Taking into consideration the experimental results we have obtained with DMSO – a product that shows a capacity for tissue and cellular diffusion, as well as the capacity of modifying the stereoisomery of fatty acids – we have now reinforced our previous hypothesis of the lipoperoxide permeability of membrane.

Zusammenfassung. Es wird gezeigt, dass das DMSO die Möglichkeit hat, die *cis-cis*-Struktur der ungesättigten Fettsäure zu ändern. Diese strukturelle Veränderung, wenn sie in den gesättigten Fettsäuren benachbarten Molekülen stattfindet, bedingt die Bildung einer Pore. Sie hängt überdies zusammen mit der Veränderung, welche die Bildung von Lipoperoxyden bedingt und ihrer möglichen Rolle in der zellulären Permeabilität.

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Research Department of 'Laboratorio P.E.V.Y.A.', Molins de Rey, Barcelona (Spain), March 29, 1965.

The Influence of Continuous Irradiation and Experimental Diphtherial Intoxication upon the Origin and Incidence of Non-Specific Necrotic Changes in the Cardiac Muscle

In the course of our research work, concerning the interrelations between continuous irradiation and experimental diphtherial intoxication[1-3], an interesting phenomenon in the myocardium has been observed. This phenomenon is described in this communication.

Material and methods. The experiments were performed on albino Wistar rats of both sexes. The mean body weight at the beginning of experiments was 160 g.

80 rats were divided into 4 groups. The first group received a relatively small dose of the diphtherial toxin – 250 MLD/150 g body weight. The second group was represented by the rats which were only continuously irradiated. In the third group the dose of the diphtherial toxin was the same as in the first group, but the animals were pretreated with continuous irradiation. The rats in the fourth group were treated with a massive dose of the diphtherial toxin: 1000 MLD/150 g body weight.

The irradiation was continuous for 23–23 1/2 h a day. The daily dose was 90 R, total dose within 25 days 2250 R. The animals were kept in special cages in the γ-field in a semicircular area around the source of Co^{60}-type irradiation with an activity of 45 C. Not a single rat died in the course of irradiation.

The diphtherial toxin used in this experiment was submitted by Biogena, Prague; series HPA, Tk-5-6, titer 115 Lf, 2500 MLD in 1 ml of the stock solution. At the end of the first week, after irradiation was finished, all rats, both irradiated and non-irradiated, received diphtherial toxin intravenously into the right jugular vein. From all 80 rats, 40 animals were selected (10 rats from every group) for the evaluation of the myocardial lesions. The animals which died or were sacrificed, were all dissected and the organs were examined by means of stereoscopic section lens. Organ specimens were fixed in the 10% neutral formalin, and the cardiac tissue was stained using acid fuchsin method. Other organs were stained with hematoxylin eosin and sudan III. These results are reported elsewhere[1-3]. The histological evaluation of the myocardial tissue was performed from several sections of every organ according to the arbitrary scale 0–3.

Results. The characteristic feature of the lesions in the cardiac muscle was the incidence of minute necroses in the myocardial fibres. These necroses were characterized by the accumulation of a strongly fuchsinophilic material in the myocardial fibres, and in the 3rd and 4th group were accompanied by the simultaneous occurrence of large foci of infarctoid necroses. The appearance of a strongly fuchsinophilic material in the sarcoplasm between the myofibrils is the earliest detectable change in the necrotizing myocardium. This change usually begins in the immediate vicinity of nuclei and then tends to spread, so that larger portions of a muscle fibre are transformed into a more or less hyaline, strongly fuchsinophilic mass. As the lesion progresses further, the nucleus becomes indistinct and eventually disappears, while an entire segment of the muscle fibre, still sharply delimited by sarcolemma and intercalated discs, is completely transformed into a fuchsinophilic tube. The fuchsinophilic material is characteristic only for the initial stages of cardiac lesion. It disappears completely from muscle fibres which have totally disintegrated and are in the process of being absorbed. Most affected was the cardiac tissue in the 4th group, where massive doses of diphtherial toxin were administered. The incidence of the lesions was slightly reduced in the 3rd group, where the animals received relatively small doses of the diphtherial toxin, but were pretreated by continuous irradiation. In the second group, where the animals were treated only with continuous irradiation, the occurrence of necrotic lesions could also be confirmed. Finally, in the first group (small dose of the diphtherial toxin) the lesions observed are practically unimportant, considering the fact that some small degree of incidence of these lesions may be found even in the normal, intact cardiac tissue. The findings are summarized in the Table and in Figure 1, and the description of the necrotic cardiac lesions in the text is illustrated by the appearance of the cardiac tissue, as shown in Figures 2 and 3.

