Results and discussion. The Table presents measurements of average nuclear area for both liver and hepatoma cell nuclei. The area of the tetraploid liver cell nuclei (49.94 μm^2) is nearly twice that of the diploid nuclei (28.56 μm^2), while the hepatoma cell nuclei are much larger than either (115.47 μm^2). To compensate for these differences in nuclear size, all the grain count data on the Table are presented as a standard unit measure: grains per 100 μm^2 nuclear area.

Proof that ^{3}H-actinomycin D binds specifically to DNA is provided by the grain count data obtained from the enzyme treated control sections. The Table shows that RNA extraction did not influence ^{3}H-actinomycin D binding, but when the DNA was extracted, no binding occurred.

The grain density over diploid and tetraploid liver cell nuclei was almost identical in all experiments (about 93 grains per 100 μm^2), showing that DNA distribution was not altered by the increase in ploidy. In marked contrast, the ^{3}H-actinomycin D binding to hepatoma cell nuclei (200 grains per 100 μm^2) was more than twice as dense as in the normal liver cells. In the Figure, silver grains are not only visible over the nuclear area, but over the cytoplasm as well, however, the latter are not background fog. Evidence that they represent ^{3}H-actinomycin D binding to cytoplasmic DNA has been documented in other publications by the present authors[4, 12].

The mouse hepatoma used in the present study is aneuploid and shows wide variations in the total amount of DNA per nucleus[4]. Aneuploidy and polyploidy have been reported in many types of tumors[13, 14]. However, the increased ^{3}H-actinomycin D binding reported above for hepatoma nuclei cannot be explained on the basis of increased ploidy alone.

It is of particular interest that heavy concentrations of silver grains were observed over chromatin masses near the nuclear membrane and around nucleoli. Heavy ^{3}H-actinomycin D binding in these heterochromatic regions was particularly prominent in the hepatoma nuclei, as shown in the Figure. High levels of heterochromatin are characteristic of many types of tumor cell nuclei[15, 16]. SIMARD[5] has reported that ^{3}H-actinomycin D binds exclusively to the heterochromatin in cultured hamster fibroblasts, and interprets his results in terms of either more DNA in heterochromatin or preferential binding of ^{3}H-actinomycin D to heterochromatin. A similar interpretation of preferential ^{3}H-actinomycin D binding to heterochromatin in the hepatoma cell nuclei has been adopted by the present authors to explain the results reported here.

Résumé. Le ^{3}H-Actinomycin D se fixe seulement sur l'ADN dans des coupes histologiques; les noyaux de l'hépatome en liant plus que ceux du foie normal. Ces observations sont interprétées en relation avec l'augmentation de l'hétérochromatine de l'hépatome.

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Radio-Protection of Arousing Ground Squirrels (*Citellus tridecemlineatus*) by Endogenous Catecholamines

Radio-protection by exogenous catecholamines has been known for many years[1] and has recently been studied in greater detail on the hibernator, *Mesocricetus auratus*[2]. The radio-protection is thought to be due to a vasoconstriction-hypoxia mechanism. That is, intense vasoconstriction leads to a local tissue hypoxia of the bone marrow which is then protected through the 'oxygen effect'. The quantities of catecholamines necessary to elicit a radio-protection response (1.5 mg/kg) are well above the physiological range, and until now radio-protection by endogenous catecholamines has not been demonstrated.

Naturally occurring radio-protection in hibernating ground squirrels has been reported[3]. It was thought that radio-protection could also occur during arousal from hibernation since severe vasoconstriction occurs at that time, thus indicating the release of large amounts of norepinephrine[4]. Vasoconstriction is strong enough to allow the thorax of the 5°C animal to warm quickly to 30°C before the rectal temperature begins to rise. Furthermore, bretylium β-TM 10 eliminates the differential warming rate and greatly prolongs the arousal process[4]. The possibility that vasoconstriction of such severity could cause radio-protection due to tissue hypoxia in blood forming organs seems likely. In addition, if the radio-protection were due to the release of norepinephrine, it should be blockable with an α-blocker such as phentolamine.

