similarly and simultaneously prepared did not display such changes.

In vitro studies of the heart muscle indicate that high concentrations of alcohol have a direct toxic effect on the membrane potential and the contractile system[8,9]. Loss of enzymes and electrolytes[10,11] from the myocardium of chronic alcoholic patients suggests that ethanol affects the permeability and metabolic pathways of the myocytes. It is thus possible that the structural changes of the intercalated disc are secondary to the metabolic changes induced by chronic ethanol consumption.

The pathogenesis or the effects of the alterations of intercalated disc on myocardial function is unknown. However, in some cardiomyopathic states of man[6] and in chronically exercised rats[5] the nonspecialized region of the intercalated disc was found to be swollen. Macula occludens or nexus has been considered as the region of lowest electrical resistance allowing electrotonic coupling between the myocardial cells[3]. What effect the changes of the intercellular space in adjacent portions of the intercalated disc have on the electrophysiological properties of macula occludens is not known. In any event, it is possible that degenerative changes in the structure of intercalated disc may affect the normal spread of excitation impulse in the heart. Interestingly, a variety of electrocardiographic alterations in association with alcoholic heart disease of man has been reported[12,13].

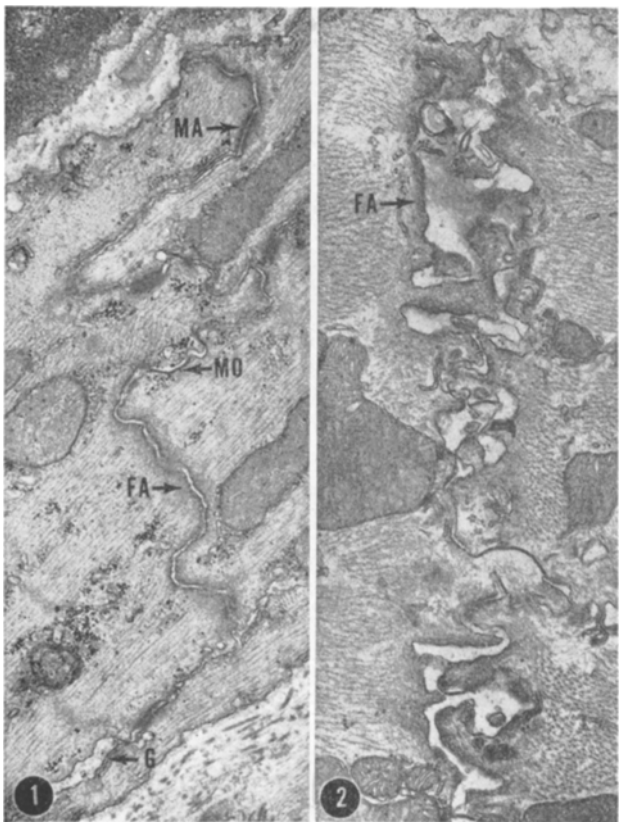

Fig. 1. Intercalated disc from the myocardium of a control mouse showing the 4 types of intercellular contact: macula adherens (MA); macula occludens (MO); fascia adherens (FA); and nonspecialized region (G). × 34,000.

Fig. 2. Intercalated disc of the myocardium of a mouse on ethanol ingestion. Note the vesiculation of fascia adherens (FA). × 23,400.

Résumé. L'examen au microscope électronique du disque intercalaire dans le tissu cardiaque de la souris, après administration orale d'alcool éthylique, ont révélé une augmentation de la distance intercellulaire. Le couplage électrotonique entre les cellules du myocarde est assuré par le disque intercalé. Par conséquent, des changements dégénératifs survenus dans le disque intercalaire diminuent la propagation de l'impulsion excitatrice dans le tissu cardiaque.

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The Effect of Various Beta-Receptor Blocking Agents on Platelet Aggregation

In 1960 Hellem found that a protein-free, heat-stable, dialysable extract of red cells could produce marked platelet aggregation in vitro[1]. This extract, or 'factor R' as Hellem called it, was subsequently identified as adenosine diphosphate (ADP) by Garder et al.[2].

ADP was found to be the most active of a number of nucleotides, lipids, catecholamines and other substances tested for their aggregating activity[3,4].

In recent years an increasing number of substances has been found – besides those with aggregating properties – that inhibit platelet aggregation in vitro. Among these substances the β-receptor blockers seem to be of particular interest, as they are used increasingly in the treatment of heart diseases.

In the following we have explored the inhibitory action of four β-receptor blockers on adenosine-diphosphate induced platelet aggregation.

Method. Blood was obtained from healthy volunteers in fasting condition by punction of an antecubital vein and collected in siliconized (silicone oil: AK 350) glass tubes. Nine parts of blood were added to one part of 3.8% trisodium citrate as anti-coagulant. Platelet-rich

plasma (PRP) was prepared by slow centrifugation at 250 *g* for 10 min, whereas platelet-poor plasma (PPP) was obtained by centrifugation at 2000 *g* for 20 min.

