acid on the synthesis of nuclear liver RNA. A 30-min pulse of [6-^{14}C]-orotic acid resulted in a higher incorporation of ^{14}C in liver RNA when borate was given intraperitoneally. This increased incorporation was diminished when the diet contained 1 ppm boron as boric acid. An attempt to determine the effect of borate on the synthesis of liver DNA, using [6-^{3}H]-thymidine, gave unsatisfactory results. It is apparent that borate increases the rate of synthesis of nuclear liver RNA.

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Induction of Tryptophane Pyrrolase and Tyrosine-α-Ketogluarate-Transaminase in Regenerating Liver of Hypophysectomized Rats.* (32536)

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It is now well established that several control mechanisms governing the synthesis of certain enzymes in normal liver are altered or entirely absent in a number of hepatomas (1). In normal liver the synthesis of cholesterol is controlled by a feedback mechanism which responds to the intracellular concentration of cholesterol. The impairment of this control mechanism in several hepatomas leads to a production of cholesterol far exceeding that of the liver cell(2). Pitot and Morris reported that the activity of the enzyme tryptophane pyrrolase which increases in normal liver after administration of cortisol is very low in the slowly growing hepatoma 5123 and does not rise under the influence of cortisol. The tyrosine-α-ketoglutarate-transaminase activity, however, was found to be high in the hepatoma and could not be further stimulated by administration of cortisol(3). In the hepatomas of adrenalectomized animals the activity of the tyrosine transaminase was still twice as high as in normal liver but considerably lower than in the hepatomas of intact animals. Furthermore, administration of cortisol to adrenalectomized animals produced a 2.5-fold stimulation of the tyrosine transaminase activity in the tumor. Pitot and

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Morris suggested that a single basic lesion involving a cellular component essential to the control of several enzymes might account for these findings as well as for the biologic malignancy expressed by the tumor. The finding that the cortisol induced tryptophane pyrrolase activity is significantly lower in regenerating liver between 12 and 48 hours after hepatectomy than in normal liver has recently raised the possibility that the alterations in the regulatory functions of the cells as observed by Pitot and Morris may be a consequence of cell division in general rather than of malignancy(4). It therefore seemed justified to study the induction of tryptophane pyrrolase and tyrosine transaminase with cortisol in regenerating liver at the various stages of regeneration and under conditions where the maximal mitotic activity occurred at different time intervals after hepatectomy. It will be shown that the base line activity of tryptophane pyrrolase is slightly lower in 24-hour regenerating liver than in normal liver and that there is a marked decrease in the induction of this enzyme by cortisol 24 hours after hepatectomy. It will also be demonstrated that the increase of tyrosine transaminase activity after cortisol administration is much more pronounced during the period of regeneration studied than it is in resting liver. Although the substitution of growth hormone to hypophysectomized rats eliminates the delay in mitotic activity which is observed in hypophysectomized animals it does not influence the time pattern of the alterations in enzyme activities described above.

Experimental procedure. Animals. Male hypophysectomized Wistar rats weighing 150 g were obtained from Charles River Farms and were maintained on a Purina lab chow and water *ad lib.* Their weight was controlled for 2 weeks after the operation and only animals which showed no increase in weight during this period were used in the experiments. Hepatectomy was performed according to the method of Higgins and Anderson (5) under ether anesthesia; control animals were sham-operated. Cortisol phosphate (5 mg per animal) was injected intraperitoneally into rats with normal livers or at various time intervals after partial hepatectomy. Six hours later the animals were sacrificed and the livers quickly removed for determination of the enzymes. Bovine growth hormone (supplied by the Endocrinology Study Section, National Institutes of Health, Bethesda, Md.) was dissolved in saline which had been adjusted to pH 8.5 with a few drops of 1 M Tris HCl of the same pH. It was injected subcutaneously in doses of 0.2 mg/100 g of body weight on 5 consecutive days before the experiments were started. The injections were continued throughout the experimental period. All animals which received growth hormone started to gain weight at a rate of 2-3 g per day.

The mitotic activity in the regenerating livers was assayed by preparing Feulgen-stained histologic slides and by counting at least 4000 cells per liver. The results were expressed as mitoses per 1000 cells.

Tryptophane pyrrolase was assayed according to the method of Knox(6) with addition of hematine(7). The values were expressed as μmoles of kynurenine formed per hour per 1 g of fresh tissue.

The *tyrosine-α-ketoglutarate-transaminase* activity was determined by a modification(8) of the procedure described by Lin and Knox (9). The values represent μmoles p-hydroxyphenylpyruvate/g tissue/hour. All enzyme assays were done in triplicate.

Results. During the first 24 hours of regeneration a small decrease of endogenous tryptophane pyrrolase activities can be observed in animals which were not treated with growth hormone. The decrease in the response of the enzyme activity to cortisol follows the same time pattern and is much more striking. Forty-eight hours after hepatectomy, tryptophane pyrrolase activities are higher than after 24 hours but have not yet reached their normal levels. There is no coincidence of the diminished induction of tryptophane pyrrolase which is observed after 24 hours and the highest mitotic activity which appears after 48 hours of regeneration. While the endogenous transaminase levels remain very stable during the first 24 hours of regeneration and show only a modest increase 48 hours after hepatectomy there is a sharp rise in the induction of this enzyme by cor-

TABLE I. Enzyme Induction in Normal and Regenerating Livers from Hypophysectomized Rats.

