SERUM HORMONE LEVELS FOLLOWING PARTIAL HEPATECTOMY IN THE RAT

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SUMMARY

Serum levels of insulin, glucagon, growth hormone (somatotrophin) and thyroxine (TT_4) were measured by radioimmunoassay following both sham operation and 70% partial hepatectomy in the rat to evaluate changes in hormone levels during liver regeneration. An eleven fold increase in glucagon was observed (from 112 $\pm$ 10 pg/ml to 1500 $\pm$ 200 pg/ml) 6 hours following partial hepatectomy but not sham operation. In contrast, insulin levels remained unchanged compared to sham controls for up to 72 hr while growth hormone fell to low levels, 6 to 48 hr after partial hepatectomy. Both total thyroxine and free thyroxine levels also fell 24-72 hours after hepatectomy. These studies suggest that growth hormone, thyroxine and insulin are not primary stimulants of hepatic regeneration although the data suggests that glucagon may modify this growth process.

INTRODUCTION

Humoral factors are believed to initiate the dramatic burst of hepatocellular proliferation that follows partial hepatectomy (1-4), but whether known hormones (5-8) or new polypeptides (9,10) are involved in this process is not clear.

A number of workers (5,6,8,11,12) have independently shown that the pancreatic hormones, insulin and glucagon may play an important role in regulating liver regeneration. For example, work by Price et al and Whittemore et al (5,6) showed that glucagon could normalize the timing of liver regeneration which is delayed in partially hepatectomized depancreatized dogs (5) and eviscerated rats (6). More recently Bucher and Swaffield (13), also working with partially hepatectomized eviscerated rats, indicated that glucagon and insulin were synergistic regulators of the rate of regeneration but were not the humoral factors which initiate liver cell proliferation.

Thyroid hormone (tri-iodo-thyronine) stimulated hepatocellular hyperplasia (14) and while growth hormone might also influence liver regeneration (15) the regenerative response to partial hepatectomy is nearly normal in hypophysectomized rats (16).

Despite the interest in the role of hormones in liver regeneration, few measurements of serum hormone levels have been made following partial hepatectomy (13). In the present report we measured simultaneous changes in serum levels of insulin, glucagon, growth hormone (somatotrophin) and thyroxine following sham operation and partial hepatectomy in the same group of rats.

MATERIALS AND METHODS

Animals - Eight male Sprague Dawley rats (200 ± 10 g (fig. 1) were housed in wire bottom cages (2 to a large cage) at a constant temperature of 37°C in air conditioned windowless rooms with a 12 hour night and day cycle. The animals were allowed free access to standard laboratory chow and water, throughout the experiments.

Surgery - Partial hepatectomy (65 ± 5%) was performed by the method of Higgins and Anderson (17) under light ether anesthesia. Sham operations consisted of an abdominal incision (1" long) and manipulation of the liver. All operations were performed between 9 and 11:00 am to diminish the influence of diurnal variations on hormone levels (18).

Experimental design - Blood (2 ml) was collected from the right or left juglar vein under light ether anesthesia in Trasylol (100 u/ml blood) (FBA Pharmaceutical, Bay Chem. Corp. N.Y.), 24 hours before and at timed intervals following surgery. Sera were collected and stored at -20°C until assayed.

All animals initially underwent sham operation (see above). 2 ml of blood were then collected from each animal at 6,24,48 and 72 hours and their weight recorded. At the end of the three days, the animals were allowed to recover for a period of six weeks.

A second control blood (2 ml) was then collected. Partial hepatectomies were performed 4 days after this blood collection. Samples of blood (2 ml) were collected and the animal's weight recorded at 6,24,48 and 72 hours following partial hepatectomy. After the second 3 day experimental period, the rats were allowed to recover for a further 9 days, and final blood samples were collected.

Hormone assays - All hormones were detected by radioimmunoassay except TT_4 which was measured using a competitive binding protein assay. Insulin by the method of Morgan et al, (19) and glucagon (20) by a modified procedure using a second antibody instead of charcoal for the separation of free and bound antibody. Growth hormone (21) was assayed in the laboratory of Dr. V. Fang and thyroxine (22,23) in the laboratory of Dr. L. DeGroot.

