hydroxylated product may also have been produced in the chemical reaction.

Other oxidizing agents also able to 12a-hydroxylate 12a-deoxytetracycline are $KMnO_4$, I_2, $K_2Cr_2O_7$, $K_3Fe(CN)_6$ and $K_2S_2O_8$. Udenfriend's hydroxylation system[6] was inactive. Reducing agents, *e.g.*, ascorbic acid, Na_2SO_3 and $FeSO_4$ inhibited consumption of 12a-deoxytetracycline in both the chemical and microbiological processes.

Grateful acknowledgment is extended to A. Green and J. H. Boothe for supplies of 12a-deoxytetracycline, to A. C. Dornbush for microbiological assays, to J. H. Martin for paper chromatographic assays and to W. Fulmor for infrared analyses.

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SEPARATION OF NITROGEN AND OXYGEN BY GAS LIQUID PARTITION CHROMATOGRAPHY USING BLOOD AS THE STATIONARY PHASE

Sir:

It has been shown[1,2,3] for a number of olefins that high efficiency of separation of compounds, boiling only 0.1° apart, can be obtained by the use of complex-forming solutions as the stationary phase in gas–liquid partition chromatography.

It appeared that the application of this type of stationary phase could be further extended to the separation of gases of very low solubility, which so far had been considered to be outside the range of gas-liquid partition chromatography. We wish now to report the separation of oxygen and nitrogen by this method.

In order to be useful in partition chromatography, complex-formation must be reversible, and reaction with the substance to be separated must proceed rapidly in both directions. Respiratory pigments fulfill these requirements for oxygen. Accordingly a column was prepared with blood as the stationary phase and, after a number of trials, conditions were found in which the separation of nitrogen and oxygen could in fact be achieved.

Sheep, cow and human blood were used. The animal blood was collected in semi-sterile bottles containing a solution of 3.2% of sodium citrate dihydrate (18 cc. of solution for 100 cc. of blood). Heparin was added to human blood to avoid coagulation.

The solid support was powdered fire brick (Johns–Mansville C-22) of 120–170 or 170–200 mesh. The blood, in the proportion of 0.5 cc. per g. of solid support, was added slowly to the powder while stirring. Mixing was continued until the mass became homogeneous and the free flowing powder was then filled into U shaped glass columns of 4 mm. diameter and 1 m. length. Helium was passed through the columns to desoxygenate the blood and the sample of air (0.4–1.0 cc.) then was injected. When not in use, the columns, filled with helium, were kept in a refrigerator, and could be reëmployed many times. The experiments were carried out with a Perkin Elmer Model 154 A Fractometer.

With a total column length of 2 m. and a temperature of 30–40° excellent separation is obtained, with the oxygen peak being nearly symmetrical. At 20–25° results are less good and no separation occurs at 13°. Also, if the rate of flow is much less than 8 cc. per min., part of the oxygen appears to be bound irreversibly. For a given column and temperature, the retention volume of oxygen will change with the characteristics of the blood, since the partition coefficient will vary with such factors, as the percentage of hemoglobin, and the affinity of hemoglobin for oxygen in the environmental conditions (*p*H, concentration of hemoglobin in the erythrocytes, etc.). Thus, *e.g.*, blood of patients having various blood diseases, and, in particular, different hemoglobin percentages and erythrocyte counts, gave different values for the retention volume of oxygen in the same chromatographic conditions (work of the authors with D. Dannon and L. R. Rosenstein). It is to be noted that the partial pressure of oxygen in the chromatographic column is of the order of a few mm. only, that is, the uptake and release of oxygen proceeds at quite different pressures than in the living organism and the degree of saturation of the hemoglobin at equilibrium is low.

Work is in progress to examine the possible applications of these findings and to extend the method to the analysis of substances other than oxygen (*e.g.*, carbon monoxide) and to complex-forming compounds other than respiratory pigments.

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IDENTITY OF THE α CHAINS OF HEMOGLOBINS A AND F

Sir:

Possible identity of portions of human fetal and adult hemoglobin was suggested by Schroeder and Matsuda,[1] who determined that fetal, like adult,[2] hemoglobin contained two polypeptide chains N-terminal in the sequence val-leu (α chains).[3] This suggested identity now has been substantiated by our present experiments which show not only that "fingerprints"[5] of the soluble portion of tryptic

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