Virchows Arch. Abt. B Zellpath. 13, 79—85 (1973)

The Pars Distalis of the Pituitary as a Source of Liver Growth Stimulating Factors during Liver Regeneration*

A. F. Badran, J. M. Echave Llanos, and J. M. Surur

Instituto de Embriología. Biología e Histología. Facultad de Ciencias Médicas
Universidad Nacional de La Plata. 60 y 120. La Plata, Argentina

Received August 26, 1972 / November 26, 1972

Summary. Plasma and pituitary extracts from hepatectomized mice as well as pure growth hormone, stimulate DNA synthesis in hepatocyte population of adult intact mouse liver. This effect is not observed with plasma and pituitary extracts from intact or sham operated mice. The corresponding effects on the litoral cell population of the liver follow the same pattern, but the results are not so clearcut as in the hepatocyte population. The possibility that growth hormone, released by STH cells of the pituitary, is one of the factors responsible for the stimulating effect of plasma during liver regeneration, is discussed on the basis of the present results and of those already reported in the literature.

The participation of the pituitary gland in liver regeneration has been repeatedly reviewed (Harkness, 1957; Weinbren, 1959; Bucher, 1963). It is generally accepted that this gland plays a permissive role, being necessary for the normal timing of proliferative events during liver regeneration.

We have already reported extensive changes in growth hormone producing cells of hepatectomized mice, indicating increased synthesis and release. These changes are well developed before the rise in DNA synthesis of regenerating liver (Echave Llanos *et al.*, 1971a). Plasma from these hepatectomized mice produces a strong stimulation of intact adult mouse liver DNA synthesis, similar to that observed with pure growth hormone (Echave Llanos *et al.*, 1971b).

In the present paper we report the results obtained assaying pituitary extracts and plasma from hepatectomized, sham operated and intact mice, respectively, on adult intact mouse liver DNA synthesis.

Material and Methods

C3H-S male mice were used. This strain, provided by Prof. J. W. Wilson (Brown University, Providence, Long Island, U.S.A.), has been maintained in our laboratory, by inbreeding, since 1966. The animals were standardized for periodicity analysis (Halberg *et al.*, 1958; Vilchez and Echave Llanos, 1971). They were living, since birth, under standard conditions of light (Fluorescent, 40 w, from 06:00 till 18:00 hours, alternating with 12 hours darkness), water, food (ad libitum) and temperature ($22 \pm 1°$ C). After weaning they were caged in groups of 8 and weighed weekly, till they were 9 weeks old. Animals with abnormal growth curves were not used in the experiments.

* Supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas de la Republica Argentina, the Comisión de Investigación de la Universidad Nacional de La Plata and the Comisión de Investigación Cientifica de la Provincia de Buenos Aires. Thanks are due to the members of the technical Staff of the Institute for their technical assistance.

When the animals were 9 weeks old they were caged singly (Echave Llanos *et al.*, 1963) in a room ad hoc, with the same conditions of light, feeding and temperature. In this room no person was allowed to enter, except at 17:50 hours, for control. Three weeks later, when they were 12 weeks old, the mice were used for the experiments.

A group of mice was separated as donors and another one as receivers. Sixty donor mice were subdivided into three groups of 20: 1) Intact donors, 2) Donors in which a sham hepatectomy was performed, with handling of the liver (Weinbren *et al.*, 1969), 3) Hepatectomized donors (Brues *et al.*, 1936). The operations were made at 16:00 hours (Echave Llanos *et al.*, 1971a).

All donors were killed by decapitation and exsanguination at 24:00 hours, 32 hours after operation (Echave Llanos *et al.*, 1971a) for the second and third group. The blood was collected at 0° C on 3% sodium citrate, giving a final concentration of 9:1. Later on it was centrifuged at 0° C and 2000 rpm, during 10 minutes and, finally, the plasma was collected on separate tubes and stored at 0° C until use.

Once the animals were bled, the skull was opened and the hypophysis removed. All the glands of each group were freezed (-17° C) and stored at -25° C until use. At this time they were homogenized with a TenBroek homogenizer, under ice bath, in buffered saline (pH:7.2), added with 3% sodium citrate (9:1), giving a final concentration of 1.5 gland per mililiter. The homogenates were stored at 0° C for successive injections. The dilution was calculated taking into account the dose to be used (0.5 gland per mouse) and, approximately, the volume to be injected (0.01 ml per gram of body weight) to mice of about 30 grams of body weight.

