hydroxylase and dopa decarboxylase activities in the brain were accelerated[13].

On the other hand, some another properties of DDC must be considered, i.e. the inhibition of enzyme activities, including those involved in morphine destruction.

Our results show that DDC potentiates the morphine analgesia and that the initial changes in CA content itself in whole brain by DDC do not seem to be responsible for this phenomenon.

Zusammenfassung. Nach DDC-Vorbehandlung wurde bei männlichen Ratten eine deutliche Potenzierung der Morphinanalgesie beobachtet. Es konnte nachgewiesen werden, dass dieses Phänomen nicht durch die nach DDC-Gabe auftretende Veränderung des CA-Gehaltes im Gehirn bedingt ist.

K. Watanabe, Y. Matsui and H. Iwata

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Osaka University, Toneyama, Osaka (Japan), 28 March 1969.

[13] T. Itoh, M. Matsuoka, K. Nakajima, K. Tagawa and R. Imaizumi, Jap. J. Pharmac. *12*, 130 (1962).

Aggression in Mice Associated with Changes in the Monoamine-Metabolism of the Brain

It is known that the administration of L-DOPA and psychotomimetic drugs may provoke an aggressive behaviour in certain animals (Valzelli[1]). However, in principal the biochemical background creating aggressive attitudes is unknown.

Previously Lycke and Roos[2] reported that the *Herpes simplex* virus (HSV) encephalitis in mice will cause raised synthesis of dopamine (DA) as well as 5-hydroxytryptamine (5-HT). These studies suggested the use of specific inhibitors for DA and 5-HT. In one series of experiments the mice were treated with the methylesterhydrochloride of DL-*p*-chlorophenylalanine (H 69/17), an effective inhibitor for the hydroxylation of tryptophane to 5-hydroxy-tryptophane.

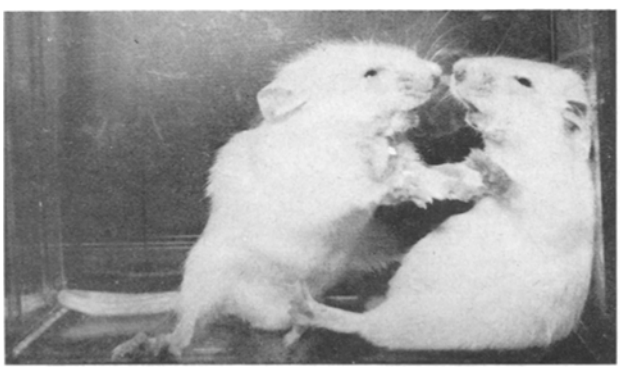
Swiss albino mice of our own laboratory breed were inoculated intracerebrally with a mouse-brain-adapted strain (St 2 Gbg 11) of HSV (15 LD_{50}). H 69/17 was given by injection, 400 mg/kg, one day prior to virus inoculation and then daily for 4 days, after which the animals were sacrificed and the brains analyzed for the contents of DA, 5-HT, homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA). Determinations of DA and HVA were made spectrophotofluorometrically according to the methods described by Carlsson and Waldeck[3] and Andén et al.[4]. Assays of 5-HT and 5-HIAA were made according to Andén and Magnusson[5] and Roos[6], respectively. Infective virus was titrated by determining the number of plaque-forming units (pfu) on monolayer cultures of GMK cells, overlayed with Eagle's complete medium containing 3% bovine serum and 1% methylcellulose.

The HSV infected animals, treated with H 69/17, demonstrated at 4 days after the virus infection marked excitation and, in addition, an aggressive behaviour. Thus, they not only revealed the characteristic jumpiness and sensitivity to light and sound effects, usually found during the excitatory stage of the disease, but were frequently found involved in fights. No aggressiveness was noted in mice only inoculated with HSV or only treated with H 69/17, although both these groups of animals showed signs of excitation. Table I presents the results of assays of monoamines and infective virus.

The rise in DA synthesis due to the herpetic encephalitis is reflected in the increased concentrations of HVA. The inhibitory effect of H 69/17 on 5-HT synthesis appears from the 5-HT as well as the 5-HIAA concentrations. No inhibitory effect on synthesis of HSV by H 69/17 was observed.

