samples of *Cannabis sativa* from 10 different countries has been determined. The procedure adopted was based on the experience of some previous authors[3,6]. The extract in petrol ether was shaken out with a solution containing 5% NaOH and 5% Na_2SO_3. The alkali extract obtained was acidified by means of diluted H_2SO_4, extracted with ether, dried in vacuum, weight and calculated as percentage in dry petrol ether extract. In order to avoid decarboxylation of cannabidiolic acid, the procedure has to be run rapidly. Duplicate analyses have indicated a good reproducibility of the results. Although the acid fraction obtained by this way might contain also some other weak acids, the results are treated as if they correspond only to the content of cannabidiolic acid, which is obviously the main constituent of this fraction. The results obtained are summarized in the Table.

As is seen, the content of acid fraction in resin ranged from 3.8% to 41.7%. It was lowest in cannabis originating in tropical regions, while highest in samples from European countries, marked in the Table as 'northern area'. Samples from Mediterranean area exhibited the properties of both the groups, mostly showing the tendency to an intermediate content of acid fraction. In spite of the lack of exact data dealing with the age of certain samples, some results show that there is a lower content of acid fraction in old resin than in fresh.

The results obtained indicate that the phytochemical process of gradual conversion of cannabinolic compounds ('ripening' of the resin) is rather advanced in varieties growing in hot regions. In contrast to this, in plants developed under unfavourable climatic conditions, the 'unripe' type of the resin predominates, containing a large amount of unchanged cannabidiolic acid. It seems probable that even fresh cannabis from tropical regions mostly belong to the 'ripe' type, as the largest part of acid is converted before harvesting to more thermostable products. However, such cannabis might still be exposed to a further slight decrease in acid content during storage. In 'unripe' type of the drug, originating from northern areas, the process of additional 'ripening' during storage seems also to occur under favourable conditions. According to the results obtained, it seems that the intermediate type, represented in our work by the samples from the Mediterranean area, exhibits the greatest variations in acid content, and is probably more affected by the time and conditions of storage than the two previous types. Some of the conclusions drawn here may confirm previous findings, based on direct ultraviolet spectrophotometry of cannabis resin[10,11].

The content of acid fraction in various groups of samples

Origin Area	Country	Age (Production year)	Number of samples analyzed	Percentage of acid fraction in resin
Tropical	Burma	old	1	3.8
Tropical	Costa Rica	old	1	7.0
Tropical	Brazil	1959	14	5.2–10.7 (mean 8.2)
Mediterranean	Greece	—	5	8.9–15.4 (mean 12.0)
Mediterranean	Yugoslavia	—	1	16.1
Mediterranean	Morocco	1960	1	18.5
Mediterranean	Cyprus	old	1	14.1
Mediterranean	Cyprus	1959	1	33.7
Northern	Switzerland	1960	1	32.4
Northern	England	1959	1	41.7
Northern	Germany	old	5	28.4–39.1 (mean 31.8)

Résumé. L'acide cannabidiolique pouvant être considérée comme la substance initiale dans la conversion graduelle des composants de la résine du chanvre (*Cannabis sativa*), le taux de la fraction acide peut servir d'indication sur l'avance du processus de «mûrissement» de la résine. La teneur en acide a été trouvé la moins élevée dans la résine du chanvre provenant de régions tropicales, ce qui s'explique par la conversion plus avancée dans des variétés dévelopées sous un climat favorable et chaud. D'après les résultats obtenus, cette conversion semble continuer pendant la conservation de la drogue.

LJ. GRLIĆ and A. ANDREC

Institute for the Control of Drugs, Zagreb (Yugoslavia), March 31, 1961.

[10] United Nations Secretariat, document ST/SOA/SER. S/2 (1960).

[11] We are indebted to the Division of Narcotic Drugs of United Nations for having kindly supplied most of cannabis samples examined in this study.

Cerebral Vascular Action of Bradykinin in the Dog

One of the most important biological effects of bradykinin is its action on the vascular system[1]; moreover the demonstration of the presence of kinin-forming enzymes in perfusates of different systems[2] supports the view that bradykinin may play a role in the regulation of local blood flow in various vascular districts[3]. The presence of these enzymes also in the perfusates of the ventricular system of the cat[4] suggests the need for a critical evaluation of the effects of bradykinin on cerebral vascular flow. Moreover, since the description of its chemical composition and its synthesis[5], investigations of its effects can be carried out with much greater reliability.

