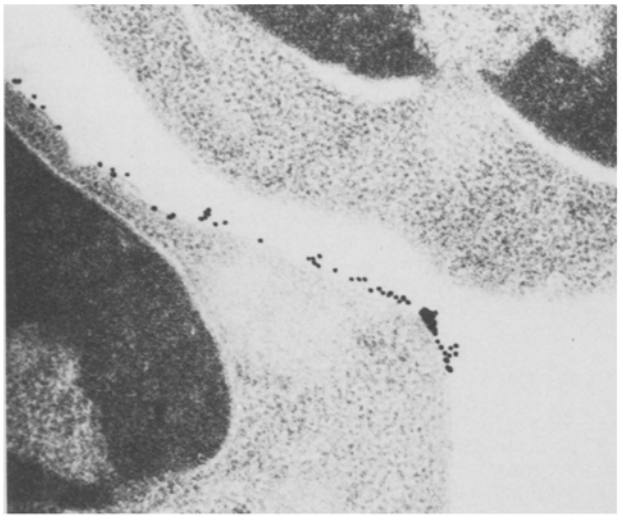

Figure 1. Demonstration of the thymosin-gold-binding sites at the plasma membrane of the thymic lymphocytes. × 33,800.

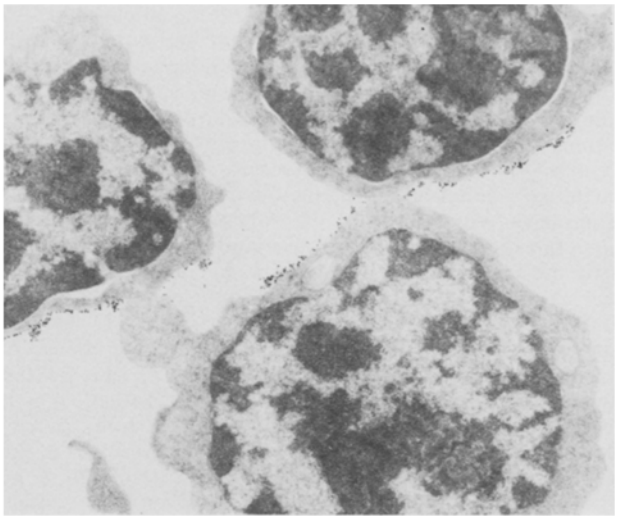

Figure 2. Thymosin-binding lymphocytes. T-Au complex covered some region of cell surface. × 10,000.

Discussion. The experiments showed that the lymphocyte surface labeling, observed in the EM, involved a specific reaction of binding thymosin peptide(s) by cell surface receptors. The reaction could be blocked by preincubating the cells with thymosin before incubation with T-Au complex. Lack of T-Au binding by erythrocytes as well as by other non-lymphoid cells in the thymus seems to exclude T-Au binding by putative free glutaraldehyde groups following glutaraldehyde fixation. Also, no labeling could be obtained when T-Au was substituted by albumin-Au or uncoated colloidal gold while similar labeling results were noted when formaldehyde-fixed or unfixed, cold treated thymocytes were labeled with T-Au. The labeled cells seem to represent a certain subpopulation of lymphocytes with free receptor sites for xenogeneic thymic hormones. If bovine and rat thymic hormones are trapped by identical receptors on rat thymocytes, the unlabeled majority of rat thymocytes may represent cells the receptors of which have been saturated in vivo by the endogenous hormone.

The morphological observations of this paper indicate that thymosin-binding lymphocytes are heterogenous both in ultrastructure and the cell cycle stage. Further characterization of such thymocytes is in progress.

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Night pineal N-acetyltransferase activity in rats exposed to white or red light pulses of various intensity and duration

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Summary. Night N-acetyltransferase activity is suppressed by red and white light; the red light intensity, however, must be 10 times higher. Short light pulses also suppress night N-acetyltransferase; the higher the light intensity, the shorter the pulse is effective.