RESULTS

Figure 1. shows the effect of sham operation and partial hepatectomy on body weight (1A), serum insulin (1B), glucagon (1C), growth hormone

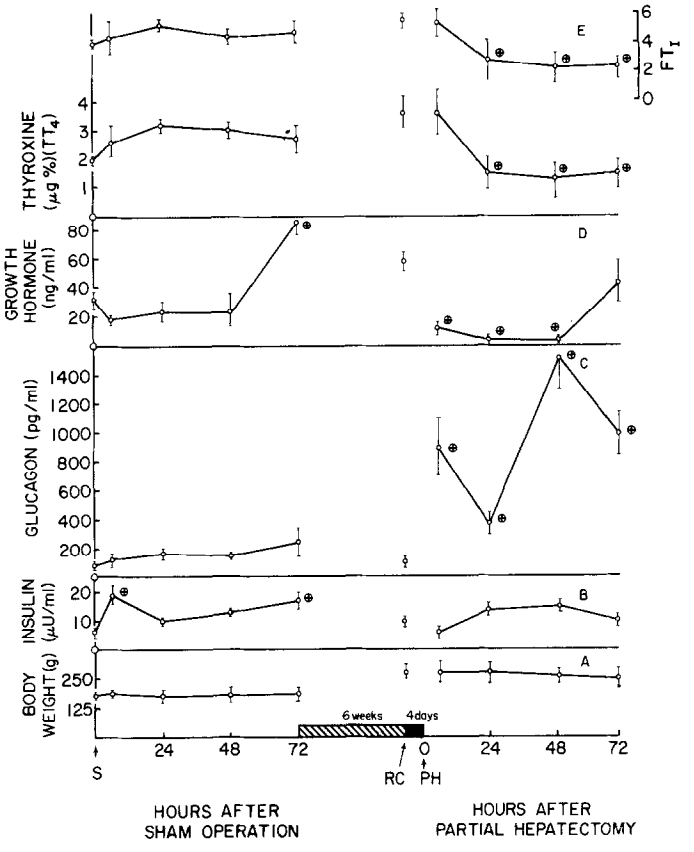

Fig. 1. Body Weight and Serum Hormone Levels in Rats Following Sham or 70% Partial Hepatectomy.

Groups of 8 rats were either sham operated or partially hepatectomized. Blood was withdrawn from these animals at the times indicated. The serum was assayed for hormones as described in the text.

The animals were first sham operated, the course of hormone changes followed for 3 days. The animals were then allowed a 6 week recovery period (the light hatched section indicated on the abscissa). Control samples of blood were withdrawn and the animals were allowed a further 4 days recovery (the dark hatched section). Partial hepatectomies were then performed and the animals followed for a further 3 days by serial blood withdrawals. The rats were then allowed to recover for a further 9 days for final control blood samples.

Data is expressed as the mean $\pm$ standard deviation, and those numbers differing from controls by P 0.01 or less in a paired Student t-test are considered significant and marked thus ⊕

A - Body weight (g)
B - Insulin levels (uU/ml)
C - Glucagon levels (pg/ml)
D - Growth hormone (somatotrophin) levels (ng/ml)
E - Thyroxine levels (total thyroxine TT_4 ug%, and free thyroxine index FT_I)
S - sham operation, RC - recovered control, PH - partial hepatectomy

(somatotrophin (1D) and both total and free thyroxine levels (1E).

Following sham operation, all animals maintained body weight but did not gain weight until the recovery period (Fig. 1A). Following partial hepatectomy all animals showed a steady weight during the experimental period (Fig. 1A).

The most dramatic change in serum hormone levels occurred with glucagon which did not rise significantly above control values following sham operation but increased 11 fold shortly after partial hepatectomy (Table 1). After falling at 24 hr. (Fig. 1C), glucagon levels rose again to a maximum of 1500 $\pm$ 200 pg/ml at 48 hours which was sustained for a further 24 hours (Fig. 1C). Serum collected 9 days after surgery still showed an elevated level of 450 $\pm$ 127 pg/ml.

Insulin levels fluctuated but were significantly increased at 6 and 72 hours following sham operation. Following partial hepatectomy there was no significant rise of the hormone level above the levels in sham controls (Table 1, Fig. 1B).

Growth hormone increased at 72 hrs following sham operation but fell to very low levels 48 hours after partial hepatectomy (Table 1). A return to control levels was seen at 72 hours (Fig. 1D).

Neither total thyroxine (TT_4) nor free thyroxine (FT_I) levels were influenced by sham operation (Table 1). However, 24 hr following partial hepatectomy, both values fell precipitously to 50% of control level and remained at a low level for the remainder of the experiment. (Table 1, Fig. 1E).

Boiling of the sera at pH 5.5 for 2 minutes destroyed the immunoreactivity of thyroxine and insulin but not growth hormone (Table 2). The glucagon level was reduced approximately four fold to levels similar to those found in the sera from sham operated animals.

