In our previous communication (*loc. cit.*) we gave an extinction coefficient of $E_{1\,cm.}^{1\%} = 385$ at 248 $m\mu$ 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 a potency of about 100 units per mg."

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RECEIVED MAY 22, 1939

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

(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).

–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.

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
MgCl₂	0.35	2 min.	0.46
CaCl₂	0.25	5–10 min.	..
NH₄Cl	1.4	10 min.	0
CH₃NH₃Cl	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-

min K in a pure or nearly pure form. The vitamin was said to give a characteristic color reaction with sodium ethylate in alcoholic solution. We hoped that this reaction would be useful in the isolation of vitamin K from alfalfa, especially because the typical color changes described by the European workers were noticed with relatively crude concentrates. However, it was found that the color reaction is not a criterion for the presence of the vitamin as illustrated by the following experiment.

One gram of a vitamin K concentrate which gave the color reaction very strongly and had a potency of 1 unit [*J. Nutrition*, **17**, 303 (1939)] in 15 γ was dissolved in petroleum ether and chromatographically adsorbed on a slightly heat-activated calcium sulfate. Washing with petroleum ether was continued until the lowest yellow zone had passed into the filtrate. The adsorbed substance was then eluted with ether and we obtained 0.3 g. of a material with a very intense color reaction but with no vitamin K activity at 15 γ. The yellow filtrate contained 0.6 g. of an oil which did not give the typical color reaction; only a slight darkening occurred with the ethylate. However, it was fully active in a dose of 8 γ, containing the entire potency of the initial preparation.

Upon further purification by several chromatographic adsorptions on a more highly activated calcium sulfate, a concentrate was obtained which behaved like a single substance chromatographically. Its potency was comparable to that of the vitamin K_1 of McKee, *et al.* [THIS JOURNAL, **61**, 1295 (1939)], assuming that the potency of their product was not 100 but 1000 units per mg., as stated in their earlier paper [*Proc. Soc. Exptl. Biol. Med.*, **40**, 482 (1939)]. It was a light yellow oil which darkened on standing even in the refrigerator and which gradually lost potency. It did not give derivatives with reagents for keto groups or for hydroxyl groups, and in spite of its high degree of unsaturation failed to react with maleic anhydride in boiling benzene. Exposure to bright sunlight caused an almost instantaneous formation of a pink coloration fading in a few minutes. During the last steps of the isolation process the sodium ethylate color reaction became again positive, although it never reached the intensity of the previously separated inactive fraction. It may be that the blue coloration is given by readily formed decomposition products of vitamin K.

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THE RELATION BETWEEN METHYL *etio*-DESOXY-CHOLATE AND THE METHYL DIHYDROXY-*etio*-CHOLANATE DERIVED FROM DIGOXIGENIN; METHYL 12-*epi-etio*-DESOXYCHOLATE

Sir:

Steiger and Reichstein [*Helv. Chim. Acta*, **21**, 828 (1938)] have degraded digoxigenin to a dihydroxy- and a diketo-*etio*-cholanic acid and a diketo-*etio*-cholenic acid. We have recently shown [THIS JOURNAL, **60**, 2824 (1938)] that these diketo acids are identical with the corresponding acids derived from desoxycholic acid. The dihydroxy acid, however, was found to be different from *etio*-desoxycholic acid in that its methyl ester melted at 180–183° while methyl *etio*-desoxycholate melted at 145–146°. Since both acids have the α configuration at C-3, epimerism at C-12 appeared to be the only plausible explanation of the difference. However, it became important to test this point in order to eliminate the possibility of a flaw in the other comparisons. The evidence now at hand confirms this explanation and furnishes additional proof for the positions of the oxygen atoms in the acids derived from digoxigenin.

Reduction of methyl 3,12-diketo-*etio*-cholanate in alcohol with platinum oxide catalyst resulted in a mixture of esters. The esters with the β configuration at C-3 were removed by precipitation with digitonin. The esters with the α configuration were separated by adsorption analysis on a column of alumina. Repeated crystallization of the fraction with higher melting point gave an ester (methyl 12-*epi-etio*-desoxycholate) which melted at 176–178°; $[\alpha]^{25}_{5461} + 49.4 \pm 2.4°$ (0.203% in alcohol). Analysis of the ester was not entirely satisfactory because of the presence of ash (Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.59. Found [corrected for ash]: C, 71.69; H, 9.81), but the 3-monobenzoate of the ester gave satisfactory values (Calcd. for $C_{28}H_{38}O_5$: C, 73.96; H, 8.42. Found: C, 74.06; H, 8.62). The monobenzoate melts at 136–138°; $[\alpha]^{25}_{5461} + 62 \pm 3°$.

Professor Reichstein very kindly compared