solubilized preparation of rat myocardial adenylate cyclase, but results in a striking increase in sensitivity to norepinephrine. This degree of sensitivity approaches that observed in intact physiological preparations (19).

Phosphatidylinositol is normally present in heart tissue. It comprises about 3.3\% of lipid-phosphorus in rat heart, 2.3\% in sheep heart, and 7.3\% in ox heart (20) or approximately 0.02 mg to 0.11 mg of lipid-phosphorus per g of heart muscle, fresh weight. It may be calculated that incubations with the particulate enzyme contained about 0.001 to 0.022 mg of phosphatidylinositol, values roughly comparable to the phosphatidylinositol required in the solubilized preparation as shown in Fig. 3.

Since competitive beta adrenergic blocking agents, such as dl-propranolol, abolish catecholamine activation of the enzyme in intact muscle and in particulate heart preparations (1, 3, 21), it would appear that beta adrenergic blockade would be a necessary prerequisite in considering the specificity of any in vitro system attempting to define the molecular nature of a beta adrenergic receptor. The data show that dl-propranolol abolished the activation of solubilized adenylate cyclase produced by norepinephrine in the presence of phosphatidylinositol.

Recently, Lefkowitz and Haber (22) isolated a partially purified cardiac beta receptor in a microsomal fraction of canine ventricle. They measured displacement of bound [H]norepinephrine by unlabeled norepinephrine and determined that 50 To \textit{X} 10^{-7} M norepinephrine by unlabeled norepinephrine and determined that 50 To \textit{X} 10^{-7} M norepinephrine by unlabeled norepinephrine and determined that 50 To \textit{X} 10^{-7} M norepinephrine by unlabeled norepinephrine and determined that 50 To \textit{X} 10^{-7} M norepinephrine by unlabeled norepinephrine and determined that 50 To 0.11 mg of lipid-phosphorus per g of heart muscle, fresh weight. This figure is similar to that obtained for half-maximal activation of the solubilized adenylate cyclase by norepinephrine. The Lefkowitz and Haber preparation also seems to provide a useful system for determining the molecular components of the cardiac beta receptor.

Acknowledgments—The author is grateful to Dr. R. Pressman for his many helpful suggestions in the preparation of this manuscript and to Mrs. Eva Ruiz and Mrs. Antoinette Schenk for their excellent technical assistance.

REFERENCES
1. Murad, F., CHI, Y.-M., RALL, T. W., AND SUTHERLAND, E. W., J. Biol. Chem., 237, 1239 (1969).
2. Murad, F., AND VAUGHAN, M., Biochim. Biophys. Acta, 18, 1033 (1969).
3. LEVEY, G. S., AND EISEN, S. E., Curr. Res., 34, 151 (1969).
4. LEVENE, L., AND LEVEY, G. S., J. Clin. Invest., 51, 1012 (1973).
5. LEVEY, G. S., AND EISEN, S. E., J. Clin. Invest., 59, 1463 (1968).
6. SUTHERLAND, E. W., RALL, T. W., AND MENON, T., J. Biol. Chem., 237, 1230 (1962).
7. WATE, D. R., Pharmacol. Rev., 49, 49 (1968).
8. EBERHARD, H., FLEISCH, J. H., AND MITTAG, T. W., Pharmacol. Rev., 31, 131 (1969).
9. LEVENE, G. S., Biochim. Biophys. Acta, 38, 86 (1970).
10. LEVENE, G. S., AND EVANS, N. Y. Acad. Sci., 185, 449 (1971).
11. LEVENE, G. S., Biochim. Biophys. Acta, 48, 105 (1971).
12. KHADRA, G., WEISS, B., AND BRODIC, B. B., J. Pharmacol. Exp. Ther., 163, 379 (1968).
13. LEVINE, G. S., BURLINGTON, C., AND EISEN, S. E., J. Clin. Invest., 45, 2044 (1969).
14. LOWRY, O. H., ROSS, R., AND RANDALL, R. J., J. Biol. Chem., 193, 265 (1951).
15. ROSS, R., AND KNOBLOCH, H. M., J. Biol. Chem., 246, 1851 (1971).
16. BURNSTADT, L., POHL, S. L., AND RODBELL, M., J. Biol. Chem., 246, 1857 (1971).
17. MACCHIA, V., AND PASTAN, I., J. Biol. Chem., 242, 1864 (1967).
18. POHL, S. L., KNOBLOCH, H. M., KOSYREFF, V., BURNSTADT, L., AND RODBELL, M., J. Biol. Chem., 246, 4447 (1971).
19. BUCCINO, R. A., SPANN, J. F., POOL, R. E., AND ROBBINS, L., J. Biol. Chem., 245, 341 (1971).
20. ANSELL, G. B., AND HAWTHORNE, J. N., Phospholipids, Chemistry, metabolism and function. Elsevier Publishing Company, New York, 1964, p. 414.
TABLE I

