in contrast to the 'normal group' where the levels in nearly all cases are below 0.11 μg/ml.

A possible source of error in this study might be that the cerebrospinal fluid in the hydrocephalic group was mostly obtained by ventricular puncture, in contrast to the non-hydrocephalic group where the liquor was withdrawn by lumbar puncture. Perhaps there is normally a difference between the level of 5-HIAA in ventricular fluid compared with lumbar spinal fluid. Further investigations were thus made in order to clarify this aspect. It is for many reasons impossible to obtain ventricular fluid from a normal child, so we had to rely upon 5 hydrocephalic children, where a double puncture could be made. The results of this separate study showed a slight decrease in the level of 5-HIAA when ventricular fluid was compared with lumbar spinal fluid. The lumbar values ranged between 0.15–0.22 μg/ml and the ventricular values between 0.13–0.28 μg/ml. In view of these data it does not seem probable that the different puncture technique has any influence upon the results. The control group is rather small but further investigations are to be carried out.

It is our impression that the values of 5-HIAA are higher the more centrally the obstruction is located. In some cases also homovanillic acid, the final metabolite of dopamine, was analysed in hydrocephalic CSF using the method of ANDÉN, ROOS, and WERDINIUS[8]. These values were also considerably increased. A more complete account will appear elsewhere.

Zusammenfassung. Die Konzentration der 5-Hydroxyindolessigsäure wurde in der Cerebrospinalflüssigkeit hydrocephalischer Kleinkinder (bis 1 Jahr) bestimmt. Die Werte waren deutlich erhöht. In einigen Fällen wurde die Konzentration an Homovanillinsäure, die ebenfalls erhöht war, bestimmt.

H. ANDERSSON and B.-E. ROOS[9]

Departments of Neurosurgery and Pharmacology, University of Gothenburg (Sweden), March 28, 1966.

[8] N.-E. ANDÉN, B.-E. ROOS, and B. WERDINIUS, Life Sci. *2*, 448 (1963).

[9] Acknowledgment: This work was supported by grants from the Swedish State Medical Research Council (40x-165-01; B66-152), Leo Ltd., Hälsingborg, Sweden and the Medical Faculty, Göteborg. For technical assistance we are indebted to Miss GUN ALFREDSSON, Mrs. MARIA BABARIN, and Mrs. ILONA OLOFSON.

On the Mechanism of Potentiation of Kinins by Inhibitors of the Fibrinolytic System

In his review on bioactive peptides of plasmatic origin, LEWIS[1] first reported that cysteine potentiates the smooth muscle stimulating action of plasma kinin. Later, PICARELLI et al.[2] made a closer investigation of this effect and suggested that the potentiation of bradykinin possibly results from an inhibition of kininase present in the guinea-pig ileum. Later still, we could demonstrate that also inhibitors of the fibrinolytic system and peptones could potentiate the bradykinin action on the ileum[3,4]. When extending our study on further kinins (kallidin, eledoisin) and other bioactive substances (angiotensin, histamine, acetylcholine) we found that only the bradykinin and kallidin effects were potentiated. Fibrinolytic inhibitors and peptones in low concentration caused potentiation, while higher concentrations, for instance $> 0.1\,M$ ε-aminocaproic acid (EACA), diminished the bradykinin and kallidin effects in the same way as they inhibited the ileum's response to other bioactive substances.

It appeared not unlikely that intestinal tissues contain kininases which act specifically against bradykinin and kallidin, so that the potentiation would be the result of kininase inhibition. But also another explanation of the effect had to be considered; namely, that the different reaction patterns of bioactive substances with smooth muscle are responsible for the controversial effects when chemical compounds, otherwise known as inhibitors, interact with the respective reactive system. Such differences of the reaction pattern may be assumed the more, since bradykinin and kallidin, so-called slow-reacting substances, can be distinguished from other bioactive substances according to the time characteristics of their response.

In order to check the first mentioned of the two possible explanations of the potentiation phenomenon, we studied the degradation of bioactive substances in the presence of intestinal tissue. Homogenates from guinea-pig ileum were incubated with bradykinin, kallidin, eledoisin, and angiotensin respectively. The mixtures were kept at 30 °C and samples were taken at regularly spaced intervals. The activities of the samples were evaluated on the standard guinea-pig ileum preparation. Figure 1 shows the results of these experiments. While bradykinin and kallidin suffered a steep drop in activity, a longer incubation time was necessary for angiotensin to lose its activity; the activity of eledoisin, however, was only slightly diminished by contact with the homogenate.

