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# THE INFLUENCE OF SUBSTANCE P ON THE NERVOUS SYSTEM OF THE EARTH WORM (LUMBRICUS TERRESTRIS)

The results of pharmacological investigations of the action and distribution of SP in the nervous system indicate a peculiar importance of this factor in nerve activity (Gaddum, 1960). The depressing, i. e. inhibitory, effects of SP in particular parts of the nervous system (Zetler, 1960), as well as its markedly tranquillizing properties (Stern and Dobrić, 1957) have already been described in medical literature.

No indications, however, could be found in the various reports on SP that its presence had been sought for in the nervous system of the earth worm. In view of the simplicity of the earth worm's nervous system and its high sensitivity to various ecological factors we have undertaken a study of the influence of SP on this organism and propose to report in the present paper some of the results obtained with SP in cerebral ganglia and the subpharyngeal ganglion in the earth worm subject to agression.

## Materials and methods

Adult earth worms of similar weight, collected in November from the same place and the same day, have been divided into four groups. Group I was kept constantly in the dark; Group II was exposed to daylight and to light of a 200-W bulb for 24 hours; Group III, again, was kept in the dark but was, at the same time, exposed to the action of SP; Group IV was exposed to both SP and light 24 hours through. All earth worms in groups I—IV were put into Petri dishes of the same size filled with the same volume of spring-water, and the equal number of worms placed into each Petri dish. 100 U./50 ml SP (9 U./mg potency) were added to the Petri dishes assigned to groups III and IV. Controls for groups I and II were kept in a vessel filled with earth in the laboratory for 24 hours. Groups I and II served for comparison to groups III and IV. The temperature of surroundings was the same for all groups and controls. In-toto fixation of the worms was carried out in Bouin's fluid. Paraffin was used for embedding. Staining of the slides was made according to methods by Florentin, Gomori and Bargmann, and the azan-H method.

## Results

### A. PHYSIOLOGICAL OBSERVATIONS

Phenomena of locomotion and peristaltic reptation are very powerful in worms exposed to light (Group II). Considerable slowing down of movements, however, was observed in those simultaneously exposed to SP (Group IV). This group, moreover, exhibited a slower reflex contraction in response to a blow on the base (vibrations of base), and the worms in this group also had a fainter coloration than those in group II.

### B. HISTOPHYSIOLOGICAL OBSERVATIONS

#### 1. Influence of darkness (Group I)

**Cerebroid ganglia.** — a-Type neurosecretory cells situated in the peripheral region of the dorso-caudal part of the ganglia are hyperchromatic and contain a homogenously phloxinophilic or chrome alum-hematoxylin-(CAH)-positive cytoplasm (Fig. 1). The nuclei of these cells are spherical, flattened or irregular and rich in chromatin. In the majority of cells the nucleoli are unequal in size and positioned centrally. The cells situated in the immediate vicinity of the intercerebral part (pars intercerebralis) are larger than those previously described, and also contain much CAH-positive secretion either formed in coarse particles, or as a homogenously confluent content of the cytoplasm.

b-Type cells with basophilic cytoplasm show irregularly distributed granulations. Their nuclei are spherical or ovoidal, the nucleoli are weakly phloxinophilic.

c-Type cells are of varying sizes, irregular shape, and a wrinkled surface. The cytoplasm is slightly basophilic, the nuclei disfigured, and frequently pycnotic. This holds for cells from the peripheral, as well as for those from the middle part of the ganglia. The intercellular spaces are edematous.

**Subpharyngeal ganglion.** — The nerve cells responded to darkness in an identical manner as those from the cerebroid ganglia. Individual neurosecretory cells are extended in one dimension, with oblong ovoid nuclei which contain a greater amount of chromatin than the controls. The neurosecretory substance is hyperchromatic (Fig. 2A). Neuroganglionic cells corresponding to c-type cells from cerebroid ganglia are also misshaped. The cytoplasm contains unequal vacuolae and is less basophilic than in controls. The edges of nuclei often show indentations, and some of them are entirely disintegrated.

