Organ weights and the succinic dehydrogenase activities in kidneys and livers of rats (mean ± standard deviation)

Treatment	No. of rats	Kidney (g) / Body wt. (g) · 10^4	Adrenal (g) / Body wt. (g) · 10^6	Enzyme activity[b] Kidney	Liver
Untreated control	17	37 ± 3.9	44 ± 8.0	147 ± 16	132 ± 11
Sham control	17	38 ± 3.5	51 ± 6.3	145 ± 14	134 ± 12
Egg-white injection	11	38 ± 4.1	53 ± 12.0	159 ± 28	223 ± 21[a]
Right nephrectomy	12	47 ± 4.7[a]	53 ± 3.5	126 ± 11[a]	143 ± 15
Combined treatment	12	49 ± 5.3[a]	66 ± 18.0	132 ± 13[a]	173 ± 37

[a] P of 0.05 or less, [b] mμg formazan/μg protein nitrogen/5 min.

Results and discussion. The left kidneys of rats subjected to right nephrectomy, with or without egg-white injections, were significantly heavier than those of the controls, whereas injection alone did not alter the weight. No significant change in the adrenal weight was observed in any group. The enzyme activity in the kidney of injected rats showed a slight increase but with no significance (P of 0.8). In contrast, the activity in the remaining kidneys of unilaterally nephrectomized rats was significantly less than in the controls, and a similar reduction was noted also in the rats of the combined treatment. The difference between these 2 diminutions, however, was not significant (P of 0.4). The enzyme activity in the livers of injected rats was significantly greater than in the controls.

The reduced enzyme activity in the remaining kidneys of the operated rats is probably related to the phenomenon of compensatory hypertrophy itself. Although both mitoses and anabolism in the renal cells of compensatory hypertrophy are above normal[7,8], the renal clearance is diminished[9]. The enzymatic functions of both regenerating and functionally impaired kidney cells are subnormal[10,11]. The lack of enzymatic change from the original level in the kidneys of both groups of injected rats does not support the view that the droplet formation involves mitochondrial enzymes[1,12]. The work of other enzymes likewise tends to cast doubt on the direct relationship[13,14]. The hepatic parenchymal cells do not develop droplets in response to protein injection[15], and the present data do not point to any meaningful relation between the enzymes of kidney and liver.

Résumé. L'activité de la déshydrogénase succinique du rein ou du foie ne peut expliquer la formation de gouttelettes dans les cellules rénales après injection i.p. de blanc d'œuf associée ou non à une néphrectomie.

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Experiments on Mitochondrial Swelling in vivo

Much work has been devoted to the study of active swelling and contraction of isolated mitochondria in vitro and a number of chemical swelling agents have been described; however, the intracellular significance of these mitochondrial changes remains to a large extent a matter of conjecture.

Mitochondria have been known to be capable of undergoing changes of movement, shape and volume, also in the living cells since the early investigations on tissue cultures[1], and more recent observations, with the aid of the phase-contrast microscope and cinemicrography, have shown that mitochondria can be induced to swell in vivo by addition to the tissue culture medium of a variety of chemical agents such as 2,4-dinitrophenol (DNP), adenosine triphosphate (ATP), ethylenediaminetetraacetate (EDTA), adrenochrome, as well as by oxygen deprivation and by illumination with intense light of 556 nm wave-length[2]. Also fluoroacetate[3] and diphtheria toxin[4,5] have been shown to induce swelling of mitochondria in vivo. These findings showed that mitochondria may undergo reversible swelling in the living cells; however, the available data indicate that marked differences must exist between the in vitro and the in vivo processes. In fact the mitochondrial swelling agents in vivo include compounds which prevent, like DNP and EDTA, or even reverse, like ATP, the active swelling of isolated mitochondria in vitro. In order to gather information on the

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volume changes of mitochondria in the living cells and their significance, it seemed of interest to test the effects in vivo of in vitro swelling agents; in particular it appeared relevant to see whether the swelling agents of natural occurrence in animal tissues, called 'physiological'[6], would provoke mitochondrial volume increases also in vivo. With the exception of Ca^{++} and, perhaps, of phosphate, none of the 'physiological swelling agents' tested caused any visible effect on the conformation of mitochondria in the living cells used in the present study.