Effect of various RNA preparations on the synthesis of anti-ovalbumin-precipitable radioactivity in the reticulocyte lysate system

| RNA added | Concentration µg/ml | Anti-ovalbumin cpm/100 µg | Total protein cpm X 10^{-4} | Percent of total |
|-----------|---------------------|--------------------------|-----------------------------|-----------------|
| Experiment 1 |                     |                          |                             |                 |
| None       | 47                  | 10.00                    | 0.05                        |                 |
| Hen oviduct polysomal | 10                 | 155                      | 10.60                       | 0.15            |
| Fraction 1 | 20                  | 277                      | 9.70                        | 0.20            |
| Fraction 2 | 40                  | 506                      | 8.50                        | 0.60            |
| Fraction 3 | 80                  | 814                      | 7.36                        | 0.56            |
| Fractions 4 + 1 | 80                | 585                      | 6.85                        | 0.87            |
| Fractions 5 + 2 | 50               | 480                      | 7.72                        | 0.62            |
| Experiment 2 |                     |                          |                             |                 |
| Hen oviduct total* | 200                | 2180                     | 7.22                        | 3.02            |
| Hen liver total* | 33                 | 0                        | 0.91                        | 0.60            |
| Hen oviduct + liver | 200              | 1380                     | 6.67                        | 2.06            |
| 400 + 300 | 1160                | 6.94                     | 1.67                        |                 |
| Experiment 3 |                     |                          |                             |                 |
| Hen oviduct total | 104                | 1350                     | 8.52                        | 1.62            |
| Hen liver total | 208                | 2180                     | 7.22                        | 3.02            |
| Hen liver fractions | 312               | 312                      | 1.42                        | 0.90            |
| 50 + 600 | 654                 | 7.45                     | 6.25                        |                 |
| 1250 + 600 | 650                 | 6.10                     | 10.63                       |                 |

*Non-specific radioactivity trapped by anti-BSA ranged from 85 to 140 cpm with an average of 110 cpm.
* Fractions 1 and 2 correspond to those shown in Fig. 3. Tissue from the magnum portion of the oviduct of an actively laying hen was homogenized with a Dounce homogenizer in 9 volumes of polysome buffer (25 mM Tris-HCl, pH 7.4, at 4°C, 25 mM NaCl, 5 mM MgCl2), containing 140 mM sucrose, 100 µg per ml of sodium heparin, 1% sodium desoxycholate, and 1% Triton X-100, and centrifuged at 27,000 X g for 5 min. Heavy polysomes were separated from lighter polysomes, monosomes, and supernatant protein by the discontinuous sucrose gradient method of Palmiter et al. (9). Migration toward the anode was from left to right. A, reaction contained no oviduct RNA and [14C]ovalbumin (9) was added as a marker. [14C]Ovalbumin and antibodies were kindly provided by Dr. Palmier. B, reaction contained 40 µg per ml of oviduct polysomal RNA, similar to Fraction 4 in Fig. 3. The radioactive peak at Fraction 28 in Gels A and B probably results from the fact that the washing procedure for antibody precipitates used for these gels was less efficient than that used for Gel C (see text). C, reaction contained 400 µg per ml of oviduct total nucleic acid (see Table I, Footnote c) and 50 PC1 per ml of [3,4-3H]leucine. Before antibody precipitation the reaction mixture was combined with [3H]leucine-labeled ovalbumin. D, 200 µg of trichloroacetic acid-precipitated protein from total reaction mixture containing no oviduct RNA.