Tissue	Tryptophane pyrrolase		Tyrosine transaminase		No. of mitoses (per 1000 cells)
	Control	Cortisol	Control	Cortisol	
Normal liver	21.0 ± 5	110 ± 7.0	440 ± 31.0	1311 ± 103	—
Regenerating 6 hr	29.2 ± 4.0	126 ± 7.5	429 ± 28.0	2829 ± 170	3.0 ± 1
" 12 "	20.8 ± 3.5	103 ± 6.8	541 ± 80.0	2606 ± 153	4.0 ± 1
" 24 "	13.7 ± 1.5	53.7 ± 4.1	409 ± 71.0	2358 ± 120	7.0 ± 2
" 48 "	15.6 ± 2.0	82.5 ± 3.0	524 ± 87.0	2292 ± 130	29.0 ± 3

Tryptophane pyrrolase values represent μmoles of kynurenine produced per hr per g of tissue. Tyrosine transaminase values are expressed as μmoles of p-hydroxyphenyl-pyruvate formed per hr per g of tissue. Mean values and standard deviations are given. Each value represents results obtained from 4 rats. In the case of normal liver 8 rats were used in each group.

tisol as early as 6 hours after the operation. This rise is maintained throughout the whole 48-hour period. No relationship between the tyrosine transaminase activities and the mitotic rate emerges from these data which are shown in Table I.

While the onset of mitotic activity of regenerating liver seems to be delayed in hypophysectomized rats by approximately 24 hours as compared to normal rats(10) the application of growth hormone brings it back to normal. In hypophysectomized rats the first mitotic wave does not seem to occur before 48 hours after hepatectomy; if growth hormone is given, the mitotic peak is observed by 24 hours after hepatectomy. These findings are in agreement with results published by Canzanelli *et al*(11) and by Higgins and Ingle(12). Surprisingly, this variation in the time course of mitotic activity had no influence on the baseline activities of tryptophane pyrrolase and tyrosine transaminase or on the response of these enzymes to cortisol. As is shown in Table II there is a sharp increase in the induction of tyrosine transaminase 6 hours after hepatectomy in the presence of growth hormone while a marked depression of the tryptophane pyrrolase activity induced by cortisol is observed after 24 hours of regeneration. These findings are identical with the results obtained from untreated hypophysectomized rats.

Discussion. The baseline activities of tryptophane pyrrolase and the response of this enzyme as well as of tyrosine transaminase to cortisol are clearly altered during liver regeneration. While there is a decrease in the absolute levels and in the induction of tryptophane pyrrolase 24 hours after hepatectomy there is a sharp rise in the induction of tyrosine transaminase which remains present throughout the whole period of regeneration studied. Neither the rate of cell division, the time of maximal mitotic activity during regeneration nor the application of growth hormone as such had any influence on the observed changes of the enzyme activities during regeneration. These findings are in agreement with the results of Seidman *et al* with respect to the induction of tryptophane pyrrolase in regenerating liver. They are, however, not compatible with the suggestion of these authors that the depression of tryptophane pyrrolase induction may be only a

TABLE II. Enzyme Induction in Normal and Regenerating Livers from Hypophysectomized Rats Receiving Growth Hormone.

Tissue	Tryptophane pyrrolase		Tyrosine transaminase		No. of mitoses (per 1000 cells)
	Control	Cortisol	Control	Cortisol	
Normal liver	19.6 ± 2.5	136 ± 8.4	370 ± 30	920 ± 61	—
Regenerating 6 hr	17.6 ± 4.0	95.0 ± 7.3	378 ± 41	1929 ± 120	2.5 ± 1.0
" 12 "	15.8 ± 3.2	106 ± 7.1	326 ± 2.1	1804 ± 102	8.0 ± 4.0
" 24 "	4.9 ± 1.5	63.0 ± 5.7	353 ± 40	1553 ± 117	35.0 ± 7.0
" 48 "	14.5 ± 3.8	81.0 ± 7.4	417 ± 91	1693 ± 105	10.5 ± 2.0

For legend see Table I.

consequence of cell division. If cell division were to be the critical event in causing either the diminution of tryptophane pyrrolase induction or the enhancement of the tyrosine transaminase induction, one would expect these events to follow the time course of mitotic activity. This does not seem to be the case: the changes in enzyme activity occur at identical time intervals after hepatectomy whether the mitotic activity is delayed (hypophysectomized rats) or normal (growth hormone treated rats).

The relative unimportance of cell division as a factor modifying the endogenous levels and the induction by cortisol of both enzymes is also emphasized by the fact that in a slowly growing tumor (5123) the tryptophane pyrrolase levels are extremely low and show no response to cortisol while in 24-hour-regenerating liver, a tissue with a much more rapid cell proliferation, the baseline level of the same enzyme is only slightly lower than in normal liver and its induction—though impaired—is still clearly present.

At present it cannot be decided whether the alterations in enzyme activities observed in the hepatoma are in any way related to the enzymic changes found in regenerating liver. The results presented here, however, allow the conclusion that neither the deletion of control mechanisms observed in hepatoma 5123 by Pitot and Morris nor the altered response of tryptophane pyrrolase and tyrosine transaminase to cortisol in regenerating rat liver is caused by cell division.

Summary. The induction of tryptophane pyrrolase and of tyrosine-α-ketoglutarate-transaminase with cortisol is altered in regenerating liver of hypophysectomized rats as compared to normal liver. While there is a decrease in induction of tryptophane pyrrolase 24 hours after hepatectomy a marked increase in the response of tyrosine transaminase activities of cortisol is found during the entire period of regeneration. The onset of mitotic activity during liver regeneration is delayed in hypophysectomized animals. By application of growth hormone it can be brought back to normal. This variation of the mitotic activity, however, has no influence on the time course of the changes in the induction of the two enzymes.

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Prevention of SV_{40} Virus Tumorigenesis in Newborn Hamsters by Maternal Immunization.*† (32537)

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Immunologic control of virus-induced cancer presents 3 possibilities, *viz.*, immunization against initial viral infection, immunization against homologous tumor-specific antigen prior to tumor appearance, or immunization

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