As the aggressiveness was assumed to be conditioned by an increased DA synthesis and a simultaneous reduction of the formation of 5-HT, a series of experiments

[1] L. Valzelli, Adv. Pharmac. *5*, 79 (1967).
[2] E. Lycke and B.-E. Roos, Experientia *24*, 687 (1968).
[3] A. Carlsson and B. Waldeck, Acta physiol. scand. *54*, 87 (1962).
[4] N.-E. Andén, B.-E. Roos and B. Werdinius, Life Sci. *2*, 448 (1963).
[5] N.-E. Andén and T. Magnusson, Acta physiol. scand. *69*, 87 (1967).
[6] B.-E. Roos, Life Sci. *1*, 25 (1962).



was performed in which L-DOPA was given to animals with reduced 5-HT turnover due to treatment with H 69/17. The latter compound was given as one dose of 400 mg/kg, i.p., to 60 mice and after various periods of time L-DOPA (500 mg/kg) was administrated to groups of 10 mice each.

It was then found that L-DOPA given 7 h or later after the injection of H 69/17 produced an immensely aggressive behaviour in the animals. Within 10 min after the L-DOPA injection the mice revealed a picture of the type generally seen after administration of L-DOPA i.e. piloerection, exophtalmus, rising of the tail and neck etc. This was, however, a transient phenomenon and was followed during the next hour by pronounced aggressive behaviour (Figures 1 and 2). The animals appeared in positions as suggesting postures for fight and indeed fights occurred constantly between groups, often containing several animals. These vigorous fights resulted in bleedings often from the nose or other parts of the heads.

Another characteristic feature was that the nose and the front of neck were wet as indicative for an increased perspiration or salivation. After about 1 h the aggressive behaviour ceased.

When an interval of 7 h between H 69/17 and L-DOPA was allowed, the concentrations of DA and 5-HT were 10.79 and 0.09, respectively, in μg/g of brain tissue. If the H 69/17 treatment followed immediately or 3 h after the injection of L-DOPA no aggressive behaviour but the usual L-DOPA effects only were observed.

The dose-response for L-DOPA in H 69/17 treated mice was studied with concentrations of L-DOPA ranging from 500–200 mg/kg and in mice pretreated for 5 days with daily injections of 400 mg/kg of H 69/17. Mice receiving 200 mg/kg of L-DOPA showed excitation but no aggressiveness. Among those to which a dose of 300 mg/kg was given, 4–5 incidents of fights were seen during the hour of observation while more than 20 fights were registered for animals given 400 mg/kg of L-DOPA. In the group receiving 500 mg/kg the fighting went on almost continuously for the whole period of observation. In this context it is of interest to mention that the pronounced excitation developed in mice treated with *p*-chlorophenylalanine and L-DOPA has been observed by CHURUSCIEL and HERMAN[7]. These authors did not observe aggressiveness in the animals, however, probably because the concentrations of L-DOPA used did not exceed 200 mg/kg.

Using H 22/54, another effective inhibitor of the 5-HT synthesis with a slight catechol-*o*-methyltransferase (COMT) inhibitory effect and with a more rapid onset of inhibition than *p*-chlorophenylalanine, the results obtained after a subsequent L-DOPA injection were in agreement with those described for H 69/17. Table II summarizes one of the experiments. 3 groups of 10 mice each received i.p. 500 mg of H 22/54 per kg. 2 h later the groups were given 100, 200 or 300 mg/kg of L-DOPA, respectively. Those mice receiving 200 or 300 mg/kg L-DOPA developed an aggressive behaviour, whereas mice to which only 100 mg/kg were given did not. The lower dose dependence to L-DOPA for mice pretreated with H 22/54 than the one for animals treated with H 69/17 is explicable if the COMT inhibitory effect of H 22/54 is kept in mind.