The platelets were counted in the phase-contrast microscope according to BRECHER and CRONKITE's method[5], but a white-blood-cell haemocytometer pipette was substituted for the red-cell pipette.

Platelet aggregation was measured in adaptation of BORN and CROSS' method[6] as follows: 3 ml PRP were filled into a cuvette of 10 mm layer thickness and the light absorption measured with a Zeiss Photometer Elko III at a wave length of 610 nm as compared with PPP. At a constant temperature and over a longer period of time there was no fluctuation in the optical density of the plasma. According to BORN and CROSS and to our own findings there is a linear correlation between platelet concentration and light absorption at this wave length. In order to carry out all measurements at fairly the same level of absorption, PRP was diluted with PPP if necessary and thus the platelet concentration was maintained between 250–350,000/mm³.

At the onset of the measurements, adenosine diphosphate (ADP) (trisodium salt of adenosine-5′-diphosphate, C. F. Böhringer-Söhne) was added to PRP up to a final concentration in the cuvette of 0.5 µg/ml. The optical density was first recorded every minute and later every 5 min after having covered the cuvette with 'Parafilm' and having overturned it sharply 6 times.

In order to study the influence of the various β-receptor blocking agents 0.1 ml of the dissolved substance was mixed with 2.9 ml PRP in the cuvette itself and incubated at room temperature for 30 min before adding ADP. In a control experiment a standard value with saline was obtained.

β-receptor blocking agents. Each of the 4 β-receptor blockers tested – propranolol, I.C.I. 50,172, LB-46 (Sandoz) and Ba-39,089 (CIBA) – has the characteristic basic side-chain. The structural formulae are shown in Figure 1. Propranolol (Inderal) has been used for several years in the treatment of arrhythmias and of angina pectoris. Unfortunately this drug is known to have a considerable depressant effect on myocardial function[7–9] and therefore its application is limited. At an equal dosage I.C.I. 50,172 shows no fall in cardiac output concomitant with the reduction of the heart rate as compared with propranolol, nor is there a rise in pulmonary artery pressure[10,11]. LB-46 (Sandoz) is characterized by an advantageous relation between the desired β-receptor blockade and the circulatory depressant effect[12,13]. Ba-39, 089 (CIBA) also features a more favorable relation between anti-arrhythmic and negative inotropic action, as pharmacological and clinical tests have shown[14,15].

Results. During the incubation of PRP with β-receptor blockers no change in optical density could be observed. The addition of ADP produced the typical aggregation and dis-aggregation curve for all substances, variations depending on the degree of concentration. Figure 2 shows the mean values of 20 curves for saline, propranolol, Ba-39, 089 and LB-46. For statistical purposes an analysis of variance (F-test) was performed. At the given concentration all 3 substances deviate considerably from the saline value after the third minute. This deviation may be interpreted as accelerated disaggregation. Difference between NaCl and propranolol (30 µg/ml) significant from 4th to 60th min ($P < 1\%$). Difference between NaCl and propranolol (6 µg/ml) insignificant. Difference between NaCl and LB-46 (6 µg/ml) significant from 4th to 60th min ($P < 5\%$). Difference between NaCl and Ba-39,089 (70 µg/ml) significant from 3rd to 60th min ($P < 1\%$). Difference between NaCl and Ba-39,089 (30 µg/ml) only significant in the 60th min ($P < 5\%$).

Ba-39,089, in a $2^1/_2$ times higher concentration, has the same inhibitory action as propranolol, whereas LB-46 still inhibits platelet aggregation at a concentration 5 times weaker than propranolol. At the same concentra-

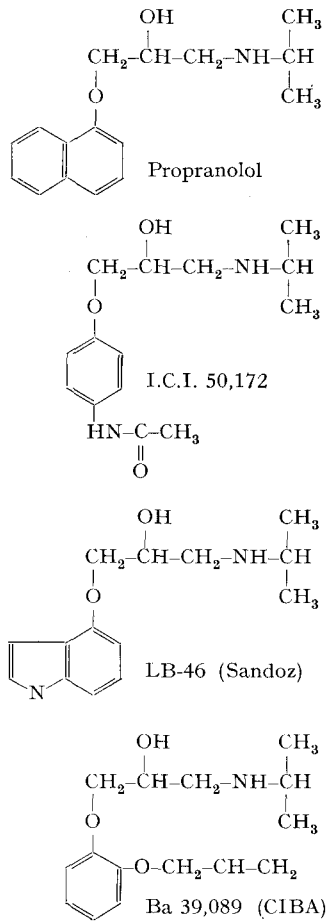

Fig. 1. The structural formulae of propranolol, I.C.I. 50,172, LB-46 und Ba-39,089.

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tion as LB-46, i.e. 6 µg/ml, propranolol showed no significant inhibitory action during the 65 min observation period. Furthermore, Ba-39,089, at a concentration equal to that of propranolol (namely 30 µg/ml), deviated significantly from the control value only after 60 min (Figure 3).

On the other hand I.C.I. 50,172, which, at the same concentration as propranolol, proves to have a comparable β-receptor blocking action in vivo, showed no influence of ADP-induced platelet aggregation in vitro whatsoever.