Methods. The radiation source used in these experiments was a U.S. Nuclear of California model Gamma 12 ^{60}Co. The description and calibration of the source has been reported previously[3]. The dose rate was 200–185 rads/min due to the decay of the ^{60}Co over the time in which the experiments were done.

The animals used for this study were 13 lined ground squirrels (*Citellus tridecemlineatus*) of either sex. They were born in our vivarium to pregnant females trapped in Kansas. The ages ranged from $1^1/_2$ to $2^1/_2$ years, and it was the first experience with hibernation for each animal.

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The animals were individually caged and placed in a cold room (5°C) to hibernate in late fall. After several weeks, the hibernating ground squirrels were divided into 3 groups of 25 animals each. The first group was injected i.p. with 10 mg/kg of phentolamine mesylate and allowed to arouse undisturbed. The second group also received 10 mg/kg of phentolamine mesylate, but were exposed to 1450 rads after they had aroused to 12°C rectal temperature. The third group received equivalent volumes of sterile, physiological saline and were also exposed to 1450 rads when they had reached 12°C rectal temperature. All arousals took place in an ambient temperature of approximately 23°C. All animals were then placed in the vivarium for observation up to 90 days, and the percent survival recorded daily.

A second experiment was done the same time of year on nonhibernating ground squirrels. A group of 20 animals was injected i.p. with 10 mg/kg of phentolamine mesylate and exposed to 1200 rads 15 min later. Another group of 20 animals was given equivalent volumes of sterile physiological saline and also exposed to 1200 rads 15 min later.

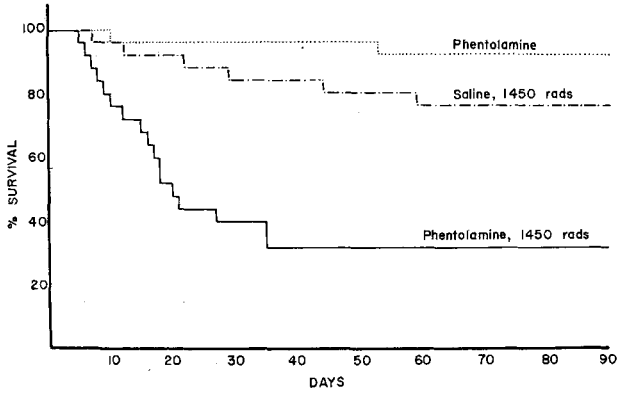

Fig. 1. The survival of phentolamine or saline treated ground squirrels exposed to 1450 rads during the arousal process and the survival of ground squirrels given phentolamine alone. N = 25 per group.

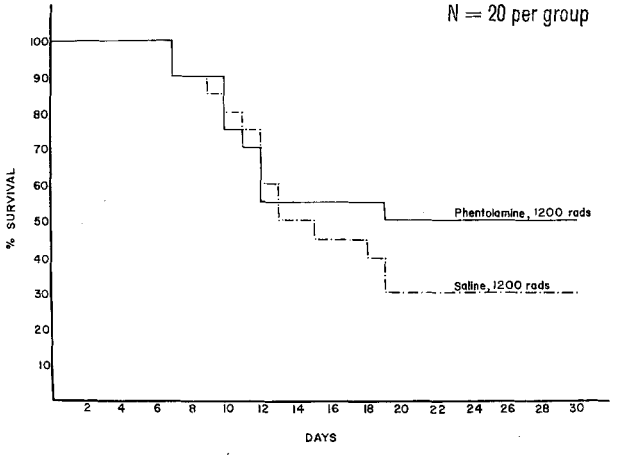

Fig. 2. The survival of nonhibernating ground squirrels exposed to 1200 rads after pretreatment with phentolamine or saline. N = 20 per group.

