Short Communications

Use of glyoxylic acid in the demonstration of autonomic nerve profiles[1]

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Summary. 2% glyoxylic acid buffered to pH 7 could be used to improve the localization of cholinergic, adrenergic and nonspecific nerve profiles both in frozen sections and stretch preparations. The results are better than those obtained by conventional techniques, exhibiting the distinct reactions, with the least possible background and diffusion. The results are quickly obtained.
Key words. Adrenergic; cholinergic; glyoxylic acid.

Since the publication of the glyoxylic acid (GA) condensation method for the histochemical demonstration of biogenic monoamines[2], numerous methods have been developed using this acid for the formation of fluorophores[3-7]. The latter investigators have reported the superiority of this method over the conventional formaldehyde-induced technique[8]. In the present study glyoxylic acid has been used for the localization of adrenergic, cholinergic and nonspecific nerve profiles.

Pieces of urinary bladders of rat, rabbit, guinea pig and camel and ileum of fowl were immediately dissected out after killing the animals, rinsed in glucose saline and then fixed in 2% glyoxylic acid buffered to pH 7 at room temperature for 5–30 min. Stretch preparations of the longitudinal smooth layer of the ileum with attached Auerbach's plexus were carefully made on glass slides. 40–60-µm-thick sections of urinary bladders and ileum were cut using a freezing microtome.

For the demonstration of cholinergic innervation the stretch preparations and the sections were incubated directly in the incubating medium[9]. Comparable and mostly better results were obtained when the GA treated preparations were incubated in a modified medium (10 mg acetylcholine, 15 ml GA, 1 ml 0.1 M sodium citrate and 2 ml 300 mM potassium ferricyanide). The cholinesterase activity was checked every 15 min under the dissecting microscope. The proper activity was developed within 1–2 h. These preparations were then dehydrated in an ascending series of alcohols, cleared in xylene and mounted in DPX. Excellent cholinesterase activity was also developed after postfixation of GA treated tissue in 10% formaldehyde.

For the localization of monoaminergic innervation the whole mounts of Auerbach's plexus and the sections (both already incubated for 5–30 min in GA) were dried at room temperature, covered by a thin film of liquid paraffin and then heated at 95 °C for 3 min. The preparations were covered by cover slips and examined under a Leitz Orthomat Fluorescence Microscope using a 200 W mercury vapor lamp as a light source, a BG 12 excitation filter and 530/nm barrier filter.

For the demonstration of nonspecific nerve profiles the sections were processed for silver impregnation using routine methods[10,11]. The duration of the various steps was reduced to half of the period mentioned in these lengthy procedures. For comparison a few sections were processed following the conventional methods[8-11]. Photomicrographs of the selected areas were taken on Kodak Panatomic-X film.

In figure 1 cholinergic nerve fibers and ganglion cells are shown in the urinary bladder of camel. Similar results are obtained in other species as far as the cholinesterase activity is concerned. The cholinesterase activity is brightly developed and well differentiated from the background muscle fibers and the connective tissue. Though the methods reported earlier for histochemical demonstration of cholinesterase activity have proved reliable[9,12,13], the simplified method described in this communication has several advantages. Fixation for long periods is not necessary; even incubation for 5 min in GA shows excellent cholinesterase activity. Moreover, there is no or the least possible background reaction, and the preparations thus made are neat and clean with no trace of unnecessary precipitates. The activity is not lost even after keeping preparations for months. In the present study glyoxylic acid-treated preparations have produced bright fluorophores both in the nerve fibers and ganglia and the results are comparable with those of the earlier investigators[2-6]. In figure 2 numerous brightly fluorescent nerve fibers are well localized and found to be better than or at least comparable with those preparations made by following the formaldehyde condensation method[8].

