Table III. Lack of stimulation of CDF_1 spleen cells by DIC-treated spleen cells in MLC

Stimulated	Stimulator	Expected cpm ± SE	Obtained cpm ± SE	Stimulation index
CDF_1	CDF_1	—	6892 ± 820	1
CDF_1	CDF_1-DIC	4190 ± 382	3962 ± 460	0.9
CDF_1	CDF_1-DIC_m	3061 ± 247	3346 ± 104	1.1
CDF_1	$C57B1/6_m$	3843 ± 106	14882 ± 1037	3.8
CDF_1	PHA	3446 ± 128	56424 ± 1891	16.3

24 h before challenge with L1210/DIC viable cells. L1210/DIC leukemia grew progressively and killed animals previously sensitized to DIC-treated skin, but was rejected in mice that had been sensitized to L1210/DIC cells (Table II).

The results of the above experiments indicate that DIC-treated skin was ineffective in sensitizing syngeneic mice or in cross-reacting with DIC-induced antigens on leukemic cells. CDF_1 spleen cells, incubated with spleen cells from DIC-treated CDF_1 mice, have not been stimulated (Table III).

The failure to detect DIC-induced 'stimulating' antigens on spleen cells is in contrast to the stimulation observed by DIC transformed leukemic cells[17]. In studies not reported here, DIC-treated spleen cells did not stimulate allogeneic lymphocytes. DIC treatment might deplete B lymphocytes which have the property of acting as stimulators of T lymphocytes in mixed lymphocyte culture[18]. If this were the case, spleen of DIC treated mice might contain responder T lymphocytes predominantly.

Discussion. The nature of drug-induced antigen(s) on tumor cells has not been investigated extensively. The studies have been concerned primarily with the occurrence of new tumor transplantation antigens. There are two major aspects of DIC activity: a) DIC induced antigen(s) in neoplastic cells. b) In the current experiments normal tissues were apparently not altered by chronic treatment with DIC which is an immunosuppressive drug. No evidence could be obtained that new antigens were expressed on normal skin following DIC treatment in vivo. The lack of response of CDF_1 lymphocytes could be interpreted in two possible ways: a) there was no antigenic alteration detectable by the MLC method for the DIC-treated spleen cells; b) if DIC treatment depleted B lymphocytes, this might still leave open the possibility that T lymphocytes could be altered by DIC treatment, although not detectable in MLC.

Therefore the hypothesis that an immunosuppressive treatment such as with DIC might induce a new autoimmune disease, clinically apparent after the discontinuation of the therapy, does not seem to be supported. The possibility that DIC may deplete preferentially B lymphocytes requires further investigation. This property would be of general interest in therapeutic research.

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Mitochondrial Derivation of Centrioles in Some Endocrine Adenomas

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Summary. Fine structural studies of various endocrine adenomas indicate that mitochondria may serve as progenitors of centrioles and cilia.

Centrioles, known to play a fundamental role in mitotic division, occur with varying frequency in different cell types and exhibit uniform, easily recognizable features by electron microscopy[2]. While studying the fine structure of surgically removed human tumors arising from various endocrine glands, several adenomas were found which contained numerous structures believed to represent forming centrioles in various phases of development providing an opportunity to investigate the subcellular aspects of centriologenesis[3].

The material consisted of 17 chief cell adenomas of the parathyroid, 7 sparsely granulated growth hormone cell adenomas, 4 mixed adenomas composed of growth hormone cells and prolactin cells, 3 undifferentiated cell adenomas of the pituitary and 1 pheochromocytoma arizing from the adrenal medulla. These tumors were selected from 76 cases because they contained numerous centrioles.

Small pieces of tumor tissue were fixed immediately after their surgical removal in 2.5% glutaraldehyde in 0.15 *M* Sorensen's buffer, postfixed in 1% osmium tetroxide in Millonig's buffer, dehydrated in graded ethanol and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and investigated with a Philips 300 electron microscope.

While attempting to reconstruct the various steps of centriole formation from the electron micrographs, a hitherto undetected sequence of events seemed to emerge: centrioles appeared to arise from mitochondria. Firstly, mitochondrial cristae disappeared and fibrillar-granular material accumulated at one pole of the mitochondria. The rest of the internal compartment was usually occupied by a clear vacuole (Figure 1). The double mitochondrial membranes exhibited increased electron density and, by further accumulation of fibrillar-granular substance, mitochondria gradually transformed into procentriolar bodies (Figures 2 and 3). Then the double membranes appeared to disintegrate followed by an asymmetrical division of the electron dense material (Figure 4). As-

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sembly of tubular structures within the divided aggregates, leading to the development of a diplosome (pair of centrioles), was already noticeable in this stage. Maturation of these precursors resulted in the formation of the 'primary' organelle (basal body) usually possessing basal feet and other satellites, and of the 'secondary' centriole.

Appearance of multiple procentriolar bodies (Figure 5) was common in parathyroid chief cell adenomas. Accumulation of centrioles, characteristic of some endocrine adenomas (Figures 6 and 7), may be associated with rudimentary cilia deriving from the basal bodies (Figure 8).

