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

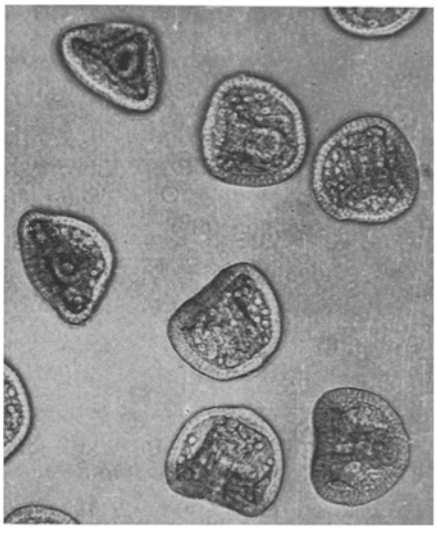

Fig. 1. Control. Late gastrulae. × 130.

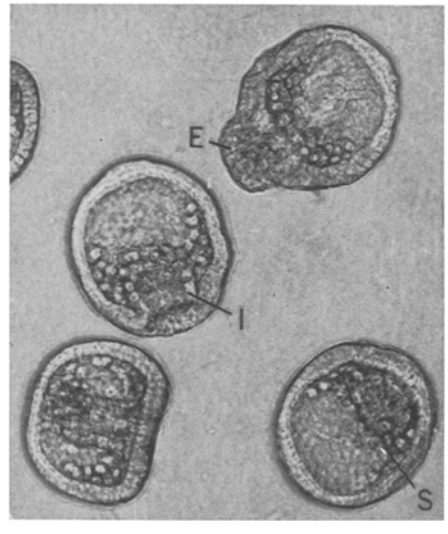

Fig. 2. Larvae from the same female as in Figure 1. Treatment from the 32-cell stage in 10^{-5} *M* tolbutamide. The formation of the intestine is more or less inhibited. One larva shows a tendency to exogastrulation (E). I, intestine; S, skeleton. × 230.

Fertilization rate experiment[10]. Eggs and sperm from *Paracentrotus lividus*

	Fertilized eggs (%)						
	Seconds after insemination						
	5	10	15	20	25	40	∞
1. Control							
Tolbutamide (2–5):	30	76	94	98	99	100	100
2. Pretreatment of sperm (2×10^{-4} *M*)	30	73	89	96	98	99	100
3. Pretreatment of sperm (2×10^{-5} *M*)	45	87	97	98	98	99	100
4. Insemination in presence of 2×10^{-4} *M*	34	67	91	96	98	99	100
5. Insemination in presence of 2×10^{-5} *M*	52	78	92	98	99	99	100

Concentration of sperm 10^5/ml. The spermatozoa were pretreated for 2 min in tolbutamide before insemination (Nos. 2 and 3), or tolbutamide was added at the moment of insemination (Nos. 4 and 5). Each percentage quoted is based on counts of 250–300 embryos. For the statistical significance of the method cf.[13].

germ layers of the embryo and that the morphogenetic effect is correlated with the developmental stage of the onset of treatment and also with the duration of the treatment.

The effect on fertilization. When eggs or spermatozoa were treated with tolbutamide before or during insemination, the formation of the fertilization membrane was normal and no tendency towards polyspermy was observed. The effect of tolbutamide was also tested in fertilization rate experiments[10]. Pretreatment of the sperm for 2 min before insemination did not appreciably harm the fertilizing capacity of the sperm even in as high concentration as 2×10^{-4} *M*. A significant increase in the fertilization rate was registered after pretreatment or in presence of low to moderate concentrations (i.e. $10^{-7} - 5 \times 10^{-5}$ *M*) of tolbutamide (Table).

The effect on differentiation. It appears that tolbutamide is not very toxic to the gametes and the cleaving embryo. Only if tolbutamide was present in concentrations above 10^{-4} *M* there was a slight retardation in cleavage, and in these high concentrations there was also a general deleterious effect on the cell. In contrast with this are the severe disturbances observed during the ensuing differentiation of the larva.

