

4°C, recentrifuged, and the cells resuspended in fresh fixative before making slide preparations by the method of BISHUN et al.<sup>5</sup> for human cells.

**Results.** Mitotic indices were calculated for each culture as the percentage of cells with visible chromosomes per 500 cells counted. The indices for each age group were averaged and the averages for the experimental and control animals compared. The average mitotic index of the 3-week-old animals treated with phytohemagglutinin 'P' was 9.8 as compared with 2.1 in the controls; that of 6-week-old animals was 6.0 as compared with 1.7 in the controls; and that of 12-week-old animals 8.9 as compared with 6.3 in the controls (Table).

The average mitotic index in each of the two younger groups was considerably higher (about four times) than

that of the untreated control animals of comparable ages, while that of the older animals was not appreciably different in the treated and untreated animals. The numbers are too few for statistical analysis. There is no clear sex difference apparent except perhaps in the older treated females, four of which showed markedly higher mitotic rates than did the males of their subgroup.

There does seem to be a definite increase in the mitotic rates for the younger animals. This might be due to a more susceptible marrow in these groups than in the older animals. If this is so it might be possible to increase bone marrow activity in young individuals by phytohemagglutinin injections while impossible in older individuals.

This study is being extended to cover a larger number and greater age range of rats, and also of other animals, to see if the action is similar in other species<sup>6</sup>.

Mitotic indices of control (C.) and experimental (Exp.) rats at 3, 6, and 12 weeks of age

Age	3 weeks		6 weeks		12 weeks	
	C.	Exp.	C.	Exp.	C.	Exp.
Females	3	24	2	13	3	15
	2	6	2	9	7	14
	2	10	3	7	8	10
	–	–	1	3	12	15
	–	–	0	5	5	6
Males	2	8	1	5	2	5
	2	1	2	3	15	7
	2	10	1	2	4	6
	–	–	3	5	3	7
	–	–	2	5	4	4
Average mitotic indices	2.1	9.8	1.7	6.0	6.3	8.9

**Résumé.** Chez des rats (mâles et femelles) auxquels on a administré de la phytohématagglutinine P (Difco) par voie intrapéritonienne, l'activité mitotique dans les cellules de la moelle des os a été notablement plus grande à l'âge de trois et six semaines qu'à l'âge de douze semaines (par comparaison avec les animaux de contrôle).

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<sup>5</sup> N. P. BISHUN, W. R. M. MORTON, and B. McLAVERTY, *Lancet* 1964 ii, 315.

<sup>6</sup> We would like to thank Dr. J. B. BRIDGES of this Department for supervising the experimental work.

### Neurosecretory Cells in *Artemia salina* L.

It is well known that physiologically active substances are produced by the neurosecretory cells located throughout the nervous system of crustaceans<sup>1</sup>. These neurosecretory cells are distributed as distinct groups at least in the eye stalk and the brain. Relatively little is known about the neurosecretory system of anostracans<sup>2,3</sup>, a primitive group of crustaceans. By analogy with what obtains in many other crustaceans the neurosecretory organs of which have been recently investigated, control of many metabolic processes in the Anostraca may be expected to be under hormonal control originating in the neurosecretory cells.

With this end in view, we have studied *Artemia* collected from the Sambhar salt lake, Rajasthan. The neurosecretory groups of cells have been identified by using the Gömöri technique. Large neurosecretory cells are seen in clusters in the supraoesophageal ganglion and in the eye stalk. Based on the shape, presence or absence of vacuoles in the cytoplasm and on the nature of secretion, the neurosecretory cells may be classified into three groups. One type of cell is large (15–20  $\mu$ ), oval in shape with vacuolated cytoplasm and large nuclei (Figure 1). The cytoplasm

of these cells is basophilic with the Nissl substance zonated. The nuclear membrane also stains basophilic, but the nucleoplasm is acidophilic. Some of these cells show large axons. The secretory granules occur in aggregates in the nucleus and also outside the Nissl zone. The second type of cell (Figure 1) is relatively small (8–12  $\mu$ ) with little cytoplasm and large spherical nuclei. These are without axons. While simulating the large cells in the secretory granules, these cells also show perinuclear concentration of neurosecretory granules. No tract from these is traceable into the eye stalk. Both these types of cells are seen in the supraoesophageal ganglion. A third type is present in the X-organ of the eye stalk (Figure 2). These cells are small (3–5  $\mu$ ) and form grape-like clusters with practically no cytoplasm.

<sup>1</sup> D. B. CARLISLE and F. G. W. KNOWLES, *Endocrine Control in Crustaceans* (Univ. Press, Cambridge 1959).

<sup>2</sup> J. H. LOCKHEAD and R. RESNER, *Proc. 15th Int. Cong. Zool.* 4, 397 (1958).

<sup>3</sup> M. MENON, *Neurosecretory System of Streptocephalus sp. (Anostraca: Branchiopoda)*. *Neurosecretion*, Mem. Soc. Endocrin. (Eds. H. HELLER and R. B. CLARK; Acad. Press, London 1962), p. 411.

