compound is sensitive to moisture and oxygen, m.p. 37–38°, b.p. 105° (15 mm.). The infrared spectrum of this compound shows an absorption band at 12.8 μ. It is soluble in most organic solvents in all proportions, but reacts with alcohol forming an insoluble solid.

It is remarkable that the tetravalent chromium compound was isolated. Additional evidence for the tetravalent state of chromium was found in another experiment. An acid solution of potassium iodide was treated with a weighed amount of this compound and the liberated iodine titrated. Thus 0.638 g. of chromium tetra-*t*-butoxide liberated 1.89 mmol. of iodine. Assuming that one electron transfer occurred as shown in equation (1), the molecular weight was calculated as 338 in good agreement with the molecular weight determined cryoscopically.

$$Cr^{4+} + I^- \longrightarrow Cr^{3+} + {}^1/_2I_2 \qquad (1)$$

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GROSS STRUCTURE OF HEMOGLOBIN H

Sir:

Human hemoglobin H has been described in some detail by Rigas, Koler and Osgood.[1] Chemical investigations of chromatographically purified[2] hemoglobin H, here presented, lead to a further understanding of its structure and of its relation to other human hemoglobins.

When DNP-globin H was prepared and examined by methods previously described,[3,4,5] the result was approximately four N-terminal valyl residues per molecule of 66,000 molecular weight[1] but only one kind of N-terminal sequence: val-his-leu. This N-terminal sequence defines β chains[5] and suggests that hemoglobin H may be represented[6] as β_4^H.

"Fingerprints"[10] of tryptic hydrolysates of hemoglobins H and A differed markedly. Peptides numbered[10] 5, 10, 11, 13, 17, 18, 23, and probably several others in regions normally poorly resolved were absent on the fingerprint of H but no new peptides were apparent. The absent peptides were present on fingerprints of isolated α^A chains. The likely conclusion that the sequence in β^H and β^A chains is identical was substantiated by the following hybridization experiment.[8,11]

Following hybridization of carbonmonoxyhemoglobin H and radioactive carbonmonoxyhemoglobin S at *p*H 11.0 at 3° for 24 hr., four hemoglobins were chromatographically isolated. These data are pertinent:

	Reactants		Products			
Zone	...	...	1	2	3	4
Mg.	22	22	5[a]	2[a]	15	7
C.p.m./mg.	0	1200	70	1100	600	1200
Identity of material	Hb-H	Hb-S*	Hb-H	β_4^S*	Hb-A*	Hb-S*
Formula	β_4^A	$\alpha_2^A\beta_2^S$*	β_4^A	β_4^S*	$\alpha_2^A\beta_2^A$	$\alpha_2^A\beta_2^S$*

[a] Precipitation that occurred during hybridization must have consisted of β^A and β^S* chains because α chains are conserved.

Identification of the products involved chromatographic studies and determination of radioactivity and for hemoglobin A also the study of sedimentation velocity and examination of N-terminal peptides[3,4,5] to show that only the α chains were radioactive. Thus, hemoglobin A and β_4^S* were formed during hybridization but there was no evidence for $\beta_2^A\beta_2^S$*. On the basis of the radioactive and material balance, it was concluded that the four β chains of hemoglobin H are identical with each other and with β^A chains.

Hemoglobin H is the first observed example of a hemoglobin composed of a single kind of polypeptide chain. Possibly, other abnormal hemoglobins or minor components in normal hemoglobin may be built on the scheme α_4, $\alpha_3\beta$, $\beta_2\gamma_2$, etc. Biologically, it suggests that hemoglobin H disease results from an imbalance in the relative production of α and β chains and hence that α and β chains are under separate biosynthetic and genetic control. This latter suggestion is further supported by experiments now in progress which show that the α^A and α^F chains are identical and that β chains are present in several minor hemoglobin components normally associated with hemoglobin A and S.

These experiments were made possible by the interest and generosity of Dr. D. A. Rigas and Dr. R. D. Koler. This investigation was supported in part by grants H-2258 and H-3394 from the National Institutes of Health, United States Public Health Service.

(1) D. A. Rigas, R. D. Koler and E. E. Osgood, *J. Lab. Clin. Med.*, **47**, 51 (1956).

(2) Extension of methods of D. W. Allen, W. A. Schroeder and J. Balog, THIS JOURNAL, **80**, 1628 (1958).

(3) H. S. Rhinesmith, W. A. Schroeder and L. Pauling, *ibid.*, **79**, 609 (1957).

(4) *Ibid.*, **79**, 4682 (1957).

(5) H. S. Rhinesmith, W. A. Schroeder and N. Martin, *ibid.*, **80**, 3358 (1958).

(6) The N-terminal sequence[5] defines the chain as α or β, the superscript denotes the hemoglobin that is the source of the chain, and the subscript has the usual chemical significance. The glycyl chains[7] of hemoglobin F are termed γ chains. Thus, hemoglobin A and S are $\alpha_2^A\beta_2^A$ and $\alpha_2^A\beta_2^S$ inasmuch as the α chains are identical.[8,9]

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

(8) J. R. Vinograd, W. D. Hutchinson, and W. A. Schroeder, *ibid.*, in press.

(9) V. M. Ingram, personal communication.

(10) V. M. Ingram, *Biochem. Biophys. Acta*, **28**, 539 (1958).

(11) J. Vinograd and W. D. Hutchinson, *Nature*, to be submitted.

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THE SYNTHESIS OF TIGOGENIN AND NEOTIGOGENIN

Sir:

We wish to report the synthesis of tigogenin (VIa) and neotigogenin (VIb), typical members of the large and important family of steroidal sapogenins.[1]

(1) *Cf.* L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publishing Corp., New York, N. Y., 3rd Edition, 1949, Chapter VIII.