In the study reported, aggressive behaviour could be induced in mice using 3 different types of experimental set-up. In common they have revealed that the appearance of aggression in the animals is associated with a high concentration in the brain of DA and a low concentration of 5-HT. Thus, an aggressive behaviour seems to follow upon an increased synthesis of DA, whether induced by a viral encephalitis (*Herpes simplex*) or the administration of L-DOPA, provided the 5-HT synthesis was reduced by the inhibitors, H 69/17 or 22/54. Moreover, it seemed obvious that it was not the absolute concentrations of the monoamines that were determining but the DA turnover relative to the one of 5-HT. Aggression thus seemed to be caused by a disturbed balance in activity between the 2 types of monoaminergic neurons. Under the experimental conditions applied, NA seemed less important for the development of aggression. The present study does not, however, exclude the possibility that also an altered NA turnover might be of importance, since no determinations of NA or normetanephrine after MAO inhibition were made. A more detailed report of the present study will follow elsewhere.

In mice the symptomatology of the HSV encephalitis seems at least partially dependent upon the rise in the monoamine synthesis. Inhibition of the DA-synthesis by administration of α-methyltyrosine will thus markedly reduce the excitatory stage in the infected animals (unpublished data). Furthermore, the capacity to affect the monoamine-metabolism is not present in all viral encephalitides. One of the viruses besides HSV which by intracerebral infection of mice will cause an increase of DA and 5-HT synthesis is rabies. With these observations

Table I. Concentrations of monoamines and *Herpes simplex* virus (HSV) in brains of mice after treatment with *p*-chlorophenylalanine (H 69/17) and inoculation with HSV

Treatment	No. of mice	μg/g Brain tissue DA	HVA	5-HT	5-HIAA	log pfu/ml
HSV	100	0.48	0.23	0.38	0.59	5.4
HSV + H 69/17	175	0.44	0.19	0.06	0.10	6.1
H 69/17	60	0.43	0.09	0.07	0.05	–
Untreated control	50	0.43	0.06	0.35	0.31	–

Mice were treated with H 69/17 for 24 h before they were inoculated with HSV (15 LD_{50}). 4 days later the brains were harvested, the concentration of dopamine (DA), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), respectively, assayed, the amount of infective virus in a 20% brain suspension titrated. The virus titer is expressed in plaque forming units (pfu) per millilitre.

Table II. Concentration of monoamines in brains of mice treated with α-propyldopacetamide (H 22/54) and L-DOPA

Treatment	μg/g Brain tissue DA	5-HT	NA
H 22/54 + L-DOPA 100 mg/kg	0.96	0.21	0.35
H 22/54 + L-DOPA 200 mg/kg	1.43	0.12	0.40
H 22/54 + L-DOPA 300 mg/kg	3.25	0.11	0.38

The mice were pretreated with H 22/54, 500 mg/kg, and received 2 h later 100–300 mg/kg of L-DOPA. The brains were harvested 30 min after the L-DOPA injection and analyzed for contents of dopamine (DA), 5-hydroxytryptamine (5-HT) and noradrenalin (NA).

[7] T. L. CHURUSCIEL and Z. S. HERMAN, Psychopharmacologia *14*, 124 (1969).

in mind it is tempting to suggest that the excitement phase and the aggressiveness of a rabies diseased animal might be due to a changed DA/5-HT ratio of the brain. Similarly, changes in the monoamine-balance of the type described might be pertinent for the understanding of the phases of excitement and aggressiveness often appearing in human cases of encephalitis[8].

Zusammenfassung. Mit *Herpes simplex* Virus i.c. beimpfte Mäuse entwickelten nach vorausgegangener Behandlung mit *p*-Chlorphenylalanin ein ausgesprochen aggressives Verhalten. Bei Mäusen, deren Serotoninsynthese durch Behandlung mit *p*-Chlorphenylalanin oder α-Propylacetamid ausser Funktion gesetzt war, konnte ausgeprägtes Aggressionsverhalten durch i.p. Injektion von L-DOPA induziert werden.

