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CHANGES IN PLASMA MEMBRANE ENZYME ACTIVITIES DURING LIVER REGENERATION IN THE RAT

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Summary

Changes in activities of plasma membrane enzymes during liver regeneration may be related to the maintenance of hepatic function or to the regulation of cell proliferation. Plasma membranes were isolated from rat livers at various times after partial hepatectomy, and the specific activities of alkaline phosphatase, $(Na^+ + K^+)$-ATPase, leucine aminopeptidase, 5′-nucleotidase, and adenylate cyclase (basal and with glucagon or epinephrine) were measured. Alkaline phosphatase and $(Na^+ + K^+)$-ATPase activity increased 3.6-fold and 2-fold respectively, during the first 48 h after partial hepatectomy. The time of onset and duration of change suggest that these increases in activity are involved in the maintenance of bile secretion. Decreases in leucine aminopeptidase activity at 48—108 h and in 5′-nucleotidase activity at 12—24 h were observed, which may be involved in the restoration of protein and accumulation of RNA. The basal activity of adenylate cyclase increased after partial hepatectomy. The response of adenylate cyclase to epinephrine showed a transitory increase between 36 and 108 h after surgery, while the response to glucagon was decreased by approximately 50% at all time points through 324 h after surgery. These changes in the hormone responsiveness of adenylate cyclase are similar to those previously observed in fetal and preneoplastic liver.

Introduction

Partial hepatectomy of rats is followed by an initial burst of rapid compensatory growth of the remaining liver lobes. After the first two or three rounds of cell division, the frequency of mitosis decreases and returns to normal by two weeks after surgery. At this time, the restoration of liver mass is essen-

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tially complete [1]. The rapid liver growth which occurs after partial hepatectomy is not regeneration, since the increase in mass is accomplished by enlargement of the residual lobes rather than by replacement of the excised lobes; however, regenerating liver is the common descriptive term for this process.

Regenerating liver provides an opportunity to study the control of cellular proliferation and has been proposed as an experimental model for neoplasia [1]. On the other hand, regenerating liver continues to carry out hepatic functions, often at rates equivalent to or higher than normal liver [2,3]. Changes in soluble enzymes of regenerating liver have been well documented (reviewed in refs. 1 and 4), but the enzymes of the plasma membrane have not been extensively studied. This study examines changes in specific activities of some plasma membrane enzymes during the course of liver regeneration in order to provide insight into their relationship to the maintenance of hepatic function or the regulation of cellular proliferation.

Methods and Materials

Partial hepatectomy of rats

Male Wistar rats weighing 250—300 g were used for all experiments. Rats were fed Wayne Lab-Blox and tap water ad libitum and were kept on a controlled lighting schedule with a 12-h dark period from 7.00 p.m. to 7.00 a.m. Partial hepatectomies and sham operations were always performed between 9.30 and 10.30 p.m. Surgery was carried out under antiseptic conditions using the procedure of Higgins and Anderson [5]. Ligation and excision of the median and left lateral lobes of liver constituted partial (70%) hepatectomy. For sham operation, these lobes were delivered to the outside and allowed to slip back into the body cavity. Rats were given in solution of 20% glucose ad libitum in place of water for the first 12 h after surgery.

Preparation of liver plasma membranes

Normal control rats (no operations), sham-operated control rats (12 and 48 h after surgery) and partially hepatectomized rats (12 through 324 h after surgery) were killed by decapitation. Livers were perfused in situ with ice-cold phosphate-buffered saline until blanched. Membranes were prepared under ice-cold conditions, using a procedure similar to that of Toda et al. [6] but employing a single four-part gradient for density gradient centrifugation instead of three successive two-part gradients. Medium A consisted of 0.25 M sucrose, 5 mM Tris, 1 mM $MgCl_2$, pH 7.4; Medium B was the same buffer without $MgCl_2$ [6]. 3.5 g of perfused liver, taken from the right lateral and caudate lobes, were homogenized in 35 ml of Medium A using 15 strokes of a loose pestle in a hand-held Dounce homogenizer. The homogenate was filtered through cheesecloth, diluted to 70 ml with Medium A, and centrifuged 10 min at $1500 \times g$. The pellet was washed twice in Medium B by homogenizing 3 strokes and centrifugation as before. The washed pellet was homogenized in 10—15 ml of Medium B, and sufficient 66% sucrose was added to make the suspension 48% (w/w) in sucrose. All sucrose solutions were buffered with 5 mM Tris, pH 7.5. The sample was divided between two tubes for the Beckman SW-27 rotor. Over the sample in 48% sucrose, a discontinuous gradient consisting of 7 ml of 45% (w/w) sucrose, 8 ml of 41% (w/w) sucrose, and Medium

B to fill the tubes, was constructed. Tubes were spun 1.5 h at 82500 × *g*. The material at the 41% sucrose : Medium B interface ($d \leqslant 1.18$) was collected as plasma membranes. For further purification, membranes were washed and suspended in Medium B and were made 45% in sucrose. The suspension was divided between two SW-27 rotor tubes and overlayed with a discontinuous gradient consisting of 10 ml of 41% sucrose and Medium B to fill the tubes. Centrifugation and collection of membranes was repeated as before. Plasma membranes were washed twice and suspended in Medium B, divided into aliquots, and stored at −50° C.

Marker and enzyme assays

DNA was used as a marker for nuclear material, succinate dehydrogenase activity as a marker for mitochondria, and glucose-6-phosphatase activity as a marker for endoplasmic reticulum. Plasma membrane enzyme activities measured were 5′-nucleotidase, alkaline phosphatase, ($Na^+ + K^+$)-ATPase, leucine aminopeptidase and adenylate cyclase.

Before determination of DNA, samples were precipitated and washed twice with ice-cold trichloroacetic acid, as described by Hutchison et al. [7]. DNA was extracted by heating at 90° C for 30 min in 0.3 M trichloroacetic acid [8]. Insoluble material was removed by centrifugation, and DNA in the supernatant was determined by the diphenylamine method of Burton [9]. DNA from calf thymus (Type V, sodium salt, Sigma Chemical Co.) was used to prepare standards.

