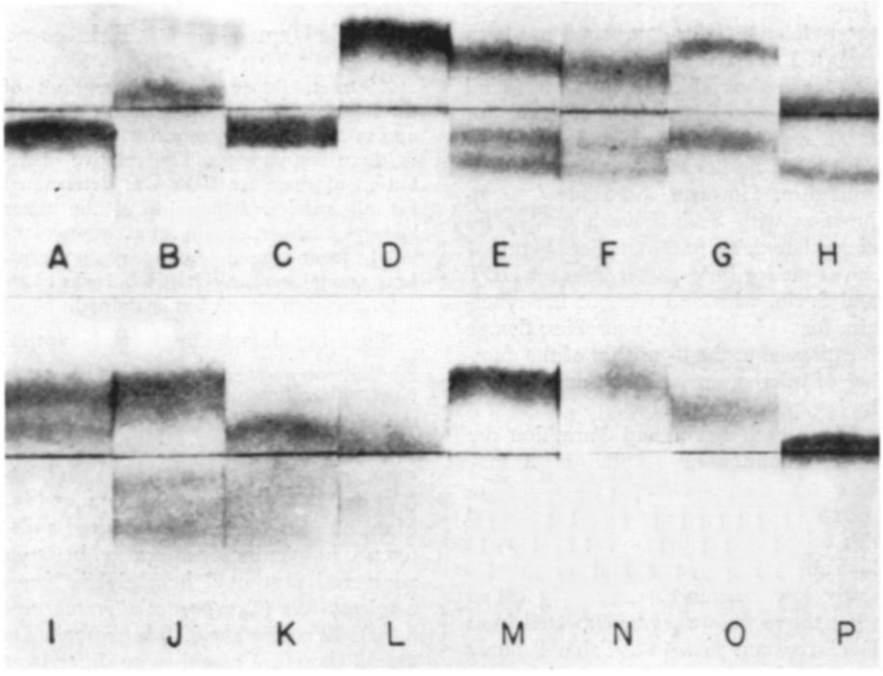

Fig. 1. Separation of fish hemoglobins in Veronal buffer (*p*H 8.8; ionic strength, 0.05): (A) *Entosphenus tridentatus* (Pacific lamprey); (B) *Lepomis macrochirus* (bluegill); (C) *Cyprinus carpio* (carp); (D) human hemoglobin A; (E) *Salmo gairdneri* (steelhead trout); (F) *S. gairdneri* (rainbow trout); (G) *Oncorhynchus nerka* (blueback salmon); (H) *Alosa sapidissma* (shad); (I) *Micropterus salmoides* (largemouthed bass); (J) *Salvelinus fontinalis* (brook trout); (K) *Oncorhynchus kisutch* (silver salmon; (L) *O. tshawytscha* (chinook salmon); (M) human hemoglobin A; (N) *Squalus acanthias* (dogfish shark); (O) *Ophiodon elongatus* (ling cod); and (P) *Ptychocheilus oregonensis* (Columbia squawfish).

fluid, after centrifugation, was stored at −10°C. Paper electrophoresis was performed in a Spinco model R apparatus at 6 ma for 6 hours; S and S 2043A paper with sodium barbital buffer (*p*H 8.8; ionic strength, 0.05) was used. Preliminary experiments had established that maximum separation occurred under these conditions. The electrophoregrams were scanned photometrically by means of a Photovolt densitometer, model 525, equipped with either a 420 or a 540 mμ filter. Moving-boundary electrophoresis of dialyzed hemoglobins was carried out at 10 ma in sodium barbital buffer (*p*H 8.6; ionic strength, 0.1) in a Perkin-Elmer model 38 apparatus operating at approximately 180 v.

In Fig. 1 the mobilities at *p*H 8.8 of hemoglobins (7) from 14 species of fish (8) are compared with that of human hemoglobin A, which served as a reference standard. Although many samples have been examined in this manner, no gross differences in mobilities have been detected in a given species with regard to sex, age, or race.

Percentage distribution of the hemoglobin, as determined by planimetry of the densitometer recordings, is given in Table 1 for those species possessing more than one hemoglobin component. In general, the largest component appears to have the highest mobility.

