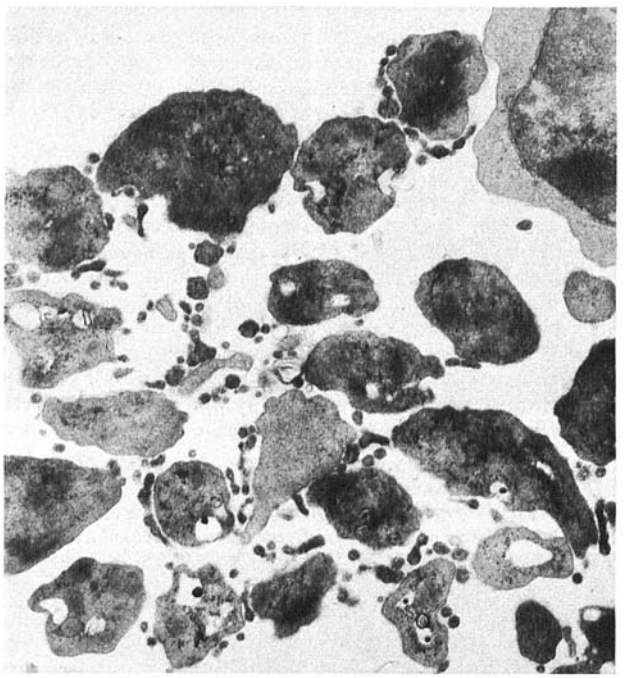

Fig. 1. Different forms of agent particles loosely attached to several platelets. Some of the platelets show degranulation and reduced density. Very dense bodies are seen within some platelet vacuoles. Part of a lymphocyte is seen in the upper right corner. × 7,500.

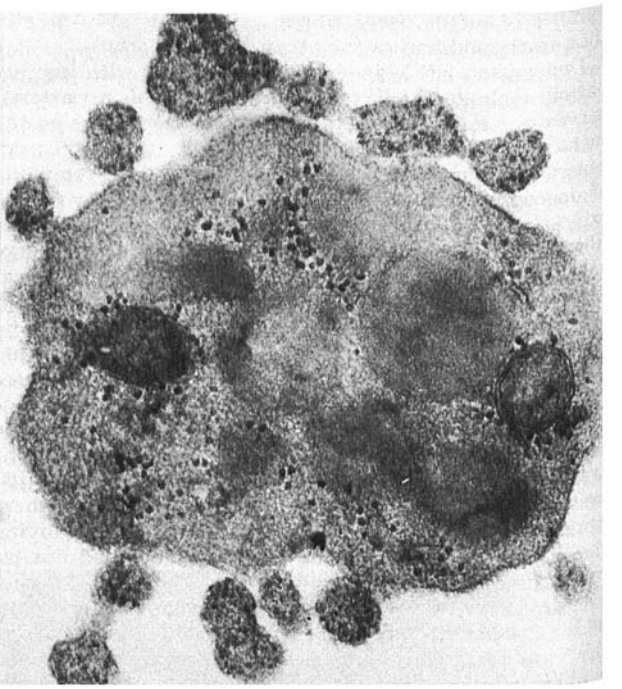

Fig. 2. Agent particles attached to a platelet. A chain form with 4 'beads' is seen above the platelet. Below this platelet, to the left of centre, a particle has almost completed its division. Ribosome-like granules are dispersed in the ground substance of the particles. The particles are bounded by a plasma membrane. × 46,000.

The apparent specificity of the agent-platelet association, if confirmed, would mean discovery of a previously unrecognized relationship between microorganisms and host cells.

Zusammenfassung. Bei einem den bovinen Thrombozyten anliegenden Mikroorganismus werden elektronenmikroskopisch beschrieben: Polymorphe 1.5 · 0.4 μ messende Gebilde mit feiner Plasmamembran an der Oberfläche und ribosomenähnlichen Strukturen im Innern, die Ähnlichkeit mit Mycoplasma, Eperythrozoen und Haemobartonellen zeigen.

