The observation that glutathione, which contains -SH groups, is affected by CAP is of significance. It is conceivable that the reduction of glutathione observed might have been reflected in the fall of induction capacity of the Hensen's node on treatment with chloroacetophenone[11].

*Zusammenfassung.* Die Organisatorregion von Hühnchenembryonen wurde mit CAP behandelt und die Wirkung des CAP auf schwefelhaltige Aminosäuren papierchromatographisch untersucht. Die Analyse ergab, dass Glutathion stärker beeinflusst wurde als Methionin. Die Reduktion des Glutathiongehaltes könnte dem Abfall der Induktionskapazität des Hensenschen Knotens entsprechen, der durch die Behandlung mit CAP hervorgerufen wird.

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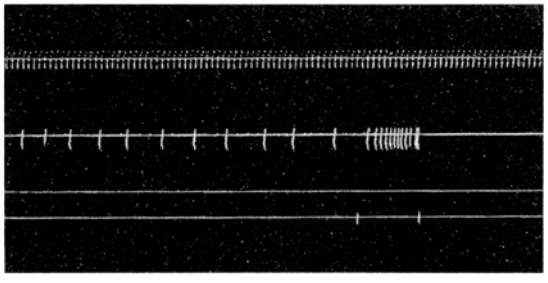
## Secretion of Saliva in the Rabbit after Postganglionic Parasympathetic Denervation

One to three days after postganglionic parasympathetic denervation the parotid gland of the cat shows a secretory activity occurring in bursts and assumed to be due to a paroxysmal release of acetylcholine from the degenerating nerve-endings[1]. A similar, although more continuous, 'degeneration secretion' has been observed in submaxillary and sublingual glands of cats[2,3] and dogs[4].

In the present experiments the effect of unilateral postganglionic parasympathetic denervation of the submaxillary and parotid glands was studied in rabbits. As in cats[3] the chorda tympani was dissected along the submaxillary duct and cut as close to the gland as possible. The parotid gland was denervated by section of the auriculotemporal nerve, which contains its parasympathetic secretory fibres[5]. Morphine-urethane was used for the former, ether anaesthesia for the latter operation.

One to five days later acute experiments in urethane anaesthesia were carried out. The parotid ducts were exposed and cannulated; the submaxillary ducts were cannulated through the mouth. The cannulae used gave about 50 drops out of 1 ml of distilled water.



'Degeneration secretion' from left parotid gland of the rabbit. Tracings from above: time in min; drops of saliva from left denervated gland; right control gland; signal. Postganglionic, parasympathetic denervation of left gland 24 h before the experiment. At first signal 100 µg eserine/kg, at second signal 100 µg Hoechst 9980/kg was given intravenously.

One to three days after section of the auriculotemporal nerve the parotid gland of the rabbit showed a 'degeneration secretion'; the flow was particularly marked on the first two days, when drops of saliva could fall with intervals of 3–5 min. The secretion appeared in paroxysms with intervals of 1–2 min. It was increased by eserine and abolished by Hoechst 9980 (αα-diphenyl-γ-piperidinobutylamide) as shown in the Figure, and must therefore be assumed to be caused by acetylcholine.

Contrary to the parotid gland, the submaxillary gland of the rabbit is normally in a permanent state of spontaneous activity[6]. One to three days after the operation a 'degeneration secretion' could be observed in the submaxillary gland, superimposed on the slow spontaneous flow. During the first two days, when the flow was particularly pronounced, drops of saliva fell every 4–6 min from the denervated gland whereas the contralateral, normal gland secreted about one drop per h. The 'degeneration secretion' was much more regular in the submaxillary than in the parotid glands; only in some few rabbits there was a tendency to paroxysmal flow from the submaxillary glands. Eserine augmented the flow and Hoechst 9980 reduced it to the level seen in the normal gland.

*Zusammenfassung.* Eine Degenerationssekretion erscheint beim Kaninchen in den ersten drei Tagen nach postganglionär-parasympathischer Denervierung der Submaxillaris- und Parotisdrüsen.

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## The 5-Hydroxytryptamine Content of the Brain and Some Other Organs of the Hedgehog *(Erinaceus europaeus)* During Activity and Hibernation

HESS[1] compares hibernation with the state of sleep and considers both these conditions to be dependent upon an autonomic central regulation. According to this opinion the functional balance in both these states is shifted towards a trophotropic predominance. SUOMALAINEN[2] considers that sympathetic hypofunction is essential to hiber-

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nation. LYMAN and CHATFIELD[3] also suggest that the central representation of the sympathetic nervous system is essential since it appears to be involved in arousal from hibernation.

It appeared to be of interest to investigate the 5-hydroxytryptamine (5HT) and catecholamine concentrations in the brain of the hedgehog during hibernation and summer activity. In the present paper the distribution of 5HT in the brain, and in some other tissues, is reported.

