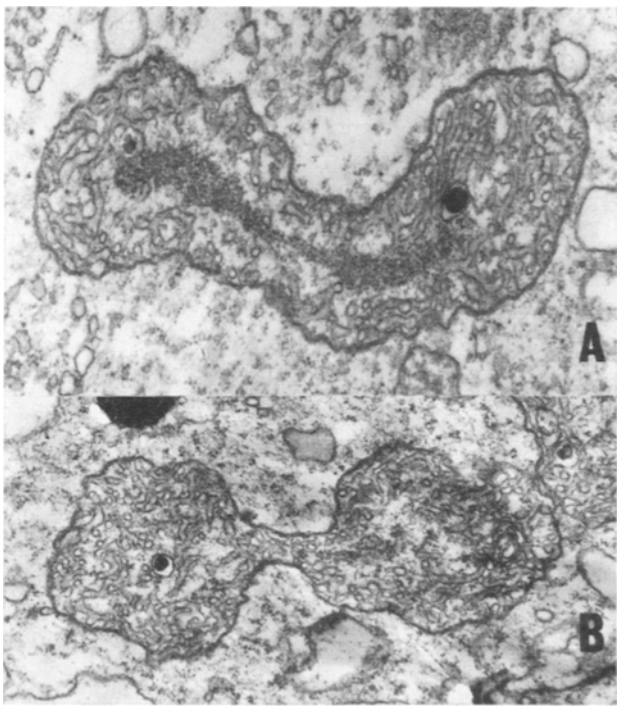

Fig. 4. Mitochondria from plasmodium treated with actinomycin. (A) Elongated mitochondrion showing delayed division of nucleoid. × 13,000. (B) Mitochondrion with narrow constriction, probably similar to that in Figure 1B. Nucleoid understained. × 17,400.

narrower constriction in the middle. They may be similar to the type shown in Figure 1B. In these mitochondria also, division of the nucleoid appears to be inhibited. Incomplete division of the nucleoid in mitochondria that had a narrow constriction in the middle was not found in untreated plasmodia.

The finding that the mitochondria of *P. polycephalum* are rich in DNA[16,17] and that they appear to have a regular division cycle involving division of a nucleoid would suggest the possibility that, during evolution, they might have retained a comparatively high level of autonomy.

The above-described division stages were not correlated with a specific stage of the mitotic cycle of the plasmodia.

Zusammenfassung. Die Form der Mitochondrien und das Verhalten des Mitochondrien-Nukleoids im Schleimpilz *Physarum polycephalum* werden durch Behandlung mit Actinomycin C in einer Weise modifiziert, die darauf schliessen lässt, dass die Mitochondrien einen regelmässigen Teilungszyklus durchlaufen, der mit der Teilung des Nukleoids korreliert ist.

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Inactivation of Tissue-Specific Inhibitors by a Carcinogen (Diethylnitrosamine)

The deletion of tissue-specific growth-regulating proteins by carcinogens has been postulated to be of primary importance during carcinogenesis[1]. Carcinogens become bound to specific proteins (h-proteins)[2–5], which are eliminated during carcinogenesis[3,5,6]. A direct inactivation of growth-regulating factors could indeed lead to neoplastic growth, provided that the deletion becomes hereditary; a model to explain such hereditary changes has been proposed[7]. Purified fractions of h-proteins were shown to inhibit reversibly cell growth in tissue-culture[8]; however it was found that liver arginase is responsible for most of the inhibition[9].

Starting from the study of wound healing in mouse skin, BULLOUGH developed the concept of tissue-specific mitosis inhibitors ('chalones') acting together with hormones, which are lacking or reduced in tumours[10].

In the following experiments we studied the effect of liver supernatants from adult rats (1:4 homogenate; S_3: 78,000 g; 2.5 h) containing carcinogen-binding h-proteins on liver explants. Despite some drawbacks (time consumption, non-uniform size, diffusion rate, internal necrosis) these explants offer a better test system for potential liver specific substances than HeLa or L-cells[8] or ascites cells[11]. Some tissue culture cells and kidney explants were used for comparison. Diethylnitrosamine (DENA) was used as a fast reacting carcinogen (10 mg/kg i.v.). Supernatants listed in experiments 1–3 in Table II and in Table I were partially purified as suggested by BULLOUGH using 50–80% ethanol precipitates[10].

Livers from new-born rats were cut into 1 mm pieces, incubated as described under Table I and processed as described elsewhere[12]. Kidney explants were similarly handled. Tissue culture cells were grown as monolayers in flasks in their proper media, distributed into roller tubes ($2.5–3.5 \times 10^5$ cells/ml and tube) and incubated overnight. The medium was removed and 0.9 ml TCM-199 and 0.1 ml supernatant were added. The monolayers were incubated for 3 h, then 1 μC ^{3}H-thymidine was added. The incorporation was stopped after 1 or 2 h by cooling and adding 0.1 ml 1.0*N* NaOH. Controls without cells were run alongside. The monolayers were broken by stirring and ultrasonicating. TCA-soluble material was

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extracted from filter paper disks as described earlier[13].