Glyoxylic acid converts various catecholamines and indoleamines to highly fluorescent compounds[2,3], and provides an improved histochemical technique for the demonstration of central catecholamine[4], peripheral stores of noradrenaline and 5-hydroxytryptamine[6] and adrenaline[5]. The excellent localization of the adrenergic nerve profiles and the bright yellowish green

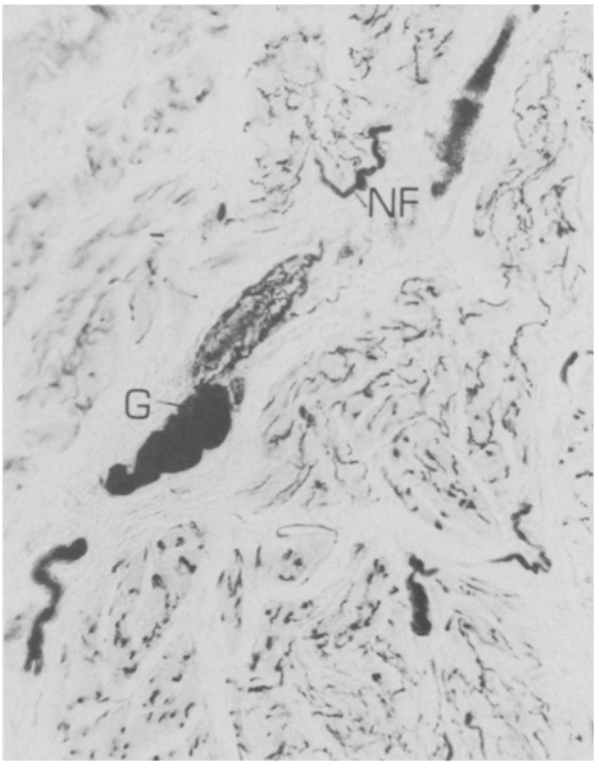

Figure 1. Photomicrograph of the GA fixed urinary bladder of camel showing distinct cholinergic nerve fibers (NF) and ganglion cells (G). Note fine localization of cholinesterase activity in nerve profiles and clear background. × 40.

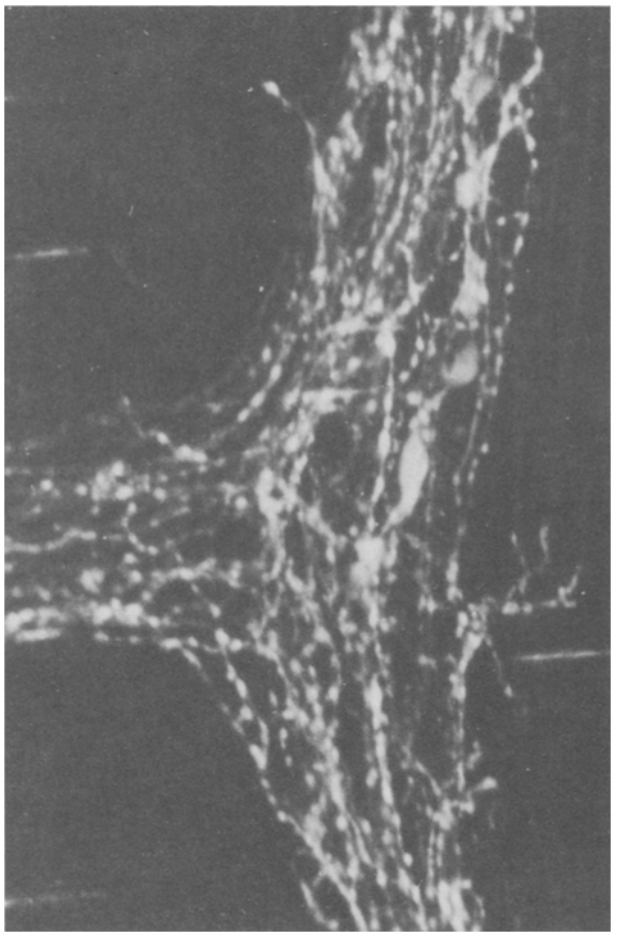

Figure 2. Photomicrograph of Auerbach's plexus of fowl showing excellent localization of adrenergic nerve terminals after G.A. treatment. × 40.