Pure bovine growth hormone (NIH, GH, B13, Bovine) was diluted (1 μg/0.01 ml) in alcaline saline (pH:9.0) and then buffered till pH 7.2. It was then added with 3% sodium citrate in a proportion of 9:1.

Intact receivers were subdivided into 8 groups of 6–8 mice each. When grouping the animals, those pertaining to the same litter, were placed in different experimental groups in order to avoid genetical or litter effect (Echave Llanos, 1967b).

The groups received intraperitoneal injections (0.01 ml per gram of body weight) at 17:00 hours, during three consecutive days (Echave Llanos, 1973) of the following materials: 1) Buffered saline, 2) Plasma from intact mice, 3) Hypophysis from intact mice, 4) Plasma from sham operated mice, 5) Hypophysis from sham operated mice, 6) Plasma from hepatectomized mice, 7) Hypophysis from hepatectomized mice, 8) Growth hormone.

After the third injection the receivers were killed at 04:00 hours of the following day (Echave Llanos *et al.*, 1971c, 1972). Sixty minutes earlier they received an intraperitoneal injection of 1 μc per gram of body weight of tritiated thymidine (New England, Boston, Mass. SA:2c/mM).

After killing, the liver was removed, fixed in buffered formalin (Echave Llanos and Sadnik, 1964), embedded in celloidin-paraffin, cut into 5 μ sections and processed for autoradiography, using Kodak AR10 stripping film, exposed 12 days and developed in Kodak D 19.

In the autoradiographs, the labelled hepatocyte and litoral cell nuclei were counted in 140 high power fields (1250×) controlling the number of cells per field in 14 fields (every 10 fields). The Labelling Index (LI) was expressed as labelled nuclei per 1000 nuclei. The results were analyzed with the Student's "*t*-test".

Results

Hepatocyte Population (Table 1 and Fig. 1)

1. Mice receiving plasma or hypophysis from intact and sham operated animals do not show a different LI than those receiving saline.

2. Mice receiving plasma or hypophysis from hepatectomized animals and growth hormone, show a significantly higher LI than those receiving saline.

Litoral Cell Population (Table 1 and Fig. 2)

1. Mice receiving hypophysis from intact and sham operated animals show a significantly lower LI than those receiving saline.

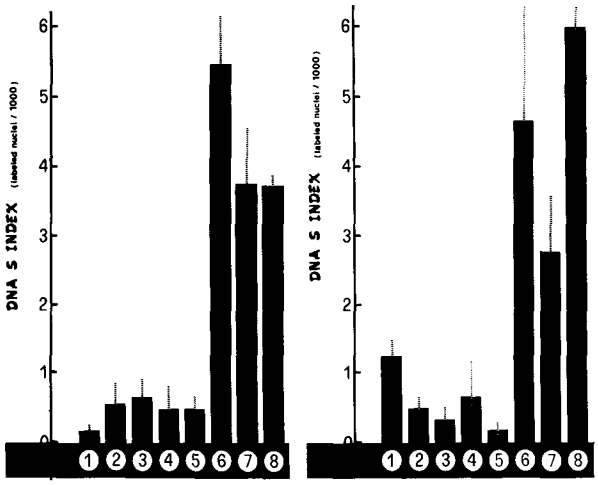

Fig. 1 Fig. 2

Fig. 1. Stimulating effect of plasma and pituitary extracts from hepatectomized mice on DNA synthesis of hepatocyte population of intact adult male mouse liver. *1* Saline. *2* Plasma from intact mice. *3* Pituitary extract from intact mice. *4* Plasma from sham hepatectomized mice. *5* Pituitary extract from sham hepatectomized mice. *6* Plasma from hepatectomized mice. *7* Pituitary extract from hepatectomized mice. *8* Growth hormone

Fig. 2. Effect of plasma and pituitary extracts from intact, sham hepatectomized and hepatectomized mice on DNA synthesis of litoral cell population of intact adult male mouse liver. The indications for the numbers are the same as in Fig. 1

2. Mice receiving plasma from intact or sham operated animals do not show a different LI from those receiving saline.

3. Mice receiving hypophysis from hepatectomized mice do not show a different LI from those receiving saline but, they have a significantly higher LI than the one observed in animals receiving hypophysis from intact and sham operated mice.

