under conditions adaptable to large-scale production.

One example is the quantitative process $B_5H_{11} + 3H_2O \rightarrow 2H_2 + B(OH)_3 + B_4H_{10}$. This represents an efficient synthesis of B_4H_{10} because B_5H_{11} is efficiently made from B_2H_6.[2] Another example is the reaction of $[(CH_3)_2N]_2BH$ with B_5H_{11}, converting nearly half of the latter to B_5H_9 and 3.6% to B_6H_{10}. This represents the best known way to obtain the hitherto very rare hexaborane, the yield of which probably can be improved further. The conversion of B_5H_{11} to B_5H_9 was not necessarily direct, for B_4H_{10} also was formed, and we have found that $[(CH_3)_2N]_2BH$ converts as much as 25% of a B_4H_{10} sample to B_5H_9. These reactions suggest interesting new ideas about the mechanisms of borane interconversions by the classical gas-phase processes.

In the experiments described all volatile products were identified by their known physical properties and hydrolytic analyses. Numbers preceding formulas represent millimole quantities.

Tetraborane from Pentaborane-11.—Nearly pure B_5H_{11} (0.509 mmole) was hydrolyzed during one minute at 0°, yielding 0.491 B_4H_{10} and 0.506 $B(OH)_3$; and 0.017 B_5H_9 impurity was recovered. Again, the hydrolysis of 0.392 B_5H_{11} gave 0.360 B_4H_{10}, 0.767 H_2, and 0.366 $B(OH)_3$, with recovery of 0.023 B_5H_9. Allowing for the B_5H_9 impurity, the yields of B_4H_{10} were 99.8 and 97.6%. The process also shows how to analyze B_5H_{11} for B_5H_9, an impurity formerly difficult to estimate.

Pentaborane-9 and Hexaborane from Pentaborane-11.—A reaction between 1.187 B_5H_{11} and 0.696 $[(CH_3)_2N]_2BH$ was noticed at −78°. During slow warming to 0°, volatile products formed at increasing rates; final stoichiometry: 0.192 B_2H_6, 0.165 B_4H_{10}, 0.583 B_5H_9, 0.009 B_5H_{11}, 0.035 B_6H_{10}, 0.074 $(CH_3)_2NB_2H_5$, and non-volatile material. The last was heated with dimethylamine, forming 0.626 $[(CH_3)_2N]_2BH$ and 1.02 $(CH_3)_2NBH_2$; final recovery of boron as volatile compounds, 91%. The 3.6% yield of B_6H_{10} was not an impurity in the B_5H_{11}, for partial hydrolysis of parallel samples would have disclosed any B_6H_{10} (easily separable from B_4H_{10} and B_5H_9).

Pentaborane-9 from Tetraborane.—An adduct empirically formulated as $[(CH_3)_2N]_2BH \cdot B_4H_{10}$ was formed at −78°. On warming to −15° during 5 hr., 0.692 mmole evolved 0.031 B_2H_6, 0.120 B_4H_{10}, 0.037 $[(CH_3)_2N]_2BH$, 0.092 $(CH_3)_2NBH_2$, and 0.116 B_5H_9. The pentaborane represented 25% of the boron in the unrecovered tetraborane.

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DEPARTMENT OF CHEMISTRY JAMES L. BOONE
UNIVERSITY OF SOUTHERN CALIFORNIA
LOS ANGELES 7, CALIFORNIA ANTON B. BURG

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THE SYNTHESIS OF HEPARIN IN MOUSE MAST CELL TUMOR SLICES

Sir:

Heparin appears to be entirely localized in the mast cells which occur generally throughout all connective tissue. This had led to the general assumption that these cells are the site of synthesis of heparin although the possibility has never been excluded that they serve only a storage or excretory function. The availability of mouse mast cell tumors,[1] 2 to 2.5 g. subcutaneous masses of essentially homogeneous tissue, made possible a study of the metabolism of heparin in mast cells, *in vitro*.

Slices of tumor were incubated with either C^{14}-glucose or S^{35}-sulfate and heparin of relatively high purity isolated.

