^{32}P exchange in fibre bundles during isometric contraction (A) and during application of rectangular quick stretches (B)

Experiment No.	A Isometric contraction	B Stretch	B/A
1	0.94	1.53	1.63
	0.80	1.47	1.84
2	1.08	1.94	1.79
	0.63	–	–
3	0.36	0.64	1.78
	–	0.38	–
4	0.31	0.52	1.71
	0.32	0.72	2.25
5	0.37	1.35	3.65
	0.32	1.70	5.32

ATP-^{32}P in $^0/_{00}$ of inorganic ^{32}P. For additional data see text.

Zusammenfassung. Wiederholte Dehnung von glycerinierten Fasern aus Insektenflugmuskeln erhöht die Rate des ^{32}P-Austausches zwischen P_i und ATP. Offenbar bildet sich eine energiereiche Zwischenverbindung im kontraktilen Protein (ADP-Myosin).

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Cocarcinogenic Croton Oil Factor A_1 Stimulates Lipid Synthesis in Cell Cultures

Croton oil factor A_1 (12-*O*-Tetradecanoyl-phorbol-13-acetate = TPA)[1,2] was found to influence protein and nucleic acid metabolism in vivo[3,4] and in vitro[5]. Effects on lysosomes and on mitochondria in cell cultures have been reported[6,7] and recently localization of TPA in plasma membranes by autoradiography was claimed[8]. These effects on cell organelles suggest that membranes may be involved in the biochemical mechanisms at action of TPA. Also certain physico-chemical properties of this compound indicate that direct interactions with cell membranes might be possible. Therefore we studied the incorporation of radioactive choline into lecithin, known to be incorporated into membranes.

HeLa- and L-cells were distributed in 1 ml portions into roller tubes and rolled overnight (12 rph) (400,000 cells/ml; HeLa-medium: 80% Gey's solution + 10% lactalbumine (2,5%) + 10% calf-serum; L-cell medium: 90% TCM-199 + 10% calf serum). The culture medium was replaced by TCM-199 containing 0.5% dimethyl-sulfoxide (DMSO) and TPA in the final concentrations indicated. After 5 h of incubation, ^3H-labeled choline (specific activity 250 mC/m*M*; Amersham/England) was added (2 μC/tube) and incubation continued for 1 additional h. The incorporation was stopped by removing the medium and cooling the roller tubes in an ice bath. A cell lysate was prepared by adding 1 ml 0.2% sodium dodecylsulfate solution per tube, shaking on a mechanical vibrator and incubation for 20 min at 37 °C. 0.1 ml aliquots were placed on filter paper disks. To process such disks a modified MANS-NOVELLI-procedure[9] was used: 3 extractions with cold 5% TCA removed non-incorporated acid soluble choline, residual TCA was removed by pressing the disks on filter paper. In later experiments, residual TCA was removed by an additional extraction with 5% cold acetic acid followed by drying at 60 °C.

As a control the same cell lysates were processed by extraction with chloroform/methanol (3/1)[10] to obtain the lipid material. From the latter, non-incorporated choline was extracted with 1 *N* H_2SO_4. The radioactivities remaining in the organic phases are practically identical with filter paper values obtained from corresponding lysates as described above. More than 95% of the radioactivity was extractable from the paper disks with chloroform/methanol. This comparison indicates that TCA-insoluble

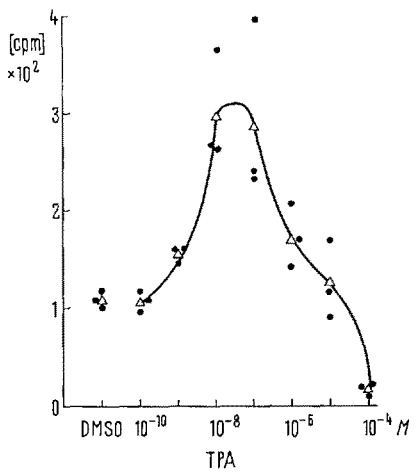

Fig. 1. Croton oil factor TPA dependent incorporation of ^3H-choline into TCA-insoluble material of HeLa-cells. For each concentration of TPA 3 tubes have been incubated for 6 h, worked up and measured (●). The average value from 3 tubes is indicated (▲).

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choline-containing membrane constituents show a behaviour similar to chloroform extractable material. Lecithin was the only radioactive compound found in thin-layer chromatography (chloroform/methanol/water 65:25:4) of the organic phases.