In the present study, the vascular reactions of the brain vessels were studied in the chloralosed dog by means of a previously developed technique consisting of the registration of the intracranial venous pressure by means of a catheter introduced in a craniad direction into the peripheral portion of the external jugular vein[6]; simultaneous tracings of arterial pressure, intracranial venous pressure, other vascular phenomena (systemic venous pressure, nasal plethysmogram[6] venous outflow from the superior sagittal sinus[7]) and respiration were registered on a 6-channel Grass Polygraph. The effects of intravenous administration

[1] M. ROCHA Y SILVA, W. T. BERALDO, and G. ROSENFELD, Amer. J. Physiol. *156*, 261 (1949).

[2] S. M. HILTON and G. P. LEWIS, J. Physiol. *128*, 235 (1955); *129*, 253 (1955). – R. H. FOX and S. M. HILTON, J. Physiol. *142*, 219 (1958). – L. F. CHAPMAN, A. RAMOS, H. GOODELL, G. SILVERMAN, and H. G. WOLFF, Arch. Neurol. *3*, 223 (1960).

[3] D. F. ELLIOT, E. W. HORTON, and G. P. LEWIS, J. Physiol. *153*, 473 (1960).

[4] L. F. CHAPMAN, A. RAMOS, A. CORRADO, and V. FORTES, Arch. Neurol. *3*, 43 (1960).

[5] R. A. BOISSONNAS, S. GUTTMANN, P. A. JAQUENOUD, H. KONZETT, and E. STÜRMER, Exper. *16*, 326 (1960). – D. F. ELLIOTT, G. P. LEWIS, and E. W. HORTON, Biochem. biophys. Res. Comm. *3*, 87 (1960). – H. KONZETT and R. A. BOISSONNAS, Exper. *16*, 456 (1960). – D. F. ELLIOT, E. W. HORTON, and G. P. LEWIS, Biochem. J. *78*, 60 (1961).

[6] D. BOVET, M. VIRNO, G. L. GATTI, and A. CARPI, Arch. int. Pharmacodyn. *110*, 380 (1957).

[7] R. C. URSILLO and A. CARPI, in preparation.

of natural crude, highly purified and of synthetic bradykinin[8] were compared with the action exerted by histamine[9].

A clear cerebral vasodilator effect of bradykinin was observed in all experiments. Bradykinin injected intravenously in doses ranging between 3 and 10 U/kg for the natural crude and between 0.5 and 3 μg/kg for the natural highly purified and synthetic preparations, regularly produced in dogs under good experimental conditions a lowering of the arterial pressure and a simultaneous increase in the intracranial venous pressure (Figure 1, 2); according to previous observations[10], as no changes in the central venous pressure were observed with these doses of bradykinin, this type of reaction must be considered the consequence of a reduction in cerebral vascular resistance; this is further demonstrated by the observation that the increase in the intracranial venous pressure provoked by bradykinin is accompanied by a rise in the intracranial venous outflow (Figure 1). The vessels of the nasal mucosa exhibited, on the contrary, a very low sensitivity to the vasodilator effect of bradykinin (Figure 2). In six experiments of this type, a slight nasal vasodilator response was observed only in one case, whereas, in the other 5, no response or a delayed and slight constriction was obtained. The effects of bradykinin on arterial pressure and cerebral vessels were accompanied by an increase in heart rate and a slight stimulation of respiration (Figure 2).

All these effects are very transient; they last no more than 30–60 sec and no tachyphylaxis is seen with repeated administration of the polypeptide. They persist with the same intensity even after the administration of doses of pyrilamine or atropine which inhibit the cerebral vasodilator effect of histamine or, respectively, of acetylcholine.

If the action of bradykinin is compared with that exerted by histamine, it is evident that a cerebral vasodilator response of similar intensity and duration can be obtained with the same or a slightly lower dose of histamine. Generally, the ratio between doses of bradykinin and of histamine equally active on the cerebral vessels ranges between 0.8 and 2. Though the intensity of the effects of bradykinin and histamine differed by not more than twofold in respect to the hypotension and tachycardia, a clear difference exists between their effects on the nasal vessels which always respond with a definite vasodilatation to histamine. In contrast to bradykinin, histamine in the doses employed did not exert any clear influence on respiration (Figure 2).

These results indicate that the cerebral vessels show a great sensitivity to the vasodilator action of bradykinin; this fact and the observation that the same sensitivity is not exhibited by the nasal vessels, which generally react to other vasoactive drugs in the same manner as the cerebral vessels[11], appears to be a good indication of the possible rôle played by bradykinin in the physiopathological control of brain circulation.

