Technical Papers

Pentobarbital Inhibition of Sulfanilamide Acetylation in Pigeon Liver Extracts

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Recently a great deal of interest has been shown in the effects of narcotic and hypnotic drugs on acetylation systems, and particularly on the system involving acetylcholine synthesis. McLennan and Elliott (*1*) have postulated that pentobarbital inhibits the choline acetylase system directly, whereas Johnson and Quastel (*2*) prefer the explanation that pentobarbital and other hypnotics interfere with oxidative synthesis of adenosinetriphosphate, thus indirectly resulting in impaired acetylcholine synthesis. In addition to using a brain choline acetylase system, the latter authors have employed an unaged fluoride containing aqueous extract of pigeon liver acetone powder added to rat brain homogenate for aerobic acetylation of sulfanilamide as well as the same pigeon liver extract alone, anaerobically. The addition of adenosinetriphosphate (ATP) to the aerobic system resulted in reduction of the considerable inhibition produced by chloretone. In the anaerobic pigeon liver system pentobarbital and other barbiturates, as well as chloretone, produced only very slight inhibition.

When one considers the acetylation of sulfanilamide by pigeon liver preparations several points must be borne in mind. Fresh pigeon liver homogenates are able to acetylate sulfanilamide aerobically. This ability is lost to extracts of acetone powders but acetylation proceeds anaerobically when ATP is added (*3*). Johnson and Quastel have succeeded in using rat brain homogenate as a source of supply of ATP.

In addition to the acetylation mechanism, unaged pigeon liver extract contains large quantities of coenzyme A (Co A) together with an enzymic mechanism for splitting the Co A molecule (*4*). Aging of pigeon liver extracts results in the removal of endogenous Co A, thus making aged extracts useful in the assay of Co A (*5*). Additionally, pigeon liver extracts have the ability in the presence of ATP to resynthesize Co A from split products including pantethine (*6, 7*) and phosphopantethine (*8*). The addition of fluoride (as employed by Johnson and Quastel) blocks dephosphorylation in the enzymic breakdown of Co A by liver (*4*).

Thus it occurred to us that Johnson and Quastel (*2*) in their aerobic system, may have not been dealing with a direct effect of ATP in removal of narcotic inhibition but actually with a replenishing of the Co A supply which in turn may have been more directly concerned with inhibition noted. Along the same line of reasoning, their failure to obtain inhibition with narcotics and hypnotics in the anaerobic pigeon liver system may have been due to the presence of adequate supply of Co A or split products which are convertible to Co A in the presence of ATP.

In this paper we wish to report the effect of added Co A on the inhibition of sulfanilamide acetylation produced by pentobarbital in aged pigeon liver extracts.

Aged bicarbonate extracts of acetone dried pigeon liver powder were prepared in the manner of Kaplan and Lipmann (*5*). A reaction mixture containing the following substances was used: sulfanilamide, 0.04*M*, 10 ml; sodium acetate, 1*M*, 2.5 ml; Na_4 ATP, 0.05*M*, 8.0 ml; sodium citrate, 0.2*M*, 10 ml.

To each tube was added the above reaction mixture, 0.3 ml; pigeon liver extract, 0.3 ml; tris-hydroxymethylaminomethane buffer 1*M*, pH 8.0, 0.1 ml; cysteine hydrocholoride, 0.1*M* (freshly prepared), 0.2 ml; coenzyme A, 0–5 u in 0.1 or 0.2 ml of aqueous solution; pentobarbital-Na when added, 0.2 ml of aqueous solution of appropriate concentration to make the final molarity required (usually 1–3 millimolar). The final volume was 1.3 ml. These tubes were incubated for 1 hr at 37° C without shaking. Free sulfanilamide was determined on the trichloracetic acid filtrates by the Bratton-Marshall (*9*) procedure. Blank tubes containing all reagents except Co A were run, including blanks containing pentobarbital. Values obtained from tubes containing pentobarbital were subtracted from the pentobarbital-containing blank value. The results are recorded as ΔD, the difference between the spectrophotometer reading of the blank and that of the Co A-containing tubes. This represents disappearance of free sulfanilamide or accumulation of acetylsulfanilamide.

As shown in Table 1, the addition of pentobarbital in final concentrations of 1–3 millimolar results in appreciable inhibition of acetylation of sulfanilamide when aged pigeon liver extracts are used under anaerobic conditions. The addition of more Co A reduces the magnitude of the inhibition. This finding does not invalidate the postulate of Johnson and Quastel, that pentobarbital depresses ATP synthesis.

TABLE 1. Sulfanilamide acetylation.

