occurs, first, a rapid dissociation into halves. In this time, there is little loss of activity and we suppose that there can be little alteration of the subunit structure. Disruption of the subunit structure does take place, but much more slowly, as exposure to pH 3.5 is prolonged. Since the value of *s* decreases only from 9.0–8.0 in the period from 1–48 h, we also conclude that disruption of the subunit structure does not cause further dissociation.

Figure 1 (c) shows that the change from 18–9.8 S could be largely reversed, after 1 h. After longer exposure to pH 3.5, the extent of regeneration of the 18-S peak decreased, with a concomitant increase in material of 8–10 S; e.g. in an aliquot portion that was adjusted to pH 7 after 6 h at pH 3.5, the area of the 18-S peak amounted to about $^1/_3$ of the total. Although there is a qualitative correspondence between the decrease in activity, the increase in viscosity, and the loss of the ability to reaggregate, these do not correspond quantitatively (cf. Figure 2). We believe this may be due to the fact that in the progress toward complete denaturation there are formed diverse 'mixed' aggregates, that contain both denatured and native subunits. This makes it impossible to interpret the data in terms of a simple model.

Many experiments were done at other concentrations and pH values, that will not be reported for lack of space. In general, they show that both pH and concentration influence the dissociation and denaturation processes. Some indicative results are (all 1 h after preparation of the solution): (1) 0.1 *M* acetate, pH 4.2, caused little dissociation; (2) 0.02 *M* acetate, pH 3.5, induced substantial but not complete dissociation to a 10-S product; (3) 0.1 *M* acetate buffer of pH 3.1 produced lower-*s* products, as exemplified in Figure 1 (d), and the activity was lost very quickly.

There have been some earlier reports in the literature concerning the existence of urease with an *s*-value of 8–12[9-11]; however, the characterization of the products mentioned in these reports is not sufficiently detailed to permit a meaningful comparison with the 10-S product described in this communication. Very recently, BLATTLER et al.[12] have reported that urease exposed to *Tris* buffer of pH 9 and 90% 1,2-propanediol is dissociated into halves and that the activity retained. It seems quite probable that this product is closely related to that obtained in the present work[13].

Riassunto. Le molecole dell'urease (peso molecolare 480,000, coefficiente di sedimentazione 18,6 S) si dividono a metà quando trattate con tampone acetato, 0,1 *M*, pH 3,5. Inizialmente, l'attività enzimatica è 40% di quella al pH 7 (tampone fosfato, 0,34 *M*) e la dissociazione è riversibile. Prolungata esposizione al pH 3,5 causa una lenta denaturazione, con perdita dell'attività e dell'abilità di ricostituirsi.

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Effect on the Monoamine-Metabolism of the Mouse Brain by Experimental *Herpes simplex* Infection

The syndrome observed after administration of the dopamine precursor L-DOPA is characterized by excitation and a pronounced peripheral sympathetic tonus. The dopamine (DA) concentration will reach very high values in the corpus striatum area. Mice infected i.c. with *Herpes simplex* virus reveal during the course of infection i.a. signs of excitation, similar to those observed after administration of L-DOPA. These symptoms precede the more severe symptoms of encephalitis, such as convulsions.

Abnormal low concentrations of dopamine are found in the neostriatum and substantia nigra areas in post mortem examinations of patients with Parkinson's disease[1]. A decrease in the concentration of 5-hydroxy-tryptamine (5-HT)[2] has also been demonstrated in the brains of such patients. It is known that Parkinson's disease might appear after time periods of varying length following an acute encephalitis. Against this background we considered it important to study experimentally the possible relationship between virus induced encephalitides and the monoamine-metabolism of the brain.

Swiss albino mice of our own laboratory breed were inoculated i.c. with a mouse-brain-adapted strain (St2Gbg 10) of *H. simplex* virus. A series of experiments were performed in which the amount of virus inoculated (30–300 LD_{50}) was adjusted to produce encephalitis but not to kill the mice during the first 5 days after the inoculation. On day 5 after inoculation, when most of the mice showed signs of encephalitis (ticks, convulsions or lethargia) 15–22 mice were selected, sacrificed by ether narcosis, and the brains were analysed for the contents of DA and homovanillic acid (HVA). In parallel, the brains of mice inoculated with an isotonic NaCl solution or brains of non-inoculated mice were analysed. As in the first 4 experiments, no differences were observed if non-inoculated mice or mice given the NaCl solution were used; only non-inoculated mice were used as controls in following experiments.

Determinations of DA and HVA were made spectrophotofluorometrically according to the method described by CARLSSON and WALDECK[3] and that of ANDÉN et al.[4].

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Assays of 5-HT and 5-HIAA were made according to ANDÉN and MAGNUSSON[5] and ROOS[6], respectively.

