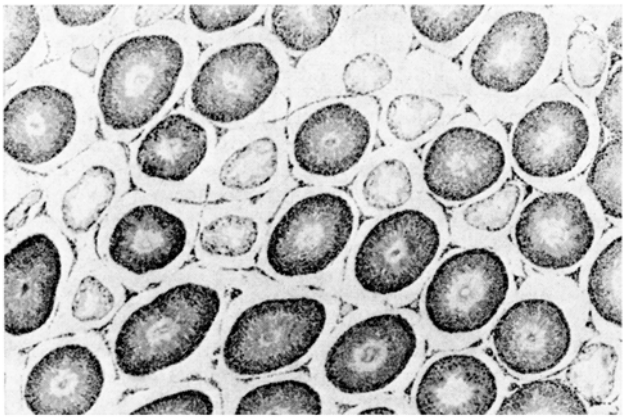

Fig. 1. Rat testis after 26 days of treatment with hyperlipidic diet + ANI. Some seminiferous tubules are markedly decreased in size, which contain few or no germinative cells. ×176.

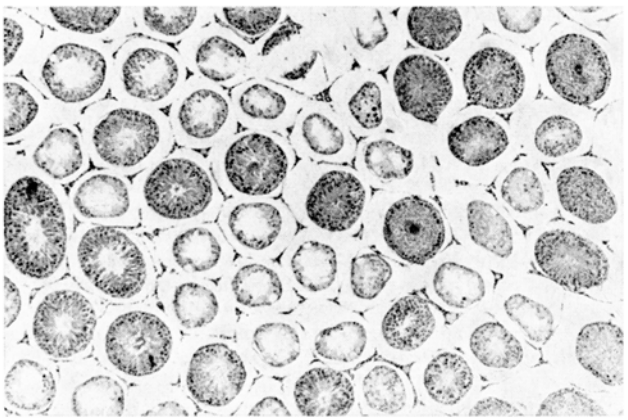

Fig. 2. Rat testis after 26 days of treatment with hyperlipidic diet + TAA. Involuted tubules containing necrotic material, few normal or degenerating spermatogonia and normal Sertoli cells. ×176.

tubules were remarkably decreased in size and contained degenerate cells, multinucleate masses sloughing in the lumina and, next the limiting membrane, a few degenerating spermatogonia. Sertoli as well as Leydig cells appeared to be normal.

It seems, therefore, that the hyperlipidic diet is a factor in allowing the toxic effect of ANI and TAA, since these substances are ineffective when fed together with an equilibrate diet.

It may be supposed that the testis lesions observed under our experimental conditions are due to cell biochemical changes, which possibly involve protein synthesis, as reported for ethionine intoxication.

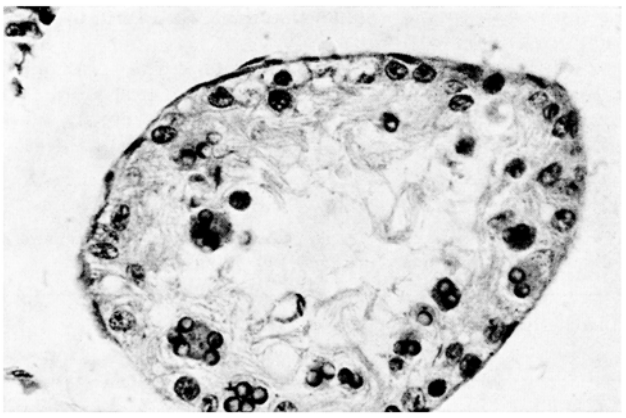

Fig. 3. Rat testis after 26 days of treatment with hyperlipidic diet + TAA. Damaged seminiferous tubule. Pathological changes are evident in the residual germinative epithelium. Some characteristic multinucleate cells can be seen. ×1760.

As far as the finding of almost completely degenerate tubules near normal ones is concerned, it has to be remembered that like features have been described during treatment with substances electively and greatly toxic for the testis. Therefore, such behaviour could be related to the rhythmic and alternate function of the various portions of the organ.

On the basis of the results obtained, from a wider point of view it can be pointed out that toxic substances may be ineffective when present in a balanced diet, whereas their toxicity may appear and be greatly increased when they are added to a non-equilibrate diet.

