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

DONALD S. WIGGANS
WILLIAM W. BURR, JR.
Biochemistry Department,
University of Texas,
Southwestern Medical School, Dallas

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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.

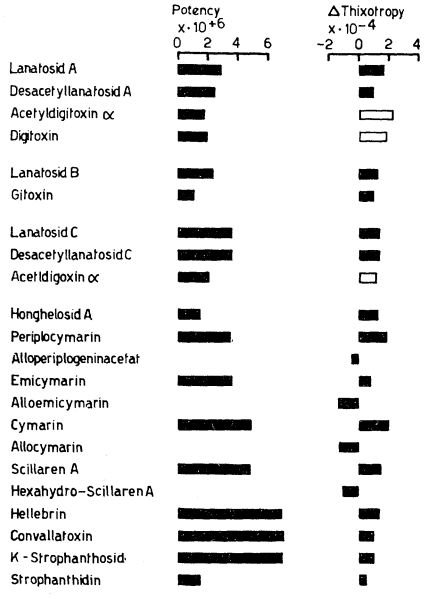

Fig. 1. Comparison of difference in thixotropy ($B_{AM} - B_{AMG}$) with biological activity.

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*).

PETER G. WASER
Department of Pharmacology,
Medical School, University of
Zürich, Zürich, Switzerland

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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

randomly divided into two groups of 50 animals each. Meprobamate (*6*) was administered orally to one group either as a 2- or 3-percent suspension in 6-percent acacia solution in a total daily dose of 1200 mg/kg (200 mg/kg at 8 A.M., 12 noon, 4 P.M., and 8 P.M., and 400 mg/kg at 12 midnight) for 6 days. Because of the development of tolerance, following the 8 A.M. treatment on day 7, each dose was increased by 50 percent to make a total daily dose of 1800 mg/kg; this higher dose level was continued for 10 days, except as indicated in the legend to Fig. 1. The other group served as a control and was given the requisite volume of 6-percent acacia solution. Low-frequency electroshock seizure threshold was determined for both groups at the times indicated in the legend to Fig. 1. Seizures were induced by means of a Grass stimulator (model S4B). The stimulus parameters employed were the same as those previously described (*7*)—namely, unidirectional pulses of 0.2-msec duration delivered for 3 seconds at a frequency of 6 pulses per second. Mice were shocked at various voltages by the staircase procedure (*8*), and the voltage required to evoke convulsions in 50 percent of each group was determined; the 95-percent confidence limits were calculated by the method of Litchfield and Wilcoxon (*9*). Results are presented as the threshold ratio (threshold of drug group/threshold of control group). In a separate series of experiments, 80 mice were divided into two equal groups. One group was given orally 300 mg/kg of meprobamate, and the other group the requisite volume of 6-percent acacia solution; the seizure threshold of each group was determined 30 minutes after drug administration. This procedure was repeated at intervals of 3 or 4 days in the same groups of mice until the seizure threshold was established 1, 2, 3, and 4 hours after drug administration.

As illustrated in Fig. 1, tolerance develops to the threshold-raising effect of meprobamate. For example, the administration of 300 mg/kg of meprobamate to nontolerant animals increased the threshold more than tenfold (see small inset in Fig. 1), whereas, after treatment for 6 days with 1200 mg/kg day and then for 10 days with 1800 mg/kg day, this same dose of drug increased the threshold only 2.5-fold.

Figure 1 also illustrates that, 4 hours after the 8 A.M. dose on day 12 and 4 hours after drug withdrawal on day 17 (see *B* and *E*, Fig. 1), the seizure threshold in meprobamate-treated mice was only approximately 0.8 that of control animals. Such hyperexcitability (but of lesser degree) was also observed 8 hours after the double midnight dose on day 13 and 8 hours after the 8 A.M. dose on day 14 (see *C* and *D*, Fig. 1). Since seizure threshold is not reduced 4 hours after the single administration of this same dose of meprobamate to nontolerant animals (see small inset in figure), the withdrawal hyperexcitability must be attributed to the chronic administration of this agent.

