In our previous communication (*loc. cit.*) we gave an extinction coefficient of $E^{1\%}_{1\,cm.} = 385$ at 248 mμ for vitamin K_1 but since that time a value of 540 has been obtained. The analyses of both preparations indicated that each was analytically pure. Our first value was probably due to instability of the vitamin on storage and toward light. For this reason we are not at present certain that 540 is the maximum value attainable. In this same communication line 38 column 1 should read "all have a potency of about *1000* units per mg." instead of, "all have potency of about 100 units per mg."

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DENATURATION OF MYOSIN

Sir:

Myosin is a protein particularly susceptible to "denaturation" by very mild chemical agents. We have employed four criteria of alteration in the molecule: alteration in –SH groups,[1] loss of double refraction of flow,[2] changes in viscosity and in solubility. Seven preparations of myosin from rabbit muscle, and one from lobster, have been studied with very consistent results.

Some of our observations are briefly summarized in Table I, from which several conclusions may be drawn. (1) The extreme asymmetry of the molecule, on which its double refraction depends, is diminished rapidly by all commonly employed denaturing agents, and by many other substances as well. (2) The content of titratable –SH groups is greatly increased in concentrated solutions of urea and even more in guanidine hydrochloride; in the presence of the latter at 16 *m*, nearly all the non-methionine sulfur of myosin[3] can be accounted for as –SH. The concentrations of urea and guanidine needed to produce marked increase in –SH content are much greater than those needed to destroy double refraction. (3) Other substances, such as lithium, calcium and magnesium chlorides, destroy double refraction but do not affect –SH groups. Substances containing an ammonium group abolish titratable –SH completely (but the full content of titratable –SH is immediately restored by adding concentrated guanidine hydrochloride). Such substances may or may not destroy double refraction (see table). There appears to be no systematic correlation between the effect of reagents on –SH groups and their effect on double refraction.

(1) Greenstein, *J. Biol. Chem.*, **125**, 501 (1938); **128**, 233 (1939).
(2) Von Muralt and Edsall, *ibid.*, **89**, 351 (1930).
(3) Bailey, *Biochem. J.*, **31**, 1406 (1937).

TABLE I

EFFECT OF REAGENTS ON DOUBLE REFRACTION OF FLOW AND ON SULFHYDRYL CONTENT OF RABBIT MYOSIN

All substances tested were added to myosin dissolved in KCl, 0.4–0.5 *n*, at *p*H 6.2–7.4. *T* denotes the time required for disappearance of double refraction of flow. –SH content is expressed as percentage cysteine.

Substance added	Molality in solution	*T*	–SH content, %
KCl	0.5	>2 weeks	0.42 ± 0.03
KBr	0.80	15 min.	..
KI	0.27	15 min.	0.46
Guanidine HCl	0.30	5 min.	0.42
Guanidine HCl	16.6	At once	1.14 ± 0.03
Guanidine HI	0.075	>5 days	..
Guanidine HI	0.14	1 hour	..
Guanidine HI	0.20	5 min.	..
Guanidine HI	0.28	<30 sec.	..
Urea	1.4	15 min.	0.42
Urea	16.6	At once	0.66 ± 0.03
LiCl	1.0	10 min.	0.46
$MgCl_2$	0.35	2 min.	0.46
$CaCl_2$	0.25	5–10 min.	..
NH_4Cl	1.4	10 min.	0
CH_3NH_3Cl	1.4	10 min.	0
Arginine mono-HCl	0.35	10 min.	0
Glycine	1.7	>1 week	0

The viscosity of myosin solutions decreases markedly on addition of reagents which destroy double refraction of flow. This is explicable on the assumption that the very long molecules of native myosin are broken up into smaller and less asymmetrical chains by the action of such reagents. This decrease in viscosity is in marked contrast to the increase produced by denaturation in solutions of "globular" proteins.[4]

The solubility of myosin is not fundamentally altered by any of the reagents studied; it retains the characteristics of a typical globulin. This is in marked contrast with the loss of solubility produced by heating myosin[5] and with the effect of all denaturing agents in decreasing the solubility of such proteins as egg albumin or hemoglobin.

(4) Anson and Mirsky, *J. Gen. Physiol.*, **15**, 341 (1932).
(5) Mirsky, *Cold Spring Harbor Symp. Quant. Biol.*, **6**, 150 (1938).

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ON THE COLOR REACTION FOR VITAMIN K

Sir:

Recently Dam, Karrer and co-workers [*Helv. Chim. Acta*, **22**, 310 (1939)] described a vita-