During the early development the first gross differentiation is seen when the cells of the primary mesenchyme invade the blastocoel after hatching, i.e. about 12 h after fertilization. The immigration of the primary mesenchyme cells apparently proceeds normally, but the following invagination of the gut is delayed and is also quantitatively disturbed. In extreme cases there is a total inhibition of the intestine resulting in blastulae containing a more or less well-differentiated skeleton (Figures 1 and 2). If a reduced gut is invaginated, the larva may develop into a pluteuslike embryo with a somewhat abnormal skeleton. In some experiments, we observed that a minor proportion of tolbutamide-treated embryos form exogastrulae[11] with strongly reduced guts (Figure 2).

If the treatment is started just before hatching, the disturbances in the differentiation become almost entirely confined to the intestine; and the closer to hatching the treatment is started, the less affected is the development of the skeleton. The negative action on the endoderm is, however, always considerable.

The effect of tolbutamide on the ultrastructure. Fixations were made of several developmental stages from cultures

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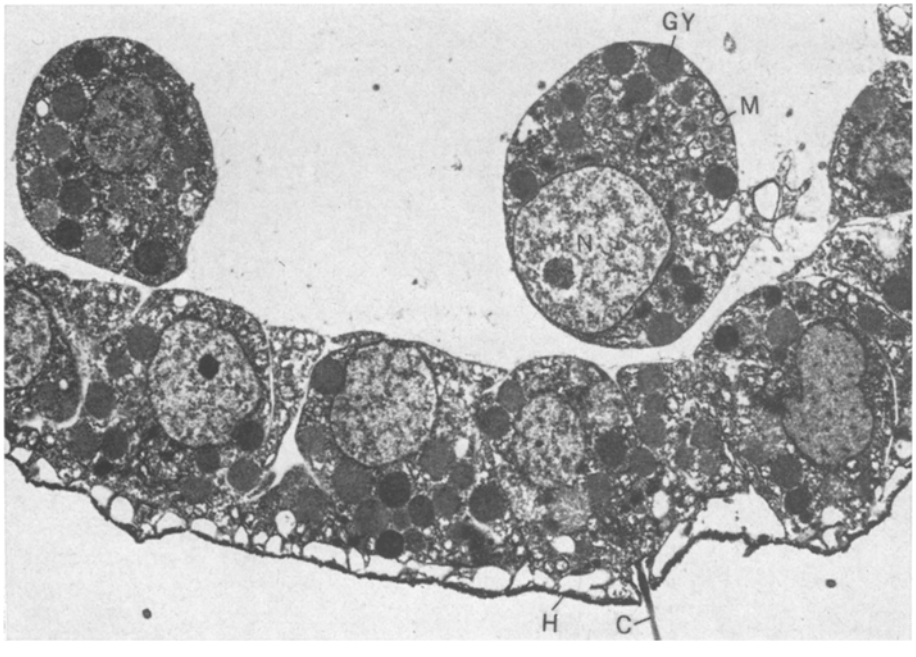

Fig. 3. Part of a control gastrula with wall and primary mesenchyme cells. C, cilium; H, hyaline layer; M, mitochondrion; N, nucleus; GY, granulated yolk granule. ×6,000.

