particularly the IHC. That the cochlea undergoes dynamic changes after noise exposure is a well established phenomenon[13,14] and its possible effect on the priming induced audiogenic seizure risk should not be ignored. The advantage of this alternative hypothesis of priming is that it takes into consideration basic information such as the innervation patterns of the OHC and IHC, differential effect of intense noise on the OHC and IHC and the dynamic changes in the cochlear function after exposure to noise. All of these are not considered by the disuse hypothesis of priming.

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Mitogenic effect of the serum of rats exposed to cold

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Summary. Serum from rats exposed for 3 days to 5 °C stimulated the multiplication of mouse heteroploid and human and rat diploid cells in culture. The response of the cells to this unspecific growth stimulation was related to the growth capacities of the different cell types.

The weight increase of the liver and kidneys of cold-acclimated rats is due to a stimulation of DNA synthesis in the beginning of acclimation, followed by increased cellularity[1,2]. Preliminary tests with mouse L cells in culture revealed that in the serum of cold-exposed rats there is a growth-stimulating activity which reached its peak on the 2nd and 3rd days of exposure and then disappeared[3]. The tissue and species specificity of these sera and the sensitivity of different cell types were studied in the following experiments.

Materials and methods. Sera of 6 adult female Wistar rats kept for 3 days at 5 °C, as described previously[2], were obtained by sterile cardiac puncture and centrifugation, pooled and stored at -20 °C. Sera of 6 corresponding controls living at 24 °C were prepared in the same way. 4 samples of experimental and 4 samples of control sera were prepared independently. Parts of the samples 1 and 2 were dialyzed for 48 h against phosphate-buffered saline and sterilized by Millipore filtration, samples 3 and 4 were inactivated for 30 min at 56 °C before use. The sera were tested in 2 sublines of heteroploid mouse L cells, L-As and LA cells[4], in phase II human diploid fetal lung fibroblasts LEP 19, in 1st-passage embryonic rat liver cells, in 5th-passage newborn rat hepatocytes and in adult rat kidney primaries. The cells were incubated for 3–8 days at 36 °C in Eagle's minimal essential medium supplemented with 5, 10

Mitogenic effect of native, inactivated and dialyzed sera from cold-exposed and control rats on different cell types in vitro

Cells	Days at 36 °C	% of serum-serum sample	Mean cell yield ± SE as % of original inoculum with C and E sera				Difference between C and E	
			C	E	CD	ED	E-C	CD-ED
L-As	3	10-2	361 ± 12.48	417 ± 10.02	293 ± 12.64	361 ± 13.12	56	68
L-As	3	10-2	360 ± 16.12	450 ± 11.48	285 ± 18.15	383 ± 18.25	90	98
L-As	3	10-2	273 ± 11.25	360 ± 14.49	158 ± 7.66	218 ± 11.51	87	60
LA	3	10-1	325 ± 10.24	398 ± 66.69	314 ± 9.71	340 ± 13.16	73	26
LA	6	5-1	129 ± 6.77	250 ± 19.09	110 ± 8.19	153 ± 6.66	100	43
LEP 19	6	20-3in	430 ± 20.21	520 ± 22.25				
LEP 19	6	10-3in	346 ± 12.16	506 ± 25.00				
NbRH	8	10-4in	86 ± 9.38	141 ± 10.12				
ERL	8	10-2	82 ± 10.11	132 ± 8.92				
ARK	8	10-4in	28	32			4	
ARK	8	10-1	23	28	21	24	5	3
ARK	8	5-1	24	30	22	25	6	3

C, serum of control rats; E, serum of rats exposed for 3 days to 5 °C; CD, dialyzed control serum; ED, dialyzed E serum; in, inactivated serum; L-As, LA, sublines of the L cell line; LEP 19, human diploid fetal lung fibroblasts; NbRH, newborn rat hepatocytes; ERL, embryonic rat liver cells; ARK, adult rat kidney cells. Numbers indicate mean cell yield ± SE.

or 20% of the sera or their non-dialyzable fractions. Usually 1×10^5 cells per 1 ml of medium were incubated in Müller flasks or test tubes, and the percentage of the initial inoculum reached by cultures with experimental and control sera was calculated from 4 vessels (8 hemocytometer counts) in repeated time-independent experiments.

