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Action of Urea Upon Hemoglobin. Spectrophotometric Study of Progress of a Protein Denaturation.

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When NaOH is added to solutions of hemoglobin, a reaction is initiated whose progress may be followed very accurately by means of spectrophotometry. The changes which occur may, for convenience, be divided into 2 successive main reactions, and may be represented as follows:

1. Ferrohemoglobin (HbO_2) + NaOH → Globinoferriporphyrin (oxidized, denatured globin hemochromogen).
2. Globinoferriporphyrin + NaOH → Ferriporphyrin hydroxide (alkaline hematin) + denatured globin.

Under the conditions which have been used the reactions are unidirectional, but the addition of a reducing agent, $Na_2S_2O_4$, shifts reaction 2 in favor of the hemochromogen, as follows:

3. Ferriporphyrin hydroxide + denatured globin + $Na_2S_2O_4$ → Globinoferroporphyrin (reduced, denatured globin hemochromogen).

When $Na_2S_2O_4$ is added to globinoferriporphyrin (reaction 1), globinoferroporphyrin also is produced. The terminology which is employed is unambiguous, and has been proposed by the writer.[1] The protein component of the hemoglobin is probably irreversibly denatured in this process. It has already been pointed out by Drabkin and Austin[2] that the first reaction takes time and is the basis for the von Krüger reaction.[3]

It has been found by the writer that essentially similar reactions to the above occur when hemoglobin is denatured by reagents other than alkali—notably urea and acetamide in high molar concentrations (4 to 6 M), and HCl. In the case of the latter reagent, denaturation results in the production of the familiar acid hematin and even with fairly strong acid (0.1M) it is well recognized that the reaction takes time. During the course of this reaction the writer has found that, if enough alkali is added to just dissolve the material precipitated at the iso-electric point as the acidified solution is neu-

1 Drabkin, D. L., *J. Biol. Chem.*, 1938, **123**, xxi.

2 Drabkin, D. L., and Austin, J. H., *J. Biol. Chem.*, 1935-36, **112**, 89.

3 von Krüger, F., *Z. ges. exp. Med.*, 1926, **53**, 233; Haurowitz, F., *Z. physiol. Chem.*, 1929, **183**, 78.

tralized, the presence of the typical globinoferroporphyrin (the reduced hemochromogen) is disclosed upon the addition of $Na_2S_2O_4$.

In studying denaturation reaction rates conditions were chosen to provide for a very slow reaction with alkali. The concentration of hemoglobin (HbO_2) in the final solution was 0.1 mM per liter (where 1 mole is equivalent to 1 mole of iron porphyrin), and the concentration of total alkali in the final solution was 0.008M. The alkali used was a mixture of 0.005M NaOH and 0.003M NH_4OH. The ammonia was included so as to more than compensate for the slight ammonia production which occurred in the experiments in which 6M urea was used with NaOH added to a concentration of 0.005M. The determinations were upon solutions in closed 1 cm cuvettes at a temperature of approximately 20°C. Under these conditions the time required for one-half completion of reaction 1 was 13 hours for horse hemoglobin (HbO_2) with alkali alone added, as above. For dog hemoglobin, under the same conditions, the reaction was somewhat more rapid.

Carbonmonoxide hemoglobin of both species is appreciably more stable to alkali and to other denaturing reagents than is oxyhemoglobin. The rate of denaturation of oxyhemoglobin in the presence of 6M urea alone is approximately of the same order of magnitude as with the 0.008M concentration of alkali, when judged by the criterion of globinoferriporphyrin formation. The rate of reaction is decreased by lowering the concentration of urea to 4M, and is appreciably increased at a temperature of 38°C instead of 20°C.

The present experiments are not inconsistent with Steinhardt's finding[4] that with horse carbonmonoxy hemoglobin in 4M urea, disaggregation of the molecules of the protein into units of one-half the original molecular weight takes place independently of the process of denaturation. According to Wu and Yang[5] dog hemoglobin, in contrast with horse hemoglobin, is not disaggregated in the presence of urea. Both horse and dog hemoglobin, however, undergo denaturation in urea, and at about the same rate in the writer's experiments. From the standpoint of the possible rôle of urea in protein denaturation the following finding of the writer is of great interest: In the presence of 6M urea and 0.005M NaOH, both horse and dog oxyhemoglobin are denatured approximately 60 times as rapidly (13 minutes for one-half completion of reaction 1) as with 6M urea alone or with a total of 0.008M concentration of alkali alone, as described above. The exposure of sulfhydryl groups in the protein in

4 Steinhardt, J., *J. Biol. Chem.*, 1938, **123**, 543.

5 Wu, H., and Yang, E. F., *Chinese J. Physiol.*, 1932, **6**, 51.

the presence of urea[6] does not appear to account for the above finding since disaggregation in acetamide is unaccompanied by exposure of sulfhydryl groups,[6] and denaturation of hemoglobin also occurs in the presence of the latter amide.

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Prothrombin Concentration in Newborn.

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The concentration of prothrombin in the blood of babies 3 to 7 days old has been found to be essentially the same as in adult blood.[1] Curiously, however, a profound fluctuation has been observed to occur during the first 48 hours of life, as shown in Table I.

It should be noted that the prothrombin level of babies 6 hours old is relatively high and not strikingly different from cord blood. At the end of 24 hours, however, it may drop to an exceedingly low level as shown by babies 5 and 6. After 48 hours the prothrombin concentration usually has begun to return to normal. In one baby

TABLE I.

			Clotting Time, Quick's Prothrombin Method		
		Age	Undiluted plasma	Diluted plasma, 50%	Prothrombin Concentration, %
Cord blood	1		13	18	71
	2		13	18½	67
	3		13	18½	67
Baby	1	6 hr	12	17	80
	2	6 ''	12½	17½	75
	3	6 ''	12	18	71
	4	1 day	12½	17½	75
	5	1 ''	53	105	7
	6	1 ''	55	—	7
	7	2 days	13	18½	67
	8	2 ''	13½	21	54
	9	2 ''	17	30	35
	10	2½ days	29	47	16
		3½ ''	30	48	15½
		5½ ''	13	17½	75

[6] Greenstein, J. P., *J. Biol. Chem.*, 1938, **125**, 501; 1939, **128**, 233.

[1] Quick, Armand J., and Grossman, Arthur M., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 647.