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THE EFFECT OF SURFACE-ACTIVE SUBSTANCES ON THE FUCHSIN REACTION OF HIGHER FATTY ALDEHYDES*

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Higher fatty aldehydes are present in considerable quantity in the lipide fraction of muscle and brain and may be intermediates in lipide metabolism (1, 2). The meager information concerning the quantitative distribution of the higher fatty aldehydes in tissues has been acquired exclusively by quantitative evaluation of the Schiff reaction, as in the procedure of Feulgen and Grünberg (3) or modifications of it (4). Serious doubt as to the reliability of the method arose when it was found that added palmitaldehyde or stearaldehyde or their acetals could not be estimated quantitatively in tissue extracts (4).

The experiments reported in this paper show that the result of the quantitative fuschsin reaction for the determination of higher fatty aldehydes depends to a large degree on the presence of surface-active lipides in the tissue extract. Naturally occurring lipides or synthetic surface-active agents inhibit the color development if added at the beginning of the reaction, and destroy the color already formed if added later. This effect of surface-active agents can be suppressed to a large degree by reducing the water content of the medium through the use of a high concentration of acetic acid.

EXPERIMENTAL

The Schiff reaction was employed in three forms, two at low and one at high concentrations of acetic acid. In all expriments palmitaldehyde glycol acetal was used as reference substance (4).

Reactions at Low Acetic Acid Concentrations (Reactions S. F. and S. F. Acid)—The Schiff reaction for the determination of higher fatty aldehydes as carried out previously in our laboratory (4) differed in three respects from the procedure used by Feulgen and Grünberg (3): (*a*) 1 ml. of 1 N HCl was added to the reaction mixture consisting of 10 ml. of fuchsin reagent, mercuric chloride solution, and 1 ml. of glacial acetic acid containing the compounds or tissue components to be tested. It was found that increased

* The higher fatty aldehydes, IV. This work was supported by grants from the Josiah Macy, Jr., Foundation and the United States Public Health Service.

acidity resulted in a greater precision. (*b*) The color was developed at 37° for 18 to 24 hours. (*c*) The color complex was extracted with capryl (4, 5) instead of amyl alcohol (3). In the present experiments this procedure (Reaction S. F. HCl) was compared with a procedure (Reaction S. F.) in which no HCl was added. The reaction mixtures contained 8.9 per cent (Reaction S. F.) and 8.1 per cent (Reaction S. F. HCl) acetic acid respectively.

Reaction at High Acetic Acid Concentration (Reaction S. A. A.)—2 gm. of basic fuchsin (National Aniline Division) were dissolved in 50 ml. of glacial acetic acid. 10 gm. of sodium bisulfite, 100 ml. of 0.1 N HCl, and 50 ml. of water were added in succession. The reagent was used after it had stood for several hours. The bisulfite did not decolorize the solution appreciably; the final reagent retained a reddish brown color. To 1 ml. of glacial acetic acid containing the compounds or tissue components to be tested 2 ml. of glacial acetic acid and 1 ml. of fuchsin reagent were added. The color was developed in sealed glass tubes (9 mm. inside diameter, 10 ml. capacity) at 50° for 18 to 20 hours. When cool, the sealed tubes were opened, 2 ml. were transferred to a 25 ml. graduated cylinder (glass-stoppered), and 10 ml. of an aqueous solution were added, containing 5 gm. of sodium bisulfite and 5 ml. of concentrated HCl in 100 ml. In the blank samples the color faded within 10 minutes to a light yellow. The solutions were extracted with 10 ml. of capryl alcohol exactly 10 minutes after the addition of the sulfite solution, and the alcoholic solution was cleared by centrifuging as described previously (4).

Substrates—The preparation of palmitaldehyde and stearaldehyde and their acetals was described previously (4). As synthetic surface-active substances the non-ionic detergents, Tweens and Spans, of the Atlas Powder Company (mono- and polyesters of sorbitan with long chain fatty acids and their polyalkylene derivatives) were used. These substances produced small and consistent color values. The crude egg yolk phosphatides were prepared according to the method of Feulgen and Grünberg (3) and dried to constant weight. The samples gave fuchsin color values (Reaction S. F. HCl) corresponding to as much as 720 mg. of palmitaldehyde per 100 gm. of lipide. In the experiments with brain extract the residue of an alcohol-ether extract of finely minced brain was dissolved in the appropriate amount of glacial acetic acid.

In all experiments reported in this paper the color density was determined with a Coleman junior spectrophotometer, model 6, in cuvettes No. 6-302 at 545 mμ. The values obtained in Reaction S. A. A. were doubled, since only half of the reaction mixture was extracted with capryl alcohol.

RESULTS AND DISCUSSION

The addition of Span 20, egg yolk phosphatides, or brain lipides to palmitaldehyde or its glyceryl acetal resulted in an inhibition of the color de-

elopment if the Schiff reaction was carried out in a medium of approxiately 90 per cent water (Reactions S. F. and S. F. HCl) (Table I). By rrying out the Schiff reaction in a medium containing 80 per cent acetic

TABLE I

Schiff Reaction of Palmitaldehyde and Its Glyceryl Acetal in Presence of Lipides and Synthetic Surface-Active Agents

