DISCUSSION

F. M. BUMPUS *(Cleveland, U.S.A.):*

Because of the present confusion concerning the structure of "venom" bradykinin (Zuber and Jaques), I should like briefly to discuss a purification of venom bradykinin by Ulla Hamberg as carried out in our laboratory. This purification of the "venom" bradykinin was carried out by a simple three step procedure:

1) Extraction into butanol from aqueous toluene sulfonic acid.
2) Adsorption on and elution from IRC-50.
3) Electrophoresis on paper.

This pure product was found to possess identical biological activity as synthetic bradykinin obtained from Dr. Boissonnas.

Likewise the N-terminal amino acid and the amino acid ratio (determined as the DNP amino acid) was found to be identical in "trypsin" and "venom" bradykinin which strongly suggests the identity of peptides from these two sources.

E. HABERMANN *(Wurzburg, Germany):*

The serum globulin fraction used by Elliott and Lewis for the production of trypsin-bradykinin contains not only kinin precursor but also a kinin releasing factor. This system can be activated by hydrochloric acid. The plasma kinin formed after neutralization can be isolated in a way similar to Elliott and Lewis' procedure. The resulting peptide corresponds to synthetic bradykinin (Sandoz) quantitatively and qualitatively with respect to stimulation of the guinea-pig ileum, lowering of the rabbit's blood pressure and raising the rabbit's capillary permeability. Our kinin could be called kallidin too, since it is produced by serum kallikrein *per definitionem.* Therefore, bradykinin and kallidin are identical.

The relative amino acid content (determined some days after the symposium) was 2·7 Pro : 2·1 Arg : 2·0 Phe : 1·1 Gly : 0·92 Ser, showing no difference against synthetic bradykinin.

D. F. ELLIOTT *(London, England):*

I was very interested to hear that Dr. Habermann has been working on the kinin produced by acid activation of plasma. We are also working on this problem and first I should like to say that we believe that the preparation of bradykinin and acid treatment to inactivate the bradykinin-destroying enzyme leaves substrate available for trypsin to work upon. In

the formation of "acid-kinin", as we call it, we dialyse the ammonium sulphate fraction in the presence of acid and this takes at least 2 hr. On neutralization and incubation full bradykinin activity results as Dr. Habermann has also found. I was intrigued to hear that Dr. Habermann was able to use methods similar to those used by us for isolation of bradykinin, for isolation of "acid-kinin". In our laboratory we found that the methods used for bradykinin were not suitable for "acid-kinin". Therefore, we have not been able to obtain this substance in a pure condition, but recently have made some progress in this problem by using alternative methods.