*Material and Methods.* We used hedgehogs which were collected in natural surroundings in summer and early autumn. They were fed on oatmeal milk, cooked meat and viscera, and water. During hibernation the animals were kept at about 4 to 6°C. The brains of a group of animals, both active and hibernating (4+4), were perfused in sodium pentobarbital anesthesia (35 mg/kg i.p.) with Locke's solution at body temperature. Other animals were decapitated without anesthesia or perfusion. Some brains were divided into the following parts: cerebellum, medulla and pons, mesencephalon, diencephalon, cerebral hemispheres and olfactory bulbs. 5HT was determined biologically by using the rat fundus strip method of VANE[4] modified as described previously[5]. Methysergid (Deseril-Sandoz) was used as a 5HT antagonist in a concentration of 2 to 5 ng/ml.

*Results.* The results concerning the 5HT content in the brain of the hedgehog during the cold season as compared with the summer season, are presented in Table I. An increase of the 5HT content occurred during the cold season. On the other hand no significant difference was found between the brain 5HT content in hibernating hedgehogs and those kept at room temperature during the cold season.

The distribution of 5HT found in the various parts of the brain shows that the highest concentrations are located in the mesencephalon, diencephalon and medulla and pons (Table I). Considerable amounts also appeared in the telencephalon. The relatively highest increase was observed in the cerebral hemispheres during the cold season and particularly during hibernation. A lower increase was observed in other parts of the brain, except in the cerebellum and olfactory bulbs, where the increase was statistically nonsignificant. The perfusion of brains did not influence the values.

The highest 5HT content of the other organs was observed in the fundal portion of the stomach, whereas the pylorus contained only about $^1/_4$ of this amount (Table II). Significantly less was found in the duodenum than in the fundus. The 5HT concentrations in peripheral organs were subject to larger variations compared with the brain. The differences found in different activity states between various peripheral tissues were not statistically significant.

*Discussion.* The results suggest that there are seasonal variations of the 5HT content in the brain of the hedgehog. No similar changes have been reported in common non-hibernating mammals. The 5HT content of the brain in the active hedgehog during the midsummer period proved to be similar to that found in the rat by using the same bioassay method[5]. The brain 5HT content of the hedgehog during cold seasons, especially in hibernation increased to a level which rarely occurs physiologically in other mammals[6]. The distribution of 5HT in the brain of the active hedgehog during summer is approximately comparable with that in canine brain[7]. The olfactory bulbs and pyriform lobes of the hedgehog are large and the neopallium is less developed[8]. The rhinencephalic structures may there-

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Table I. Distribution of 5-hydroxytryptamine in the brain of the hedgehog during the warm and cold season. Numbers of animals in parentheses

| Part of the brain | 5-Hydroxytryptamine content of the brain | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | During the summer | | During the cold season[a] | | | | | | | |
| | In active state | | In active state | | Increase | | In hibernation | | Increase | |
| | μg/g ± S.E. | | μg/g ± S.E. | | % | P | μg/g ± S.E. | | % | P |
| Whole brain (excluding cerebellum) | 0.56 ± 0.026 | (8) | 0.85 ± 0.056 | (8) | 52 | <0.05 | 0.96 ± 0.045 | (8) | 71 | <0.001 |
| Cerebellum | 0.05 ± 0.004 | (3) | 0.07 ± 0.02 | (5) | 40 | >0.3 | 0.06 ± 0.02 | (8) | 20 | — |
| Medulla and pons | 0.78 ± 0.02 | (3) | 1.00 ± 0.07 | (5) | 28 | <0.05 | 1.21 ± 0.08 | (8) | 55 | <0.001 |
| Mesencephalon | 1.37 ± 0.04 | (3) | 1.76 ± 0.07 | (5) | 29 | <0.01 | 1.79 ± 0.08 | (8) | 31 | <0.01 |
| Diencephalon | 1.00 ± 0.05 | (3) | 1.25 ± 0.08 | (5) | 25 | <0.05 | 1.22 ± 0.06 | (8) | 22 | <0.05 |
| Cerebral hemispheres | 0.37 ± 0.04 | (3) | 0.75 ± 0.08 | (5) | 103 | <0.01 | 0.89 ± 0.06 | (8) | 141 | <0.001 |
| Olfactory bulbs | 0.27 ± 0.08 | (3) | 0.41 ± 0.04 | (5) | 52 | >0.1 | 0.47 ± 0.04 | (8) | 74 | >0.05 |

[a] From September 15 to May 31.