Experimental. Primary 18–22 h monolayer cultures on glass coverslips in Leighton tubes from trypsinized hearts of 6-day-old chick embryos were used. The cells were grown in Hanks' BSS containing 3% of fresh inactivated calf serum and 0.5% of lactalbumin hydrolysate and incubated at 38 °C. The experiments were performed by substituting the medium with the same volume (2 ml) of Hanks' BSS (with or without calf serum) containing dissolved in it the substance to be tested. Controls were treated in the same manner with plain Hanks' BSS (with or without calf serum). Chemicals of the highest grade available were used; solutions were made immediately before use in glass bidistilled water and neutralized either with NaOH or HCl if necessary. Each agent was tested at various concentrations and on different batches of cultures. After 30 or 60 min incubation at 38 °C, the coverslips were mounted on a slide and examined at the phase-contrast microscope. Ten fields of the cells layer were examined at random; on the average each field contained at least 4 cells. Mitochondria were classed into 3 groups: filamentous, thickened and swollen, and each field was graded: (1) if all the cells present in it contained filamentous mitochondria; (2) if 1 or more cells showed thickened mitochondria; (3) if 1 or more cells had swollen mitochondria. Thus each culture was graded from 10–30; normal untreated and control cultures showed mainly elongated thin mitochondria in agreement with classical description and their values ranged from 10–13. In nearly all instances the results were recorded by photomicrography.

Results. Preliminary experiment showed that fluoroacetate caused mitochondrial swelling in the cells grown

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Compound	Concentration range	Cultures tested	Cells examined[a]	Effect after 30 min	Effect after 60 min
None	–	42	1680	12	13
Fluoroacetate	m*M*	3	120	23	27
L-Cystine	Saturated solution	5	200	13	12
L-Cysteine	m*M*–100 m*M*	9	360	13	12
Glutathione (GSH)	m*M*–100 m*M*	7	280	12	12
L-Ascorbic acid	m*M*–200 m*M*	7	280	12	12
L-Thyroxine	μM–m*M*	10	400	12	11
L-3,5,3′-Triiodothyronine	10 μM	2	80	13	12
Phosphate	100 μM–10 m*M*	9	360	15	15
$CaCl_2$	0.1 μM	1	40	15	17
$CaCl_2$	μM	1	40	13	16
$CaCl_2$	2 μM	3	120	19	23
$CaCl_2$	10 μM	3	120	22	25
$CaCl_2$	100 μM	6	240	22	24
$CaCl_2$	200 μM	2	80	13	18

[a] Average figures on the base of 4 cells/microscope field.

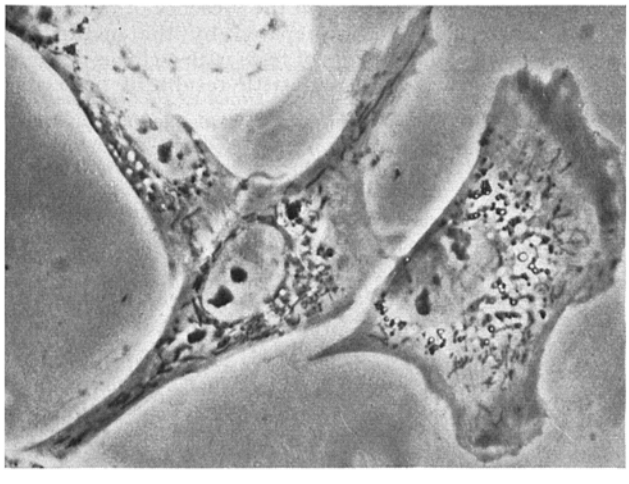

Fig. 1. Two normal, untreated myoblasts. Several filamentous mitochondria are visible. Phase-contrast microscope. About × 1000.

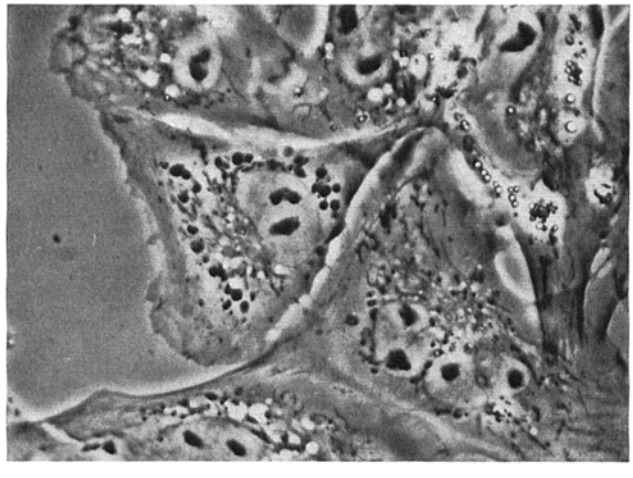

Fig. 2. A myoblast from a culture treated with Ca^{++} ($CaCl_2$ 10 μM). Several round swollen mitochondria are visible in the cytoplasm. Phase-contrast microscope. About × 1000.