#### 2. Influence of light (Group II)

**Cerebroid ganglia.** — Structural changes in response to the action of light are completely different in character from those observed in Group I. The majority of neurosecretory cells are devoid of any CAH-positive contents. In some of them there are tiny granulations, either

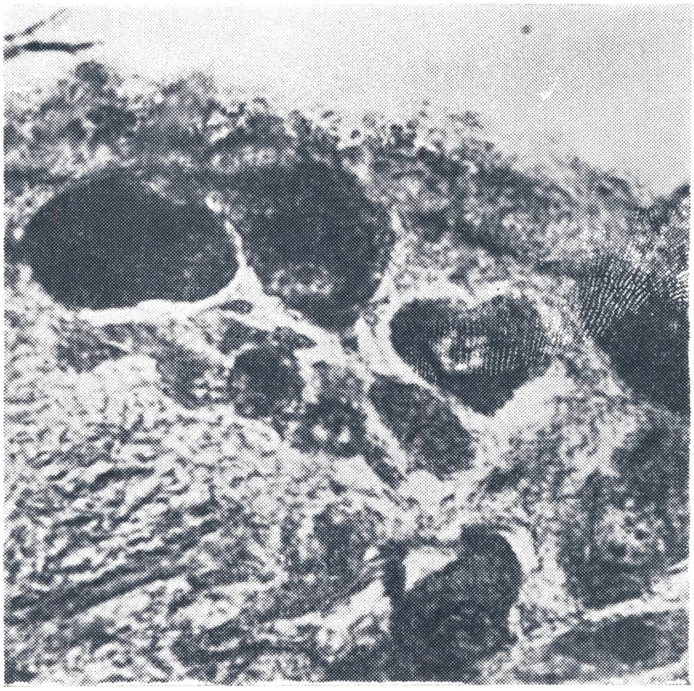

FIG. 1

Cerebroid ganglion from earth worm, Group I.
Cells filled with CAH-positive secretion (Bouin, Gomori-Bargmann, oc 8, obj. imm.)

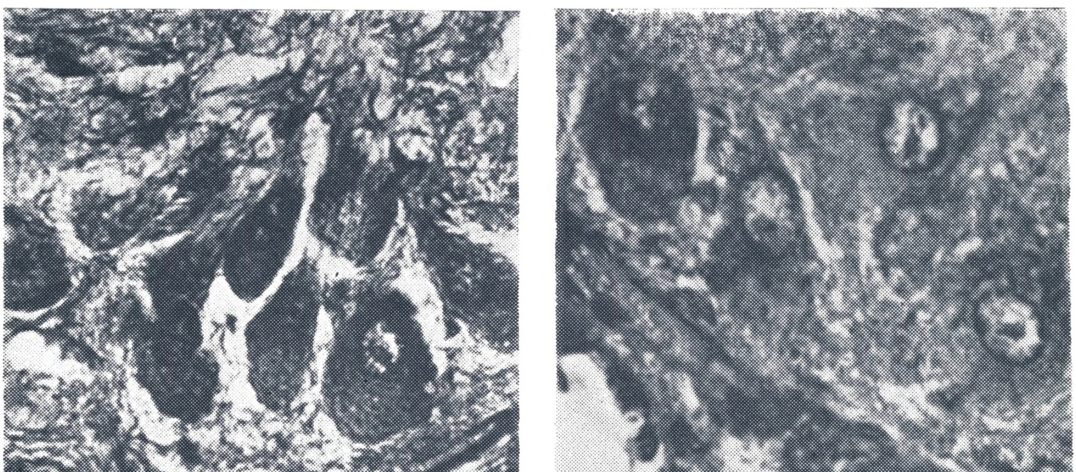


FIG. 2

Subpharyngeal ganglion from earth worm.
A, Group I, extended cells, well delimited; cytoplasm contains much CAH-positive secretion. — B, Group III, cell limitation unclear, nuclei hypertrophied, cytoplasm vacuolised; reduced amount of neurosecretion (Bouin, Gomori-Bargmann, oc. 8, obj. imm.).