Discussion. Adding adenosine diphosphate to a platelet suspension produces a characteristic and well-reproduceable clumping of the platelets. This two-phase process can be observed directly by measuring the optical density of the plasma at 610 nm. The first phase begins immediately after addition of ADP and consists in clumping the platelets; it reaches its climax 3–5 min later. The intensity of this process, which is also known as platelet aggregation, depends on the degree of ADP concentration in this mixture[6]. Immediately thereafter the dis-aggregation phase takes place and lasts for about 1 h. Our experiments confirm the results of HAMPTON et al.[16], who were able to trace an effect on the in vitro-behaviour of platelets in a series of vasoactive substances, one of which being propranolol.

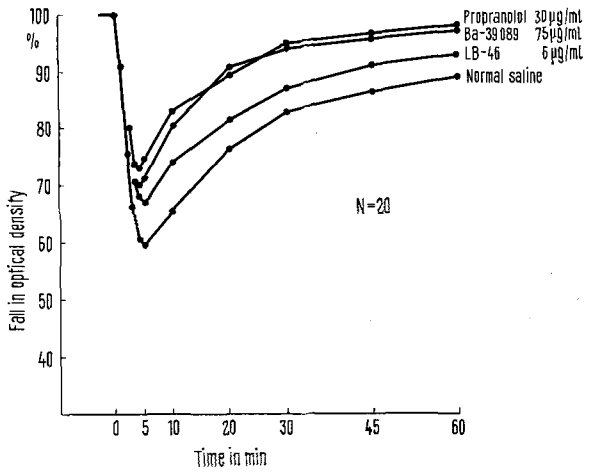

Fig. 2. Mean percentage fall in optical density produced by adding ADP to platelet-rich plasma in presence of 3 different β-blocking agents compared with normal saline control.

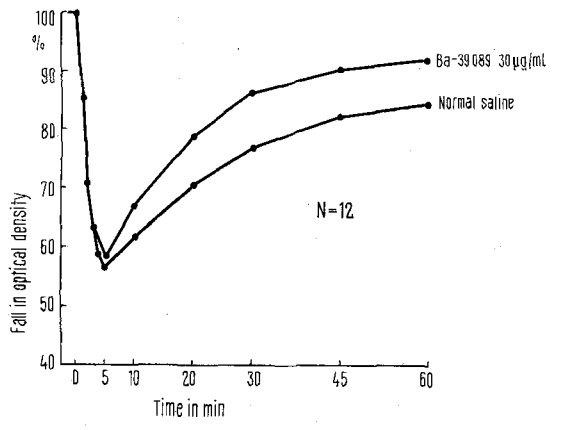

Fig. 3. Mean percentage fall in optical density produced by adding ADP to platelet-rich plasma in presence of Ba-39,089 (30 µg/ml) compared with normal saline control.

Of the 4 substances considered to be β-receptor blocking agents because of their chemical structure and for pharmacological reasons, propranolol, LB-46 and Ba-39,089 proved to inhibit ADP-induced platelet aggregation in vitro to a significant degree. The only difference between the various substances is found in the relation between dose and effect. The activity of LB-46 on platelet aggregation in vitro seems to be 5 times higher than that of propranolol, whereas Ba-39,089 is only half as effective.

The figures clearly show that the addition of an aggregation-inhibiting substance does not prevent the clumping in itself but enhance dis-aggregation. This leads to the conclusion that the bonds between the individual platelets which produce clumping in vitro under the influence of ADP are either weaker or of shorter duration. HAMPTON et al.[16] found that ADP and catecholamines increase the negative charge of the platelets. They also observed that this rise in negative charge is reversed by various substances, one of which being propranolol. Such facts favour the assumption that the β-receptor blocking agents possibly attack the cell membrane directly in vitro and thus modify the flow of ions. As a result the elimination of a platelet clumping agent from the cells or their surface would be easier.

Until today there are no indications that these findings could be of direct clinical importance, i.e. that the administration of β-receptor blockers would influence the role of blood platelets in the formation of thrombi. Nonetheless, HAMPTON et al. were able to prove that after oral administration of propranolol the circulating platelets show no more increase in negative charge after having been treated with ADP or noradrenaline[16]. These observations indicate that drugs decisively influence the in vitro behaviour of platelets, a fact which should be taken into account while studying platelet function in patients with vascular disease[17,18].

Zusammenfassung. Vier betarezeptorenblockierende Substanzen wurden auf ihre Fähigkeit untersucht, die Adenosindiphosphat-induzierte Thrombozytenaggregation in vitro zu hemmen. Dabei erwies sich die Dosis-Wirkungs-Relation bei LB-46 (Sandoz) am günstigsten, indem diese Substanz eine etwa fünfmal stärkere Aktivität als Propranolol aufweist. Ba-39 089 ist gegenüber Propranolol etwa zweimal weniger wirksam. Für I.C.I. 50 172 konnte bei gleicher Dosierung wie Propranolol überhaupt keine Hemmung festgestellt werden.

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