H hepatoma. Furthermore, the fast-growing tumor exhibited only insignificant oscillations of DNA synthesis values. Thus, it is apparent that this hepatoma is far less sensitive to the factors that control the circadian variations of its mitotic activity than the slow-growing one.

According to our observations, the presence of the SS1-K hepatoma revealed a clear influence on the fine structure of STH cells. The endoplasmic reticulum showed changes indicative of an increase in growth hormone synthesis, especially conspicuous in the 2 first stages of the tumor development. It is interesting to note the association of a dilated endoplasmic reticulum with modified mitochondria at 00.00 and 12.00 h in group I (figure 1). Features of an enhancement of secretion release, such as variations in the Golgi complex or exocytosis, could not be detected. This latter process is not frequent in normal STH cells, and it has not been described in adenohypophysis from tumor-bearing animals.

The decreased content of STH secretion granules observed during the last stages of tumor growing would indicate a less amount of detectable storaged hormone. Considering the lack of evidence of an intense release, the reduced number of secretion granules would be the result of a diminished synthesis of the hormone, in addition to a possible granulolysis of that material. The presence of numerous secondary lysosomes in group III would give support to this assumption, considering the implication of lysosomes in the elimination of cytoplasmic granules and membranes[15].

The STH cells of control mice showed time variations in their organelles, which were in keeping with previous and more extensive observations[4,13]. From the present experiment, even accepting the existence of time variations in STH cells of hepatoma-bearing animals, it has become evident that such variations did not always present clearly, and were not coincident with the changes found in control mice. It is apparent that the slow-growing SS1-H hepatoma has been shown to possess an effect on the pituitary which is more sensitive to undergo circadian variations[4], when compared with the fast-growing SS1-K hepatoma, which would not exert an action clearly related to the light regimen. In this regard, there exists accordance with the mitotic activity and DNA synthesis in both tumors, as mentioned before.

From the morphological changes which occurred during the 3 stages studied, it seems likely that the stimulating action of the hepatoma upon the STH cells decreases in parallel as the tumor grows. This is in agreement with Afanasjev et al.'s[3] findings in other types of transplantable tumors. However, changes in other pituitary cell types, as well as extreme degrees leading to cell disruption, could not be found in our material.

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2 A.F. Badrán, R.E. Nash and J.M. Echave Llanos, Z. Zellforsch. *118*, 525 (1971).
3 Y.I. Afanasjev, B.D. Gusova and A.N. Yatskovski, Archs Anat. Histol. Embryol. *65*, 42 (1973).
4 C.L.A. Gómez Dumm, M. Souto, R.E. Nash and J.M. Echave Llanos, Chronobiologia *5*, 127 (1978).
5 C.A. Vilchez and J.M. Echave Llanos, J. interdisc. Cycle Res. *2*, 73 (1971).
6 J.M. Echave Llanos and R.E. Nash, J. natl Cancer Inst. *44*, 581 (1970).
7 G. Millonig, J. appl. Phys. *32*, 1637 (1961).
8 B.G. Barnes, Endocrinology *71*, 618 (1962).
9 C.L.A. Gómez Dumm and J.M. Echave Llanos, Acta anat. *82*, 254 (1972).
10 P.W. Coates, E.A. Ashby, L. Krulich, A.P.S. Dhariwal and S.M. McCann, Am. J. Anat. *128*, 389 (1970).
11 D.L. Wilbur, W.C. Worthington, Jr, and R.R. Markwald, Neuroendocrinology *19*, 12 (1975).
12 J.M. Echave Llanos, C.L.A. Gómez Dumm and A.C. Nessi, Z. Zellforsch. *113*, 29 (1971).
13 C.L.A. Gómez Dumm and J.M. Echave Llanos, Experientia *26*, 177 (1970).
14 R.E. Nash and J.M. Echave Llanos, J. natl Cancer Inst. *47*, 1007 (1971).
15 R.E. Smith and M.G. Farquhar, J. Cell Biol. *31*, 319 (1966).