4. Mice receiving plasma from hepatectomized mice and growth hormone show a significantly higher LI than those receiving saline.

Discussion

The present experiments demonstrate a growth stimulating effect of plasma and hypophysis from hepatectomized mice and of growth hormone on the hepatocyte population of intact adult liver (Fig. 1).

The effects observed on litoral cell population are not so clear. Although they appear with the same pattern, the scatter of the figures is much greater and only some differences appear as significant (Fig. 2). This would indicate a more complex situation and, probably, the migratory behaviour reported for macrophages (Wilson *et al.*, 1950; Easton, 1952; Fabrikant, 1968; Echave Llanos, 1972) would introduce a variable which could rise or lower, independently, the LI of this cell population observed in the liver. Perhaps the control of this parameter in a wider area of this population, including, for example, the spleen and lung, would give

Table 1. Effect of plasma and hypophysis from intact, sham hepatectomized and hepatectomized mice on DNA synthesis of hepatocyte and litoral cell populations of intact adult mouse liver. Number of H3Tdr-labelled cells per 1000 cells = LI

Group	Treatment	*N*	Hepatocytes LI	Litoral cells LI
			$\bar{x} \pm$ SE	$\bar{x} \pm$ SE
1	S	8	0.19 ± 0.09	1.25 ± 0.25
2	IP	6	0.58 ± 0.30	0.50 ± 0.17
3	IH	6	0.67 ± 0.28	0.33 ± 0.21
4	SP	6	0.50 ± 0.34	0.67 ± 0.50
5	SH	6	0.50 ± 0.18	0.17 ± 0.17
6	HP	6	5.50 ± 0.72	4.67 ± 1.73
7	HH	6	3.78 ± 0.82	2.78 ± 0.81
8	GH	8	3.75 ± 0.15	6.00 ± 0.31
P values (Student's *t* test)			1–6 < 0.001 1–7 < 0.001 1–8 < 0.001 2–6 < 0.001 3–7 < 0.01 4–6 < 0.001 5–7 < 0.01	1–3 < 0.01 1–5 < 0.01 1–6 < 0.05 1–8 < 0.001 2–6 < 0.05 3–7 < 0.02 4–6 < 0.05 5–7 < 0.01

N: Sample size. S: Saline. IP: Intact mouse plasma. IH: Intact mouse hypophysis. SP: Sham hepatectomized mouse plasma. SH: Sham hepatectomized mouse hypophysis. HP: Hepatectomized mouse plasma. HH: Hepatectomized mouse hypophysis. GH: Growth hormone.

more consistent results. No conclusions can be drawn from the present results until such controls are made.

Since Christensen and Jacobsen (1950) reported the appearance of a growth stimulating effect in the blood of hepatectomized animals, several papers supported this suggestion while several others reported negative or even opposite results (see Goss, 1964, for review).

Recent experiments have strengthened the evidence that both-growth stimulating (Moolten and Bucher, 1967) and inhibiting (Badrán *et al.*, 1972) factors, are present simultaneously, in the blood after hepatectomy. Their demonstration depends on the model and the experimental design used for the assay. Nevertheless these experiments cannot prove the hepatic origin of these factors, nor that the effect observed is due to single substances.

The changes already reported in the ultrastructure of the growth hormone producing cells of the pituitary of hepatectomized mice (Echave Llanos *et al.*, 1971a), as well as the present results, suggest that one of the stimulating factors appearing in the blood after hepatectomy is growth hormone. As liver regeneration, however, takes place in hypophysectomized-hepatectomized animals (Weinbren, 1959), growth hormone cannot be the only, or the principal liver growth stimulating factor during regeneration.

The evidence obtained about the site of origin of liver growth stimulating factors others than growth hormone, is still not conclusive. Some cross circulation experiments (Fisher *et al.*, 1971b) show that liver growth stimulation in the unoperated partner is maximal after 100% hepatectomy of the operated one. This result points to an extrahepatic origin of liver growth stimulating factors and, partially, support our own results. Experiments by the same group (Fisher, 1971a) suggest that the intestine could also be the site of origin of liver growth stimulating factors.

