

extracts on isolated organs (guinea pig ileum, rat uterus, hen rectal caecum, rabbit jejunum) and on rabbit blood pressure was the same in each case. The activity of both preparations was destroyed by trypsin. After separation on Al_2O_3 columns, both preparations consisted of Zetler's fraction F_a . The Euler-Gaddum extract contained in addition a small amount of F_b . Some differences between the extracts could be found after separation in paper chromatography and electrophoresis.

Four regions of the brain containing different amounts of substance P were extracted with both methods; the activity ratios between the regions were the same with each method. After the extraction with chloroform-methanol, no substance P activity remained in the tissue.

Separation of the chloroform-methanol extract by lipid extraction methods shows that the activity goes with the phosphatide fraction. From these experiments it is concluded that substance P in the brain tissue is bound to phosphatides.

25. Plasma Kinins and the Sympathetic Nervous System. GRAHAM P. LEWIS (*CIBA Laboratories, Horsham, Sussex, England*).

No abstract received.

26. The Influence of Substitution or Omission of an Amino Group on the Hypotensive Activity of the C-Terminal Sequences of Eledoisin. K. LÜBKE and E. SCHRÖDER (*Schering AG, Hauptlaboratorium, West Berlin, Germany*).

Most of the analogues of eledoisin described in the literature were obtained by substituting amino acids in the peptide chain or by shortening it. Only little is known about the influence of non-peptidic substituents on the activity. Therefore we investigated the hypotensive activity of several acyl derivatives of the C-terminal eledoisin sequences. The C-terminal heptapeptide H-Asp-Ala-Phe-Ileu-Gly-Leu-Met-NH₂ is approximately four times more active than the C-terminal hexapeptide H-Ala-Phe-Ileu-Gly-Leu-Met-NH₂. But this increase in activity does not depend on original aspartic acid residue in position 5. A number of other α -amino acid residues also cause an increase (two- to fourfold). To clarify the question whether an extension of the peptide chain enhances the activity, we synthesized acyl hexapeptides with the formyl-, caprinoyl-, palmitinoyl-, chloroacetyl-, succinoyl-, hydroxyisovaleryl-, *p*-amino benzoyl-, nicotinoyl-, and *n*-butylcarbonyl group as acyl residues. The importance of the free amino groups is examined with the Lys⁶-heptapeptide H-Lys-Ala-Phe-Ileu-Gly-Leu-Met-NH₂. The possible acetyl derivatives (α -acetyl-, ϵ -acetyl-, and α, ϵ -diacetyl-) and the possible des-amino derivatives (des- α -amino = ϵ -amino caprinoyl-,

des- ϵ -amino = norleucyl, and des- α, ϵ -diamino = caprinoyl-) were synthesized.

The hypotensive activity of all the described derivatives will be discussed with regard to the influence of the structure on the biological activity.

27. The Measurement of Kinin-Releasing Enzymes in Plasma. J. MARGOLIS (*Children's Medical Research Foundation, Royal Alexandra Hospital for Children, Sydney, Australia*).

The rapid phase in the release of kinin by plasma kallikrein(s) is a specific reaction which utilises a susceptible part (approximately 25%) of kininogen complex (component B) and involves two distinct components of the releasing enzyme system: component A and Hageman factor (HF). These were measured in terms of $\mu\text{g}/\text{min}$ of bradykinin-equivalent (BK-*eq*) produced from a suitable substrate. Removal of inhibitors by various methods resulted in marked slowing of the reaction and apparent loss of specificity but, with the addition of EDTA and corrections for dilution, temperature, and kininogen content, reproducible results were obtained on intact plasma. In this substrate, activated HF is a potent releasing agent. By fractional elution from kaolin, at pH 11.6, stable preparations were obtained, capable of producing more than 80 $\mu\text{g}/\text{min}$ BK-*eq*/mg enzyme protein at 22° in undiluted systems. This is equivalent to 200 units of kallikrein ('Glumorin' F.B.A.) per mg. Activated HF is inactive in 'B-depleted' plasma which is still a satisfactory substrate for glandular kallikreins. Component A is necessary for the formation of soluble kallikrein in plasma or fractions activated by contact. It was assayed in samples treated with glass or kaolin and an excess of activated HF. The results were expressed either as per cent activity relative to a standard or in absolute units ($\mu\text{g}/\text{min}/\text{ml}$). Unexpected discrepancies in the latter led to a re-examination of the kinin-releasing mechanisms in plasma.

