Effects of X-Irradiation During Hibernation on Tissue Catecholamine Contents[1]

Hibernation is said to delay the onset and progression of the biological effects of radiation[2]. However, the mechanism of this 'protection' is not clear. We have demonstrated increased levels of catecholamine in the liver and kidneys of hibernating squirrels[3], and others[4] have reported that epinephrine and norepinephrine offer limited protection to certain radiosensitive tissues. It therefore appeared desirable to compare the catecholamine content of organs from active and hibernating animals exposed to radiation. Results of such experiments may indicate the extent to which the sympathetic nervous system is implicated as a factor in the protective mechanism of hibernation in animals subjected to radiation.

Methods. Ground squirrels, *Citellus tridecemlineatus*, were collected in Northern Illinois and were maintained in the laboratory in an air-conditioned room at 23°C. The animals had free access to water and to Rockland guinea-pig diet and supplements of raw carrots were given twice weekly. The experiments were conducted in early December 1961, i.e. during the animal's natural period of hibernation. Both males and females, body weights ranging from 140 to 230 g, were used. Hibernation was induced by transferring the animals to a cold room (5°C) where they were deprived of food and water.

Fifty specimens of ground squirrels were equally divided into five groups and treated as follows: Group I, active animals selected at random from laboratory stocks, served as controls; in Group II were hibernating animals, 7 to 10 days at 5° to 10°C; in Group III were active animals X-irradiated, and sacrificed 20 h post-irradiation; in Group IV were hibernating animals which were X-irradiated and kept under hibernal conditions and sacrificed 20 h post-irradiation, 8 of these animals had elevated body temperatures indicative of lessened or lack of hibernal torpor at the time of sacrifice; lastly, Group V consisted of animals which were irradiated during hibernation, maintained under hibernal conditions for 20 h and then transferred to animal room conditions where they were permitted to awaken for an additional 20 h prior to sacrifice.

The squirrels were subjected to a single, total-body exposure of 2000 r of X-rays (250 kv, 35.6 cm target distance, 0.5 mm copper and 3.0 mm bakelite filters, 1.5 mm copper half-value layer, and 130 r pro min).

Each animal was spinalectomized. The heart was excised and blotted free of blood; both kidneys were extirpated and a segment of liver was removed. These tissues were rapidly frozen at the temperature of dry ice. Samples were assayed for catecholamine by a fluorimetric method[5]. Oxidations were carried out at pH 4,9 so that equivalent readings for epinephrine and norepinephrine were obtained. The micro-Kjeldahl method with Nesslerization was used to determine tissue nitrogen.

Results. The levels of catecholamine in heart, kidney and liver in active and hibernating, both irradiated and non-irradiated ground squirrels are summarized in Figures 1 and 2. The catecholamine values in active non-irradiated and active irradiated animals are similar. The kidney and liver of hibernating ground squirrels have significantly greater ($P = 0.05$) concentrations of catecholamine than does the kidney and liver of active animals. Catecholamine levels in tissues of irradiated hibernating ground squirrels (Group IV) are comparable to those of the irradiated active animals (Group III), however, they are lower than those of non-irradiated hibernators (Group II). In particular, kidney and liver catecholamine concentrations are significantly decreased ($P = 0.05$).

The squirrels which were irradiated during hibernation and then aroused have tissue catecholamine levels comparable to those of irradiated active and irradiated hibernating animals, but are lower than non-irradiated hibernators (Figure 1).

When the tissue catecholamine concentrations are expressed on a wet weight basis (Figure 1) then a comparison of the cardiac and hepatic levels from non-irradiated hibernators (Group II) with animals from each of the irradiated Groups shows decrease in each of the latter.