Results. (Table.) The yield of the L-As and LA cells after 3 days of incubation was higher with the sera from cold-exposed rats than with the sera of controls. Dialysis lowered the cell yield, but the difference between the experimental and control sera did not disappear. In the combination L-As cells-sera 2, the effect was practically the same for native and dialyzed sera. In the system LA cells-sera 1, the response to dialyzed experimental serum was lower than to native serum, but still present. Decreased serum concentration in the medium decreased the total cell yield, but did not influence the result. For human diploids LEP 19, native sera were toxic, but with inactivated sera, the cell yield was the same as in mouse L cells, only after a longer incubation. Higher serum concentration improved the cell yield. The difference between the experimental and control sera was in the range of mouse heteroploids. 1st-passage embryonic rat liver cells and 5th-passage newborn rat hepatocytes were not able to multiply with the control sera, but did so with the experimental sera. Their response was lower than that of mouse heteroploids and phase II human diploids. Adult rat kidney primaries unable to overcome rapidly the stress of explantation suffered great cell losses, but even here slight evidence of a positive reaction was found.

Discussion. The mitogenic effect of the serum of cold-exposed rats was neither species nor tissue specific. The serum stimulated multiplication of mouse, human and rat cells, fibroblastic and epithelial, heteroploid and diploid. It differed thus from the tissue specific growth- and metabolism-stimulating sera of rats after hepatectomy[5] and uninephrectomy[6]. The absence of tissue specificity was to be expected in view of the in vivo induction of DNA synthesis and the increase in cell number in many organs of cold-acclimated rats[1,2,7,8]. The high sensitivity of the quickly growing cell types and the low sensitivity of those growing slowly was in agreement with the known positive correlation between the proliferative capacity of cultured cells and their requirements for serum and its growth-stimulating macromolecules[9].

The nature of the mitogenic stimulus in the sera of cold-exposed rats is not known. One might speculate about some hormones which take part in the development of cold acclimation, for instance triiodothyronine, a stimulator of liver thermogenesis in vivo and in cultured hepatocytes[10]. Triiodothyronine also stimulates the production of growth hormone whose mediators are the growth-promoting somatomedins[11]. Another mitogenic peptide, the epidermal growth factor (urogastrone), was recently shown to induce DNA synthesis and hepatocyte proliferation in the presence of insulin and glucagon[12]. Nothing is known about the production and activity of these or other growth-stimulating factors in cold acclimation, but, as assays for at least some of them are now available, the induction of organ growth in cold-exposed rats is open to further analysis.

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Effects of 2-deoxy-D-glucose, glucose and insulin on efferent activity in gastric vagus nerve[1]

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Summary. Intracarotid injection of 2-deoxy-D-glucose and insulin increased the efferent activity in the gastric vagus nerve of anesthetized rats, while glucose injection transiently decreased vagus activity.

2-Deoxy-D-glucose (2-DG) and insulin are powerful stimulants of vagal gastric secretion[2,3]. Since 2-DG is converted by phosphorylation to 2-DG-6-phosphate but is not metabolized further, this product competes with glucose-6-phosphate and inhibits its phosphorylation[4-6] resulting in glucopenia in the cell. 2-DG causes glucopenia in neurones in the lateral hypothalamic area which have been shown to initiate vagally mediated gastric secretion[7]. Little work has been done in studying the effect of 2-DG on efferent activity of the gastric vagus nerve. The present experiments were designed to investigate this aspect.

Materials and methods. Experiments were carried out on 28 male rats weighing 250–400 g. The animals were fasted for 18 h but had free access to water. Animals were anaesthetized with sodium pentobarbital (45 mg/kg, i.p.). A fine filament dissected from the central cut end of the vagus nerve innervating the stomach was placed on a pair of silver wire electrodes. Efferent nerve activity was amplified with a differential amplifier and was integrated after converting the spikes to standard pulses by a window discriminator and presented as vertical deflections. Spontaneous activity is expressed as mean spikes per 5 sec over 50 sec (i.e. mean of 10 samples) just before the injection of the drugs. Responses to 2-DG and insulin are indicated by the