normal alkanes from C_{12} to C_{14}, proportionately greater accommodation greatly reduces the distribution coefficients so that the hydrocarbon in the gas phase cannot be detected by this method.

This proposed reduction in the distribution coefficient is supported by data derived from measurement of the activities of K^+ and Na^+ in soap solutions (*7*). Constant values of activity coefficients for these cations were observed up to the critical micelle concentrations; above the critical micelle concentrations, a marked reduction in the activity coefficients occurred with constant cation concentrations.

Solubilities of the alkane hydrocarbons which are gases at 25°C and atmospheric pressure were measured at a pressure of 1 atm (*2*). Multiplication of this measured solubility at 1 atm by the vapor pressure of the hydrocarbon at 25°C gives a calculated solubility value for each normally gaseous hydrocarbon. The vapor pressures at 25°C and calculated solubilities are: C_1, 289 atm, 7060 ppm; C_2, 38.5 atm, 2320 ppm; C_3, 9.34 atm, 583 ppm; and C_4, 2.35 atm, 147 ppm. Although the critical point for methane is less than 25°C, an assumed vapor pressure of 289 atm is obtained by extrapolating the plot of P^0 against $1/T$ through the critical point (*8*). A curve in which these calculated solubilities for the alkanes from C_1 to C_4 and the measured solubilities for the alkanes from C_5 to C_{10} (liquid at 25°C) are plotted against carbon number shows a remarkably constant proportionate decrease in solubility with increasing carbon number (Fig. 1).

Figure 1 also shows values for the solubilities of normal alkanes reported by other investigators. From C_{12} on, the normal alkanes are accommodated in water in increasingly higher concentrations than anticipated from extrapolation of solubility measurements of alkanes from C_1 to C_{10}. It appears that a change from a state of true solubility to one of accommodation occurs at C_{11}. Baker (*6*) attributes this higher accommodation to intermolecular association, which gives rise to clusters or aggregates of normal paraffin hydrocarbons, comparable to the micelles formed in dilute solutions of colloidal electrolytes.

The low concentration of hydrocarbons in the gas phase beginning with the normal C_{11} alkane (and, by inference, the high concentration in the water phase) is an independent indication of a change from a state of true solubility to one of accommodation between normal C_{10} and C_{12} alkanes, as indicated by measurements of Franks (*3*) and as predicted by extrapolation of the data of McAuliffe (*2*) and Baker (*6*).

Peake and Hodgson (*4, 5*) generally found a very much higher accommodation of normal alkanes from C_{12} to C_{28} in water than did other investigators (Fig. 1). Filtration of the aqueous alkane solutions greatly reduces the hydrocarbon concentrations.

Because of its high sensitivity, this gas equilibration method is excellent for the determination of low concentrations in aqueous media of dissolved alkane hydrocarbons having between one and ten carbon atoms. The technique permits measurement of the solubility in water of the normal C_9 and C_{10} alkanes with good precision.

CLAYTON MCAULIFFE
Chevron Oil Field Research Company, La Habra, California

References

1. C. McAuliffe, *Nature* **200**, 1092 (1963).
2. ———, *J. Phys. Chem.* **70**, 1267 (1966).
3. F. Franks, *Nature* **210**, 87 (1966).
4. E. Peake and G. W. Hodgson, *J. Amer. Oil Chem. Soc.* **43**, 215 (1966).
5. ———, *ibid.* **44**, 696 (1967).
6. E. G. Baker, in *Fundamental Aspects of Petroleum Geochemistry*, B. Nagy and U. Colombo, Eds. (Elsevier, Amsterdam, 1967), p. 299.
7. M. E. Feinstein and H. L. Rosano, *J. Colloid Interface Sci.* **24**, 73 (1967).
8. J. H. Hildebrand and R. L. Scott, *The Solubility of Nonelectrolytes* (Reinhold, New York, 1950), p. 241.