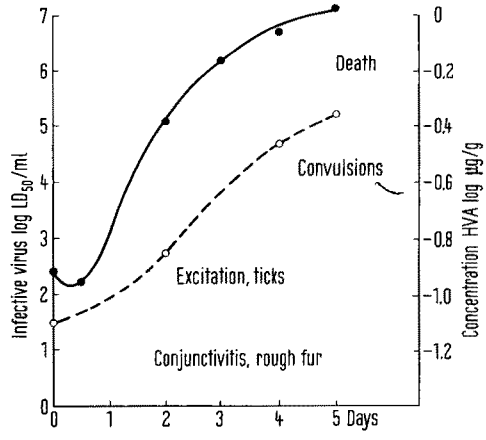
The results, listed in Table I, demonstrate that the contents of HVA in brains of *Herpes* virus infected mice were almost 10 times that of the non-infected control brains. On the other hand, essentially the same values of DA were recorded for infected as for non-infected animals.

In another type of experiment, the HVA concentration was studied in relation to the synthesis of infective virus and the symptomatology of infected mice. A number of mice were inoculated i.c. with 30 LD_{50} of virus. Infectivity titrations were performed by serial dilutions of suspended, homogenized brain material and assayed by the i.c. inoculation of mice. The Figure illustrates that the concentration of HVA in the *Herpes* virus infected brains increased logarithmically with time. The curve of HVA concentration thus demonstrated evident similarities with the virus growth curve, although the data available were too limited to reveal an initial log phase in HVA formation. The HVA concentration of non-infected brains never exceeded 0.11 μg/g of brain tissue and, again, the DA values of infected and non-inoculated mouse brains indicated no essential differences, the mean value of the infected brains being 0.32 and that of the non-infected brains 0.28 μg/g of brain tissue. The Figure also gives onset and end of certain clinical symptoms. An increase in HVA concentration seemed to be discernible at an early stage when the mice showed signs of excitation only.

The correctness of this latter observation was verified in the following experiment. 75 mice were inoculated i.c. with 30 LD_{50} of the *H. simplex* virus strain used. 3 days after the inoculation 2 groups of mice were selected, one consisting of seemingly normal mice, the other comprising mice with hyperactivity, marked jumpiness, and abnormal sensitivity to light and sound effects. None of the groups contained mice which reacted with convulsions, not even after repeated provocations by spinning the animals by the tail. The group of seemingly normal mice gave a HVA value of 0.11 μg/g of tissue whereas the corresponding figure for the group of excitated mice was 0.20. 2 batches of brain tissue from non-inoculated control mice gave values of 0.05 and 0.11 μg/g, respectively.

Table II gives results obtained with varying concentrations of virus. The brains were harvested on day 5 after inoculation and the amounts of HVA were determined. As indicated by the Table a positive correlation between the dose of infective virus inoculated and the amount of HVA found was demonstrable, stressing the causal relationship between the herpetic infection and the finding of increased concentrations of HVA in the infected brains.

A herpetic infection in 21 mice with none or only single cases of brain stem infected animals caused no significant increase of HVA. Thus, s.c. infected mice (300 LD_{50}), harvested 12 days after inoculation, gave a HVA value of 0.11 μg/g of brain tissue, compared with 0.08 μg/g found for non-infected control mice. Of infected mice 4 had



The relationships between virus formation and HVA synthesis in the mouse brain and the symptomatology in mice inoculated i.c. with *H. simplex* virus. Swiss albino mice were inoculated with 30 LD_{50} of *H. simplex* virus. 1 h after the inoculation and then after intervals indicated in the Figure, 25 mouse brains were harvested, pooled, homogenized and tested for contents of infective virus (left abscissa, filled circles) and HVA activity (right abscissa, open circles). The results of these assays were plotten against time after inoculation. In addition onset and end of certain clinical symptoms observed are indicated in the Figure.

Table I. Contents of DA and HVA in brains of mice infected with *H. simplex* virus and in uninfected mouse brains

Experiment	No. of mice	Non-infected DA	Non-infected HVA	Infected DA	Infected HVA
1	18	N.D.	0.07	N.D.	0.41
2	22	0.34	N.D.	0.43	N.D.
2a	18	0.45	N.D.	0.43	N.D.
3a	6	0.59	N.D.	0.57	0.36
4	15	0.46	0.10	0.48	0.41
7	20	N.D.	0.01	N.D.	0.50
8	19	N.D.	0.02	N.D.	0.44
9	19	N.D.	0.06	N.D.	0.53
11	18	N.D.	0.04	N.D.	0.23
	Means	0.46	0.05	0.48	0.44

The mice were inoculated i.c. each with 0.02 ml (30 LD_{50}) of the virus suspension, except in experiments 2a and 3a when an inoculum corresponding to 300 LD_{50} was employed. The brains were harvested on day 5 after the inoculation. The number of harvested brains are given. A matching number on non-inoculated or NaCl-inoculated mice were used as non-infected controls. DA- and HVA-values are expressed in μg/g brain tissue. N.D. means not done.

Table II. Contents of HVA in brains of mice infected with varying concentrations of *H. simplex* and in non-infected mouse brains

No. of LD_{50} inoculated	No. of mice	HVA Non-infected	HVA Infected
30	19	0.06	0.53
15	19	0.09	0.35
8	20	0.07	0.29
4	21	0.10	0.29

The mice were inoculated i.c., each with 0.02 ml of the virus suspension diluted to contain the concentrations of virus indicated in the Table. The number of brains harvested on day 5 after the inoculations are given. A matching number of non-inoculated mouse brains served as non-infected controls. HVA-values are expressed in μg/g brain tissue. N.D. means not done.

