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.

(1) B. W. Bradford, D. Harvey and D. E. Chalkley, *J. Inst. Petrol.*, **41**, 80 (1955).

(2) E. Gil-Av, J. Herling and J. Shabtai, *J. of Chromatog.*, **1**, 508 (1958).

(3) J. Shabtai, J. Herling and E. Gil-Av, *J. of Chromatog.*, in press (1959).

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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

(1) W. A. Schroeder and G. Matsuda, THIS JOURNAL, **80**, 1521 (1958).

(2) H. S. Rhinesmith, W. A. Schroeder and L. Pauling, *ibid.*, **79**, 4682 (1957).

(3) The two γ chains of fetal hemoglobin terminate in glycine[1] and the two β chains of adult hemoglobin in val-his-leu.[4]

(4) H. S. Rhinesmith, W. A. Schroeder and N. Martin, THIS JOURNAL, **80**, 3158 (1958).

(5) V. M. Ingram, *Biochim. Biophys. Acta*, **28**, 539 (1958).

hydrolysates of α^A and α^F chains are grossly indistinguishable[6] but also that α chains may be transferred by hybridization of hemoglobin F with other hemoglobins.

All hemoglobins were isolated from hemolysates by column chromatography[7] to remove minor components normally present. Zone F_{II},[7] the main component of normal full-term cord blood, was taken as hemoglobin F.

The reversible dissociation of hemoglobin F into molecular sub-units is similar but not identical to hemoglobin A[8,9] at both alkaline and acid pH. Thus hybridization[10,11] or recombination[10] with other hemoglobins might be expected. Initial hybridization of hemoglobin F_{II} ($\alpha_2^F\gamma_2^F$) and radioactive (C^{14}-labelled leucine) hemoglobin S ($\alpha_2^{A*}\beta_2^{S*}$) at pH 11.0 or 11.2 at 3° for 1 to 6 days showed less transfer of radioactivity to hemoglobin F than would have been expected from a hybridization of the type

$$2\,\alpha_2^{F=A}\gamma_2^F + 2\,\alpha_2^{A*}\beta_2^{S*} \rightarrow \alpha_2^{F=A}\gamma_2^F + \alpha_2^{A*}\gamma_2^F + \alpha_2^{A*}\beta_2^{S*} + \alpha_2^{F=A}\beta_2^{S*} \quad (1)$$

if α^F and α^A are identical. Nevertheless, hemoglobin F_{II} after chromatographic isolation was radioactive in the α chains only, as shown by investigation of the N-terminal peptides.[2,4,12] Likewise, when radioactive hemoglobin F_{II} and hemoglobin A were hybridized at pH 4.7 at 3° for 12 hr., transfer of radioactivity to hemoglobin A was 53% of that calculated from an equation like (1). These experiments do not determine the number of identical α chains in hemoglobin F. However, after approximately equal amounts of hemoglobin F_{II} and hemoglobin H (β_4^A)[13] had been hybridized at pH 4.7 at 3° for 12 hr., chromatography separated three hemoglobins, an "H-like" hemoglobin, "F_{II}", and "A." From the ratio of F_{II} to A which was very close to 1:2, it was calculated that hybridization according to the equation

$$3\alpha_2^{F=A}\gamma_2^F + 3\beta_4^A \rightarrow 2(2\beta_2^A + \gamma_2^F) + \alpha_2^{F=A}\gamma_2^F + 2\alpha_2^{F=A}\beta_2^A \quad (2)$$

was essentially complete (97%): thus, two identical α chains are present in hemoglobin F. The newly formed hemoglobin A was characterized by its chromatographic and starch electrophoretic behavior. The nature of the "H-like" hemoglobin from the hybridization is unknown at present. At pH 11.2, the hybridization of F_{II} and H was about 20% in 12 hr.

From the incompleteness of hybridization under certain conditions, the process clearly is a complex one. As more information becomes available, it should give an insight into the apparently different forces between the chains of the various hemoglobins.

The conclusion that the α^F and α^A chains are identical strongly suggests a related genetic control of hemoglobins A and F and is an extension of the idea derived from the structure of hemoglobin H that the α and β chains of adult hemoglobin are under separate genetic control.[13,14] It is clearly apparent that these ideas would be substantiated by the detection of an abnormal fetal hemoglobin in a newborn child who, in adult life, will produce an abnormal hemoglobin that has an aberration in the α chain.

This investigation was supported in part by grants H-2558 and H-3394 from the National Institutes of Health, United States Public Health Service.

(6) J. A. Hunt (*Nature*, **183**, 1373 (1959)) likewise concludes that α^A and α^F chains are identical on the basis of a somewhat more extensive study of "fingerprint" data which included examination of the chymotryptic digest of the insoluble tryptic residue. We wish to thank Mr. Hunt for sending us a copy of his manuscript prior to publication.

(7) D. W. Allen, W. A. Schroeder and J. Balog, THIS JOURNAL, **80**, 1628 (1958).

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(14) H. A. Itano, *Adv. Prot. Chem.*, **12**, 260 (1957).

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MICROBIOLOGICAL HYDROXYLATION OF 12a-DEOXYTETRACYCLINE

Sir:

We wish to report on the ability of *Curvularia lunata* (NRRL 2380) to form tetracycline by introduction of the 12a-hydroxyl group into the compound 12a-deoxytetracycline.[1]

Curvularia lunata was grown for 40 hours in shaker flasks containing a medium consisting of 2% corn meal, 1% N-Z Amine B, 0.2% Difco yeast extract, 0.1% K_2HPO_4 and 0.5% $CaCO_3$. To 1.4 liters of culture filtrate was added 1.5 g. of 12a-deoxytetracycline as a 20 mg./ml. aqueous solution (pH to 1.5 with HCl). The final pH of the reaction mixture was 6.0. The reaction mixture was incubated at 28° for 24 hr. At this time, spectrophotometric assay indicated that 89% of the 12a-deoxytetracycline had been consumed. Microbiological assay of the harvest sample suggested a 23% yield of tetracycline. For isolation purposes the pH of the reaction mixture was raised to 8.5. It was then extracted three times with half volume portions of 1-butanol. The butanol extracts were combined, adjusted to pH 2.6 and concentrated to about 20 ml. The precipitate obtained by adding 200 ml. of petroleum ether was chromatographed on a Celite 545[2] column employing the solvent system ethyl acetate:1-butanol:H_2O (68:12:20) adjusted to pH 2.1 with HCl. Ultraviolet absorption of the effluent was continuously measured at 270 mμ and fractions representative of three peaks were collected. The crystalline product obtained from fraction three proved identical by ultraviolet and infrared ab-

(1) Details for the preparation of 12a-deoxytetracycline are to be published by A. Green and J. H. Boothe. The suggestion concerning the possibility of microbiological 12a-hydroxylation was made by A. Green.

(2) A grade of diatomaceous earth produced by the Johns-Manville Co.