Rat pineal N-acetyltransferase (NAT) activity increases more than 100 times within a few hours after evening lights off[2]. When lights are kept on, the evening increase does not occur. Dark-induced high NAT activity in rats exposed either to prolonged light[3,4] or to a 1-min light pulse[5] at night declines rapidly and within 20 min it reaches 1/10 of its original value. Photoreception of red by rats is supposed to be poor or absent and hence all handling and killing of rats at night is usually done under red light[6,7]. However, entrainment by red light of running activity rhythm of

albino rats was recently reported[8]. This study attemps to determine what minimal intensity of white or of red light inhibits the increase and induces decline of night NAT activity, and what minimal duration of light pulse is required to trigger the decline of night NAT activity.

Materials and methods. We used 50–60 days old Wistar-Konárovice male rats in all groups except for those exposed to red light of intensity 5 lx, in which females of the same age were used. Before the experiment rats were maintained for at least 3 days in a room lit between 06.00 and 18.00 h by 40 W Tesla fluorescent tubes (light of intensity 50–120 lx). When the rats were exposed to light at night 15 or 100 W Tesla bulbs were used as a source of white light and 15 W red Tesla photographic bulb as a source of red light (less than 5% of emitted light was shorter than 600 nm). The bulbs were screened by a black photographic paper to get the required light intensity (measured by Luxmeter Meopta type MDL 818514). Rats were rapidly killed by decapitation, in darkness under red light of intensity under 1 lx. Pineal glands were dissected immediately and stored in Petri dishes on solid CO_2. Within 48 h they were assayed for NAT activity by a modification[9] of the method of Deguchi and Axelrod[10]. Differences between means were analyzed for significance by Student's t-test.

Results and discussion. At 24.00 h, NAT activity was more than 150 times higher than at 18.00 h in rats kept either in darkness or in red light of intensity 2.5 lx (fig. 1,A). A significant increase in NAT occurred also in rats kept from 18.00 h in white light of 0.5 lx intensity or in red light of 5 lx, intensity but it was lower than that in rats kept in darkness. White light of intensity 2.5 lx or higher or red light of intensity 8 lx suppressed the NAT increase. High NAT activity markedly declined in rats exposed after midnight for 20 min to white light of intensity 0.2 lx or higher, or to red light of intensity 2.5 lx or higher (fig. 1,B). Only in rats exposed to red light of intensity 1 lx no decline occurred. Thus, red light intensity had to be about 10 times higher than that of white light to have the same effect on night NAT activity.

Exposure of rats to white light of 200 lx intensity for only 0.1 min was sufficient to induce the decline of high night NAT activity (fig. 2). When light of lower intensity, i.e. 5 or 0.5 lx was used, the significant decline of NAT occurred only in rats exposed for at least 1 min. Decreased intensity of light must be thus compensated by increased time of exposure to have the same effect.

These results show that red light of sufficient intensity and duration suppresses night NAT activity, which may be important from a methodological point of view. Using red light at night is obviously not quite 'safe' and can bring about artefacts. Photoreception of red might be either due to the presence of red sensitive cones in albino rat retina[11] or because the rods of the rat retina do respond to red light[12]. Comparison of part A and B of fig. 1, indicates that higher intensity of light is required for inhibiting the evening increase than for inducing the decline of night NAT activity. This difference might be due to the decreased sensitivity of photoreceptors in the beginning of the night caused possibly by long exposure of rats to high intensity light during day time[13]. Finally we have shown a reciprocal relation between intensity of light and duration of light exposure: a very short light pulse of high intensity has the same effect as longer exposure to light of lower intensity.