Succinate dehydrogenase activity was measured by a modification of the method of King [10]. The assay mixture contained 0.1 M sodium phosphate buffer, 40 mM succinate, pH 7.8, 0.1% bovine serum albumin and 2 mM $K_3Fe(CN)_6$. Concentrations of $K_3Fe(CN)_6$ above 2 mM were found to be inhibitory for the unpurified enzyme. The decrease in absorbance at 420 nm, followed at 25° C in a Gilford-DU spectrophotometer, was converted to μmol of succinate oxidized by the method of King [10].

All other enzyme assays were conducted at 37° C with shaking, in the presence of saturating substrate. Except where stated otherwise, assay solution (final concentrations given) and sample were pre-incubated 3 min before the addition of substrate. Time and protein concentration were chosen such that linear initial reaction rates were measured. Reagent blanks to correct for spontaneous hydrolysis of substrate were tubes to which sample was added after incubation and termination of reaction. All tubes for assays involving measurement of inorganic phosphate were acid-washed before use.

Glucose-6-phosphatase activity was measured according to the method of Baginski et al. [11] using glucose-6-phosphate, disodium salt (Sigma).

5′-Nucleotidase activity was determined in an assay mixture containing 80 mM Tris, 10 mM $MgSO_4$ and 5 mM AMP (disodium salt, Boehringer-Mannheim) [12] at pH 7.5, the experimentally determined pH optimum. After 7—15 min incubation, the reaction was stopped by the addition of ice-cold Chen reagent [13]. For determination of phosphate, color was developed at 45° C for 20 min, particulate material was removed by centrifugation and the absorbance of the supernatant at 820 nm was measured. Protein in the concentrations used did not interfere with the determination of phosphate.

Alkaline phosphatase activity was determined using 0.2 M Tris, 5 mM $MgSO_4$, 70 mM KCl, pH 8.9 [14], and 5 mM *p*-nitrophenylphosphate (Sigma 104 phosphatase substrate). The reaction was stopped after 20 min by adding 0.2 M NaOH. Particulate material was removed by centrifugation and the absorbance of the supernatant at 420 nm was measured. E_{420} for *p*-nitrophenol was 14 $mM^{-1} \cdot cm^{-1}$.

$(Na^+ + K^+)$-ATPase was determined in a mixture containing 80 mM Tris, 5 mM $MgSO_4$, 0.2 mM EDTA, 3 mM ATP (sodium salt, Boehringer-Mannheim), pH 7.5, in the presence or absence of 10 mM KCl and 100 mM NaCl. After 10 min incubation, the reaction was terminated by adding ice-cold trichloroacetic acid to a final concentration of 10% and placing the tubes on ice. After centrifugation, inorganic phosphate was determined in the supernatant by the method of Bonting et al. [15]. $(Na^+ + K^+)$-ATPase activity was taken to be the difference between total ATPase (with KCl and NaCl) and Mg^{2+}-ATPase (without KCl and NaCl).

Leucine aminopeptidase activity was measured by the method of Goldbarg and Rutenburg [16], using L-leucyl-β-naphthylamide (Sigma) as substrate.

Adenylate cyclase basal activity was measured in an assay mixture containing 40 mM Tris, 5 mM $MgCl_2$, 10 mM theophylline (Sigma), 3 mM ATP, pH 7.5 [17], and an ATP-regenerating system consisting of 10 mM phosphoenolpyruvate (tricyclohexylammonium salt, Gibco) and 15 units/ml of pyruvate kinase (Boehringer-Mannheim). Polypropylene tubes were used throughout. Sample was not pre-incubated before the addition of substrate. For studies of hormone responsiveness, the following substances (final concentrations given) were dissolved in 1 mM HCl and added in a volume of 5 μl immediately before substrate: 1 μM glucagon (Eli Lilly and Co.), 100 μM L-epinephrine bitartrate (California Diagnostics) and 1 mM D,L-propranolol (Sigma). Addition of 5 μl of 1 mM HCl alone had no effect on basal activity. After 15 min incubation, the reaction was stopped by immersing tubes in boiling water for 3 min. After centrifugation, adenosine 3′ : 5′-cyclic monophosphate (cyclic AMP) was determined in an aliquot of supernatant using a cyclic AMP assay kit from Diagnostic Products Corp. This assay is based on a modification by Tovey et al. [18] of the method of Gilman [19].

Protein was measured by the method of Lowry et al. [20] using crystallized bovine serum albumin (Sigma) as standard.

Results and Discussion

Yield and purity of plasma membranes

The average yield of plasma membranes from normal liver was 1.05 ± 0.19 mg/g wet weight of liver (mean ± S.D. of 8 preparations) and from regenerating livers was 1.12 ± 0.18 mg/g (30 preparations). These were membranes which had undergone two density gradient centrifugations, as described in Methods and Materials. To assess the purity of membranes prepared by this method, DNA and marker enzyme activities for plasma membranes, endoplasmic reticulum, and mitochondria were measured in homogenates and plasma membranes from normal rat livers, as shown on Table I. The specific activities of 5′-nucleotidase and alkaline phosphatase were increased 38-fold and 21-fold,

TABLE I

MARKER ENZYME ACTIVITIES AND DNA CONTENT OF LIVER HOMOGENATES AND PLASMA MEMBRANES

Homogenates and plasma membranes were prepared from livers of normal rats, and assays were carried out as described in Methods and Materials. Specific activities of 5'-nucleotidase and glucose-6-phosphatase are expressed as μmol phosphate released per min/mg protein. Specific activity of alkaline phosphatase is expressed as μmol *p*-nitrophenol released per min/mg protein. Specific activity of succinate dehydrogenase is expressed as μmol succinate oxidized per min/mg protein. One unit of activity is one μmol of product released or substrate oxidized per minute. DNA content is expressed as μg/mg protein and as mg/g liver. Numbers represent mean ± S.D. for preparations from 3 rats (DNA in homogenate) or 5 rats (DNA in plasma membranes and all enzyme activities).

