triglyceride concentration. The changes in the rats fasted for 22 hours were similar to the changes observed previously in rats fasted for 24 hours (1).

In the rats fasted overnight (group b), the relative proportion (wt. %) of phospholipid arachidonate was significantly higher, and the wt. % of linoleate was significantly lower than in the fed rats (group a). If calculated in terms of milligrams of fatty acid per liver, these changes represented a small increase in arachidonate and a 24% decrease in linoleate (88 mg of arachidonate, group b cf. 84 mg of arachidonate group a; 25 mg of linoleate, group b cf. 33 mg of linoleate, group a).

Comparison of group a (8:30 a.m. fed) and group d (8 p.m. fed) gives an indication of diurnal fluctuations in liver lipid composition. In group d, the wt. % of phospholipid arachidonate was slightly higher and that of linoleate slightly lower.

With respect to triglyceride composition, the rats fasted overnight (group b) had significantly higher proportions of arachidonate and linoleate and significantly lower proportions of oleate, palmitate, and palmitoleate than did the fed rats at 8 a.m. (group a), although the total amount of triglyceride fatty acid per liver was not significantly changed. Fasting 22 hours caused a significant decrease in total triglyceride, with additional small decreases in the relative proportions of oleate and palmitate, together with an increase in linoleate. In the fed rats killed at 8 p.m., the proportion of triglyceride fatty acids was quite similar to that of the 8 a.m.-fed rats, except for an increase in linoleate.

In summary, these results show that fasting for only 12 hours can produce significant changes in liver fatty acid composition. Consequently, feeding or fasting procedures should be carefully regulated in studies of liver lipid composition if comparable results are to be obtained in different experiments.

Summary. Fasting rats overnight from 8 p.m. to 8:30 a.m. produced significant changes in liver weight and liver fatty acid composition in comparison with fed rats killed at 8 p.m. and 8:30 a.m. Feeding and fasting procedures and time of sacrifice of animals should be carefully standardized in studies of liver lipid composition to minimize variations related to the eating patterns of the animals.

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Effect of Severe Hemorrhage on the Hemoglobins of a Virginian White-Tailed Deer (*Odocoileus virginianus*)* (32616)

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Since the original observation that sheep, homozygous or heterozygous for hemoglobin A (Hb-A), will produce a new Hb-type (Hb-C) during severe anemia (5,13) a similar phenomenon has been shown to occur in goats (7), but not in cattle (4). Structural studies have indicated that the two hemoglobins of

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each species have the same α-chains in common but differ in their β-chains, and that the differences reside in a number of substitutions (7,14,6). It seems that in both species the production of normal β-chains in the anemic animal is suppressed and replaced by the production of a different β-chain, the β-C chain. The β-C chains of the sheep and the goat are apparently similar, since only one structural difference has been observed so far (7).

Of the other ruminants, it has been reported that as many as seven hemoglobin types have been observed in deer (10). The most frequent combination found in the Virginian white-tailed deer is that of two types, designated as Hb-I and Hb-III (10,11). Analyses of the quantities of these two types in five deer (% Hb-I: 28.6; 33.6; 28.5; 16.0; 13.5) suggest that the proportions may vary between animals and perhaps within one animal dependent on its hematological status. The present work extends these observations by studying the hemoglobin components of a Virginian white-tailed deer with the Hb-types I and III during severe blood-loss anemia.

Material and Methods. A female deer, aged about 8 months, was bled for 8 weeks 400–600 ml twice a week from the jugular vein. Blood samples were collected for examination before each bleeding. A male deer of the same age served as control. Packed cell volume (PCV in %), total hemoglobin (Hb in %), white and red blood cell counts (WBC $\times$ 10^3/mm^3; RBC $\times$ 10^6/mm^3), and reticulocytes (in %) were determined using standard procedures. The hemoglobin of red blood cell hemolyzates was examined by starch gel electrophoresis, at pH 8.1 (8), and at pH 6.5. At pH 6.5 electrophoresis was carried out at 4°C; the sodium phosphate buffer used for the preparation of the gel was 0.02 *M* while a 0.01 *M* phosphate buffer was placed in the electrode vessels. Quantitative measurements were made by DEAE-Sephadex chromatography (9) using the beaded form of resin (A-50, medium; Pharmacia Fine Chemicals). The same procedure, but on a larger scale, was used for the isolation of larger amounts of the two major hemoglobin fractions. Each hemoglobin fraction was converted into globin using the acid-acetone procedure of Anson and Mirsky (1), after which the total amino acid composition was determined by analyzing acid hydrolyzates (prepared by hydrolysis of 5 mg of globin with 6 *N* HCl at 110°C for 24 and 72 hours in evacuated sealed tubes) using a Spinco model 120 amino acid analyzer. Oxygen equilibria experiments of various blood samples with PCV values adjusted to 28–32% were carried out using the procedure of Astrup *et al.* (2,3,12). The results were expressed as log P-50 values, i.e., the logs of the oxygen pressures required for 50% oxygenation. The partial pressures of CO_2 varied from 0 to 71.6 mm Hg; the temperature was maintained at 37°C.

