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.

F. PARADISI[9]

Istituto di Patologia Generale della Università di Modena (Italy), 19th April 1967.

[7] E. CARAFOLI, J. gen. Physiol., in press.

[8] Acknowledgment: the skilled technical assistance of Miss MADDALENA FRUGGERI is gratefully acknowledged. This work was supported by the Italian Consiglio Nazionale delle Ricerche.

[9] Present address: Clinica Malattie Infettive, Università di Napoli (Ospedale Gesù e Maria), Napoli (Italy).

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.

[1] J. SVOBODA, Nature *186*, 980 (1960); J. SVOBODA, Folia biol., Praha 7, 46 (1961); C. G. AHLSTÖM and N. JONSSON, Acta path. microbiol. scand. *54*, 145 (1962).

[2] D. ŠIMKOVIČ, J. SVOBODA and N. VALENTOVA, Folia biol., Praha *9*, 82 (1963); J. SVOBODA, P. VESELY, M. VRBA and L. JIRÁNEK, Folia biol., Praha *11*, 251 (1965).

[3] J. SVOBODA and P. CHYLE, Folia biol., Praha *9*, 329 (1963).

[4] P. VESELY and J. SVOBODA, Folia biol., Praha *11*, 78 (1965).

[5] R. G. HAM and T. T. PUCK, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1962). Vol. 5, p. 90.

[6] J. SVOBODA, Folia biol., Praha *8*, 215 (1962); J. SVOBODA, Natn. Cancer Inst. Monogr. *17*, 277 (1964); D. ŠIMKOVIČ, Natn. Cancer Inst. Monogr. *17*, 351 (1964).

[7] M. RYCHLIKOVA and J. SVOBODA, Virology *10*, 545 (1960).

The stability of the morphotype of the transformed cells was also studied. As shown in Figure 1, a colony of transformed cells contained round basophilic cells with a marked tendency to grow in clumps. In contrast, a stabilized line of rat fibroblasts LWF-1 [8] formed colonies of typical fibroblastic cells (Figure 2). This line was selected for comparison because its culture history resembled that of B-mix cells, except that it had not been exposed to RSV.

Under the given experimental conditions, B-mix cells and LWF-1 cells had PEs of 90–100% and of 70–80% respectively. These percentages remained the same when the cells were seeded together on Petri dishes. Such reconstruction experiments showed that transformed cells did not inhibit the formation of colonies of non-transformed cells.

Two thousand colonies of B-mix cells obtained at a high PE were screened, and no typical fibroblastic colonies were found. It follows that spontaneous revertants which have lost the morphological characteristics of RSV-transformed cells were not detectable by the present tests, and if they occur at all, their incidence must be less than 10^{-3}.

Testing of clones isolated from rat cells B-mix transformed by RSV in vitro

Experiment No.	Designation of clone	Injection of 3.10^6 live cells into chicks	Injection of 3.10^6 sonicated cells into chicks	Injection of cell-free tissue culture fluid into chicks	Injection of live cells into rats
1.	K_{10}	2/5[a]	0/5	0/5	4/4[c]
	K_{13}	2/5	0/5	0/5	4/4
	K_{14}	1/5	0/5	0/5	4/4
	K_{15}	3/5	0/4	0/5	4/4
	K_{16}	3/5	0/5	0/5	4/4
2.	K_{30}	1/4	0/5	0/5	4/4[d]
	K_{31}	1/13 1/2[b]	0/5 0/3[b]	0/5	3/3
	K_{32}	2/5	0/5	0/5	4/4
	K_{33}	1/5	0/5	0/5	4/4
	K_{34}	2/5	0/5	0/5	4/4
3.	K_{54}	1/5	0/4	0/4	4/4[c]
	K_{55}	2/5	0/5	0/4	4/4
	K_{56}	1/5	0/5	0/4	8/8
	K_{57}	5/15	0/5 0/6[b]	0/5	4/4
	K_{58}	1/5	0/5	0/4	4/4

[a] Numerator: No. of animals with tumour. Denominator: total No. of inoculated animals. [b] 10^7 cells injected. [c] 10^4 cells/animal. [d] 10^3 cells/animal. Clones K_{10}–K_{16} were obtained by the microdrop technique under paraffin oil. Six of 20 drops gave rise to colonies, of which 5 were tested. Clones K_{30-34} were isolated according to [5] from colonies growing on a 50 mm Petri dish after seeding of 100 cells. The plating efficiency in this experiment was 100%. Clones K_{54-58} were obtained by the same method, with 10% rabbit anti-PR-RSV serum added to the medium. Cells used for cloning had been seeded in an amount of 10^3 in the medium with antiserum into a Legroux flask, where they grew for 4 days. The antiserum used caused a 100-fold reduction of the pock-forming activity of RSV. Ten-day-old White Leghorn chickens sensitive to infection by RSV and 1 to 4-day-old outbred Wistar rats were used in the experiments. A suspension of live cells in 0.5 ml of medium and 0.5 ml of culture fluid centrifuged for 30 min at 2000 *g* was administered i.m. to the chickens. Sonicated cells were administered in the same amount of citrate buffer. Cells were sonicated in an ice bath for 1 min on an MSE Mullard ultrasonic disintegrator at ultrasonic frequency 20 Kc/sec. Rats were injected s.c. with 0.1 ml cells suspended in culture fluid.

