10–12 min after turning off $CO_2$ flow, both the waves show a continuous recovery phase. However, the slope of this phase, marked with broken lines in figure 2, indicates that the 'b' wave had a significantly slower recovery rate than 'a' wave.

*Discussion.* The effect of $CO_2$ on ERG in scorpion is somewhat similar to that reported in worker honeybee[3]. However, in this preparation, the 'off' effect of the retinal action potential completely disappeared during the exposure and reappeared after turning off $CO_2$. Based on this differential sensitivity of the sustained negativity and the 'off' effect, Goldsmith proposed that these 2 waves have different origins. As the effects of $CO_2$ are pronounced on second and higher order neurons, the 'off' effect which was totally abolished by the same is considered to have a more central origin. Such selective poisoning of certain neural pathways leaving others intact was attempted by other workers, using such agents as cocaine and procaine[5]. While studying the effect of cocaine, Bernhard suggested that the negative component of the retinal action potential in Dytiscus originates in the layer of the retinula cells[6]. Swihart demonstrated that procaine, when introduced into the eye via a corneal hole in Danaus plexippus L. quickly removes positive components from the ERG waveform[7]. This selective action of procaine on the positive components was considered as proof for their origin distal to the basement membrane. In the present results, it is significant that the recovery of 'b' wave not only proceeds at a much slower rate but is also delayed by 2–3 min, when compared to the recovery process in 'a' wave. Even a duration of 2–3 min may be quite significant in terms of electrical events in neural structures. It appears therefore that $CO_2$ has a more pronounced effect on the 'b' wave and may possibly originate, therefore, at a locus more central to the same from where 'a' wave originates. This conclusion finds corroboration in the results of the depth-recording in this eye with the microelectrode[8]. Thus the present study possibly indicates that the temporal aspects of the effect of exposure to and recovery from $CO_2$ may be helpful in delineating the peripheral electrical events, even if such exposure does not result in total suppression of one or more components.

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## Occurrence of 5-hydroxytryptamine in chick retina

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*Summary.* 5-Hydroxytryptamine (5-HT) was found in chick retina. 5-HT level in chick retina was increased by the administration of pargyline and decreased by reserpine, but remained unchanged with tryptophan.

The existence of catecholamine[2–6], $\gamma$-aminobutyric acid[7–10] and acetylcholine[11,12] as putative neurotransmitters in the retinas of several species of animals is well documented. In regard to 5-hydroxytryptamine (5-HT) in the retina, however, comparable information is lacking. While there are a few reports[13,14], including the determination of 5-HT in the retinas of some vertebrates, Häggendal and Malmfors[3] could not detect any 5-HT in rabbit retina. By the histochemical method of Falck and Hillarp, Hauschild and Laties[15] suggested the occurrence of 5-HT in a special type of cells in chick retina. The present paper deals with quantitative analysis of 5-HT in chick retina, and the effects of some drugs on its level.

*Material and methods.* Chicks of the White Rock breed weighing approximately 1 kg were used. Animals were injected i.p. with pargyline-HCl (100 mg/kg) 2 h, reserpine (5 mg/kg) 5 h or L-tryptophan (200 mg/kg) 2 h before killing. Immediately after decapitation, the eye was enucleated, and the retina was rapidly removed and frozen on dry ice. More than 300 mg of retinal tissue was collected for a sample. 5-HT was analyzed within 24 h after the sacrifice. The fluorometric assay using ninhydrin reaction[16] was adopted for the determination of 5-HT in the retina, for the method is quite specific for 5-HT and excludes the contamination of other indoleamines[17]. The retina was homogenized in 5.0 ml of cold acidified n-butanol[18] by using a glass homogenizer fitted with a Teflon pestle and

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5-HT in chick retina

| Treatment | 5-HT (ng/g wet wt) |
|---|---|
| None | 176 ± 12 |
| Pargyline | 223 ± 19* |
| Reserpine | 109 ± 7** |
| L-Tryptophan | 163 ± 22 |

Pargyline-HCl (100 mg/kg) was injected i. p. 2 h, reserpine (5 mg/kg) 5 h and L-tryptophan (200 mg/kg) 2 h before killing. Means ± SD are given. Each value was obtained from 4 experiments. Significance from the value obtained without treatment (Student's t-test); *$p < 0.02$; **$p < 0.001$.