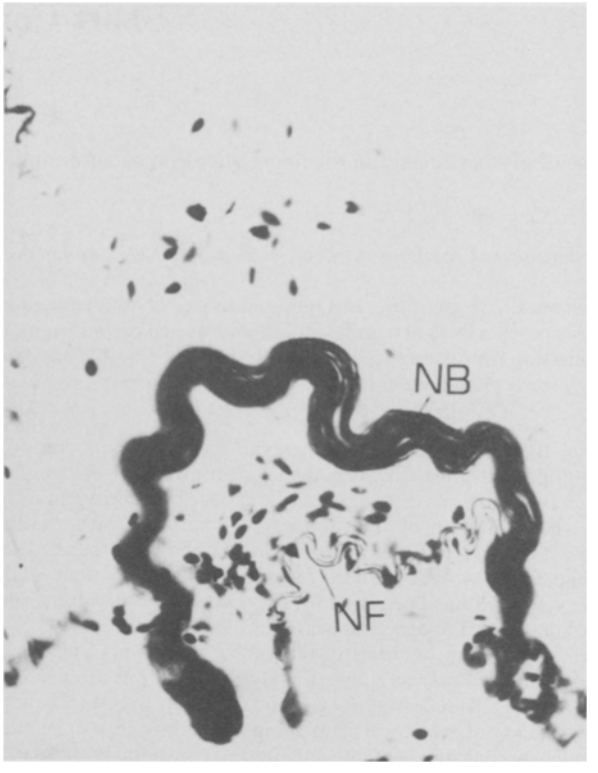

Figure 3. Photomicrograph of the section of GA fixed urinary bladder of camel showing both thick nerve bundle (NB) and fine nerve fibers (NF) after silver impregnation. Nerve profiles are distinctly demonstrated with no background staining. × 100.

fluorescence developed in the preparations of our study are in agreement with those of these investigators.

Glyoxylic acid probably reacts as an aldehyde, through the Pictet-Spengler condensation reaction[14], to form weakly fluorescent tetrahydron-carbolines or tetrahydroisoquinoline which are then converted to the equivalent highly fluorescent dihydro compounds[2,15]. In figure 3 nonspecific nerve fibers are shown in the urinary bladder of camel. The nerve fibers and ganglion cells are well impregnated with silver after fixation in GA and the nerve profiles are well differentiated from the surrounding tissue. Moreover, the background is clear and the preparations do not show the unnecessary precipitates which are frequently seen in preparations of formaldehyde-fixed tissue. Almost the same reaction has been observed in other species studied in the present investigation. Glyoxylic acid-fixed tissue has shown excellent silver impregnation with the technique employed in this study[10,11]. Of course, there are many advantages in using GA as fixative for the localization of peripheral nerve profiles, as mentioned in the preceeding paragraph. The most significant advantage is that the adjacent sections from a single piece could be processed for the demonstration of cholinergic, adrenergic and nonspecific nerve profiles. But the noticeable disadvantage is that the GA treated tissue becomes very loose, causing much inconvenience in handling. This difficulty could be overcome by using GA in combination either with glutaraldehyde (1%) or formaldehyde (4%). By addition of these aldehydes the tissue becomes hardened, which facilitates proper and convenient handling. It is concluded that GA could be used as a suitable fixative for the demonstration of cholinergic, adrenergic and nonspecific nerve profiles.

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Lithium suppresses hibernation in the Turkish hamster

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Summary. Daily uptake of lithium salt (LiCl) in the drinking water at a rate of over 100 µg/g b.wt (or 2.4 mEq/kg) reduced or suppressed natural torpidity (hibernation) in the Turkish hamster *(Mesocricetus brandti)*. The data indicate a direct influence of lithium on clock-related functions controlling the hibernation process rather than indirect effects by preventing gonadal regression and thereby also hibernation.

