periment was terminated after the last observation which was made at 123 days.

Results. Each measurement was corrected for background and, in the case of ^{81m}Kr and ^{99m}Tc-MDP. The results are shown in figure 2. It can be seen that this ratio increases for both agents, the increase being greater in the case of ^{99m}Tc-MDP. It reaches a maximum at about 14 weeks after the osteotomy, thereafter returning to baseline levels. The increase in ^{81m}Kr is more or less constant throughout except that it shows a slight diminution towards the end of the experimental period.

Discussion. Bone blood supply has been shown to increase following a complete osteotomy[13]. Bone blood flow also increases and reaches a maximum of 4 times the control value at 10 days following an experimentally induced tibial fracture[14]. This increase in flow produces an increase in capillary surface area from recruitment and dilatation of new capillary beds. The result of the capillary recruitment is that the radioactive tracers are distributed over a larger surface area, with a corresponding increase in the number of bone mineral binding sites which are available for exchange.

The mechanism of uptake of isotopes in bone containing tumour has been examined and it was suggested that there are 2 stages of uptake, a rapid vascular stage and a selective concentration in immature new bone[15]. In an osteotomy at 10 days, although there is a great deal of osteoblastic activity with periosteal new bone formation, there is also proliferation of blood vessels with re-establishment of the medullary circulation.

These experiments have confirmed the findings that following an osteotomy blood flow to bone increases. This increase however, is shown to be proportionately less than the increase in extraction of ^{99m}Tc-MDP. The high increase in extraction of the latter agent following the osteotomy supports the two-stage theory of uptake mentioned previously[15], i.e. an increase in flow followed by an increase in extraction by newly formed bone. The extraction reaches a peak at about 14 weeks by which time healing and bone remodelling has neared completion.

It can be concluded therefore, that the effect of a partial osteotomy is to produce both an increase in bone blood flow as shown by ^{81m}Kr, as well as an increase in mineral extraction of the bone-seeking agent, ^{99m}Tc-MDP. These results have also shown that it is the increased efficiency for extraction of ^{99m}Tc-MDP by the newly formed bone which is the more important of the 2 factors.

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Rapid development of acoustic trauma-induced audiogenic seizure risk in 3 strains of seizure-resistant mice[1]

C.-S. Chen

Department of Psychology, Monash University, Clayton (Victoria 3168, Australia), 17 December 1979

Summary. Audiogenic seizure risk can be induced in genetically seizure resistant mice by exposure to an intense noise a few days prior to testing for seizure. This experiment demonstrates that the priming induced seizure risk can develop within 6–16 h after priming. It was argued that this finding suggested an alternative hypothesis of priming involving peripheral auditory mechanisms.

Certain strains of inbred mice, such as C57BL/6J or BALB/c mice are genetically non-susceptible to audiogenic seizure in the sense that they rarely exhibit seizure responses on the very first exposure to an intense noise. However, audiogenic seizure risk can be induced in these so-called seizure-resistant mice by exposure to an intense noise a few days before testing for audiogenic seizure[2,3]. It has been suggested that the seizure risk develops because the priming exposure has triggered development of a hyper-reactive state similar to the well-known physiological phenomenon of disuse or denervation supersensitivity in the higher auditory system[4]. This hypothesis argues that the priming exposure causes stimulation damage to the mouse cochlea. Consequent to this damage the afferent input to the higher auditory structures is reduced leading to development of a supersensitive state in these structures. When the primed mice are re-exposed to an intense noise, the supersensitive structures are stimulated and audiogenic seizure responses are precipitated.

The disuse hypothesis of priming implies that susceptibility to audiogenic seizure should take time to develop. Indeed, available evidence indicates that the priming induced seizure risk generally takes 1.5–3 days to develop[2,3,5,6]. However, results showing unusually rapid development of seizure risk have occasionally been reported[5,7]. Since the developmental rate of the seizure risk could be an important limiting factor for the credibility of the disuse hypothesis of priming, the present study was designed to obtain additional information regarding this temporal dimension of the priming phenomenon.

The animals used were BALB/c mice, C57BL/6J mice and priming prone mice selectively bred in this laboratory. The acoustic stimulus used for priming and testing for audiogenic seizures was a repeated impulse noise with a repeti-

Incidence of audiogenic seizure response as a function of prime-test intervals in 3 strains of inbred mice

	Prime-test interval	Priming duration (sec)	N(a)	Incidence of seizure responses (%) Wild Running	Clonic	Tonic
Priming prone mice	3 h	120	12 (10)	33	25	25
	6 h	120	12 (10)	67	33	25
	24 h	120	11 (10)	100	91	91
C57BL/6J mice	6 h	60	12 (11)	42	25	0
	16 h	60	10 (8)	90	60	40
	24 h	60	8 (8)	88	88	88
BALB/c mice	6 h	60	20 (10)	20	15	15
	16 h	60	20 (10)	55	50	40
	24 h	60	18 (10)	78	56	39

The number in brackets represents numbers of sham-primed mice used.

tion rate of 222 pulses/sec and a sound pressure level of 125 dB (re: 0.0002 dyne/cm^2). The impulse noise was generated from a Wavetek Pulse Generator (Model 802). The generator output (330 μse width) was amplified by a 50-W audioamplifier and the sound source was a Coral HD-55 tweeter speaker (frequency response: 4–20 kHz).