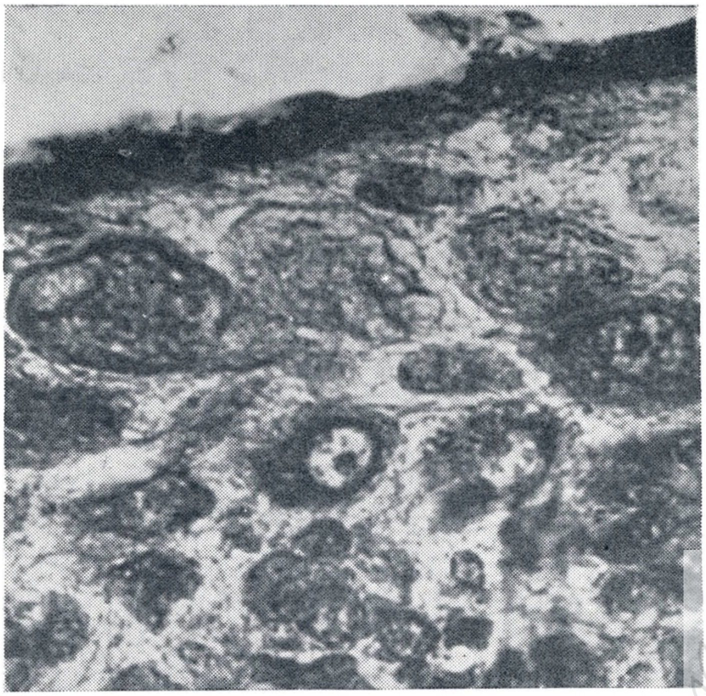

FIG. 3

Cerebroid ganglion from earth worm, Group II.
Large neuroganglionic cells with vacuolised cytoplasm (Bouin, Gomori-Bargmann, oc. 8, obj. imm.).



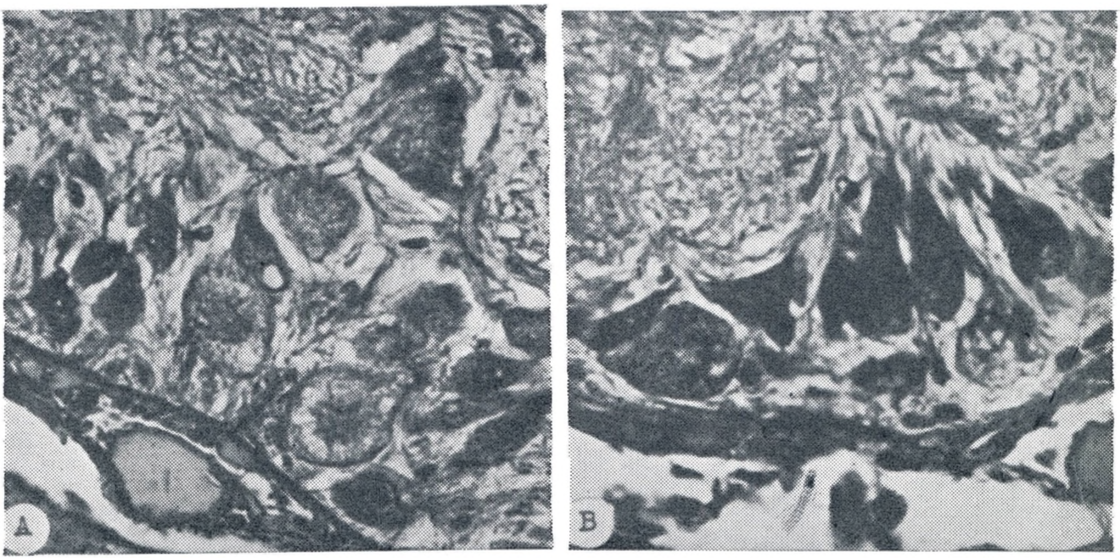


FIG. 4

Subpharyngeal ganglion from earth worm.
A, Group II, hypertrophy of neuroganglionic cells, vacuolised cytoplasm; sparse cells contain neurosecretion. — B, Group IV, shrunk neuroganglionic cells, neurosecretion blocked (Bouin, Gomori-Bargmann, oc. 8, obj. imm.).