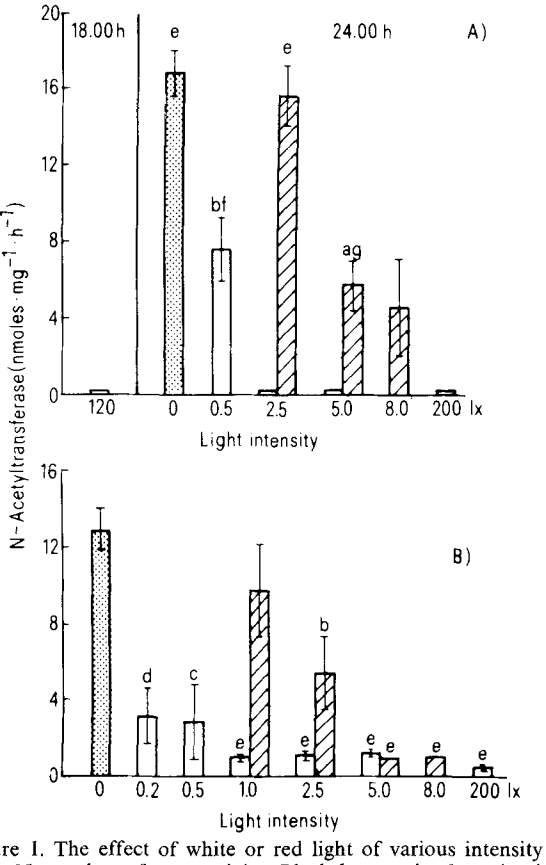

Figure 1. The effect of white or red light of various intensity on night N-acetyltransferase activity. Black bars, animals maintained and killed in darkness; empty bars, animals exposed to white light; cross hatched bars, animals exposed to red light. Each bar represents mean (±SE) from 3 to 5 rats; where SE is omitted, it is less than 0.1 nmoles · $mg^{-1} \cdot h^{-1}$. *A* Inhibition of increase of N-acetyltransferase activity by light. At 18.00 h, before lights off control rats were killed and experimental animals were transferred either to darkness or to white or red light of various intensity and they were killed at 24.00 h. $^{a}p \leq 0.01$; $^{b}p \leq 0.005$; $^{c}p \leq 0.0001$ vs animals killed at 18.00 h; $^{f}p \leq 0.02$; $^{g}p \leq 0.002$ vs animals killed at 24.00 h in darkness. *B* Decrease of night N-acetyltransferase activity induced by light. Between 24.00 and 03.00 h rats were killed in darkness or they were exposed for 20 min to white or red light of various intensity and killed at the end of this 20-min period. $^{b}p \leq 0.005$; $^{c}p \leq 0.002$; $^{d}p \leq 0.001$; $^{e}p \leq 0.0001$ vs animals killed in darkness.

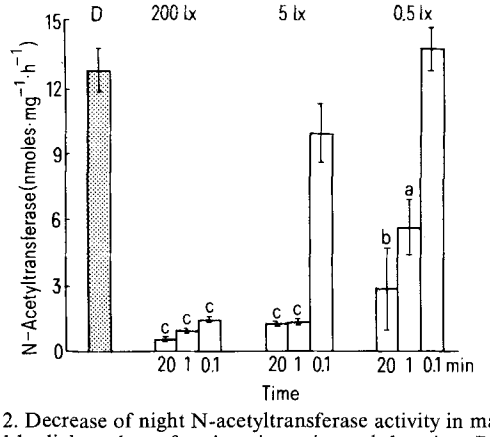

Figure 2. Decrease of night N-acetyltransferase activity in male rats induced by light pulses of various intensity and duration. Between 24.00 and 03.00 h, rats were transferred from darkness to white light of intensity 200, 5, or 0.5 lx and either killed in the light 20 min later or after 1 min or 0.1 min exposure they were returned to darkness and killed 20 min later. D, control rats maintained and killed in darkness. Each bar represents mean (±SE) from 4 animals. $^{a}p \leq 0.005$; $^{b}p \leq 0.002$; $^{c}p \leq 0.0001$ vs control animals maintained in darkness.

1 The authors are grateful to Mrs Marie Svobodová for her skillful technical assistance.
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Effect of gentamycin on insulin release and ^{45}Ca net uptake by isolated islets[1]