Fig. 1. SDS-acrylamide gel electrophoresis of anti-ovalbumin-precipitable radioactivity (A to C) and total reaction mixture (D). Antibody precipitates from 200 µl of reaction mixture were subjected to electrophoresis as previously described by Palmiter et al. (9). Migration toward the anode was from left to right. A, reaction contained no oviduct RNA and [14C]ovalbumin (9) was added as a marker. [14C]Ovalbumin and antibodies were kindly provided by Dr. Palmier. B, reaction contained 40 µg per ml of oviduct polysomal RNA, similar to Fraction 4 in Fig. 3. The radioactive peak at Fraction 28 in Gels A and B probably results from the fact that the washing procedure for antibody precipitates used for these gels was less efficient than that used for Gel C (see text). C, reaction contained 400 µg per ml of oviduct total nucleic acid (see Table I, Footnote c) and 50 PC1 per ml of [3,4-3H]leucine. Before antibody precipitation the reaction mixture was combined with [3H]leucine-labeled ovalbumin. D, 200 µg of trichloroacetic acid-precipitated protein from total reaction mixture containing no oviduct RNA.

From the size of the ovalbumin molecule (387 amino acid residues (11)) one can calculate a minimum molecular weight of 350,000 for ovalbumin mRNA, and estimate a sedimentation coefficient of approximately 14 S (12). RNA was prepared from hen oviduct polysomes, and a fraction sedimenting between about 11 S and 17 S was tested in the cell-free protein-synthesizing system (Table I, Experiment 1). Increasing concentrations of RNA produced nearly linear increases in the percentage of total radioactivity precipitated by anti-ovalbumin. The 28 S ribosomal RNA species (Fraction 1 in Table I) failed to produce anti-ovalbumin precipitable radioactivity. To rule out the possibility...
tive activity was due to inhibitory substances that interfere with mRNA detection, this fraction was tested in the presence of 11 to 17 S RNA (Experiment 1, Fractions 4 + 1). Some inhibition was observed, both in antibody-precipitable radioactivity and in percentage of total incorporation, but activity was not completely suppressed, indicating the absence of ovalbumin mRNA activity in the 28 S rRNA fraction. Experiment 2 shows that detection of ovalbumin mRNA activity does not require a highly purified RNA fraction. Total phenol-extracted nucleic acid from hen oviduct was active in directing the synthesis of anti-ovalbumin precipitated radioactivity, although hen liver nucleic acid obtained by the same procedure was inactive. A mixing experiment similar to that described above showed that the liver nucleic acid preparation partially suppressed the translation of ovalbumin mRNA, but not completely. To test whether the reticulocyte lysate system could be saturated with ovalbumin mRNA, the concentration dependence of ovalbumin synthesis was investigated at high levels of oviduct nucleic acid. Experiment 3 shows that the system continued to give a linear response at nucleic acid concentrations as high as 1.2 mg per ml where the radioactivity in ovalbumin represented more than 10% of that in total protein.

Two experiments were performed to characterize the protein synthesized under the direction of oviduct RNA. The molecular size of the antibody-precipitable radioactivity was determined by SDS-acrylamide gel electrophoresis (9). When oviduct polysomal RNA was added to the cell-free system the major portion of radioactivity migrated in a single peak (Fig. 1B). The mobility of this peak was similar to that of [14C]ovalbumin prepared in oviduct explants (9), Fig. 1A). [3H]Ovalbumin was subjected to electrophoresis with [14C]ovalbumin as an internal marker. The protein synthesized in the reticulocyte lysate had a slightly higher mobility (Fig. 1C). A possible explanation for this is that the ovalbumin molecule synthesized in the cell-free system lacks the carbohydrate moiety (mol wt approximately 1500 (11)). Fig. 1D shows that in the absence of oviduct RNA the reticulocyte lysate does not synthesize detectable amounts of protein having the electrophoretic mobility of ovalbumin.