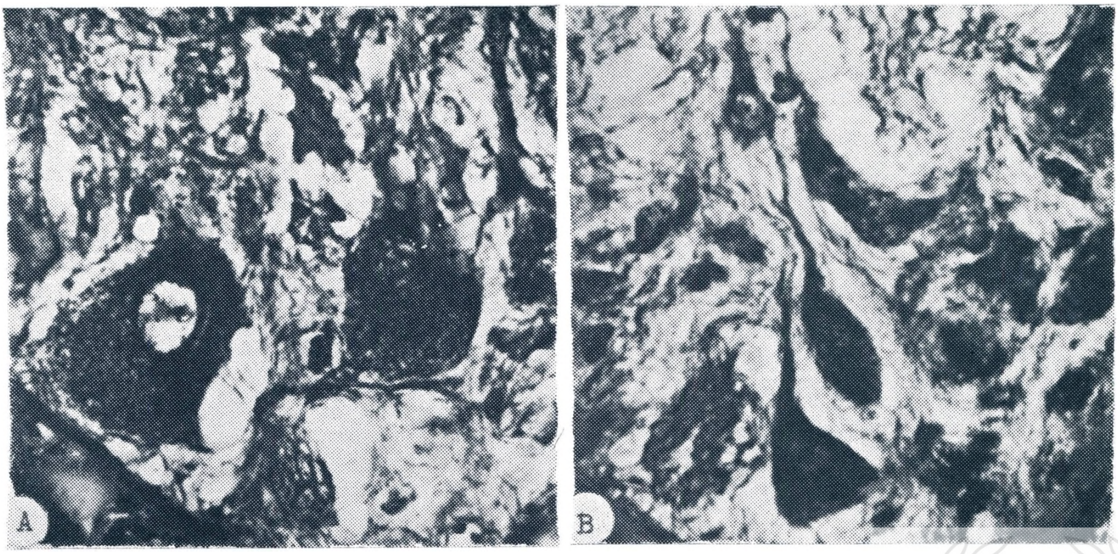

FIG. 5

Subpharyngeal ganglion from earth worm.
A, Group II, — a-type cell with peripheral vacuolae. — B, Group IV, disfigured a-tupe cells, its extensions filled with hyperchromatic neurosecretion (Bouin, Gomori-Bargmann, oc. 8, obj. imm.).



