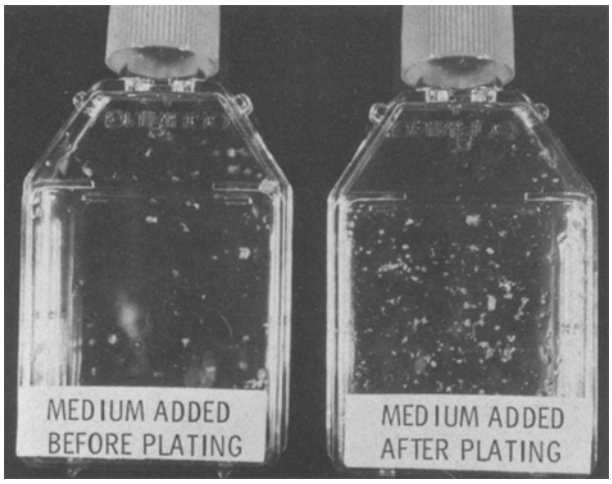

Fig. 1. The adherence of trypsin-treated organ fragments with complete culture medium added either before or after the fragments were decanted into the flask.

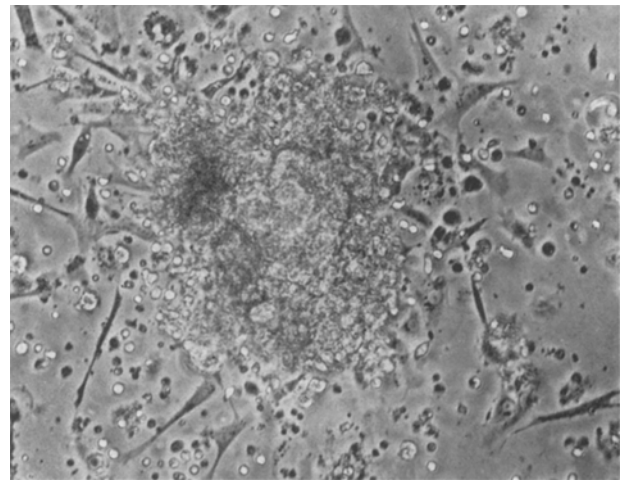

Fig. 2. Outgrowth from a walking catfish kidney explant initiated 1.5 days previously. × 50.

natant was then decanted and fresh trypsin added to the organ fragments; this was repeated 3–4 times. After the last trypsin treatment, the supernatant was decanted and the fragments placed into plastic culture flasks (Corning Glass Works, Corning, New York).

Culture medium consisted of a modified Ham's F-12 in which all components were present at 52% of the standard recommended concentration. The medium contained 23 mM HEPES, 25 mM sodium bicarbonate, 9% fetal bovine serum, penicillin G (200 units/ml), streptomycin sulfate (200 µg/ml) and amphotericin B (5 µg/ml). During initial attempts to establish cell cultures, fragments were mixed with culture medium and decanted into the vessel. The flasks were slowly tilted into an upright position and left for 2 h so that the fragments remained on the growth surface without medium[7].

Results and discussion. The procedure as described above was of limited success, as most of the fragments usually failed to adhere to the surface of the culture flask. Fragments which had apparently adhered usually detached even though the medium was carefully added. If the trypsin-treated fragments were added to the flask without first adding culture medium, more than 90% of all the organ pieces adhered within seconds (figure 1). Under these conditions, the fragments remained firmly attached after the addition of culture medium.

The cultures could be immediately examined without dislodging the fragments. Cells began migrating from the explants within 36 h (figure 2). The attachment was so tenacious that fragments were difficult to remove once they had contacted the surface of the flask for only a few sec. Cultures initiated in this manner were confluent within 1–2 weeks, depending on the organ used. 2 of these cultures, from gonad and kidney, have been passaged in excess of 70 times.

1 Adapted from a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Florida Atlantic University.
2 Acknowledgment. This work was carried out in the laboratory of J.X. Hartmann, whom I thank for his generosity and helpful discussion.
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GABA stimulates the rabbit corneal endothelial fluid pump[1]

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Summary. GABA and its analogues were shown to activate the rabbit corneal endothelial fluid pump in the micromolar range. The stimulation was abolished by bicuculline and chlorpromazine, but not by picrotoxin.