These results differ from recently published data [9] which show the formation of revertants early after transformation of a stable line of hamster BHK cells by RSV. There are however, several significant differences between both experimental set-ups, especially in that B-mix cells were obtained by transformation of a primary culture of rat fibroblasts and the appearance of revertants was studied after long-term cultivation in vitro.

The results presented show that RSV-transformed rat cells cultivated for long periods are virogenic and permanently retain the viral genome, which is regularly

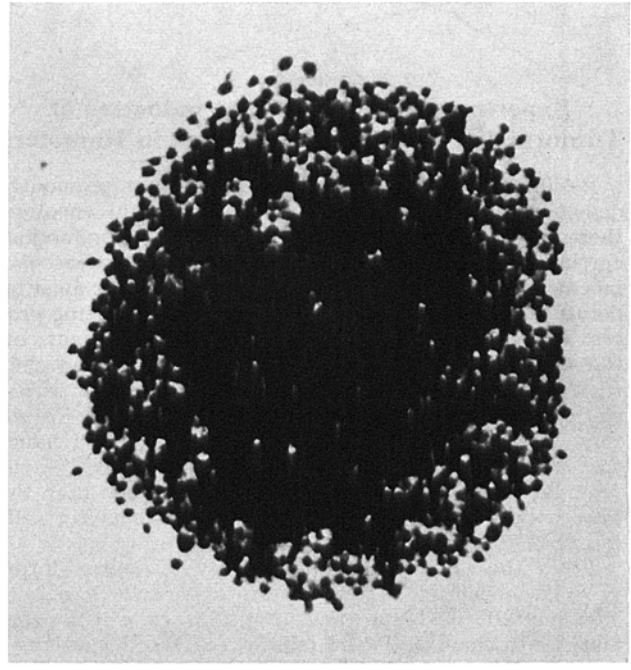

Fig. 1. Typical colony of basophilic RSV-transformed rat cells B-mix. Haem.-eosin stain. × 120.

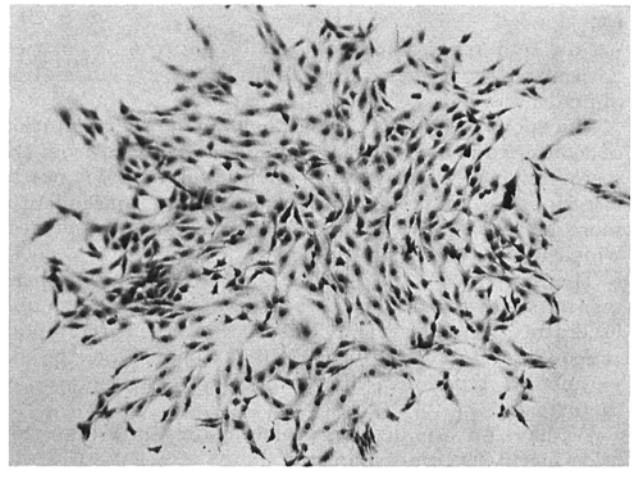

Fig. 2. Colony of control fibroblastic rat cells LWF-1. Haem.-eosin stain. × 120.

[8] P. Vesely, in preparation.

[9] I. Macpherson, Science *148*, 1731 (1965).

transmitted in the cell population without reinfection. This genome, in view of its stability, is also a suitable cellular marker.

Riassunto. Il virus del sarcoma di Rous (RSV), ceppo Praga, transforma le cellule di ratto mantenute in vitro: le cellule così transformate, dopo selezione clonale contengono il genoma virale, non risultante però da reinfezione. Tutte le cellule dei cloni transformati dal RSV hanno caratteristiche morfologiche di malignità. Inoltre dalle cellule transformate non si riottengono cellule con le caratteristiche morfologiche proprie delle cellule normali. Le cellule di ratto transformate dal RSV sono utili per lo studio dell'interazione virogenica.

J. SVOBODA[10] and P. VESELY

Institute of Experimental Biology and Genetics, Czechoslovak Academy of Sciences, Prague (Czechoslovakia), 20th February 1967.

[10] Present address: Imperial Cancer Research Fund, Division of Experimental Biology and Virology, London N.W.7.

