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HISTOCHEMICAL STUDIES OF ENZYMATIC BEHAVIOUR IN THE HEPATIC CELLS OF THE SENILE RAT LIVER

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As a part of the investigation on the senile changes, histochemical findings of some enzyme activities in the resting and regenerating liver have been compared between senile and young adult rats.

In the resting senile liver, succinate dehydrogenase, cytochrome oxidase and glucose-6-phosphatase exhibited a tendency to decrease in activity, especially in the central and mid-zonal areas of the lobule, but peripheral hepatic cells rather showed a high activity. Acid phosphatase activity was slightly weak. TPN-linked glucose-6-phosphate dehydrogenase activity was remarkably stronger throughout the lobule in the senile case, and DPN-linked lactate dehydrogenase activity was also higher especially in the periportal area. Alkaline phosphatase activity was also more pronounced in the senile case.

In the liver regenerating after partial hepatectomy, the early decrease in succinate dehydrogenase activity was more marked, and the restoration of the activity was delayed in the senile case. The TPN-linked glucose-6-phosphate dehydrogenase and the DPN-linked lactate dehydrogenase in the regenerating liver showed similar changing patterns between the young and senile cases, and their activities in the senile which were higher than those in the young in the resting stage were surpassed by those in the young at the peak of mitotic activity.

On the contrary, the changing pattern of glucose-6-phosphatase activity in the senile and that in the young adult differed from each other. The activity decreased in the young adult and increased in senile case early after hepatectomy, and it reached the minimum in the former and the maximum in the latter at the peak of mitotic activity.

It is also noted that the giant-nuclear and binucleate hepatic cells which were observed more frequently in the senile liver showed usually a high level in succinate dehydrogenase, TPN-linked glucose-6-phosphate dehydrogenase, DPN-linked lactate dehydrogenase, DPN diaphorase, cytochrome oxidase and glucose-6-phosphatase activities.

Numerous papers have been published on the problem of senile changes of the tissues and cells. However, there are still many problems to be solved in the essential nature of senile changes of the parenchymal cells.

Tauchi and his coworker[45)75)] examined the livers of human autopsy cases

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of different ages and reported that characteristic senile change of the liver was its weight loss, which was due to decrease not in volume but in number of hepatic parenchymal cells with advancement of age, and they reported that increases in volume of the cells, especially of their nuclei and also in number of giant-nuclear and binucleate hepatic cells were noted in the aged.

They considered that some cells of the aged seemed to be, in a sense, rather active. They[34)35)39)77)78)79)80)81)] also reported that electron microscopic findings on the hepatic cells in senility were characterized by appearance of microbodies, decrease in number of mitochondria, increase in their volume, and lumina formation of endoplasmic reticulum.

The investigation was then designed to correlate the morphological pictures with functional behaviours of the hepatic parenchymal cells of different ages. The present author has made histochemical studies on the enzymatic behaviour of the resting and regenerating hepatic cells of the rats of different ages, and some interesting findings are presented here.

MATERIALS AND METHODS

All rats used in these studies were of Wistar strain and were bred and maintained in our laboratory; their ages were accurately recorded. Twenty-eight rats of 6 months of age were used as the young adult ones, and 28 rats of age above 24 months as senile ones and they were fed with the standard pellet and water *ad libitum* at resting stage and after partial hepatectomy.

Partial hepatectomy was performed according to the technique of Higgins and Anderson[29)] without anesthesia and with customary sterile precautions. In this study, the median and the left lateral hepatic lobes, roughly two thirds of the liver, were removed.

Some of enzymatic activities of the liver tissue were histochemically demonstrated at resting and regenerating stages of 6, 24, 30, 36, 48, 60, and 72 hours after partial removal. The time of performance of the partial hepatectomy was determined so as to all animals could be sacrificed between 10 A.M. and 2 P.M., in consideration of the report that very large diurnal variation in PAS-positive substance in parencnymal cells and rate of mitosis in regenerating liver was observed[31)76)].

