Table 1. Mutagenic activity of NG[a] on *Salmonella typhimurium* Strain TA100 in the presence of different tissue homogenates

Tissue or sample preparation	Concentration [mg protein/plate][b]					
	0.0	3.7	7.4	11.1	14.8	18.5
Uninduced rat liver	20000[d]	900[c]	481[c]	364[c]	197[c]	101[c]
Aroclor-induced rat liver	20000[d]	702[c]	201[c]	155[c]	100[c]	22[c]
Phenobarbital-induced rat liver	20000[d]	697[c]	598[c]	69[c]	1[c]	−15[c]
3-Methyl cholanthrene-induced rat liver	20000[d]	478[c]	415[c]	54[c]	23[c]	−10[c]
Aroclor-induced rat liver without NADPH	20000[d]	20000[d]	20000[d]	20000[d]	20000[d]	20000[d]
1S maize-tissue homogenate	20000[d]	20000[d]	20000[d]	20000[d]	20000[d]	20000[d]
9S maize-tissue homogenate	20000[d]	20000[d]	20000[d]	20000[d]	20000[d]	20000[d]
Bovine serum albumin (fraction V)	20000[d]	20000[d]	20000[d]	20000[d]	20000[d]	20000[d]

[a] NG concentration was 294 µg/plate in all tests

[b] Protein concentrations were determined by the methods of Lowry et al. [10]

[c] All data are given as revertants/plate minus spontaneous revertants/plate from a corresponding control plate. A control was run for each sample tested using equivalent amounts of tissue preparation for each separate experiment. All data given as average number of revertants/plate (3 plates/test)

[d] Estimate based upon microscopic field analysis of test plates

Table 2. Mutagenic activity[a] of selected chemicals on *Salmonella typhimurium* Strain TA100 in the presence of Aroclor-induced rat-liver homogenates

Chemical agent (concentration [µg/plate])	S9 [mg protein/plate][b]					
	0.0	3.7	7.4	11.1	14.8	18.5
Water control	103	98	109	118	142	163
Aflatoxin B_1 (50)	97	332	350	427	632	781
2-Anthramine (25)	152	433	926	1306	1037	775
Ethyl methanesulfonate (100)	750	687	782	1004	1102	1046
ENG (294)	2470	1078	886	637	690	491
Streptozotocin (50)	5760	5327	5714	5169	5048	4987
NG (294)	20000	907	421	201	131	97

[a] All data are given as average number of revertants/plate (3 plates/test)

[b] Protein determination were made according to the methods of Lowry et al. [10]

with great caution. In other words, since the mutational activity of a known and powerful carcinogen such as NG can be modified or eliminated by simplistic means in standard mutation assays, it may be premature to accept routinely the results of mutagen-carcinogenic potency correlations. In this connection it has been pointed out that in order to reduce false negatives or false positives in the Ames test, the general protocol should include the routine testing of positive mutagen controls not only in the absence but in the presence of all modifying agents, e.g., S9 and the sample being tested [6, 16].

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Disturbances of Early Sea-Urchin Development by the Tumor Promoter TPA (Phorbol Ester)

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In recent times phorbol esters have been tested not only on mouse skin but also in different cell cultures. Several effects caused by the cocarcinogens were observed in vitro as recently reviewed [1]: phenotypical alterations of cells as though they were transformed by carcinogens but without expressing malignancy; reversible inhibition of terminal differentiation in a variety of cell types; mutagenic activity in most cell types treated with active phorbol esters; activation of latent viruses [2]. As to the molecular processes influenced by the esters in the cells, no detailed mechanisms are known. Disappearance of cell-surface proteins has been reported [3] as well as increased prostaglandin-E_2 production [4] upon treatment of macrophages with active esters.

