hoffii by DEAE-cellulose chromatography, a proteinase with bradykinin-destroying activity was separated from the bradykinin-releasing enzyme and was found to be identical with proteinase b. Proteinase b, one of the three proteinases with hermorrhagic activity in the venom, hydrolyzed the glycyl-phenylalanyl-R linkage of the bradykinin. When the partially purified bradykinin-releasing enzyme, which had no detectable proteolytic activity, was incubated with physicochemically pure bradykininogen, the bradykinin liberated was destroyed very slowly. The bradykinin-destroying enzyme which was found in the partially purified bradykinin-releasing enzyme preparation was different from proteinase b and hydrolyzed the phenylalanyl–seryl–R linkage of the bradykinin.

The arginine ester hydrolase in the venom of *A. halys blomhoffii* was separated into three entities: bradykinin-releasing, clotting, and capillary permeability-increasing enzymes. The content of arginine ester hydrolytic activity of the purified bradykinin-releasing enzyme was unexpectedly low and was less than 5% of the total activity of the venom. The enzymatic properties of the purified bradykinin-releasing enzyme were similar to those of pancreatic kallikrein. Trasylol inhibited only the activity of the bradykinin-releasing enzyme. The enzymatic mechanisms of liberation of bradykinin from bradykininogen will also be presented.

43. A Possible Role for the Plasma Kinins in Pancreatitis and Shock. ALAN P. THAL (*Robert S. Marx Laboratories of the Dept. of Surgery, Wayne State Univ. School of Medicine, Detroit, Mich., U.S.A.*).

The initial symptoms in human acute hemorrhagic pancreatitis are intense pain, accumulation of edema fluid in and around the pancreas, and accumulation of peritoneal fluid. The local features of edema and pain appear to result from local kinin release. The hypotension which follows appears to be largely the result of fluid loss, but in addition toxic substances emanating from the pancreas may be important. The present study will deal with the measurement of plasma kinins, their precursors, and kininases in acute experimental pancreatitis. Evidence for a proteolytic process will also be supported by measurements of plasma TAME esterase activity in both the experimental and clinical situation.

In other forms of shock as well, evidence will be presented in the experimental animal that endotoxin shock is associated with initiation of the proteolytic process. It will also be shown that endotoxin encourages intracellular and intravascular proteolysis with resultant liberation of plasma kinins. A possible role for these agents in shock and pancreatitis will be discussed. (Supported by Grant AM 06385-02 from the National Institute of Health, the Michigan Heart Association, and the Receiving Hospital Research Corporation.)

44. Kininogenases, Kininases, and Their Inhibitors. I. TRAUTSCHOLD, H. FRITZ and E. WERLE (*Klinisch-Chemisches Institut an der Chirurgischen Klinik der Universität, Munich, Germany*).

Some properties of a pure pancreatic kallikrein preparation will be presented, together with its amino acid composition and kinetic data of the proteolytic and esterolytic action of the enzyme.

Kallikreinogen was prepared from pig serum by a new method, and the possibilities of its activation were studied. New aspects of the already known kininogenase inhibitors such as the trypsin–kallikrein inhibitor from bovine tissues or the submandibular inhibitor from dogs will be discussed. Pancreas from mammals contains a specific trypsin inhibitor which is different from the well-known beef pancreatic trypsin inhibitor of Kunitz. This new inhibitor was isolated and its properties studied. The characteristics of the serum kallikrein inhibitor were also investigated. There is only little evidence for a physiological role of the naturally occurring kininogenase inhibitors in the control of the liberation of kinin.

Under physiological conditions the kinin action is controlled by the kininase system, which is much more active in nearly all organs than in blood. The enzymes that take part in the kinin destruction may be differentiated by selective inhibitors.

45. Effects of Bradykinin on the Specialized Ventricular Conducting System of Dog Heart. R. L. VICK, W. KRIVOY and D. C. KROEGER (*Depts. of Physiology and Pharmacology, Baylor Univ. College of Medicine, and Dept. of Pharmacology, Univ. of Texas Dental Branch, Houston, Texas, U.S.A.*).

Bradykinin (BK), 0·1 to 1·0 μg/ml, has been shown to decrease the action potential duration of the Purkinje, or specialized ventricular conducting system (SCS), of dog heart, with relatively little effect on the action potentials of myocardial cells (*Fed. Proc.* **24**, 137, 1965). By means of KCl-filled micropipettes and direct-current recording techniques, additional effects of the same concentrations of BK have been recorded. In SCS tissue undergoing repetitive stimulation, the maximal diastolic potential and the membrane potential prior to excitation are reduced, and the average conduction velocity is decreased. In spontaneously beating SCS tissue, BK decreases the frequency and stabilizes the membrane potential at a level between maximal diastolic potential and threshold. All these effects are more

pronounced in SCS cells with prominent diastolic depolarization. These observations could be accounted for by a specific increase in K^+ conductance in cells in which the K^+ equilibrium potential is less than maximal diastolic potential. (Supported by U.S. Public Health Service Grants HE 08372-02, HE 05435-05, and MH 3477.)