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The Electron Microscope Laboratory, University of Helsinki, Helsinki (Finland), October 17, 1966.

Correlation Between Functional and Morphological Heart Changes Due to Isoproterenol

In experiments concerning isoproterenol-induced heart necroses (LESZKOVSZKY and GÁL, to be published) the question was raised whether some alterations could be detected in the hearts damaged with isoproterenol before the appearance of histologically detectable necroses. From this point of view the observation of ECG tracings seemed not to be an appropriate method, since it is known from HILL et al.[1] that ECG changes caused by isoproterenol-induced heart necroses are considerably less marked than those due to myocardial infarctions of dietary origin. More was expected from a test with i.v. administered vasopressin, which induces hypoxia and ischaemia of cardiac muscle with corresponding ECG changes (elevation of T waves). The incidence of this phenomenon depends on the dose of vasopressin; thus dose-effect curves can be plotted in this way (TARDOS and LESZKOVSZKY[2]). Isoproterenol-induced heart necroses are generally believed to be the consequence of myocardial ischaemia, so it can be expected that the animals' sensitivity to a vasopressin-induced hypoxic-ischaemic state could be altered without the development of morphological alterations. Therefore it was examined whether the sensitivity of rats to vasopressin was increased by doses of isoproterenol smaller than those inducing histologically detectable necroses.

Male albino rats of an inbred colony weighing 150–250 g were used in the experiments. The animals were given

[1] R. HILL, A. N. HOWARD, and G. A. GRESHAM, Brit. J. exp. Path. *41*, 633 (1960).

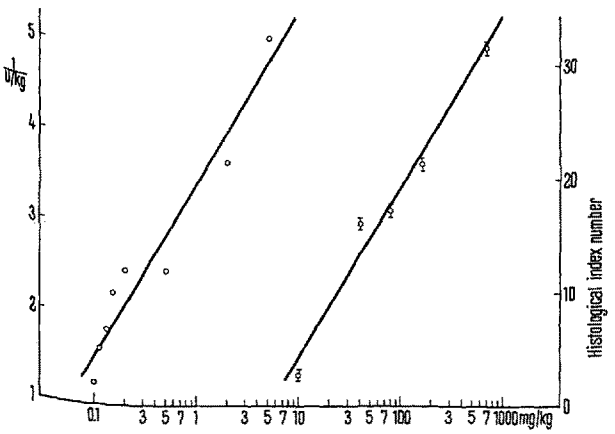
[2] L. TARDOS and G. LESZKOVSZKY, Archs int. Pharmacodyn. Thér. *145*, 293 (1963).

s.c. injections of the same dose of D,L-isoproterenol hydrochloride freshly dissolved in isotonic saline in a volume of 0.2 ml/100 g body weight. 24 h passed between the 2 injections and tests were performed 24 h after the second injection. The effective doses (ED_{50}) of vasopressin causing myocardial ischaemia (i.e. causing T-wave elevation of at least 1 mm) were determined according to the method of LITCHFIELD and WILCOXON[3] on groups of 40 rats treated with the same dose of isoproterenol. Vasopressin was administered in the form of a posterior pituitary extract (Glanduitrin® injection 10 IU/ml; Richter, Budapest) diluted with isotonic saline to the volume of 0.2 ml/100 g body weight. Morphological changes were evaluated in paraffin sections stained with hematoxylin and eosin by means of a quantitative histological method developed by us (GÁL et al.[4]). The rats were sacrificed by ether over-anaesthesia 24 h after the second injection of isoproterenol.

The sensitivity of the rats to myocardial hypoxia is increased by pretreatment with isoproterenol: the doses of Glanduitrin required to produce appropriate ECG changes are considerably decreased. The ED_{50} value in rats treated with $2 \cdot 0.2$ mg/kg isoproterenol is already significantly smaller than that in non-pretreated controls. Detailed results are shown in the Table.