Enzyme activity or DNA	Homogenates		Plasma membranes	
	Specific activity or μg/mg protein	Units/g liver or mg/g liver	Specific activity or μg/mg protein	Units/g liver
5'-Nucleotidase	0.037 ± 0.004	5.83 ± 0.56	1.41 ± 0.16	1.54 ± 0.23
Alkaline phosphatase	0.0056 ± 0.0002	0.879 ± 0.032	0.120 ± 0.021	0.129 ± 0.012
Glucose-6-phosphate	0.023 ± 0.004	3.52 ± 0.62	0.022 ± 0.004	0.026 ± 0.005
Succinate dehydrogenase	0.046 ± 0.005	7.29 ± 0.89	0.021 ± 0.006	0.025 ± 0.006
DNA	9.1 ± 0.4	1.43 ± 0.06	none detected	

respectively, in plasma membranes as compared to homogenates, while the recoveries of these enzymes in plasma membranes were 26% and 15% of total units of homogenate. Differences in the purification and recovery of these two plasma membrane marker enzymes have been observed previously [6,21,22] and may reflect the presence of alkaline phosphatases in other subcellular organelles or the selective loss during membrane preparation of plasma membrane vesicles derived from the bile canaliculi, a region enriched in alkaline phosphatase [22]. Glucose-6-phosphatase activity was not enriched, and less than 1% of the total activity of homogenate was recovered, in the plasma membrane preparation. Based on published values for glucose-6-phosphatase activity in purified microsomes, this specific activity represents about 5% contamination by endoplasmic reticulum [23]. The specific activities of 5'-nucleotidase, alkaline phosphatase and glucose-6-phosphatase in membranes prepared by this method are very similar to those found in plasma membranes prepared by other methods [6,21,23,24]. Less than 0.5% of the total units of the mitochondrial marker succinate dehydrogenase was recovered in the plasma membrane preparation and the specific activity was approximately half that of homogenate. These results are similar to those of Brown et al. [25] who measured succinate dehydrogenase using a different method. DNA was not detected in plasma membranes under assay conditions which would have detected 3 μg of DNA in 200 μg of membrane protein.

Due to the requirements for large amounts of plasma membrane material in assays for DNA, glucose-6-phosphatase and succinate dehydrogenase, it was not practical to measure these markers for contaminants in each preparation of membranes throughout the course of liver regeneration. Contamination by mitochondria was judged to be the most serious potential problem, since as much as 30% of total succinate dehydrogenase activity was routinely found in the 1500 × *g* pellet, as compared to less than 5% of total glucose-6-phosphatase

activity (data not shown). Therefore, only succinate dehydrogenase activity was measured in each preparation of control and regenerating rat liver plasma membranes before freezing. The average specific activity in regenerating rat liver plasma membranes was 0.024 ± 0.006 μmol/min per mg protein (mean ± S.D. of 30 preparations), and in no case was the specific activity in liver membranes from a single time point during regeneration statistically different from that of control membranes.

Changes in activities of plasma membrane enzymes after partial hepatectomy

Table II shows the effect of sham operations on enzyme activities in rat liver plasma membranes. No effect of sham operation was seen except on $(Na^+ + K^+)$-ATPase activity, which was significantly decreased at 12 and 48 h after sham operations.

Figs. 1—5 show changes in specific activities of plasma membrane enzymes from livers obtained at intervals between 12 and 324 h (13.5 days) after partial hepatectomy.

Alkaline phosphatase activity (Fig. 1) increased to a maximum of 3.6 times control activity at 30—36 h after partial hepatectomy. Specific activity at all times from 12 to 60 h was significantly greater than control. This result confirms an earlier observation of an increase in activity of alkaline phosphatase in regenerating liver homogenates [26], as well as single time-point observations in liver plasma membranes [21,27] from partially hepatectomized rats.

5′-Nucleotidase activity (Fig. 2) was significantly lower than control values ($P \leqslant 0.05$) in membranes isolated from rat livers at 12, 18 and 24 h after partial hepatectomy. This result is consistent with the finding of Sirica et al. [21] that 5′-nucleotidase activity in plasma membranes isolated at 24 h after surgery is decreased. In contrast, Masuda and co-workers [28], measuring enzyme activ-

TABLE II

EFFECT OF SHAM OPERATIONS ON PLASMA MEMBRANE ENZYME ACTIVITIES

Liver plasma membranes from unoperated rats and sham-operated rats 12 and 48 h after surgery were prepared as described in Methods and Materials, omitting the second sucrose density gradient centrifugation. Enzyme assays were performed as described in Methods and Materials. Specific activities of 5′-nucleotidase and $(Na^+ + K^+)$-ATPase are expressed as μmol phosphate released per min/mg protein. Specific activity of alkaline phosphatase is expressed as μmol *p*-nitrophenol released per min/mg protein. Specific activity of leucine aminopeptidase is expressed as μmol β-naphthylamine formed per min/mg protein. Specific activity of adenylate cyclase is expressed as pmol of cyclic AMP formed per min/mg protein. Activities are given as mean ± S.D. for plasma membranes from 6 rats (no operation) or 3 rats (sham operations).

Enzyme activity	No operation	Sham operations	
		12 h	48 h
5′-Nucleotidase	1.29 ± 0.15	1.09 ± 0.14	1.44 ± 0.24
Alkaline phosphatase	0.103 ± 0.015	0.120 ± 0.047	0.104 ± 0.022
$(Na^+ + K^+)$-ATPase	0.29 ± 0.04	0.16 ± 0.02 *	0.18 ± 0.10 *
Leucine aminopeptidase	0.100 ± 0.023	0.108 ± 0.013	0.130 ± 0.032
Adenylate cyclase (basal)	23 ± 5	not done	23 ± 3
Adenylate cyclase (+ glucagon)	779 ± 12	not done	624 ± 71

* Significantly different from unoperated group at $P < 0.05$.

ities in plasma membranes isolated at daily intervals after partial hepatectomy, detected no decrease at one day but observed a significant increase in 5'-nucleotidase activity at 3—5 days. A slight increase in activity at 108 and 156 h (4.5 and 6.5 days) was seen in the present study, but this change was not statistically significant.