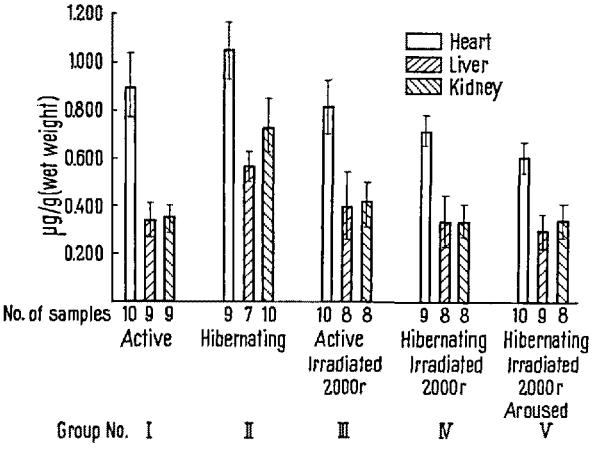

Fig. 1. Catecholamine contents of heart, kidney and liver in active and hibernating, irradiated and non-irradiated ground squirrels, *Citellus tridecemlineatus*. Results are expressed in µg/g wet weight.

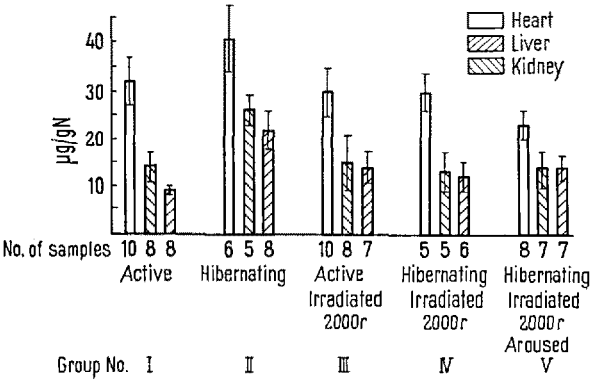

Fig. 2. Catecholamine contents of heart, kidney and liver in active and hibernating, irradiated and non-irradiated ground squirrels, *Citellus tridecemlineatus*. Results are expressed in µg/g nitrogen.

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[2] J. F. THOMSON, *Radiation Protection in Mammals* (Reinhold Publishing Corporation, New York 1962).

[3] X. J. MUSACCHIA, M. JELLINEK, and T. COOPER, Proc. Soc. exp. Biol. Med. *110*, 856 (1962).

[4] Z. M. BACQ and P. ALEXANDER, *Fundamentals of Radiobiology* (Pergamon Press, London 1961).

[5] J. R. CROUT, C. R. CREVELING, and S. UNDENFRIEND, J. Pharmacol. exp. Therap. *132*, 269 (1961).

However, if the catecholamine values are expressed on the basis of nitrogen content (Figure 2), in order to compensate for hydration due to experimental procedure, then the difference in myocardial concentration of catecholamine is minimized, the hepatic difference is sustained and the kidney catecholamine levels appear significantly decreased in all irradiated groups ($P = 0.05$).

Nitrogen contents of the specimens are graphically shown in Figure 3.

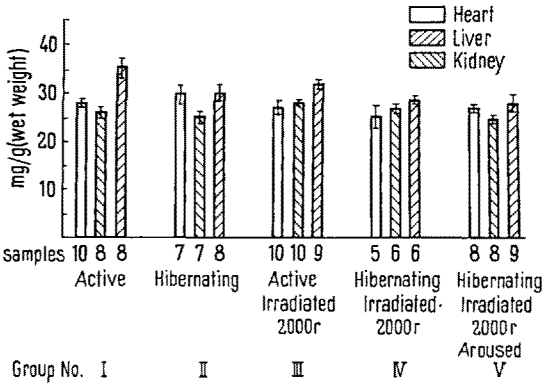

Fig. 3. Nitrogen contents of heart, kidney and liver in active and hibernating, irradiated and non-irradiated ground squirrels, *Citellus tridecemlineatus*.

Discussion. These results suggest that the changes in extra-adrenal catecholamine stores during the period following X-irradiation are associated with arousal, rather than direct effects of the irradiation. This conjecture is supported by reports that X-irradiation has been demonstrated to induce arousal from sleep[6]. In our experience hibernating ground squirrels, properly handled, do not ordinarily arouse in experimental circumstances such as these. However, they often display arousal following irradiation, despite the fact that they are maintained in cold hibernacula during and following X-irradiation.

The absence of additional gross changes in catecholamine storage in animals from Group V, during the 40 h period following radiation, is not surprising in view of the fact that lethal changes which appear subsequent to radiation require extended periods for manifestation in this species[7]. This view is supported by reports that sympathetic nerves, the alleged sites of catecholamine storage[8], are said to be radio-resistant[9].