Effect of synthetic polynucleotides on the growth of transplantable tumours in BALB/c mice[1]

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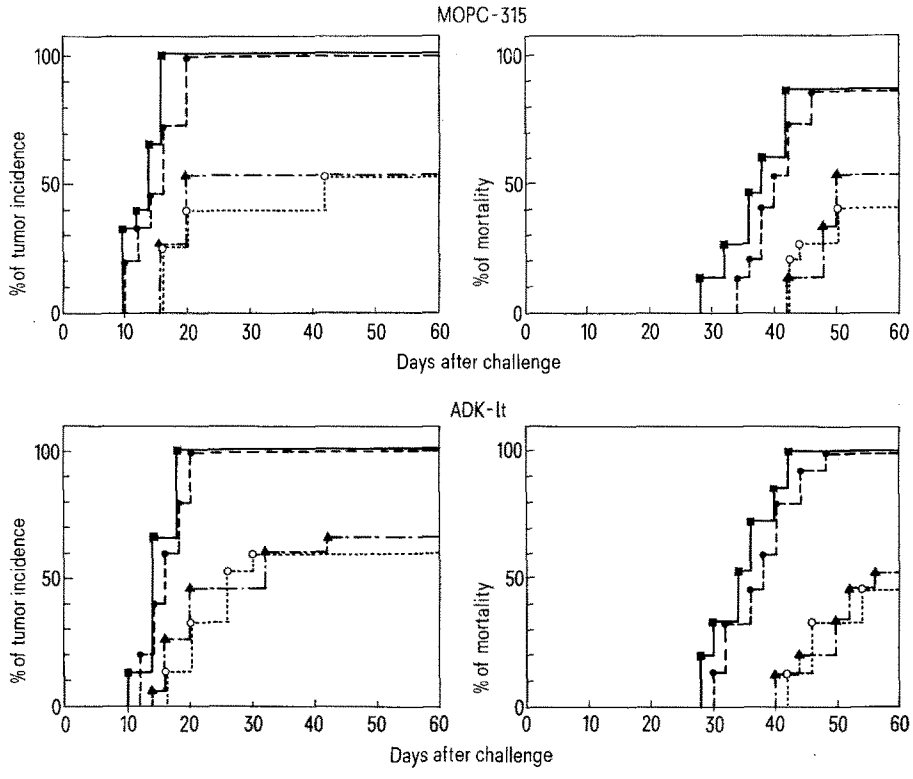
Summary. The effect of BALB/c mice pretreatment with tumour cells (a mammary adenocarcinoma, ADK-1t and an IgA secreting plasmocytoma, MOPC-315) adsorbed with poly I:C, poly I and poly C is examined. Only mice pretreated with cells of both tumours adsorbed with poly I:C and poly C proved to be extensively protected against challenge by homologous untreated tumour cells, whereas this was not so in the case of poly I. A possible explanation of this phenomenon is discussed.

It has been shown[2] that small amounts of poly I:C adsorbed on tumour cell membranes can enhance their immunogenicity. We therefore decided to see to what extent this power is also possessed by the single strand polynucleotides that form poly I:C. Syngeneic BALB/c mice were pretreated with cells from an IgA-secreting plasmocytoma (MOPC-315)[3], or from a spontaneous adenocarcinoma (ADK-1t)[4], adsorbed with poly I: C, poly I and poly C prior to challenge with the corresponding untreated tumour cells. This type of study is relevant in that these polynucleotides display different immunological behaviour[5,6] and biological effects[7,8]. In addition, the membrane affinity of poly C is very weak compared with that of poly I:C, and still more so with that of poly I[9].

Materials and methods. Animals. Randomized groups of 10-week-old male syngeneic BALB/c mice weighing about 20 g were used.

Tumours. 2 different syngeneic tumours were employed. A chemically induced IgA-secreting plasmocytoma (MOPC-315)[3] and an adenocarcinoma (ADK-1t) that arose spontaneously in our BALB/c colony[4] and was maintained by s.c. transplants for several generations before use in the experiments reported. The tumour cells were obtained by



Tumour incidence and mortality rate in mice pretreated with 1×10^3 MOPC-315 and ADK-1t tumour cells adsorbed with poly I:C (▲-·-·-·-▲), poly I (●——●), poly C (○·····○), and 1×10^3 tumour cells only (■——■), and 8 days later challenged with untreated tumour cells (1×10^5 MOPC-315 cells or 3.5×10^4 ADK-1t cells). Polynucleotide adsorption was performed at 37 °C for 20 min by mixing 5×10^6 cells of both tumours and 500 µg of the polynucleotides in 1 ml of Eagle MEM. All the experimental and control groups consisted of 15 animals each.

Hemagglutinin titer in sera of mice pretreated with 1×10^3 sheep RBC adsorbed with the same doses of polynucleotides as those employed for tumour cells*.

Polynucleotides	Titer HA**
Poly I:C	3.64± 1.01
Poly I	3.52± 1.50
Poly C	3.40± 1.00
Controls	3.34± 1.60

* 8 days later the animals were sacrificed and blood collected. Hemagglutinin titer was evaluated by mixing equal volumes of inactivated sera, diluted in microtiter V plates and 0.25% sheep RBC. ** Geometric mean in $\log_2$ and SD.

mechanical teasing. The percentage of live cells was determined by the exclusion of trypan blue[10].

