# DISCUSSION

P. STERN:

Wir haben gesehen dass Bradykinin eine starke Entzündung am "granulome-pouch" verursacht, was in gutem Zusammenhang mit der antagonistischen Wirkung des Aspirins steht.

G. P. LEWIS (*London, England*):

I should like to emphasize a point which Dr. Collier has already made. That is, that although analgesic-antipyretic drugs such as aspirin or phenylbutazone specifically inhibit bradykinin bronchoconstriction they do not specifically inhibit those actions of bradykinin which would be involved in inflammation. It would therefore be wrong to conclude from your experiments that an analgesic-antipyretic drug acts in inflammation by inhibition of the actions of bradykinin.

A. L. COPLEY (*New York, U.S.A.*):

I should like to make some comments to several of Dr. Schachter's findings which interest me greatly. We found also that plasmin has little or no significant effect in increasing capillary permeability. However, we noted that plasminogen, the precursor of plasmin or fibrinolysin, present in the circulating blood increases markedly capillary permeability in guinea-pigs and rats, the two species which we employed in our studies. I wonder whether histamine or some other mediator, such as bradykinin, may have been released following the intradermal injection of plasminogen.

The electron microscopic observations of Dr. Schachter appear to some extent to be similar to certain hitherto unpublished studies made recently in Professor Palade's laboratory in New York by G. Majno and G. E. Palade (*J. Exp. Med.*, in press). I can briefly acquaint you with some of their major findings. They employed histamine and serotonin in a new preparation of the capillary bed, the cremaster muscle in the rat's scrotum. As Prof. Palade told me, he and his associates did not do any work with bradykinin or other mediators. Majno and Palade found also a gap between the endothelial cells, as Dr. Schachter reported. Moreover, they found, in using certain substances as tracers, that the basement membrane is the main filtration barrier. The basement membrane, a fibrillar felt-like structure present in the blood vessels of all tissues, is acellular and about 400 – 600 Å in width.

On the basis of new work wihch I shall report in a few days in Vienna before the 8th Congress of the European Society of Haematology, I pro-

pose that the basement membrane contains fibrin in different stages of polymerization A. L. Copley, *Proc. 8th Congr. Europ. Soc. Haemat. Vienna* 1961. *Wien. Med. Akad. f. aerztl. Fortbildung,* in press). In this connection our new findings appear to be of significance, i.e., fibrinolysis opposes increased capillary permeability produced by histamine or serotonin. This has been found both intradermally and intravenously in guinea-pigs and rats by the blueing reaction and also in preliminary experiments using the more sensitive method of measuring protein extravasation, which was published last March by E. Aschheim and B. W. Zweifach (*Circulation Research* **9** 349, 1961). The course of the inflammatory reaction can be followed with this method by assaying radioactive changes of the skin of rats injected intravenously with radio-iodinated human serum albumin. We found together with Dr. Aschheim that the histamine injected site accumulates up to 9 times more of the previously administered radio-iodinated albumin.

We found in preliminary experiments the anti-inflammatory action not merely with different preparations of plasmin, activated by streptokinase or by chloroform, and of streptokinase, but also with other proteolytic enzymes, such as trypsin, chymotrypsin and papain. Since fibrinolysis is considered to be a physiologic process occurring during life in all blood vessels closest to the endothelial wall and regulating the maintenance of the endoendothelial fibrin film, proposed first in 1953 by Copley and developed ever since (A. L. Copley, *Proc. 8th Internat. Hematol. Congr., Tokyo,* 1960. Tokyo, Maruzen Co., 1961, in press), the antagonistic action of fibrinolysis in decreasing capillary permeability, induced by histamine or serotonin, may well be of physiologic significance.

Since Majno and Palade found changes in the smallest capillaries only after extremely high doses of histamine or serotonin, while such changes occurred usually in the larger capillaries 10–20 μ in diameter and on the venous side of the capillary bed, the permeability of the smallest capillaries and of those larger ones on the arterial side may be affected by mediators other than histamine and serotonin. Further electron microscopic studies may well establish that different mediators may affect selectively different vessels in the capillary bed.

In some preliminary experiments we found bradykinin antagonistic to histamine, but it remains to be established whether this effect is consistent. Because of the recent date of our studies on bradykinin, now in progress, it is too early for me to come to any conclusions regarding the role of bradykinin in capillary permeability.

G. P. LEWIS *(London, England)*:

We should not discuss plasmin as being one of the enzymes in blood which forms a plasma kinin. There is an important difference in plasma kinin function by plasmin and by kallikrein. Plasmin forms plasma kinin

slowly and as plasma and the substrate used in assays of enzyme activity contains a kinin destroying enzyme, it is possible that during the slow kinin formation, the kinin is destroyed as quickly as it is formed, so masking its true plasma-kinin forming activity.

E. G. ERDÖS (*Pittsburgh, U.S.A.*):

An enzyme in human plasma fraction IV-1, which destroys bradykinin was characterized. The decrease in the activity of bradykinin was assayed on the isolated rat uterus with the help of a new device. $CoCl_2$ was the only activator found, eleven other agents inhibited the enzyme. Chelating compounds like 1,10-phenantroline or EDTA are effective inhibitors, however the addition of $CoCl_2$ overcomes this block and restores the activity of the enzyme. Compounds which usually inhibit trypsin, chymotrypsin and or kallikrein were ineffective against plasma fraction IV-1. The enzyme was further characterized with the help of high-voltage paper electrophoresis, paper chromatography and Spinco aminoacid analyser. The experiments indicated that the enzyme in plasma fraction IV-1 inactivated bradykinin by removing its C-terminal arginine, thus it behaved like a carboxypeptidase. The suggestion was made to name this enzyme not after the whole peptide chain, but after the bond it breaks, and call it carboxypeptidase N.

A more detailed account of these studies will appear as "Enzymes that inactivate polypeptides" in *Enzymes and other factors in drug metabolism.* (Edited by B. B. Brodie and E. G. Erdös). *Proceedings of the First International Pharmacological Meeting* Vol. 6, Pergamon Press, 1962.