	Pentobarbital Concentration					
	1 mM		2 mM		3 mM	
	ΔD	% inhibition	ΔD	% inhibition	ΔD	% inhibition
Co A 1 u	0.104		0.109		0.099	
Co A 1 u + PB	0.089	14.4	0.073	33.0	0.050	49.5
Co A 5 u	0.230		0.244		0.226	
Co A 5 u + PB	0.210	8.7	0.208	14.8	0.159	29.7

However, pentobarbital evidently does have an inhibitory effect on the acetylation system when small amounts of Co A are used, in the presence of preformed ATP. The lessening of inhibition when more Co A is added strongly suggests that pentobarbital has a direct effect on the acetylation system and that any effect on ATP synthesis may be in addition to its effect on the coenzyme A-apoenzyme complex or the formation thereof. It is possible that the lack of inhibition noted by Johnson and Quastel in the unaged pigeon liver system may be due to a large excess of Co A in the system. The failure of Co A to be irreversibly broken down in their preparation may be due to the presence of fluoride or the instability of the catabolic enzyme (*5*). In the case of the coupled rat brain-pigeon liver system of Johnson and Quastel, one must postulate a lower level of Co A, since ATP is effective in reducing the inhibitions described. This is borne out by the assays of Co A in tissues reported by Kaplan and Lipmann (*5*).

References

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Depigmentation of Hair as a Biological Radiation Dosimeter

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Exposure of large numbers of mice to graded doses of whole-body ionizing radiation at the Eniwetok weapon tests in 1951 gave an opportunity to evaluate radiation-induced depigmentation of fur as a biological dosimeter. The feasibility of such a study was indicated by earlier experiments in which decolorization of hair had been correlated with the dosage of x-radiation (*1–3*).

As early as 3 mo postirradiation graying of fur was detected in many of the more heavily irradiated LAf_1 mice; their normal coat is brown over the entire body. As the animals were genetically uniform males and females of approximately the same age, irradiated simultaneously, the degree of depigmentation appeared to be correlated with the dose of radiation. Accordingly, random samples from a colony of over 3000 mice exposed at 16 dose levels, were examined and the pattern of graying recorded as follows. The coat of each mouse was divided into 6 regions: (1) top of head (scalp), (2) nuchal region, (3) supraclavicular area, (4) anterior chest and abdomen, (5) scapular area, and (6) lumbo-sacral region. Each area was observed grossly for coat color, and the degree of depigmentation was graded on an integral scale from 0 to 4, zero indicated no change from the normal brown color, and 4 designated pure white, or complete depigmentation. Repeated examinations of the same mice, made at intervals of 2 mo, revealed that the decolorization progressed during the passage of time. Therefore, an attempt was made to fit a linear function to the date, which would best relate the depigmentation of the 6 regions specified to the dose at a given time postirradiation.

The function postulated assumed the form

$$d = \lambda_0 + \lambda_1 p_1 + \lambda_2 p_2 + \lambda_3 p_3 + \lambda_4 p_4 + \lambda_5 p_5 + \lambda_6 p_6,$$

where p_i is the degree of depigmentation at region i ($i = 1, 2, \cdots, 6$) and λ_i is a fitted, constant coefficient, so chosen as to maximize the ratio of the differences between means of the dose groups to the variation within each group. The procedure followed considered each region separately, then all combinations of 2 regions, then 3, and so forth, until the variation about the line or plane was not significantly reduced by the addition of another variable.

Representative data, covering the examinations made 7 and 17 mo postirradiation, are presented. Two hundred and thirty-nine mice, exposed at 16 dose levels, including nonexposed controls, were surveyed at 7 mo. At 17 mo the surviving 174 mice of this sample were re-examined. In each case the top of the head (scalp) was the region of the least variation about the fitted line. However, this function was not completely satisfactory, because those animals receiving high doses of radiation were uniformly white over the top of the head ($p_1 = 4$) and those exposed to low doses did not exhibit evidence of depigmentation ($p_1 = 0$). The animals in the extreme dose groups were, therefore, omitted and the procedure was repeated for the 138 and 116 mice examined at 7 and 17 mo, respectively. They received between 287 and 687 r. The radiation was a mixture of gamma rays and neutrons of varying energies, predominantly the former. Again a significant decrease in the variation resulted from consideration of the top of the head alone. The 2 resulting equations are of the form

$$d = \lambda_0 + \lambda_1 p_1,$$

where λ_1 represents the average increase in the dose per unit increase in depigmentation and λ_0 may be interpreted as the upper bound for the dose received when there is no depigmentation. The parameters estimated from the data are summarized in Table 1 and the lines are exhibited graphically in Fig. 1. The slopes, or λ_1's, of the lines do not differ statistically, but there is a definite significant statistical difference between the intercepts, or λ_0's.

These findings represent a shift of the line downward and to the right during the 10 intervening mo. This is interpreted as indicating a uniform increase in degree of depigmentation among dose groups dur-