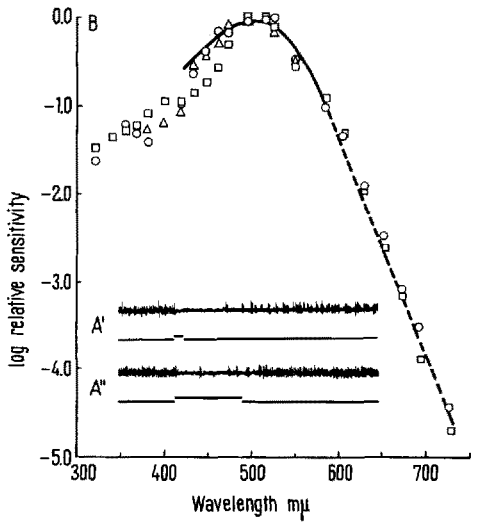
at some places near the midline. Following exposure to strong light, the threshold luminance which evokes a response is greatly increased as compared to the dark adapted threshold, 0.75 lm/m². After cessation of light adaptation the threshold falls exponentially by 2 log units after about 20 sec of dark adaptation, and 5 log units within 30 min. Full dark adaptation of the pineal vesicle in the rainbow trout requires about 1 h or even more.



Rainbow trout (*Salmo irideus*). *A:* Microelectrode recording from the exposed pineal organ (epiphyseal vesicle) showing the activity of several sensory units. Exposure to light indicated by upward deflection of the lower beam. Stimulus duration 0.1 sec (A'), 0.65 sec (A''). *B:* Relative spectral sensitivity, after dark adaptation, of the photic response of the pineal organ. Measurements made at wavelengths between 321 and 727 mμ by determining the energy causing the smallest perceptible decrease of impulse frequency. Equal quantum intensity spectrum. Three different animals indicated by different symbols. For comparison the absorption curve of visual pigment 505 mμ[8] is shown (continuous line).

Spectral sensitivity of the inhibitory response of the pineal organ in *Salmo irideus* is highest in the blue-green and declines towards either side of the spectrum (Figure, B). A comparison of the sensitivity function with the absorption spectrum of known photopigments shows that visual pigment 505 mμ[8] fits the sensitivity data fairly well. Recently, it was found that visual pigment solutions prepared from the lateral eyes of the rainbow trout, *Salmo irideus*, contained a mixture of two photosensitive pigments, one with λ max at 533 mμ, the other with λ max at 507 mμ[9]. From the present measurements it is tempting to conclude that only visual pigment 507 mμ may be responsible for the action of light on the pineal organ in the rainbow trout[10].

Zusammenfassung. Mikroelektrodenableitung der freigelegten Epiphyse der Regenbogenforelle, *Salmo irideus* (Gibbons), zeigt eine spontane Impulsaktivität, die bei Belichtung des Organs gehemmt wird und auch nach Entfernung der lateralen Augen bestehen bleibt. Nach Helladaptation des Pinealorgans an ein starkes Licht ist während des nachfolgenden Dunkelaufenthalts eine Empfindlichkeitszunahme um das 10^5-fache festzustellen. Die Spektralsensitivität der Hemmung ähnelt der Absorptionskurve eines Photopigments mit Maximum bei 505 mμ.

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[9] C. D. B. Bridges, J. Physiol. *134*, 620 (1956).
[10] The author is indebted to the Deutsche Forschungsgemeinschaft for support in this work.

The Effect of Incubation in Phosphate Buffer of Different pH on the Transplantability of the Mouse Ascites Tumor of Ehrlich

As part of a study concerned with the effect of specific environmental conditions on the viability of mouse ascites tumor cells, samples of these cells were incubated in phosphate buffer solutions of different pH before being injected into recipient mice.

Ascites fluid was withdrawn from tumor-bearing white Swiss mice 7–8 days after inoculation with Ehrlich ascites tumor cells, centrifuged for 10 min at 4000 rpm, and then washed three times with triple volumes of 0.85% saline. 1 cm³ of these packed cell preparations contained about 34×10^7 tumor cells (counted in a hemocytometer after dilution with 0.1% citric acid). The packed cells were then suspended in an equal volume of 0.85% saline and 0.48 *M* phosphate buffer was added to make each preparation 0.16 *M*. Buffers of pH 6.1, 6.4, 6.8, 7.1 and 7.4 were used. After the addition of 1000 units of penicillin, each mixture was incubated in Erlenmeyer flasks at 37° while being constantly shaken. Mixtures were incubated for periods from ½ to 7 h. Following incubation, each preparation was brought to pH 6.1 by the addition of 0.1 *N* HCl, and diluted with 0.85% saline to different cell concentrations. 1 cm³ of this suspension was then injected intraperitoneally into 10 white Swiss mice (weighing 20–22 g) and their survival followed for 40 days.

The effect of pH on the viability of the incubated tumor cells is illustrated in the Figure. On incubation for 2 h survival of the tumor cells decreased with increasing pH, until at pH 7.4 no mice died of tumor within 40 days when an inoculum of 1.7×10^6 cells was used.

The Table shows a comparison for 20 representative experiments between the viability of non-incubated cells and cells incubated at pH 6.1 and 7.4 respectively for various periods of time. Whereas 1.7×10^6 cells produced between 50 and 100% mortality, even after incubation for 5 h at pH 6.1, the inoculation of as many as 51×10^6 cells, incubated at pH 7.4 for 2 h, produced no tumor in 40 days. In 8 of the 20 experiments with cells incubated at pH 6.1 the 'break' in viability occurred between 3 and 5 h, while in the remaining 12 the 'break' was between 5 and 7 h.