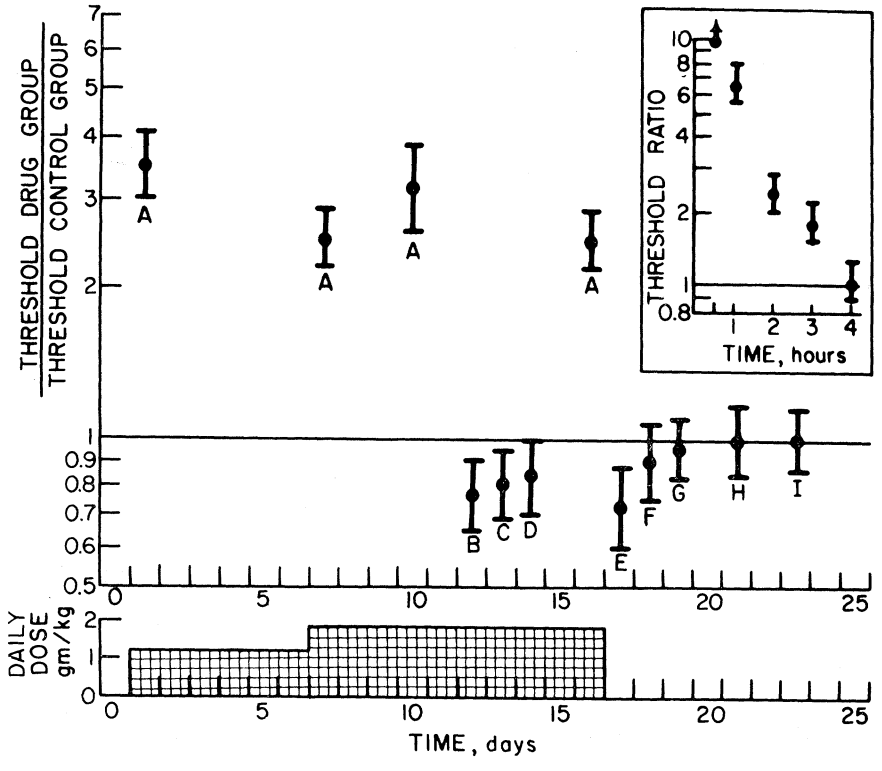

Fig. 1. Effects of chronic administration of meprobamate on threshold for low-frequency electroshock seizures in mice. Time in days is indicated on the abscissa. Seizure threshold ratio (threshold of drug group/threshold of control group) is shown on the upper ordinate, and total daily dose administered is shown on the lower ordinate. Vertical bracketed lines indicates 95-percent confidence limits. *A*, *B*, and *D* indicate the ratio at ½, 4, and 8 hours, respectively, after an 8 A.M. dose; *C* indicates the ratio 8 hours after the double midnight dose; *E*, *F*, *G*, *H*, and *I* indicate the ratio at 4, 28, 52, 100, and 148 hours, respectively, after the final dose of the drug. The inset in the upper right-hand corner illustrates the duration (in hours) of the effect of a single dose of meprobamate (300 mg/kg) on seizure threshold.

McQuarrie and Fingl (*2*) have shown that hyperexcitability in mice following the chronic administration of either ethanol or phenobarbital is usually not present 24 hours after drug withdrawal, is maximal on day 2 or 3, and declines progressively over the next 2 to 4 days. In the studies reported here, hyperexcitability in mice following chronic administration of meprobamate is detectable 4 and 8 hours after drug withdrawal and subsides within 28 hours. At the clinical level, tremors and nervousness may be experienced during the first day of abstinence from either ethanol or barbiturates, but the most severe withdrawal symptoms appear later, spontaneous convulsions usually occurring between the second and the fifth day after ingestion of the drug has been stopped (*4*, *5*). In contrast, hyperexcitability climaxed by a grand mal convulsion has been noted 10 hours after meprobamate withdrawal (*1*). Thus, the time course of hypersusceptibility to induced seizures following cessation of chronic administration of either ethanol, barbiturates, or meprobamate in mice appears to parallel that of withdrawal hyperexcitability in man.

The abstinence syndrome which occurs in man following the chronic abuse of barbiturates is characterized by hyperexcitability, which may progress to convulsions, and by psychosis. This withdrawal syndrome is qualitatively similar to that which occurs upon withdrawal of various other central nervous system depressants, including ethanol, chloral hydrate, and paraldehyde (*4*, *10*). However, the relative prominence of these major withdrawal symptoms, and particularly the time course, vary with the addicting drug. Clinical observations such as those of Lemere (*1*) suggest that meprobamate should be added to the list of drugs that cause withdrawal syndromes, and the experiments in mice described herein support this view. The meprobamate abstinence syndrome differs from the others by its more rapid development and briefer duration. Whether psychosis can occur as a feature of the meprobamate withdrawal syndrome remains to be explored. Like-

wise, the minimal dosage schedules which induce withdrawal phenomena have yet to be established.

Two important clinical implications merit brief mention. First, the qualitative similarity of the meprobamate and the barbiturate abstinence syndromes implies that precautions should be taken to minimize both the development of physical dependence on meprobamate and the severity of the withdrawal syndrome; such precautions as those currently used in the case of barbiturates could be expected to be effective. In particular, following a period of chronic administration of large doses of the drug, medication should never be stopped abruptly. The second implication is that demonstration of physical dependence and an abstinence syndrome following abuse of meprobamate suggests that all members of the new classes of tranquilizing agents should be held suspect until definitely proved to be devoid of such undesirable properties.

