einer auf 37 °C erwärmten O_2-gesättigten Tyrodelösung bei einem Druck von 40–50 mm Hg durchströmt.

Phentolamin (1–2 mg/kg) in das isolierte Gefässgebiet 90 sec vor Bradykinin gegeben, schwächt die Vasokonstriktion um 40% ab.

Durch Vorbehandlung mit Dichlorisoproterenol (1–10 mg/kg) bzw. Nethalid (2–20 mg/kg) kommt es ebenfalls zu einer Verminderung der Vasokonstriktion durch Bradykinin um mehr als die Hälfte. Diese Befunde sind durchaus mit einer Katecholaminfreisetzung durch Bradykinin in Einklang zu bringen.

Dauerinfusion von Iproniazid (10 mg/kg/min) verändert die Reaktivität der Gefässe auf Bradykinin nicht. Kokain, das die Wirkung von indirekt wirkenden Sympathicomimetica abschwächt, die von direkt wirkenden Sympathicomimetica jedoch verstärken kann, führt in einer Dosierung von 10–20 mg/kg zu einer wesentlichen Steigerung der durch Bradykinin ausgelösten Gefässverengung; 8–10 min nach Gabe von Kokain ist sie nahezu verdoppelt. Guanethidin (15–20 mg/kg) ist fast ebenso wirksam wie Kokain und verstärkt den Bradykinineffekt. Schliesslich fanden wir an katecholaminverarmten Katzen (5 mg/kg Reserpin i.p. 24 h vor dem Versuch) die Vasokonstriktion unverändert und regelmässig auslösbar. Eine Zusammenfassung unserer Befunde ergibt die Tabelle.

Vasokonstriktion durch Bradykinin

	Einfluss	n
Phentolamin	–	4
Dichlorisoproterenol	–	8
Nethalid	–	6
Iproniazid	0	3
Kokain	+	9
Guanethidin	+	4
Reserpin[a]	0	3

– = Hemmung. + = Steigerung. 0 = Ohne Einfluss. n = Zahl der Versuche. [a] 24 h vor Versuch i.p.

Die Ergebnisse zeigen, dass unter unseren Versuchsbedingungen Bradykinin möglicherweise an adrenergen Strukturen des Gefäßsystems direkt angreift und die Vasokonstriktion in der Hinterextremität der Katze nicht durch Vermittlung von Katecholaminen zustande kommt. Ob diese Vorstellungen auch für andere Gefässgebiete zutreffen, wird von uns überprüft. Eine ausführliche Darstellung unserer Befunde ist an anderer Stelle vorgesehen.

Summary. The divided hindlimb of the cat was perfused through the femoral artery with tyrode solution. Synthetic bradykinin administered via the artery elicits a vasoconstriction. α- and β-sympathicolytics decrease the vasoconstriction, cocain and guanethedin emphasize it, whilst iproniacid and reserpin have no influence on it. It is assumed that bradykinin acts directly on adrenergic structures of the vessels of the hindlimb of the cat.

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Lysosomal Modifications after Administration of Radioprotective Drugs

The protective activity of sulfhydryl compounds is limited in time[1]. For most of them, the protective action is lost about 1 h after their administration; a large part of the drugs are catabolized in the liver and excreted in the kidney[2]. 20 min after injection of ^{35}S-tagged 2-β-aminoethylisothiourea bromide (AET), the amount of protein-bound ^{35}S is great but varies from one organ to the other[3]. Moreover, it has been observed that these sulfhydryl drugs are responsible for morphological changes of mitochondria and ergastoplasm, at least in the stem cells of duodenal crypts of mouse[4] and in the tubular cells of the rat[5]; these alterations are observed from 15–60 min after the administration of the drugs.

Recently, one of us[6] demonstrated the good protective activity of a mixture of glutathione, AET, and serotonin, which allows one to raise the LD 100/30 by a factor of 2.4. Since the fundamental mechanism of action of AET and glutathione does not seem different, it is assumed that the better protection obtained with this cocktail is due, in part, to the fact that glutathione allows more AET to become available in the cell for protection of radiosensitive structures.