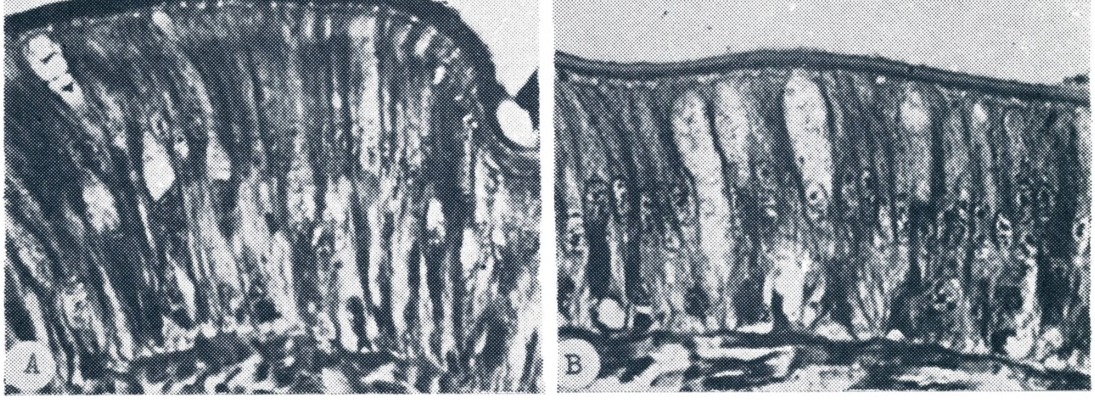

FIG. 6

Epidermis from earth worm.
A, Group II, long epithelial cells from dorsal part. — B, Group IV, epithelial cells from dorsal part shortened, cuticle thinner than in preceeding worm (Bouin, Florentin, oc, 8, obj. 60).

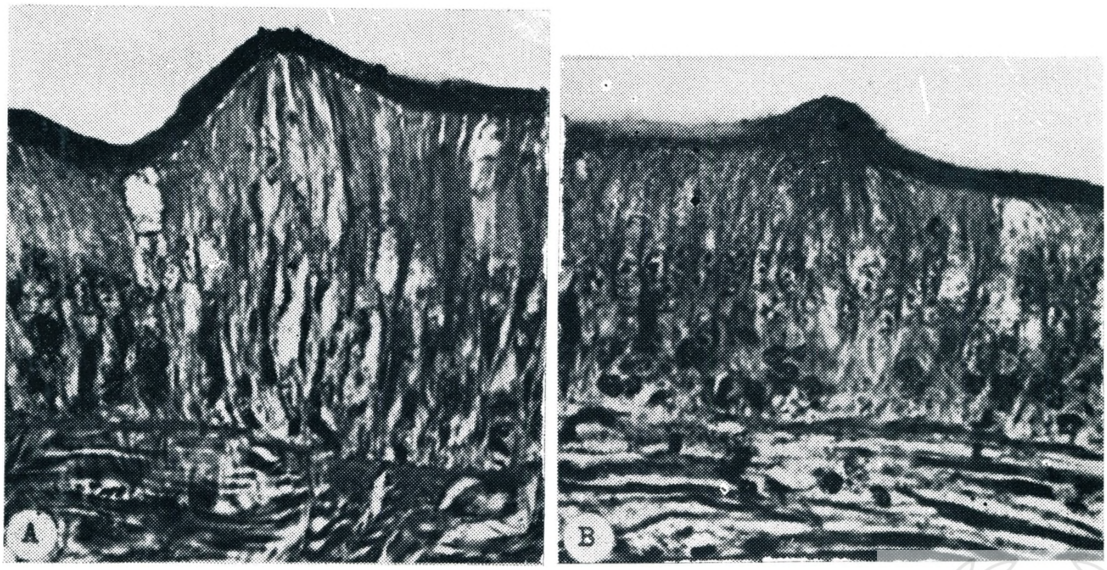


FIG. 7

Epidermis from earth worm.

A, Group II, disjoined tacto-receptor cells with atrophic, extended nuclei. — B, Group IV, compact tacto-receptor cells in a shortened epidermis (Bouin, Florentin, oc. 8, obj. 60).

diffusely distributed, or grouped around the nuclei; among these cells those with phloxinophilic cytoplasm and hypertrophied, excentrically positioned nuclei prevail. The nucleoli are also hypertrophied, hyperphloxinophilic, and totally displaced towards the periphery, adjacent to the cell membrane. Individual cells contrast the rest by their dimensions and possess a homogenously stained or granular acidophilic cytoplasm. These cells lie close to the capillaries, which in turn show widened lumina. b-Type cells are also hypertrophic. The presence of large ganglionic cells with vacuolised cytoplasm, having a greyish tint when treated according to Gomori and Bargmann, represents one of the essential distinctions in the reactivity of cerebroid ganglia to light (Fig. 3). The nuclei of these cells, frequently carrying transcellular capillaries, are bubble-shaped, pallid, and have a very thin membrane. Around the periphery of these cells there is a chain of vacuolae larger than those from the inner part of the cytoplasm. Some of these vacuolae burst and pour out their contents into the neuropil. More numerous phloxinophilic fibres are observed than in the preceeding group. The net of capillaries is highly ramified and the lumina of capillaries dilatated.