[5] N.-E. ANDÉN and T. MAGNUSSON, Acta physiol. scand. *69*, 87 (1967).

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pareses in muscles of the extremities or the trunk and 2 developed convulsions after provocation by tail spinning.

The virus had to be of a mouse-brain-adapted strain in order to produce abnormal amounts of HVA. Virus strains adapted to chorioallantoic membranes of embryonated chicken eggs, gave no significant increase of HVA.

With the brain-adapted strain used, Syrian hamsters produced after i.c. inoculation (300 mouse LD_{50}) HVA concentrations (0.49 µg/g of brain tissue) equivalent to what was detectable in mice. The HVA activity on non-inoculated hamster brains was assayed to 0.06 µg/g of tissue.

Results of determinations of 5-HT and 5-HIAA in *H. simplex* infected mouse brains are listed in Table III. No effect on the 5-HT concentration was encountered by the herpetic infection and for non-infected brains essentially the same amounts of 5-HT and 5-HIAA were recorded. However, the virus encephalitis caused a significant increase in the 5-HIAA concentration, the mean-values being almost twice as high for infected as for non-infected brains.

It is known from previous studies that an increase of HVA or 5-HIAA with unchanged levels of DA or 5-HT might reflect an increased synthesis of DA and 5-HT in the monoaminergic neurons[7,8]. A retarded outflow of monoamines could also be possible. However, there are no obvious reasons why the herpetic encephalitis should cause a less effective outflow via the brain-blood barrier.

Table III. Contents of 5-HT and 5-HIAA in brains of mice infected with *H. simplex* virus and in non-infected mouse brains

Experiment	No. of mice	Non-infected 5-HT	Non-infected 5-HIAA	Infected 5-HT	Infected 5-HIAA
12	22	N.D.	0.45	N.D.	0.65
13	25	N.D.	0.28	N.D.	0.41
14	10	0.30	0.47	0.37	0.93
15	19	0.34	0.36	0.39	0.60
16	22	0.38	0.30	0.41	0.93
17	12	0.44	0.33	0.38	0.63
18	14	0.36	0.32	0.28	0.81
19	22	N.D.	0.39	N.D.	0.50
Means		0.36	0.36	0.37	0.68

The mice were inoculated i.c., each with 0.02 ml (30 LD_{50}) of the virus suspension. The brains were harvested on day 5 after the inoculation. The number of harvested brains are given. Brains of a matching number of non-inoculated mice were used as controls. 5-HT and 5-HIAA values are expressed in µg/g brain tissue. N.D. means not done.

On the contrary, an inflammatory condition will tend to increase the transport through the brain-blood barrier[9,10]. An increased monoamine synthesis seems, therefore, to be the most probable background for the findings of increased HVA and 5-HIAA concentrations in the *H. simplex* virus infected mouse brains.

For the mechanism behind a raised synthesis of monoamines in herpetic encephalitis, one possibility as a result of the viral infection would be that aminergic receptors were subjects to a blocking, an effect comparable to that evoked by chlorpromazin[7]. Such an explanation is, however, not compatible with the excitatory effect of the infection.

Another possibility which should be considered is that one or more of the synthesizing enzymes, e.g. the tyrosin hydroxylase which transfers tyrosin to DOPA, and which are rate limiting, are influenced by the infection. The synthesis of these enzymes may well be controlled by normally occurring inhibitory factor set out of action by the herpetic infection. It is tempting to suppose that the possible inhibitor has the effect of a repressor preventing the function of an operon and that, as a result of the virus synthesis, proteins are formed which combine with the repressor. As a consequence this would lead to an uncontrolled production of the monoamine synthesizing enzymes[11].

Zusammenfassung. Es wird nach i.c. Applikation von *Herpes-simplex*-Virus eine erhöhte Synthese von Monoaminen gefunden. Der Nachweis gelang auf Grund der erhöhten Konzentrationen der sauren Metabolite wie Homovanillinsäure und 5-Hydroxyindolessigsäure. Eine positive Korrelation zwischen der Synthese von infektiösem Virus und der Bildung von Homovanillinsäure wurde festgestellt.

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Stimulatory Action of Secretin on Gastric Pepsin Secretion

Since Greenlee's demonstration[1] that i.v. secretin markedly diminished gastric acid secretion from the Heidenhain pouch, the inhibitory effect of secretin on acid secretion has been extensively studied in dogs and man. There is no study, however, on the action of secretin on pepsin secretion in either species.

Recently Jorpes and Mutt prepared highly purified porcine secretin[2] and reported the amino acid composition and primary sequence for its 27 amino acid residues[3].

We have found that the i.v. administration of 3 units Jorpes secretin/min during continuous histamine stimulation (1 mg/h) caused a sharp rise in pepsin output from the Heidenhain pouch to a level 8 times that obtained during the control period.

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