28. Automated Peptide Synthesis. R. B. MERRIFIELD (*The Rockefeller Institute, New York, N.Y., U.S.A.*).

In an effort to simplify and accelerate the synthesis of peptides, a new approach to the problem was devised. It was called solid-phase peptide synthesis, and was based on the idea that peptides could be assembled in a stepwise manner while attached at one end to an insoluble solid particle. With the peptide securely bound in the solid phase it was possible to purify each of the intermediates simply and quickly by thorough washing, rather than by recrystallization or other tedious procedures. The method was applied to the synthesis of bradykinin, methionyl-lysyl-bradykinin, and angiotensin. The products were obtained in good

yields and were shown to be chemically pure and biologically active. Solid-phase peptide synthesis has now been automated in collaboration with Dr. John Stewart. All the reactions were carried out in a single reaction vessel and each of the manipulations was performed in the proper sequence under the control of a pre-set programmer. A peptide chain can be lengthened by six amino acid residues per day completely automatically without manual attention. The process was tested on the synthesis of bradykinin and gave satisfactory results.

29. Human Salivary Kallikrein and Liberation of Colostrokinin. HIROSHI MORIYA (*Laboratory of Physiological Chemistry, Tokyo College of Science, Shinjuku-ku, Tokyo, Japan*).

The isolation and purification of kallikrein from human pooled saliva (Japanese) has been studied. The kallikrein content in saliva was 1–2 Frey U/ml. Acetone-dried powder of human mixed saliva was found to be suitable as starting material for purification. A highly purified fraction was obtained by using ion-exchange column chromatography, acetone fractionation, and Sephadex filtration. The activity of kallikrein was assayed by measuring the esterase activity with TAME substrate and the increase in arterial blood flow of dogs. The purest preparation obtained had 200 Frey U/mg with a yield of 0.2–0.5 FU/ml of original saliva. Human salivary kallikrein liberates colostrokinin from bovine colostrum as measured on the rat uterus and dog blood pressure. Human salivary kallikrein was labeled with ^{131}I ; its effect on absorption through the intestinal wall was also studied.

30. Bradykinin in the Carcinoid Syndrome. JOHN A. OATES, WILLIAM A. PETTINGER and R. B. DOCTOR (*Division of Clinical Pharmacology, Depts. of Medicine and Pharmacology, Vanderbilt Univ. School of Medicine, Nashville, Tenn., U.S.A.*).

Previously we have demonstrated that a kinin peptide is released into the circulation of some patients with carcinoid syndrome after the injection of epinephrine. The present investigations were carried out to characterize this peptide. By gradient elution chromatography on CM-Sephadex, it was possible completely to separate microgram amounts of bradykinin from kallidin. The elution characteristics of the carcinoid kinin in this system were identical with those of authentic bradykinin. On both high-voltage electrophoresis at pH 3.5 and paper chromatography with butanol:acetic acid:H₂O, the carcinoid kinin had the same mobility as bradykinin. The rate of inactivation during incubation with chymotrypsin for 12 min at 12° was the same for both the carcinoid kinin (22.7%) and bradykinin (22%). Very little in-

activation of either occurred during incubation with trypsin for 60 min at 38°. The pharmacologic effects of the carcinoid kinin and bradykinin on the rat uterus, guinea pig ileum, rabbit blood pressure, and rat duodenum were also similar. All these studies indicate that the kinin found in the hepatic vein blood of patients with carcinoid syndrome is bradykinin. There is additional evidence suggesting that the tumor kallikrein initially forms kallidin which is rapidly converted to bradykinin in plasma.

31. Observations in vivo of the Peripheral Circulation During Bradykinin Infusion by Transilluminating Quartz-Rod Technique (colored motion picture). G. PELLEGRINI and C. PIOVELLA (*Istituto di Patologia Medica dell'Università di Pavia, Italy*).

The terminal circulation of the mesentery and liver of rats and frogs was studied during and after bradykinin infusion. After an initial vasoconstriction, a large dilatation of the small arteries was observed, together with a spastic vasoconstriction. The irregular shape of the capillaries with an increase in permeability was demonstrated by fluoroscopy.

32. Bradykininogen in the Blood of Women During Pregnancy, Labor, and Puerperium. P. PERITI and F. GASPARRI (*Istituto di Farmacologia, and Istituto di Clinica Ostetrica e Ginecologia, Università di Firenze, Italy*).

Bradykininogen (BKG) of the plasma has been assayed with a biological method using rat uterus *in vitro*. During pregnancy BKG increases and at the ninth month reaches above normal levels with an arteriovenous ratio significantly greater than unity.

At the onset of labor BKG decreases progressively as the uterine contractions become more intense. The decrease reaches its maximum in the expelling stage. Within a few hours of the delivery, BKG values return to normal.

Twelve to twenty-four hours after the delivery the level of BKG starts a slow, progressive decrease which lasts three to four days, reaching in some cases below normal values. Within eight to ten days after the delivery BKG returns to normal in women who have had no puerperal complication.

The fetus is born with a BKG content of the blood markedly lower than the normal average value in the adult. During the first week of life, BKG slowly rises toward a higher level.

The hypothesis is suggested that the behavior of BKG in the woman during pregnancy and labor is connected with the uterine muscular mass and with its prolonged rhythmic contraction in the dilating and expelling stage of labor. In puerperium