In a further series of experiments we studied the effect of EACA, as a representative of the fibrinolytic inhibitors, on the degradation process. The same procedure as above was utilized, with the exception that EACA was present in the incubation mixture. It can be seen (Figure 2) that EACA from $0.1\,M$ concentration upwards inhibits the degradation depending on the concentration. We found no significant difference in the inhibitory exertion between bradykinin and kallidin on the one hand, kinins susceptible to potentiation by low concentrations of EACA, and on the other hand eledoisin and angiotensin, which are refractory to potentiation.

[1] G. P. LEWIS, Physiol. Rev. *40*, 647 (1960).

[2] Z. P. PICARELLI, O. P. HENRIQUES, and M. C. F. OLIVEIRA, Nature *18*, 77 (1962).

[3] W. DOLESCHEL, W. AUERSWALD, and H. SCHUBERT, Wien med. Wschr. *115*, 575 (1965).

[4] W. DOLESCHEL and W. AUERSWALD, Wien. med. Wschr. *116*, 130 (1966).

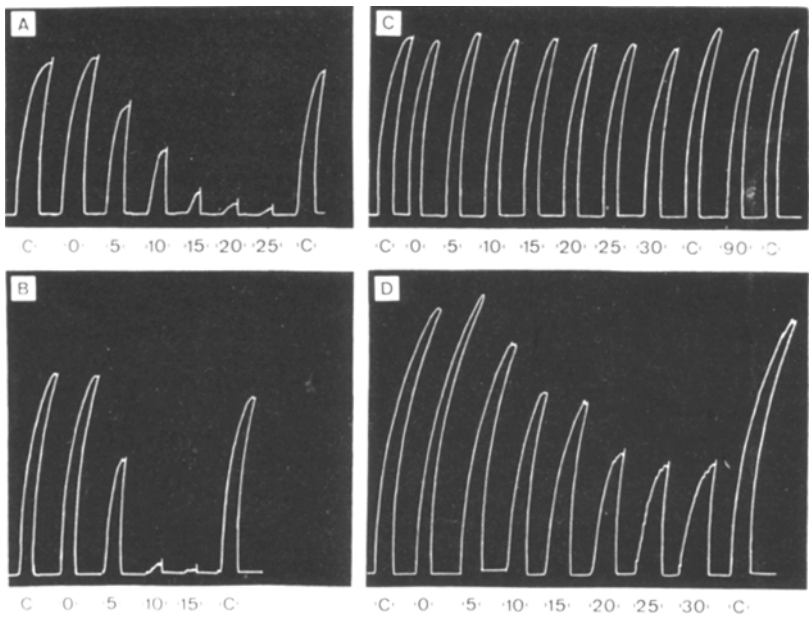

Fig. 1. Responses of a standard guinea-pig ileum preparation to different bioactive substances (A = bradykinin; B = kallidin; C = eledoisin; D = angiotensin), which had been incubated previously with intestinal homogenates for increasing time periods. (Numbers at the bottom of the diagrams indicate the respective applied incubation times in minutes; C indicates the response to the non-incubated control sample.)

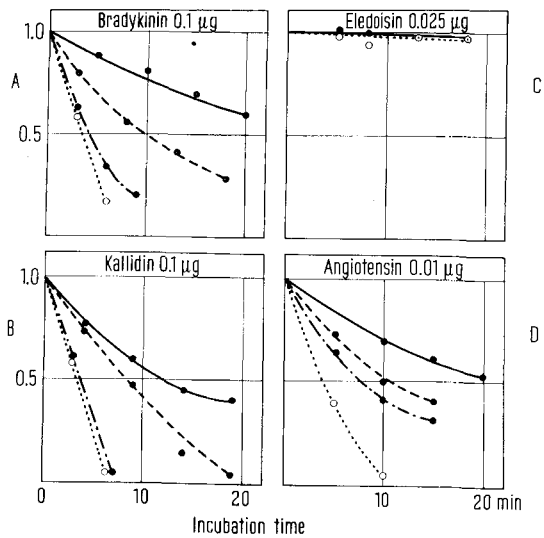

Fig. 2. Decrease of activity of bioactive substances (A = bradykinin; B = kallidin; C = eledoisin; D = angiotensin) during their incubation with intestinal homogenate in the presence of various concentrations of EACA. Ordinates: response to incubated sample/response to sample prior to incubation. o·····o = *control (without EACA)*; ●·····● = with 0.1 molar EACA; ●——● = with 0.5 molar EACA; ●———● = with 1.0 molar EACA.