$(Na^+ + K^+)$-ATPase activity (Fig. 3) was significantly greater than control ($P \leqslant 0.05$) in membranes isolated 30—48 h after partial hepatectomy, with a

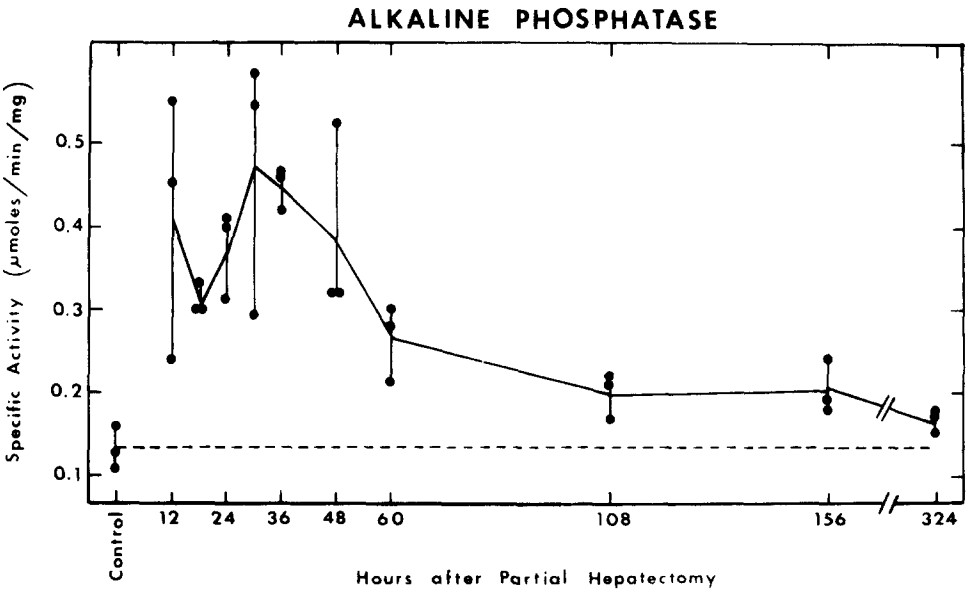

Fig. 1

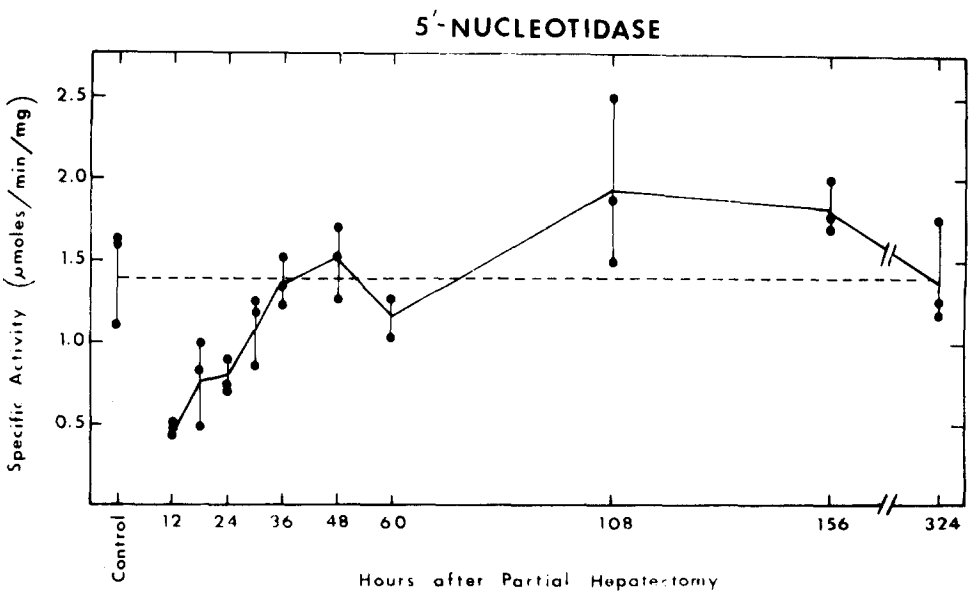

Fig. 2

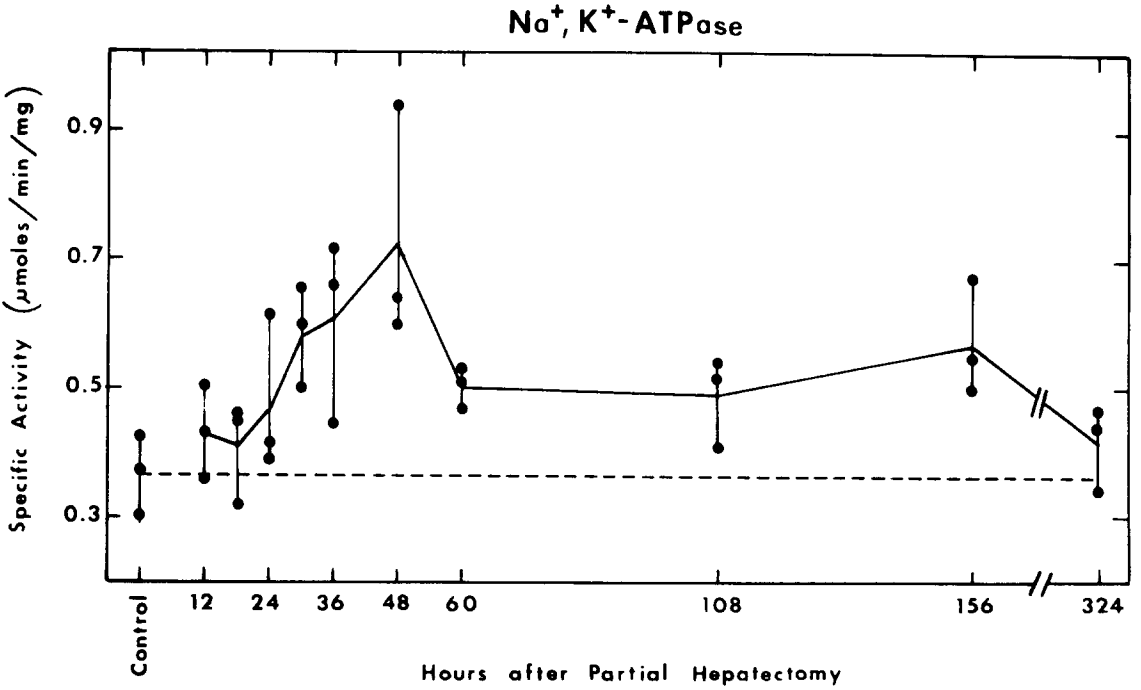

Fig. 3

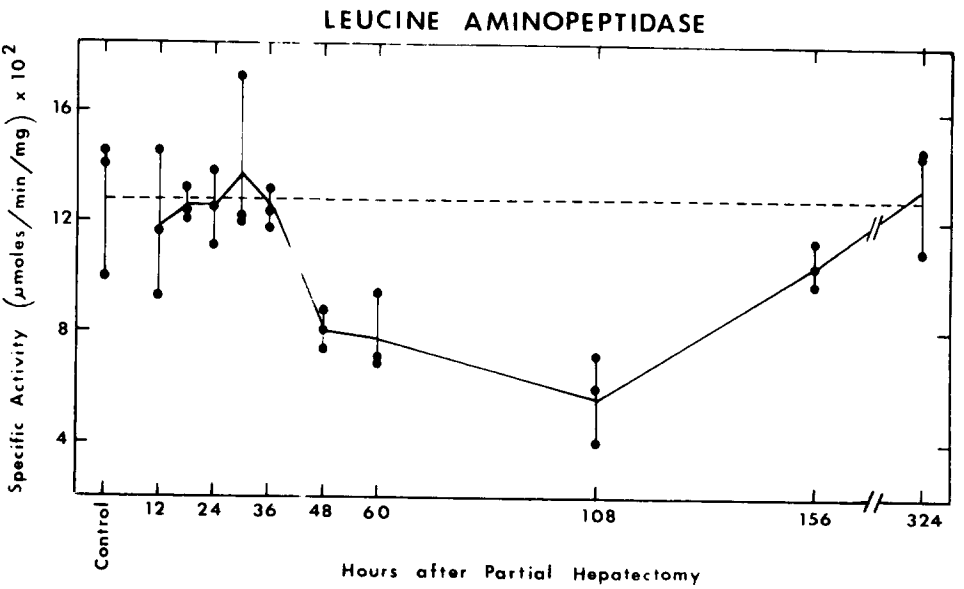

Fig. 4

Figs. 1—4. Activities of enzymes of liver plasma membranes isolated at various times after partial hepatectomy. Plasma membranes were isolated from livers of unoperated control and partially hepatectomized rats as described in Methods and Materials. Specific activities are expressed as μmol of product formed per min/mg protein (see Table II). Each solid circle represents the average of triplicate determinations of enzyme activity in membranes from a single liver. Vertical lines connect the specific activity values for membranes from rats sacrificed at each time point. The solid horizontal line passes through the average specific activity for each time point. The dashed horizontal line, showing the average specific activity in liver plasma membranes from unoperated control rats, is included for reference.

maximum specific activity of two times control at 48 h. This result is consistent with a previous report of increased activity in rat liver homogenates at 1—6 days after partial hepatectomy and increased ability