the distribution of the reaction product was uneven in the matrix of the individual granules (figure 2).
The light microscopical N-acetyl-β-glucosaminidase reaction was stronger, apparently all the granules reacted, the JG cells rich in granules gave an almost diffuse reaction (figure 3). Electron microscopical localization of this enzyme has not been carried out. The nonspecific esterase, α-hydroxy acid oxydase and peroxydase reactions gave negative results in the JG cells.
Thus, in addition to the already known protease (renin), acid phosphatase and β-glucorinidase, 2 additional lysosomal enzymes, aryl sulphatase and N-acetyl-β-glucosaminidase, were demonstrated in the JGC granules.

Inspite of the close histochemical similarity between JGC granules and lysosomes, it has not been possible to demonstrate a functional similarity between them. After the administration of sucrose, iron sorbital-citric acid complex, horseradish peroxydase or thorium dioxide to the experimental animals, none of these substances was found to enter the JGC granules[16]. These functional differences do not exclude the possibility, that the ancestors of the JGC granules are lysosomes and that the lysosomal enzymes may have a role in the still incompletely understood mechanism of renin release.

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Ultrastructural study of somatotroph cells from mice bearing a fast growing transplanted hepatoma, in different periods of the tumor development

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Summary. The presence of a transplanted, fast-growing hepatoma (SS1-K) produces conspicuous ultrastructural changes in pituitary STH cells of C3H-S male mice. These changes are suggestive of an increased secretion of growth hormone only during the first stages of the tumor development. The hepatoma influence does not seem to be clearly related to the illumination regimen or time of killing.

Badrán et al.[2] have studied the ultrastructure of the pars distalis of the pituitary in mice bearing transplanted hepatomas. Their observations revealed changes in somatotroph (STH) cells suggesting an enhancement of their secretory activity. Besides, the fine structure of adenohypophysis from rats with transplanted tumors was found to present variations, depending on the degree of the tumor growth[3].
Recently, we have reported circadian variations in STH cells of mice bearing a slow-growing transplanted hepatoma[4]. The present paper attempts to analyze the electron microscopy of STH cells in the same strain of mice after transplantation of a fast-growing hepatoma (SS1-K), studying the pituitary at 3 time-points of different stages of the tumor development.
Material and methods. C3H-S male mice were used for the experiment. The animals were kept under standard conditions for periodicity analysis[5] and single-caged at a temperature of 25 °C, with water and food ad libitum. They were illuminated from 06.00 to 18.00 h, alternating with 12 h darkness.
An SS1-K Wilson hepatoma was transplanted in the interscapular region of 45 normal 4-week-old male mice. The SS1-K hepatoma is fast growing and reaches a mean diameter of about 2 cm in 30 days. This tumor kills 50% of the mice in 40 days, and it can be considered a poorly differentiated carcinoma. The biological behavior and histomorphology of SS1-K hepatoma has already been studied in our laboratory[6].
The 45 mice were separated into groups of 5 animals each. Normal intact mice were used as controls for each group. The animals were killed 13 (group I), 27 (group II) and 43 days (group III) after transplantation. The mean weights of the tumors were 0.137 g, 1.039 g and 11.882 g, respectively. The mice were sacrificed at 00.00, 12.00 and 16.00 h in each different period of the tumor growing, taking into account previous data about time variations[4]. The animals were killed by decapitation and exanguination, the pituitaries were removed and their lateral wings were separated and sliced into small pieces. The material was fixed 90 min in 1% osmium tetroxide according to Millonig[7], dehydrated in ethanol and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop I electron microscope.
Results. The STH cells were easily identified in the pars distalis and presented the general aspect previously reported in mice[8,9]. The presence of the SS1-K hepatoma produced conspicuous changes in the fine structure of these cells. The rough endoplasmic reticulum appeared more extended, irregular and dilated in nearly all the animals, at different times (figure 1). The changes were found in the

3 stages of the tumor development, but they were stronger in groups I and II, especially in the animals killed at 00.00 and 12.00 h. At these times, the dilatation of the endoplasmic reticulum was prominent. In the control mice, this organelle only showed a moderate dilatation at 16.00 h.

In mice bearing hepatomas, the Golgi complex did not exhibit important variations depending on either the tumor growth or the time of killing (figure 3). It was more extended and slightly dilated only in some STH cells of the animals from group I killed at 12.00 h. In control mice, the Golgi complex also appeared more developed at 12.00 h.

The presence of the hepatoma also produced changes in STH cells lysosomes. These organelles, predominantly of the autophagic vacuole type, were abundant in group III at 16.00 h (figure 3). Control mice did not present lysosome changes.

Mitochondria were found clearly modified in STH cells of mice from group I (figure 1). They were frequently larger than normal and very irregular in shape. Short cristae in oblique position or disorderly located were common findings. Mitochondrial alterations appeared especially at 00.00 and 12.00 h, in coincidence with a strongly dilated endoplasmic reticulum. Important changes were not found in mitochondria of control groups.