The histochemical techniques employed were of three types: (I) methods to demonstrate activities of dehydrogenases (succinate, TPN-linked glucose-6-phosphate, DPN-linked lactate dehydrogenase and DPN diaphorase), (II) method to demonstrate activity of cytochrome oxidase, and (III) methods to demonstrate hydrolytic enzyme activities (acid, alkaline, and glucose-6-phosphatase).

Almost all the histochemical reactions were performed on liver tissue quenched as rapidly as possible, after removal from the living animal, in the bottle cooled to $-70°C$ with acetone and solid carbon dioxide.

Sections 10 microns thick were cut in a Coons-type cryostat[18)] at $-20°C$,

mounted on clean coverslips, allowed to dry for 2 minutes, lightly fixed for 5 minutes in cold acetone (−20°C) for better cytological localization of enzymes and then placed immediately in the incubating medium. Cold acetone fixation was omitted for the histochemical demonstration of cytochrome oxidase.

For the demonstration of alkaline phosphatase activity, cold acetone (4°C)-fixed paraffin 4 microns sections were made.

In order to make a strict comparison of the enzymatic activity between two or three pieces of the tissues, they were placed on a same block holder, and preparation of the specimens was performed simultaneously by the same procedures. In addition, sections incubated in a substrate-free medium and those boiled before incubation in the full medium served as the controls in every experiment.

Succinate dehydrogenase activity was demonstrated by nitro-BT method by Nachlas *et al.*[47] For the demonstration of TPN-linked glucose-6-phosphate dehydrogenase activity, nitro-BT method of Karnovsky[32], a modification of Hess *et al.*[27] was employed, and DPN-linked lactate dehydrogenase activity was demonstrated by a modification of Hess *et al.*[27] (substrate: lithium lactate).

For DPN diaphorase activity, the technique of Scarpelli *et al.*[66] modified by Mizutani[44] was ued.

For the cytochrome oxidase activity, Nadi-method[54] was employed.

Glucose-6-phosphatase activity was demonstrated by the Wachstein and Meisel[83] of the modification of the method of Chiquoine[17].

For the acid phosphatase activity, sections were incubated at 37°C for 2 hours in the medium prepared by azo-dye method of Burstone[14] and alkaline phosphatase activity was demonstrated by calcium method of Takamatsu[54].

RESULTS

I) Histochemical findings in the resting stage (Table 1)

1) Succinate dehydrogenase:

In young adult rats, the cytoplasm of the hepatic parenchymal cells contained extremely fine granular deposits of formazan at the site of enzymatic activity and their deposits were somewhat concentrated around the nuclear membrane, and the activity was somewhat strong in the periportal areas of the hepatic lobule, moderate in the mid-zonal areas, and weak in the central portion; these histochemical findings were very similar to those in many other reports[43)44)49)50)51)58)64)70)84)].

In senile cases, the activity decreased in the central and mid-zonal areas of the lobule, and rather increased in the periportal areas; so the hepatic cells with high activity level were localized in relatively limited fields of periportal area. In other words, in the senile case the hepatic cells with a high activity seemed generally to decrease in number, but the cells in relatively limited fields of periportal area exhibited a higher activity and there was especially

TABLE 1. Enzymatic Activity in Resting Liver

Enzyme		Area of hepatic lobule	Young adult	Senile
Enzyme of the Krebs' cycle:	Succinate dehydrogenase	Central	++++	++
		Mid-zonal	+++ +++	++++
		Peripheral	++++ ++++	+++++ +++++
Enzyme related to electron transport to oxygen	Cytochrome oxidase	Central	+	±
		Mid-zonal	++	+
		Peripheral	+++	++++
Diaphorase	DPN diaphorase	Central	++++	++++
		Mid-zonal	+++	+++
		Peripheral	+++	++++
Enzyme related to glycolysis	DPN-linked lactate dehydrogenase	Central	+++	+++
		Mid-zonal	+++	+++
		Peripheral	++	++ ~ ++ + ~ ++
Enzyme of the hexose shunt	TPN-linked glucose-6-phosphate dehydrogenase	Central	+++	++++
		Mid-zonal	+++	++++
		Peripheral	++++	+++++
Hydrolytic enzymes	Alkaline phosphatase	Central	±	±
		Mid-zonal	+	++
		Peripheral	++	++
	Acid phosphatase	Central	++	+
		Mid-zonal	++	+
		Peripheral	+++	++
	Glucose-6-phosphatase	Central	+++	++
		Mid-zonal	++++	+++
		Peripheral	++++	++++ ~ +++++

The number of plus signs can only be compared for the same enzyme, and represents relative activities.

marked activity in the giant-nuclear and binucleate hepatic cells, which were frequently seen in periportal area in the hepatic lobules of the senie liver[34)79)].