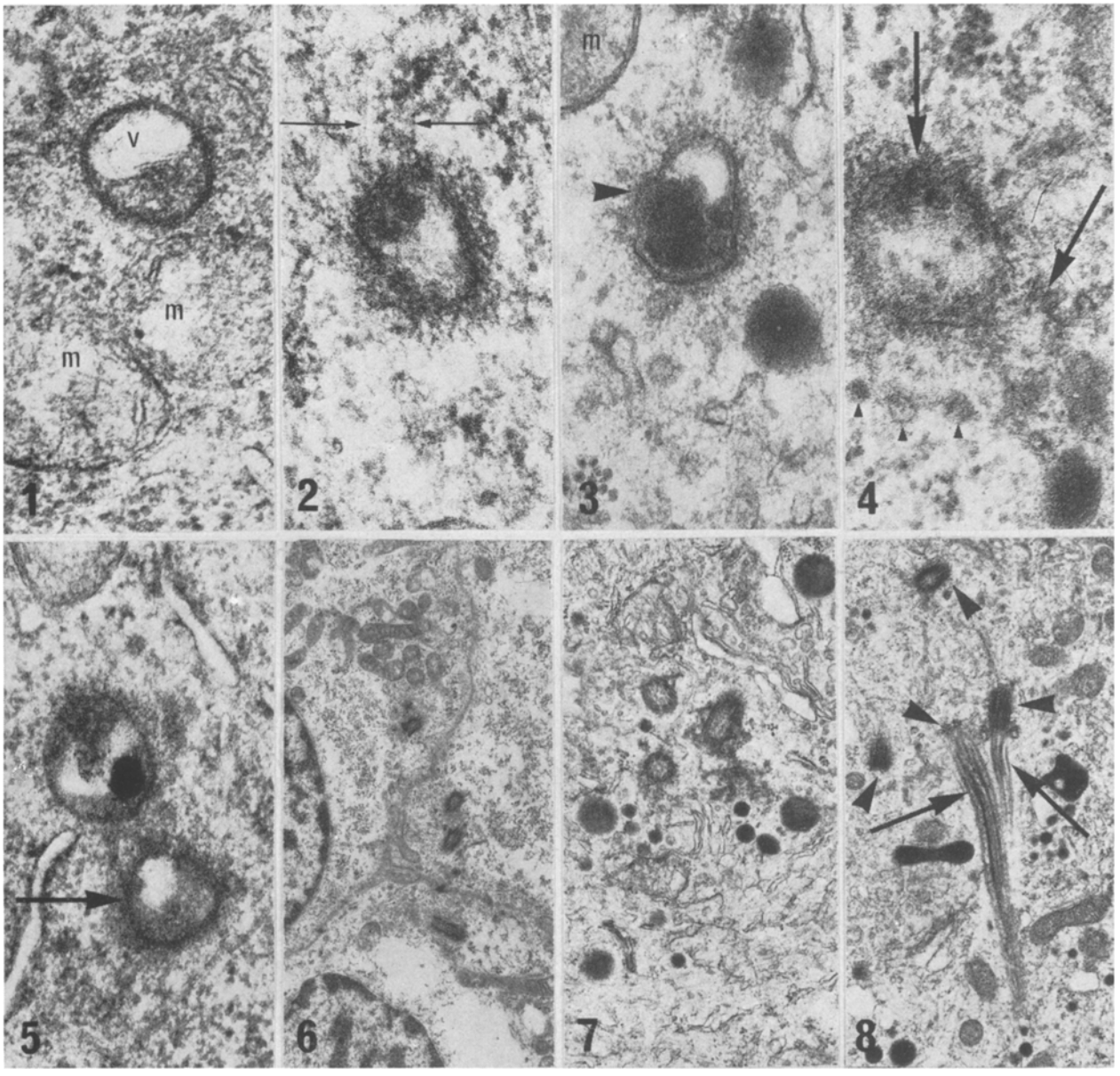

Fig. 1. Early procentriolar body showing light accumulation of fibrillar-granular material at one pole of mitochondrion and appearance of a clear vacuole (v) at the other pole. The mitochondrial double membranes are still clearly distinguishable but, compared to the double membranes of adjacent mitochondria (m), have markedly increased electron density. Parathyroid chief cell adenoma. × 67,000.

Fig. 2. Early procentriolar body. The double membranes became indistinct and exhibit spiky appearance. Note the presence of cytoplasmic microtubules, appear to be in contact with the body (arrows). Sparsely granulated growth hormone cell adenoma. × 73,400.

Fig. 3. Procentriolar body displaying marked polar accumulation of electron dense substance. Compared to the neighboring mitochondrion (m) the limiting membranes are dense but still distinct except for one part (arrowhead) where they have already started to disintegrate. Parathyroid chief cell adenoma. × 65,900.

Fig. 4. Procentriolar body in advanced phase showing signs of division into a larger and a smaller part (arrows). Note the presence of centriolar satellites nearby (arrowheads). Parathyroid chief cell adenoma. × 67,000.