of regenerating liver slices to take up ^{86}Rb, as compared to normal liver slices [29]. In contrast, Masuda et al. [28] observed no change in $(Na^+ + K^+)$-ATPase activity over a 10-day period after partial hepatectomy.

Leucine aminopeptidase activity (Fig. 4) remained unchanged through the first 36 h after surgery, but was significantly lower than control ($P \leqslant 0.05$) in membranes isolated at 48—108 h after partial hepatectomy. This observation is the opposite of the result of Masuda et al. [28], who reported an increase in activity at 1—3 days.

The basal activity of adenylate cyclase (Fig. 5) was significantly greater than control ($P \leqslant 0.05$) in membranes isolated at all time points between 24 and 324 h after partial hepatectomy, except for the 30-h time point. Adenylate cyclase activity was not stimulated by epinephrine in control membranes or membranes from livers 12—30 h after partial hepatectomy, as shown in Fig. 5. Stimulation of activity occurred in membranes isolated 36—108 h after surgery, with maximum stimulation of 1.7-fold at 60 h. The effect of epinephrine on adenylate cyclase activity in 60-h regenerating rat liver plasma membranes was blocked by the addition of the β-adrenergic antagonist propranolol to the assay mixture (specific activity = 36 ± 6 pmol/min per mg protein). Propranolol alone had no effect on adenylate cyclase activity.