**Subpharyngeal ganglion.** — Individual neuroganglionic cells are considerably larger than in controls. This is true likewise of cells with basophilic, and of extended cells with vacuolised cytoplasm. The nuclei of the latter are bubble-shaped with a hyperphloxinophilic large nucleolus. There are less CAH-positive cells than in controls.



### 3. Influence of darkness and SP (Group III)

**Cerebroid ganglia.** — The tissue pattern described in Group I is considerably modified by the presence of numerous cells without distinct limitation. Individual nuclei are larger than in Group I, excentrically positioned, bubble-shaped, with little chromatin. The nucleoli are strikingly larger than in Group I and also peripherally positioned. Neurosecretory cells are recognized by a perinuclear CAH-positive zone. Coarsely vacuolised, honneycomb-shaped cells are found in the median region near the intercerebral part. They are most intimately connected by capillaries. These cells also pour out the contents of their peripheral vacuolae into the neuropil. The net of capillaries is more marked than in Group I.

**Subpharyngeal ganglion.** — Cells and nuclei are larger than in Group I, with less chromatin in the nuclei. The nucleoli are also considerably larger. These changes are observed in both CAH-positive neurosecretory cells and those with basophilic, vacuolised cytoplasm. The delimitation of the latter cells is less visible.

### 4. Influence of light and SP (Group IV)

**Cerebroid ganglia.** — The general character of changes in ganglionic structure in response to light and SP is represented by smaller dimensions of the cells in comparison to those in Group II. The nuclei, too, have a smaller volume, and show an ovoid or oblong form with irregular edges. They contain more chromatin and strikingly smaller sized, less phloxinophilic, nucleoli. Especially the vacuolised cells are fewer in number and smaller in size, but this is also the case with the cells containing acidophilic cytoplasm. The capillaries are less twisted and show a narrower lumen.

**Subpharyngeal ganglion.** — The structural characteristics are identical with the involutive changes described for the cerebroid ganglia. There are more cells with CAH-positive content in the cytoplasm than in Group II, and the number of vacuolised neuroganglionic cells is strikingly smaller (Figs. 4 and 5).

## Discussion

The cytological characteristics of cerebroid and subpharyngeal ganglionic cells in Group I are opposite to those in Group II. They are involutive in the former, and progressive in the latter. Thus, darkness acts depressively, and light acts stimulatively not only on neuroglandular, but also on other nerve cells which, under normal circumstances do not show any neurosecretory activity. In trying an interpretation of these findings it is necessary to have in view that the experiments were carried out with earth worms in a fluid medium, whereas the controls were only kept in moist earth. To the experimental animals the staying in a fluid medium represents in itself a reactive change of surrounding conditions, which, in order to keep up the osmo-regulating balance, calls forth the response of primarily those neuroglandular cerebroid ganglionic cells playing some rôle in the osmo-regulating mechanism (Aros and Bodnar, 1960). The results obtained with the first two groups must, therefore, be interpreted as a combined effect of darkness, light, and change of surroundings.

As in Groups I and II the neuro-cytological responses in Groups III and IV, which in addition to the influence of light and darkness have been submitted to the action of SP, are also opposite. The hypertrophy of nuclei and nucleoli, the mobilisation of neurosecretory activity, the vacuolisation of the cytoplasm and the hyperemia observed in Group III reflect the stimulated activity of neuroganglionic cells; the shrinking of nuclei and nucleoli, the block of neurosecretion, and the homogenisation of cytoplasm in Group IV are indicative of certain inhibitory actions. Hence the neurotropic properties of SP are of a stimulating character in the dark, and of an inhibitory character in light. These findings point in favour of a conditioned neurotropic activity of SP, i. e. the effects of SP in one and the same nervous substrate will be different depending on the influence of various ecologic factors. Insofar as the neuroglandular cells of cerebroid and subpharyngeal ganglia are concerned, SP is a stimulant under influence of darkness, and an inhibitor under influence of light. The biological effects of SP in these experimental conditions thus depend on the physiological state of the responding organ.