DISCUSSION

This study suggests that insulin, growth hormone, and thyroxine are

Table 1. Control Values and Peak Changes in Body Weight and Serum Hormone Levels in Sham and Partially Hepatectomized Rats.

Animal Treatment	Insulin (uU/ml)	Glucagon (pg/ml)	Growth Hormone (ng/ml)	Total (TT_4) Thyroxine (ug%)	Free (FT_I) Thyroxine
Normal	5.8 ± 0.9	72.7 ± 5.3	32.0 ± 4.9	2.1 ± 0.6	4.0 ± 0.1
Sham	18.7 ± 1.6 (72)*+	262.5 ± 74.0 (72)**	87.5 ± 10.5 (72)**	3.3 ± 0.2 (24)**	5.3 ± 0.3 (24)**
Recovered Control	10.2 ± 1.0	112.5 ± 10.3	69.3 ± 4.1	3.7 ± 0.3	5.7 ± 0.2
Partially Hepatectomized	15.2 ± 2.3 (24-48)**	1500 ± 200 (48)*+	4.5 ± 1.4 (48)*+	1.3 ± 0.6 (24)**+	2.4 ± 1.1 (72)**+
Post 9 Day Recovery Period		450 ± 127+	23 ± 2	1.9 ± 0.3	3 ± 1

* Animal treatments are described in the text.

** Figures in parentheses indicate the time of the peak change after surgery. (expressed in hours)

\+ Indicates a p value of 0.01 or less by a paired Student t-test.

Table 2 - The Effect of Boiling on Rat Serum Hormone Levels

Hormone	Untreated Serum*	Boiled Serum**
Insulin	36.8 ± 3.3	2.5 ± 0.1+
Glucagon	450 ± 127	105 ± 18+
Growth Hormone	23.0 ± 2.0	17.0 ± 3.1
Thyroxine (TT_4)	1.95 ± 0.3	0.4 ± 0.02+
Thyroxine (FT_I)	3.0 ± 1.0	0.5 ± 0.1+

* Collected at end of the final 9 day recovery period
** Sera were adjusted to pH 5.5 with 1 N HCL and boiled for 2 minutes, followed by centrifugation. The supernatant solution was used for hormone assays.
\+ Significantly lower than controls ($p < 0.01$ in a Student t-test)

not primary stimulators of cell proliferation in the liver since serum insulin levels did not rise significantly in the first 72 hours after partial hepatectomy while growth hormone and thyroxine actually fell (Fig. 1, Table 1). However, the large increases in glucagon levels following removal of two thirds of the liver suggest that this hormone might influence liver regeneration, an observation which would be consistent with previous concepts that pancreatic hormones play a role in this process (5,6,12,13). Data of Leffert (12) and Bucher and Swaffield (13) suggests that insulin and glucagon interact in a synergistic fashion to facilitate liver regeneration. However, we observed only a small rise in insulin levels after partial hepatectomy which were not significantly different from those observed after the sham operation. Our first measurements were obtained 6 hours after partial hepatectomy, and earlier changes might be different. Bucher and Swaffield observed a fall in portal insulin to negligible levels 2 hours after removal of two-thirds of the liver in the rat and therefore concluded that insulin was not necessary for the very early events of liver regeneration, despite the demonstration that both insulin and

glucagon normalized the timing of the regenerative response that was markedly delayed but not abolished in the partially hepatectomized eviscerated rat (13).

It is also possible that changes in serum levels of hormones are a secondary response to the removal of two thirds of the liver. The fall in FT_I values (with a concomitant increase in resin uptake ratio (RUR)- not shown) suggests that there is a decline in binding protein production by the liver remnant, while the decrease in free thyroxine may reflect a disturbance in the pituitary axis leading to a reduction in release of the thyroid stimulating hormone from the pituitary and therefore a decrease in thyroxine production by the thyroid (22).

Diminished binding of hormones to liver plasma membranes might also influence serum hormone levels and could independently account for the rise in serum levels of glucagon. Leffert (24) has found that liver plasma membrane preparations from 24 hr regenerating liver tissue bind 40-60% less I^{125}-glucagon than liver plasma membranes from sham operated animals, which would support this hypothesis. Furthermore, it is well known that the liver is an important organ for the degradation of both glucagon (25) and insulin (26). The marked increase in the former but not the latter hormone is of interest in this regard and suggests that the feedback control of insulin through circulating glucose and amino acid levels may be more precisely regulated than that of glucagon.