The standard technique of study of the rabbit corneal endothelial fluid pump is to pre-swell the cornea from its normal thickness (less than 400 µm) to about 550 µm and to follow the extent of deturgescence as a function of time at 37 °C. It was shown[5] that the deturgescence depends on the presence of sodium and bicarbonate ions and is improved by reduced glutathione[5]. Later on oxydized glutathione and cystine were shown to improve pumping and survival as well[6]. In such solutions, the survival time is about 3 h, but the deturgenscence is always only partial[5,6]. In the presence

Effect of GABA, its analogs and antagonists on corneal deturgescence*

Material	Concentration (μM)	No. of experiments	Final thickness (μm)	Survival time (h)
1) Salt solution***		4	480±5	2.5±0.3
2) 1+oxydised glutathione	100	6	460±5	3.5±0.2
3) 1+adenosine	5000	15	400±5	6.0±0.3
4) 1+GABA	1	4	410±4	3.5±0.2
5) 1+GABA	10	5	390±7	6.2±0.3
6) 1+muscimol	10	4	385±6	6.0±0.3
7) 6+bicuculline	≃50**	4	480±6	2.0±0.2
8) 5+chlorpromazine	5	4	480±6	3.0±0.2
9) 5+picrotoxin	50	4	400±4	5.0±0.2
10) 1+glycine	100	4	460±5	3.0±0.2

* The method of study used is described by Dikstein and Maurice[5]. We used the modification of Anderson et al.[6]. ** Saturated solution. *** Does not contain glucose.

of 5 mM adenosine - an unphysiological concentration - the deturgescence reaches near completion and the survival time is increased to 6 h[5-7].

Many metabolites were tested unsuccessfully, including sugars, glycolytic intermediates, Krebs cycle intermediates, agents influencing c-AMP formation and prostaglandins[8], with the aim of finding the natural agent permitting full temperature reversal.

We wish to report that GABA, in the range of 10 μM, causes complete reversal with a survival time of 6 h. The GABA analog muscimol is about as active as GABA (table). The action of GABA cannot effectively be blocked by picrotoxin, which in other preparations blocks the chloride ionophore of the receptor[9] in line with the lack of importance of chloride ions for the corneal endothelial fluid pump[10]. On the other hand, the effect of GABA and muscimol is blocked by chlorpromazine and bicuculline respectively in line with the known blocking effect of these agents on the target area of the receptor[11]. Glycine at much higher concentrations than GABA had no effect.

The importance of our finding is: a) It provides an easily and continuously monitorable, single layer uniform cell preparation, in which the receptor stimulated fluid transport can be conveniently studied. Indeed the advantages of this preparation over others have already been pointed out[10,12]. b) It shows that GABA might have an important function outside the nervous or invertebrate neuromuscular systems. c) It increases the chance of developing a long-term preserving fluid for corneal transplantation.

1 This work was supported by PHS-NIH Grant No. 1R01 EY 00965, to S.D.
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λ-Prophage induction by furocoumarin photosensitization

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Summary. Furocoumarin photosensitization induces λ-prophage from lysogenic *Escherichia coli* cells; this effect is clearly due to the photoreaction that furocoumarins give with DNA, and it appears connected with the formation of monoadducts rather than of diadducts (cross-links).

Photosensitizing furocoumarins are a group of natural or synthetic drugs which, by irradiation with long wave UV-light, are able to link covalently to the pyrimidine bases of DNA[2]. Furocoumarins having an angular molecular structure, such as angelicin derivatives, behave as monofunctional reagents, forming only monoadducts (1 furocoumarin molecule + 1 base molecule)[3]. Linear furocoumarins, i.e. psoralen derivatives, form both monoadducts and diadducts (1 furocoumarin molecule + 2 base molecules), giving in this latter case interstrand cross-links[4,5].

The biological consequences of such damage has been studied by several authors on very different substrates, in such a manner that a high number of biological effects of furocoumarin photosensitization are now known. We remember the erythemas on human or guinea-pig skin[2], the killing[6] and the mutations[7] of bacteria, or of mammalian cells grown in vitro[8], the loss of the tumor-transmitting capacity for experimental tumors of the mouse[9,10], the inactivation of some DNA viruses[11] and so on.

In this paper we report on a new effect of photosensitizing furocoumarins, that is the induction of the λ-prophage from lysogenic *E. coli* cells. Lambda is the most studied among the temperate phages, which, upon infection, insert their DNA into the host chromosome, producing a new bacterial strain (lysogen), carrying the virus genome (prophage). These virus genes, generally inactive, can be induced, with production of new infectious phage particles, by serious damage afforded to bacterial DNA[12].