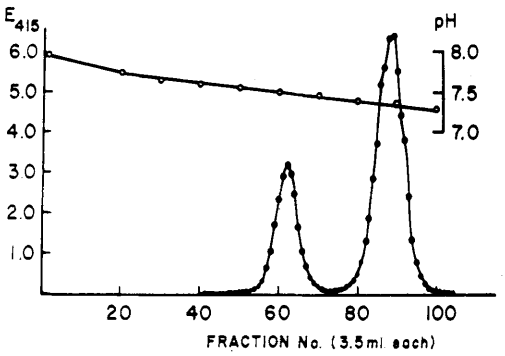

FIG. 1. Chromatographic separation of the deer hemoglobins I and III using DEAE-Sephadex, A-50 medium.

Results. It has been observed in our experiments and by others (10, 11) that deer Hb-II does not separate from Hb-III in starch gel electrophoresis at pH 8.1, while Hbs-II and -I showed identical electrophoretic mobilities at pH 6.5. The definite identification of the hemoglobin pattern of the deer was determined by reexamination of chromatographically isolated hemoglobin components on starch gel both at pH 8.1 and at pH 6.5. In each case, the individual components migrated as a single band. Figure 1 presents an example of chromatograms obtained. The first peak (eluted at pH 7.50) was identified as Hb-I, and the second (eluted at pH 7.42) as Hb-III.

No significant changes in the elution pH values were observed throughout the entire experiment. No newly formed Hb-type was noted.

The experimental bleeding produced a severe anemia (Fig. 2). The maximal de-

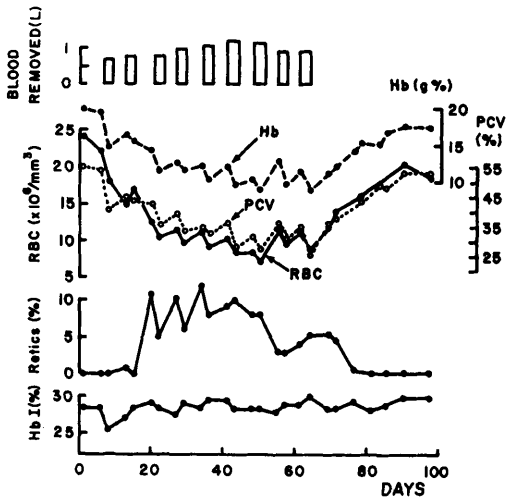

FIG. 2. The quantities of Hb-I and the variations in hematological parameters in a deer with the hemoglobins I and III before, during, and after severe blood-loss anemia.

creases in total Hb and PCV values were about 50%, and that of the RBC counts 60–65%. White cell counts showed little change. Two to 3 weeks after the start of bleeding reticulocytes appeared in quantities up to 10%. The relative quantities of Hb-I and Hb-III remained the same during the entire duration of the experiment (error of estimination: 2%). The recovery was rapid and uneventful.

It has been demonstrated that sickling of erythrocytes of deer can be produced by either an increase in pH or by oxygenation. The presence of deer Hb types V and VII may prevent the sickling phenomenon (10, 11). A definite form of sickling is also observed in supravital stains. During the routine staining for reticulocytes, it was noted that sickling of almost all red cells occurred before the anemia was advanced, but was virtually absent during the anemia, and returned slowly during the recovery period (Fig. 3). However, when the blood samples from anemic deer and from the normal control animal were oxygenated at a slightly increased pH in a tonometer, the red cells from both animals could be sickled.