Previous studies from our laboratory have described the appearance of a polypeptide (s) in serum, 12-36 hrs following partial hepatectomy in rats, which stimulates hepatic DNA synthesis and which is resistant to boiling and dialysis. (RF_1) (9). The present study indicates that this factor is not related to growth hormone, insulin, or thyroxine since these hormones do not increase in serum from partially hepatectomized animals during this time period. Although changes in

glucagon levels parallel the appearance of RF_1, glucagon itself is not a very effective stimulant of DNA synthesis in the intact rat (14). In addition, although immunologic and biologic activity can not always be equated, glucagon immunoreactivity is reduced four fold by boiling of the sera (Table 2) suggesting that it also is not related to the heat stable humoral factors from the partially hepatectomized rat which stimulate hepatic DNA synthesis. However, plasma glucagon has recently been shown to comprise a number of components (27), and further work will be required to determine whether one or more of these components is resistant to boiling and may rise disproportionately following partial hepatectomy.

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REFERENCES

1. Moolten, F.L. and Bucher, N.L.R. Sci. (1967), 158, 272.
2. Grisham, J.W., Leong, G.F., Albright, N.L. and Emerson, J.D. Cancer Res. (1966), 26, 1476.
3. Levi, J.U. and Zeppa, R. Ann. Surg. (1971), 174, 364.
4. Sakai, A. Nat. (Lond) (1970), 228, 1186.
5. Price, J.B., Takeshige, K., Max, M.M. and Vorhees, A.B. Surgery (1972), 72, 74.
6. Whittemore, A.D., Kasuya, M., Fodor, P.B. and Price, J.B. Surg. Forum. (1973), 24, 384.
7. Bucher, N.L.R. and Swaffield, M.N. Cancer Res. (1973), 33, 3189.
8. Starzl, T.E., Francavilla, A., Halgrinison, C.G., Francavilla, F.R., Porter, K.A., Brown, T.H. and Putnam, C.W. Surg. Gynec. Obstet. (1973), 137, 179.
9. Morley, C.G.D. and Kingdon, H.S. Biochim. Biophys. Acta (1973), 308, 360.
10. LaBrecque, D.R. and Pesch, L.A. (1975), J. Physiol (Lond), 248, 273.
11. Orloff, M.J., Lee, S., Charters, A.C., Chandler, J.G., Sgro, J.C., Grambort, D.E. and Broelsch, C.E. (1974), Gastroenterol. 66, 755.
12. Leffert, H.L. (1974) J. Cell. Biol., 62, 792.
13. Bucher, N.L.R. and Swaffield, M.N. Proc. Nat. Acad. Sci. (U.S.) (1975), 72, 1157.
14. Short, J., Brown, R.F., Husakova, A., Gilbertson, J.R., Zemel, R., and Lieberman, I. J. Biol. Chem. (1972), 247, 1757.
15. Echave-Ilanos, J.M. and Surur, J.M. Experentia, (1971), 27, 574.
16. Bucher, N.L.R. Int. Rev. Cytol. (1963), 15, 245.
17. Higgins, G.M. and Anderson, R.M. Arch. Pathol. (1931), 12, 186.
18. Hopkins, M.A., Campbell, M.A., Barbiroli, B., and Potter, V.R. Biochem. (1973), 136, 115.
19. Morgan, C.R. and Lazarow, A. (1963), Diabetes, 12, 115.
20. Ungar, R.H., Aguilar-Parada, E., Muller, W.A., and Einsenstraut, A.M. (1970) J. Clin. Invest. 49, 837.

21. Fang, V.S., Refetoff, S. and Rosengield, R.L. Endocrinol. (1974), 95, 991.
22. Murphy, B.E.P. and Jachan, C. J. Lab. Clin. Med. (1965), 66, 161.
23. Robin, N.I., Hagen, S.R., Collaco, F., Refetoff, S. and Selenkow, H.A. Hormones (1971), 2, 266.
24. Leffert, H.L. Proc. Soc. Exp. Biol. Med. (1975) In press.
25. Lefebre, P.J. and Ungar, R.H., (eds), Glucagon: Molecular Physiology Clinical and Therapeutic implications. (1972), p.47 Pergamon Press, Oxford, U.K.
26. Rubenstein, A.H., Pottenger, L.A., Mako, M., Getz, G.S. and Steiner, D.F. (1972) J. Clin. Invest. 51, 912.
27. Kuku, S.F., Zeidler, A., Emmanouel, D.S., Katz, A.I., Levin, N.W., Tello, A. and Rubenstein, A.H. (1975). Abstract in press.