are not restricted to 5-hydroxy indoles. We therefore propose the name "hydroxyindole oxidase" for the enzyme.

The rapid oxidation of psilocine, together with the development of the deep blue colour, is of particular interest. It is suggested that in the enzymic reaction of the 4-hydroxyindole an *o*-quinonoid compound is formed. The oxidation of the 5-hydroxy and the 6-hydroxy indoles may lead to the formation of *p*-quinones.

Acknowledgements—We are grateful to Dr. A. Hofmann, of Sandoz A.G., Basel, who gave us most of the substances used in this study, and to Dr. R. B. Barlow, of Edinburgh University, for the bufotenine. This work has been supported in part by the U.S. Air Force Office of Scientific Research.

University Department of Pharmacology
Oxford

H. BLASCHKO
W. G. LEVINE*

REFERENCES

1. H. BLASCHKO and A. S. MILTON, *J. Physiol.* **148**, 54 *P* (1959).
2. A. HOFMANN and F. TROXLER, *Experientia* **15**, 101 (1959).

* Postdoctoral Fellow on Interdisciplinary Grant from the National Institute of Mental Health, U.S. Public Health Service.

Cerebrospinal fluid and bradykinin release

(*Received* 15 *February* 1960)

A RECENT investigation[1] showed a correlation between bradykinin release and the activation of plasmin in rat and bovine plasma. Various human body fluids contain an activator of plasminogen or its precursor.[2] Normal human cerebrospinal fluid was found to contain only the proactivator of the fibrinolytic system.[2] More recently Chapman and Wolff[3] showed that cerebrospinal fluid from patients with an active central nervous system (CNS) disease contracted smooth muscle or developed such activity when incubated with a bovine globulin fraction. Bioassay showed similar properties to vasodilator peptides[4] derived from plasma proteins, such as bradykinin[5] which is released by protease action. It became of interest to decide whether cerebrospinal fluid from patients with CNS disease contains sufficient amounts of protease to release bradykinin or whether the reaction involves activation of a fibrinolytic type.[6]

Cerebrospinal fluid from five patients* was investigated for protease activity using the synthetic substrates† benzoyl-1-arginine methylester (BAMe) and toluenesulfonylarginine methylester (TAMe) and the fibrin plate method.[8, 9] At most, only traces of protease activity could be detected with the synthetic subtrates. In one case, however, (multiple sclerosis) a protease/esterase activity was observed with 0·01 M-TAMe (no activity with BAMe) and 0·08 ml per ml of the cerebrospinal fluid in 0·02 M-phosphate pH 7·7. trypsin† (5 μg per ml), approximately ten times higher activity was obtained. When trypsin was added after incubation of the substrate with the cerebrospinal fluid no activity developed, showing that the substrate was used up and that although low this sample had a detectable

* The author wishes to thank Dr. E. Hillbom and Dr. Y. O. Alanen, the Psychiatric and Neurological Clinic of the University of Helsinki for their interest and co-operation.

† The substrates were kindly supplied by the Research Division of the Cleveland Clinic Foundation and the trypsin by Dr. T. Astrup, the Biological Institute of the Carlsberg Foundation, Copenhagen.

protease action. No inhibitory activity towards these substrates was found to be present in the cerebrospinal fluids when added to samples incubated with trypsin in concentration up to 0·2 ml per ml.

When applied to the fibrin plates a weak protease activity appeared in some of these cases, showing that, if present, the amount of free protease acting on fibrin was very low (the fibrin plate was sensitive to 0·01 μg trypsin in 0·03 ml applied).[8] However, when streptokinase was added to the cerebrospinal fluids a strong activation appeared on the standard plates.[8] On the heated fibrin plates[9] activity appeared only after addition of a bovine plasminogen preparation.[1] While the standard fibrinogen preparation contains plasminogen,[8] the latter is destroyed by heating the clotted fibrin plates.[9] The results therefore indicate that while free protease is absent or low in the cases of cerebrospinal fluid tried, a precursor of a plasminogen activator is present, in agreement with the findings of Albrechtsen *et al.*[2]

It could now be demonstrated (Fig. 1) that the presence of all three components, cerebrospinal fluid, streptokinase and plasminogen, was necessary to develop smooth muscle contracting activity from a bovine plasma substrate.[1] As previously shown, this substrate contains plasminogen that can

