FIG. 1. Five types of designs for sequential preference test, $\alpha=0.05$, $1-\beta=0.95$. The sample path starting at 0 moves 1 unit N-E if A is preferred; otherwise (if B-preference is stated), it moves 1 unit S-E. When the sample path crosses the upper boundary the null hypothesis is rejected, and when it hits the lower boundary the null hypothesis is not rejected. Non-preference is to be neglected in plotting.

Finally the lower boundary points are settled on a 45° line starting at the point 2 units lower than the upper boundary point at N .

The designs given in this paper may be further modified to obtain improved designs or skew designs (1).

REFERENCES

- 1) ARMITAGE, P.: *Sequential Medical Trials*, Blackwell Sc. Pubs., Oxford (1960); 2) WALD, A.: *Sequential Analysis*, John Wiley & Sons, Inc., New York (1947)

Added in Proof: Dr. Armitage and Mr. McPherson have kindly calculated the exact α and β values from their computer program. The values are :

θ_1	.75	.80	.85	.90	.95
α	.0452	.0432	.0471	.0477	.0483
$1-\beta$	.9536	.9550	.9598	.9507	.9845

FAILURE OF KININ FORMATION BY GLASS POWDER IN RAT PLASMA AFTER INJECTION OF BROMELAIN

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It was reported from this laboratory that pretreatment of rats with stem bromelain (BM) prevents lung edema produced by adrenaline infusion, but not with papain (PAP), although both enzymes are similar thiol-

proteinases of plant origin (1). In the course of study on the mechanism of the preventive action of BM, it was observed that the plasma of rats treated with intravenous injection of BM failed to form kinin after glass activation.

Wistar strain male albino rats of 250-380 g were anesthetized by sodium pentobarbital (50 mg/kg, i.p.). Blood was aspirated by cardiac puncture with a siliconized needle and polythene syringe 10 minutes after an intravenous injection of crude BM (10 mg/kg) or PAP (10 mg/kg). Control samples were collected by the same procedure without injection of the enzymes. The plasmas of untreated and of either BM- or PAP-injected rats were incubated in parallel at 37°C in the following way to test kinin formation. The incubation mixture contained (a) 0.3 ml of plasma, 1, 10-phenanthroline (ph.th., 3×10^{-4} g/ml), *p*-hydroxymercuri-benzoate (pmb, 8×10^{-5} g/ml) and 0.1 M phosphate buffer (pH 7.4) up to 1.2 ml, with 0.3 g of glass powder, (b) 0.3 ml of plasma and 0.1 M phosphate buffer up to 1.2 ml, with 0.3 g of glass powder. Fifteen minutes later rat saliva with ph.th. and pmb was added to (b). pMb was used to inhibit a potent kininase activity of both BM and PAP. Kinin formed was assayed on the isolated uterus of rats treated by hexestrol.

FIG. 1. Kinin formation in rat plasma. Responses of the isolated rat uterus to bradykinin (doses given in ng/bath) and to aliquots of incubation mixtures. At the arrows, glass powder or saliva with or without the kininase inhibitors was added to the incubation mixture at zero time. The times at which 0.05 ml of aliquots were taken and added to the organ bath are given in minutes. For details see text.

Cont. plasma: untreated plasma, BM plasma: plasma of rat injected with bromelain, PAP plasma: plasma of rat injected with papain, ph.th.: 1,10-phenanthroline and pmb: *p*-hydroxymercuri-benzoate.

As shown in the upper tracing of Fig. 1, the plasma of BM-injected rats failed to form kinin after glass activation even in the presence of ph.th. and pmb. However, after depletion of a kininogen which should be consumed after glass activation, the same plasma was able to form kinin with rat saliva as much as the untreated plasma did. On the contrary, the plasma of PAP-injected rat formed kinin after glass activation as well as with rat saliva in the same amount as did the control plasma (lower tracing).

In addition, BM initiated the kinin formation in rat plasma *in vitro*. The formation was inhibited by soya bean trypsin inhibitor (10^{-4} g/ml). Furthermore, when the total kininogen in rat plasma was measured by Diniz's method (2), it was reduced by 40–50% in the plasma of BM-injected rats, but no reduction or only a slight reduction was observed in the plasma of PAP-injected rats.

Recently two kininogens have been separated in many species (3, 4). A kininogen which should be used up by the kinin forming enzyme activated by glass powder is designated as kininogen I, having a larger molecular weight than kininogen II (3, 4).

Consequently it is feasible to interpret the present observation in such a way as BM activated the kinin forming enzyme of plasma and depletes kininogen I in the rat plasma.

The relationship of the present findings to the prevention of the rat lung edema by the enzyme is under investigation.

REFERENCES

- 1) SHIGEI, T., SAKUMA, A., ENOMOTO, T., OH-ISHI, S. AND HATANO, R.: THIS JOURNAL **17**, 591 (1967);
- 2) DINIZ, C.R., CARVALHO, I.F., RYAN, S. AND ROCHA E SILVA, M.: *Nature, Lond.* **192**, 1194, (1961); 3) JACOBSEN, S.: *Brit. J. Pharmac. Chemother.* **26**, 403 (1966); *Ibid.* **28**, 64 (1966); 4) YANO, M., NAGASAWA, S., HORIUCHI, K. AND SUZUKI, T.: *J. Biochem.* **62**, 504 (1967)

COMPARATIVE STUDY OF THE FINE-STRUCTURAL CHANGES OF ALVEOLAR WALL IN ADRENALINE- AND ANTU-PULMONARY EDEMA OF THE RAT

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Adrenaline- and ANTU-pulmonary edema were induced in rats, and ultrastructural alterations of the alveolar walls were examined. The present paper describes the typical changes which were determined on the basis of survey observation on many pictures taken by low-powered magnification. Particular emphasis is put on the finding that adrenaline tends to affect groups of alveolar cells selectively.

Male rats of Wistar strain, 280–350 g, were used. Adrenaline-pulmonary edema was induced in 5 anesthetized rats following the procedure described previously (1). ANTU (α -naphthylthiourea), 200 mg/kg, was injected intraperitoneally in 5 unanesthetized rats, as a suspension in olive oil, and the animals were sacrificed 2 to 3 hours after injection.

Endothelium of the alveolar capillaries: In both adrenaline- and ANTU-lungs, the endothelial linings were always continuous with intact, tight intercellular junction. No increase in pinocytotic vesiculation, nor porous formation of the very thinly attenuated cellular processes was observed. Therefore in either case, hardly any defects of the endothelial barrier were found, which would permit passage of solid elements of the blood.

Interstitial edema of the alveolar wall: There was a marked quantitative difference between the two kinds of edema. In ANTU-lungs, prominent subendothelial accumulation of plasmatic liquid led to strong focal