Cross perfusion experiments (Levi and Zeppa, 1971) show that, from a liver isolated 18 to 24 hours after hepatectomy, liver growth stimulating factors pass to an isolated, intact liver and increase its DNA synthesis. There is a strong suggestion of the existence of liver growth stimulating factors of hepatic origin in these experiments. Nevertheless the evidence is not conclusive as some extra hepatic factor, fixed by the liver cells during the 18–24 hours previous to isolation, could have been washed out by the perfusion liquid.

Cell population unspecific growth stimulation appearing after hepatectomy, would support the hypothesis that growth hormone is one of the liver growth stimulating factors appearing in the blood during liver regeneration. There is not much information on this problem (Paschkiss, 1958) and the existing one is somewhat contradictory. Mitotic activity appears to increase in the cornea but not in pinna epidermis 4 days after hepatectomy (Paschkiss *et al.*, 1959). The control in these experiments has been made a long time after hepatectomy and an apparent growth stimulation in the cornea could be only the homeostatic reaction to an earlier inhibition (Echave Llanos, 1967a). Contrarily, the existence of cell population specificity of some liver growth stimulating factor would not be the proof that it is originated in this cell population. Specific growth stimulating effects are produced by substances which, like thyrotrophine, are originated in a cell population different from the target organ. This important problem of specificity deserves a more detailed and careful analysis.

In summary, the existence of liver growth stimulating factors of hepatic origin is still not clearly established, while the existence of liver growth stimulating factors originating in sites other than the liver is well established. Growth hormone appears to be one of them.

The fraction of cells in S phase (Labelling Index) of the hepatocyte population of intact adult liver, increases and decreases, following circadian rhythms, with peaks at about 02:00–04:00 hours (Echave Llanos *et al.*, 1972). This circadian rhythm in cell gain which, in some experiments, is represented by $3^0/_{00}$ labelled hepatocytes at the peak time might be related to a circadian rhythm in cell loss in the normal hepatocyte population.

This rhytmic loss and gain of specific cytoplasm along the 24 hour scale would be a result of the rhythmic alternation of darkness, activity, feeding, light, rest, and fasting periods (Vilchez *et al.*, 1971). The feeding period makes hepatocytes increase its function and, with all probability, is followed by a certain amount of aged cells loss. Thereafter, the needs of the organism (Goss, 1964) until the next feeding period, would require the cell gain observed.

The introduction of growth stimulating materials changes the form, butnot the phase, of the circadian curve of the labelling index, enhancing the peak follow-

ing the injection (at 04:00 hours) and propably, as a homeostatic reaction, lowering the peak of the next circadian period. This was the case with inhibiting factors (Echave Llanos, 1967a) which inhibited the peak of mitotic activity after the injection, and made the peak of the next circadian period higher than normal.

The present results and the circadian rhythm of growth hormone produced by the pituitary (Echave Llanos, 1973) would also suggest that growth hormone plays some role in the mechanism of appearance of the circadian rhythm in cell proliferation of hepatocyte population in the normal liver.

References

Badrán, A. F., Surur, J. M., Balduzzi, R., Echave Llanos, J. M.: Assay of plasma from intact and hepatectomized mice on mouse regenerating liver DNA synthesis. Virchows Arch. Abt. B **10**, 176–178 (1972).

Brues, A. M., Drury, D. R., Brues, M. C.: A quantitative study of cell growth in regenerating liver. Arch. Path. **22**, 658–673 (1936).

Bucher, N. L. R.: Regeneration of mammalian liver. Int. Rev. Cytol. **15**, 245–300 (1963).

Christensen, B. G., Jacobsen, E.: Studies on liver regeneration. Acta med. scand., Suppl. **234**, 103–108 (1950).

Easton, T. W.: The role of macrophage movements in the transport and elimination of intravenous thorium dioxide in mice. Amer. J. Anat. **90**, 1–34 (1952).

Echave Llanos, J. M.: Liver tissue growth factors and circadian rhythms in liver regeneration. In: H. Teir and T. Rytömaa (eds.), Control of cellular growth in the adult organism, p. 209–220. New York-London: Academic Press 1967a.

