

kept constant at 35° C. Four experiments gave concordant results. Table I records the results of one typical experiment together with those of Némec.

TABLE I.

Duration	Némec					Author			
	Control		Potassium urate			Control		Potass'm urate	
	H ₂ SO ₄ *	NH ₃	H ₂ SO ₄ *	NH ₃	NH ₃ from urate	H ₂ SO ₄	NH ₃	H ₂ SO ₄	NH ₃
hours	cc.	mg.	cc.	mg.	mg.	cc.	mg.	cc.	mg.
24	8.7	5.31	23.4	14.30	8.99	0.4	0.14	0.5	0.17
72			30.6	18.69		0.7	0.24	0.7	0.24
120	11.8	7.21	31.8	19.43	12.22	0.6	0.20	0.7	0.24
144	11.3	6.90	34.2	20.89	13.99	0.6	0.20	0.8	0.27

* 1 cc. of acid equals 0.000611 grams NH₃.

These results show no evidence of the presence of any appreciable amount of uricase in soy beans.

¹ Némec, A., *Biochem. Z.*, 1920, cxii, 286.

² Horvath, A. A., *J. Biol. Chem.*, 1926, lxxviii, 343.

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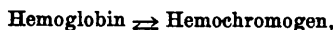
Denaturation of Hemoglobin.

HSIEN WU AND KUO-HAO LIN.

From the Department of Biochemistry, Peking Union Medical College.

The present study arose from an inquiry into the question: Is the denaturation of hemoglobin reversible? According to general experience denaturation of proteins is irreversible, but reversion of denatured hemoglobin has been reported by Anson and Mirsky.^{1, 2}

These workers found that when an alkaline solution of hemochromogen was neutralized, a small amount of hemoglobin was obtained. On the basis of this observation they concluded that there is a reversible equilibrium between hemochromogen and hemoglobin:



and that the position of the equilibrium depends on hydrogen ion concentration. Adding acid or alkali to hemoglobin converts it into

hemochromogen, while neutralizing hemochromogen turns it into hemoglobin.

The term hemochromogen is used by these authors to designate, not the reduced iron pyrrol complex which they called hem, but a compound of this substance with globin. Since the solubility of hemochromogen is similar to that of denatured proteins, and since it is formed under conditions which would cause denaturation of proteins, they considered hemochromogen as denatured hemoglobin.

The work of Anson and Mirsky was entirely qualitative, and they stated that the evidence for hemochromogen equilibrium was not conclusive.

We denatured different forms of hemoglobin with acid, alkali, heat and alcohol. We allowed the products so obtained to revert in faintly alkaline solution in which they were soluble. Our work definitely establishes the reversibility of the hemoglobin-hemochromogen equilibrium, but our interpretation differs from that of Anson and Mirsky.

Our findings are as follows:

1. Reduced hemoglobin when treated for a short time with dilute HCl is completely precipitated on neutralization. The precipitate, when dissolved in faintly alkaline solution, reverts to hemoglobin. Under suitable conditions the reversion is practically quantitative. If the acid-reduced hemoglobin solution is exposed to the air before it is allowed to revert, no reversion is obtained. If the exposed solution is reduced with hydrosulphite, reversion can again occur.
2. Reduced hemoglobin subjected to the action of alkali, alcohol or heat also shows reversion, but it is much less complete than in the case of acid treated solution. The behavior of the "denatured" hemoglobin towards air in these cases is the same as in acid solution.
3. Methemoglobin behaves towards acids just like reduced hemoglobin. Methemoglobin "denatured" by alkali, alcohol or heat shows no reversion. But if the "denatured" methemoglobin is reduced by hydrosulphite, some reversion is obtained.
4. Oxyhemoglobin "denatured" by acid shows partial reversion. The hemoglobin formed by reversion in this case is methemoglobin.
5. Oxyhemoglobin is only partially converted into methemoglobin by the action of acid. This explains the finding of Roaf and Smart³ and of Quagliariello⁴ that acid liberates from oxyhemoglobin only half as much oxygen as does ferricyanide, and brings it into harmony with the conclusion reached by Conant⁵ from electrochemical studies, that methemoglobin bears the same relation to hemoglobin as ferric iron does to ferrous iron.

6. Carboxyhemoglobin behaves just like reduced hemoglobin. The hemoglobin recovered is carboxyhemoglobin.

7. Prolonged action of acid or other agents of denaturation on hemoglobin decreases the percentage of reversion.

From the above findings it is concluded that hemoglobin undergoes two kinds of change when subjected to denaturation. One is reversible and the other is irreversible. The two changes overlap when hemoglobin is heated or is treated with alkali or alcohol. But when hemoglobin is treated with acid the velocities of the two changes are so different that they can be separately studied.

Since the reversible denaturation, which we propose to call pseudo-denaturation, involves only the prosthetic group, it is a reaction peculiar to hemoglobin and is fundamentally different from the denaturation of proteins in general.

This is a preliminary paper.

¹ Anson, M. L., and Mirsky, A. E., *J. of Physiol.*, 1925, **lx**, 50.

² Anson, M. L., and Mirsky, A. E., *J. of Gen. Physiol.*, 1925, **ix**, 169.

³ Roaf, H. E., and Smart, W. A. M., *Biochem. J.*, 1923, **xvii**, 579.

⁴ Quagliariello, G., *Arch. sc. Biol.*, 1923, **v**, 193.

⁵ Conant, J. B., and Scott, N. D., *J. of Biol. Chem.*, 1926, **lxix**, 575.

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The Rôle of the Platelets in Blood Clotting.*

C. A. MILLS.

From the Department of Medicine, Peking Union Medical College.

Blood was obtained from a dog by the method of Lim,* which absolutely excludes admixture of tissue juice. It was kept in contact only with paraffin, was immediately cooled and centrifuged. By differential centrifuging platelets were obtained free of red and white cells. They were washed well in saline and then dissolved in water. This platelet solution was tested for thrombin, prothrombin cephalin and tissue fibrinogen. On treating it with active cephalin and calcium and letting the mixture stand for 15 minutes at room temperature, it was unable to clot a citrated solution of

* A detailed report of this work and of Dr. Lim's method will appear in the July number of the *Chinese Journal of Physiology*.