Figure 1 shows the incorporation of ^{3}H-choline into TCA-insoluble material of HeLa-cells as a function of the final concentrations of croton oil factor TPA indicated on the abszissa: A dramatic increase was observed beginning at as low as $10^{-9}M$, reaching a 300% maximum at 10^{-8} and finally decreasing to zero at the 'toxic concentration' of $10^{-4}M$. Already 1 h after the addition of TPA to the cell culture, increased incorporation of choline was observed. A much less conspicious stimulation of lipid synthesis was observed in L-cells, with a maximum at $10^{-7}M$ TPA (Figure 2). A concentration of $10^{-8}M$ TPA corresponds to the extremely small amount of only 0.6×10^{-2} µg per ml. A stimulation of lipid synthesis by Tween 80 has been reported earlier[11], however Tween was given to Ehrlich ascites cells at comparatively high dose levels ($3\times10^{-4}M$).

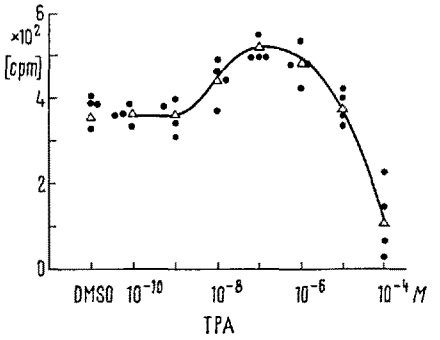

Fig. 2. Croton oil factor TPA dependent incorporation of ^{3}H-choline into TCA-insoluble material of L-cells incubated for 6 h. For further explanation see legend of Figure 1.

The data presented suggest that TPA stimulates membrane metabolism, perhaps by direct interaction with membrane constituents. It would be premature to draw any conclusion as to the relationship of this membrane effect with the cocarcinogenic potency of this compound. However, it appears reasonable to assume that membrane changes lead to a changed tissue regulation[12], which in turn would explain the hyperplasiogenic effect of the croton oil factor TPA. Thus, the stimulation of choline incorporation observed deserves more detailed studies on different cell types using other cocarcinogenic and non-cocarcinogenic derivatives of the phorbol series.

Zusammenfassung. Der tumor-promovierende Crotonölfaktor 12-*O*-Tetradecanoyl-phorbol-13-acetat (TPA, früher A_1) stimuliert den Einbau radioaktiven Cholins in HeLa-Zellen bis zu 300% bei $10^{-8}M$ Endkonzentration (0,006 µg/ml). Der Einbau wurde mit einer neuen Variante der Papierfiltermethode (MANS-NOVELLI[9]) gemessen.

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Effect of Succinate Administered in Combination with Progesterone Chlorpromazine and Chloramphenicol on the Stability of Liver Lysosomes of Rats Fed on Different Diets

Recently it was found that succinate (ST) protects rat liver lysosomes from the injurious effect of chlorpromazine (CPZ)[1]. The results presented in this paper indicate that CPZ administered to rats fed on diets with a great amount of yeast (20%) slightly labilizes lysosomes while ST strengthened this effect. The data obtained suggest that the intake of a great amount of glutathione (G-SH + GSSG) from the yeast is the most probable cause for the inversion of the action of ST on lysosomes. The in vivo effect of ST in combination with one of agents inhibiting mainly the oxidation of $NADH_2$ (CPZ, progesterone[2] and chloramphenicol[3]) on liver lysosomes of rats fed on different diets was studied.

Male and female Wistar rats of 120–150 g body weight were used, divided into 7 groups and fed on diets given in Table I in the course of 5–6 days prior to the experiments. After 12 h of starvation, the rats were treated as shown in Table II, and kept at temperature of 18–20 °C. 5 h after the first treatment they were killed by decapitation. Livers were rapidly removed and cooled in an ice-cold isotonic sucrose solution. Preparation of homogenates and their centrifugal fractionation were done as described earlier[1]. The rate of release of acid phosphatase from the granular fractions was used as criterion for the lysosome membrate stability (experimental conditions are given in the text for the Figure).

The results from the experiments on rats included in group I and II (Table I) and treated with CPZ and CPZ + ST are given in the Figure A. CPZ administered to rats from group I at a rate of 1 mg/100 g body weight, induced a significant labilization of lysosomes. The administration of CPZ in the same doses to rats from group II, did not cause any well-expressed labilization of these particles. In this case ST strengthened the lysosome labilizing action of CPZ. It is interesting that ST applied to rats from group II, in combination with other agents inhibiting mainly the oxidation of NADH, also led to a labilization of lysosomes (Figure B).

The fact that CPZ applied to rats fed on diet rich in yeast caused much slighter damage to lysosomes than in the case of rats fed on ordinary diet was not suprizing.

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