With the further progress of the experiment (5th–10th injection) abnormal fibroblasts became less conspicuous. The site of injection and surrounding zones exhibited numerous normal-looking fibroblasts arranged in irregular bundles or foci, the latter suggesting pre-neoplasia. Throughout all 3 experiments a variable lymphocytic and histiocytic infiltrate was present. It was most marked in the case of BEI.

Temporary suppression of actively growing tissue appears to be the initial effect of carcinogenic agents. For example, 20-methylcholanthrene, 1,2,5,6-dibenzanthracene and other carcinogenic polycyclic hydrocarbons have been reported to inhibit the connective tissue response when implanted in paraffin wax pellets or injected s.c. in oil, in both rats and mice[4–7]. X-irradiation of a surgically inflicted wound in rats, 28 h after injury, reduces the fibroblastic response by 53%[8]. Oral administration of aromatic mustards, aminostilbenes and carcinogenic hydrocarbons retards the growth rate of young rats and mice[9] and inhibits the growth of malignant tissues[10], whilst N-2-fluorenylacetamide depresses mitotic activity in regenerating liver after hepatectomy[11]. Our results, indicating that the fibroblastic response to injury is inhibited by water-soluble carcinogens are in keeping with these related observations.

Nuclear abnormalities similar to those reported here have been observed in the nuclei of hepatocytes after treatment with a variety of chemical carcinogens[12, 13].

The evidence of a local inhibitory effect on fibroblastic proliferation and granulation tissue formation by carcinogens is in strong contrast to the proliferative lesions induced by surface-active model compounds, e.g. sodium lauryl sulphate, and food colourings, e.g. Blue VRS[14] (Figure 3), or by hypertonic solutions of glucose[1, 15] or by acidic solutions[3] and supports the hypothesis that sarcoma induction in rats could result from 2 distinct mechanisms: one representing a true carcinogenic response, the other leading to malignant change as a result of continued stimulation of fibroblastic proliferation in response to repeated trauma.

Zusammenfassung. Nach subkutaner Injektion einiger sogenannter wasserlöslicher Karzinogene (N-Methyl-N-Nitrosoharnstoff, Nitroquinolin-N-Oxyd und Butyryl-ethylenimin) erfolgt eine lokale Nekrose der subkutanen Gewebe mit verzögerter Regenerierung des Bindegewebes.

J. Hooson and P. Grasso

The British Industrial Biological Research Association, Woodmansterne Road, Carshalton (Surrey, England), 19 November 1969.

[4] E. Hval, *Om 1:2:5:6 Dibenzantracensarkomenes Utvikling* (Klaus Hansens Fond. XIV, Bergen 1937).
[5] J. W. Orr, J. Path. Bact. *49*, 157 (1939).
[6] L. M. Shabad, Z. Krebsforsch. *42*, 295 (1935).
[7] S. B. Wolbach, Arch. Path. *22*, 279 (1936).
[8] H. C. Grillo, Ann. Surg. *157*, 453 (1963).
[9] A. Haddow, Proc. R. Soc. *44*, 263 (1951).
[10] A. Haddow, G. A. R. Kon and W. C. J. Ross, Nature *162*, 824 (1948).
[11] J. O. Laws, J. Cancer *13*, 669 (1959).
[12] B. A. Afzelius and R. Schoental, J. Ultrastruct. Res. *20*, 328 (1967).
[13] D. Svoboda and J. Higginson, Cancer Res. *28*, 1703 (1968).
[14] P. Grasso and L. Golberg, Fd Cosmet. Toxicol. *4*, 269 (1966a).
[15] N. Takizawa, Gann *34*, 1 (1940).