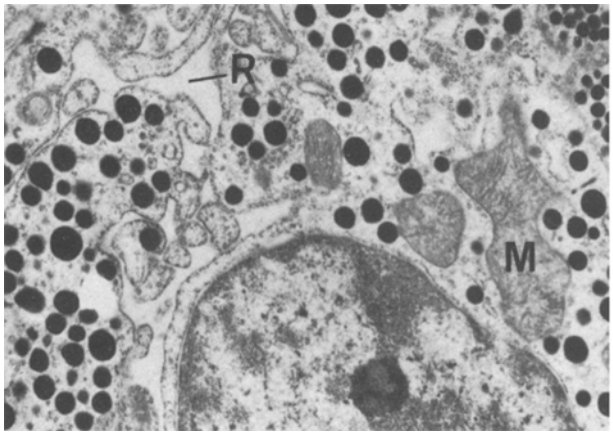
The secretion granules of STH cells were especially numerous during the first stage of tumor development (figure 2) and were more abundant at 16.00 h. A diminishing of secretion material was noticed in groups II and III, without a clear time variation (figure 3). Changes in granulation of STH cells from control groups were not detected.

Discussion. A clear ultrastructural picture of increased secretory activity in STH cells has been made possible by using direct stimulation, by means of hypothalamic extract[10] or growth hormone-releasing hormone[11]. Other than these methods, only a few experimental conditions such as partial hepatectomy[12] and presence of transplanted tumors [2-4], have led to similar results.

Changes in STH cells suggestive of an increased synthesis and release of growth hormone were described by Badrán et al.[2] in mice bearing transplanted hepatomas. Besides, Afanasjev et al.[3] studied the fine structure of the adenohypophysis of rats after transplantation of an epithelial tumor. They found an increase in the functional activity of several cell types, including somatotrophs, during the first 2 weeks of tumor growth. An inhibition associated with destructive alterations would have been evident in the last half of tumor development.

In a previous paper[4], we have studied the electron microscopy of STH cells at different times of a circadian period in mice bearing a slow-growing transplanted hepatoma (SS1-H). On the basis of changes found in the Golgi complex, endoplasmic reticulum and lysosomes, our results were consistent with increase of growth hormone secretion, especially remarkable at 12.00 and 16.00 h (light period). In intact mice, similar but slighter variations also appeared during the light period[4,13].

It is interesting to take into account some data regarding the cell proliferation in transplantable hepatomas. The slow-growing SS1-H hepatoma has been reported to show significantly higher values in mitotic activity during the light period of a 24-h cycle[6]. This tumor also presented a circadian rhythm in DNA synthesis[14], which could be correlated with the mitotic activity. On the other hand, the fast-growing SS1-K hepatoma also showed an enhancement in the mitotic activity during the light period, but its circadian variation was lower than the one observed in SS1-



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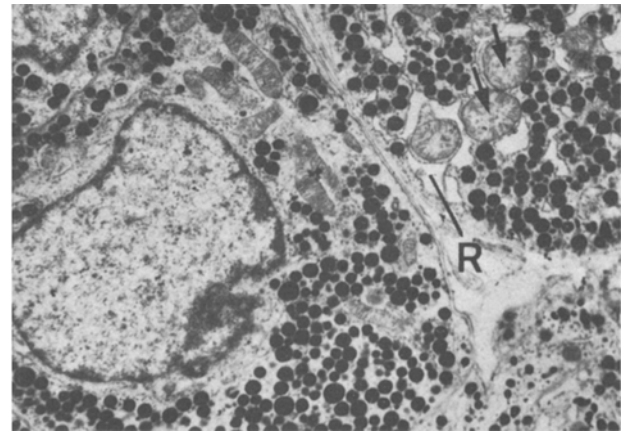

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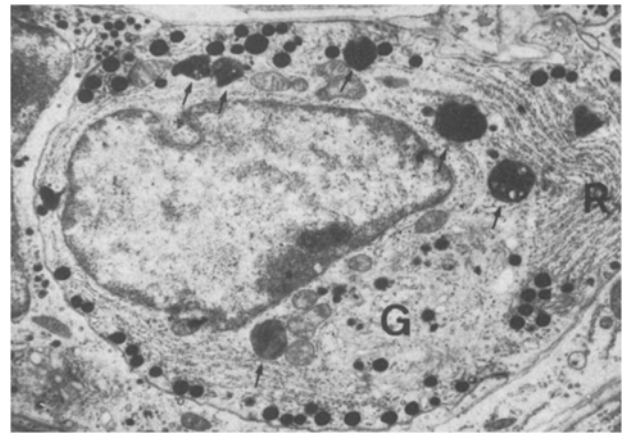

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Fig. 1. STH cell from a hepatoma-bearing mouse (group I, 00.00 h), showing irregular dilated endoplasmic reticulum (R) and a large bizarre mitochondrion (M). × 10,500.

Fig. 2. 2 STH cells from a hepatoma-bearing mouse containing numerous secretion granules. Some large mitochondria (arrows) and dilated endoplasmic reticulum (R) are also shown (group I, 00.00 h). × 5800.

Fig. 3. STH cell from a mouse bearing a hepatoma (group III, 16.00 h), containing abundant lysosomes (arrows) and scarce secretion granules. The Golgi complex (G) and a well developed endoplasmic reticulum (R) are also seen. × 7000.

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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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