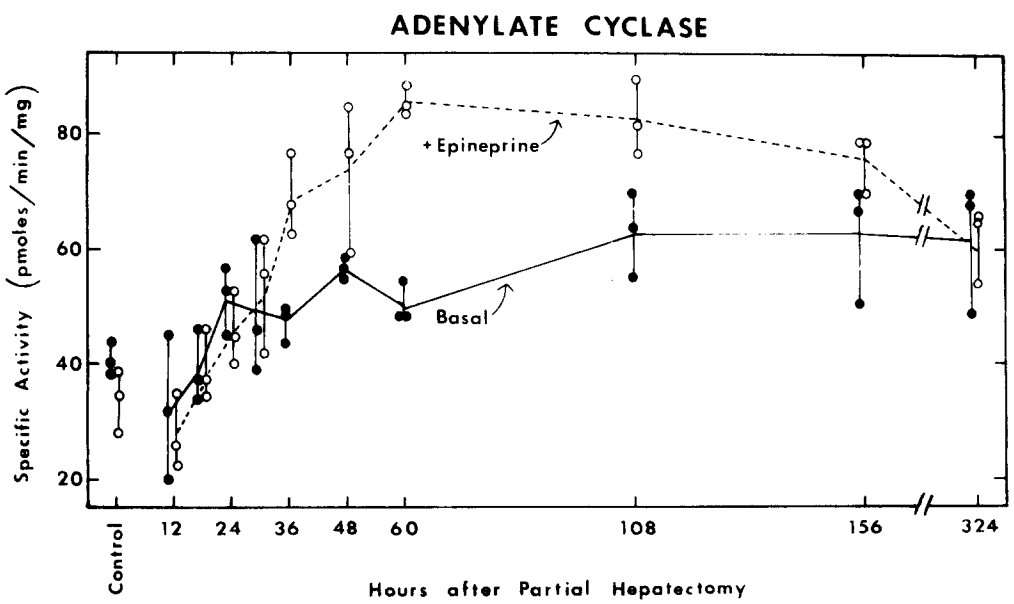

Fig. 5. Basal (●) and epinephrine-stimulated (○) activity of adenylate cyclase in liver plasma membranes isolated at various times after partial hepatectomy. Plasma membranes were isolated from livers of unoperated control and partially hepatectomized rats as described in Methods and Materials. Specific activity is expressed as pmol of cyclic AMP formed per min/mg protein. Each circle represents the average of duplicate determinations of enzyme activity in membranes from a single liver. Vertical lines connect the specific activity values for membranes from rats sacrificed at each time point. Solid and dashed horizontal lines pass through the average specific activity for each time point.

A decrease [30] or a loss [31] of epinephrine response of adenylate cyclase in plasma membranes, as compared to liver homogenate, has been observed by others. Basal and epinephrine-stimulated adenylate cyclase activities were therefore measured in the homogenates from which control and 60-h regenerating liver plasma membranes were isolated. As shown in Table III, the basal activity of adenylate cyclase in homogenates of 60-h regenerating rat liver was nearly three times that in unoperated control homogenates, which is consistent with the elevation of basal activity in membranes isolated from the 60-h homogenate. Control homogenates show a 5.6-fold stimulation of adenylate cyclase activity by epinephrine, while regenerating liver homogenates show a 7.3-fold increase.

Leoni et al. [32] found an increase in basal activity of adenylate cyclase in liver plasma membranes isolated 4 and 15 h after partial hepatectomy, accompanied by a decrease in epinephrine sensitivity. The present observation of an increase in basal activity beginning at 24 h with no effect of epinephrine before 36 h does not necessarily conflict with that result, since the sensitivity of normal rat liver adenylate cyclase to epinephrine was lost during the isolation of plasma membranes. In contrast, Weber [33] reported a decrease in basal adenylate cyclase activity in 24-h regenerating liver.

The activity of adenylate cyclase in liver plasma membranes in the presence of glucagon is shown in Table IV. The response to glucagon (fold-stimulation of basal activity) in plasma membranes isolated 12 h after partial hepatectomy was less than half that in control membranes, and remained low throughout 13.5 days. This result is consistent with the report of Leffert et al. [34] of a decreased glucagon-binding capacity in plasma membranes from 24-h regenerating liver.

In summary, shown here for the first time are (a) a decrease in leucine aminopeptidase activity and (b) changes in basal activity and hormone responsiveness of adenylate cyclase in liver plasma membranes isolated at times later than 24 h after partial hepatectomy. Other results presented here generally agree with previously published reports, with the exception of the recent time-course study of Masuda and co-workers [28]. Experimental differences in sex and strain of rats, husbandry, time of surgery or method of membrane isolation may contribute to these differences in results.

TABLE III

EFFECT OF EPINEPHRINE ON ADENYLATE CYCLASE ACTIVITY IN HOMOGENATES OF UNOPERATED CONTROL AND 60-h REGENERATING RAT LIVERS

Homogenates were prepared and enzyme assays were carried out as described in Methods and Materials. Specific activity is expressed as pmol of cyclic AMP produced per min/mg protein and is given as mean ± S.D. for three livers.

Source of homogenate	Adenylate cyclase activity		Average fold-stimulation by epinephrine
	Basal	+ Epinephrine	
Control liver	0.5 ± 0.0	2.7 ± 0.6	5.6
60-h regenerating liver	1.4 ± 0.4 *	9.4 ± 0.9 *	7.3

* Different from control at $P \leq 0.01$.

TABLE IV

EFFECT OF GLUCAGON ON ADENYLATE CYCLASE ACTIVITY IN PLASMA MEMBRANES FROM CONTROL AND REGENERATING RAT LIVER

Plasma membranes were isolated from livers of unoperated control rats and partially hepatectomized rats as described in Methods and Materials. Specific activity is expressed as pmol of cyclic AMP produced per min/mg protein and is given as mean ± S.D. for membranes from three livers.

Hours after partial hepatectomy	Adenylate cyclase activity		Average fold-stimulation by glucagon
	Basal	+ Glucagon	
Control (no operation)	41 ± 3	927 ± 63	23
12	32 ± 12	297 ± 39	10
24	52 ± 6	694 ± 166	13
36	48 ± 3	640 ± 28	13
48	54 ± 4	754 ± 199	14
60	50 ± 4	587 ± 48	12
156	63 ± 10	787 ± 81	13
324	62 ± 12	815 ± 165	13

Physiological significance of results

The possible significance of changes in activities of plasma membrane enzymes after partial hepatectomy must be considered from the standpoint of regulation of cellular proliferation during regeneration and from the standpoint of maintenance of liver function following a large loss of mass. Since the physiological functions of some plasma membrane enzymes are not known, insight into their roles in liver regeneration may be provided by the time of onset, direction, and duration of change in specific activity after partial hepatectomy.