Discussion. If we have to explain the pathogenesis of the large, infarctoid foci in the myocardium of our experimental rats, then it is necessary to pay attention to the simultaneous incidence of these minute, miliary necroses in the cardiac tissue. Under these conditions we assume the pathogenesis of infarctoid lesions in the myocardium as a non-specific stress process, which is initiated by the disturbance of the electrolyte metabolism in the isolated fibres of the cardiac muscle. This may be caused by the direct action of the diphtherial toxin upon the electrolyte

[1] J. Vašků, M. Praslička, and E. Urbánek, Rev. Canad. Biol., in press.
[2] J. Vašků, E. Urbánek, M. Praslička, and O. Chlebovský, Z. inn. Med., in press.
[3] E. Urbánek, J. Vašků, O. Chlebovský, and M. Praslička, Radiation Res., in press.

cardiac metabolism on the one hand, and by the enhanced secretion of the autologous corticosteroids under the influence of the diphtherial toxin on the other hand. It is a well known fact that the essential component in the mechanism of the diphtherial intoxication is the stimulation of the hypophyseal and adrenal activity[4]. Thus, in this respect this cardiac necrotizing process is very similar to the pathogenesis of the electrolyte and steroid cardiopathies (ESCN) with necroses of SELYE and BAJUSZ[5–8]. The microscopic evidence of miliary, minute necrotic lesions was found by SELYE in all animals treated with $NaHSO_3$, Na_2CO_3, sodium acetate and sodium urate, despite the almost uniformly negative autopsy findings. But the necrotic process did not proceed further unless the animals received some cortisol treatment (Me-Cl-COL or F-COL). In the rats treated in this way, various stresses tend to increase this disseminated fibre necrosis and they may even produce large miliary necrotic foci detectable with the dissecting lens. Thus the situation is very similar to the observed non-specific action of the

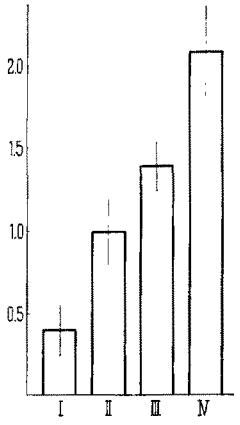

Fig. 1. The incidence of myocardial lesions. 0.5–2 indicates the mean values of lesions. I, Small dose of the diphtherial toxin only. II, Continuous irradiation only. III, Continuous irradiation and subsequent diphtherial intoxication with 250 MLD/150 g body weight. IV, High dose of the diphtherial toxin – 1000 MLD/100 g – only.

[4] E. TONUTTI, Symposium on the Mechanism of Inflammation, Montreal (September 1953).
[5] H. SELYE, *The Chemical Prevention of Cardiac Necroses* (The Ronald Press Company, New York 1958).
[6] H. SELYE, *The Pluricausal Cardiopathies* (Charles C. Thomas, Springfield 1961).
[7] E. BAJUSZ, *Conditioning Factors for Cardiac Necroses* (S. Karger, Basel-New York 1963).
[8] E. BAJUSZ, *Nutritional Aspects of Cardiovascular Diseases* (Crosby, Lockwood and Son Ltd., London 1965).