It is interesting to note that many fish hemoglobins are characterized by a low anodal mobility at *p*H 8.8 and are consequently shifted to the cathode side of the origin by the electroosmotic buffer flow. Mobility measurements made from moving-boundary electrophoresis patterns showed, for example, that at *p*H 8.6 the mobilities of the three hemoglobin components of rainbow trout were -2.39×10^{-5}, -1.24×10^{-5}, and -0.70×10^{-5} cm²/v sec. The low mobility at this *p*H and apparent high isoelectric point associated with many fish hemoglobin fractions may prove to be a characteristic unique among vertebrates.

Although the physiological significance of the occurrence of multiple hemoglobins in fishes is at present not known, subsequent investigation may establish a correlation of this heterogeneity with phylogeny.

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6. No major protein contaminant was found either in boundary patterns or in dyed paper strips.
7. Preliminary studies showed no difference in mobilities of fish carbonmonoxyhemoglobin and oxyhemoglobin; consequently, all of the work described in this report was carried out with oxyhemoglobin.
8. We are indebted, for specimens, to C. H. Elling and A. Henslip, Bonneville Experimental Laboratory, North Bonneville, Wash.

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Analysis of Gas in Biological Fluids by Gas Chromatography

Abstract. Combining a vacuum extraction method with gas chromatography makes possible accurate, reproducible determination of small quantities of permanent gases contained in biological fluids. One useful application is demonstrated by determining the oxygen tension in 1 ml of human plasma.

Chromatography as a technique for separation of closely related substances has been known for many years. However, the more recent use by James and Martin (*1*) of gas-liquid partition chromatography has resulted in a renewed interest in the technique and the development of modern instrumentation which makes possible rapid separation and accurate quantitative analysis of most of the biologically important permanent gases when they are in the gas phase. However, it has not previously been possible to analyze the permanent gases in terms of their concentration in biological solutions, for two reasons: (i) the columns used for separation of permanent gases are adsorption columns and lose their useful characteristics when they are wet, and (ii) attempts to rapidly volatilize gases in biological solutions have been unsuccessful because of the coagulation of protein and because of the oxidation and the resultant change in gas concentrations which occur with heat. These problems have been solved by combining a vacuum extraction method with the technique of gas chromatography.

The instrumentation and apparatus consist of a gas chromatograph (*2*), a 1-mv, 1-sec full-scale strip-chart recorder (*3*), and a Van Slyke volumetric extraction chamber (*4*), the waste arm of which is connected to the carrier-gas stream of the gas chromatograph proximal to the chromatograph column. Helium is used as the carrier gas.

An aliquot of the fluid is admitted to the Van Slyke reaction chamber via a between-line Van Slyke pipette and washed in with mercury. The upper three-way stopcock is closed, the lower

stopcock is opened to the mercury reservoir, a vacuum is produced by lowering the mercury to the 50-ml mark, and the gases are extracted by gentle shaking for 2 to 3 minutes. The fluid sample is then drawn into the reservoir chamber; following this, mercury is admitted to the reaction chamber from the mercury reservoir. When the mercury level reaches the 0.2-ml mark in the reaction chamber, the upper three-way stopcock is opened to the waste-arm side and the extracted gases are injected into the carrier-gas stream by moving the fluid level to the junction of the waste arm and carrier-gas tubing. After a wait of 5 seconds, the small amount of fluid and mercury can be returned to the reaction chamber; this is closed off from the column gas, and the sample is reextracted to remove any gas that may have redissolved as the vacuum was released following the first extraction. The sample fluid is removed from the Van Slyke chamber, via the same port through which it was admitted, by filling the reaction chamber with mercury from the reservoir.

The sample gases thus placed in the carrier-gas stream are then carried through the chromatograph column and eluted as separate components; the length of time required depends upon the characteristics of the gas and the column material. Each component is detected, and the quantity of the gas present is determined by a thermal conductivity reference and by sensing cells; the signals are amplified and recorded, in time, on the strip chart recorder.

The use of a 4-ft, 5-A molecular sieve column 0.25 in. in diameter allows the oxygen component of a sample of permanent gases to be eluted first from the column; this is followed by nitrogen and carbon monoxide. Carbon dioxide is permanently absorbed. All permanent gases with the exception of argon, which at present cannot be separated from oxygen by known column materials, can be separated and analyzed by means of a molecular sieve column, a silica gel column, or a charcoal and firebrick column (*5*).