Mice were first exposed to the impulse noise for either 30 sec, 60 sec or 120 sec (priming) and then tested for audiogenic seizures by re-exposure to the same noises for 30 sec or until seizure occurred. The prime-test interval used ranged from 3 to 24 h. Types of seizure responses, i.e., wild running, clonic seizure and tonic seizure, were noted during testing. Each strain of mice was primed at ages known to be most sensitive to the priming manipulation: BALB/c mice at 20 ± 1 days, C57BL/6J at 17 ± 1 days, and priming prone mice at 18 days of age. Relevant sham-primed control groups were also run.

Results show that none of the sham-primed mice exhibited any kind of seizure responses at test. However, high incidence of audiogenic seizures was obtained in the primed mice tested 24 h after priming, irrespective of the duration of priming exposure. The proportion of animals showing wild running were 59% (30-sec condition), 78% (60-sec) and 65% (120-sec) for BALB/c mice; 78% (30-sec), 100% (60-sec) and 100% (120-sec) for priming prone mice; 56% (30-sec), 88% (60-sec) and 78% (120-sec) for C57BL/6J mice. All differ significantly from their respective sham-primed groups (Fisher exact probability test: $p_s < 0.01$). The proportions of animals showing clonic seizure were: 47% (30-sec), 56% (60-sec) and 35% (120-sec) for BALB/c mice; 78% (30-sec), 91% (60-sec) and 91% (120-sec) for priming prone mice; 33% (30-sec), 88% (60-sec) and 44% (120-sec) for C57BL/6J mice. These results demonstrate that the priming induced seizure risk can develop as early as 24 h after priming.

The table summarises the experimental conditions under which the best priming effect was obtained for each strain of the inbred mice tested at shorter prime-test intervals. Results for less effective conditions were not presented; it is sufficient to say that the 30-sec exposure condition was less effective than the 60-sec condition and the 120-sec condition, and that the difference between the 60-sec condition and the 120-sec condition was often small. The results of the table indicate that when tested 3 h after priming, 33% of the priming prone mice had a wild running response and 25% of them showed clonic and tonic seizures. The increase in the seizure incidence was not statistically significant, probably due to the small number of subjects used. Nevertheless, these results suggest that seizure risk could develop in some subjects as early as 3 h after priming.

When tested 6 h after priming, a significant increase in the incidence of wild running was shown by priming prone mice ($p < 0.01$), by C57BL/6J mice ($p < 0.05$) but not by BALB/c mice ($p > 0.05$), suggesting a strain difference in the developmental rate of the seizure risk. A significant increase in the incidence of wild running and clonic seizure was obtained in both C57BL/6J and BALB/c mice ($p_s < 0.05$) when tested 16 h after priming; the priming prone mice were not run under this condition as high incidence of seizure has already been observed under the 6-h condition.

These results have demonstrated 3 important developmental characteristics of the priming induced audiogenic seizure risk: a) it can develop rapidly, b) it increases in degree over post-priming time, and c) it develops at a differential rate among different strains of animals.

Demonstrations of rapid development of seizure risk in this and other studies[5,7] appears to pose problems for the disuse supersensitivity hypothesis. To account for these results it is necessary to assume that the postulated supersensitive state develops within a few hours after priming exposure which however, is not consistent with the general view that denervation supersensitivity in the nervous system usually takes days if not weeks to develop[8]. An alternative hypothesis worth speculating upon is to assume that the priming-induced seizure risk is related to the functional state of the cochlear outer hair cells (OHC) and inner hair cells (IHC) at the time of testing: being positively related to the degree of the OHC dysfunction but negatively related to that of the IHC dysfunction.

Formulation of this hypothesis was based on the following considerations: 1. Histological evidence has shown that the major effect of priming exposure is to cause substantial structural damage to the OHC with little effect on the IHC[9]. 2. Some kind of interaction between the OHC and the IHC has been assumed to exist by auditory neurophysiologists and there are speculations suggesting that dysfunction of the OHC could result in greater afferant input[10,11]. 3. Major components of the afferent auditory input to the CNS come from the IHC, because up to 90–95% of the afferent fibres are originated from the IHC[12]. Thus dysfunction of the IHC could result in reduced afferent input and hence reduce the tendency of the animal to show audiogenic seizure responses. In fact it has been reported that seizure response was not shown by primed mice with substantial OHC and IHC damage, whereas damage occurred almost exclusively in the OHC of the seizure-stricken primed mice[9].

According to this hypothesis, rapid development of seizure risk after priming would occur if the priming stimulus used is such that it could cause sufficient damage to the OHC but minimum deleterious effect on the IHC function. Increase in seizure risk over the post-priming period as observed in the present study and others[2,3,5,7] is to be explained in terms of the pattern of dynamic changes in the function of the OHC and IHC after priming exposures: continuous deterioration of severely affected structures, particularly the OHC, and recovery of less affected ones,

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

E. Holečková

Institute of Physiology, Czechoslovak Academy of Sciences, Videňská 1083, 142 20 Prague (Czechoslovakia), 27 December 1979

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.