Fig. 5. A pair of procentriolar bodies in a parathyroid chief cell adenoma. The double membranes are still distinguishable at certain parts (arrow). × 45,300.

Fig. 6. 4 mature centrioles shown in a parathyroid chief cell adenoma. × 6800.

Fig. 7. 4 mature centrioles situated in the Golgi region in a sparsely granulated growth hormone cell adenoma. × 19,700.

Fig. 8. 2 centrioles (basal bodies) of the 4 (arrowheads) observed in a sparsely granulated acidophil adenoma, gave rise to rudimentary intracytoplasmic cilia (arrows). × 15,400.

According to current views, centrioles are self-replicating organelles[4–6]. Present study, however, seems to indicate that mitochondria may serve as progenitors of centrioles. Preliminary findings suggest that transformation of mitochondria into centrioles is apparent not only in certain endocrine adenomas but occasionally also in non-tumorous tissue. The fact that only a limited number of mature centrioles can be detected in non-neoplastic endocrine glands[7–10] obviously restricts the investigation of sequence of events of centriologenesis by electron microscopy in non-tumorous material. It is evident that in certain endocrine adenomas, centriologenesis is strikingly abnormal resulting in accumulation of centrioles, including immature forms. No explanation can be offered regarding causes of defective centriologenesis. It may well be that in certain endocrine adenomas centriologenesis is accelerated, or alternatively, retarded or arrested. These defects could account for the accumulation of precursors.

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Teratogenic Effect of Tolbutamide on the Development of the Sea Urchin Embryo (*Paracentrotus lividus* Lamarck)

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Summary. The hypoglycemic agent tolbutamide was tested for its action on the cleavage and differentiation of the sea urchin embryo. Tolbutamide effects a strong selective action on the endoderm which becomes suppressed.

In previous studies we investigated a number of substances of pharmacological significance in order to establish a preclearance testing system using the gametes and embryos of sea urchins as material[1,2]. The present study is also a contribution to the knowledge of the morphogenetic and subcellular effects of drugs, and deals with tolbutamide, i.e. N′-4-methylbenzenesulfonyl-N″-butylurea[3]. This substance is an oral hypoglycemic agent which has proved to be very useful as a therapeutic. However, evidence has been produced that hypoglycemic drugs must be included among the increasing number of substances with known or suspected teratogenic action[4–9].

Material and methods. The experiments were carried out at 'Stazione Zoologica', Naples, using eggs and sperm from *Paracentrotus lividus* (Lamarck) as material. The concentration of tolbutamide was varied between 10^{-7} *M* and 10^{-3} *M*, but most of the experiments were made in concentrations of about 10^{-5} *M*. The time of treatment was 3 h in short-time experiments; in long-time experiments we indicate the start of the treatment, e.g. hatching, whereupon the substance was acting on the larvae until the pluteus stage of the control. Further details about the technique were described elsewhere[1].

Results. The experiments with tolbutamide indicate that this substance exerts selective effects on the different

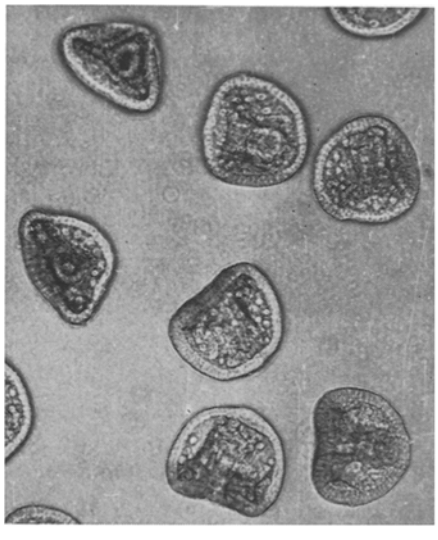

Fig. 1. Control. Late gastrulae. × 130.

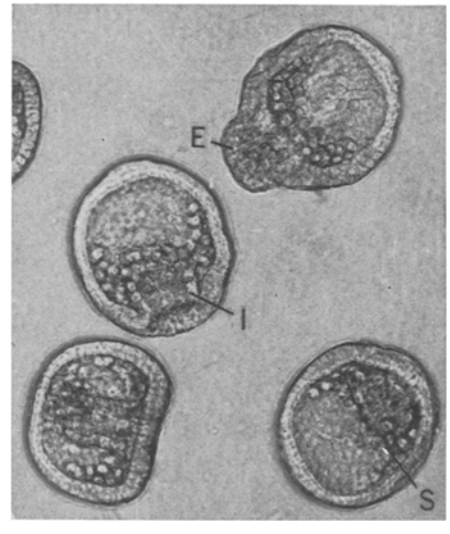

Fig. 2. Larvae from the same female as in Figure 1. Treatment from the 32-cell stage in 10^{-5} *M* tolbutamide. The formation of the intestine is more or less inhibited. One larva shows a tendency to exogastrulation (E). I, intestine; S, skeleton. × 230.