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 ($3 \times 10^{-4}M$).

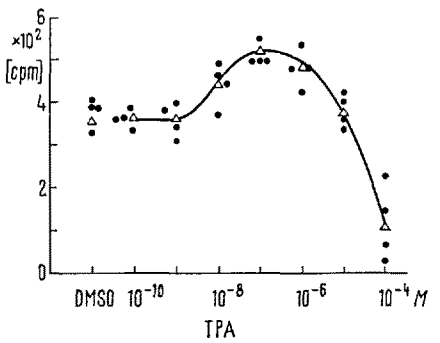

Fig. 2. Croton oil factor TPA dependent incorporation of ^{3}H-choline into TCA-insoluble material of L-cells incubated for 6 h. For further explanation see legend of Figure 1.

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.

R. Süss, V. Kinzel and G. Kreibich

Deutsches Krebsforschungszentrum, Institut für Experimentelle Pathologie, Berliner Strasse 29, D-69 Heidelberg (Germany), and Deutsches Krebsforschungszentrum, Biochemisches Institut, D-69 Heidelberg (Germany),
7 August 1970.

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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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Table I. Diets of different group of experimental rats

Compounds (%)	I	II	III	IV	V	VI	VII
Casein[a]	14	14	14	14	14	14	14
Corn starch	35	21	35	35	35	35	35
Sucrose	35	35	35	35	35	35	35
Dried Baker's yeast	6	20	6	6	6	6	6
Olive oils	6	6	6	6	6	6	6
Mineral mixture[6]	4	4	4	4	4	4	4
Glutathione (SH)[b]	—	—	4	5	—	—	—
Glutathione (SS)[b]	—	—	1	—	—	—	—
L-Cysteine hydrochlor.[b]	—	—	—	—	—	—	30–50
L-Cystine[b]	—	—	—	—	2–3	10	—
Vitamins A, D, E and K in adequate quantity	+	+	+	+	+	+	+

[a] According to HAMMARSTEN. [b] mg/100 g body weight per day.

It has been found that at least one factor contained in considerable amount in yeast (riboflavin) protects lysosomes from the injurious action of CPZ[4]. In this case the inversion of the effect of succinate is of interest. Recently it was shown that the lysosome labilizing action of CPZ applied in combination with ST + malonate is much higher[1]. On the basis of this fact it might be suggested that in the case of serious disturbances in the functioning of the oxidizing pathways (both the NADH oxidizing and the ST oxidizing ones), ST may cause a labilization of lysosomes by certain indirect mechanism as yet unknown. When rats were fed on a diet rich in yeast, probably an inhibition of succindehydrogenase

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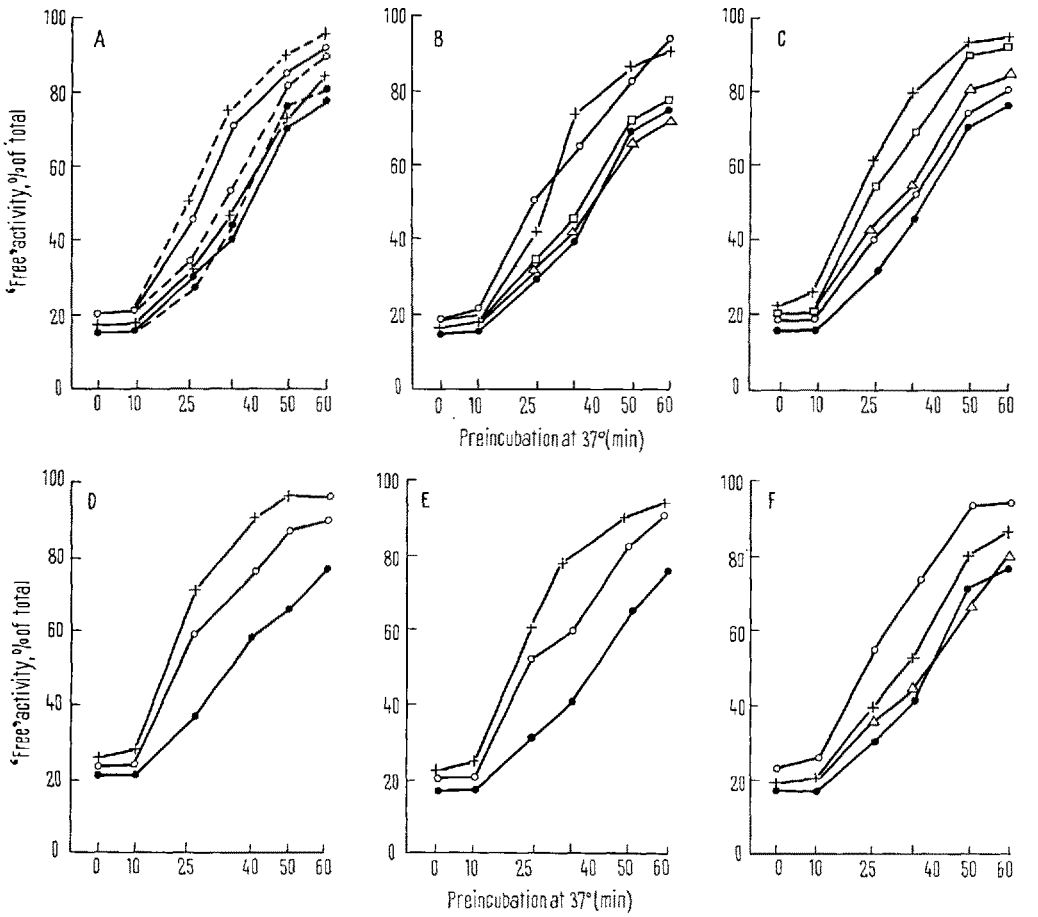