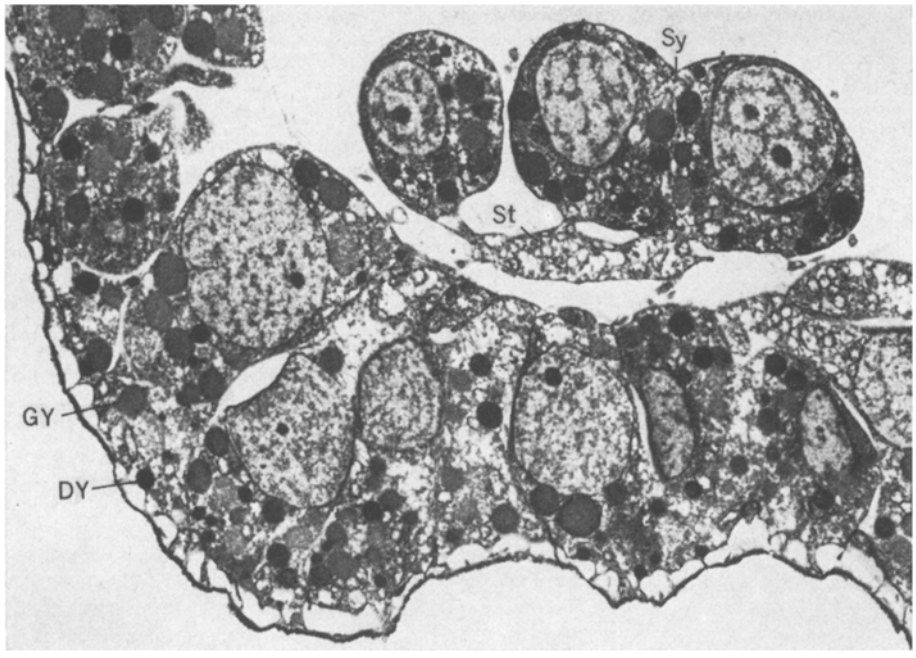

Fig. 4. Larva from the same female as in Figure 3. Treatment from before hatching in 10^{-4} *M* tolbutamide, fixed 18 h later. Gastrula with primary mesenchyme cells forming a syncytium (Sy) and a cytoplasmic stolon (St)[12]. Two kinds of yolk granules (dense yolk, DY; granulated yolk, GY) can be identified. × 6,000.

treated according to the descriptions given above. The ultramicrographs revealed that the structural changes inflicted by tolbutamide are small and in most respects there are no deviations from the normal structure.

However, the yolk granules seem to become changed and there appears to be two distinct kinds of yolk; one type of granules is very electron dense and appears dark in the micrographs, whereas the other type of granules have the normal greyish granulated appearance (Figures 3 and 4, DY resp. GY).

Counts were also made of the different cell organelles. Blastulae treated in 10^{-4} *M* tolbutamide for 4 h before hatching contain considerably more yolk granules than control embryos of the same age. In tolbutamide the hatching blastulae were found to have almost 30% more yolk granules than the larvae of the corresponding control. This difference is entirely confined to the yolk since the number of mitochondria is equal in control and tolbutamide.

Discussion. The general impression of the action exerted by tolbutamide is in most respects positive. In concentrations below 10^{-5} *M* there is no deleterious effect on cleavage and hatching is enhanced and facilitated. The studies of fertilization indicate that tolbutamide promotes fertilization.

With this background it is therefore of interest that tolbutamide has a clear deleterious influence on the differentiation of the endoderm. The fact that the formation of the skeleton takes place more or less unharmed, whereas the intestine is clearly affected, intimates that there is a selective action of tolbutamide on the endoderm. The skeleton develops in a fairly normal way, whereas the endoderm, which is differentiating a little after the appearance of the primary mesenchyme, becomes reduced. We therefore conclude that tolbutamide acts specifically on the growth and the differentiation of the endoderm. This is rather exceptional, since, as a rule, most substances have an unspecific inhibiting effect on differentiation, whereas observations of a specific effect on some clearly defined part of the embryo are uncommon.

It is still unclear if the teratogenic action of tolbutamide in warmblooded animals is inflicted by the drug or a metabolite of the drug, or if it is related to a general metabolic change in the pregnant female. In our experiments the action of tolbutamide is probably a direct one exerted by the drug itself.

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OECOLOGICA HUMANA

Redactional remark. The founder of the Experimental Gerontology Fritz Verzár gives to Experientia for the rubric 'Oecologica' humana some fundamental reflections concerning the phenomenon life – death, evoked by the 10th International Congress of Gerontology.

Basic Research in Experimental Gerontology

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Gempenweg 1, 4144 Arlesheim (Switzerland), 18 March 1976.

The concept of basic research is an expression of the most important task of the biological sciences for the future. But it cannot be said to describe the present day program of those sciences. Over 4,000 titles in the Year Book of the biological publications show an immense diversity of themes of a physico-chemical nature of ever-increasing complexity, as a reflection of the day-to-day publications in the different scientific journals.