

cristae were seen (Figures 1 and 2). In animals injected with saline only, no mitochondrial changes were observed. A preliminary study with rat liver mitochondria examined with an oximeter after addition of wasp venom registered an immediate arrest of oxidative phosphorylation, also indicating a functional lesion. The effect of hymenopterae venom on the function of mitochondria has recently been reviewed by HABERMAN<sup>6</sup>. As numerous lipolytic enzymes are present in the venom, functional and morphological mitochondrial changes are expected.

In numerous conditions, alterations in the internal structure of muscle mitochondria have been reported. These were observed in human muscle diseases<sup>7</sup>, in human rhabdomyoma<sup>8-10</sup> under environmental conditions influenced by acid pH<sup>11</sup>, under the influence of estrogenic hormones<sup>12</sup>, and in vitamin E deficiency<sup>13,14</sup>. Lately changes in mitochondrial ultrastructure in Nickel-Sulfide-induced rhabdomyosarcoma was reported<sup>15</sup>.

It seems, therefore, that muscle mitochondria exposed to many different noxious agents manifest morphological changes. The observation that wasp venom cause both morphological and functional changes in muscle mitochondria may explain the paralyzing effect of the venom.

**Résumé.** Du venin de *Vespa orientalis*, injecté dans la lèvre inférieure du cobaye, a donné une réaction inflammatoire non spécifique mais aiguë. Dans les mitochondries du muscle contigu strié de graves changements apparaissent. Les cristae se sont agglutinées à mi-hauteur et les mitochondries agglutinées ont été disloquées et prirent l'aspect d'une matière granulaire amorphe. Après l'arrêt immédiat de phosphorylation oxydative on con-

state que la lésion mitochondriale pourrait expliquer l'effet névrotique du rénin de la guêpe.

U. SANDBANK, J. ISHAY and S. GITTER

*The Department of Pathology, Beilinson Medical Center, and the Department of Physiology and Pharmacology, Tel-Aviv University, Medical School, Tel-Aviv (Israel), 17 September 1970.*

- <sup>1</sup> T. PIEK and R. T. SIMON THOMAS, *Comp. Biochem. Physiol.* **30**, 13 (1969).
- <sup>2</sup> J. H. BARNARD, *J. Allergy* **45**, 92 (1970).
- <sup>3</sup> S. SHULMAN, C. LANGLOIS and C. E. ARBESMAN, *J. Allergy* **35**, 446 (1965).
- <sup>4</sup> S. SHULMAN, C. LANGLOIS and C. E. ARBESMAN, *J. Allergy* **36**, 109 (1965).
- <sup>5</sup> H. VON SCHALLER, *Schweizer Arch. Neurol. Psychiat.* **94**, 92 (1964).
- <sup>6</sup> E. HABERMAN, *Rev. Physiology, Biochemistry and exp. Pharmacology* (Springer Verlag, Berlin 1968), vol. 60, p. 220.
- <sup>7</sup> M. G. SHY, *Ann. N.Y. Acad. Sci.* **138**, 232 (1966).
- <sup>8</sup> J. L. CORNOG and N. K. GONATAS, *J. Ultrastruct. Res.* **20**, 433 (1967).
- <sup>9</sup> B. CZERNOBILSKY, J. L. CORNOG and H. T. ENTERLINE, *Am. J. clin. Path.* **49**, 782 (1968).
- <sup>10</sup> A. I. FREEMAN and W. W. JOHNSON, *Cancer Res.* **28**, 1490 (1968).
- <sup>11</sup> R. CAREJO-SANTALO, *Can. J. Biochem.* **44**, 695 (1966).
- <sup>12</sup> L. S. DIETRICH, J. J. FRIEDLAND and R. C. CEFALU, *Proc. Soc. exp. Biol. Med.* **107**, 168 (1961).
- <sup>13</sup> N. F. CHEVILLE, *Int. J. Vetr. Path.* **3**, 208 (1966).
- <sup>14</sup> J. F. VAN VLEET, B. V. HALL and J. SIMON, *Am. J. Path.* **51**, 815 (1967).
- <sup>15</sup> P. K. BASRUR, A. K. SYKES and J. P. W. GILMAN, *Cancer* **25**, 1142 (1970).

## Ultrastructural Response of the Neural Plate Cells of Chick Embryos to Dithiodiglycol

It has been reported<sup>1,2</sup> that in amphibians the morphogenetic movement leading to the closure of the neural tube was linked with the reversible denaturation of a fibrous protein. It seems that as -SH groups in the protein are being oxidized into -SS- groups the fibrous molecule is transformed into a globular one and changes in cell shape<sup>3</sup> thus follow. This valuable hypothesis led to a series of studies of the effect on morphogenesis, in a variety of biological species, of a number of sulphhydryl reagents including mercaptoethanol, a strongly reducing sulphhydryl reagent<sup>4-6</sup>, and dithiodiglycol, its oxydation product<sup>3,7</sup>.