Release of acid phosphatase from large granule fractions isolated from liver homogenates of control and treated rats.

Amounts of the granular fractions (sedimented between 1500 and 20,000 *g* and washed once) used for determining the enzyme activity were preincubated for 0, 10, 25, 35, 50 and 60 min in a medium containing 0.25 *M* sucrose and 0.1 *M* acetate buffer with pH 5, at 37 °C. Substrate (Na β-glycerophosphate) ensuring a concentration of 0.05 *M* was added at the end of the period of time indicated for preincubation after which the incubation continued for 10 min. The 'free' activity is expressed in percentages to the total one determined in the presence of Triton X-100 (0.1% v/v). The method of GIANETTO and DE DUVE was used for determining the activity of the acid phosphatase[7].

Fig. A. —, rats from group I (Table I); – – –, rats from group II; ●—●, controls (6); ○—○, treated with CPZ (10); +—+, treated with CPZ + ST (11); ·– – –·, controls (4), ○– – –○, treated with CPZ (7); +– – –+, treated with CPZ + ST (7).

Fig. B. Rats from group II. ·—·, controls (5) and treated with chloramphenicol (5); △—△, treated with ST (5); □—□, treated with progesterone (6); ○—○, treated with progesterone + ST (6); +—+, treated with chloramphenicol + ST (6).

Fig. C. Rats from group III. ·—·, controls (6); △—△, treated with CPZ (6); +—+, treated with CPZ + ST (5); rats from group IV; ·—·, controls (6); ○—○, treated with CPZ (5); □—□, treated with CPZ + ST (6).

Fig. D. Rats from group VII. ·—·, controls (5); ○—○, treated with CPZ (7); +—+, treated with CPZ + ST (7).

Fig. E. Rats from group V. ·—·, controls (5); ○—○, treated with CPZ (6); +—+, treated with CPZ + ST (6).

Fig. F. Rats from group VI. +—+, controls (4), ○—○, treated with CPZ (6); △—△, treated with CPZ + ST (4); ·—·, controls from group I (6).

Number of experiments are given in brackets.

Table II. Subcutaneous treatment of experimental rats with various substances

Substances (mg or ml/100 g body weight)	At zero h	After first treatment 1.5 h	3 h
1. Controls	–	–	–
2. Chlorpromazine[a]	1 mg/100 g	–	–
3. Chlorpromazine Succinate[b]	1 mg/100 g 0.2 ml/100 g	– 0.2 ml/100 g	– 0.2 ml/100 g
4. Progesterone[c]	10 mg/100 g	–	–
5. Progesterone Succinate	10 mg/100 g 0.2 ml/100 g	– 0.2 ml/100 g	– 0.2 ml/100 g
6. Chloramphenicol[d]	20 mg/100 g	–	–
7. Chloramphenicol Succinate	20 mg/100 g 0.2 ml/100 g	– 0.2 ml/100 g	– 0.2 ml/100 g

[a] Rats from group III and IV (Table I) are treated with CPZ in doses of 0.2 mg/100 g. [b] 0.4 *M* solution of disodium succinate. [c] Dissolved in 0.1 ml dioxane. [d] Introduced into stomach by stomach tube as aqueous suspension.

