since assays of the ventricular myocardium at the end of each experiment revealed that considerable amounts of norepinephrine were still present in the nerve endings. The four hearts studied 2 hours after the injection of metaraminol contained a mean concentration of 0.55 μg of metaraminol per gram of tissue (wet weight) and 0.80 μg/g of norepinephrine. In the three hearts studied at 17 to 20 hours, these values were 0.46 μg/g and 0.31 μg/g, respectively. It is interesting that the amount of metaraminol released was much greater in hearts studied at the early time period (Fig. 1), although the concentration of metaraminol in the left ventricular myocardium was nearly the same in both groups. This suggests that metaraminol taken up by the heart can shift with time from an "available" to a "less readily available" pool, as suggested for norepinephrine (*8*).

In other studies it has been observed that tyramine, an amine which is known to release norepinephrine from adrenergic nerve endings (*9*), can also release metaraminol from the perfused cat heart. This provides additional evidence for the view that metaraminol is bound in the nerve endings at sites ordinarily occupied by norepinephrine. Muscholl and Maitre (*10*) have recently reported that α-methylnorepinephrine, a metabolite of α-methyl-dopa (*3*), can also be released from the heart by stimulation of the sympathetic nerves. Their study and this one therefore provide the first direct evidence that synthetic compounds can be released as neurohumoral transmitters from adrenergic endings. Since metaraminol is much less potent than the normal transmitter, norepinephrine, it is possible that more extensive replacement of norepinephrine by metaraminol than was achieved in these experiments could produce a form of adrenergic nervous system blockade. It is commonly observed that patients given large doses of metaraminol by infusion are hypotensive for varying periods of time after the infusion; perhaps a "transmitter substitution" mechanism plays a role in the pathogenesis of this type of hypotension.

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21 AUGUST 1964

References and Notes

1. S. Udenfriend and P. Zaltzman-Nirenberg, *J. Pharmacol.* **138**, 194 (1962).
2. G. L. Gessa, E. Costa, R. Kuntzman, B. B. Brodie, *Life Sci.* **1962**, 605 (1962).
3. A. Carlsson, M. Lindqvist, *Acta Physiol. Scand.* **54**, 87 (1962).
4. D. C. Harrison, C. A. Chidsey, E. Braunwald, *Ann. Internal Med.* **59**, 297 (1963).
5. P. A. Shore and H. Alpers, *Federation Proc.* **23**, 350 (1964).
6. J. R. Crout, C. R. Creveling, S. Udenfriend, *J. Pharmacol.* **132**, 269 (1961).
7. P. A. Shore and H. Alpers, *Life Sci.* **1964**, 551 (1964).
8. U. Trendelenburg, *J. Pharmacol.* **134**, 8 (1961); C. A. Chidsey and D. C. Harrison, *ibid.* **140**, 217 (1963); R. Montanari, E. Costa, M. A. Beaven, B. B. Brodie, *Life Sci.* **1963**, 232 (1963); J. R. Crout, *Arch. Exptl. Pathol. Pharmakol.* **248**, 85 (1964).
9. J. H. Burn and M. J. Rand, *J. Physiol. London* **144**, 314 (1958); C. A. Chidsey, D. C. Harrison, S. Braunwald, *Proc. Soc. Exptl. Biol. Med.* **109**, 488 (1962); J. Axelrod, E. Gordon, G. Hertting, I. J. Kopin, L. T. Potter, *Brit. J. Pharmacol.* **19**, 56 (1962).
10. E. Muscholl, L. Maitre, *Experientia* **19**, 658 (1963).
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Thysanuran Median Frontal Organ: Its Structural Resemblance to Photoreceptors

Abstract. *The median frontal organ of* Thermobia domestica *(Packard) and the median ocellus of* Tricholepidion gertschi *Wygodzinsky were compared with respect to location, innervation, and fine structure. The data suggest that the two organs are homologous. Elementary neurosecretory granules were not found in the median frontal organ, but multivesicular bodies are present in both the frontal organ and ocellus.*

Because the Thysanura (Bristle-tails) seem more closely related to pterygote insects than do any of the other extant apterygote orders (*1*) there is a distinct possibility that clarification of hormonal mechanisms operative in this group may provide clues toward an interpretation of comparative arthropod endocrinology. Experimental studies on thysanuran endocrine systems were commenced only recently (*2*) and emphasize the need for detailed anatomical information.