Experimental Model for the Induction of Tumoral Lymph Node Metastases in Hamsters

Besides metastases arising from a tumour previously developed in the same host, in experimental oncology there are also so-called metastases with the tumours occurring in various organs following the intravascular inoculation of tumoral triturates[1-8]. However, it must be pointed out that in this latter case the metastasizing process is only partially reproduced in the experiment: on the one hand, because the intravasculary injected cells that are retained by the capillaries of various parenchymas do not detach themselves spontaneously from a tumour of the respective animal, and on the other hand, the spontaneous emission of neoplastic cells from a tumour takes place on much more complex bases than the purely mechanical phenomena occurring when free cells (monerocytes[8]) are obtained by trituration.

From the immunological point of view, also, intravascular inoculation of homologous cancer cells represents a homograft, while in metastases the cell which penetrates into the vessels and migrates is a cell previously developed for many generations in the host's tumour: hence an autologous graft.

In other words, part of the resistance which a primary tumour homograft has to overcome before beginning to proliferate, no longer exists for the metastasizing tumour cells.

This represents an important difference as compared with homologous metastases obtained by i.v. inoculation of neoplastic cells.

The spontaneous detaching phenomena and migration of mother tumour cells thus cannot be excluded from the metastasizing experimental model, if the model is not to lose an essential compound of itself and consequently more or less of its similarity with the natural process which it tries to reproduce.

Therefore, we have endeavoured to perform experimental metastasizing models for various tumours and laboratory animals, where this process is entirely spontaneous, and at a frequency which would allow the experimental study of the pathogeny and therapy of metastases.

We have already described such models for Walker 256 and Guérin T8 tumours in rat[9-12].

In the present paper we present the results obtained with the hamster H 10 sarcoma[13].

Material and methods. We used 60 hamsters (*Mesocricetus aureus*), from our own Institute animal colony. The tumour is a spontaneous hamster sarcoma maintained by serial s.c. passages in the Oncological Institute since 1960. When grafted s.c. it yields no metastases but grows inside a capsule within 20–30 days, up to sizes sometimes equal to that of the host animal. In our attempts to obtain the metastases, we inoculated strictly intratesticular 0.1–0.3 ml H 10 broiate in saline. The inoculations were done bilaterally or unilaterally with a 16–18 gauge needle.

Results. Seven of the 60 injected hamsters died without being examined. Of the remaining 53 (17 bilaterally, 36 unilaterally injected), 52 (98%) presented testicular tumours up to the size of a dry nut, as well as individualized ganglionic paraaortic and pararenal metastases of varying sizes (bean, cherry or even a nut).

The unilaterally injected animals developed metastases in an average period of 30 days. The bilaterally inoculated animals developed symmetrically localized metastases in 15–20 days. In one animal metastases were also found in the lung and, in another, in the liver.

Due to the high percentage of metastases and their rather rapid growth rate, we consider this model of ganglionic tumoral metastases in hamsters as being useful in the experimental study on the pathogeny, prophylaxis or therapy of the tumoral metastasizing.

Résumé. Le travail décrit une technique expérimentale qui permet d'obtenir de grosses métastases ganglionnaires chez le hamster, après l'inoculation intratesticulaire de la tumeur H 10. Ce modèle peut être utilisé dans diverses études pathogéniques et thérapeutiques des métastases.

A. RIVENZON and V. COMISEL

Oncological Institute, Bucharest (Rumania), 17th March 1967.

[1] D. AGOSTINO and E. E. CLIFFTON, Tumori *2*, 89 (1964).
[2] O. COSTACHEL, Oncologia si Radiologia *5*, 467 (1963).
[3] O. COSTACHEL and I. POPP, in *Hommage to Ion Enescu* (Ed. R. ALINESCU; R.S.R. Acad., Bucharest 1966).
[4] O. COSTACHEL and I. POPP, Nowotwory *14*, 89 (1964).
[5] A. ENGESET, Cancer Res. *19*, 277 (1959).
[6] H. S. N. GREENE, Cancer Res. *24*, 799 (1964).
[7] B. KELLNER, Therapia hung. *3*, 105 (1964).
[8] A. C. WALLACE and N. E. HOLLENBERG, Br. J. Cancer *19*, 338 (1964).
[9] O. COSTACHEL, A. RIVENZON and V. COMISEL, Oncologia, Basel *19*, 273 (1965).
[10] A. RIVENZON and V. COMISEL, Oncologia si Radiologia *5*, 243 (1966).
[11] A. RIVENZON, C. r. Séanc. Soc. Biol. *155*, 65 (1961).
[12] A. RIVENZON, N. VOICULETZ and T. POPP, C. r. Séanc. Soc. Biol. *156*, 279 (1962).
[13] I. POPP and T. PREDETEANU, Neoplasma *8*, 363 (1960).