Dithiodiglycol was shown to be an efficient inhibitor of neurulation in amphibians<sup>8</sup> and in chick embryos<sup>7</sup>. This oxidizer of -SH groups was also reported<sup>9</sup> to favor the isolation of the mitotic apparatus of sea urchins through its stabilizing effect on the apparatus, and to inhibit cell division in cleaving amphibian eggs<sup>10</sup> by opposing the regression of the achromatic figure.

The purpose of this note is to give an account of the ultrastructural response of the neural plate cells of chick embryos when neurulation is inhibited with dithiodiglycol.

**Abbreviated materials and methods.** Chick embryos were explanted at stage 7+ or 8- of Hamilton's table and cultivated for 5.5 h at 38°C on Spratt's medium, but with 10<sup>-3</sup>M dithiodiglycol added. Control embryos, growing on standard media, were cultivated, fixed and embedded at the same time as the treated ones.

All embryos were fixed first in phosphate buffered glutaraldehyde and then in osmium tetroxyde. Blocks

were embedded in epon or araldite and sections double stained with uranyl acetate and lead.

**Principal observations.** Control embryos were comparable to those previously described in detail<sup>11</sup>.

In experimental animals, thin sections cut transversely in front of the first pair of somites showed a wide open nervous system still appearing in the form of a plate. The cellular contours were identical to those observed in controls. After a 5.5 h treatment all cellular organelles, save microtubules, appeared entirely comparable to those seen in control embryos.

Microtubules were apparently the only structures affected by the treatment. In control embryos microtubules follow a straight line or occasionally show a slightly wavy and smooth undulatory path (Figure 2). Under dithiodiglycol treatment similar outlines were

- <sup>1</sup> J. BRACHET, in *Embryologie chimique* (Deseor, Liège 1944).
- <sup>2</sup> L. RAPKINE and J. BRACHET, *Bull. soc. chim. biol.* **33**, 427 (1951).
- <sup>3</sup> J. BRACHET, *J. exp. Zool.* **142**, 115 (1959).
- <sup>4</sup> J. BRACHET, *Devl. Biol.* **7**, 348 (1963).
- <sup>5</sup> F. DESCOTILS-HEERNU, J. QUERTIER and J. BRACHET, *Devl. Biol.* **3**, 277 (1961).
- <sup>6</sup> V. POHL and J. QUERTIER, *J. Embryol. exp. Morph.* **11**, 293 (1963).
- <sup>7</sup> V. POHL and J. BRACHET, *Devl. Biol.* **4**, 549 (1962).
- <sup>8</sup> J. BRACHET and M. DELANGE-CORNIL, *Devl. Biol.* **1**, 79 (1959).
- <sup>9</sup> D. MAZIA and K. DAN, in *Sulfur in Protein* (Academic Press, New York 1958).
- <sup>10</sup> S. LIMBOSCH-ROLIN and J. BRACHET, *Expl. Cell Res.* **24**, 120 (1961).
- <sup>11</sup> P. E. MESSIER, *J. Embryol. exp. Morph.* **21**, 309 (1969).

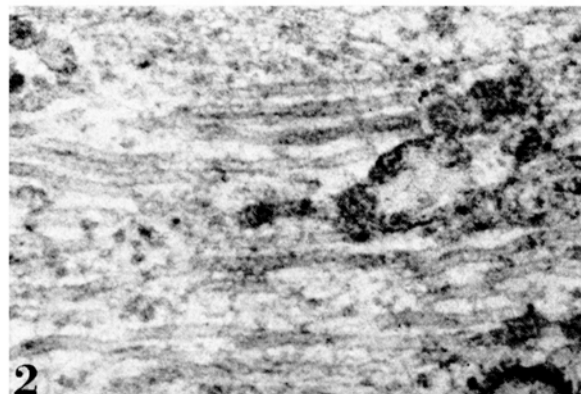
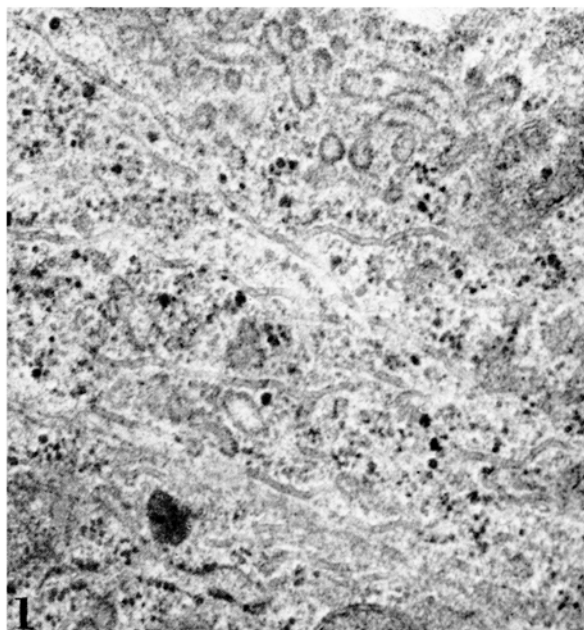