The cephalic endocrine system in this group of insects was reviewed by Watson (*3*). In addition to the paired lateral frontal organs which consist of components with structural and tinctorial characteristics of neurosecretory cells, an unpaired median frontal organ has been described (*4–8*). The latter structure seems to be absent in members of the family Machilidae, but present in many Lepismatidae (*4, 6*). Since it has been postulated that the median frontal organ may serve an endocrine function (*5*), we decided to compare the fine-structural features of this organ in *Thermobia domestica* (Packard) with those of known neurosecretory cells (*9*).

In *Thermobia*, the median frontal organ (Fig. 1) lies just beneath the anteromedial region of the frons, above the epistomal suture, and between the frontostomodaeal muscles (*8*). We were able to distinguish the location of this subellipsoidal organ by external examination of living specimens with a dissecting microscope, for it is light tan and visible through the transparent frontal cuticle after removal of the scales. On the basis of its gross anatomy as seen in frontal section, one could subdivide the median frontal organ of *Thermobia* into a medial body and two transverse processes (*5*), but there was no cytological distinction between the cells of these subdivisions as far as we could determine. Histological examination revealed an unpaired nerve which leaves the organ, passes medioposterior-

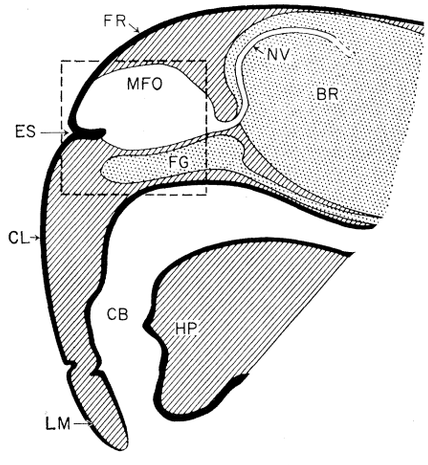

Fig. 1. Diagram of a sagittal section through the head of *Thermobia*. An area comparable to that shown in Fig. 2*A* is indicated by broken lines. (*BR*, brain; *CB*, cibarium; *CL*, clypeus; *ES*, epistomal suture; *FG*, frontal ganglion; *FR*, frons; *HP*, hypopharynx; *LM*, labrum; *MFO*, median frontal organ; *NV*, protocerebral nerve of *MFO*).

ly just beneath the dorsal cortex of the protocerebrum (Fig. 1), and appears to terminate in the neuropile above and behind the protocerebral bridge (*4*). Although we examined serial sections, we were not able to confirm the report (*8*) that each of the lateral projections of the median frontal organ is innervated by a component arising from the dorsal roots of the frontal ganglion.

The bicellular nature of the constituents of the median frontal organ has frequently been described (*3–7*). Each pair of cells seems to enclose a disk which, after fixation with Helly's fluid, stains intensely purple with Dawson's (*10*) modification of Halmi's paraldehyde-fuchsin (Fig. 2*A*), and slightly with the phloxine counterstain of Gomori's chrome alum hematoxylin method. The disks are some 15 to 20 μ in diameter and 3 to 4 μ thick. Occasional preparations reveal minute outpocketings of their lateral margins. After staining with paraldehyde-fuchsin, small fuchsinophil inclusions are found in the cytoplasm of the median frontal organ cells. They are particularly abundant at the lateral margins of each disk, in males as well as in females. Accumulations of "Gomori-positive" inclusions, such as those seen in the lateral frontal organs, are not found in the cells of the median frontal organ.