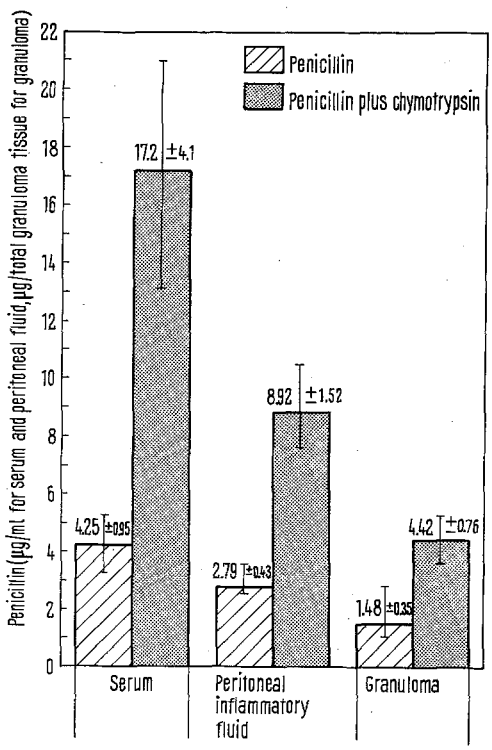
E. Lycke, K. Modigh and B.-E. Roos

Departments of Virology and Pharmacology, University of Göteborg (Sweden), 29 April 1969.

[8] We are indebted to Dr. H. Corrodi, AB Hässle, Göteborg, for having kindly provided the substances H 69/17 and H 22/54, and to Miss Gun Alfredsson, Miss Ann-Britt Björkman, Miss Anna-Karin Brorson, Mrs. Gun Lundblad and Mrs. Margareta Rosenkvist for skilful technical assistance. The study was supported by grant No. K 69-14X-2728-01A from the Swedish Medical Research Council.

Enhancement of Drug Activity by Chymotrypsin. Penicillin Penetration into Granulomatous Lesions and Inflammatory Fluids

In an earlier publication Wohlman, Syed and Ronchi[1] showed that the administration of penicillin G combined with the proteolytic enzyme chymotrypsin yields significantly higher serum, eye and brain levels of penicillin than does the administration of the antibiotic alone. It was suggested that chymotrypsin may be of considerable value in enhancing the penetration of penicillin into relatively inaccessible tissues thereby making it possible to eliminate penicillin-sensitive bacteria that have become localized in these areas. This suggestion has been experimentally tested by ascertaining the level of penicillin achieved in granulomatous and inflammatory tissues after the administration of penicillin alone and penicillin in combination with chymotrypsin.



Effect of chymotrypsin on the concentration of penicillin in serum, granuloma and inflammatory fluid.

Material and methods. The formation of granulomas in Wistar rats was accomplished by the insertion of a sterile cotton pellet into subcutaneous tissues of the axillary region. The incision was sutured and the cotton pellet allowed to remain in place for 5 days to insure complete development of granulomatous tissue surrounding the pellet. 5 days after implanting the cotton pellet the control animals received 1 ml of saline (i.p.) 30 min prior to the administration of 100 mg/kg of penicillin G (i.m.). Test animals received 10 mg/kg of pure chymotrypsin in 1 ml of saline (i.p.) 30 min before the administration of 100 mg/kg of penicillin G (i.m.) (penicillin potency: 1595 U/mg). 2 h after the administration of penicillin the rats were sacrificed. The granuloma and enveloped pellet were excised, homogenized in bovine serum albumin-phosphate buffer (pH 4.5) and centrifuged. The resultant supernatant was collected and frozen at −20 °C.

Peritoneal inflammations were induced in Wistar rats by the i.p. administration of 0.1 ml of turpentine. 15 min after the turpentine injection control animals received 1 ml of saline (i.p.) while the test animals received 10 mg/kg of pure chymotrypsin in 1 ml of saline (i.p.) 30 min after the administration of saline or chymotrypsin both control and test animals were given 100 mg/kg of penicillin G (i.m.) The rats were sacrificed 2 h after the penicillin administration and peritoneal inflammatory fluid was collected and frozen at −20 °C.

Granuloma extracts and inflammatory fluids were thawed and assayed 1 day after the experiment had been performed. The samples were appropriately diluted with bovine serum albumin-phosphate buffer (pH 4.5) in preparation for the microbiological plate assay[2]. The size of the zones of inhibition of *Staphylococcus aureus* was used as a measure of penicillin concentration. The base agar layer consisted of Difco Bacto Antibiotic Medium 2 and the seed layer consisted of Difco Bacto Antibiotic Medium 1 containing a 3% suspension of *Staphylococcus*

[1] A. Wohlman, M. Syed and M. Ronchi, Can. J. Physiol. Pharmac. *46*, 815 (1968).

[2] D. C. Grove and W. A. Randall, *Assay Methods of Antibiotics* (Medical Encyclopedia Inc., New York 1955), p. 7.