Chemicals. Poly I and poly C were supplied by PL Biochemicals, Milwaukee, Wisconsin, USA. Poly I:C was obtained by mixing equal volumes of poly I and poly C according to Field et al.[11].

Adsorption with the polynucleotides and procedure inoculation. 5×10^6 tumour cells were adsorbed for 20 min at 37 °C in 1 ml of Eagle MEM alone or containing 500 µg poly I:C, poly I or poly C. Then the cells were washed twice in Eagle MEM and resuspended at a concentration of 5×10^3 cells/ml; 0.2 ml of each solution were then inoculated s.c. in groups of 15 animals. Previous work had shown that this priming dose is too low to induce tumours under our experimental conditions[2]. 8 days later, the mice were challenged with 1×10^5 MOPC-315, or 3.5×10^4 ADK-1t live, untreated cells. These doses produced tumours in 100% of the controls within 15–20 days. Tumour incidence was evaluated every 2 days. Mortality rate was also determined.

Hemagglutinin titers in sera of mice pretreated with sheep RBC adsorbed with polynucleotides. 1×10^3 sheep RBC adsorbed with the same doses of polynucleotides employed for tumour cells were inoculated in BALB/c mice. After 8 days, the animals were sacrificed and their blood collected. Then the sera, inactivated at 56 °C for 20 min, were diluted in microtiter V plates and equal volumes of 0.25% sheep RBC were added to each dilution. The results were read after 12 h at 4 °C.

Results. The upper graphs of the figure show the tumour incidence (on the left) and mortality rate (on the right) for the experiment with MOPC-315. It can be seen that animals pretreated with cells adsorbed with poly I:C and poly C were considerably protected to about the same degree: tumour incidence was only 53% and 40%, respectively, on the 20th day, and 53% for both groups on the 60th day (i.e. the end of the experimental period). By contrast, mice pretreated with MOPC-315+poly I were not protected: tumour incidence, in fact, was 100% as in the controls. The mortality rate shows the same pattern: 87% in the poly I group and the controls after 46 and 42 days, respectively (followed by no further deaths), and only 53% and 40% by the 60th day in those pretreated with poly I:C and poly C.

The same pattern was observed in the case of ADK-1t (the lower graphs). In this case, too, only mice pretreated with tumour cells adsorbed with poly I:C and poly C were protected from challenge, whereas ADK + poly I pretreated animals were not. On the 20th day (graph on the left), tumour incidence was 13% in ADK+poly C pretreated mice and 27% in ADK+poly I:C pretreated ones, while controls and ADK+poly I pretreated mice showed 100% incidence. On the 40th day, tumour incidence was 60% in the former 2 groups of animals. No further increase followed during the whole period of observation in ADK+poly C pretreated mice, whereas by the 42th day, the percentage reached 67% in ADK+poly I:C pretreated mice. With regard to mortality percentage (graph on the right), ADK+poly I pretreated animals and controls begin to die about the 30th day, whereas ADK+poly I:C pretreated animals do not exceed 53% mortality even on the 60th day, and ADK+poly C pretreated animals do not exceed 47%.

To see whether the immunological effect observed in our experiment was the result of a humoral response, we adsorbed the same polynucleotide doses on sheep RBC, and then inoculated mice with 1×10^3 of these treated cells. As the table shows, after 8 days no significant difference in hemagglutinin titer between the treated groups and the controls was noted. Moreover, the sera from the groups of animals pretreated with the ADK-1t cells adsorbed with the polynucleotides and from the controls were tested for cytotoxic antibodies, in terms of the trypan blue dye exclusion test. In no instance were tumour-specific antibodies directly demonstrable in response to immunization with tumour cells, polynucleotides-treated or not.

Discussion. Our results suggest that the protective effect observed by mice pretreatment with tumour cells adsorbed with poly I:C is essentially due to the poly C component, because this is the only strand that enhances immunogenicity when used alone. By contrast, it has been demonstrated that poly I is more important than poly C in the poly I:C induction of Interferon[7,8]. This observation shows that 2 phenomena are distinct. The different effect of this polynucleotides on tumour cells immunogenicity could, of course, be ascribed to the different membrane affinity[9]. It is therefore possible that poly I, which has a higher membrane affinity compared with poly I:C and especially poly C[9], ends up by masking rather than modifying important structures on the surface of tumour cell membrane. Moreover, the different antigenic characteristics of the 3 polynucleotides should not be overlooked[5,6].