Time and Dose Dependent Inhibition and Enhancement of Thymidine Incorporation into Fibroplasts by Prednisolone

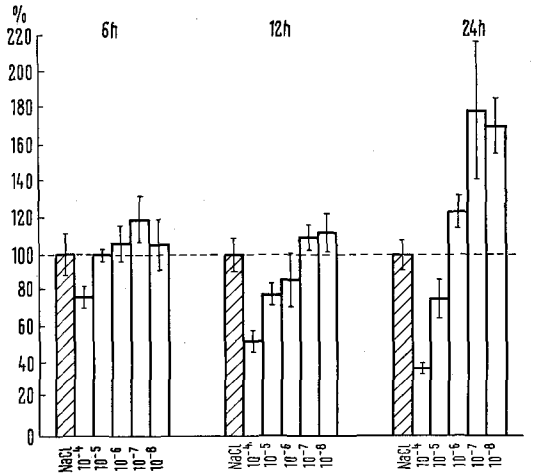
Glucocorticoids have been found to inhibit growth of fibroblasts in vitro[1–7] as well as to stimulate proliferation[1, 8–12]. Fibroblasts have been used as an assay system for these hormones because the activity in depressing the rate of growth of fibroblasts reflects their clinical efficiency as anti-inflammatory agents[1]; moreover, fibroplasts may be considered as indicator cells for cytostatic effects on lympoid cells[3], effects which are exploited in leukaemia treatment and in immunosuppression.

In monolayers of L-cells we investigated the influence of Solu-Decortin-H®[13] (Prednisolone) on the incorporation of tritiated thymidine[14]. We employed a wide range of concentrations of the hormone at different time intervals. L-cells were grown in flasks (10 ml medium: 70% Hanks, 20% calf serum and 10% chicken embryo extract). They were harvested with trypsin and distributed in fresh medium into (80–90) roller rubes (1 ml/tube containing 500,000 cells). After 20–27 h the medium was replaced by TCM '199' (Difco) and 2 h later Prednisolone was added in saline to the final concentrations given in the Figure (moles/1.1 l). At the time points indicated, 2 μC thymidine were added in 20 μl saline and the L-cells were incubated for 2 h. The reaction was stopped by adding 0.1 ml 1.0*N* NaOH at ice temperature. The lysate was pipetted in 100 μl aliquots on to filter paper disks (3 disks per tube) and extracted with cold TCA according to Mans and Novelli[15]. The residual radioactivity was measured in a liquid scintillation counter.

The Figure represents a typical experiment out of four: At 10^{-4} and 10^{-5} *M* a pronounced inhibition of thymidine

[1] A. G. Ruhmann and D. L. Berliner, Endocrinology *76*, 916 (1965).
[2] R. Seifert and H. Hilz, Acta Endocrin. *53*, 189 (1966).
[3] W. B. Pratt and L. Aronow, J. biol. Chem. *241*, 5244 (1966).
[4] L. Berliner and A. G. Ruhmann, J. invest. Derm. *49*, 117 (1967).
[5] A. G. Ruhmann and D. L. Berliner, J. invest. Derm. *49*, 123 (1967).
[6] B. Rasche, L. D. Leder and W. T. Ulmer, Z. ges. exp. Med. *144*, 322 (1967).
[7] O. Wieser and A. H. Taifour, Experientia *25*, 841 (1969).
[8] B. Rohde, H. E. Schreiner and A. Vogel, Arzneimittelforsch. *11*, 392 (1961).
[9] C. W. Castor and R. K. Prince, Biochem. biophys. Acta *83*, 165 (1964).
[10] P. Farnes and B. E. Barker, Metabolism *14*, 75 (1965).
[11] A. Macieira-Coelho, Experientia *22*, 390 (1966).
[12] B. Rasche and W. T. Ulmer, Z. Zellforsch. *84*, 506 (1968).
[13] Solu-Decortin-H® E. Merck = Prednisolone-21-Succinat Sodium.
[14] Thymidine-6-T (*n*) 5 Ci/m*M*. The Radiochemical Centre, Amersham.
[15] R. J. Mans and G. D. Novelli, Arch. Biochem. Biophys. *94*, 40 (1962).

incorporation was found (down to 38% of the control value). At 10^{-7} and $10^{-8}M$, however, the incorporation was stimulated up to 180%. The degree of inhibition and of enhancement became more pronounced after 24 h. Enhanced incorporation was observed even at concentrations as low as $10^{-9}M$. Maximal enhancement occurred either after 12 or 24 h, obviously dependent on the pre-treatment of L-cells in the flask culture. The Table summarizes the incorporation data for the control cultures used in the Figure.