We wanted to know whether the actions of the active 12-O-tetradecanoyl-phorbol-13-acetate (TPA) are limited to mammalian cells only or whether the substance

also displays similar or other distinct effects on nonmammalian eucaryotic cell types. In addition and for comparison we tested the cocarcinogenic inactive compounds 4-O-methyl-12-O-tetradecanoylphorbol-13-acetate (MeTPA) and 12-O-acetylphorbol-13-acetate (PDA). The early sea-urchin embryo was chosen as an object which can be regarded as a special kind of tissue culture in which sequential differentiation processes are occurring.

Eggs of the species *Sphaerechinus granularis* were fertilized and cultured as described [5]. The substances were presolved in dimethyl-sulfoxide and added to sea water immediately before the experiment (DMSO-sea water 0.05%). The sedimented morulae were incubated in these solutions at a density of 300 individuals/ml medium. In Figure 1a the results of the first experiment are shown. Morulae were continuously incubated in three TPA concentrations until the pluteus stage. Samples were evaluated at different stages. As Figure 1a shows, $1 \cdot 10^{-7}\,M$ TPA not only completely blocked cell cleavage, but even caused the embryos and their cells to lyse. A concentration of $10^{-8}\,M$ TPA did not prevent cell cleavage. However, the rates were reduced and differentiation of the embryos proceeded abnormally resulting in anomalous gastrulae; skeletal development and differentiation of the gut were suppressed. Development was less disturbed under the influence of $10^{-9}\,M$ TPA, with a certain differentiation of the gut being apparent. The larvae also seemed more prism-like, although only a rudimentary skeleton was generated. At a concentration of $10^{-10}\,M$, development essentially resembled that of the control. Lower concentrations tested (down to $10^{-13}\,M$ TPA) showed no effects.

In the second experiment, the actions of MeTPA and PDA were examined using the same procedures as above. In contrast to TPA, $1 \cdot 10^{-6}$ and $1 \cdot 10^{-7}\,M$ MeTPA permitted cell cleavage and lysis was observed only at $10^{-5}\,M$ and higher concentrations. Development even progressed with occurrence of irregular plutei as shown in Figure 1b. Lower concentrations did not show any effects. PDA only caused irregular development in concentrations $>10^{-5}\,M$ (not shown). Thus the noncocarcinogenic phorbol derivatives were at least a hundred times less harmful than TPA.

In a further experiment the embryos were incubated in TPA as described above but for 1 h only; development continued in pure sea water. The evaluation of the samples showed that the embryos were similarly damaged by the same concentrations as under permanent treatment. Whether the 1-h incubation period was sufficient to damage irreversibly the embryos by specific reactions, or whether the substance was still present in the embryos even after several washing steps and thus might have permanently acted as in the first experiment, is still an open question.

According to all results described (these could be verified in four independent series