These animals were placed in the vivarium and observed for 30 days. The dose of 1200 rads was selected for this experiment because it is near the $LD_{50(30)}$ for active ground squirrels. Radio-protection or radio-sensitization can be detected by this type of experiment since the control will be near the center of the survival scale.

Results and discussion. The results of the first experiment, shown in Figure 1, are 32% survival for the phentolamine treated irradiated group, 76% for the saline treated irradiated group, and 92% for the phentolamine treated, non-irradiated group. The 76% survival of the saline treated group represents a substantial amount of radio-protection compared with nonhibernating animals since it has been established in previous experiments that active ground squirrels cannot survive doses of 1250 rads or greater[3]. The phentolamine pretreatment resulted in a significant (χ^2 test at 5% level) reduction in the radio-protection seen during the arousal process. The reduction seen could be explained by 3 mechanisms. First, it could be due to a specific lethal effect of α receptor blockade during the arousal period; second, it may be due to a separate, direct radio-sensitizing effect of phentolamine itself; and third, the decreased survival could be due to blockade of radio-protection by release of endogenous catecholamines.

The high percent survival of the phentolamine treated, non-irradiated group indicates that the phenotlamine does not have a specific lethal effect when given to ground squirrels arousing in an ambient temperature of 23°C. This eliminates the first proposed mechanism. This possibility was checked because the sympathetic nervous system plays an integral part in the arousal process, and blockade of a portion of its effects may result in failure of the animal to survive hibernation[5].

If phentolamine were a radio-sensitizer as proposed in the second mechanism, the effect should be seen in the active animal as well as the arousing one. The results of the second experiment (Figure 2) show that phentolamine has no significant effect as a radio-sensitizer on nonhibernating ground squirrels. The survival was 50% for the phentolamine treated group and 30% for the saline treated group. The difference is not statistically significant, but in any case the greater survival of the phentolamine group indicates that phentolamine is not a radio-sensitizer.

The third proposed mechanism remains. That is, the reduction in survial of the irradiated, arousing ground squirrels treated with phentolamine must be due to blockade of stimulation of the α-receptors that occurs during the arousal process. It is highly likely that these receptors are stimulated by the release of norepinephrine from the sympathetic nerve terminals as demonstrated by LYMAN and O'BRIEN[4]. The mechanism of action would then be the same vasoconstriction-hypoxia mechanism that occurs when exogenous doses of norepinephrine are given.

The radio-protection that remains after treatment with phentolamine (32% survival) may be due to the shift of the hemoglobin-oxygen dissociation curve to the left at the low body temperature[6]. This forces the tissue to a much lower pO_2 in order to obtain the oxygen needed for metabolism and would also contribute to the 'oxygen effect'.

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In conclusion, the radio-protection seen in arousing ground squirrels appears to be due to a combination of release of endogenous catecholamines and a shift of the hemoglobin-oxygen dissociation curve to the left. Both mechanisms lead to radio-protection through the 'oxygen effect'.

[7] We thank MARYLIN SPATE for her technical assistance.
[8] Supported by NASA grant No. NGL 26-004-021, S5 and 6, USPHS grant No. GM 41418-03, and the Dalton Research Center, University of Missouri.

Résumé. Au cours des phases du réveil d'hibernation, la radio-protection des spermophiles (*Citellus tridecemlineatus*) est réduit d'une manière significative par la phentolamine (agent bloquant le récepteur alpha). Cette découverte implique la libération des catécholamines par les fibres nerveuses à la source de la radio-protection.