In an attempt to follow the morphological action of this mixture on the stem cells of the duodenal crypt of the mouse, we have observed that the number and the size of some dense vacuoles increase greatly in the first hours following injection; a cytochemical study has shown that these structures contain acid phosphatase and may be considered as lysosomes.

Adult, fasting, male mice of BALB/C+ strain receive 16 mg glutathione per os; after 15 min, 8 mg of AET are injected intraperitoneally, followed 5 min later by 1 mg of serotonin. The animals are sacrificed at different time-periods after the last injection. The duodenum is fixed in osmium tetroxide 1%, embedded in Epon, and thin sections are examined in an EM 200 Philips electron microscope; for the cytochemical study, the fixation is carried out in glutaraldehyde 6%; frozen sections are incubated in a slightly modified Gomori medium for 15 min and post-fixed in OsO_4 2%. The present report deals with results obtained 3 and 24 h after the injection.

In the stem cells of the duodenum of the control mouse, no more than 3–4 dense bodies are observed per cell profile; these structures are loaded with acid phosphatase and are assumed to be lysosomes; a few Golgi vesicles present reaction product; no large vacuoles are encoun-

[1] Z. M. Bacq, *Chemical Protection against Ionizing Radiation* (Charles Thomas, Springfield, USA, 1965).
[2] B. Shapiro, E. Schwartz, and L. Hollmann, Radiat. Res. *19*, 17 (1963).
[3] L. Hollmann, B. Shapiro, and E. Schwartz, Radiat. Res. *20*, 17 (1963).
[4] J. Hugon, J. R. Maisin, and M. Borgers, C. r. Soc. Biol. *158*, 201 (1964).
[5] H. Firket, personal communication.
[6] J. R. Maisin, Nature *204*, 196 (1964).

tered[7]. 3 h after the injection of the radioprotectors, a great number of dense bodies with dark fibrillar content, interpreted as residual bodies[8], are observed in the mid-part of the cell above the nucleus (Figure 1); at 24 h, most of these bodies are dilated and their electron opaque material seems pushed onto the edge of the vacuole by a less dense product filling the structure (Figure 2); often dilated endoplasmic reticulum lies close to these vacuoles. In the sections treated for acid phosphatase activity, it appears that the number of reactive sites is much higher than in the controls; at 3 h, many Golgi vesicles show fine precipitate of lead phosphate; large vacuoles are also heavily charged with reaction product; they are situated in clusters of 3–5 bodies (Figure 3); these structures are similar to those observed after osmium fixation and may tentatively be considered as enlarged lysosomes.

The increase in number and the dilatation of lysosomes take place not long after the fading of the radioprotective action of the injected drugs. NOVIKOFF[9] has suggested that lysosomes are formed by a regional dilatation of ergastoplasm in which enzymes are concentrated. Now, the ultrastructural study of the early modifications of the

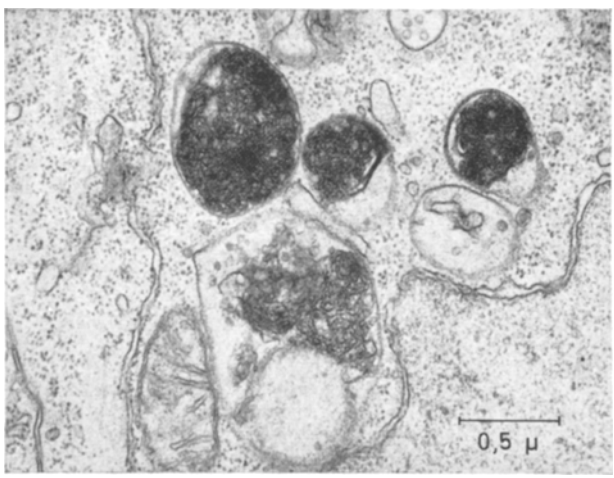

Fig. 1. Cluster of residual bodies and clear vacuoles in a stem cell of the duodenum, 3 h after the administration of radioprotective mixture. × 47,000.