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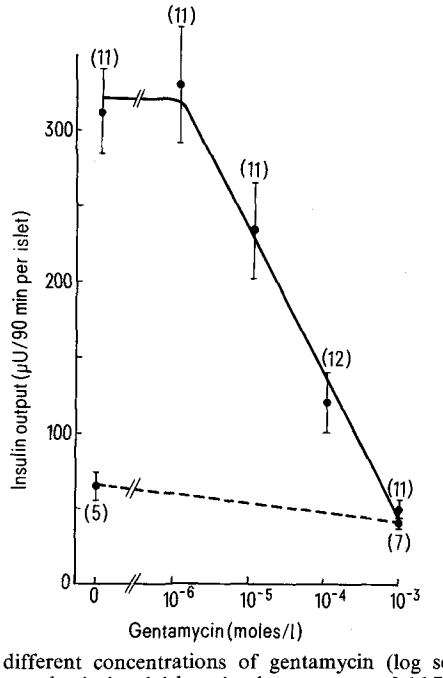
Summary. In isolated islets, gentamycin reduced both the ^{45}Ca net uptake and insulin release induced by glucose, but failed to inhibit the insulin secretion provoked by the combination of Ba^{2+} and theophylline. This indicates that the inhibitory effect of gentamycin is due to a reduction of Ca^{2+} entry into B-cells instead of to a harmful effect upon the integrity of the effector system, responsible for the extrusion of the insulin containing granules.

Recent reports have indicated that the inhibitory action of some aminoglycoside antibiotics on synaptic transmission, cardiovascular functions and smooth muscle contraction is related to their calcium antagonistic properties[4-6]. Because Ca^{2+} plays a crucial role in the mechanism of glucose-induced insulin release[7], this investigation was conducted to determine a possible inhibitory effect of gentamycin, a representative aminoglycoside antibiotic, upon the insulin release and ^{45}Ca uptake by isolated islets.

Materials and methods. The experiments were done with isolated islets obtained by the collagenase procedure[8] from pancreas of fed Wistar rats. The medium used in all experiments was a bicarbonate-buffered solution, enriched with 3 mg/ml of bovine serum albumin and equilibrated with a mixture of 95% O_2: 5% CO_2. In some experiments the medium was deprived of Ca^{2+} and enriched with Ba^{2+} and theophylline. The medium also contained, when required, 16.7 mM glucose. For insulin release, groups of 4 islets each were incubated at 37 °C under constant shaking in 1.0 ml of medium. After 90 min of incubation, an aliquot of the medium was drawn and stored at −20 °C for subsequent insulin dosage[9]. For measurement of ^{45}Ca net uptake, groups of 100 islets each were incubated for 90 min in the presence of ^{45}Ca (100 μCi/ml). The islets were then extensively washed with a nonradioactive medium and examined in sub-groups of 8 islets each for their ^{45}Ca content as described elsewhere[10]. All results were expressed as the mean (± SE) together with the number of individual experiments.

Results and discussion. Gentamycin inhibited the glucose-induced insulin release by isolated islets in a dose-related manner (fig.). In the 10^{-5} M range, gentamycin provoked a 24% reduction of the insulin release with respect to the control ($p < 0.05$). In the presence of 10^{-3} M gentamycin, the rate of glucose-induced insulin release was not significantly different from the basal value found in the absence of glucose. The ED_{50} for the inhibitory effect of this drug is close to 5.5×10^{-5} M. The figure also shows that 10^{-3} M gentamycin significantly reduced the insulin secretion in the absence of glucose ($p < 0.05$). According to Malaisse-Lagae and Malaisse[10] and Henquin and Lambert[11] there is a strict correlation between the insulin release and the ^{45}Ca net uptake by isolated islets. These autors showed that insulin secretion decreases more rapidly than the ^{45}Ca net uptake and that secretion is almost abolished when the ^{45}Ca net uptake is lowered below 60% of the control value, obtained in the presence of 16.7 mM glucose. Recently, Malaisse and others[12] suggested that the mechanism of insulin release requires the maintenance of an internally located Ca^{2+} pool which exhibits a high fractional turnover rate. Probably this pool is responsible for the above 60% Ca^{2+} threshold and corresponds to the so called La^{3+} nondisplaceable Ca^{2+} pool of Hellman et al[13]. In the presence of 16.7 mM glucose, gentamycin 10^{-4} and 10^{-3} M



Effect of different concentrations of gentamycin (log scale) on insulin release by isolated islets, in the presence of 16.7 mM of glucose (●——●) or in the absence of the sugar (●---●). Each point represents mean ± SE of the number of observations indicated in parentheses.