portance is intensified in certain instances and should be considered in any situation where multiple recrystallization is to be encountered.

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Changes of Thixotropic Behavior in Actomyosin Solutions Induced by Cardiac Glycosides

Actomyosin solutions are thixotropic, which literally means that they "change by touching." An internal structure formed by the fibrous protein molecules is partially broken by agitation of the fluid, and, for example, the viscosity is accordingly diminished. As long as the flow remains constant, the structure will not reform. As soon as flow stops, thixotropic structure is built up again.

For measuring changes in viscosity, the thixotropy has to be kept at a steady state. In making these measurements with an Ostwald viscosimeter, we found that numerous active and inactive cardiac glycosides reduce the viscosity of actomyosin solutions parallel to the degree of biological fixation on heart muscle. The binding of some glycosides to actomyosin was proved by ultrafiltration and determination of the dissociation constants. One receptor group per molecule of actomyosin (molecular weight taken as 10^6) was found, probably belonging to myosin (*1*).

The thixotropy of actomyosin that had been extracted from rabbit skeletal muscle was studied in a rotational viscosimeter at 0°C. Eighteen milliliters of protein solution (3 mg/ml in Weber-Edsall fluid with barbiturate buffer) was placed in a narrow cylindrical slit in which another cylinder was rotated with constant speed. The breaking force on the cylinder exerted by the viscous solution was counteracted by a spring and was measured on a dial. It was proportional to the viscosity of the fluid.

We measured the coefficient of "thixotropic breakdown with time" (*2*):

$$B = \frac{U_1 - U_2}{\ln \frac{t_2}{t_1}}$$

where U_1 is the viscosity at time t_1 and U_2 is the viscosity at time t_2. The times t_1 and t_2 were 1 and 10 minutes, respectively. Both the coefficient for actomyosin (B_{AM}) and for actomyosin of the same concentration with 10^{-6} *M* glycoside (B_{AMG}), were determined. No alcohol was used to increase solubilities, for we found that even traces of alcohol caused fundamental changes in actomyosin. The difference in thixotropy ($B_{AM} - B_{AMG}$) was then compared with the biological activity (see Fig. 1). Potency is expressed as reciprocal of lethal Hatcher dose (moles per kilogram) determined in cats by W. R. Schalch of Sandoz AG. Basel. The probability that Δ-thixotropy ($B_{AM} - B_{AMG}$) is significant (< 0.02 to 0.05) was computed from the variances of the determinations of $B_{AM} - B_{AMG}$ using the *t* distribution.

There exists an interesting correlation between these two properties. Cardioactive glycosides diminish the thixotropy, whereas biologically inactive glycosides (potency = 0) augment it. Activities of lanataglycosides are approximately proportional to the decrease of thixotropy. In three cases (open bars in Fig. 1: digitoxin, acetyldigitoxin α, and acetyldigoxin α) in which the ion concentration in the solution was diminished by adding the glycosides in water instead of buffer solution, the effect was larger than was expected from the biological activities. This may be connected with the poor water solubility of these three glycosides, or else with the decrease of the ion concentration. A change in the active ion concentration or some other charge phenomenon along the actomyosin fiber may be responsible for the observed effect. Preliminary studies on the dialysis of actomyosin in 0.6*M* K solution have shown the concentration of free potassium ions to be diminished by adding 10^{-6} *M* glycosides (lanatosid A, B, C, scillaren A, cymarin, and strophanthin) but not by the inactive glycosides hexahydroscillaren A and allocymarin.

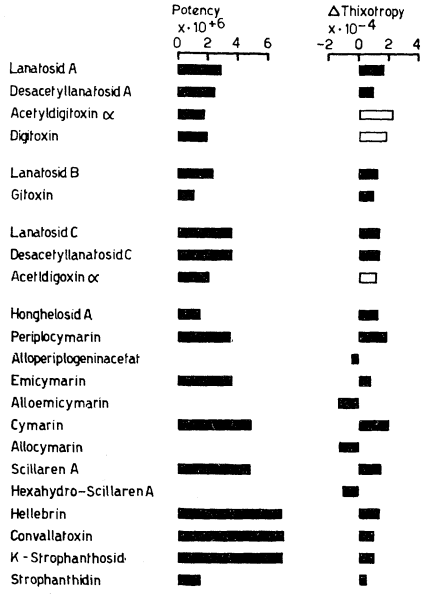

Fig. 1. Comparison of difference in thixotropy ($B_{AM} - B_{AMG}$) with biological activity.

It is therefore possible that there exists a direct mode of action of cardiac glycosides on actomyosin within the cell and not only in the cell membrane phase, as was suggested by previous workers (*3*).

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Withdrawal Hyperexcitability Following Chronic Administration of Meprobamate to Mice

Because of known side effects and potential "behavioral" toxicity, concern over the indiscriminate use of the so-called "tranquilizing agents" is readily justified. However, the added possibility that these drugs might also possess addiction liability has been little, if at all, explored. Recently, Lemere (*1*) reported that one patient who took 6.4 g of meprobamate (Equanil, Miltown) daily for 30 days exhibited a grand mal convulsion 10 hours after therapy was discontinued. In view of this report, the effects of the chronic administration of this agent and its abrupt withdrawal on the excitability of the central nervous system of mice, by use of the experimental design developed by McQuarrie and Fingl (*2*), were studied.

Since this investigation (*3*) was planned merely to determine whether tolerance develops during the chronic administration of meprobamate and whether abrupt withdrawal is followed by increased excitability of the central nervous system, only high dose levels were employed. This choice of dosage was justified also on the bases that large amounts are taken by patients who abuse drugs and that excessive quantities are usually necessary to demonstrate physical dependence on ethanol and barbiturates in man (*4, 5*). It must be emphasized that final assessment of the addiction liability of any drug must be based on quantitative comparison of the dosage schedules that induce withdrawal phenomena with those that are required to obtain therapeutic results.

Male albino mice (Carworth Farms, CF #1 strain, 25 to 35 g in weight) were