Zusammenfassung. Die Wirkung von natürlichem und synthetischem Bradykinin auf den cerebralen Kreislauf wurde beim Hund in Chloralosenarkose durch die Registrierung des intracraniellen Venendruckes und Blutausflusses und des Nasenhöhlenplethysmogrammes untersucht. Bradykinin erzeugt in Dosen von 0.5–3 μg/kg i.v. eine deutliche Erweiterung der Hirngefässe, analog der durch eine gleiche oder etwas niedrigere Menge von Histamin hervorgerufenen. Im Gegensatz zum Histamin übt Bradykinin in dieser Dosierung keine erweiternde Wirkung auf die Nasenhöhlengefässe aus. Die durch Bradykinin erzeugte Zunahme der Gehirndurchblutung wird durch Neoantergan oder Atropin nicht gehemmt.

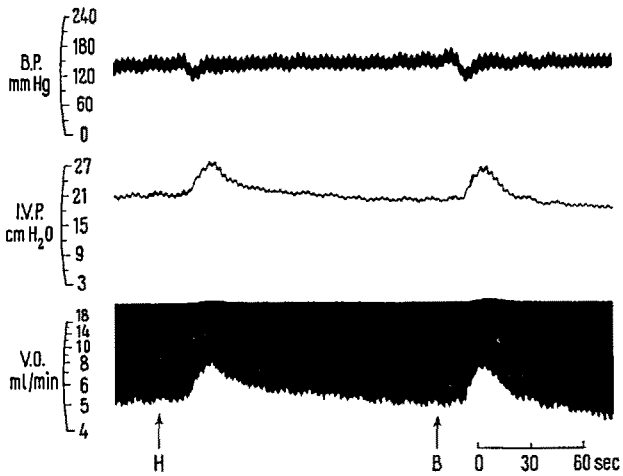

Fig. 1. Cerebral vasodilator action of natural crude bradykinin (B = 3 U/kg i.v.) and of histamine (H = 1 μg/kg i.v.). B.P. = femoral blood pressure (mm Hg). I.V.P. = intracranial pressure (cm H_2O). V.O. = outflow from the superior sagittal sinus (ml/min). Female dog, 19.5 kg (10/21/60) chloralose (100 mg/kg i.v.).

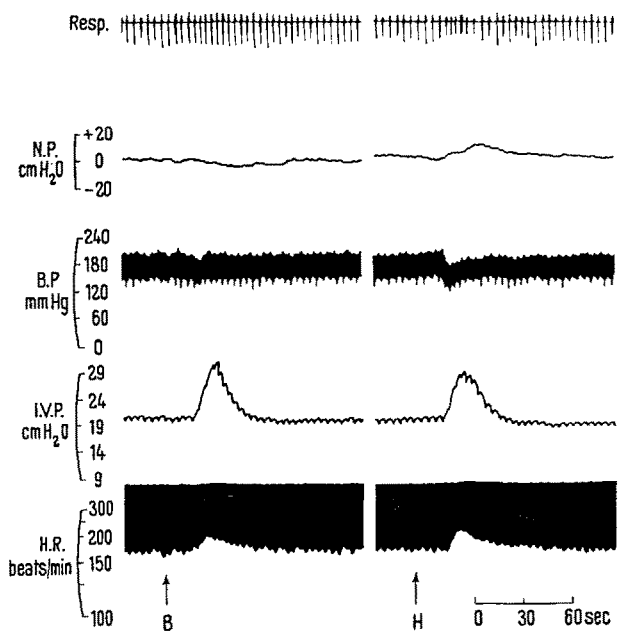

Fig. 2. Cerebral vasodilator effect of natural highly purified bradykinin (B = 1.5 μg/kg i.v.) and of histamine (H = 1 μg/kg i.v.). Resp. = Respiration. N.P. = nasal plethysmogram. B.P. = femoral arterial pressure (mm Hg). I.V.P. = intracranial venous pressure (cm H_2O). H.R. = heart rate (beats/min). Male dog, 16 kg (11/28/60) chloralose (100 mg/kg i.v.). The tracing shows a vasodilator effect both on the cerebral and nasal vessels with histamine, but only on the cerebral vessels with bradykinin.

A. Carpi and A. Pinto Corrado[11]

Istituto Superiore di Sanità, Roma (Italy), April 4, 1961.

[8] We should like to express our thanks to Prof. M. Rocha y Silva for the supply of the natural crude bradykinin, to Dr. G. P. Lewis of the National Institute for Medical Research (London) for the natural highly purified sample and to Dr. A. Cerletti of Sandoz Ltd. (Basle) for the synthetic preparation.

[9] M. Virno, S. B. Gertner, and D. Bovet, J. Pharmacol. exp. Therap. *118*, 63 (1956).

[10] D. Bovet, A. Carpi, and M. Virno, Exper. *16*, 1 (1960).

[11] Present address: Department of Pharmacology, University of Riberao Preto, S. Paulo (Brazil).