The oxygen dissociation curves of blood samples from the anemic and the control deer were identical. The log P-50 values were 1.430–1.450 (anemic deer) and 1.420–1.450 (normal deer) at pH 7.30 and at 37°C. The Bohr effects were not different when determined over a pH range of 7.10–7.80 ($P\text{-}CO_2$ varying from 71.6 to 0 mm Hg). The Δ log P-50/ΔpH values were 0.83 and 0.80, respectively.

The total amino acid compositions of globin I and globin III were determined after isolation from blood samples collected on days 1 and 50 of the bleeding experiment. The results are presented in Table I. The results show no apparent difference between either the normal and anemic component I or between the normal and anemic component III. Our data for component III correspond closely with those reported by Kitchen *et al.* (10). Only slight and inconclusive differences were observed between the components I and III.

Discussion. These experiments have demonstrated that likely no new hemoglobin type is produced in deer with the hemoglobins I and III, when made anemic. There also appears not to be any change in the relative quantities of these two Hb types. The reason for the apparent change in sickling properties of the erythrocytes during anemia was not ascertained. It seems unlikely that a change in type of hemoglobin is responsible for this phenomenon, and the possibility of membrane differences in the younger red blood cells should be considered. Our study certainly does not

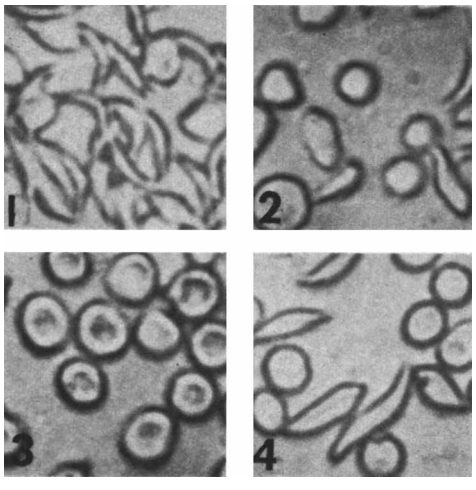

FIG. 3. The sickling phenomenon observed in reticulocyte stains. Samples were collected on days 1 (sample 1), 8 (sample 2), 41 (sample 3), and 97 (sample 4).

TABLE I. The Amino Acid Compositions of the Deer Globins I and III Isolated before and during Severe Anemia.[a]

	Component I		Component III	
Amino acid	Normal	Anemic	Normal	Anemic
Lysine	21.20	21.08	20.63	20.61
Histidine	16.98	17.02	17.34	17.37
Arginine	9.02	8.75	9.36	9.28
Aspartic acid	27.79	28.53	27.83	28.46
Threonine	13.65	14.10	14.80	14.20
Serine	12.20	12.40	13.10	13.30
Glutamic acid	15.59	16.05	16.00	16.08
Proline	12.26	11.91	11.56	11.48
Glycine	21.40	21.45	21.10	21.45
Alanine	35.60	35.37	34.19	34.61
Valine	32.00	31.57	30.35	29.91
Methionine	3.19	3.14	3.42	3.19
Isoleucine	0	0	0	0
Leucine	37.05	37.09	36.07	35.64
Tyrosine	4.01	3.84	4.00	3.93
Phenylalanine	18.47	18.48	18.14	17.70
Tryptophan	not determined			
Half-cystine	not determined			

[a] The values are the means of duplicate analyses of 24-hour and 72-hour hydrolyzates and are expressed as numbers of residues per half molecule (mol. wt. 33,000). The values for threonine and serine are corrected for partial losses observed during acid hydrolysis; those for valine are the means of duplicate analyses of 72-hour hydrolyzates only.

exclude the possibility that deer with other hemoglobin types may show changes in the proportions of these hemoglobin types during the anemic states. It might be of interest to examine deer with the hemoglobin type, designated Hb-V, because of certain structural resemblance with the Hb-C of the goat and sheep (14,6).

Summary. Electrophoretic, chromatographic, and limited structural analyses of the hemoglobins of a deer with two distinct hemoglobin types, Hb-I and Hb-III, failed to demonstrate any change in hemoglobin type as well as in the proportions of the hemoglobins already present when the animal was made severely anemic. Oxygen equilibria also failed to demonstrate any differences in the oxygen affinities and Bohr effects of blood samples from the severely anemic animal and from the nonanemic deer. The sickling of the erythrocytes, which was observed in supravital stains, was absent during the period of bleeding.

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