In addition to the findings above mentioned, a slight irregularity in the activity level among neighbouring hepatic cells was noted in the senile liver.

2) TPN-linked glucose-6-phosphate dehydrogenase:

The hepatic cells showed diffuse cytoplasmic staining for this enzyme activity, and the activity was relatively stronger in the peripheral area than in the central portion of the hepatic lobule both in the young adult and the senile cases.

The activity of the enzyme in each area of the lobule was remarkably stronger in the senile liver than in the young adult liver, and it was especially strong in the giant-nuclear and binucleate hepatic cells, which were more often found in the senile liver[39].

In the senile rat liver there was also a slight irregularity in the activity level among neighbouring hepatic cells.

3) DPN-linked lactate dehydrogenase:

In the young adult, as some authors reported[49][50], the activity was histochemically demonstrated to be most prominent in the central area of the lobuIe, and less in the periphery. In the senile case, however, the hepatic cells in the peripheral portion also exhibited relatively high activity level, and an elevated activity was observed especially in the giant-nuclear and binucleate hepatic cells which were often found in the senile rats.

From this histochemical finding, this enzyme activity seemed to increase in the senile rats. But in the senile rat liver, a slight irregularity in the activity level among neighbouring hepatic cells was usually noted.

4) DPN diaphorase:

In the young adult rats, this enzyme showed a relatively higher activity level in the centrilobular hepatic cells than in the peripheral ones as some investigator[70][84] reported. In the senile case, the activity did not decrease, and even a tendency of increase in activity was often found, and a strong activity was noted especially in the giant-nuclear and binucleate hepatic cells, which were more frequetly seen in the periportal area of the lobule of the senile liver. In the senile liver there was also a slight irregularity in the activity level among neighbouring hepatic cells.

5) Cytochrome oxidase:

In general, the result on this enzyme paralleled that on succinate dehydrogenase. In the young adult liver, the hepatic cells in the periportal area exhibited a higher activity than in the central portion, and the active hepatic cells showed a heavy perinuclear staining as some authors reported[13][33][50].

In the senile rat liver, the activity decreased in the central and mid-zonal areas of the lobule, and generally rather increased in the periportal area; so the hepatic cells with a high activity level were localized in relatively limited fields of periportal area, and the giant-nuclear and binucleate hepatic cells showed a marked activity.

There was a slight irregularity in the activity level among neighbouring hepatic cells in the senile case.

6) Glucose-6-phosphatase:

In the young adult liver, this enzyme activity was nearly evenly distributed within the lobule, but somewhat pronounced in the periphery.

This enzyme was concentrated around the nuclear membrane as was previously reported[17)49)50)83)].

In the senile case, this actvity was markedly lowered in the central area and moderately lowered in the mid-zonal area, and rather increased in the periphery of the lobule, and the giant-nuclear and binucleate hepatic cells exhibited a high activity.

7) Acid phosphatase:

In the young adult case, as seen in some papers[23)49)50)84)], the activity was relatively higher in the periportal area, and staining for this enzyme was located in cytoplasmic granules oriented closely around the bile canaliculi, and occasional granules were found more deeply in the liver cell cytoplasm. In addition, some stained granules of various sizes were seen in sinusoidal lining cells.

Judging from staining reaction, the activity of the senile liver was slightly depressed[34)39)79)] as compared with that of the young adult, but the histochemical distribution of this enzyme was not influenced by age difference.

8) Alkaline phosphatase:

In the young adult rat liver, the activity was stronger in the periportal area as seen in some literatures[84)], and in the senile case, the activity was more pronounced without any difference of its distribution in the lobule.