Key words. Lithium; hibernation; gonadal cycle; circadian and circannual rhythms.

The principal effect of lithium treatment on manic-depressive disorder (MDD) is prophylactic: manic as well as depressive phases become attenuated, shorter, rarer, or are entirely prevented[1]. The fact that this prophylactic effect often begins only after 6–12 months of treatment suggests a mode of action involving a long-term metabolic adaptation or the interference of the drug with long-ranging physiological and behavioral programs, possibly involving circadian and/or circannual rhythms[2–4].

In this context a hypothesis of the German psychiatrist Johannes Lange from 1928[5] deserves interest. The phasic course of MDD, the accumulation of depressive phases in the winter half-year and its characteristic symptoms of conservation-withdrawal[6]: reduced appetite, weight loss, reduced motor activity, social withdrawal and anhedonia, led Lange not only to state that there are phenomenological similarities between phasic depression and hibernation, but, moreover, to speculate about the two states being physiologically homologous. An even more interesting analogy exists between hibernation and the symptomatology of a subgroup of depressives, first described by Kraepelin and recently by Rosenthal and coworkers[7] as suffering from 'seasonal affective disorder'. These recurrent mostly bipolar depressions take place in winter and show 'atypical' signs of increased appetite, weight gain and hypersomnia. The marked seasonality of these patients may be an exaggeration of the normal circannual rhythmicity which has been proven for sleep and a broad spectrum of other physiological processes[8–10].

However, the absence in depression of two of the most characteristic features of hibernation, hypothermia and a sleep-like state, shows the limits of comparing the two states. Moreover, many symptoms which are often part of the depressive syndrome, like increases in blood pressure, pulse and respiration rate, muscle tension, body temperature, cortisol secretion, and behavioral agitation bear more resemblance to the arousal reaction by which a hibernation period is terminated than to hibernation itself. As hibernation is regularly initiated via slow-wave sleep (SWS) – both states are considered to be physiologically homologous[11] – the profound disturbance of SWS in general as well as in winter depression[7] may be a further sign of the 'fight against falling into hibernation'. Taking this into account, depression could be understood as a combination of two opposite tendencies: the pathological tendency to fall into a hibernation-like lethargy and the attempt to withstand it (Lange: useless efforts of repair). Feierman and coworkers[3] were the first to draw experimental consequences from the speculative analogy between phasic depression and hibernation. They investigated the influence of the tricyclic antidepressant imipramine on hibernation in ground squirrels *(Citellus lateralis)* and found the circannual torpidity significantly reduced. More recently, Zvolsky and coworkers[12] found similar effects of imipramine on hibernation in golden hamsters *(Mesocricetus auratus)* but only ambiguous influences of lithium salt because of methodological problems.

The present pilot study rests on the hypothesis that phasic depression and hibernation are controlled by similar temporal (clock-related) functions which can be influenced by lithium. Two groups of Turkish hamsters *(M. brandti)* were tested. This species has a well defined hibernation season with intermittent periods ('bouts') of torpor which last between 2 and 6 days depending on an internal timing program as well as on photoperiod and temperature[13].

Group 1: Seven female hamsters, born under natural daylight conditions during the previous summer, were kept under a constant 10L:14D light-dark cycle (100:0.01 lx) and temperature (10 ± 1 °C) from 4 January until 9 May 1984. Three animals received 1.5 g lithium chloride (LiCl) in 1 l of drinking water (= 35.4 mEq/l) from 4 January until 12 April (one animal only until 29 February). About 15 mmoles sucrose was added to 1 l of the LiCl solution to improve the taste. Three hamsters served as controls.