centrifuged (3000 rpm, 5 min). The supernatant was decanted into a glass centrifuge tube, shaken vigorously with 5.0 ml of the borate buffer solution[19] (pH 10.0) saturated with NaCl, and centrifuged (3000 rpm, 5 min). A 3.0-ml aliquot of the supernatant was transferred to another centrifuge tube containing 1.5 ml of 0.05 M sodium phosphate buffer (pH 7.0) and 6.0 ml of n-heptane, shaken vigorously and centrifuged (3000 rpm, 5 min). 1 ml of the aqueous phase was transferred to a test tube containing 0.2 ml of 0.1 M ninhydrin solution, heated at 75°C for 30 min in an oil bath, cooled with tap water, and left at room temperature for 15 min. The fluorescence of the solution was measured with excitation at 385 nm and emission at 490 nm[16].

*Results and discussion.* The result is shown in the table. An appreciable amount of 5-HT was found in the retina of the untreated chick, though the level is much lower than that in the brain. The 5-HT level was increased by the administration of pargyline, a potent inhibitor of monoamine oxidase. This should be compared with the result obtained with the pineal body, because of the similarity in embryological origins of the retina and pineal body[20]. In the pineal body, 5-HT localizes in pinealocyte cytoplasm and plays exclusively the role of the precursor of melatonin[21]. The level of 5-HT in pineal body is not increased by the administration of a monoamine oxidase inhibitor[22]. Thus, the increase in 5-HT found in chick retina after the administration of pargyline suggests the role of 5-HT in the retina other than the precursor of melatonin synthesis[23].

5-HT in the retina was decreased by the administration of reserpine. This result indicates the presence of the reserpine-sensitive storage mechanism[24] of 5-HT in chick retina. This result also supports the idea that 5-HT in the retina might act as a neurotransmitter.

5-HT in chick retina remained unchanged after the administration of tryptophan. This result indicates that the level of 5-HT in chick retina is not dependent on the level of tryptophan, probably due to the higher concentration of tryptophan in untreated chick retina[25] than that in the brain, which may cause the saturation of tryptophan hydroxylase with its substrate.

Chick retina seems to be an appropriate material for the study of the physiological role of 5-HT in the retina, since 5-HT level in chick retina seems to be much higher than that in rat or rabbit retina, and in addition, chick retina is relatively large in size and can be easily excised. The possible role of 5-HT in the retina is under investigation in our laboratory.

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## Polypeptides of cerebral subcellular fractions of differentially-housed mice[1]

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*Summary.* The difference in protein of cerebral nerve-ending fractions caused by differential housing of male mice is of a quantitative nature and might reflect a change in the number of nerve-endings.

Recent studies have revealed that synaptosomal fractions of the brains of aggressive 'isolated' mice contain less protein than those of their 'aggregated' counterparts[2,3]. This environmentally-induced change has been correlated with changes in the 'binding' of putative central neurotransmitters (e.g., $\gamma$-aminobutyric acid, glycine, acetylcholine[4,5]) and psycho-active agents (e.g., d-amphetamine and $Li^+$[6,7] to subcellular structures of the brain. Such findings have led to the contention that individual housing of mice causes a decrease in the number, size, or development of cerebral nerve-endings[8]. To elucidate further this postulated morphological change, the protein contents and polypeptide profiles of cerebral nerve-ending fractions of differentially-housed mice have been examined.

*Materials and methods.* Male, Swiss albino mice were differentially-housed from weanling age (21–22 days) for 6–7 weeks[9]. After decapitation, their brains (rostral to the inferior colliculi; excluding cerebellum) were excised, weighed, and homogenized at 0°C in 20 vol. isomotic (0.32 OsM) sucrose solution. Portions (5 ml) of homogenates were used to prepare 'synaptosomal' ($P_2$) fractions[10]. These were resuspended in 1.5 ml of 0.32 M sucrose solution, and 1.2-ml aliquots were centrifuged at 53,000×g, 1 h, on discontinous gradients consisting of (from top to bottom): 3.0 ml 0.6 M, 3.0 ml 0.8 M, 3.0 ml 1.0 M, 3.0 ml 1.2 M and 4.5 ml 1.6 M sucrose solutions. Gradient fractions (0.5 ml) were collected and their protein contents were estimated[11]. For polyacrylamide gel electrophoresis, the apparatus described by Studier[12]