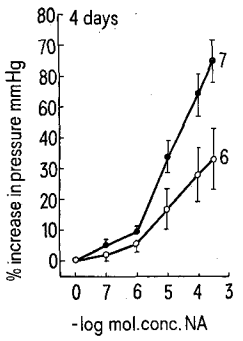

Fig. 2. Log concentration-response curves for the perfusion pressure response to noradrenaline in isolated femoral arteries from control rats (●) and from rats exposured to cold environment for 4 days (○). Initial pressure is 30.7 mm Hg for the cold exposed group and 29.7 mm Hg for the control group. Further explanation as in figure 1.

A comparison between the figure 1 C and E shows that the subsensitivity to NA induced in rats by the cold environment was exactly as great as response ro PHE, thus indicating lowered alpha-adrenergic response in femoral arteries. This result is similar to that found in isolated atria from cold-exposed rats, where the temporary alpha-adrenergic subsensitivity was developed in the cold within 4 days[2]. This induction time is also in agreement with the results concerning supersensitivity. The nonspecific postjunctional supersensitivity to NA was shown to develop in the dog heart in 1–3 days of treatment with reserpine[13,14]. In rabbit aortic strip, significant supersensitivity was found 24 h after a single dose of reserpine and was maximal after 3 daily doses[16]. Contradiction among the previous results[6–9] on vascular sensitivity induced in cold are probably due to the differences of acclimatisation time used. This type of adaptive change seems to be temporary, becoming compensated later by other factors.

Earlier[2–4] we had assumed that the enhanced adrenergic activity in the rat could be a reason for the subsensitivity in the heart. The present results indicate that this inductive effect is not only limited to the heart but is also present in the vascular system and perhaps in all adrenergically innervated effectors.

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The action of ouabain in promoting the release of catecholamines

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Summary. It is suggested that ouabain promotes catecholamine release by causing a rise in intracellular Na^+ which, in turn, causes an elevated steady-state level of intracellular Ca^{2+}. It is suggested that the Na^+-K^+-ATPase is not directly involved in exocytosis at either adrenergic or cholinergic synapses.

Katsuragi and Suzuki[1] in a recent paper in Experientia have shown clearly that ouabain (the inhibitor of the Na^+-K^+-ATPase) at a concentration of 10^{-5}M is effective in releasing extraneuronal catecholamine in the guinea-pig vas deferens. This study complements their earlier findings[2] in which they suggested that ouabain was also able to facilitate catecholamine release from the neuronal site. Ouabain has been known to promote catecholamine release since the earlier studies of Banks[3] on spontaneous release from bovine adrenal gland. Katsuragi and Suzuki have suggested that the Na^+-K^+-ATPase is essential for storage at both extraneuronal[1] and neuronal sites[2], catecholamine release taking place when the ATPase is inhibited by ouabain. This hypothesis is similar to that proposed by Garcia and Kirpekar[4,5] who have shown that ouabain causes a dose-dependent release of noradrenaline from cat spleen slices and suggest[4] that the Na^+-K^+-ATPase serves to maintain the integrity of the axonal membrane; procedures that depress enzyme activity (e.g. intracellular accumulation of Ca^{2+}, Garcia et al.[5]) would cause transmitter release by producing a temporary disturbance in the membrane.

Comparable suggestions concerning the involvement of the Na^+-K^+-ATPase in exocytosis have been advanced for the release of transmitters from synaptosomes[6] and of acetylcholine in the myenteric plexus of the longitudinal

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muscle of guinea pig ileum[7] and in cortical slices from rat brain[8], but it seems to be important to emphasize that there might be an alternative explanation for this effect of ouabain on catecholamine release.

Release of catecholamines has many features in common with the release of acetylcholine from presynaptic terminals[9,10] and it is clear that Ca^{2+} acts as the link in stimulus-secretion coupling in both systems[11,12]. It has also been suggested that the frequency of the spontaneous rate of release of quanta of acetylcholine (recorded as the miniature endplate potentials, MEPPs) at the amphibian neuromuscular junction is largely determined by $[Ca^{2+}]_i$ at the presynaptic terminals[13–17]. Factors that serve to elevate $[Ca^{2+}]_i$ produce an increase in MEPP frequency. Cardiac glycosides also cause a rise in the rate of spontaneous release at the frog neuromuscular junction[18–20] and similar effects are found when Li^+ is allowed to accumulate intracellularly at the presynaptic terminals[21–25]. Such experiments suggest that a rise in the intracellular concentration of either Na^+ (by the action of ouabain in suppressing Na^+-efflux) or Li^+ (which is not readily removed by the cation pump) promotes spontaneous release at the neuromuscular junction and there is now evidence that a rise in $[Li^+]_i$ or $[Na^+]_i$ causes a rise in $[Ca^{2+}]_i$. Such effects have been reported in synaptosomes[26], Chironomus salivary gland cells[27] and in the isolated islets of Langerhans, apparently by release of Ca^{2+} from intracellular stores[28]. It is noteworthy that both Na^+ and Li^+ have been shown to promote Ca^{2+} release from isolated heart mitochondria[29]. It therefore seems probable that the action of ouabain in promoting catecholamine release is also achieved indirectly by a rise in $[Ca^{2+}]_i$ and that one need not necessarily postulate that the Na^+-K^+-ATPase is directly concerned with exocytosis.

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Sucrose activation of mitosis in lemon fruit explants (Citrus limon L.)

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Summary. Lemon fruit explants manifest mitotic activity when incubated on a single component nutrient medium consisting solely of an aqueous sucrose solution.

Excised juice vesicle stalks from mature lemon fruits were observed to be capable of manifesting mitotic activity when incubated on a calcium-potassium-sucrose-agar nutrient medium[2]. Although this nutrient medium was far simpler in composition than any used previously for bringing about mitotic activity in lemon fruit explants, the possibility could not be ruled out that Oxoid Agar No. 3 may have supplied the tissue explants with other mineral nutrients known to be essential for plant growth[2]. The manifestation of mitotic activity in lemon fruit explants incubated on sucrose alone on an all-glass physical substratum is reported here.

Materials and methods. Entire juice vesicles (sac plus stalk) were removed aseptically from firm mature yellow lemon fruits with green buttons (Citrus limon L.) as described previously[3] and floated on the surface of sterile glass-distilled water in 'Pyrex' Petri dishes immediately upon removal from the fruit. The stalks were severed from the sacs, the sacs were discarded, and the stalks were transferred to 'Pyrex' Petri dishes lined with Whatman GF/A glass fibre paper saturated with a) sterile glass-distilled water, and b) sterile 4% sucrose solution. The Petri dishes of both treatments were sealed with 'Parafilm' and placed in continuous darkness at 26–27 °C.

After 6 days of incubation, all stalks of both treatments were fixed in Lillie's AAF solution[4], dehydrated with isopropanol and embedded in paraffin wax as described previously[5], and sectioned at 10 μm thickness. The sections were mounted on glass microscope slides without any adhesive, dewaxed in xylene, brought down to water through a graded isopropanol series, and stained with 1% aqueous Safranin-O for 30 min. The Safranin-stained sections were rinsed thoroughly with glass-distilled water, stained with 1% alcoholic Fast-Green FCF for 5 sec, and the Fast-Green staining action stopped by quickly immersing and gently agitating the slide in absolute isopropanol. The sections were thoroughly dehydrated in 2 more changes of absolute isopropanol and mounted in Euparal.

Results and discussion. There was no evidence of mitotic activity in any of the explants incubated on glass-distilled water (treatment a) whereas mitotic figures repre-