Group 2 consisted of eight male hamsters that had previously been used in another experiment. They were born during summer 1982 and were maintained in continuous light (ca 100–150 lx) from 5 December 1982 until 3 May 1984. Ambient temperature was constant (10 ± 1 °C) from 15 September 1983 until the end of the test. From 5 December 1983 until 12 April 1984, four hamsters received 1.5 or 2.0 g LiCl per l of water (= 35.4 resp. 47.2 mEq/l) without sucrose (one animal only until 27 February). Three hamsters were used as controls. For estimating the reproductive state, the testes of all hamsters were palpated under light ether anesthesia at 4-week intervals.

To all hamsters food pellets (Altromin) and tap water were given ad libitum. For measuring activity, a photoelectric device was mounted on both sides in the middle of the cages. Whenever the animal crossed the red-light beam, a contact was closed on an event-recorder channel. The length of each torpor bout was calculated as the time difference between the last activity count preceding, and the first count following a period of inactivity of at least 12 h. The amount of drinking was continuously measured over approximately 3-day periods and the daily uptake of LiCl per unit b.wt was determined. Body weight was measured about every 3 weeks.

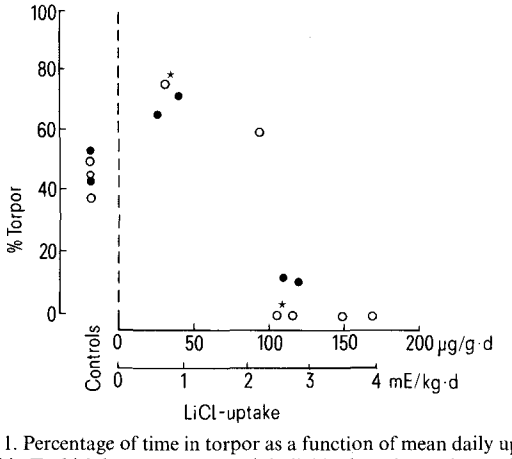

Figure 1. Percentage of time in torpor as a function of mean daily uptake of LiCl in Turkish hamsters. For each individual total experimental time is 100%. Closed circles: female hamsters kept under a 10L: 14D light-dark cycle (group 1); open circles: male hamsters kept in continuous light (group 2). Ambient temperature was 10 ± 1 °C for both groups. Values indicated by an asterisk are from the same individual.

Figure 1 presents the percentage of torpor for each individual of the total experimental period (= 100%) as a function of the mean daily LiCl-uptake. The data for both groups of hamsters show that the time each animal spent in torpor was reduced or even totally suppressed in the six hamsters that obtained LiCl at a daily rate of over 100 µg per g b.wt (or 2.4 mEq/kg), whereas in 4 hamsters which received less than 100 µg daily, torpor was the same or slightly more than in the five controls. One hamster is represented by two data points (indicated by asterisks). During the first 9 weeks of the test, this animal obtained approximately 100 µg/g daily and did not enter torpor. When the LiCl concentration was increased from 1.5 to 2.0 g/l H_2O, it drank much less (30 µg/g per day or 0.8 mEq/kg) and spent nearly 80% of the time in torpidity during the following 3 weeks until it died on 13 March.