Fig. 1. Microtubules in a neural plate cell of a treated embryo. They follow a sinuous and rather tortuous path.  $\times 41,000$ .

Fig. 2. Microtubules in a neural plate cell of a control embryo.  $\times 77,000$ .

Fig. 3. Tortuous microtubules of treated embryos exhibiting notched walls (arrows).  $\times 77,000$ .

seen but highly sinuous, tortuous and rather crooked microtubules were frequently observed (Figure 1). In such cases, especially when the tubules exhibited a sharp change in direction, the microtubular wall showed small characteristic notches (Figure 3). Their number did not seem to vary and other attributes, such as their width and density to the electrons, were not modified. It could not be ascertained, however, whether these peculiarities affected the shape of the cells in any way.

**Comments.** The only ultrastructural alteration observed in the neural plate cells of dithiodiglycol-inhibited chick embryos was localized in microtubules. The usual slightly wavy path and smooth tubular wall became tortuous and irregular. It is possible that the oxidizing effect of dithiodiglycol could affect the thiol groups reportedly involved in the polymerization of the tubular element<sup>12</sup>.

It could not be ascertained from our experiments whether the observed microtubular modifications had, in any way, a bearing on the inhibition of the morphogenetic movements. Complex biochemical events, such

as interference with -SH containing enzymes for instance, could have occurred and not be detected by our technical approach<sup>13</sup>.

**Résumé.** La neurulation a été inhibée chez des embryons de poulet par des traitements au dithiodiglycol ( $10^{-3}M$ ). L'analyse ultrastructurale révèle que seuls les microtubules subissent des modifications.

PAUL-EMIL MESSIER<sup>14</sup>

Département d'Anatomie, Faculté de Médecine,  
Université de Montréal, Case postale 6128,  
Montréal 101 (P. Québec, Canada), 12 August 1970.

<sup>12</sup> D. MAZIA, Symp. Int. Soc. Cell Biol. 6, 39 (1967).

<sup>13</sup> This work was initiated while at Prof. J. BRACHET's Laboratory in Bruxelles and supported by a fellowship of the Medical Research Council of Canada.

<sup>14</sup> Scholar of the Medical Research Council of Canada.

### Autoradiographic Evidence for Cytoplasmic DNA Synthesis During the Early Final Growth Period in the Oocytes of the Japanese Quail (*Coturnix coturnix japonica*)

In previous studies<sup>1,2</sup> we have established the existence of peculiar subcortical cytoplasmic organelles, appearing in the oocytes of regularly laying Japanese quails just before and/or at the beginning of yellow yolk formation. By an autoradiographic study<sup>1</sup> these RNA-rich organelles<sup>2</sup> have been found labelled 90 min after an i.p. injection of <sup>3</sup>H-thymidine. In the present experiment, laying Japanese quails received an i.p. injection of 1 mCi of thymidine 6-<sup>3</sup>H (14 Ci/mM, 1  $\mu$ Ci/ $\mu$ l) followed 1 h later by a second injection of 1 mCi of the same precursor. 1 h after the second injection, the birds were killed by decapitation and the germinal discs of oocytes, ranging from 4 to 6 mm diameter, fixed in

acetic acid-alcohol (1:3 v) for 3 h. After dehydration, the germinal discs were embedded in paraffin and cut at 7  $\mu$ m thickness in a direction perpendicular to the plane of the germinal disc. Alternating sections were placed on different slides. These paraffin sections were screened under the dark ground microscope in order to select those sections containing some part of the germinal vesicle. Only these sections, which also contain part of the subcortical cytoplasmic organelles, are employed in this autoradiographic study. After deparaffination, the slides supporting the alternating sections are distributed into 4 groups: 1. Slides without any pre-autoradiographic treatment. 2. Slides immersed in 3% perchloric acid at