

conditions while heparin is largely resistant.⁴ After hydrolysis in 6 *N* HCl and chromatography according to Stoffyn and Jeanloz,⁵ a radioactive arabinose spot (derived from glucosamine) was present. (7) Hydrolysis of the S³⁵-labeled heparin in 2 *N* HCl at 100° for 1 hour removed 95%, and hydrolysis in 0.04 *N* HCl at 100° for 2.5 hours 47% of the original radioactivity indicating approximately equal incorporation of the labeled sulfate into amide and ester groups.⁶

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(5) P. J. Stoffyn and R. W. Jeanloz, *Arch. Biochem. and Biophys.*, **52**, 373 (1954).

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LABORATORY OF CELLULAR PHYSIOLOGY
AND METABOLISM

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RECEIVED JANUARY 31, 1958

N-TERMINAL RESIDUES OF HUMAN FETAL HEMOGLOBIN

Sir:

Porter and Sanger¹ and Masri and Singer² have found, respectively, 2.6 and 2 N-terminal valyl residues in human fetal hemoglobin. We wish to report that N-terminal glycyl residues are also present.

After red cells from umbilical cord blood of white infants had been washed with saline and hemolyzed, the hemoglobin was dinitrophenylated in aqueous solution³ and the heme was then removed.³ When this DNP-globin was hydrolyzed for one hour in refluxing 6 *N* hydrochloric acid, 1.12 N-terminal residues of DNP-glycine per molecule, 0.22 of DNP-valine, and 1.61 of DNP-val-leu were isolated from the ether extract of the hydrolysate. The quantities were 0.25 residues of DNP-glycine and 2.12 of DNP-valine after 24 hr. of hydrolysis. These compounds were isolated and identified by procedures previously described.³ The calculations of the N-terminal residues per molecule assume that fetal and adult hemoglobin have essentially equal molecular weights,⁴ and that, as does adult DNP-globin, 0.1 g. of air-dried fetal DNP-globin contains 1.14 μ moles of DNP-protein.

The above results from pooled clotted cord blood were substantiated by examination of a second sample of pooled clotted blood and a sample of individual unclotted blood. A difference lay in the DNP-glycine, which was 1.28 and 1.44 residues, respectively, in one-hr. hydrolysates. The difference is a reflection of the variation in the amounts of adult hemoglobin and of other components that are present and can be detected by chromatography.⁵ Consequently, to obtain more definite results, the main component of cord blood hemo-

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globin (termed F_{II})⁵ was isolated by chromatography⁵ on the ion exchange resin IRC-50 with Developer No. 4. DNP-Globin from F_{II} gave these results:

	DNP-Glycine	DNP-Valine	DNP-Val-leu	Sum of DNP-valine and DNP-Val-leu
1	2.04	0.34	1.62	1.96
4	1.45	0.68	1.06	1.74
24	0.27	1.58		
	0.33	1.65	0.12	1.77

If we assume that DNP-glycine is released within the first few minutes of hydrolysis and that its destruction is by a pseudo first-order reaction, a very approximate reaction rate constant is 0.08 hr.⁻¹ and the quantity at zero time is 2.06 residues.

Thus, the main fetal component contains an equal number (probably 2) of glycyl and valyl N-terminal residues. It is of interest that the N-terminal sequence val-leu- is present in both adult human and fetal hemoglobin. Perhaps the two hemoglobins have two identical chains with this N-terminal sequence and differ in two other chains which have N-terminal glycine in fetal hemoglobin and the N-terminal sequence val-his-leu in normal adult hemoglobin.⁶

This investigation was supported in part by a research grant (RG-4276(C2)) from the National Institutes of Health, Public Health Service.

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RECEIVED JANUARY 9, 1958

THE STRUCTURE OF CHAKSINE, A MONOTERPENE ALKALOID

Sir:

The alkaloid chaksine isolated from the seeds of *Cassia absus* Linn by Siddiqui and Ahmad¹ has been the subject of many studies, in the course of which it has been assigned various functional group systems and structures (*cf.* ref. 2).

We now wish to report evidence, which together with previously reported data, permits the assignment of structure I to chaksine iodide. (Found: C, 36.60; H, 5.87; N, 11.62; O, 11.23; I, 35.19. Calculated for C₁₁H₂₀O₂N₃I·0.5 H₂O: C, 36.47; H, 5.85; N, 11.60; O, 11.05; I, 35.04; infrared (KBr pellet) 1720, 1670, 1600, 1572 cm.⁻¹; ρK_a = 11).

Chaksine has no N-alkyl and O-alkyl group, and gives a negative iodoform test. A Kuhn-Roth determination on the free base gave a value corresponding to one C-alkyl group.

Hydrolysis of chaksine with 2 *N* sodium hydroxide gave a low yield of the ureido-hydroxy acid II, C₁₁H₂₀N₂O₄, m.p. 122-123°. (Found: C, 53.88; H, 8.27; N, 11.45; O, 26.27. Calculated: C, 54.07; H, 8.25; N, 11.47; O, 26.20). The infrared spectrum of the oily ester of II (CCl₄) showed bands at 1740 cm.⁻¹ (ester) and 1710 cm.⁻¹ (five-membered cyclic urea).

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(2) S. K. Guha and J. N. Ray, *J. Ind. Chem. Soc.*, **33**, 225 (1956).