The dramatic increase in alkaline phosphatase activity by 12 h after partial hepatectomy suggests that the enzyme may be involved in the regulation of DNA synthesis, which begins at 16—18 h after surgery, or mitosis, which reaches an initial peak at about 36 h when surgery is performed near the beginning of the dark period [35]. However, using an experimental system similar to regenerating liver, other investigators have shown that increases in alkaline phosphatase are not necessary for DNA synthesis [27,36]. Histochemical studies reveal high activity of alkaline phosphatase at the bile canaliculi [37,38]. Since phosphorylcholine is a substrate for the enzyme, the increase in activity may contribute to the increased biliary choline secretion observed after partial hepatectomy [27]. Similarly, increases in $(Na^+ + K^+)$-ATPase activity may also be involved in maintaining the bile secretory function of liver. This hypothesis is supported by the following observations: (a) an inhibitory effect of cardiac glycosides on bile output [39], (b) a correspondence in temperature dependence of bile flow and $(Na^+ + K^+)$-ATPase activity [40] and (c) the similarity between the change with time of $(Na^+ + K^+)$-ATPase activity in the present study and the pattern of increase of bile production per g of liver after partial hepatectomy [41].

The decrease in leucine aminopeptidase activity reflects the overall decrease in protein catabolism after partial hepatectomy [42,43]. Since the increment in protein necessary to replace lost liver mass is accomplished primarily through

decreased protein breakdown [43], the decrease in leucine aminopeptidase may contribute to the restoration of plasma membrane protein.

Several enzymes involved in RNA degradation have been demonstrated in plasma membrane preparations [44—46]. The present observation of a decrease in 5′-nucleotidase activity beginning at 12 h after partial hepatectomy correlates with observations of decreased ribonuclease activity in regenerating liver [47,48]. One may speculate that decreases in the RNA-degrading capacity of liver at early times after partial hepatectomy may contribute to the accumulation of messenger RNA species necessary for synthesis of proteins involved in cell division. Alternatively, there is evidence that some liver plasma membrane 5′-nucleotidase may be externally oriented [49,50]. Changes in extracellular concentrations of adenosine, formed by hydrolysis of AMP, may be involved in the regulation of adenylate cyclase activity [51].

Hormonal regulation of liver regeneration has been indicated in several studies [52—55]. Cyclic AMP levels increase during the first 24 h after partial hepatectomy [56], but have not been measured at later times. Three waves of cyclic AMP production occur during this early period [54,55]; the second wave, at 12 h after surgery, is blocked by propranolol and appears to be required for the initiation of DNA synthesis [54]. However, the role of cyclic AMP in the control of cell proliferation is still controversial [57]. The overall effect on liver cyclic AMP levels of the observed increase in basal activity, decrease in glucagon sensitivity, and increase in epinephrine sensitivity after 24 h, is difficult to predict. The decrease in glucagon response would tend to counteract the increase in serum glucagon which has been observed for at least three days after partial hepatectomy [34,58].

An increase in epinephrine response [59] and a decrease in glucagon response [60] have also been demonstrated for adenylate cyclase of preneoplastic liver. Fetal liver adenylate cyclase exhibits great sensitivity to epinephrine, which appears to be mediated through a β-receptor and which decreases to adult levels shortly after birth [61,62]. The glucagon response of adenylate cyclase may be lower in fetal liver than in adult liver [63]. Such similarities in hormonal regulation of regenerating, preneoplastic, and fetal liver adenylate cyclase lend support to the hypothesis that some degree of metabolic dedifferentiation may accompany, or be required for, rapid cellular proliferation [64,65].