In a second experiment [3H]anti-ovalbumin precipitate and authentic [14C]ovalbumin were digested with trypsin, and peptides were partially resolved by ion exchange column chromatography (Fig. 2A). The correspondence of each [3H] peak with a
\( ^{14} \)C peak further suggests the identity of the protein synthesized under the direction of oviduct RNA as ovalbumin. As a control, total \(^{3} \)H reticulocyte protein from a reaction mixture containing no oviduct RNA was combined with \(^{14} \)C ovalbumin and subjected to tryptic digestion and chromatography (Fig. 2B). The fact that \(^{3} \)H and \(^{14} \)C peaks do not coincide rules out the possibility that the correspondence seen in Fig. 2A is due to contamination by total protein.

In an initial attempt to purify ovalbumin mRNA and to estimate its molecular size, polysomal RNA was fractionated by sucrose gradient sedimentation and tested in the reticulocyte lysate system (Fig. 3). The fraction corresponding to the low molecular weight part of the 18 S rRNA peak had the highest specific activity, as would be expected from the predicted size of ovalbumin mRNA.

Relatively few eukaryotic mRNAs have been identified in a functional assay. Evidence has been presented that hemoglobin mRNAs from several species can be translated in a variety of protein-synthesizing systems (8, 15–17). The rabbit reticulocyte lysate system used by Lockard and Lingrel (8) was also used for the detection of a mouse immunoglobulin light chain by Stavnezer and Huang (18). Our results along with those mentioned above strongly argue against an absolute requirement for protein- or species-specific initiation factors.

The apparent ease with which we have been able to obtain ovalbumin in the reticulocyte lysate system suggests that it can be used for the assay of any number of specific mRNAs, provided that a technique such as immunoprecipitation is available for the isolation of the specific product. Our finding that a specific mRNA can be translated in the presence of total cellular nucleic acid may eliminate the necessity for isolating polysomes in order to measure the mRNA content of a tissue. The use of this assay should facilitate the isolation of ovalbumin mRNA in undegraded form and make possible the quantification of ovalbumin mRNA in various hormonal and developmental states of the oviduct.

REFERENCES

1. Brant, J. W. A., and Nalbandov, A. V., Poultry Sci., 35, 692 (1956).
2. Kohler, P. O., Grimley, P. M., and O’Malley, B. W., J. Cell Biol., 40, 9 (1969).
3. Oka, T., and Schimke, R. T., J. Cell Biol., 41, 816; 43, 123 (1969).
4. Palmiter, R. D., Biochemistry, in press.
5. Palmiter, R. D., Christiansen, A. K., and Schimke, R. T., J. Biol. Chem., 245, 823 (1970).
6. Adamson, S. D., Herbert, E., and Godchaux, W., III, Arch. Biochem. Biophys., 135, 671 (1968).
7. Evans, M. J., and Lingrel, J. B., Biochemistry, 8, 829 (1969).
8. Lockard, R. E., and Lingrel, J. B., Biochem. Biophys. Res. Commun., 37, 204 (1969).
9. Palmiter, R. D., Oka, T., and Schimke, R. T., J. Biol. Chem., 246, 724 (1971).
10. Bollum, F. J., Methods Enzymol., 12B, 169 (1968).
11. Neuberger, A., and Marshall, R. D., in A. Gottschalk (Editor), Glycoproteins, Vol. 5, BBA Library, American Elsevier Publishing Company, New York, 1966, p. 299.
12. Gerber, A., Z. Naturforsch. Teil B, 13, 477 (1958).
13. Heywood, S. M., and Ngwagwu, M., Biochemistry, 8, 839 (1969).
14. Hirs, C. H. W., Methods Enzymol., 11, 197 (1967).
15. Laycock, D. G., and Hunt, J. A., Nature, 221, 1118 (1969).
16. Heywood, S. M., Proc. Nat. Acad. Sci. U. S. A., 67, 1782 (1970).
17. Gurdon, J. B., Lane, C. D., Woodland, H. R., and Marbaix, G., Nature, 233, 177 (1971).
18. Stavnezer, J., and Huang, R. C. C., Nature, 239, 172 (1971).