

of the saline injected sides was less than the increase of the prolactin injected sides, i.e., the level $\times$ time interaction was significant ($p < 0.01$).

The basis of the daily cropsac variation remains to be explained. However, thyroxine, prednisone, and growth hormone enhance the responses of hypophysectomized young pigeons to prolactin⁹. Daily rhythms in hormone levels have been found in many vertebrates, e.g., TSH in rats^{10,11}; corticosterone in mice¹², rats¹², humans¹², and sparrows¹³; prolactin in rats¹⁴, hamsters¹⁵, and sparrows¹⁶. The rise and fall during the day of hormones auxiliary to a cropsac response might account for a sensitivity rhythm. As suggested for white-throated

sparrows¹⁶, sensitivity rhythms in pigeons may be involved in the temporal regulation of prolactin-dependent phenomena. Also, the existence of a marked daily variation in the response of cropsacs requires investigators to consider the time of day when cropsac assays are made¹⁷.

Zusammenfassung. Es wird festgestellt, dass die Ansprechbarkeit des Taubenkropfsackes auf Prolactin in Abhängigkeit vom hormonalen Zustand des Versuchstieres von Tag zu Tag verschieden ist.

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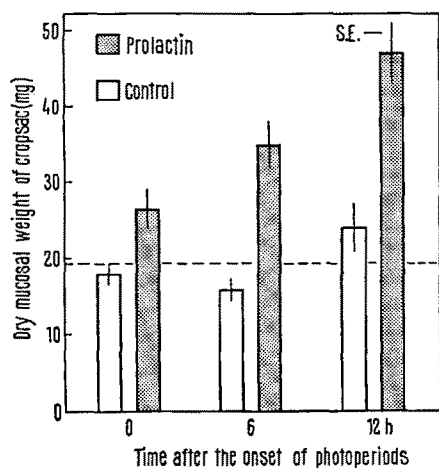


Fig. 2. Daily variations in responses of the cropsac mucosa to intradermal injections of prolactin. Prolactin (50 mg total) and saline were given in 4 equal daily injections at one of 3 different times during the day (0, 6 or 12 h after the onset of the daily photoperiod, 07.30–19.30 h). The broken line represents the mean level (19.2 mg) of the combined controls.

Growth Hormone Release After Hepatectomy¹

Contradictory reports on the participation of the pituitary gland in liver regeneration have appeared in the literature for some time^{2–4}; but recent work has thrown some light on the role played by this gland in this process^{5,6}.

With the aim of obtaining more information on this subject, and with a different approach, we have, as a first step, looked for changes in the growth hormone-producing cells of the pituitary gland of hepatectomized mice and we have observed morphological changes indicating a massive release of the hormone⁷. These changes reached a maximum at midnight of the second day after hepatectomy, some hours before the appearance of the first peak of DNA synthesis in the regenerating liver (ECHAIVE LLANOS et al. unpublished results).

In a second step of which the results are reported here, we have assayed the plasma of hepatectomized mice, taken at midnight of the second day after hepatectomy, the time of maximal release changes in the growth hormone-producing cells, on the DNA synthesis index of the liver of intact adult mice. Its effect was compared with the action of saline, plasma from intact mice killed at midnight and pure bovine growth hormone.

Seventy-two 90-day-old C3H-S inbred male mice were used for the experiment. They were standardized for periodicity analysis⁸. 20 of them, hepatectomized⁹ at

16.00 h⁷, were killed by decapitation at midnight of the second day of regeneration, together with 20 intact mice. The blood of each group was collected as a pool on 1 ml of 3% sodium citrate and then centrifuged at 3000 $\times g$ and 0°C. The plasma obtained was stored for 16 h at 0°C until injection.

Four groups of 8 intact mice were used as receivers. They were injected i.p. at 16.30 h for 3 consecutive days, with 0.01 ml/g body weight of the following solutions: 1. Buffered saline (pH: 7.2) with 3% sodium citrate (9:1). 2. Plasma from intact mice killed at midnight. 3. Plasma from hepatectomized mice killed at midnight of the second day after hepatectomy. 4. Growth hormone (NIH-GH-B13. Bovine) dissolved (1 μg /0.01 ml) in alkaline medium, buffered to pH 7.2 and finally added with 3% sodium citrate (9:1). All the animals were killed after the third injection at 02.00 h of the following day, having received, 1 h previously, an i.p. injection of tritiated thymidine (From New England, Chicago. Specific activity: 2 C/mM) in a dose of 1 μC /g body weight. The liver was removed and processed for autoradiography. The DNA synthesis index was determined and expressed as labelled nuclei/10,000 nuclei.

In this experimental design (Figure), the time for the injection of the hormone (16.30 h) was chosen bearing in mind the results of HALBERG¹⁰ who demonstrated

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¹⁷ Our study was supported by a Public Health Service Research Grant No. 5 R01 AM13400 and a Public Health Service Research Career Development Award No. GM-17,898, to ALBERT H. MEIER. The ovine prolactin was a gift of the Endocrinology Study Section of the National Institutes of Health. We thank Dr. P. E. SCHILLING of the Computer Center at Louisiana State University for making the statistical evaluations.

that growth hormone stimulates mitotic activity of immature growing liver, when injected at this time, in comparison with injections made at midnight, which are not effective. This effect was observed by controlling the mitotic activity at noon of the following day¹¹, the peak time of mitotic activity of normal immature growing liver^{12, 13}.

