

solved by using a 95% glycerol solution to provide and regulate a low humidity within the exposure box. About 200 ml of the solution is placed in a container filled with cotton. The container is then placed in the exposure box. Under these conditions the humidity drops from about 80% to 30% in a day and is held constant at 30%. Pre-drying and excessive handling is eliminated.

Following exposure the radioautographs were developed by the method of KOPRIWA and LEBLOND<sup>2</sup>. The slides were usually stained with hematoxylin<sup>2</sup> or a saturated solution of indio-carmin. When mitotic cells were to be observed, the radioautographic slides were stained with a basic fuchsin and picro-indigo carmine staining procedure<sup>6</sup>.

These radioautographic modifications using Peel-A-Way slide holders, large exposure box and the glycerol-water solution provide a more convenient and reproducible technique than those described previously.

*Zusammenfassung.* Es wird eine einfache Methode für die Vorbereitung und Belichtung von Objektträgern zur Autoradiographie beschrieben. Eine individuelle Behandlung der Objektträger sowie deren Vertrocknung vor der Belichtung wird vermieden und die Feuchtigkeitskontrolle der Emulsion ist gesichert.

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## A Method for the Determination of Thrombocyte Aggregation in Circulating Rat Blood

Various methods have been developed to study the thrombocyte aggregation both in vivo and in vitro. However, so far, it has not been possible to measure the degree of aggregation in circulating blood. Using a microfilter, introduced by SWANK et al.<sup>1</sup> for in vitro experiments, we developed a method to measure the thrombocyte aggregation and des-aggregation in circulating blood continuously and during a prolonged period, without any loss of blood. The experimental set-up is shown schematically in Figure 1.

Via a polythene aorta-prosthesis, inserted between the spermatic- and iliolumbar arteries, the filter (F) (nickel, pore size 20  $\mu$ m, ex Veeco, Eerbeek, The Netherlands) is connected to the blood circulation of rats. Before and behind this filter the blood pressure is measured. Solutions can be infused before the filter. Filter and connecting tubes are siliconized and kept at 37.5°C. The blood stream can be led via a by-pass or through the filter by means of 3 clamps. Aggregation which occurs in the blood stream may obturate the filter, as a result of which the pressure behind the filter (the experimental pressure, EP) drops and the pressure before the filter (the reference pressure, RP) increases (see Figure 2). The degree of aggregation, designated by the aggregation-index A, is

obtained by expressing the minimal ratio of the experimental and reference pressure after ADP administration ( $EP_2:RP_2$ ) as a percentage of the ratio between experimental and reference pressure at the beginning of the

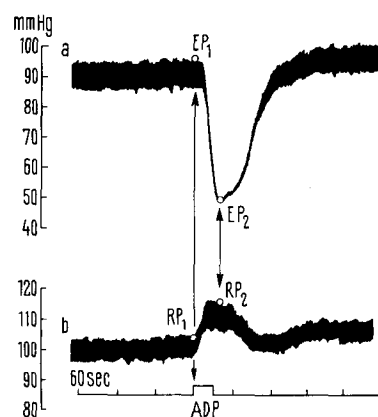


Fig. 2. Recording of an aggregation-measurement. (a) Experimental pressure; (b) reference pressure.

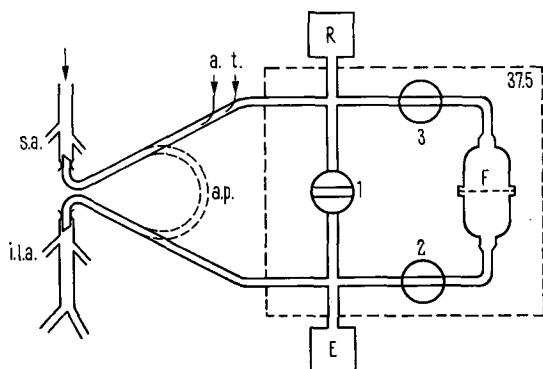


Fig. 1. Scheme of set-up for measuring thrombocyte aggregation. a.p., Aorta-prosthesis (cut through); s.a., spermatic artery; i.l.a., iliolumbar artery; R, recording of reference pressure; E, recording of experimental pressure; F, filter; a.t., administration of test solutions; 1, 2 and 3, clamps.

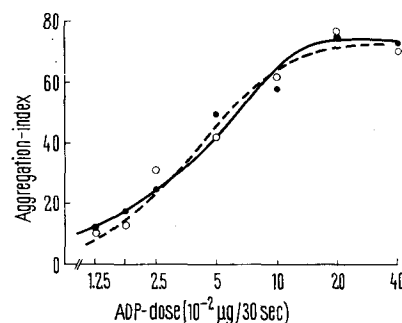


Fig. 3. Relation between ADP-dose and aggregation index at the first (full line) and second (dashed line) measurement (mean of 10 experiments per dose).