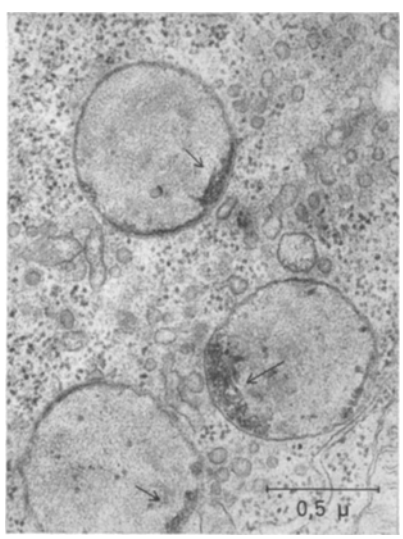

Fig. 2. Three dilated vacuoles with fibrillar remnants (arrow) 24 h after the administration of the drugs. × 58,700.

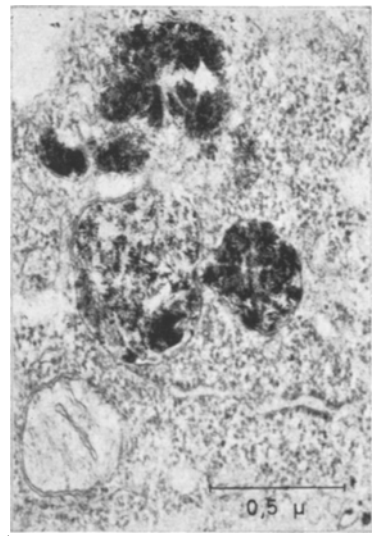

Fig. 3. Cluster of bodies containing heavy deposits of lead phosphate from the Gomori reaction, 3 h after the administration of the radioprotectors. × 73,200.

cells after injection of the radioprotective mixture has shown that endoplasmic reticulum is greatly dilated in the first hours[10]. It is possible that the radioprotectors and their metabolites, once liberated from the internal structures of the cell, are collected in special parts of the ergastoplasm and form vacuoles into which lysosomal enzymes are poured; the fate of the injected Triton W.R. 1339 seems similar[11].

These observations show, moreover, that radioprotective drugs are not easily metabolized by the cells. In the mixture studied, it is likely that the drug liable for the increased lytic activity is AET; neither glutathione nor serotonin, given separately, provokes similar lesions; but when AET is injected alone, a faint increase of dense bodies is noted after 3 h. These results seem also to be in good agreement with our hypothesis; an increasing amount of AET is retained in the cell after the injection of our mixture of radioprotective drugs[12].

Résumé. L'examen ultrastructural de l'épithélium de la crypte duodénale de souris, effectué à 3 et 24 h après l'injection d'un mélange de radioprotecteurs, a révélé l'existence de nombreuses inclusions vacuolaires. La présence de phosphatase acide les a fait considérer comme des lysosomes dilatés. Cette observation est discutée en fonction du temps d'action des substances radioprotectrices.

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Laboratoire de Microscopie Electronique. Radiobiologie, C.E.N.-S.C.K., Mol (Belgium), December 16, 1965.

[7] J. HUGON and M. BORGERS, J. Histochem. Cytochem. *13*, 524 (1965).
[8] C. DE DUVE, *Lysosomes* (Ciba Foundation Symposium, 1963), p. 1.
[9] A. NOVIKOFF, personal communication.
[10] J. HUGON, J. R. MAISIN, and M. BORGERS, Symposium on Radioprotective Drugs, Liège, June 1965.
[11] A. WATTIAUX, M. WIBO, and P. BAUDHUIN, *Lysosomes* (Ciba Foundation Symposium, 1963), p. 176.
[12] This work was done thanks to the contract A.I.E.A./C.E.N. No. 347 RB and thanks also to grants from the Fonds de la Recherche Scientifique Fondamentale Collective.