Additions of supernatants (as described above) from normal liver significantly inhibited DNA synthesis in liver explants as measured by ^{3}H-thymidine incorporation into TCA-insoluble material (Table I B and Table II B).

However, not only liver explants were inhibited; kidney explants and tissue culture cells as Walker-carcinoma (Figure), HeLa cells, amelanotic melanoma and L-cells were inhibited as well.

If supernatants from DENA-treated rats (4–6 h after injection) were used, liver explants and tissue culture cells (as well as kidney explants) behaved differently. The inhibition of the used tissue culture cells was maintained, or even slightly higher (Figure). The same was found for the kidney explants. However, the depression of DNA synthesis in liver explants was significantly reduced (Table I C, II C).

Liver supernatants obviously contain factors which inhibit DNA synthesis in liver explants and which can be inactivated by DENA. In addition, liver supernatants contain substances which depress DNA synthesis in kidney explants and in tissue culture cells, but which are not influenced by the DENA. Obviously these 'unspecific' factors are not acting on liver explants, otherwise the inhibitory action of liver supernatants on liver explants could not be fully restored by the DENA treatment (Table I C, II C). A possible inactivation of arginase by DENA appears to be improbable because of the unchanged effect on tissue culture cells. In all experiments using extracts from livers of animals which had been injected with DENA 16 h prior to killing, lost inhibitory ability was restored (Table II D). This might indicate that the growth-inhibiting factors temporarily inactivated by DENA are rapidly replaced.

In contrast to BULLOUGH's findings[10], in our systems no hormones were necessary. This difference might indicate that 'chalones' acting on DNA synthesis and 'chalones' acting on mitosis, as studied by BULLOUGH[10] react differently.

TERAYAMA and SASADA found a similar, reduced inhibition of carcinogen-treated rat liver[14]. However, the use of ascites cells makes these studies difficult to compare with our results. It is obviously open to discussion if these reduced 'growth-regulating' capacity of carcinogen-treated liver cells has something to do with carcinogenesis.

Table I. Effects of liver supernatants on DNA synthesis of liver explants (experiment 1 of Table II)

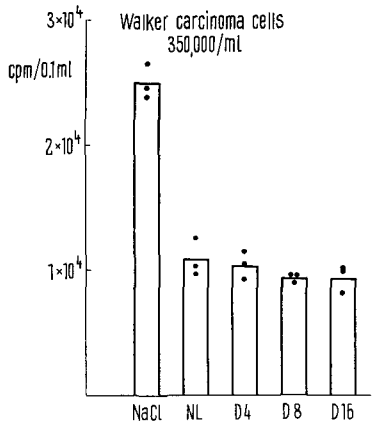
A	B	C
Control (NaCl)	Supernatant[a] normal liver	Supernatant[a] DENA liver (6 h after injection)
814	503	678
759	401	796
721	705	952
609	576	807
804	675	–
Mean 741	572	808
% 100	77	118

Liver explants prepared as described in the text were incubated for 2 h with supernatant preparations (10 mg). 2.5 μC ^{3}H-thymidine was added and after 2 h the tubes were placed into ice, the explants washed with cold saline, homogenized with 0.5 ml 0.15 *N* NaCl and aliquots placed on filter paper disks. TCA-soluble material was extracted as described earlier[12,13]. The values given (counts/min · 15 γ protein; $\alpha < 0.05$, F-distribution). [a] 50–80% ethanol precipitate.

Table II. Effect of liver supernatants on DNA synthesis of liver explants (summary)

A	B	C	D
Experiment	Supernatant normal liver	Supernatant DENA liver (4–6 h after injection)	Supernatant DENA liver (16–24 h after injection)
1	77	118	84
2	75	107	87
3	55	95	76
4	77	103	62
5	53	100	47

Listed are different experiments similar to that given in Table I. Experiments 1–3 refer to ethanol fractions, 4–5 to non-purified supernatants (0.1 ml/tube). The values given are percentages of ^{3}H-thymidine incorporation without supernatants (NaCl-control).



Effect of liver supernatants on DNA synthesis of Walker carcinoma cells in vitro. Walker cells were incubated and processed as described in the text. Values given represent 1 tube (mean of 3 determinations). NL, normal liver; $D_{4, 8, 16}$, DENA-treated liver 4, 8, 16 h after injection.

Zusammenfassung. Der Einbau von Thymidin in Leberexplantate wird durch lösliche Leberproteine gehemmt. Diese Hemmung ist aufgehoben, wenn die Proteine aus Lebern karzinogenbehandelter Tiere präpariert wurden. Dieser Effekt ist organspezifisch.

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