It should be stressed that there was no appreciable autolysis of these cells during incubation at pH 7.4 for 2 h or at pH 6.1 for 7 h. The cellular DNA content, analyzed according to Schmidt and Thannhauser[1], remained

[1] G. Schmidt and S. J. Thannhauser, J. biol. Chem. *161*, 83 (1945).

% accumulated deaths after 40 days following inoculation of Ascites rumor cells incubated at pH 6.1 and pH 7.4

h of incubation	Number of cells inoculated × 10^6									
	1.7		8.5		17		34		51	
	pH 6.1	pH 7.4	pH 6.1	pH 7.4	pH 6.1	pH 7.4	pH 6.1	pH 7.4	pH 6.1	pH 7.4
0	100	100	100	100						
1/2		50–80		70–80		100				
1		0–10		50–70		60–90				
2	100	0		0		0		0		0
3	100		100		100		100		100	
5	50–100		70–100		100		100		100	
7	0		0		0		0		0	

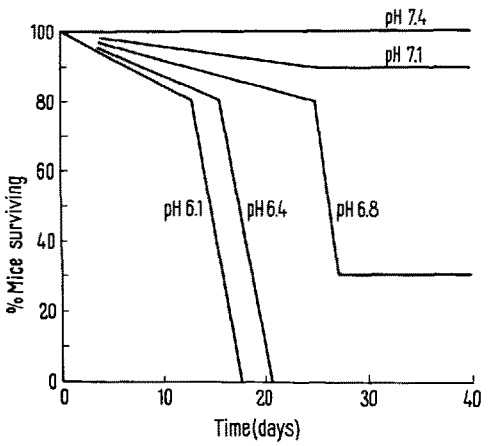
about the same. The DNA phosphorus ranged from about 1.6 to 2.2 10^{-9}/mg cell in different preparations. Cell counts carried out at 0 h and after incubation revealed no appreciable change in cell numbers.

In 6 experiments cytological preparations from representative incubation mixtures were examined without knowledge on the part of the examiner as to the previous treatment given the mixtures. One set of preparations from each mixture was fixed in equal parts of ethyl alcohol and ether, then stained by the Papanicolaou method. A duplicate set was fixed at the same time in 10% neutral formalin, then stained with hematoxylin and eosin. Depending on the appearance of the nuclei, cells were considered to be either viable or non-viable and were tabulated in these two categories.

The control specimens from non-incubated samples of ascites tumor cells showed from 60–90% 'viable' cells. After incubation at pH 7.4 for 1 h 5–30% of the cells were called 'viable', and after 2 h from 5–25% were considered to have remained 'viable'. From the microscopic examination no appreciable difference in growth potential between the 2 groups of incubated cell population would have been expected. Reinjection into new hosts, however, revealed a great difference in their ability to produce ascites tumors (Table). Apparent viability was also assessed microscopically by the eosin-staining technique of SCHREK[2]. The 0 h preparations showed from 93–97% eosin-resistant cells. Cell mixtures incubated at pH 7.4 for 1 and 2 h showed 2–22% and 3–16% eosin resistant cells respectively. Cell preparations incubated at pH 6.1 for 3 h showed 75–80% eosin-resistant cells, after 5 h 70–80%, and after 7 h 25-50% eosin-resistant, i.e. 'viable' cells. Reference to the Table shows that 25–50% of 'viable' cells in the case of injection of 51×10^6 cells should have been sufficient to produce ascitic tumors. The technique of SCHREK is also incapable of differentiating between truly viable and non-viable cells.

However, the possibility remained that the 'non-viable' part of the tumor cell preparations had an inhibitory effect on the seemingly 'viable' part, the term 'viable' taken in a morphological sense. In model experiments we found no evidence that the tumor producing ability of freshly drawn tumor cells (1.7×10^6) was inhibited by the presence of previously incubated cells (51×10^6). The tumor mortality of mice inoculated with mixtures either of freshly drawn cells plus cells incubated for 7 h at pH 6.1 or of fresh cells plus cells incubated for 2 h at pH 7.4 was the same as that of fresh tumor cells alone.

It is of interest that optimal growth of the mouse ascites tumor cells in tissue cultures depends on an acid pH[3]. The above experiments suggest that not only the multiplication of these cells but also the multiple processes which determine their survival under conditions of nutritional deprivation, their all-over maintenance metabolism, is favorably influenced by an acid milieu. A comparative study of cell constituents and biochemical activities of fresh tumor cells and tumor cells incubated at pH 6.1 and pH 7.4 may be useful for determining factors which may control tumor viability in the biological sense.



Survival of mice after i.p. inoculation of 1.7×10^6 ascites tumor cells incubated for 2 h in phosphate buffer of different pH.

Zusammenfassung. Nachweis, dass Ehrlich-Ascites-Tumorzellen, die in Phosphat-Puffer bei einer Temperatur von 37°C inkubiert werden, in saurem pH des Milieus viel länger leben als beim neutralen.

Morphologische Kriterien für Zellschädigung werden diskutiert und erweisen sich als unzulänglich zu Voraussagen über die biologische Wachstumsfähigkeit dieser Krebszellen.

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Departments of Medicine and Pathology, Boston University School of Medicine, Boston (Mass., U.S.A.), July 22, 1963.

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[3] S. GRAFF, Transaction, N.Y. Acad. Sci. *21*, 508 (1959).