Source of aldehyde	Amount	Addition	Amount	Schiff reaction* optical density			Analytical recovery		
				Reaction S. F.	Reaction S. F. HCl	Reaction S.A.A.†	Reaction S. F.	Reaction S. F. HCl	Reaction S.A.A.
	γ		mg.				per cent	per cent	per cent
Palmitalde-	30				0.11	0.24			
hyde	30	Span 20	4		0.02	0.23		18	96
	30	" 20	8		0	0.22		0	93
	40			0.16	0.16	0.24			
	40	Span 20‡	20	0.09	0.03	0.25	56	20	105
	35					0.26			
	35	Phosphatides	16			0.20			77
	20			0.12	0.09				
	20	Phosphatides	20	0.05	0.02		40	22	
	20				0.07	0.12			
		Brain lipides	14§		0.07	0.24			
	20	" "	14		0.10	0.32		44	66
Palmitalde-	82			0.31	0.28	0.68			
hyde gly-	82	Span 20	20	0.02	0.05	0.64	6	18	94
ceryl	30					0.26			
acetal	30	Phosphatides	15			0.26			100
	40				0.16				
	40	Phosphatides	1		0			0	
	61					0.54			
		Brain lipides	11			0.32			
	61	" "	11			0.78			85

* In all experiments in which Span 20 or egg phosphatides were added the values are corrected for the densities given by these substances alone.

† Optical density for palmitaldehyde glyceryl acetal: 20.4 γ, 0.17; 40.8 γ, 0.35; 61.2 γ, 0.52; 81.6 γ, 0.66; 102 γ, 0.82; for palmitaldehyde 20.2 γ, 0.15; 40.5 γ, 0.30; 81 γ, 0.54; 101 γ, 0.66.

‡ Span added 5 hours after the start of the reaction.

§ Weight of wet brain.

id (Reaction S. A. A.) the effect of the surface-active agents was minized and the recoveries of added aldehyde or acetal amounted to 66 to 00 per cent. The recovery of total color resulting from the aldehyde or cetal plus that from various compounds which were added amounted in eactions S. F. and S. F. HCl to 0 to 74 per cent and in Reaction S. A. A. o 88 to 100 per cent.

The color reaction at high acetic acid concentration is approximately twice as sensitive as that carried out at low acetic acid concentrations. Of the latter reactions the one with the higher acidity (Reaction S. F. HCl) is slightly less sensitive and more susceptible to the action of surface-active agents than the one with lower acidity (Reaction S. F.).

In agreement with previous observations, equivalent quantities of different aldehydes or of the same aldehyde on different days did not yield the same color density. The same aldehyde sample tested in different concentrations does not follow Beer's law (*cf.* foot-note, Table I) probably because of the difficulty in obtaining monomeric aldehydes, and therefore the results of experiments carried out on these substrates are variable. With acetal a better linear relation is obtained (*cf.* foot-note, Table I).

In an experiment reported in Table I, 1 mg. of egg yolk phosphatide inhibited completely the color developed by 40 γ of acetal (Reaction S. F. HCl). However, when 0.5 mg. of phosphatide was added, definite inhibition of color development was found in some experiments, but in others the color values coincided (within the error of the method) with the control value of acetal alone. This finding may explain the result reported previously (4) that addition of 1 mg. of egg yolk phosphatides, prepared according to Feulgen and Grünberg, does not affect the 18 hour color value of acetal. In that experiment the phosphatide preparation used was not dried to constant weight and the actual amount may have been considerably less than 1 mg.

The effect of surface-active agents can also be demonstrated on the aldehydes present in tissue extracts. 20 mg. of Tween 85 were added to a solution of brain lipides in glacial acetic acid. Measurements at different time intervals from 30 minutes up to 18 hours after mixing the reagents gave color values corresponding to 10 to 16 per cent of the simultaneously determined control values. Similar results were obtained in experiments in which Span 20 was used as the surface-active agent.

The addition of detergent after full color had developed with palmitaldehyde (Reaction S. F. HCl) caused fading. On the other hand if Reaction S. A. A. was used, full color was obtained when the detergent was added 5 hours after mixing of the reagents (Table I).

The effect of surface-active agents on the Schiff reaction of the higher fatty aldehydes in aqueous medium supports the point of view expressed previously (4), that analytical results obtained with the fuchsin method probably do not represent the true concentrations of higher fatty aldehydes in tissue extracts. The fact that consistent values have been obtained may be a reflection of relatively constant ratios between higher fatty aldehydes and surface-active lipides. An apparent variation, found by the fuchsin method in aqueous media, in the aldehyde concentrations in a tissue under

physiological or pathological conditions may be the result of a change in concentration of aldehydes, of surface-active lipides, or of both (5, 6). The form in which the aldehydes are present in the extracts is not known with certainty, and the lipide composition occurring in an extract obtained from tissues with an organic solvent cannot be duplicated experimentally. It is, therefore, difficult to decide how well model experiments with detergents, free aldehydes, and acetals approximate conditions in tissue extracts. But it appears that the new procedure elaborated for the use of the Schiff reaction as presented in this paper may eliminate one of the potential errors in the determination of the higher fatty aldehydes in tissue extracts

SUMMARY

Naturally occurring lipides and synthetic surface-active substances inhibit the color development of the higher fatty aldehydes and their acetals in the fuchsin test as proposed by Feulgen and used in the original or modified form by others. If the surface-active substances are added after color has developed, rapid fading occurs. Addition of synthetic detergents in the determination of aldehydes present in tissue lipides also suppresses the development of color to a marked degree. These findings cast serious doubt on the usefulness of the fuchsin method for the quantitative determination of the higher fatty aldehydes as carried out with the Feulgen method or its modifications.

The effect of surface-active, naturally occurring or synthetic agents is suppressed to a large degree if the Schiff reaction is carried out in a medium containing a high concentration of acetic acid.

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