<sup>1</sup> R. L. SWANK, J. G. ROTH and J. JANSEN, *J. appl. Physiol.* 19, 340 (1964).

ADP-infusion ( $EP_1:RP_1$ ) and subtracting it from 100, so:  $A = 100(1 - EP_2/RP_2 \times RP_1/EP_1)$ . Microscopical examination of the material on the filter, as well as blood smears and thrombocyte-counting before and behind the filter during ADP-infusion, showed that occlusion of the filter is almost exclusively caused by thrombocytes.

To induce thrombocyte aggregation, an isotonic ADP solution is infused before the filter at a rate of 0.1 ml/min for 30 sec. After completion of the ADP administration, the pressure behind the filter rapidly returns to its initial level, usually within a few minutes. This indicates a cleaning of the filter as a result of des-aggregation. The time between 2 consecutive measurements has been standardized at 10 min.

The degree of thrombocyte aggregation depends on the dose of ADP. Figure 3 shows the relationship between the ADP-dose and the aggregation-index after the first as well as the second measurement. Statistical examination showed that in all ADP-doses tested there were no significant differences between the first and second aggregation index, induced by the same dose of ADP in the same animal; this suggests that complete recovery of

platelet-response to ADP had occurred. By mixing an aggregation-inhibiting agent with the second dose of ADP, the inhibiting effect can therefore simply be calculated by subtracting the second from the first aggregation-index ( $\Delta A = A_1 - A_2$ ). This was done with Prostaglandin  $E_1$  ( $PGE_1$ ), a very active aggregation-inhibitor in vitro as found by KLOEZE<sup>2</sup>. At the first measurement 0.06  $\mu$ g ADP per 30 sec was infused, while in the second determination this amount of ADP was mixed with several doses of  $PGE_1$ . Figure 4 shows the relationship between the  $PGE_1$ -dose and its aggregation-inhibiting action. In the doses tested, this relationship is rectilinear.

This 'filter-loop' technique is particularly suitable for testing aggregation inhibitors after injection anywhere in the body. In the first place, degree and duration of the inhibition can be determined, but also any effects on the blood pressure.

**Zusammenfassung.** Das Ausmass einer experimentell induzierten Thrombozytenaggregation im strömenden Blut wird kontinuierlich bestimmt durch Messung des Blutdrucks vor und hinter einem extrakorporal in den arteriellen Kreislauf der Ratte aufgenommenen Filter. Die Aggregation wird durch Infusion von Adenosindiphosphorsäure (ADP) vor dem Filter induziert. Die Aggregate schliessen das Filter teilweise ab, was zu Änderungen im Blutdruck vor und hinter dem Filter führt. Aus diesen Änderungen lässt sich das Mass der Aggregation ermitteln. Es wurde gefunden, dass diese durch die ADP-Gabe bedingt wird.

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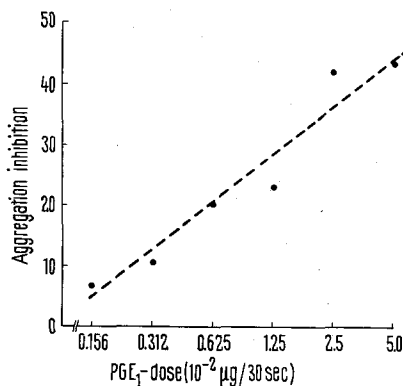


Fig. 4. Relation between  $PGE_1$ -dose and aggregation inhibition (mean of 8 experiments per dose).

<sup>2</sup> J. KLOEZE, in *Prostaglandins* (Eds. S. BERGSTRÖM and B. SAMUELSSON; Almqvist and Wiksell, Stockholm 1967), p. 241.

## ACTUALITAS

### International Cell Research Organization (ICRO)

**1. Training Courses.** One of the main activities of ICRO is the organization of training courses on topics of high novelty and on modern techniques in cellular and molecular biology: Principles and techniques of tissue and organ culture; Genetics and Physiology of Bacterial viruses; Energy transducing systems on the sub-cellular level; Methods in mammalian cytogenetics; Membrane Biophysics; DNA-RNA Hybridization; Biogenesis of Mitochondria; Embryology and Epigenetics; Interaction between Animal Viruses and host cells, application of computers to experimental work in biology and chemistry; Methods in molecular biology, etc. The courses generally last 3–5 weeks, and include 16–20 young participants (sometimes more). The ICRO courses are fully inter-

national, both the teaching staff and the participants coming from the largest possible number of countries.

**2. The Problem of Developing Countries.** Most of the past ICRO courses have been organizing in European countries – east and west – but the demand from developing countries is increasing steadily. ICRO activities in developing countries may tend to give preference to topics of potential economic usefulness, such as applied microbiology, microbial protein production, fermentation industries, soil microbiology, plant genetics, etc.

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