Since BALB/c mice pretreated with sheep RBC adsorbed with polynucleotides do not show a serum hemagglutinin titer variation when compared to controls, and cytotoxic antibodies to ADK-1t cells in animals pretreated with tumour cells adsorbed with the same polynucleotides are not detectable, it is possible that the immunogenic effect of poly C adsorbed to tumour cell membrane is primarily related to the cell-mediated response. In previous work[2], in fact, we have demonstrated that lymph node cells from mice immunized with poly I:C-treated tumour cells were significantly more active (about twice) than those from mice immunized with untreated cells.

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2 O.J. Plescia, G. Cavallo, A. Pugliese, G. Forni and S. Landolfo, in: Control of Neoplasia by Modulation of the Immune System, p. 501. Ed. M.A. Chirigos. Raven Press, New York 1977.
3 M. Potter, in: Methods in Cancer Research, vol. 2, p. 105. Ed. H. Bush. Academic Press, London and New York 1967.
4 G. Forni and P.M. Comoglio, Br. J. Cancer *27,* 120 (1973).
5 G. Cavallo, A. Negro Ponzi and G. Forni, Atti Accad. naz. Lincei *12,* 1 (1974).
6 E. Seaman, H. van Vunakis and L. Levine, Biochemistry *4,* 1312 (1965).
7 W.A. Carter, P.M. Pitha, L.W. Marshall, I. Tazawa, S. Tazawa and P.O.P. Ts'O, J. molec. Biol. *70,* 567 (1972).
8 W.A. Carter and E. De Clercq, Science *186,* 1172 (1974).
9 A. Pugliese, P. Cappuccinelli and G. Cavallo, G. Batt. Virol. Immun. *69,* 22 (1976).
10 T. Takahashi, L.J. Old and E.A. Boyse, J. exp. Med. *131,* 1325 (1970).
11 A.K. Field, A.A. Tytell, G.P. Lampson and M.R. Hilleman, Proc. nat. Acad. Sci. USA *58,* 1004 (1967).

Elaunin fibres in the basement membrane of sweat gland secretory coil are rich in disulfide-groups

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Summary. Disulfide-groups of elaunin fibres of sweat gland basement membrane are demonstrated a) by thiosulfation/aldehyde-fuchsin staining or thiosulfation/Alcian Blue + 0.8 M $MgCl_2$ staining, and b) by identifying SH-groups after reduction with sodiumthioglycollate. Elaunin fibres share this staining behaviour with 'elastic fibre microfibrils' and with oxytalan fibres.

The elastic system of the dermis is composed of 3 different types of fibres: oxytalan, elaunin and elastic fibres[1]. Elaunin fibres stain with aldehyde-fuchsin, resorcin-fuchsin and orcein, but not with Verhoeff's hematoxylin which, on the other hand, stains true elastin[2]. The basement membrane of the sweat gland secretory coil has attracted attention because elaunin fibres are present in this region, but no elastin[3].

At the fine structural level, oxytalan fibres are represented by bundles of microfibrils, 10–12 nm in diameter[4]. Elaunin fibres consist of similar bundles of microfibrils, but centrally located fibrils are embedded in few amorphous material which morphologically resembles elastin[4]. Elastic fibres are seen in the EM as amorphous material with 'elastic fibre microfibrils' attached to their periphery[5]. Chemical analysis of 'elastic fibre microfibrils' revealed 48.2 half-cystine residues/1000, whereas elastin contains only 4.1 half-cystine residues/1000[5]. This remarkable cystine content has been used histochemically to identify 'elastic fibre microfibrils' with acidic dye solutions (Alcian Blue or aldehyde-fuchsin) after thiosulfation[6] of disulfide-groups[7,8]. Methods to demonstrate disulfide-groups have been applied to basement membranes of sweat glands to get information whether microfibrils of elaunin fibres are also characterized by high cystine content. This would be a further indication that microfibrils of elaunin fibres are identical with 'elastic fibre microfibrils'.

Freshly obtained material from 3 baboons and human autopsy material was used. Biopsies were taken from toes and finger tips within 3 h p.m., fixed in unbuffered formalin and embedded in Paraplast. 10 µm sections were submitted to the following procedures: a) Resorcin-fuchsin staining[8], b) Aldehyde-fuchsin staining either without pretreatment or after thiosulfation[7] or permanganate oxidation[8], c) Verhoeff's hematoxylin staining[8], d) Alcian Blue 8GX staining, using a 1% solution in 3% acetic acid with 0.8 M $MgCl_2$ added to prevent staining of negatively charged groups other than $-SO_3^-$ or $-S.SO_3^-$, sections were stained without pretreatment, after thiosulfation[6], or after thiosulfation followed by methylation[8], e) SH-groups were identified by means of the ferric-ferricyanide method in untreated sections or after reduction with sodium thioglycollate; blockade of SH-groups was performed with N-ethylmaleimide[8].