But there is a further question of importance for the interpretation of the results described, namely that about the behaviour of the SP-preparation itself. It is possible that the polypeptidic components are subject to photochemical degradation and the effects of the preparation are, in consequence, different in light and darkness. There is furthermore a possibility of other components present as impurities to act in light, but not in dark, or vice versa. Only further studies with purest SP will enable us to elucidate this side of the problem.

In discussing the present experiments one must keep in mind that they have been performed with worms reacting under a stress. Light as the stress-producing factor is especially interesting in this respect. Its nocive action on the earth worm and the sensitivity of the latter to intense light irradiation is well known (Avel, 1959). The light stimuli are transmitted through photoreceptive cells situated in the worm's epidermis to the cerebroid and subpharyngeal, as well as to ventral ganglia (Avel, l. c.). By an articulation of these first sensory or afferent neurons to the correlated efferent or motor neurons in the subpharyngeal ganglion simple reflex arcs are formed through which the mechanism of retarded contraction takes place in order to enable locomotion. Likewise, by articulation of photo-receptor neurons to efferent neurons from the cerebroid ganglia, whose axon endings enter the giant fibres (»neurocordes«), other reflex arcs are constituted, which, in contrast to the former, are intended for fast transmission in order to produce locomotion, i. e. reptation. Now light accelerates the movements of the worms, whereas SP, under the same conditions, i. e. in light, retards them as well as the morphodynamics of neuroganglionic cells described before. Thus one could raise the question about the point of attack of SP in the reflex arcs. Epithelial and glandular cells in the epidermis are the ones which show maximum shrinkage under influence of SP (Figs. 6). The CAH-positive cellular secretion accumulates most and is eliminated least from these cells. On the other hand photo-receptor cells, after exposition to light, are better conserved in earth worms treated with SP than in untreated ones. Thus SP protects epidermal photo-receptor cells from light, i. e. it protects the integrity of the sensory neuron. This effect of SP is, however, opposite to that on transmittory, i. e. the correlated motor neurons in animals exposed to light. All these histo-physiological data indicate an anti-stress action in SP. The action of SP in the adaptation syndrome to light favours the conservation of the integrity of the sensory neuron, and, at the same time, inhibits the activity of the effector neuron. This means that the distinctly sensory photo-receptor cells are receptors of SP.

In analogy to the considerations about the rôle of photo-receptor cells we also have to consider the reaction of worms to tactile stimuli (vibrations of base) in the presence or absence of SP. Untreated worms in light react to a shock of the base by strong rapid movements. These movements are sensibly reduced in worms treated with SP. As with photo-receptor cells a protective action of SP could be observed in tacto-receptors in the epidermis. These devices include cells the structure of which, after exposition to light, is better conserved in worms treated with SP than in untreated worms (Fig. 7). Thus here again the depressive effect of SP on movements caused by sensory stimuli (light, vibration) is due to inhibition of effector, i. e. motor neurons. From all this it may be concluded that SP interferes with the transmission of sensory stimuli in the earth worm reacting under stress; its effects on sensory neurons are protective and those on the correlated effector neurons inhibitory.

The pigment particles below the epidermis, as well as in connective tissue, and especially in circular muscles are more numerous in worms exposed to light. In worms treated with SP the number of pigment particles following exposition to light is greatly reduced. Since the cumulation of pigment (porphyrin) is a response to the stress-producing effect of light in worms, and, on the other hand, the pigment metabolism is related to neurohormonal adaptive regulation, this finding is one more proof for the anti-stress activity of SP in the earth worm.