In addition to the observations with homogenates, which allow free interaction with otherwise not easily accessible enzymes, we wanted to clarify whether the degradation could be demonstrated also in the presence of intact cell membranes. We therefore repeated the previous experiments in a modified form. Instead of homogenate, fragments of intact ileum tissues which had been freed from all adherent matter by thorough washing (Tyrode solution, gassed with air, 4 h) were utilized. These fragments were incubated with the various bioactive substances in the absence or in the presence of EACA. Evaluation of the activity losses over a period of time showed no difference from the results obtained with homogenate as summarized in Figure 2. This can be regarded as proof that there is also degradation of kinins during their exposure to intact intestinal cell surfaces; this degradation is prevented by EACA in a similar way to the degradation mediated by tissue homogenate.

Regardless of whether kininases of the cell surface are chiefly responsible for the splitting of bioactive substances, or whether permeation of kinins into the cell interior also plays a role, our results make it evident that potentiation of bradykinin and kallidin by fibrinolytic inhibitors in low concentrations cannot be attributed to inhibition of kininases, since inhibition takes effect only at a higher concentration than potentiation. It therefore remained to investigate the other possible reason of the potentiation, namely that the inhibitor makes the muscle's excitatory system more sensitive to certain kinins. The considerations of EDERY[5] appeared here of value to elucidate the phenomenon. He found that chymotrypsinogen and chymotrypsin potentiates the ileum's response to bradykinin and kallidin, but has no effect on the response to angiotensin and eledoisin. He suggested that chymotrypsin facilitates the penetration and subsequent attachment of bradykinin and kallidin to their receptors. He supported his view with the observation that under the influence of chymotrypsin the latent period of the response to bradykinin is reduced.

According to EDERY's hypothesis, we asked the following question: Would EACA also facilitate the access of bradykinin and kallidin to their specific receptors? This would find its manifestation in a shortened latent period. We therefore examined the influence of increasing concentrations of EACA on the ileum's response to both kinins on a fast-revolving kymograph. But we could not demonstrate any shortening of the latent period in the presence of those concentrations of EACA which cause potentiation. With increasing concentrations from 0.1 *M* upwards, however, we observed progressive prolongation of the latent period that we could also produce by lowering the kinin concentration.

[5] H. EDERY, Br. J. Pharmac. *22*, 371 (1964).

The results of the present study seem at first sight to be disappointing. No evidence was found of how fibrinolytic inhibitors really potentiate the bradykinin and kallidin effects. But when considering all the steps in the series of events leading to myoplasmic activity, our results at least permit us to narrow the range of the possible roles of these inhibitors. Specific kininase inhibition could be excluded as a reason of potentiation, but also no proof of a sensitizing influence of the inhibitor on the excitability of the smooth muscle was revealed by the latent period evaluation. It remains, therefore, only to focus on the excitation-contraction coupling process. Current theories suggest that the coupling mechanism regulates the tension-generating process, whereby a soluble relaxing substance together with transphosphorylating enzymes plays a key function[6,7]. This system is supposed to increase considerably the amount of calcium which must be released before actomyosin can be fully activated. It could be imagined that fibrinolytic inhibitors interfere even at low concentrations with this regulating mechanism and thereby enhance the activation of the contractile substances. However, this fact alone would not give a sufficient explanation for the potentiation phenomenon.

With fast-reacting bioactive substances reacting rapidly with the smooth muscle, the presence of fibrinolytic inhibitors cannot induce potentiation. Only through the coincidence of the specific type of reaction, as it is triggered by slow-reacting kinins such as bradykinin and kallidin, and the suggested modification of the coupling process by the fibrinolytic inhibitor, can the conditions be created which result in the potentiation phenomenon[8,9].

Zusammenfassung. Die Potenzierung des Bradykinin- und Kallidin-Effektes durch Fibrinolyse-Hemmer ist nicht Folge spezifischer Kininasehemmung, sondern der Hemmkörperwirkung auf die besondere Muskelerregungsform hervorgerufen durch «langsam-reagierende» Kinine.

W. DOLESCHEL and W. AUERSWALD

Department of Physiology, University of Vienna School of Medicine, A 1090 Wien (Austria),
March 29, 1966.

[6] F. N. BRIGGS, Fed. Proc. Am. Socs exp. Biol. *23*, 903 (1964).
[7] L. LORAND, Fed. Proc. Am. Socs exp. Biol. *23*, 905 (1964).
[8] This investigation was supported fully by Public Health Service Research Grant HE 07085-03 (HEM) from the National Heart Institute.
[9] Technical cooperation of ELFRIEDE KOFFER and H. CRANZ is thankfully acknowledged.