Riassunto. L'α-naftil-isotiocianato e la tioacetamide somministrati al ratto albino con una dieta equilibrata non inducono nessuna alterazione significativa a livello dei testicoli. Tuttavia, quando vengono associati ad una dieta iperlipidica, causano notevoli lesioni a carico dell'epitelio germinale.

L. MAZZANTI, M. LOPEZ and M. DEL TACCA

Department of Pharmacology, University of Pisa (Italy), 12 June 1970.

1 M. LOPEZ and L. MAZZANTI, J. Path. Bact. *69*, 243 (1955).
2 M. R. MCLEAN and K. R. RESS, J. Path. Bact. *76*, 175 (1958).
3 O. FITZHUGH and A. A. NELSON, Science *108*, 626 (1948).
4 I. TAKI, K. KAWAI, F. VENTURA, H. KITAMURA and Y. SOYAMA, Gann *43*, 116 (1952).
5 M. LOPEZ, Experientia *12*, 185 (1956).
6 D. KOCH-WESER and H. POPPER, Proc. Soc. exp. Biol. Med. *79*, 34 (1952).
7 E. FARBER, Cancer Res. *16*, 142 (1956).
8 M. ALZIZOURI and S. WARREN, Arch. Path. *57*, 130 (1954).
9 V. BECKER, Verh. dt. path. Ges. *40*, 247 (1956).

New Mitochondrial Inclusion Revealed by Negative Staining

Numerous mitochondrial inclusions have been described in a wide variety of mitochondria in thin sections of normal and pathological cells examined in the electron microscope. The inclusions, which occur most commonly in the mitochondrial matrix compartment, have been classified on the basis of structure as either granular, crystalline or fibrous[1,2].

The inclusion described here has been observed in mitochondria obtained from developmental stages of the blowfly *Calliphora erythrocephala*. Most of the observations were confined to mitochondria in larval and early pupal fat bodies, but the inclusions have also been seen in mitochondria in the eggs.

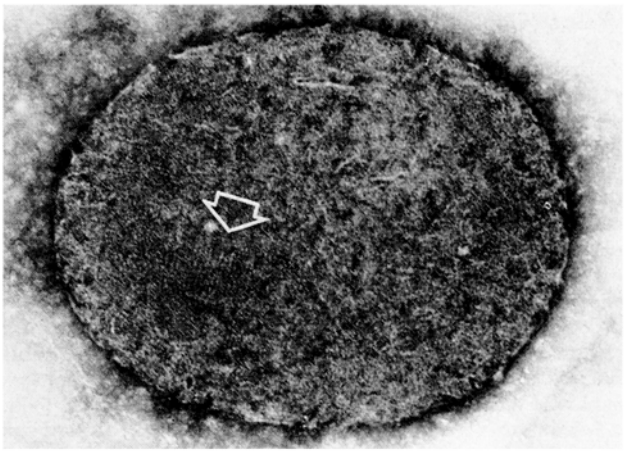

Fig. 1. Electron micrographs of specimens negatively stained with 5% ammonium molybdate. Fairly intact mitochondrion containing a small group of the inclusions (arrow). ×65,000.

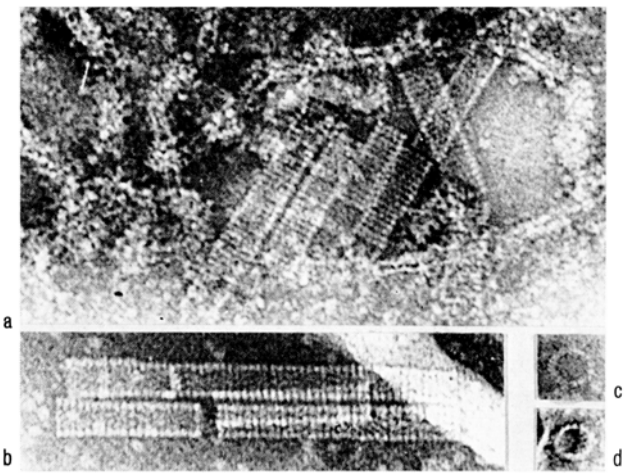

Fig. 2. a) and b), groups of the inclusions showing the prominent transverse striations (×154,000); c) and d), 2 individual rings thought to be the components from which the cylindrical inclusions are formed. ×190,000.