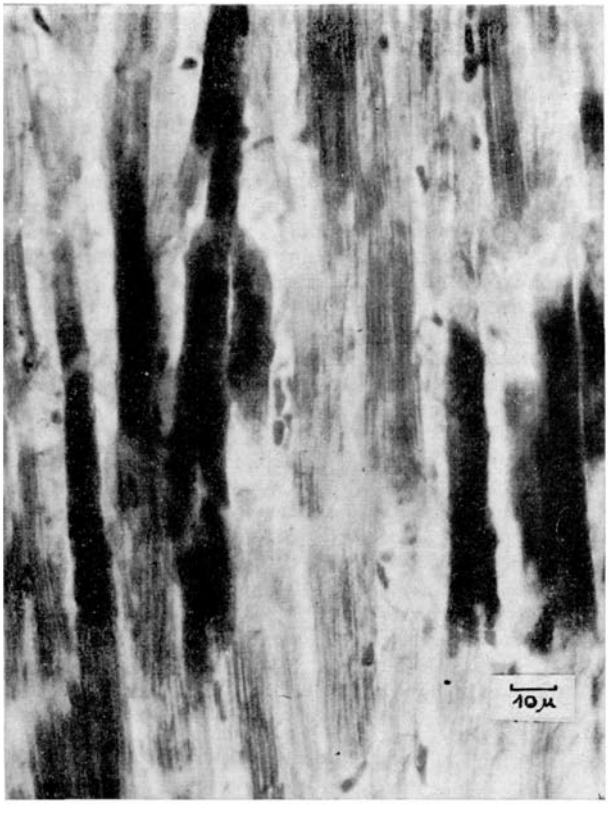

Fig. 2. Appearance of fuchsinophilic degeneration in the heart of a rat (No. 62), treated with a high dose of the diphtherial toxin (group IV). Fuchsinophilia of the isolated myocardial fibres is regarded as early index of myocardial damage. Acid Fuchsin stain, × 550.

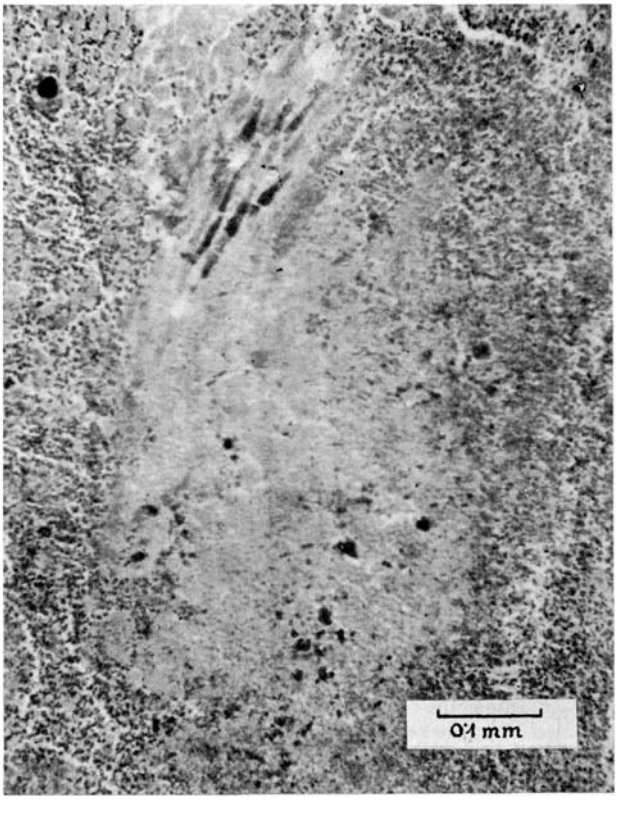

Fig. 3. Necrotic patch in the heart of a rat (No. 5), pretreated with continuous irradiation and then subsequently treated with a small dose of the diphtherial toxin (250 MLD/150 g). In this stage of necrosis, fuchsinophilia in the heart fibres disappears. Hematoxylin-eosin, × 135.