The catecholamine content of the hearts tended to be lower as a result of irradiation (Group I vs. III; Group II vs. IV; Group I vs. V) although the differences do not prove statistically significant at the $P = 0.05$ level (Figure 1). It is of interest, however, that the decrease in myocardial catecholamine in the irradiated groups is not apparent when the results are expressed in relation to the nitrogen content of the same specimen (Figure 2), rather than the wet weight. This suggests that the observed decrease in concentration of catecholamine was not due to an absolute decrease in the store of the neuro-transmitter, but rather to an increase in water content of the specimen. These measurements emphasize the usefulness of expressing catecholamine results on the basis of nitrogen content, in addition to the conventional manner, μg/g wet weight.

It is not clear from these studies whether the decrease observed in the amount of catecholamine in the liver and kidney represents increased destruction or decreased formation, or merely increased utilization due to arousal. The results of these experiments point to the need for further analysis of factors influencing storage of catecholamines in extra-adrenal sites in hibernators, particularly during irradiation and arousal from hibernation.

Zusammenfassung. 20 h nach Bestrahlung mit letaler Röntgenstrahlendosis (2000 r) zeigten winterschlafende *Citellus tridecemlineatus* Herabsetzung der Konzentration von Catecholaminen in Leber und Niere. Werden Veränderungen des Wassergehaltes im Gewebe mitberücksichtigt, so erscheint der Gehalt an myokardialen Catecholaminen unverändert.

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[6] E. L. Hunt and D. J. Kimeldorf, Science *137*, 857 (1962).
[7] D. E. Smith, Bull. Mus. comp. Zool. Harv. *124*, 493 (1960).
[8] U. S. von Euler, *Noradrenaline* (Charles C. Thomas, Publisher, Springfield, Illinois 1956).
[9] J. Q. Griffith and E. P. Pendergrass, Radiology *23*, 463 (1934).

Immunoelectrophoresis of Soluble Proteins Isolated from Cellular Fractions of Regenerating Rat Liver

Soluble and insoluble proteins of rat liver subcellular fractions have been extensively studied by means of agar electrophoresis[1-6] and immunochemical techniques[7-14]. In this note, the immunochemical properties of soluble proteins isolated from rat regenerating liver cells have been studied.

Material and Methods. Mitochondrial and cytoplasmic liver proteins from partially hepatectomized rats have been prepared in the customary way[15]. Immune-sera against blood serum, liver mitochondrial and cytoplasmic soluble proteins were separately prepared in rabbits employing mixed antigens of normal and regenerating liver. Immunization outline was: a first intravenous injection

[1] M. Kessel, Naturwissenschaften *45*, 365 (1958).
[2] M. Kessel, Proc. 7th Coll. Bruges (Elsevier Publ. Comp., Amsterdam 1959), p. 55.
[3] M. Kessel, Clin. chim. Acta *4*, 142 (1959).
[4] D. Nachkov and O. Nachkova, Bull. Soc. Chim. biol. *1*, 159 (1959).
[5] I. Goronov and J. Todorov, Nature *184*, 64 (1959).
[6] S. Sorof, B. Claus, and P. P. Cohen, Cancer Res. *11*, 873 (1951).
[7] E. Clerici and P. Scalaffa, Lo Sperimentale *3*, 115 (1961).
[8] P. Vogt, Z. Naturforschung *4*, 213 (1960).
[9] P. Vogt, Nature *182*, 1807 (1958).
[10] P. Perlmann and V. D'Amelio, Nature *181*, 491 (1958).
[11] P. Perlmann and V. D'Amelio, Exp. Cell Res. Suppl. *7*, 279 (1959).
[12] P. Perlmann and V. D'Amelio, Exp. Cell Res. Suppl. *19*, 383 (1960).
[13] P. Jungblut, N. Heimburger, and F. Turba, Hoppe Seyler's Z. *314*, 250 (1959).
[14] W. S. Morgan, P. Perlmann, and T. Hultin, J. Biophys. Biochem. Cytol. *3*, 411 (1960).
[15] G. H. Hogeboom, *Methods in Enzymology* (Academic Press Inc., New York 1955), vol. 1, p. 16.