Die therapeutische Wirkung von Adrenalin und Alupent nach letaler Röntgenganzkörperbestrahlung

In vorangegangenen Versuchen konnten wir über die Wirkung einer Reihe von Substanzen wie Compound 48/80[1], Anaphylatoxin[2], Endotoxin des *E. coli*[3] berichten, die nach letaler Röntgenganzkörperbestrahlung (RGKB) zum Überleben eines erheblichen Teils der Versuchstiere führt. Wir bezeichnen diese Stoffe zur Vermeidung von sprachlichen Missverständnissen als «Strahlenschadengegenmittel»[2].

Auch durch die Auslösung einer Antigen-Antikörper-Reaktion (AAR) mit Kälberserum[4], Rinderserum, humanen Gammaglobulinen[4] und komplettem Freundschem Adjuvans[5] konnten wir bei sensibilisierten letal bestrahlten Meerschweinchen, Mäusen und Ratten Überlebensraten von 20–66% erzielen.

Sowohl nach der AAR wie auch nach Verwendung von C 48/80[1], Anaphylatoxin[2] und Endotoxin[3] entstand ein schockartiges Zustandsbild. Da solche Stoffe zum Teil zu toxischen Erscheinungen führten, wurden neue schockauslösende Mittel untersucht, wie Adrenalin und Alupent, ein Adrenalin-Derivat.

Zu den nachfolgend beschriebenen Versuchen verwendeten wir 282 je 200–250 g schwere Albinoratten und 20 je 400–450 g schwere Meerschweinchen. Die Bestrahlung erfolgte im Zentralröntgeninstitut des Wilhelminenspitals in Wien (Vorstand Prof. R. PAPE), wofür wir auch an dieser Stelle danken. Meerschweinchen erhielten 550 R (180 kV, 15 mA, 0,5 Cu, Abstand 57 cm, Feld 20 · 24 cm, Zeit 10 min, 10 sec), Albinoratten 800 R (180 kV, 15 mA, 0,5 Cu, Abstand 60 cm, Feld 20 · 26 cm, Zeit 17 min). Mit 0,4 ml einer 1:1000 verdünnten Adrenalin-Bitartratlösung (Fa. Sanabo, Wien) überlebten von 15 Ratten 6 (39%), wenn das Mittel etwa 5 min nach einer RGKB von 800 R s.c. verabfolgt wird. Mit 0,2 ml verendeten 8 von 10 Ratten, mit 0,3 ml 7 von 10 Tieren. Mit 0,5 ml starben von 15 Ratten 8 im Schock, von den restlichen 7 überlebten 4. Zwanzig Kontrolltiere verendeten zwischen dem 7. und 16. Tag nach der letalen Bestrahlung. Mit 0,3 ml L-Adrenalinlösung überwanden 6 von 10 Meerschweinchen den letalen Strahlenschaden, während 10 Kontrollmeerschweinchen nach 550 R RGKB zwischen dem 9. und 14. Tag zugrunde gingen.

Figur 1 zeigt die therapeutische Wirksamkeit von Alupent (Fa. C. H. Boehringer & Söhne, Ingelheim

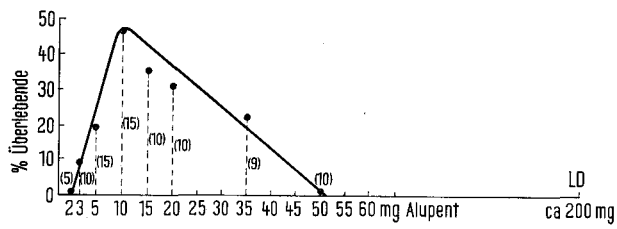

Fig. 1. Therapeutische Wirkung des Alupents bei Albinoratten. Überlebensraten in % bei den einzelnen Alupentgaben (s.c.) etwa 5 min nach letaler Röntgenganzkörperbestrahlung (800 R). $LD_{100/30}$ d. In Klammer die Anzahl der Tiere pro Versuchsreihe.

[1] E. GABLER und W. STÖCKL, Gem. Tag. d. Ges. f. Biophysik d. österr. Ges. f. reine u. angew. Biophysik und der Schweiz. Ges. f. Strahlenbiol., Tagungsber. (Wien 1964), p. 445.
[2] E. GABLER und W. STÖCKL, Strahlentherapie *126*, 590 (1965).
[3] E. GABLER und W. STÖCKL, Strahlentherapie *127*, 287 (1965).
[4] E. GABLER, Wien. Z. inn. Med. *43*, 105 (1962).
[5] E. GABLER und W. STÖCKL, Z. Immun. Allergieforsch. *128*, 333 (1965).