Examination of adult and immature specimens with the electron microscope clearly showed that the "enclosed disk" is a microvillar elaboration of the apposed surfaces of two, rarely three, cells (Figs. 2*B* and 3*B*). Transverse sections of these closely-packed tubules with double membranes reveal roughly hex-

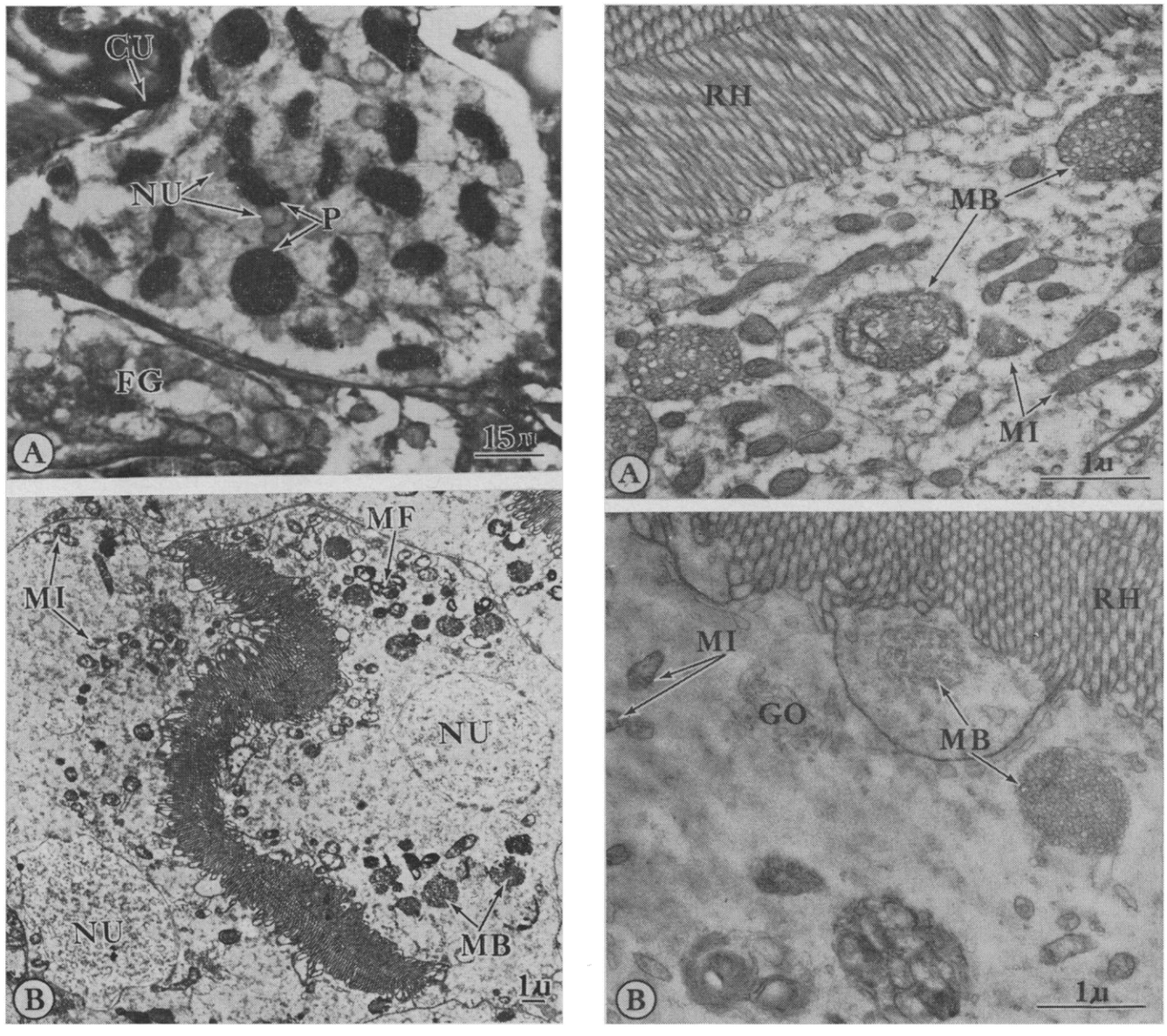

Fig. 2 (left). (*A*) Longitudinal paraffin section of *Thermobia* head fixed with Helly's fluid and stained with Dawson's modification of Halmi's paraldehyde-fuchsin (PAF). (*B*) Low-magnification electron micrograph of one of the bicellular components of the median frontal organ (*MFO*) shown in (*A*). Fixed in 1 percent OsO_4, embedded in Maraglas-Cardolite, and stained with lead citrate. (*CU*, cuticle; *FG*, frontal ganglion; *MB*, multivesicular bodies; *MF*, "myeloid" inclusion; *MI*, mitochondria; *NU*, nuclei of *MFO* cells; *P*, PAF-positive disks.) Fig. 3 (right). Electron micrograph of parts of a photoreceptor cell of the median ocellus of *Tricholepidion* (*A*) and of a cell from the median frontal organ of *Thermobia* (*B*). Both tissues were fixed with 1 percent OsO_4 and stained with lead hydroxide. The tissue in (*A*) was embedded in methacrylate; the tissue in (*B*), in Maraglas-Cardolite. (*GO*, possibly a Golgi body; *MB*, multivesicular bodies; *MI*, mitochondria; *RH*, rhabdomeres.)

agonal units some 650 to 1000 Å in diameter. The cytoplasm contains scanty, granular, endoplasmic reticulum, a feature correlated with the presence of a low concentration of cytochemically detectable RNA (*6*). Mitochondria are sparsely scattered throughout the cytoplasm, and multivesicular bodies are common. The latter are composed of spheres some 300 to 650 Å in diameter, are sometimes limited by a membrane, and are frequently closely associated with the basal zone of the microvilli (Fig. 3*B*). They were never seen within the nerve which leaves the median frontal organ. The size (about 1 to 2 μ), location, and abundance of the multivesicular bodies suggest that they are the small fuchsinophil inclusions visible with the light microscope. Membranous configurations reminiscent of the Golgi complex are present, and are occasionally associated with microvesicles similar to those constituting the multivesicular bodies.