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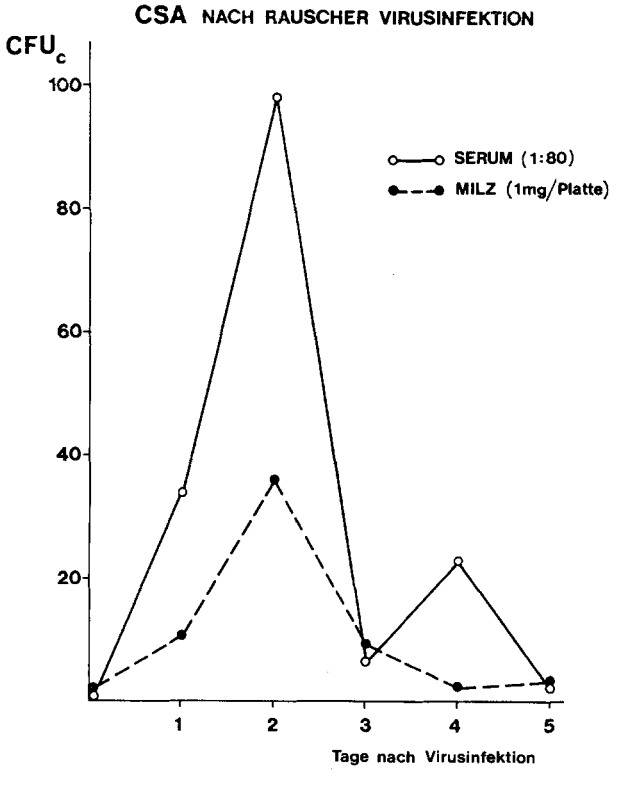
«Colony-stimulating activity» im Serum und der Milz nach Rauscher Virusinfektion[1].

Colony stimulating activity in serum and spleen after Rauscher virus infection.

Durch Infektionen wird die Granulozytopoese stimuliert, nach Injektionen von Endotoxinpräparaten steigt die Aktivität des «colony stimulating factor» (CSF bzw. CSA, «colony stimulating activity») im Serum und verschiedenen Organen bei Mäusen an. Dieser Faktor spielt für die Regulation der Granulozytopoese wahrscheinlich eine entscheidende Rolle. Wir haben die Aktivität des CSA bei CBA/J Mäusen nach Infektion mit einer Standardpräparation des Rauscher Virus untersucht, die Ergebnisse werden hier dargestellt, da bisher noch keine derartigen Daten vorliegen.



CSA aus dem Serum wurde nach Inaktivierung in der Verdünnung von 1:80 zu 1×10^5 Knochenmarkzellen in der Agarkolonietechnik (ohne CSF) gegeben; die Milz wurde zur Gewinnung des CSA homogenisiert und nach SHERIDAN und METCALF[2] präpariert; 1 mg des Organs wurde in wässriger Lösung zur Kultur addiert. Einzelheiten der Technik siehe ITURRIZA und SEIDEL[3]. Die «colony stimulating activity» ergibt sich aus der Zahl der in der Kultur auswachsenden Kolonien, die nach 7 Tagen bestimmt wird.

Die Ergebnisse sind in der Figur dargestellt. Im Serum und in der Milz kommt es zu einem starken Anstieg von CSA mit dem Maximum am 2. Tag und einer Rückkehr zu Kontrollwerten nach 5 Tagen. Die Werte am 2. Tag entsprechenden in ihrer Höhe denen, die nach Endotoxininjection ebenfalls als Maximum beobachtet werden, da allerdings bereits nach wenigen Stunden (SHERIDAN und METCALF[2]). Erst wenn gleichartige Untersuchungen für viele andere Viren und Bakterien vorliegen, kann entschieden werden, inwieweit der hier mitgeteilte Ablauf generell gültig ist oder ob er irgendwelche für ein leukämogenes Virus spezifische Besonderheiten aufweist.

Summary. Colony-stimulating activity (CSA) of serum and spleen was studied in CBA/J mice 1–5 days after Rauscher virus infection, using the agar culture system with normal mouse bone marrow cells as target cells. A sharp increase of CSA was observed with a peak after 2 days in both sites; after 5 days control levels are reached.

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