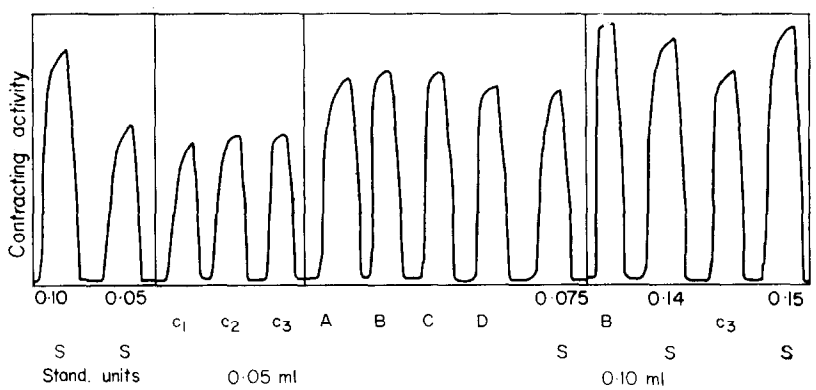

FIG. 1. Cerebrospinal fluid from patients with schizophrenia (A, B) and multiple sclerosis (C, D) was incubated with a bovine plasma substrate[1] in the presence of streptokinase (4 hr at 38°, pH 7·8). After an alcohol extraction smooth muscle (guinea pig ileum) contracting activity was assayed in comparision to standard bradykinin units[10]. In control experiments with substrate and cerebrospinal fluid (schizophrenic) (c_1), substrate alone (c_2), and substrate and streptokinase (c_3), 42·5 per cent less smooth muscle contracting activity was released. (Plasma concentration 0·25 ml per ml.)

be activated to form free plasmin. With an incomplete fibrinolytic system (Fig. 1, controls) significantly less bradykinin was formed. This is apparently due to the slowed spontaneous activation of plasminogen in the bovine substrate.[1]

A freshly prepared globulin fraction of bovine plasma precipitated at 50 per cent ammonium sulphate concentration and dialysed[5] was also used to study the effect of the cerebrospinal fluid upon bradykinin release. On incubation with an equal volume of the globulins (0·5 ml) for 13 minutes at 38° pH 7·7, no smooth muscle contracting activity developed. In a control experiment normal bradykinin activity developed (approximately 8·5 units per ml plasma) when released with 1 mg heated *Bothrops jararaca* venom (containing a protease/esterase).[10, 11] After about 10 min of incubation all activity disappeared owing to the presence of a bradykinin destroying enzyme in a fresh globulin preparation. Since longer incubation time could not be applied, the study of low protease action upon the bradykinin precursor in this type of substrate would be unsuitable. The acid heated plasma substrate applied above contains less inhibitors and no bradykinin destroying activity. From this substrate bradykinin can be released with relatively small amounts of trypsin or snake venom or by the activation of plasminogen.[1]

Cerebrospinal fluid had no effect directly upon the isolated guinea pig ileum (sensitive to 0·05 units of bradykinin) when applied in amounts up to 0·5 ml in a 3–4 ml bath of Tyrode solution. Nor did

any effect appear upon the addition of streptokinase (200 mg) and globulin preparation (0·5 ml). When, after 10 min incubation of the globulins with streptokinase and cerebrospinal fluid from a schizophrenic, snake venom was added, smooth muscle contracting activity appeared normally after 1 min of incubation[10] then slowly disappeared.

These experiments do not exclude the possibility that free protease may appear under certain conditions in the cerebrospinal fluid of patients with CNS disease. In the cases tried, however, the protease activity if present would not be sufficient to release bradykinin under the above conditions. A detailed account of this work will be published elsewhere.

Acknowledgement—I am indebted to Dr. Irvine H. Page for his interest and helpful criticism of this work.

Laboratory of the Foundation for Chemical Research,
Biochemical Institute and
Department of Pharmacology,
University of Helsinki,
Finland.

ULLA HAMBERG*

* Present address Cleveland Clinic Foundation, Research Division, Cleveland, Ohio, U.S.A.

REFERENCES

1. U. HAMBERG, *Biochim. Biophys. Acta* **36**, 296 (1959).
2. O. K. ALBRECHTSEN, O. STORM and M. CLAASSEN, *Scand. J. Clin. Lab. Investig.* **10**, 310 (1958).
3. L. F. CHAPMAN and H. G. WOLFF, *Science* **128**, 1208 (1958).
4. S. M. HILTON and G. P. LEWIS, *Brit. Med. Bull.* **13**, 189 (1957).
5. M. ROCHA E SILVA, W. T. BERALDO and G. ROSENFELD, *Am. J. Physiol.* **156**, 261 (1949).
6. T. ASTRUP, *Blood* **11**, 781 (1956).
7. G. H. SCHWERT, H. NEURATH, S. KAUFMAN and J. E. SNOKE, *J. Biol. Chem.* **172**, 221 (1948).
8. S. MÜLLERTZ and T. ASTRUP, *Arch. Biochem. Biophys.* **40**, 346 (1952).
9. M. LASSEN, *Acta Physiol. Scand.* **27**, 371 (1952).
10. U. HAMBERG and M. ROCHA E SILVA, *Arch. Int. Pharmacodyn.* **110**, 222 (1957).
11. U. HAMBERG and M. ROCHA E SILVA, *Experientia* **13**, 489 (1957).