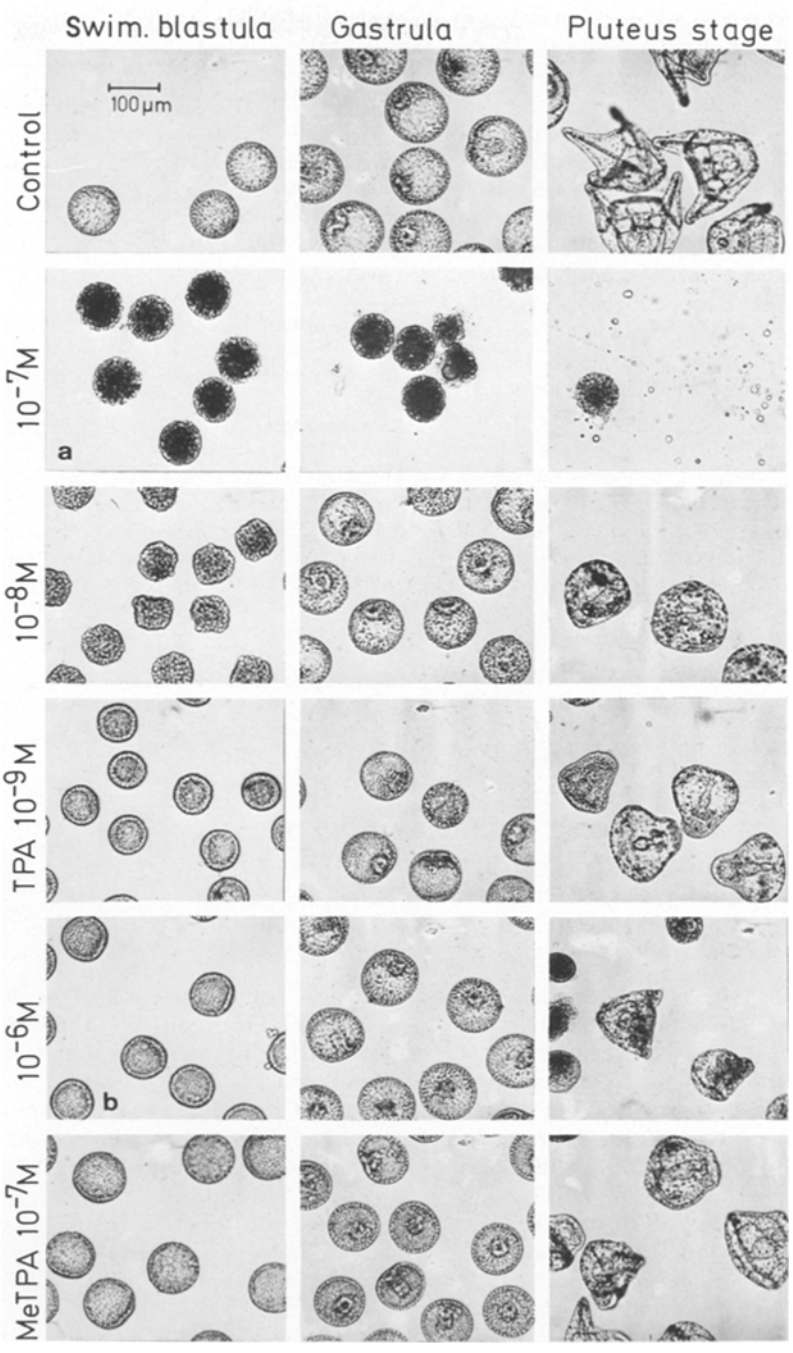

Fig. 1. (a) Continuous incubation of *S. granularis* embryos in TPA. Embryos developed in pure sea water until they reached the early morula stage (6 h after fertilization), when incubation was continued in TPA-sea water. Hatching 11 h after fertilization. Examination: swimming blastula 15 h, gastrula 26 h, pluteus stage 65 h after fertilization. (b) Incubation of the embryos in MeTPA under the same procedure as above

of experiments with gametes from different animals), the most powerful cocarcinogen from the group of the phorbol esters irreversibly influenced development even in nanomolar concentrations. Thus, the sea-urchin embryo reacted at least as sensitively as the mammalian cells tested so far. These cells were unaffected or only reversibly affected by TPA in that concentration range. The action of TPA on the embryo cells must be a specific one as MeTPA and PDA showed a remarkably lower activity, and like many other tested organic and water-insoluble substances, for example alkaloids [6, 7], alcohols [8], anesthetics [6], carcinogens [8, 9], pesticides [5], or phenols [6, 7] acted in the micromolar range but showed no effects in nanomolar concentrations. Since the embryotoxic activity of the three phorbol derivatives tested ran parallel to their cocarcinogenic activity, the hypothesis might be postulated that the primary mechanisms by which the cocarcinogen acts on mammalian cells and on the embryo cells are the same or at least very similar. The sea-urchin embryo, therefore, might represent a useful model for elucidating the molecular action of the phorbol esters in the cells.

