fractionated by centrifugation. In contrast to other homogenates, adenyl cyclase appeared in non-nuclear fractions on centrifugation. Most of the nuclei appeared intact.

2. Fractions from homogenates of non-nucleated dog erythrocytes and pigeon nucleated erythrocytes were collected between 600 x g and 78,000 x g. The major component of each migrated in a sucrose density gradient in a similar manner with peaks at density of 1.16 and 1.18, respectively. The cyclase activity of the pigeon erythrocyte fraction followed closely the distribution of the major component. A fraction from rat liver homogenate was collected between 10,000 x g and 78,000 x g and was centrifuged in a similar sucrose density gradient. Highest adenyl cyclase activity was found in the density range of 1.18 to 1.21.

3. Electron microscopy revealed structures similar to cell membranes when dog erythrocytes and rat liver fractions were studied. Pigeon erythrocyte fractions had been shattered to a fine debris.

4. All experiments to date are compatible with the localization of adenyl cyclase in cell membranes. In the two tissues in which localization has been studied most intensively, i.e. pigeon erythrocytes and rat liver, it seems probable that all, or almost all, adenyl cyclase is in the cell membranes.

### References

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