Myocardial lesions in the rats A–K of the groups I–IV

Group	A	B	C	D	E	F	G	H	I	K	Mean	S.E.	Statistical significance
I, Diphtheria toxin (250 MLD/150 g)	1	1	0	0	0	1	0	0	0	1	0.4	0.16	
II, Irradiation	1	1	1	0	1	1	1	0	2	2	1.0	0.20	$0.02 < P < 0.05$
III, Irradiation and diphtheria toxin (250 MLD/150 g)	1	2	1	2	1	1	2	1	2	1	1.4	0.16	$P < 0.001$
IV, Diphtheria toxin (1000 MLD/100 g)	1	1	3	2	3	2	3	2	3	1	2.1	0.28	$P < 0.001$

diphtherial toxin upon the myocardium. It is further shown that continuous irradiation, which was previously administered, sensitizes the cardiac tissue for incidence of these miliary fibre necroses, which can, under such conditions, be elicited by much smaller doses of diphtherial toxin. The non-specificity of these lesions is further documented by the observation of ZHDANOV[9], who has established the miliary fuchsinophilic fibre necroses in cases of hypertensive disease, regardless of the degree of coronary atherosclerosis[10].

Zusammenfassung. Grosse Gaben von Diphtherietoxin ohne vorhergehende chronische γ-Strahlung rufen vielfache kleine fuchsinophile Nekrosen in den Fasern des Myocards hervor. Eine solche chronische γ-Strahlung sensibilisiert das Myocard gegen das Auftreten von Nekrosen vom selben Typus. Dabei erweisen sich kleine Dosen von Diphtherietoxin als pathogen völlig ausreichend, indem sie kleine Brennpunkte der Schädigung bilden und Herde infarktoider Nekrosen verschiedenen Ausmasses.

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[9] V. S. ZHDANOV, *Arterial Hypertension.* The Collection of Works, 15th Scientific Session of the Institute of Therapy, Academy of Medical Sciences USSR (January 23–24, 1964).

[10] The authors wish to express their sincere thanks to Mrs. V. HADERKOVÁ, A. PEKÁRKOVÁ, and H. URBÁNKOVÁ for their excellent technical assistance.

Caractérisation de la β-galactosidase d'*Helix pomatia* par immunoélectrophorèse

Le suc digestif d'*Helix pomatia* possède une importante activité hydrolysante à l'égard du lactose et des galactosides synthétiques[1]. Il semblait donc intéressant de lui appliquer la méthode de caractérisation des enzymes par des réactions spécifiques après immunoélectrophorèse, afin de dénombrer et de caractériser les protéines responsables de cette action.

Dans une expérience préliminaire, le suc digestif est soumis à une électrophorèse à pH 8,6 sur cellogel (tampon véronal sodique, $\mu = 0{,}05$, 90 min, 20 V/cm, + 4°C). La bande est ensuite partagée longitudinalement: une moitié est colorée par l'amidoschwartz (Figure 1), l'autre est découpée en segments de 5 mm de largeur. Après élution de chaque segment, l'activité hydrolytique sur le *o*-nitrophényl-β-D-galactoside (ONPG) est dosée selon la technique de CONCHIE et al.[2]. Si la complexité protéique du suc entraîne une mauvaise résolution électrophorétique, l'activité enzymatique se répartit cependant en trois pics distincts: l'un migre sensiblement comme les γ-globulines du sérum, un second plus élevé au niveau des α_2-globulines et un troisième très faible, de migration plus rapide (Figure 1).

L'ONPG ne peut être utilisé pour la caractérisation après immuno-électrophorèse, car le nitrophénol libéré après hydrolyse est soluble et diffuse rapidement à travers la gélose.

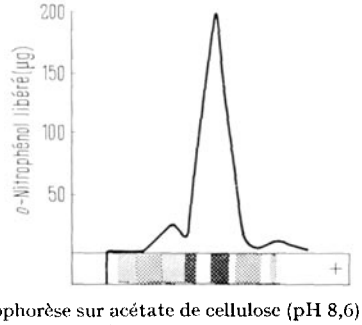

Fig. 1. Electrophorèse sur acétate de cellulose (pH 8,6) du suc digestif d'*Helix pomatia*. Révélation des protéines par l'amido-schwartz et courbe de répartition de l'activité sur l'ONPG après électrophorèse.

[1] R. GOT, A. MARNAY, P. JARRIGE et J. FONT, Nature *204*, 686 (1964).

[2] J. CONCHIE, J. FINDLAY et G. A. LEVVY, Biochem. J. *71*, 319 (1959).