Echave Llanos, J. M.: Experimental sources of variation in the study of liver regeneration. Epatologia **13**, 117–132 (1967b).

Echave Llanos, J. M.: Circadian rhythms in DNA, cell reproduction and growth hormone. In: R. O. Scow (ed.) Proceddings of the IV International Congress of Endocrinology. Symposium on Endocrine Rhythms. Amsterdam: Excerpta Medica (in press) (1973).

Echave Llanos, J. M., Bade, E. G., Bordín, C. I.: Standardization for periodicity analysis and scatter of values in the study of hepatic regeneration. Acta physiol. lat.-amer. **13**, 385–387 (1963).

Echave Llanos, J. M., Badrán, A. F., Surur, J. M.: Circadian variations in DNA synthesis and mitotic activity in intact adult mouse liver. Unpublished results 1972.

Echave Llanos, J. M., D'Aloissio, M., Souto, M., Balduzzi, R., Surur, J. M.: Circadian variations of DNA synthesis, mitotic activity and cell size of hepatocyte population of young immature male mouse growing liver. Virchows Arch. Abt. B. 8, 309–317 (1971c).

Echave Llanos, J. M., Gómez Dumm, C. L., Nessi, A. C.: Ultrastructure of STH cells of the pars distalis of hepatectomized mice. Z. Zellforsch. **113**, 29–38 (1971a).

Echave Llanos, J. M., Gómez Dumm, C. L., Surur, J. M.: Growth hormone release after hepatectomy. Experientia (Basel) **25**, 574 (1971b).

Echave Llanos, J. M., Sadnik, I. L.: Preparation of liver tissue for the evaluation of mitotic activity. Stain Technol. **39**, 289–294 (1964).

Fabrikant, J. I.: The kinetics of cell proliferation in regenerating liver. J. Cell Biol. **36**, 551–565 (1968).

Fisher, B.: Discussion to the paper by Levi and Zeppa. Ann. Surg. **174**, 369 (1971a).

Fisher, B.: Szuch, P., Levine, M., Fisher, E. R.: A portal blood factor as the humoral agent in liver regeneration. Science **171**, 575–577 (1971b).

Goss, R. J.: Adaptive growth, p. 221–233. New York: Logos Press, Academic Press 1964.

Halberg, F., Barnum, C. P., Silber, R. H., Bittner, J. J.: 24-hour rhythms at several levels of integration in mice on different lighting regimens. Proc. Soc. exp. Biol. (N. Y.) **97**, 897–900 (1958).

Harkness, R. D.: Regeneration of the liver, Brit. med. Bull. **13**, 87–93 (1957).

Levi, J. U., Zeppa, R.: Source of the humoral factor that initiates hepatic regeneration. Ann. Surg. **174**, 364–369 (1971).

Moolten, F. L., Bucher, N. L. R.: Regeneration of rat liver: Transfer of humoral agent by cross circulation. Science **158**, 272–274 (1967).

Paschkiss, K. E.: Growth promoting factors in tissues. A review. Cancer Res. **18**, 981–991 (1958).

Paschkiss, K. E., Goddard, J., Cantarow, A., Adibi, S.: Stimulation of growth by partial hepatectomy. Proc. Soc. exp. Biol. (N. Y.) **101**, 184–186 (1959).

Vilchez, C. A., Echave Llanos, J. M.: Circadian rhythm in the feeding activity of Oxymycterus Rutilans. Role played by light and food availability. J. interdiscipl. Cycle Res. **2**, 73–77 (1971).

Weinbren, K.: Regeneration of the liver. Gastroenterology **37**, 657–668 (1959).

Weinbren, K., Arden, G. M., Stirling, G. A.: Increased incorporation of 3H thymidine into rat liver DNA after handling of liver lobes. Brit. J. exp. Path. **50**, 540–547 (1969).

Wilson, J. W., Leduc, E. H., Corner, J. A.: Movements of macrophages in the liver of the mouse after injection of thorotrast. Anat. Rec. **106**, 260 (1950).

Prof. J. M. Echave Llanos
Instituto de Embriología,
Biología e Histología
Facultad de Ciencias Médicas
60 y 120 — La Plata
Argentina