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References

1 Bresnick, E. (1971) in Methods in Cancer Research (Busch, H., ed.), Vol. VI, pp. 347—397, Academic Press, New York

2 Mutschler, L.E. and Gordon, A.H. (1966) Biochim. Biophys. Acta 130, 486—492

3 Henderson, P.T. and Kersten, K.J. (1970) Biochim. Pharmacol. 19, 2343—2351

4 Bucher, N.L.R. and Malt, R.A. (1971) Regeneration of Liver and Kidney, pp. 1—176, Little, Brown and Co., Boston
5 Higgins, G.M. and Anderson, R.M. (1931) Arch. Pathol. 12, 186—202
6 Toda, G., Oka, H., Oda, T. and Ikeda, Y. (1975) Biochim. Biophys. Acta 413, 52—64
7 Hutchison, W.C., Downie, E.D. and Munro, H.N. (1962) Biochim. Biophys. Acta 55, 561—570
8 Webb, J.N. and Lindstrom, H.V. (1965) Arch. Biochem. Biophys. 112, 273—281
9 Burton, K. (1956) Biochem. J. 62, 315—323
10 King, T.E. (1967) in Methods in Enzymology (Estabrook, R.W. and Pullman, M.E., eds.), Vol. X, pp. 322—331, Academic Press, New York
11 Baginski, E.S., Foa, P.P. and Zak, B. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), Vol. 2, Second English Edition, pp. 876—880, Academic Press, New York
12 Song, C.S. and Bodansky, O. (1967) J. Biol. Chem. 242, 694—699
13 Chen, P.S., Toribara, T.Y. and Warner, H. (1956) Anal. Chem. 28, 1756—1758
14 Emmelot, P. and Bos, C.J. (1966) Biochim. Biophys. Acta 121, 375—385
15 Bonting, S.L., Simon, K.A. and Hawkins, N.M. (1961) Arch. Biochem. Biophys. 95, 416—423
16 Goldbarg, J.A. and Rutenburg, A.M. (1958) Cancer 11, 283—291
17 Emmelot, P. and Bos, C.J. (1971) Biochim. Biophys. Acta 249, 285—292
18 Tovey, K.C., Oldham, K.G. and Whelan, J.A.M. (1974) Clin. Chim. Acta 56, 221—234
19 Gilman, A.G. (1970) Proc. Natl. Acad. Sci. U.S. 67, 305—312
20 Lowry, O.H., Rosebrough, N.J., Farr, A.J. and Randall, R.J. (1951) J. Biol. Chem. 195, 265—275
21 Sirica, A.E., Goldblatt, P.J. and McKelvy, J.F. (1975) J. Biol. Chem. 250, 6464—6468
22 Wisher, M.H. and Evans, W.H. (1975) Biochem. J. 146, 375—388
23 Ray, T.K. (1970) Biochim. Biophys. Acta 196, 1—9
24 Lesko, L., Donlon, M., Marinetti, G.V. and Hare, J.D. (1973) Biochim. Biophys. Acta 311, 173—179
25 Brown, A.E., Lok, M.P. and Elovson, J. (1976) Biochim. Biophys. Acta 426, 418—432
26 Schlaeger, R. and von Seydlitz, H. (1975) in Liver Regeneration after Experimental Injury (Lesch, R. and Reutter, W., eds.), pp. 189—194, Stratton Intercontinental, New York
27 Pekarthy, J.M., Short, J., Lansing, A.I. and Lieberman, I. (1972) J. Biol. Chem. 427, 1767—1774
28 Masuda, Y., Nishimura, T., Nojiri, T. and Murano, T. (1976) Biochim. Biophys. Acta 426, 335—338
29 Bakkeren, J.A.J.M. and Bonting, S.L. (1968) Biochim. Biophys. Acta 150, 467—472
30 Pohl, S.L., Birnbaumer, L. and Rodbell, M. (1970) J. Biol. Chem. 246, 1849—1856
31 Leray, F., Chambaut, A.-M., Perrenoud, M.-L. and Hanoune, J. (1973) Eur. J. Biochem. 38, 185—192
32 Leoni, S., Luly, P., Mangiantini, M.T., Spagnuolo, S., Trentalance, T. and Verna, R. (1975) Biochim. Biophys. Acta 394, 317—322
33 Weber, G. (1975) in Liver Regeneration after Experimental Injury (Lesch, R. and Reutter, W., eds.), pp. 103—118, Stratton Intercontinental, New York
34 Leffert, H., Alexander, N.M., Faloona, G., Rubalcava, B. and Unger, R. (1975) Proc. Natl. Acad. Sci. U.S. 72, 4033—4036
35 Barbason, H. and Van Cantfort, J. (1975) in Liver Regeneration after Experimental Injury (Lesch, R. and Reutter, W., eds.), pp. 309—314, Stratton Intercontinental, New York
36 Short, J., Brown, R.F., Husakova, A., Gilbertson, J.R., Zemel, R. and Lieberman, I. (1972) J. Biol. Chem. 247, 1757—1766
37 Sulkin, N.M. and Gardner, J.H. (1948) Anat. Rec. 100, 143—157
38 Novikoff, A.B. and Noe, E.F. (1955) J. Morphol. 96, 189—221
39 Erlinger, S., Dhumeaux, D., Berthelot, P. and Dumont, M. (1970) Am. J. Physiol. 219, 416—422
40 Brauer, R.W., Leong, G.F. and Holloway, R.J. (1954) Am. J. Physiol. 177, 103—112
41 Leong, G.F., Pessotti, R.L. and Brauer, R.W. (1959) Am. J. Physiol. 197, 880—886
42 Swick, R.W. and Ip, M.M. (1974) J. Biol. Chem. 249, 6836—6841
43 Skornik, O.A. (1975) in Liver Regeneration after Experimental Injury (Lesch, R. and Reutter, W., eds.), pp. 156—168, Stratton Intercontinental, New York
44 Touster, O., Aronson, N.N., Dulaney, J.T. and Hendrickson, H. (1970) J. Cell Biol. 47, 604—618
45 Franklin, J.E. and Trams, E.G. (1971) Biochim. Biophys. Acta 230, 105—116
46 Yanarell, A. and Aronson, N.N. (1973) Biochim. Biophys. Acta 311, 191—204
47 Shortman, K. (1962) Biochem. Biophys. Acta 61, 50—55
48 Maor, D. and Alexander, P. (1968) Biochim. Biophys. Acta 157, 627—629
49 Gurd, J.W. and Evans, W.H. (1974) Arch. Biochem. Biophys. 164, 305—311
50 Benedetti, E.L. and Delbauffe, D. (1971) in Cell Membranes, Biological and Pathological Aspects (Richter, G.W. and Scarpelli, D.G., eds.), pp. 54—83, Williams and Wilkins, Baltimore
51 Clark, R.B. and Seney, M.N. (1976) J. Biol. Chem. 251, 4239—4246
52 Starzl, T.E., Halgrimson, C.G., Francavilla, F.R., Francavilla, A., Porter, K.A., Brown, T.H. and Putnam, C.W. (1973) Surg. Gynec. Obstet. 137, 179—199
53 Bucher, N.L.R. and Swaffield, M.N. (1975) in Advances in Enzyme Regulation (Weber, G., ed.), Vol. 13, pp. 281—293, Pergamon Press, New York
54 McManus, J.P. and Whitfield, J.F. (1974) Prostaglandins 6, 475—487

55 Thrower, S. and Ord, M.G. (1975) Biochem. Soc. Trans. 3, 724—727
56 Miura, Y. and Fukui, N. (1976) in Advances in Enzyme Regulation (Weber, G., ed.), Vol. 14, pp. 393—405, Pergamon Press, New York
57 Pastan, I.H., Johnson, G.S. and Anderson, W.B. (1975) in Annual Review of Biochemistry (Snell, E.E., ed.), Vol. 44, pp. 491—522, Annual Reviews Inc., Palo Alto, Claifornia
58 Morley, C.G.D., Kuku, S., Rubenstein, A.H. and Boyer, J.L. (1975) Biochem. Biophys. Res. Commun. 67, 653—661
59 Christoffersen, T., Morland, J., Osnes, J.B. and Elgjo, K. (1972) Biochim. Biophys. Acta 279, 363—366
60 Boyd, H., Louis, C.J. and Martin, T.J. (1974) Cancer Res. 34, 1720—1725
61 Christoffersen, T. and Oye, I. (1974) Acta Endocrinol. Scand. Suppl. 191, 67—71
62 Sherline, P., Eisen, H. and Glinsmann, W. (1974) Endocrinology 94, 935—939
63 Bär, H.P. and Hahn, P. (1971) Can. J. Biochem. 49, 85—89
64 Walker, P.R. and Potter, V.R. (1972) in Advances in Enzyme Regulation (Weber, G., ed.), Vol. 10, pp. 339—364, Pergamon Press, New York
65 Coggin, Jr., J.H. and Anderson, N.G. (1974) in Advances in Cancer Research (Klein, G. and Weinhouse, S., eds.), Vol. 19, pp. 105—165, Academic Press, New York