Isoproterenol mg/kg s.c.	Glanduitrin, U/kg ED_{50}	Fiducial limits
	0.86	0.51 –1.45
$2 \cdot 0.10$	0.83 (0.68 / 0.98)	0.402–1.150[a] / 0.617–1.560[a]
$2 \cdot 0.11$	0.62	0.386–0.998[a]
$2 \cdot 0.13$	0.54	0.302–0.966[a]
$2 \cdot 0.15$	0.45	0.237–0.856[a]
$2 \cdot 0.2$	0.40	0.238–0.671[b]
$2 \cdot 0.5$	0.40	0.247–0.647[b]
$2 \cdot 2$	0.272	0.185–0.400[b]
$2 \cdot 5$	0.198	0.124–0.314[b]

[a] $p > 0.05$ as compared with the control group. [b] $p < 0.05$ as compared with the control group.



Dose-effect curves of sensitivity to vasopressin (left) and histopathological changes (right) as the functions of the 2 s.c. injections of isoproterenol.

The changes in the sensitivity to vasopressin are shown graphically in the Figure as the function of the 2 doses of isoproterenol injected into the rats. Doses of isoproterenol are plotted on a logarithmic scale and the sensitivity to vasopressin is expressed by the reciprocal value of the effective dose (ED_{50}) of Glanduitrin. By this means a dose-effect curve proportional to the increases in log doses is obtained. Its mathematical formula is as follows: $Y = 1.8875 \log x + 3.4400$. The dose-effect curve of the morphological alterations based on the mean values and S.E. of the index numbers of severity counted in the different groups is demonstrated in the same figure. $2 \cdot 10$ mg/kg of isoproterenol already caused well-marked necroses; with increasing doses their severity increased, the equation of the curve being $Y = 15.0647 \log x - 10.8089$.

The 2 curves presented in the Figure are nearly parallel but there is a considerable horizontal shift between them. The slope of the curve of the morphological changes begins at $2 \cdot 10$ mg/kg; the sensitivity to hypoxia is already maximal below this dose level. Thus, our results suggest that small doses of isoproterenol, which do not induce detectable histological changes, do cause some damage to the myocardium. So this test with vasopressin injection seems to be a more sensitive method for detecting the pathological state of the heart than the histological examination.

According to BEZNÁK and HACKER[5] histological changes in the myocardium precede the functional disturbances studied by them. Nevertheless, by adequate tests, e.g. with i.v. administered vasopressin, functional disturbances can be detected without the development of histological changes. This is in accordance with the ideas explaining the pathogenesis of isoproterenol-induced myocardial necroses by ischaemia (RÓNA et al.[6], ROSENBLUM et al.[7]) and with results of measurements of metabolism and heart rate of isoproterenol-treated rats (STRUBELT and BREINING[8]).

Zusammenfassung. Intravenös verabreichtes Vasopressin (Hypophysen-Hinterlappen-Extrakt) verursacht hypoxisch-ischaemische EKG-Veränderungen an mit Isoproterenol vorbehandelten Ratten (wesentlich kleinere Dosen als bei Normaltieren). Dieser Vasopressin-Test erlaubt den Nachweis einer herzschädigenden Wirkung von Isoproterenol bereits mit Dosen, die noch zu keiner histologisch erkennbaren Veränderung (Nekrose) des Myokardiums führten.

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Pharmacological Research Laboratory, Chinoin Pharmaceutical and Chemical Works, Budapest (Hungary), September 9, 1966.

[3] J. T. LITCHFIELD JR. and F. WILCOXON, J. Pharmac. exp. Ther. *96*, 99 (1949).
[4] G. GÁL, G. LESZKOVSZKY, and J. LENDVAI, Medna Pharmac. exp. *14*, 563 (1966).
[5] MARGARET BEZNÁK and P. HACKER, Can. J. Physiol. Pharmac. *42*, 269 (1964).
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[8] O. STRUBELT and H. BREINING, Arzneimittel-Forsch. *14*, 1196 (1964).