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.

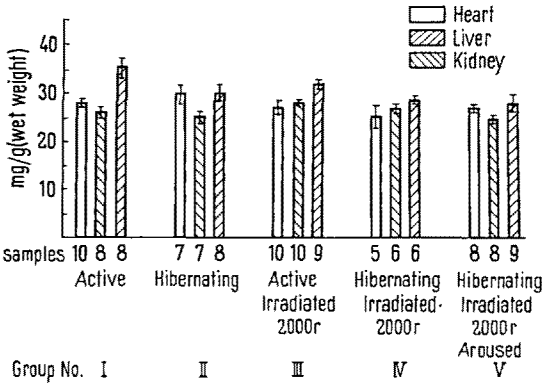

Fig. 3. Nitrogen contents of heart, kidney and liver in active and hibernating, irradiated and non-irradiated ground squirrels, *Citellus tridecemlineatus*.

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.

X. J. Musacchia, M. Jellinek, and Th. Cooper

Department of Biology and the Center for Cardiovascular Research, Saint Louis University, Saint Louis (Missouri, U.S.A), February 25, 1963.

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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

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of 50 mg of proteins was followed by seven subcutaneous injections (each of 10 mg of proteins with complete Freund's adjuvant) every 3 days.

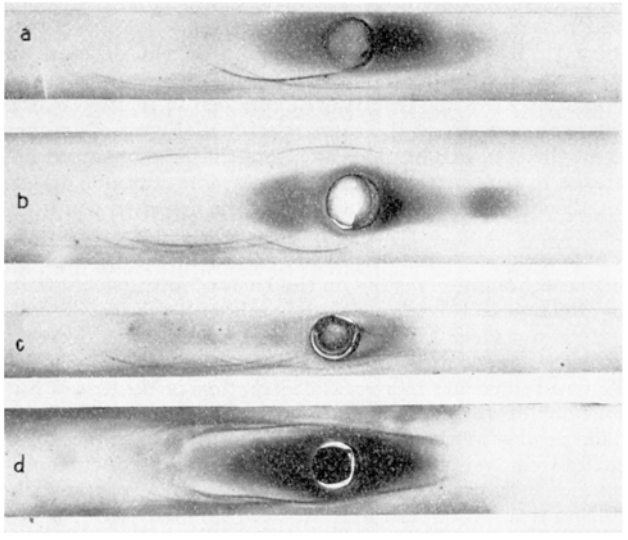

Fig. 1. Immunoelectrophorctic patterns of soluble proteins isolated from the mitochondrial and cytoplasmic fractions of regenerating liver cells.—Buffer: sodium diethylbarbiturate 0.065 *M*, HCl 0.017 *M*, pH 8.2, ionic strength 0.05 μ. Potential gradient: V/cm 3 (mA/cm 1.7) for 3 h, then V/cm 6 (mA/cm 3.5) for 5 h.—*a,b:* mitochondrial proteins and rabbit immunserum against mitochondrial proteins, absorbed by rat blood serum. *c,d:* cytoplasmic proteins and rabbit immunserum against cytoplasmic proteins, absorbed by rat blood serum and mitochondria. *a* and *c:* normal liver; *b* and *d:* regenerating liver.—Staining with red thiazine R and light green SF. Sense of migration from the left to the right.

35 days after the last administration, two intramuscular injections of 50 mg of proteins followed. The immune-sera were concentrated for γ-globulin content by saturated ammonium sulphate, dialyzed and stored at −60°C. Anticytoplasmic immune-serum was absorbed by normal rat serum and highly purified mitochondrial preparations; antimitochondrial one, by serum.

Results. A marked increase of the prealbumin and albumin-like[16] components has been observed in the rat regenerating liver by means of simple agar electrophoresis, according to our previous results[17]. The immunoelectrophoretic pattern of the cytoplasmic fraction showed, in normal rats, a various number of lines, from 7 to 10, according to results reported for total liver extracts[1,4,7]. According to [7] we have found 3 groups of lines: one in the prealbumin and albumin zone (A, B, C, D, Figure 2), a second in the α and β zone (E, F, G, H, I), the last in and after γ zone (L, M). In the regenerating liver A, B, D are frequently absent, G is always absent, while the line C and especially line H present a greater development. No differences have been observed for the arcs I, L, M.

In the mitochondrial fraction, the increase of the prealbumin and albumin-like components has been confirmed for the regenerating liver by means of simple agar electrophoresis, while the increase of the less dispersed protein fractions, as observed at a different time in guinea-pigs, did not yet appear. The immunoelectrophoretic pattern of the mitochondrial soluble proteins is characterized, in normal rats, by 8 different arcs, distributed in 3 zones; in the regenerating liver the most striking results were the appearance of a new line (no. 8) which is constantly absent in the normal patterns, and the different morphology of the line 4, which corresponds to the principal protein component of the normal mitochondria and shows a weak diffusion rate. The prealbumin and albumin-like components are weak antigens and do not give appreciable precipitation lines.

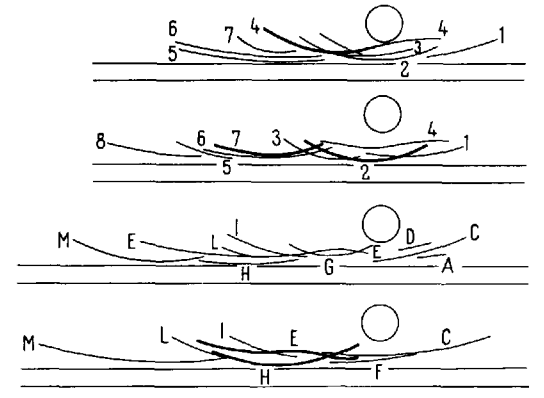

Fig. 2. Schematic diagram shown in the Figure 1. From the upper to the lower: normal liver mitochondria, regenerating liver mitochondria, normal liver cytoplasmic fraction, regenerating liver cytoplasmic fraction. Numbers and letters have been applied to the mitochondrial and respectively cytoplasmic arcs according to their situation in several patterns; therefore some of them may be lacking in a single run. In future reports, the same nomenclature will be used.

From the results reported above, it is interesting to note the increase of fast-moving protein components, both in the cytoplasmic and mitochondrial fraction of the regenerating liver. The appearance or the increase of a new component between β and γ zones in the cytoplasmic fraction of regenerating liver cells[18] (corresponding to disappearance of the arc G), and the greater development of the line H, could be explained by immunochemical changes of some liver globulins. In the mitochondrial fraction, in the same zone of G and H, is located the line 4, which takes a completely different aspect in the regenerating liver: probably this corresponds to a greater lability of a protein complex, present with the same antigenic determinants in different cell particles.

Detailed results on this subject will be reported elsewhere.

Riassunto. Gli autori hanno studiato, mediante elettroforesi su agar ed immunoelettroforesi, il comportamento delle proteine solubili isolate dalle frazioni citoplasmatica e mitocondriale del fegato rigenerante di ratto. Sono state rilevate alcune modificazioni delle proprietà immunochimiche di taluni componenti proteici.

P. P. GAZZANIGA, G. DI MACCO and F. R. SONNINO

Istituto di Patologia Generale and Istituto di Patalogia chirurgica della Università di Roma (Italy),
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