15 November 1968

Prostaglandins: Enzymatic Analysis

Abstract. *By means of a specific nicotinamide-adenine dinucleotide-dependent prostaglandin dehydrogenase from swine lung, an enzymatic method has been developed for the assay of prostaglandins. The method permits analysis with a lower limit of* 10^{-12} *mole of prostaglandin.*

We have described the purification and properties of a prostaglandin dehydrogenase [nicotinamide-adenine dinucleotide (NAD^+)-15-hydroxyprostanoate oxidoreductase] from swine lung (*1*). This enzyme catalyzes the oxidation of the secondary alcohol group at carbon-15 to a ketone in all known prostaglandins with exception of those containing the dienone structure absorbing at 278 nm (that is, prostaglandin B compounds and their 19-hydroxylated analogs). The specificity appears to be confined exclusively to the prostaglandins since none of a variety of hydroxy compounds occurring endogenously were substrates for the enzyme (*1*).

Since the equilibrium favors oxidation of the prostaglandin, the enzyme appeared suitable for an enzymatic assay based on measurement of the reduced pyridine nucleotide. Such a method would combine the selectivity of the enzyme with the high sensitivity attainable with fluorometric measurements of pyridine nucleotides. We now describe the enzymatic fluorometric analysis of prostaglandins and show that amounts as low as 10^{-12} mole (0.35 ng) can be measured.

Two types of assay were used. In the range of 10^{-10} to 10^{-8} mole of substrate, the native fluorescence of the stoichiometrically formed reduced NAD (NADH) was measured. The measurement of prostaglandin E_1 (PGE_1) with the direct method is shown in Fig. 1.

For higher sensitivity, the reaction catalyzed by prostaglandin dehydrogenase (PGDH) was coupled to an enzymatic cycling system (Fig. 2). In

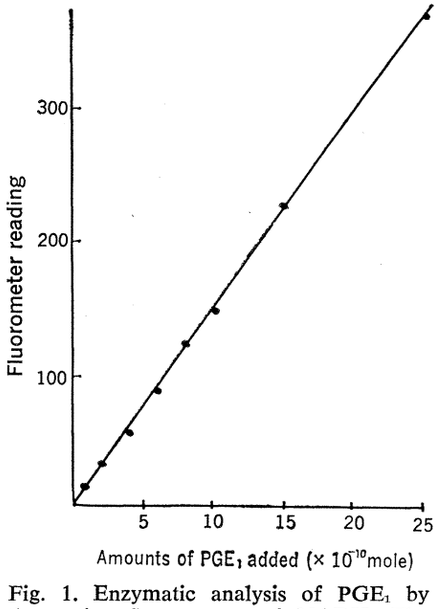

Fig. 1. Enzymatic analysis of PGE_1 by the native fluorescence of NADH. The PGE_1 was incubated at room temperature for 30 minutes in 0.05*M* tris buffer (*p*H 9.0), 1 m*M* Cleland's reagent, 1 m*M* NAD^+, and enough enzyme to oxidize 10^{-10} mole of PGE_1 per minute at 25°C (0.1 milliunit). Incubation was carried out in 100 μl at 25°C. The native fluorescence of NADH was read in microcuvettes in the Farrand model A2 fluorometer. The reagent blank was equivalent to 6×10^{-7}*M* NADH.