occurs and, hence, of ST oxidizing pathway, due to an intake of a comparatively great amount of glutathione (G-SH + GSSG). It seems that both types of glutathione may inhibit succindehydrogenase, GSSG directly, and G-SH after an oxidation[5]. The administration of ST into animals fed on a diet containing high levels of yeast and treated with agents inhibiting mainly the oxidation of NADH, led to a labilization of lysosomes. This effect was not observed when CPZ, progesterone and chloramphenicol were absent. Therefore, only when both oxidizing pathways were inhibited, ST might act as a lysosome labilizer. The results in Figure C indicate that the suggestion concerning the role of glutathione in the inversion of the effect of ST was confirmed. In addition, it was shown that this effect might be attained when other –SH and SS compounds are used (Figure D, E and F). At present, one can hardly say whether these compounds are the only cause for the effect observed, and whether the mechanism suggested is correct. There is no evidence that the presumed metabolic situations really exist in the living cells of experimental rats under the different conditions of nutrition and treatment described in this paper. If it is accepted on the basis of previous[1] and present results that, under the respective experimental conditions, there are disturbances in the functioning of ST and NADH oxiziding pathways, the degree of these disturbances, being undubtedly of great significance with a view to the manifestation of an effect, is fully unknown. The results in Figure C and D indicate that it is easier to reproduce labilization of lysosomes by ST when SH compounds are used. Depending on the dose of cystine, 2 types of action might be observed to be exerted by ST. When the rats received small amounts of cystine, ST in combination with CPZ increased lysosome labilizing action of CPZ, but when ST in combination with CPZ was applied to rats fed on a diet with great amount of cystine, an effect similar to that obtained on rats fed on ordinary diet was observed. To clear up this question, further experiments are necessary.

In this study only 2 batches of dried baker's yeast were used. It is not known, however, if all kinds of yeast would produce the same effect.

Zusammenfassung. Im Gegensatz zu Ratten bei Normaldiät, bei denen Sucinat einen Stabilisierungseffekt auf isolierte Leberlysosomen hat, vergrössert Succinat die Lysosomenpermeabilität bei Ratten mit einer Diät reich an Hefe oder ergänzt mit SH-Komponenten.

Ch. S. Popov

Higher Institute of Veterinary Medicine, Department of Chemistry and Biochemistry, Sofia 13 (Bulgaria), 29 April 1970.

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Renal Pressor Activity and Kidney Weight Responding to Angiotensin in Hypertensive Rats

Although angiotensin is known to cause a strong renal vasoconstriction[1,2], a loss of renal vasoconstrictor activity of angiotensin was observed during renal ischemia[3]. An increase[4,5] or a decrease[6] in juxtaglomerular cell granularity was observed in normal animals injected with angiotensin, but no similar observation was made in animals with experimental renal hypertension. The present study was undertaken to observe the changes in renin content and in granularity of the juxtaglomerular apparatus of an ischemic kidney exposed in vivo to long-acting angiotensin.

Material and methods. 58 female rats of Wistar-King strain weighing approximately 100 g were used. Left renal artery clipping was achieved with and without accompaniment of right nephrectomy, in 17 and 18 rats, respectively. For the respective control, right nephrectomy was done in 14 rats and no nephrectomy in 9 rats. All rats were fed a commercial rat diet (production of Oriental Yeast Manufacturing Co., Japan) and tap water ad libitum. Blood pressure was determined at weekly intervals by tail sphygmography following the surgery. In the sixth week the rats were injected s.c. either with 10 µg of synthetic angiotensin II-asp[1]-β-amide (Hypertensin, CIBA) suspended in 0.2 ml of sesame oil or with 0.2 ml of sesame oil once everyday for 6 days and sacrificed. The 58 rats were thus divided into 7 groups as follows: group I, right nephrectomy + oil (6 rats); group II, right nephrectomy + angiotensin in oil (8 rats); group III, right nephrectomy + left renal artery clipping + oil (9 rats); group IV, right nephrectomy + left renal artery clipping + angiotensin in oil (8 rats); group V, both kidneys untouched + angiotensin in oil (9 rats); group VI, left renal artery clipping + oil (9 rats); group VII, left renal artery clipping + angiotensin in oil (9 rats). When sacrificed, heart and kidney were weighed. 2 coronal sections of the kidney were made through the hilus at a distance of about 1.5 mm and the middle section was processed for the determination of juxtaglomerular granular index (JGI) according to the method of Hartroft and Hartroft[7]. Renal renin content was deter-