in monolayer just as in the ones cultured by the hanging-drop method. The results obtained with the 'physiological swelling agents' are summarized in the Table: it will be seen that only Ca^{++} and to a much less extent phosphate produced an appreciable effect on the conformation of living mitochondria. Each agent was tested at the concentration active on isolated mitochondria in vitro and also at higher ones. Figure 1 illustrates a cultured myoblast from a control culture treated with plain culture medium. This treatment did not produce any morphological effect and the cells were just as viable as the ones which received no treatment; mitochondria were mainly rod-like and conformed to the classical description.

Figure 2 shows a Ca^{++} ($CaCl_2$ 10 μM) treated cell; this agent caused nearly all the mitochondria to swell and change into spherical structures of different size and refringence.

Comment. The cells were entirely surrounded by the solution containing the swelling agents and it is highly probable that all of them reached the mitochondria in the cytoplasm. The compound tested were all of natural occurrence in cellular and extra-cellular compartments of the animal body and it seems unlikely that the negative results were due to a lack of penetration of the agents in the cells. This assumption is supported by the fact that the same cells were permeable to the foreign compound fluoroacetate which caused in them the same swelling effect on mitochondria as in hanging-drop cultures. Thus it is probable that the majority of the agents tested are devoided of swelling properties in vivo, at least in the type of cells used. With regard to thyroid hormones, it cannot be excluded that the mitochondria were not sensitive to them because of the early embryonic stage of the tissue from which the cells were cultured.

The swelling caused by Ca^{++} resembled that produced by the cation on isolated mitochondria; the round shape of the swollen mitochondria could be due to damage of the mitochondrial membranes. Recent work from this Laboratory has shown a specific concentration of injected $^{45}Ca^{++}$ in the mitochondria of the liver and other tissues of the rat[7]: our results seem to indicate that a similar concentration of Ca^{++} in mitochondria may occur also in cultured embryonic myoblast[8].

Riassunto. E stato provato l'effetto sui mitocondri in vivo dei seguenti composti che producono rigonfiamento dei mitocondri isolati in vitro: L-cistina, L-cisteina, glutatione, L-ascorbato, L-tiroxina, L-3, 5, 3'-triiodotironina, fosfato e Ca^{++}. Solo il Ca^{++} ha provocato un aumento di volume dei mitocondri nelle cellule provate, i mioblasti coltivati in vitro.

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Virogenic Lines of RSV-Transformed Rat Cells

The permanent presence of the genome of Rous sarcoma virus (RSV) in rat cells transformed by the Schmidt-Ruppin and Prague strains of this virus (SR-RSV and PR-RSV) has already been described[1]. All clones so far tested and obtained at a low plating efficiency (PE) contained the viral genome[2].

In the present paper a detailed clonal analysis of a population of rat cells, line B-mix[3] transformed by PR-RSV in vitro is given. This analysis was made under conditions ensuring monocellular origin of clones, high PE, and preventing reinfection of cells by virus. Cells were cultivated in PM_4 medium[4]. Eagle's basal medium supplemented with 10% tryptose phosphate broth (Difco), 8% calf serum and 2% fetal calf serum was used for cloning. Five clones were obtained by the microdrop technique under paraffin oil (experiment 1) and 5 by PUCK's method (experiment 2)[5]. As is shown in the Table, live cells of the clones obtained by both methods gave rise to typical Rous sarcomas after transfer to chickens, which indicates that after association with chicken cells in vivo, infectious RSV is produced[6]. Sonicated cells and culture fluid were inactive. Cells of all tested clones implanted into newborn rats gave rise to tumour growth.

In view of a 100% PE of cells in experiment 2, the 5 clones tested may be regarded as sufficiently representative of the population. Inspite of the finding that all tested clones were virogenic, there remains, as in every clonal experiment, the possibility that in the B-mix population cells are present in which the RSV genome is not activated by transfer into a chicken cell.

The permanent presence of the genome of RSV in the cell population and in all clones could not be attributed to reinfection of cells by RSV, since this virus was neither produced nor released in detectable quantities in lines of rat cells transformed by SR-RSV or PR-RSV[3,6]. In spite of this the next experiment (No. 3) was performed with a culture grown and cloned in the presence of rabbit antiserum against PR-RSV prepared by the method described earlier[7]. The results of tests with 5 clones obtained in this way were consistent with results of preceeding cloning experiments.

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