The stability of the instrumentation, including the molecular sieve column, was tested by repeated injections, over a 4-hour period, of a constant volume and concentration of oxygen and nitrogen in the gas phase through an injection port placed in the carrier-gas stream. Reproducibility of the extraction technique for oxygen was determined by analyzing the amount of oxygen contained in 1 ml of sterile distilled water which had been equilibrated at 37°C with the oxygen and nitrogen in room air. A syringe tonometer and a constant-temperature water bath were used for the equilibration. Double extraction analyses were performed on each of 35 samples over a 2-day period. The application of the technique to biological fluids was tested in the same way, human blood plasma being substituted for water. The results of these determinations are shown in Table 1.

Table 1. Oxygen tension of water and plasma as determined by gas chromatography. Column, 4-ft. 5A molecular sieve; current, 360 ma; flow pressure, 20 lb/in.^{2}; carrier gas, helium; column temperature, 100°C for water and 70°C for air and plasma. Standard deviations are given in columns 4 and 6.

Type	Sample		Peak height (mm)	O_2 (ml)	P_{O_2} (mm-Hg)
	No.	Size (ml)			
Room air	15	0.03	122 ± 0.17	.0059	140
Water	35	1.0	124 ± 0.23		148 ± 0.28
Plasma	27	1.0	92 ± 1.04	.0044	148 ± 1.68
Plasma (duplicate analysis)	13	1.0	92 ± 0.70	.0044	148 ± 1.13

The accuracy of oxygen analysis has been tested in two ways. First, both water and plasma have been equilibrated with different concentrations of oxygen, and the graphic plots of the resulting peak heights relative to the oxygen contents form a precise straight line through zero. Second, equal amounts of oxygen, whether initially in solution or initially in the gas phase, produce the same peak heights.

The extraction method presents two difficulties. (i) Biological fluids tend to foam and trap gas bubbles with vacuum extraction of gases. It is possible to collapse the bubbles, however, by pulling them through the lower stopcock before the vacuum is released. (ii) The peak heights of the oxygen curve may vary considerably if the rate of injection of the extracted gas into the carrier stream is varied (a very little practice allows one to keep this injection rate steady). However, the area under curves distorted by variable injection rates is constant. Mechanical integration of the strip chart record can remove this variable if it is so desired.

In addition to the exceptionally good stability, linearity, and reproducibility of this method, it is useful because of its accuracy and speed. The time required to introduce the plasma sample in the carrier-gas stream is less than 5 minutes. The time required for elution from the column is less than 2 minutes, and during this time another sample can be extracted.

The remarkable sensitivity of the instrumentation to minute changes in oxygen content in relatively small samples of biological fluids, the excellent stability of the instrumentation, and the accuracy of the method offer opportunity for its widespread use in biological research and clinical medicine. The method makes possible accurate analysis, on small samples, of the concentration of biologically important gases present, or changing in time, in biological solutions. It seems likely that many determinations previously made by means of the Van Slyke, Haldane, Scholander, and Warburg methods can now be made with equal or better accuracy and simplicity and in less time. The oxygen tension of arterial blood can easily be calculated from the oxygen content of plasma as determined in this manner. Such determinations previously required exceptional technical skill and even then were less accurate and more time-consuming than determinations made according to the method described (*6*).

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References and Notes

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2. A Beckman GC-2 gas chromatograph was used.
3. The strip chart recorder was secured from Minneapolis-Honeywell, Brown Instruments Division, Philadelphia, Pa.
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5. The three columns are Beckman columns No. 17298, 70019, and 70028, respectively.
6. This work was supported by public Health Service grant No. H-2798.
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Tritium Tracer in Arctic Problems

Abstract. Tritium measurements in the Arctic Ocean confirm a marked near-surface stratification. Sea water at 400 meters' depth, however, is less than 3 years old, suggesting a contribution to this water mass due to sinking along the continental shelf. The top ice layer on the Ward Hunt Island ice field is older than 25 years.

Tritium (H^3; half life, 12.26 years) is ideally suited as a natural tracer in the study of polar mechanisms of exchange and mixing among the various phases of H_2O. A steady state of cosmic-ray produced tritium has probably been achieved. Recently, however, water of relatively high specific activity has been introduced into the atmosphere by ther-