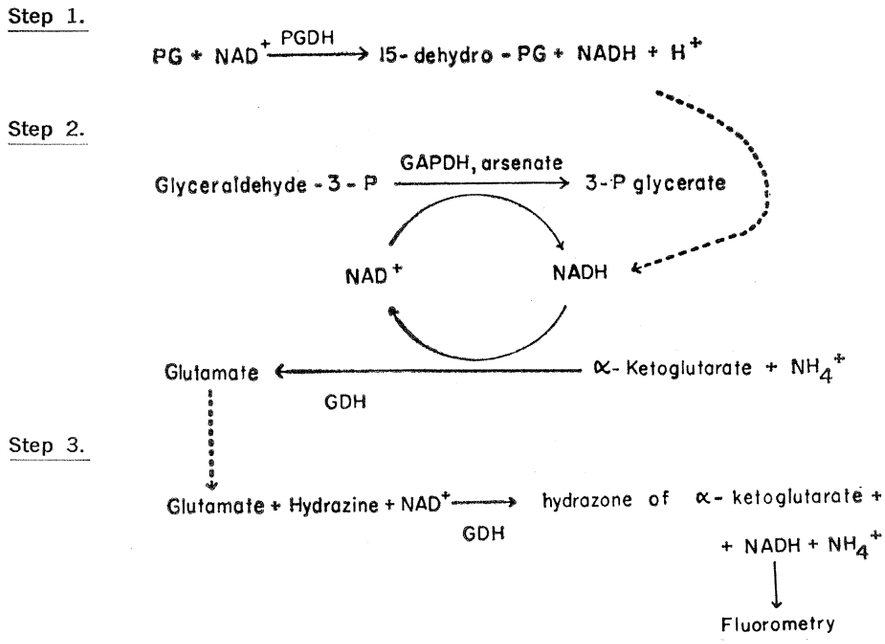

Fig. 2. Principles for the enzymatic assay of prostaglandins by enzymatic cycling of pyridine nucleotides.

cycling systems, pyridine nucleotides are used as cofactors to a pair of dehydrogenases, so that the pyridine nucleotides are oxidized by one enzyme and reduced by the other in a cyclic process (Fig. 2). If suitable experimental conditions are chosen, the cycling factor can be varied from a few hundred to about 10,000 in 1 hour. We have modified the original method (*2*) so that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) instead of lactate dehydrogenase (LDH) is used in the cycling system with NADH. The enzyme GAPDH has more favorable kinetic properties than LDH, and cycling is therefore linear over a wider concentration range of pyridine nucleotides. With a cycling factor of 1000, every molecule of NADH added from the first reaction generates 1000 molecules each of 3-phospho-glycerate and glutamate. In the last step (indicator reaction), the amount of glutamate is measured by the addition of NAD+ and glutamic dehydrogenase (GDH). The NADH fluorescence is measured after the reaction has gone to completion. The results of an experiment where PGE_1 in amounts of 1.3×10^{-12} to 2.7×10^{-11} mole/liter was measured is shown in Fig. 3. All samples, as well as controls containing NADH but not prostaglandin, were carried through the procedure in duplicate. There was a linear relation between the fluorescence and the amount of added NADH or PGE_1. The recovery of PGE_1, as compared to standardized NADH recovery, was usually about 80 percent. This indicates that the prostaglandin is not completely oxidized.

Experimentally, prostaglandins are released into the blood or perfusion medium either spontaneously or in response to drugs or nerve stimulation (*4*). The concentrations of prostaglandin in these fluids and in tissues are likely to be in the range of 10^{-10} to 10^{-8} mole/liter. Moreover, they are present together with large amounts of other biologically active substances. A prerequisite for a method for the analysis of prostaglandins in physiological amounts is therefore high specificity and sensitivity. The use of the enzymatic method for the assay of prostaglandins, coupled with a suitable chromatographic procedure to separate individual prostaglandins, makes it possible for positive identification as well as quantitative measurement in the nanogram range to be made in the same analysis.