Because the microvillar elaborations of the median frontal organ clearly resemble retinal photoreceptor elements found in many other animals (*11*), and because the location and innervation pattern of this organ suggested homology to the median ocellus of insects, a comparison with the fine structure of a thysanuran median ocellus seemed in order. Fortunately, several living specimens of *Tricholepidion gertschi* Wygodzinsky (*12*) were made available to us (*13*). The rhabdomere subunits of the median ocellus of this species and the microvillar elaborations of the median frontal organ of *Thermobia* are strikingly similar in form and size (Fig. 3*A*, *B*). In addition, both types of cells possess multivesicular bodies, and lack a well-developed granular endoplasmic reticulum. Among the more salient differences between the two is the formation of the *Tricholepidion* rhabdom by the apposition of three cells rather than the usual two seen in the median frontal organ. It also seems that the ocellar receptor cells of *Tricholepidion* possess a greater concentration of mitochondria.

Although the question of whether or not a cell is functioning as an endocrine gland cannot be answered by cytological examination per se, previous comparative studies have consistently revealed the presence of characteristic elementary neurosecretory granules in cells known or suspected of serving a neuroendocrine function (*9*). Such granules were not found in the median frontal organ of *Thermobia*. The only formed elements which might represent secretory products are the multivesicular bodies, and this interpretation seems doubtful in view of the widespread occurrence of such bodies in nonendocrine organs. On the other hand, the morphological evidence that the median frontal organ may be a photoreceptor is convincing. The location of this organ in *Thermobia*, its innervation, and its fine structure all indicate that it is homologous to the median ocellus of other Thysanura. The absence of a median frontal organ in those Thysanura which possess a median ocellus (*4*) also favors this interpretation.