EWART A. SWINYARD
LINCOLN CHIN
EDWARD FINGL
Department of Pharmacology, University of Utah College of Pharmacy and College of Medicine, Salt Lake City

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Enlargement of Avian Eye by Subjecting Chicks to Continuous Incandescent Illumination

During studies on the effect of different durations of diurnal artificial illumination on the growth of chicks, it was observed that the eyes of chicks subjected to continuous light appeared to be flattened. This condition was manifested by reduced depth of the anterior chamber and the apposition of the periphery of the iris to the cornea.

Because of these observations, the eyeballs of chicks from two different lighting treatments were subjected to certain measurements. Eight chicks that were exposed to continuous incandescent illumination and eight chicks that received such light for 12 hours daily provided the material for the study. Each pen, from which all natural light was excluded, received artificial light from a 60-watt, frosted incandescent lamp with a light intensity of 2 to 3 ft-ca measured at the bird height. The chicks were 6 weeks of age when they were sacrificed, and they had been on the two different light treatments since the day after hatching.

The average weight of eyeballs removed from chicks that had received continuous light was about 38 percent greater than the weight of eyeballs of chicks that had received only 12 hours of artificial light each day (Table 1). When the weight of the eyeballs is expressed as a percentage of body weight, there is still a large difference between the two treatments. Thus the data show that an enlargement of the eyeballs of chicks was produced by continuous artificial light.

It was of interest to determine whether the increased size of the eyeballs was brought about largely by an increase in tissue water content or by an increase in both water and tissue dry matter. When fluid was drawn from the posterior chamber of the eye by a hypodermic syringe, about twice as much fluid could be extracted from the eyeballs of chicks that had been subjected to continuous light (0.80 ml per eye) as could be extracted from the eyeballs of chicks that had been subjected to 12 hours of diurnal light (0.45 ml per eye). When the dried weight of the eyeballs is expressed as a percentage of the live body weight, there is only a slight difference between the two treatments. These results show that the increased size of the eyeballs of chicks that received continuous light was caused primarily by an increased accumulation of fluid.

It was found that the average diameter of eyeballs enucleated from chicks that received continuous light was 2.4 mm larger than that of eyeballs from chicks that had not received continuous light. Although average depth was also slightly increased, it appeared that the major change was in the diameter of the eyeballs.

A peculiar enlargement of eyeballs in chicks caused by feeding them a high level of glycine was reported by Groschke *et al.* (*1*). In these studies, the eyes were greatly enlarged by the addition of 8 percent free glycine to a purified diet. When a similar amount of glycine was added in the peptide form, no such enlargement was observed.

The data presented in this report (*2*) indicate that investigations on the effect of continuous illumination on the eye might well be expanded to determine the mechanism of the development of the abnormality. A study of the relationship of this light-induced abnormality to that of eye abnormalities in other species, including man, would be of interest.

Table 1. Effect of length of diurnal incandescent illumination period on eyes of chicks.

Item	Daily illumination period	
	24 hours	12 hours
No. of chicks	8	8
Av. body weight at 6 wk (g)	760	733
Av. weight of eyeballs (g)	4.83	3.50
Av. weight of dried eyeballs (g)	0.515	0.465
Av. diameter of eyeball (mm)	17.6	15.2
Av. depth of eyeball (mm)	12.9	12.1

LEO S. JENSEN
Department of Poultry Science, State College of Washington, Pullman
WALTER E. MATSON
Department of Agricultural Engineering, State College of Washington, Pullman

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28 January 1957

Effects of Embryonic Age on Potency in Tissue Culture of Embryonic Nucleoprotein Fractions

In the preparation of various biological growth media, and especially those for tissue culture, it is customary to use whole embryo extract as a source of growth-promoting factors (*1*). The choice of age of chick embryo used for making the extract is commonly one of convenience, usually embryos of 8 to 11 days (*2*). However, Gaillard (*3*) and Fowler (*4*) found that growth of chick heart fibroblasts in plasma culture was stimulated more by saline extracts of 14- to 18-day chick embryos than by similar extracts of 8- to 11-day embryos.

Recently a streptomycin-precipitation procedure was developed using 12-day chick embryos for isolation of the high-molecular-weight growth factors from whole-embryo extract (*5*). It seemed of interest to determine whether this active nucleoprotein fraction (NPF) would show a similar increase in potency or specific activity—that is, biological activity at the same concentration with in-