## Summary

The neuroganglionic cells of cerebroid and subpharyngeal ganglia in the earth worm undergo depressive changes in darkness and progressive ones in light. SP, under the same experimental conditions, exerts the opposite effects. This may be due either to the physiological condition of the reacting organ or to metabolic changes of SP itself as a result of photochemical reactions.

In light the earth worm reacts to shock of base by intensified and accelerated movements. SP depresses this response.

From morphodynamics of afferent, sensory neurons (photo- and tacto-receptor cells in the epidermis) and the efferent, motor neurons constituting simple reflex arcs with the former, observed in the earth worm under stress, it is concluded that SP exerts an »anti-stressogenic« effect.

In light-produced stress SP protects the sensory neurons and inhibits the correlated effector neurons.

Sensory cells in the epidermis are receptors of SP.

### *UTJECAJ SP NA NERVNI SISTEM KIŠNE GLISTE*

*Neuroganglijske ćelije cerebroidnog i subfaringealnog gangliona kišne gliste pokazuju depresivne promjene u tami, a progresivne u svjetlu. Pod istim eksperimentalnim uvjetima SP izaziva suprotne efekte. To može biti posljedica bilo fizioloških uvjeta u kojima se nalazi organ koji reaguje bilo metaboličkih promjena same SP uslijed fotokemijskih reakcija.*

*U svjetlu kišna glista reagira na potres baze pojačanim i ubrzanim pokretima. SP smanjuje ovu reakciju.*

*Iz morfodinamike aferentnih, senzornih, neurona (foto-receptorske i takto-receptorske ćelije epiderme) i eferentnih, motornih, neurona koji s prvima sačinjavaju jednostavne refleksne lukove, opaženih pod stresom, zaključuje se da SP ima antistresogeni efekat.*

*Kod stresa prouzrokovanog djelovanjem svjetlosti, SP zaštićuje senzorne neurone i djeluje inhibitivno na korelativne efektorske neurone.*

*Senzorne ćelije epiderme su receptori SP.*

## REFERENCES


AROS B. AND F. BODNAR (1960) — Symposia Biol. Hungar. 1, 191.

AVEL M. (1959) — in Grassé T. V. Traité de Zoologie.

GADDUM J. H. (1960) — in Polypeptides, Proceedings of Symposium, Pergamon Press, Oxford.

HUBL H. (1953) — in Roux' Archiv für Entwicklungsmechanik 146, 421.

STERN P. AND V. DOBRIĆ (1957) — in Psychotropic Drugs. Elsevier, Amsterdam.

ZETLER G. (1960) — in Polypeptides. Proceedings of Symposium, Pergamon Press, Oxford.


## DISCUSSION

LEMBECK: Is SP present in the earth worm? — What are the effects of ACh, noradrenaline and 5-HT, e. g., on motility and neurosecretion? Have you compared the effects of pineal gland extracts and melatonin?

MILIN: According to the literature there have been no attempts to look for SP in the earth worm. In examining 5-HT we obtained completely opposite effects to those of SP on both motility and neurosecretion. We have made no comparison between the effects of aqueous pineal gland extracts and melatonin.

UMRATH: Do the cells in the earth worm exposed to light in presence of SP resemble those in worms kept in the dark treated with SP?

MILIN: No. The cytodynamics of the photo-receptor cells in the epidermis, as well as the glandular cells under influence of SP depends on this physiological condition: the cells react differently in light and darkness.

VOGT: Have you tried to apply hypnotics to the earth worm exposed to light?

MILIN: Such experiments have not been carried out. I want to emphasize finally that a study of the relation of SP and pineal gland deserves a particular interest. The results of preliminary examinations made in collaboration with professor Stern and his colleagues indicate the presence of SP in the pineal gland. Other results obtained about the part played by the pineal gland in the adaptation syndrome show clearly the importance of this gland in the biology of SP, and particularly in the study of stress.