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References and Notes

1. A. D. Imms, *A General Textbook of Entomology* (Methuen, London, 1957).
2. A. Bart, *Gen. Comp. Endocrinol.* **3**, 398 (1963); K. Yashika, *Mem. Coll. Sci. Univ. Kyoto, Ser. B* **27**, 83 (1960); J. A. L. Watson, *J. Insect Physiol.* **10**, 305, 399 (1964).
3. J. A. L. Watson, *J. Morphol.* **113**, 359 (1963).
4. B. Hanstrom, *Kgl. Svenska Vetenskapsakad. Handl.* **18**, 1 (1940).
5. B. De Lerma, *Arch. Zool. Ital.* **32**, 251 (1947).
6. M. Gabe, *Bull. Soc. Zool. France* **78**, 177 (1953).
7. P. Cazal, *Bull. Biol. France Suppl.* **32**, 1 (1948).
8. J. Chaudonneret, *Ann. Sci. Nat. Zool.* **12**, 145 (1950).
9. H. A. Bern, *Gen. Comp. Endocrinol. Suppl.* **1**, 117 (1962); ———, R. S. Nishioka, I. R. Hagadorn, in *Neurosecretion, Society for Endocrinology, Memoir No. 12*, H. Heller and R. B. Clark, Eds. (Academic Press, London, 1962), p. 21.
10. A. B. Dawson, *Anat. Rec.* **115**, 63 (1953).
11. R. M. Eakin, in *General Physiology of Cell Specialization*, D. Mazia and A. Tyler, Eds. (McGraw-Hill, New York, 1963), p. 393; J. J. Wolken, *J. Opt. Soc. Am.* **53**, 1 (1963).
12. P. Wygodzinsky, *Ann. Entomol. Soc. Am.* **54**, 621 (1961).
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Tumor-Promoting Activity of Extracts of Unburned Tobacco

Abstract. *Both an aqueous $Ba(OH)_2$ extract and an acetone-benzene extract of unburned cigarette tobacco produced skin tumors when painted on mice previously treated with 125 μg of 7,12-dimethylbenz[a]anthracene. The extract from as little as 0.5 cigarette per day was effective. The data are of interest in connection with epidemiological evidence that tobacco chewing is a cause of oral cancer. The data also suggest that tumor-promoting agents in cigarette smoke may have their source in the unburned tobacco.*

Clinical observations that tobacco chewing is associated with oral cancer have been reported for many years (*1*). This relation is most striking in the Far East, where tobacco is often chewed in conjunction with betel nut, flavorings, and lime (*2*, *3*). In the United States, where tobacco chewing is less extensive, similar observations have been reported, but the association of tobacco chewing with oral cancer is much less striking (*4*). Peacock *et al.* suggest that tobacco chewing alone is not carcinogenic but acts as a tumor-promoting stimulus to men exposed to other carcinogenic agents (*5*). Regardless of mechanism, it is noteworthy that most tobacco chewers or snuff dippers place the quids at the same site for many years, and it is at this site that the cancers develop.

Extracts of unburned tobacco have not been tested extensively for carcinogenic activity. Wynder and Wright

Table 1. Tumor promotion by extracts of unburned tobacco.

Treatment	Tobacco equivalent (cigarettes per day)	Ratio of No. of tumors to No. of mice with tumors*	
		30 weeks	36 weeks
DMBA and acetone extract	2.5	8/5	16/7
DMBA and conc. $Ba(OH)_2$ extract	0.5	19/7	18/8
DMBA and dilute $Ba(OH)_2$ extract	.5	11/2	6/2
DMBA alone	0	0	0
Acetone extract alone	2.5	0	0
Conc. $Ba(OH)_2$ extract alone	0.5	0	0
Dilute $Ba(OH)_2$ extract alone	.5	0	0
Untreated controls	0	0	0

* Thirty mice per group, 36 weeks of promoting stimulus.