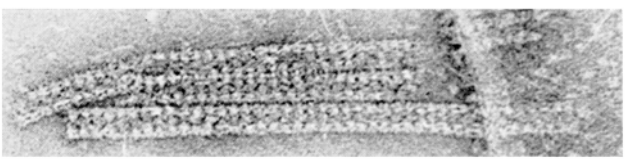

Fig. 3. One of the narrow tubular forms of polymeric ox liver glutamate dehydrogenase. ×185,000.

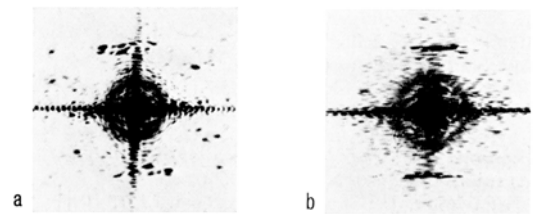

Fig. 4. Optical diffraction patterns obtained from electron micrographs of a) glutamate dehydrogenase of the form shown in Figure 3, and b) the mitochondrial inclusion.

The blowflies were cultured at 23 to 28 °C, the larvae being provided with fresh lean meat and the adults witn sugar, yeast extract and water[3]. Eggs, or fat bodies dissected from the larvae or early pupae were homogenized in a Potter-Elvehjam-type homogenizer in a medium containing 0.13 *M* NaCl, 10 m*M* potassium phosphate, pH 6.3, or directly in the negative stain (2–5% ammonium molybdate, pH 6.9, or sodium phosphotungstate, pH 7.1). Preparations were examined in a Siemens Elmiskop (generously provided by the Wellcome Trust) or an AEI EM6B electron microscope.

The inclusions, which are thin-walled, open-ended cylinders, were associated exclusively with mitochondria and were seen in all those in which the inner membrane was sufficiently damaged to allow penetration of the negative stain into the matrix space; they frequently occured in bundles (Figure 1). Each inclusion is 260 Å wide and about 500 Å up to 3600 Å long and has a regular transverse cross striation with a repeat of 65 Å (Figures 2a and b). It appears that the cylinders are formed from stacks of rings and individual rings were occasionally seen lying free (Figures 2c and d). Each ring is composed of 12 subunits with dimensions of about 45 Å by 45 Å. One of the known components of the matrix compartment of mitochondria (at least from liver) is glutamate dehydrogenase[4]. The inclusions bear a superficial resemblance to one of the narrow tubular forms of polymeric ox liver glutamate dehydrogenase[5] (Figure 3). Although unlike the enzyme (from ox liver) it did not dissociate into subunits when negatively stained with sodium phosphotungstate rather than ammonium molybdate the mitochondrial inclusion described here had an optical diffraction pattern similar to that given by electron micrographs of this tubular form of glutamate dehydrogenase[5] (Figure 4). Positive identification of the inclusion must await its isolation.

This is thought to be the first demonstration of a new mitochondrial inclusion by negative staining (although Parsons[6] has examined previously described 'matrix granules' by this technique). One of the features of the negative staining technique is that the whole length and depth of each mitochondrion can be examined at one time[7]; it is considered unlikely that this particular inclusion would have been discovered by the thin sectioning technique.

Résumé. En appliquant une technique de coloration négative, une nouvelle inclusion consistant en un cylindre ouvert aux deux extrémités, de paroi mince, a été observée dans la matrix mitochondriale de tissus d'insectes en cours de développement. Cette inclusion ressemble à l'une des formes tubulaires de la polymère-glutamate deshydrogénase, une enzyme apparaissant dans la matrix des mitochondries.

E. A. Munn

Biochemistry Department, Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge (England), 17 July 1970.

1 T. Suzuki and F. K. Mostofi, J. Cell Biol. *33*, 605 (1967).
2 E. A. Munn, in *Handbook of Molecular Cytology* (Ed. A. Lima-de-Faria; North-Holland Publishing Company, Amsterdam 1969), p. 875.
3 E. A. Munn and G. D. Greville, J. Insect Physiol. *15*, 1935 (1969).
4 K. R. Norum, M. Farstad and J. Bremer, Biochem. biophys. Res. Comm. *24*, 797 (1966).
5 E. A. Munn, Proc. R. microsc. Soc. *4*, 170 (1969), and unpublished work.
6 D. F. Parsons, Science *140*, 985 (1963).
7 E. A. Munn, J. ultrastruct. Res. *25*, 362 (1968).