Thymidine incorporation into mouse fibroblasts under the influence of Prednisolone. Incubation procedures are described in the text. Given are % inhibition or increase compared to control values (saline). Each column represents the average of 5 roller tubes (3 measurements per tube), standard deviations are indicated.

Absolute thymidine incorporation into fibroblasts control cultures

	6 h	12 h	24 h
Cpm/0.1 ml culture	2971 ± 11%	7431 ± 9.9%	4465 ± 8%

Experimental details are given in the text.

Increase and decrease of thymidine incorporation appear as a function of the concentration of Prednisolone: high concentrations inhibit, low concentrations stimulate 'DNA-synthesis', no detectable effects in between. The concentrations given in the Figure are true concentrations, because no protein was present. Protein-binding, which normally reduces the concentration of free hormones, is therefore avoided[16].

Both effects, stimulation and inhibition are possibly independent of each other: enhancement of thymidine incorporation occurs without preceding inhibition and vice versa. It may be that the inhibition simply reflects toxic effects of Prednisolone. Thymidine incorporation, as measured by the method described, depends on cell number and/or the rate of actual DNA synthesis. Our experiments do not allow a separation of these 2 parameters; an enhancement of DNA synthesis, however, definitely follows from the data presented, because cell number itself is a function of DNA-synthesis, provided no polyploidization occurred. The bimodal action of Prednisolone, as found in our experiments, may give a hint how to explain the contradictory results of cortisol therapy in the treatment of tumours[17].

Zusammenfassung. Prednisolon wirkt auf L-Zellen in vitro bimodal: Der Einbau von ^{3}H-Thymidin wird in Abhängigkeit von der Hormonkonzentration gehemmt beziehungsweise gefördert.

CHR. ACHENBACH, R. SÜSS, V. KINZEL, O. WIESER and H. A. STURM

Deutsches Krebsforschungszentrum, Institut für experimentelle Pathologie, D-69 Heidelberg (Germany), 29 October 1969.

[16] H. J. HÜBENER and W. H. STAIB, *Biochemie der NNr-Hormone* (Thieme, Stuttgart 1965), p. 98.
[17] J. WOLF, P. SPEAR, R. YESNER and M. E. PATNO, Am. J. Med. *29*, 1008 (1960).

Embryonic Cell Surface: Electrophoretic Mobilities of Blastula Cells

In embryogenesis cells undergo ordered movements during gastrulation. If these movements are related to surface charge densities then cells from different embryonic regions might be expected to vary in this respect. Herein we report differences in electrophoretic mobilities (EPM) of presumptive ectodermal, mesodermal and endodermal cells from blastulas of *Xenopus laevis*. The EPM of early embryonic cells have to our knowledge not been reported previously.

Materials and methods. Eggs were collected in BROWN and CASTON's[1] saline with penicillin-streptomycin. Embryos (stage 8–9)[2] were dejellied in 1% cysteine:papain. Following transfer to 0.002M EDTA in $Ca^{++}Mg^{++}$ free STEARNS'[3] pH 8.0, vitelline membranes were removed manually and embryos allowed to dissociate for 1 h. A discontinuous density gradient of Ficoll ranging in density from 1.07 to 1.13 g/ml was prepared. Cells were layered on the gradient and centrifuged at 500 g, resulting in the formation of 6 bands (Figure 1). To identify the origin of these layers, 100 embryos were micro-dissected into 3 regions: animal pole, marginal zone and vegetal pole. These regions were dissociated separately and centrifuged in identical density gradients. Banding patterns for each region are shown in Table I. Ectodermal cells sedimented in the lightest regions (bands A and B), mesoderm in the intermediate bands (C, D) and endoderm in the densest regions of the tube (E, F). Some overlap, occurred presumably due to the technique of microdissection.

[1] D. D. BROWN and J. D. CASTON, Devl. Biol. *5*, 412 (1962).
[2] P. D. NIEWKOOP and J. FABER, *Normal Table of Xenopus laevis* (North Holland Pub. Co., Amsterdam 1967).
[3] R. N. STEARNS, in *Chemical Basis of Development* (Johns Hopkins Press, Baltimore 1958).