The time chosen for control of DNA synthesis is based on the results by BARNUM and HALBERG¹⁴, and our own results in young C3H-S young mice¹³, in which the peak time of incorporation of tritiated thymidine is about 03.00 h. Notwithstanding the fact that adult liver shows a very low labelling at this or any other time of a circadian period, it was assumed that, if any effect were to appear, it would appear at this circadian time.

The results (Table) show that the plasma of hepatectomized mice stimulates the synthesis of DNA in hepatocytes as well as litoral cells of the liver of intact adult mice. This effect is not observed with the plasma of intact mice, being the figures of the corresponding animals not different from those of the animals injected with saline. Pure bovine growth hormone, at the dose level used, stimulates strongly the synthesis of DNA in both cell populations of intact adult liver. The labelled hepatocyte nuclei in the animals injected with plasma from hepatectomized mice as well as in those injected with growth hormone appeared in the periportal located areas.

The fact that the stimulating effect of plasma from hepatectomized mice appears some hours before the DNA synthesis peak of the regenerating liver of donors, and coincides with the maximal changes in the growth hor-

mone-producing cells of the pituitary⁷, fits quite well with the fact that the timing of this first peak is pituitary-dependent and appears to be delayed in the regenerating liver of hypophysectomized animals⁶.

The appearance of the labelling in the periphery of the liver lobule, after the injection of plasma from hepatectomized mice or growth hormone, coincides with this same location of labelling⁶ or the mitotic activity that follows² in the first proliferating peak of regenerating liver.

All this gives support to the hypothesis that the effect of plasma from hepatectomized mice in the present experiment is due to its higher content in growth hormone. If this is true, the existence of a hypothetical growth-stimulating factor from liver origin is no longer necessary to explain the stimulation of mitotic activity or DNA synthesis in the non-operated partner of a parabiotic¹⁵ or cross circulation¹⁶ pair. The negative results with growth hormone, which failed to stimulate thymidine incorporation into adult liver DNA¹⁷, could be due to a wrong experimental design with respect to the time of injection of the hormone and control of the synthesis of DNA.

Resumen. El plasma de ratones hepatectomizados, recogido a la medianoche del segundo día de la regeneración, estimula, al igual que la hormona de crecimiento pura, la síntesis de ADN de los hepatocitos y células litorales del hígado del ratón adulto intacto. Este efecto no se observa con el plasma de ratones intactos sacrificados a medianoche ni con solución fisiológica. Se sugiere que el factor estimulante del plasma de ratones hepatectomizados en hormona de crecimiento.

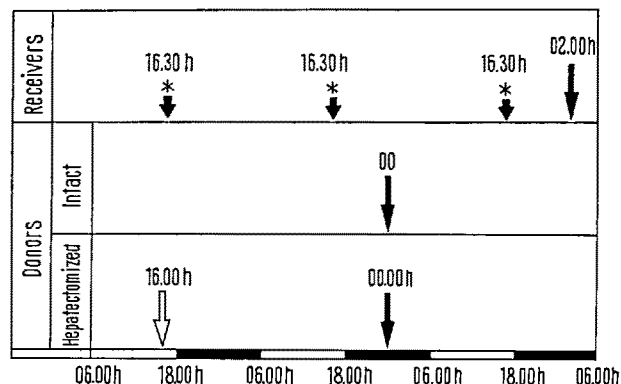
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The action of saline, plasma from intact mice, plasma from hepatectomized mice and growth hormone on the DNA S-index (labelled nuclei/10,000 nuclei) in mouse intact liver

Group	Treatment	N	Hepatocytes $\bar{x} \pm S.E.$	Litoral cells $\bar{x} \pm S.E.$
1	Saline	8	1.87 $\pm$ 0.91	12.50 $\pm$ 2.50
2	Intact mice plasma	8	4.00 $\pm$ 1.01	13.75 $\pm$ 2.73
3	Hepatectomized mice plasma	8	11.00 $\pm$ 1.29	23.75 $\pm$ 4.20
4	Growth hormone	8	37.50 $\pm$ 1.54	60.00 $\pm$ 3.05
P values (Student's <i>t</i> -test)			1-3 < 0.001	1-3 < 0.05
			1-4 < 0.001	1-4 < 0.001
			2-3 < 0.001	2-3 < 0.05
			2-4 < 0.001	2-4 < 0.001
			3-4 < 0.001	3-4 < 0.001

N, sample size; $\bar{x} \pm S.E.$, arithmetic mean $\pm$ standard error.



Arrows with asterisks, time of injections. Black arrows, time of sacrifice. White arrow: time of hepatectomy. The illuminating regimen, LD₁₂₋₁₂, is indicated in the lower line.

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