Table II. 5-Hydroxytryptamine content in some peripheral tissues of the hedgehog in different activity states (μg/g of fresh tissue ± S.E.) Numbers of animals in parentheses

| Season and state | Fundus | Pylorus | Duodenum | Spleen | Liver | Lung (perfused) | Kidney |
|---|---|---|---|---|---|---|---|
| During summer | | | | | | | |
| Active | 24.65 (2) | 4.63 (1) | 5.92 ± 1.5 (5) | 3.20 ± 1.1 (5) | 2.40 (1) | 0.71 ± 0.08 (4) | 0.21 (1) |
| During cold season | | | | | | | |
| Active | 22.59 ± 2.1 (5) | 5.66 ± 1.4 (3) | 12.22 ± 1.1 (5) | 4.30 ± 0.3 (5) | 1.46 ± 0.24 (6) | — | 0.59 ± 0.29 (4) |
| Hibernating | 18.92 ± 1.8 (8) | — | 8.74 ± 1.4 (8) | 8.59 ± 2.4 (8) | 2.79 ± 0.71 (8) | 0.94 ± 0.10 (3) | 0.49 ± 0.10 (4) |

fore constitute a relatively larger part of the hemispheres in the hedgehog than in the dog. Particularly in rhinencephalic structures 5HT is contained preferentially[9].

The immediate cause of the brain 5HT increase is probably due to such changes in enzyme activities that relatively more 5HT is synthetized than destroyed. The oxygen consumption of the tissues is greatly decreased during hibernation[10]. The monoamine oxidase activity is largely dependent upon oxygen tension[11]. The amino acid decarboxylases, on the other hand, are more efficient in anaerobic conditions[12]. Variations in the oxygen and carbon dioxide tensions, however, may not be the cause of 5HT increase, since ANDERSON and BONNYCASTLE[13] found no effect of deprivation of oxygen or accumulation of carbon dioxide, or of both, on the brain 5HT in rats. Direct evidence is so far not available concerning the possible changes of the activities of enzymes forming or destroying 5HT in different states of activity in hedgehogs.

The physiological significance of the changes of the brain 5HT is difficult to interpret. The predominant appearance of bound 5HT in synaptic vesicles of nerve terminals in the guinea-pig brain seems strongly to suggest its participation in synaptic transmission[14]. Several observations suggest that 5HT more suitably functions as a trophotropic than as an ergotropic transmitter[6,15,16]. Our own observations with hibernating hedgehogs point in the same direction. The 5HT precursor, 5-hydroxytryptophan (40 mg/kg of *dl*-5HTP s.c.) did not arouse the hibernating hedgehog, although it was decarboxylated in the brain and increased the brain 5HT content. On the other hand, the equivalent dose of *l*-DOPA started the arousal mechanism, and the hedgehog was aroused within a few hours[17].

According to AZZALI[18], SUOMALAINEN, and NYHOLM[19] the neurosecretion from the supraoptic nuclei is enhanced during the cold season, especially during the last period of hibernation. This is an important observation since it demonstrates that not all brain functions are depressed during hibernation. From this it may be concluded that also the production of an agent functioning as a transmitter at the supraoptic nuclei is probably enhanced during the cold season. PICKFORD[20] and DUKE et al.[21] have suggested that acetylcholine (ACh) is this transmitter in the dog. It would be attractive to suggest a possibility of the activation of a central cholinergic transmitter system in the hedgehog during the cold season and hibernation. Parallel changes in the 5HT and ACh contents in the brain have been reported in different activity states. Thus the ACh content of the brain is higher during sleep than in wakefulness, being at its highest during anesthesia[22,23]. The brain 5HT also increases in rats during anesthesia[13] and during the sleep period in mice[24].

The electric activity in the brain is greatly decreased during hibernation, 90% in the ground squirrel according to STRUMWASSER[25]. The biochemical processes taking place in synaptic transmission, however, need not be decreased to the same extent. Our experiments with precursor amino acids and monoamine oxidase inhibitors show a considerable amine formation in the brain and some other organs, especially in the kidney and liver of the hibernating hedgehog. The animals were aroused within a few hours after the administration of monoamine oxidase inhibitors[17].

Our studies on the catecholamine content in the brains of hedgehogs in different activity states indicate that the noradrenaline content is decreased during profound hibernation[17]. For the present we pay attention only to the opposing changes of 5HT and noradrenaline in the hibernating brain. In conclusion, we suggest that these supposed transmitter substances belong to functionally different systems in the brain, one of which may be functioning more effectively while the other is less active[26].

*Zusammenfassung.* Der 5-Hydroxytryptamingehalt des Igelhirns während der kalten Jahreszeit und besonders während des Winterschlafs war stets höher als mitten im Sommer. Die grösste Zunahme wurde in den Grosshirnhemisphären gefunden. Mechanismus und physiologische Bedeutung der Änderung wurde diskutiert und dabei angenommen, dass diese im Zusammenhang mit einer autonomen Regelung des Hibernationszustandes stehe.

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## *In vitro* Exploration of a Circadian Rhythm in Adrenocorticotropic Activity of C Mouse Hypophysis

*In vitro*[1], as well as *in vivo*[2,3] a circadian (circa, dies[4–6]) rhythm characterizes the reactivity of certain C mouse adrenals to adrenocorticotropic hormone (ACTH). Thus, the amount of corticosterone produced as a result of

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