hibition by chloramphenicol and lack of sensitivity to cycloheximide, but differ from the cytoplasmic ribosomes isolated from the same tissue. Whether this resemblance extends to the location of the chloramphenicol-binding site, which is the 50*S* subunit in the case of bacterial ribosomes (*19*), remains to be investigated.

R. J. ELLIS*
Department of Botany, University of Aberdeen, Aberdeen, Scotland

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* Present address: Department of Biological Chemistry, University of Aberdeen.

2 October 1968

Solubility in Water of Normal C_9 and C_{10} Alkane Hydrocarbons

Abstract. *A new method for equilibrating water containing alkane hydrocarbons with a gas phase and analyzing the gas for hydrocarbon content by gas chromatography extends analytical sensitivity to better than 0.1 part per billion. The solubilities at 25°C of the normal C_9 (220 parts per billion) and C_{10} (52 parts per billion) alkanes decrease with increasing carbon number. A discontinuity occurs at the normal C_{11} alkane, probably because of a change from true solubility (molecular dispersion) to accommodation (aggregation).*

The solubilities in water of normal alkane hydrocarbons (*1*, *2*) decrease linearly from the normal C_4 alkane through the normal C_8 alkane, if the logarithm of the hydrocarbon solubility is plotted as a function of the molar volume (carbon number) of the hydrocarbon. It has been suggested that, if the relation is a continuous function, the solubilities in water of longer-chain normal alkanes could be predicted by extrapolation. However, recent measurements (*3*–*6*) indicate that the solubilities (or accommodation) of the normal alkanes from C_{12} to C_{36} are very much greater than would be predicted.

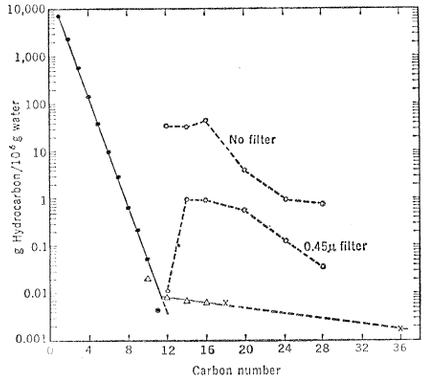

Fig. 1. Solubilities (or accommodations) of normal paraffin hydrocarbons in water at ≃ 25°C as a function of carbon number; ●, data from McAuliffe (*2*) and this work; ⊙, from Peake and Hodgson (*5*); △, from Franks (*3*); ×, from Baker (*6*).

The sensitivity of the present analytical method used to measure the solubilities of normal alkanes from C_1 to C_8 in water (*2*) is limited to about 0.1 ppm (by weight) because of the relatively small sample of water (50 μl) analyzed. The method reported here increases the sensitivity of the gas chromatographic method; the hydrocarbon content of the gas phase is measured after equilibration with 5 ml of hydrocarbon-saturated water. From the vapor pressures and solubilities of individual alkanes, the partitioning of the hydrocarbon between the water and gas phases can be calculated from the distribution coefficient

$$K = C_{HC(g)}/C_{HC(w)} = M_{(g)} \times N_{HC(g)}/M_{(w)} \times N_{HC(w)}$$

where C_{HC} and N_{HC} are the concentration and mole fraction of the hydrocarbon in the gas (g) and water (w) phases, and M is the number of moles.

Calculation of the distribution coefficients for alkanes from methane through normal octane indicates that from 96 percent (C_2) to over 99 percent (normal C_7 and C_8) of the hydrocarbon partitions into the gas phase when water saturated with that hydrocarbon is equilibrated with an equal volume of gas. The calculated distribution coefficients were experimentally confirmed for alkanes from methane through octane.

Normal alkanes from C_9 to C_{14} were equilibrated with distilled water at 25°C (*2*). Glass vials (30 ml) were filled with this hydrocarbon-saturated water; half of the water was then displaced with a glass rod. The displaced volume was replaced by air. Each vial was then quickly sealed with a metal screw cap. After the vials were shaken on a "wrist-action" paint shaker for 2 minutes, the gas phase was displaced through the 5-ml sample loop of a gas chromatograph by means of a sharpened stainless steel point 0.635 cm (0.25 inch) in diameter inserted through the cap. The stainless steel point has two openings; hydrocarbon-free water can be added through one port, and the gas phase can be displaced through the other. The puncturing device seals against the top of the metal screw cap and has suitable O-ring seals on the probe to prevent gas leakage (*2*).

The measured solubilities of the normal alkanes, expressed as parts per billion (by weight), are C_9, 220 ±21; C_{10}, 52 ± 4.3; and C_{11}, 4.4 ± 1.8. Although the analytical technique could measure less than 0.1 ppb, normal C_{12}, C_{13}, and C_{14} alkanes could not be detected in the gas phase. A predicted solubility for the normal C_{12} alkane, obtained by extrapolation, is 3.4 ppb. The value for the normal C_{11} alkane is also lower than predicted (14 ppb).

The low solubilities for normal alkanes beginning with C_{11} may well be due to the presence of hydrocarbon aggregates (*6*) or micelles arising from impurities in the water phase. During equilibration with the gas phase, the hydrocarbon aggregates or micelles compete with the gas phase for the individually dispersed hydrocarbon molecules in the water, which results in a decrease in the concentration in the gas phase. For the normal C_{11} alkane, the proportion of aggregates or micelles appears to be relatively small, resulting in a decrease in measured solubility from 14 to 4 ppb. However, for the

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

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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

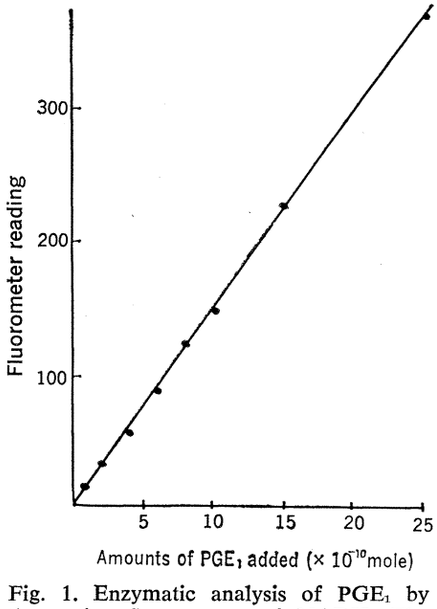

Fig. 1. Enzymatic analysis of PGE_1 by the native fluorescence of NADH. The PGE_1 was incubated at room temperature for 30 minutes in 0.05*M* tris buffer (*p*H 9.0), 1 m*M* Cleland's reagent, 1 m*M* NAD^+, and enough enzyme to oxidize 10^{-10} mole of PGE_1 per minute at 25°C (0.1 milliunit). Incubation 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.