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The Liver as a Barrier Against Antigens in Viral Hepatitis Infection

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Present knowledge of the pathogenesis of hepatitis type B indicates that the disease is caused by damage to the hepatocyte following viral infection [1, 2]. Other observations show that the hepatitis type B virus (HBV) may not be damaging the liver cell. Intact hepatocytes overloaded with hepatitis type B surface antigen (HBsAg), the surface component of HBV, have been found in patients under immunosuppression [2, 3]. Electron microscopical studies on the intracellular distribution of HBsAg have shown that the antigen was mainly present in the proliferative degranulated endoplasmic reticulum. Hepatitis type B core antigen (HBcAg), the inner component of HBV, predominated in the nuclei and was occasionally found associated with HBsAg in the cysternae of the endoplasmic reticulum [4, 5]. The degranulated endoplasmic reticulum is the hepatocellular instrument of 'biotransformation' of products of foreign origin or resulting from decay of cellular substances in preparation for their elimination [1, 6]. This may indicate that the hepatitis type B antigen (HBAg) is brought into the liver via the blood stream after multiplying elsewhere. Experimental data were therefore sought on the intrahepatic distribution of HBsAg and HBcAg, and on the infectiousness of liver-cell-associated HBAg by in vitro cultivation of HBAg-positive hepatocytes obtained from experimentally infected chimpanzees.

The distribution of HBAg in the liver. Perimembranous distribution of HBsAg on hepatocytes has been described [5, 7]. HBcAg, on the other hand, is typically associated with the nuclei of the cells [4]. This distribution pattern was confirmed by analysis of cryostatic sections of a human HBAg-positive autopsy liver. Figure 1 shows the distribution of HBsAg and HBcAg as revealed by direct immunofluorescence staining. Two HBsAg distribution patterns were found. Perimembranous (pericellular) HBsAg deposition predominated, while intracytoplasmic distribution was more rarely found. The HBsAg and HBcAg were found in focal areas in the tissue which did not overlap in frozen sections of the liver simultaneously incubated with both antibodies.

Elution of HBsAg from liver tissue by dispersion into single cells. Primary hepatocyte monolayer cultures were prepared from liver biopsies obtained during the recovery phase from two chimpanzees experimentally infected with HBV [8]. The suspension medium volume for the disclosure of the tissue represented an approximately 340fold dilution of the original tissue volume. It was tested for HBsAg by radioimmunoassay (RIA) after sedimentation of

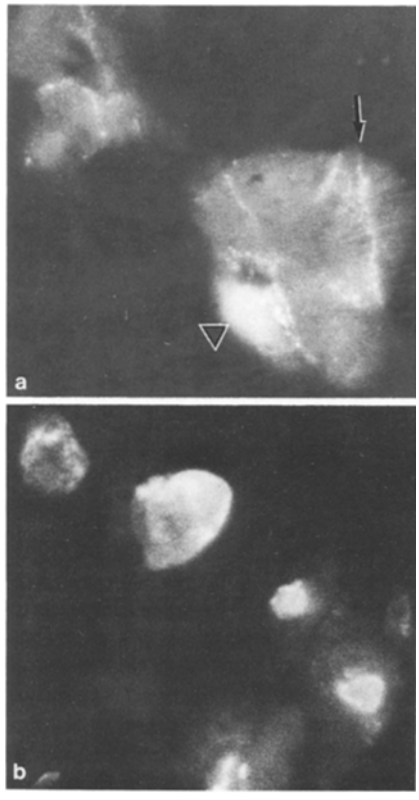

Fig. 1. HBsAg (a) and HBcAg (b) distribution in a frozen section of a human autopsy liver of a patient with chronic aggressive hepatitis type B. (a) Incubation with FITC-conjugated anti-HBs gamma globulin raised in a rabbit and diluted 20fold in phosphate buffer. Specificity of anti-HBs was determined by neutralization with purified HBsAg prior to staining. Arrow indicates perimembranous (pericellular) distribution, triangle intracytoplasmic distribution. (b) Incubation with FITC-conjugated human anti-HBc gamma globulin diluted 80fold in phosphate buffer. Specificity of anti-HBc was determined by competition against an excess of non-labelled human anti-HBc gamma globulin from a different donor. Magnification 250×

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