TABLE I

Approximately 5 g. of tissue slices of mouse mast cell tumor were incubated in Krebs–Ringer phosphate buffer with either C^{14}-glucose (5.5 μM./ml., 0.36 μC/μM.) or S^{35}-sulfate (1.5 μM./ml., 3 μC/μM.) at 37° in an atmosphere of O_2. The tissue was then homogenized, boiled, cooled, adjusted to *p*H 8.5 and incubated with 25 mg. of pancreatin overnight. The samples were then dialyzed for 18 hours against running tap water, the insoluble material was removed by centrifugation and sufficient NaCl added to the supernatant solutions to make them 1 *M*. Heparin was precipitated by the addition of an excess of cetyltrimethylammonium bromide; the complex was then redissolved in 4 *M* NaCl and re-precipitated by dilution to 1 *M*. This was repeated three times. Finally, the heparin complex was washed well with water, dissolved in 2 *M* NaCl and the quaternary amine removed as the insoluble thiocyanate salt. The solution of heparin was thoroughly dialyzed against distilled water before counting. Heparin was determined by metachromatic and carbazole assays using commercial heparin (Upjohn, 120 units/mg.) as a standard. The assays were in good agreement and approximately 70% of the weight was accounted for by heparin.

Expt.	Radioactive precursor	Incubation time, hours	Heparin Mg.	Heparin C.p.m./mg.
I	Glucose	0	2.1	14
	Glucose	1	2.0	4,040
	Glucose	2	2.0	7,560
II	Sulfate	0	2.0	20
	Sulfate	2	2.0	34,000

The following evidence supports the view that, in both experiments, the radioactive compound isolated was heparin. (1) A crude chondroitin sulfate fraction, which was also isolated, was found to have less than 20% the radioactivity and less than 15% the specific activity of the heparin. (2) When a ten-fold excess of unlabeled heparin and chondroitin sulfate were added to radioactive samples over 90% of the radioactivity was re-isolated with the heparin. (3) Shaking an aqueous solution of radioactive material with chloroform:octanol[2] (9:1) did not remove any detectable protein or radioactivity. (4) Upon electrophoresis in 0.02 *M* citrate buffer, *p*H 4.5 for 4 hours at 500 volts, single metachromatic and radioactive spots, which exactly corresponded, were observed. (5) After digestion with microbial heparinase[3] no radioactivity was recoverable upon the addition of carrier heparin. (6) After hydrolysis of the C^{14}-labeled heparin for 20 minutes at 100° in 7.5% H_2SO_4 70% of the radioactivity remained non-dialyzable. Both chondroitin sulfate and hyaluronic acid are completely degraded under these

(1) T. B. Dunn and M. Potter, *J. Natl. Cancer Inst.*, **18**, 587 (1957). Dr. Dunn has kindly provided the tumor tissue used in these experiments.

(2) A. S. Jones, *Biochem. et Biophys. Acta*, **10**, 607 (1953).

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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 S^{35}-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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LABORATORY OF CELLULAR PHYSIOLOGY AND METABOLISM
NATIONAL HEART INSTITUTE EDWARD D. KORN
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

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N-TERMINAL RESIDUES OF HUMAN FETAL HEMOGLOBIN

Sir:

Porter and Sanger[1] and Masri and Singer[2] 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 solution[3] 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 μmoles 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 hemoglobin (termed F_{II})[5] was isolated by chromatography[5] 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]

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CONTRIBUTION NO. 2300 W. A. SCHROEDER
GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA GENJI MATSUDA

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THE STRUCTURE OF CHAKSINE, A MONOTERPENE ALKALOID

Sir:

The alkaloid chaksine isolated from the seeds of *Cassia absus* Linn by Siddiqui and Ahmad[1] 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 $C_{11}H_{20}O_2N_3I \cdot 0.5\ H_2O$: C, 36.47; H, 5.85; N, 11.60; O, 11.05; I, 35.04; infrared (KBr pellet) 1720, 1670, 1600, 1572 $cm.^{-1}$; $pK_a = 11$).

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, $C_{11}H_{20}N_2O_4$, 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 (CCl_4) showed bands at 1740 $cm.^{-1}$ (ester) and 1710 $cm.^{-1}$ (five-membered cyclic urea).

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