46. Synthesis of New Isomers of Bradykinin. K. VOGLER (*Hoffmann-LaRoche, Inc., Basle, Switzerland*).

In this study the synthesis of new isomers of the natural hormone containing D-amino acids is reported. Both all-D-bradykinin and all-D-retro-bradykinin were obtained in a pure state. The former represents the first synthetic antipode of a polypeptide hormone. The biological properties of these new compounds are discussed in the light of stereochemical considerations.

47. Demonstration of the Presence in Human Plasma of Two Separate Kinin-Forming Systems. W. VOGT (*Medizinische Forschungsanstalt der Max Planck Gesellschaft, Göttingen, Germany*).

It is well established that human plasma contains plasma kallikrein which, by acting on "kininogen", liberates plasma kinin. In addition another kinin-forming enzyme is present for which the term "kininogenase II" is suggested. It acts specifically on a substrate different from that utilized by kallikrein. This substrate (kininogen II) is not attacked by plasma kallikrein (suggested name "kininogenase I") or by pancreas kallikrein or trypsin. Kininogenase II is present in plasma as an inactive precursor and is activated by trypsin or by contact with glass. Active kininogenase II activates kininogenase I and hastens the subsequent destruction of the latter in plasma.

It seems likely that kinonogenase I is not activated by glass contact (or by Hageman factor) directly, but only through activation of kininogenase II. This would explain why dog plasma cannot be induced to form kinins on contact with glass. Dog plasma contains kininogenase I but practically no kininogenase II. In contrast, rat plasma contains only the kinin-forming system II. It is therefore a poor substrate for trypsin but well activated by contact with glass.

After glass activation of human plasma (B-depletion) kininogenase II and kininogen I are left, kininogenase I being inactivated and kininogen II being used up. For this reason in such plasma no kinin can be formed on further incubation, although by different methods an enzyme as well as a substrate can be demonstrated.

48. Polypeptide Receptor Mechanisms; Influence of pH and Heat. EDWARD J. WALASZEK and DONALD C. DYER (*Dept. of Pharmacology, Kansas Univ. Medical Center, Kansas City, Kans., U.S.A.*).

The biological testing of polypeptides was carried out on the isolated guinea pig ileum in a modified Tyrode's solution at 35°. The effect of pH was studied on the contractile potency of four polypeptides: bradykinin, eledoisin, angiotensin, and substance P. The solution used was Tyrode's without $NaHCO_3$, the pH of the solution adjusted with 0·1 N HCl or 0·1 N NaOH. The pH values were varied from 5 to 10. When bradykinin, eledoisin, and angiotensin were tested together, a clear dichotomy could be seen: at low pH eledoisin was least potent, whereas bradykinin was the most potent, peptide. As the pH was increased eledoisin increased in potency very markedly, while bradykinin declined in potency. Angiotensin tended to remain reasonably constant. It was sometimes found that at pH 10 bradykinin had no effect, whereas eledoisin was still very active. We suggest that it is possible to differentiate eledoisin from bradykinin by this procedure. It would appear that the positive charge is more important for bradykinin activity than it is for eledoisin activity. This could then be interpreted that they are acting on two different receptors. Substance P resembled eledoisin in these studies.

In another phase of this study the effect of heat on actions of different polypeptides on the guinea pig ileum were observed. These results will be reported in detail.

49. The Physiological and Pathological Role of the Kallikrein–Kallidin System. MARION E. WEBSTER (*Laboratory of Cardiovascular Physiology, National Heart Institute, Bethesda, Md., U.S.A.*).

The biochemical characterization of the kallikrein–kallidin system has led to speculation that the endogenous release of these powerful vasodilator polypeptides may play a role in regulating local blood flow. Infusion of the polypeptides directly into the kidney, coronary circulation, skeletal muscle, etc., is associated with an increase in blood flow independent of any systemic effects. The demonstration, however, that infusion of a naturally occurring substance can influence local blood flow, does not necessarily indicate that it alters blood flow under physiological conditions. Thus, for example, the vasodilatation produced in skeletal muscle by muscle contraction, arterial occlusion, and lumbar cholinergic sympathetic nerve stimulation is not altered in the presence of carboxypeptidase B, although that produced by infused kallidin is effectively blocked. The kallidins, therefore, do not appear to contribute to the vasodilatation seen under these conditions.