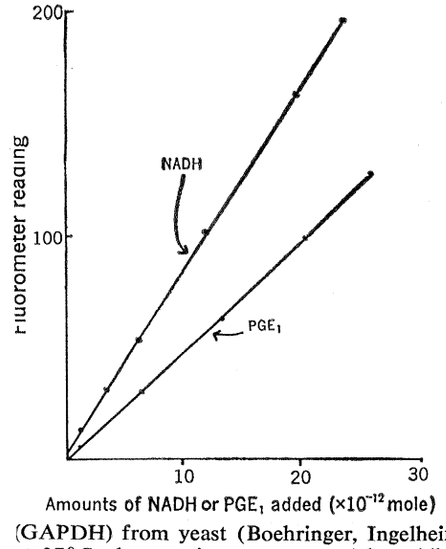

Fig. 3. Measurement of PGE_1 by prostaglandin dehydrogenase and enzymatic cycling of pyridine nucleotides. The PGE_1 or NADH was incubated in 6 μl of 0.1*M* tris buffer (*p*H 8.0) containing 0.03 percent bovine serum albumin, 2 m*M* mercaptoethanol, 0.2 m*M* NAD^+, and 0.05 milliunit of PGDH (step 1, Fig. 2). After 40 minutes at 37°C, 1 μl of 1*N* NaOH was added, and the tubes were heated at 60°C for 20 minutes. A portion (4.9 μl) from each tube was added to 50 μl of the cycling reagent (step 2, Fig. 2), which was composed of 0.1 m*M* sodium arsenate, 5 m*M* glyceraldehyde-3-phosphate, 5 m*M* α-ketoglutarate, 0.3 m*M* adenosine diphosphate (ADP), 10 m*M* ethylenediaminetetraacetate, 5 m*M* mercaptoethanol, 0.02 percent bovine serum albumin, 5 m*M* ammonium acetate, 50 μg/ml of glutamate dehydrogenase (GDH), and NAD^+-free glyceraldehyde-phosphate dehydrogenase (GAPDH) from yeast (Boehringer, Ingelheim, Germany). After cycling for 60 minutes at 37°C, the reaction was stopped by addition of 5 μl of 5*N* HCl (*3*) and by boiling for 2 minutes. Then 1 ml of glutamate assay reaction mixture was added (step 3, Fig. 2). The composition of this was 0.2*M* hydrazine buffer (*p*H 9.0), 1 m*M* NAD^+, 0.5 m*M* ADP, and 30 μg of GDH per milliliter. After 1 hour at room temperature the fluorescence was read. The overall blank was equivalent to 0.6×10^{-12} mole of NADH. The cycling factor was 1.100.

Experimentally, prostaglandins are released into the blood or perfusion medium either spontaneously or in response to drugs or nerve stimulation (*4*). The concentrations of prostaglandin in these fluids and in tissues are likely to be in the range of 10^{-10} to 10^{-8} mole/liter. Moreover, they are present together with large amounts of other biologically active substances. A prerequisite for a method for the analysis of prostaglandins in physiological amounts is therefore high specificity and sensitivity. The use of the enzymatic method for the assay of prostaglandins, coupled with a suitable chromatographic procedure to separate individual prostaglandins, makes it possible for positive identification as well as quantitative measurement in the nanogram range to be made in the same analysis.

ERIK ÄNGGÅRD
Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden
FRANK M. MATSCHINSKY
Department of Pharmacology, Washington University School of Medicine, St. Louis, Missouri
BENGT SAMUELSSON
Department of Medical Chemistry, Royal Veterinary College, Stockholm

References and Notes

1. E. Änggård and B. Samuelsson, *Ark. Kemi* **25**, 293 (1966).
2. O. H. Lowry, J. V. Passonneau, D. W. Schulz, M. Rock, *J. Biol. Chem.* **236**, 2746 (1961).
3. Recently, 1.5 percent H_2O_2 has been included in the HCl. Peroxide destroys α-ketoglutarate, providing optimum recovery and linearity in the indicator assay for glutamate [F. M. Matschinsky, C. Rutherford, L. Guerra, *Proceedings of the 3rd International Congress of Histochemistry and Cytochemistry* (Springer, New York, 1968)].
4. For references see S. Bergström, L. A. Carlson, J. R. Weeks, *Pharmacol. Rev.* **20**, No. 1 (1968).
5. Supported by grants 14X-221 (EÄ) and 13X-217 (BS) from the Swedish Medical Research Council and from Stiftelsen Svensk Näringsforskning. We thank Dr. H. U. Bergmeyer for the supply of NAD^+-free GAPDH and the Upjohn Company for prostaglandins.

30 August 1968; revised 12 November 1968