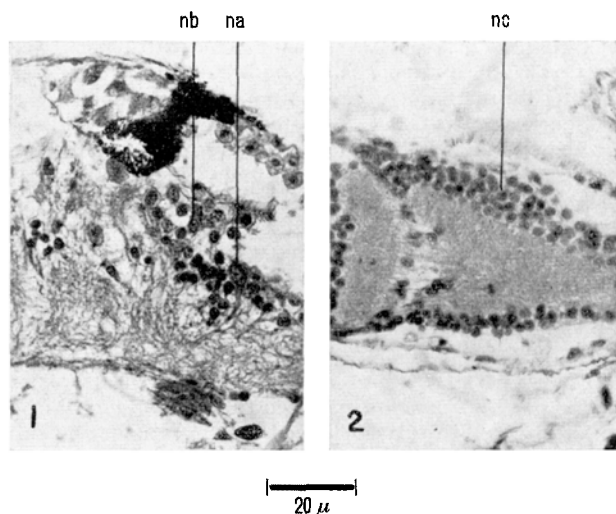


Fig. 1. Horizontal section of the supraoesophageal ganglion of *Artemia* showing large neurosecretory cells (na) and small cells (nb), Gömöri.

Fig. 2. Horizontal section of the eye-stalk in the region of the X-organ of *Artemia* showing the third type of neurosecretory cells (nc), Gömöri.

Release of granules appears to take place both through the cell membrane and by axon transport. In the middle of the brain ventrally, there is a deeply staining region receiving axons from the large cells described above. This is probably a storage organ like the Y-organ of the eye stalk of decapod crustacea.

The study of neurosecretion in *Artemia* would seem to offer a new and profitable approach to many problems of its physiology and ecology. Since this race of *Artemia* of Sambhar lake is parthenogenetic, the relation of their neurosecretory activities to their reproduction raises problems of unusual interest. Work on these lines is in progress.

**Zusammenfassung.** Mit der Gömöri-Technik wurden bei *Artemia* drei Typen neurosekretorischer Zellen nachgewiesen. Im Gehirn sind grosse, ovale Zellen mit vakuolisierendem Cytoplasma vorhanden, von denen einige grosse Axone besitzen. Der 2. Typus ist kleiner und weist keine zum Augenstiel führenden Fortsätze auf. Der 3. Typus liegt als traubenförmige Gruppen von Zellen im X-Organ des Augenstiels. Wahrscheinlich ist ventral am Gehirn ein Depotorgan für das Sekret des 1. Typus vorhanden. Die Beziehung dieser Zellen zur parthenogenetischen Vermehrung der Art wird untersucht.

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### Immuno-electrophoretic Characteristics of Plasma from Rats with Adjuvant Arthritis

Adjuvant arthritis in rats is an experimental syndrome which can be partially or completely inhibited by treatment with various anti-inflammatory agents<sup>1</sup>. There are currently no reports in the literature pertaining to alterations in plasma immuno-electrophoretic patterns of arthritic rats, nor of the influence of anti-inflammatory agents on these patterns. In this communication, the immuno-electrophoretic characteristics of plasma from both treated and non-treated adjuvant-injected rats are reported.

A total of 27 male albino rats, derived from the Wistar strain, were used in this experiment. Group I (9 rats) received an intradermal injection in the mid-tail region of 0.8 mg heat-killed *Mycobacterium butyricum* in 0.1 ml mineral oil; Group II (9 rats) received the same but were injected daily with 10 mg/kg hydrocortisone acetate (Hydro-Adreson, Organon) subcutaneously, starting on the day of adjuvant injection; Group III (9 rats) received 0.1 ml of mineral oil only. 21 days after adjuvant injection, all animals were anaesthetized with ether, and blood was obtained by heart puncture with heparinized syringes. Plasmas were separated by centrifugation and stored at  $-20^{\circ}\text{C}$  until use.

Antisera were obtained by immunization of rabbits with a 1:1 suspension of serum from normal rats and complete Freund's adjuvant. The immunization schedule involved intramuscular injection of 0.4 ml of the suspension the first week and 0.6 ml suspension for each of the succeeding 3 weeks. Rabbits were bled 10 days after the

last injection and the separated antisera were stored at  $-20^{\circ}\text{C}$  until use.

A micro-immuno-electrophoretic procedure, using microscope slides and an apparatus designed by WIEME<sup>2</sup>, was used. A constant current of 30 mA per slide at  $10^{\circ}\text{C}$  was employed, and the duration of electrophoresis was 45 min. After electrophoresis, antisera grooves were filled and the slides were allowed to set for 36 h in a moist chamber at room temperature. Following the washing and drying procedures, the slides were stained with Ponceau S.

During the second and third weeks after adjuvant injection, 7 out of 9 animals in Group I developed mild to very severe arthritis. The symptoms included swelling of the joints of the legs, nodular lesions on the external ears, and extensive necrotic areas on the tail. Animals which were treated with hydrocortisone had no joint swelling or gross manifestations of arthritis, the ear lesions were absent, and the tail lesions were less severe than those of the untreated animals.

Typical immuno-electrophoretic patterns of plasmas from animals of the three groups are shown in the Figure. Patterns from animals of the vehicle-control group had a minimum of one rather broad band for albumin, two  $\alpha$ -1 glycoproteins, a prominent  $\alpha$ -2 protein, one rather long and dense  $\beta$ -globulin line near the antiserum groove and another shorter and finer one nearer the electrophoretic axis, and a very faint  $\gamma$ -globulin precipitin line. Patterns

<sup>1</sup> B. B. NEWBOULD, Brit. J. Pharmacol. Chemother. 21, 127 (1963).

<sup>2</sup> R. J. WIEME, Clin. chim. Acta 4, 317 (1959).