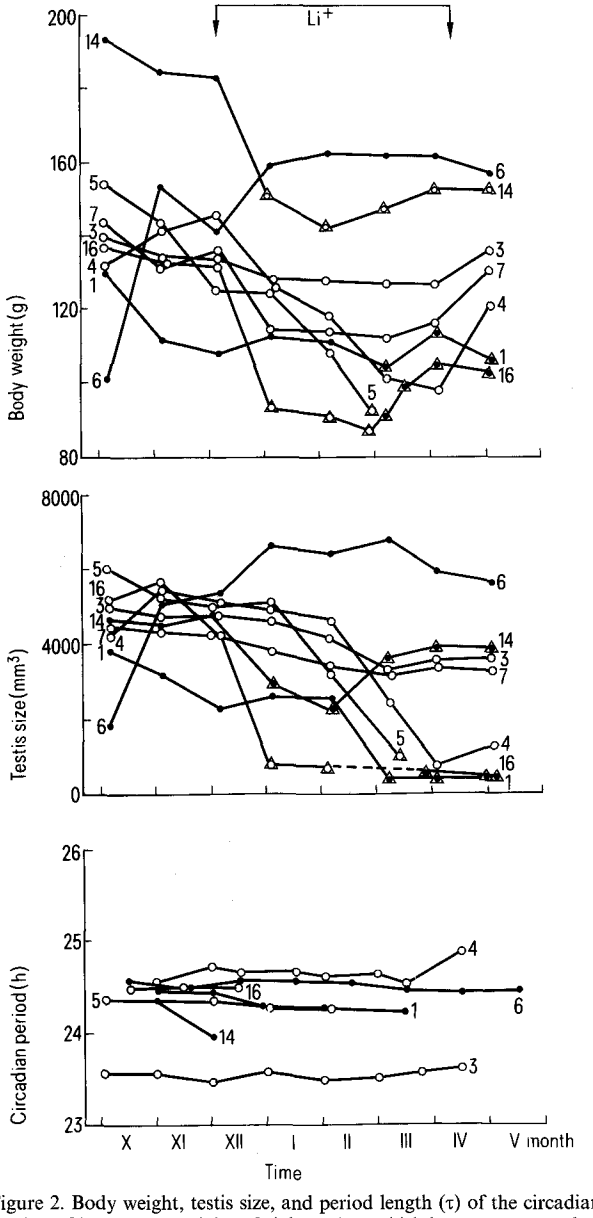

Figure 2. Body weight, testis size, and period length (τ) of the circadian rhythm of locomotor activity of eight male Turkish hamsters exposed to continuous light (100–150 lx) and constant temperature (10 ± 1 °C). Open symbols: animals treated with LiCl from 5 December to 12 April; closed symbols: controls. (Animal no 16 obtained LiCl until 27 February.) Triangles indicate hibernation. Testis size averaged from measurements of both testes (width² × length). In animal No. 7, τ could not be determined due to arrhythmia.

In the group of male hamsters that were kept in continuous light (group 2), b. wt, testis size, and the period length (τ) of the circadian rhythm of locomotor activity were measured (fig. 2). Body weight and testis volume correspondingly decreased in all hamsters, except in one individual (No. 6) which went through a second reproductive period during the test and was therefore not expected to hibernate. The changes cannot be attributed to lithium only, since they occurred even before its administration in some animals. As animals Nos 1, 5, 14 and 16 hibernated, it can be assumed that, at least in these animals, the decrease in b. wt and testis volume was caused by an endogenous (circannual) periodicity. All hamsters had been exposed to constant light since December 1982 and had already completed their first hibernation and reproductive (gonadal) cycles before the beginning of the test. The circadian period length was not influenced either by the change in gonadal development or by lithium.

Since all hamsters that received lithium, hibernating as well as non-hibernating animals, showed gonadal regression, it is unlikely that the primary action of the drug was to prevent gonadal regression[4] and thereby also hibernation. This could have been assumed in view of the finding that testicular growth inhibits hibernation in male Turkish hamsters[14] and that gonadal regression generally precedes hibernation[15,16]. Our results could be explained by a direct influence of lithium on clock-related functions governing the hibernation process: The effect in two female hamsters in which hibernation was not totally suppressed (cf. fig. 1) was a delay as well as a shortening of the hibernation season. In this case lithium should exert similar 'preventive' influences on other circannual rhythms, like avian migratory behavior, etc. Alternatively, both hibernation and gonadal development may be independently influenced by a third lithium-sensitive system.

The observed effect of lithium salt on natural torpidity is remarkable as it occurred in two groups of hamsters which differed in age, sex, and experimental conditions. Experiments are under way to confirm the present result (which is based on a small number of animals) and to test the dose-effect relationship seen in this study.

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