II) Histochemical findings in the regenerating stage after partial hepatectomy (Table 2)

1) Succinate dehydrogenase:

Both in the young adult and senile rats, the activity was rapidly decreased in the early stage after partial hepatectomy, and a definite diminution of the activity was noted in the central and mid-zonal areas of the hepatic lobule at 6 hours after partial hepatectomy, but in the periportal area there was no decrease or very slightly increase in the activity.

Twenty-four hours after operation, no sign of activity could be noted in the hepatic cells throughout the lobule with exceptions of a few cells in the periportal area in the young adult case and still fewer in the senile case. Then the activity began to increase after 24 hours of regeneration in the young adult, 30 hours in the senile case, and the activity appeared to recover about the normal level at 72 hours of regeneration. This increase in activity was always seen first in the periportal area, and then extended into the mid-zonal

and centrilobular areas.

During regenerating process, there was generally an increase in number of giant-nuclear and binucleate hepatic cells which showed a higher level of activity. Their increase was more marked in the senile case. And histochemical distribution of the activity within the lobule was more irregular in regenerating liver, and this irregularity was more marked in the senile case than in the young adult.

2) TPN-linked glucose-6-phosphate dehydrogenase:

In the early stage of regeneration, both in young adult and senile cases, this enzyme activity decreased, especially in the centrilobular hepatic cells, and the decrease in activity was more marked in the senile case than in the young adult.

The recovery of the activity began at 24 hours of regeneration, and became more rapid after 30 hours of regeneration, and the activity was noticed to be higher than in the resting stage after 36 hours. This increase in activity was less marked in the senile cases as compared with that in the young adult, and after 36 hours the activity in the former was lower than that in the latter. The recovery began in the periportal area and was extended into the midzonal and central areas.

In regenerating liver a high activity was observed not only in the giant-nuclear and binucleate hepatic cells in periportal area but also in the parenchymal cells of smaller size. And histochemical distribution of activity within the lobule was more irregular in regenerating liver, and this irregularity was more marked in the senile case than in the young adult.

3) DPN-linked lactate dehydrogenase:

After partial hepatectomy, the activity was gradually increased, reaching the maximum at 36 hours of regeneration, and was slowly decreased thereafter. These changes in activity were generally less marked in the senile case as compared with the young adult. The increase in activity started in the central areas and spread toward the periphery of the hepatic lobule. During regenerating process, there was generally an increase in number of giant-nuclear and binucleate hepatic cells which showed a higher activity level. This increase in their number was more marked in the senile case. This activity was also demonstrated even in the cells in mitotic phase.

The histochemical distribution of activity within the lobule was more irregular in regenerating liver, and this irregularity was more marked in the senile case than in the young adult.

4) DPN diaphorase:

The activity slightly decreased after partial hepatectomy and remained depressed until 24 hours of regeneration in the young adult and until 36 hours

TABLE 2. Changes of Enzymatic Activity After Partial Hepatectomy

Hours after hepatectomy→ / Enzyme ↓	Area of hepatic lobule	Young adult						Senile					
		0	24	30	36	48	72	0	24	30	36	48	72
Succinate dehydrogenase	Central	++++	±	+~++	++	++ ≀ +++	++ ≀ +++	++	−~±	±	+	+~++	++
	Peripheral	++++ ++++	+++	++++	+++ ++	+++ +++	++++ +++	++++ ++++ ++	+~++	++	+++ ≀ ++++	++++ +++	++++ ++++
DPN-linked lactate dehydrogenase	Central	+++	++++	+++ ++	++++ +++	++++ + ≀ +++ +++	+++ ++	+++	++++	++++ ≀ ++++ +	++++ +	++++	++++
	Peripheral	++	++	+++	+++ ≀ ++++	++++	+++ ≀ ++++	+++ ≀ ++++	++++	++++	++++ ≀ ++++ +	++++ ≀ ++++ +	+++ ≀ ++++
TPN-linked glucose-6-phosphate dehydrogenase	Central	+++	+	++	+++	+++	+++	++++	+	++	++ ≀ +++	+++	++++
	Peripheral	++++	++	+++	++++ +++	++++ ++++ +	++++ ++++ ++	++++ +	+++	++++	+++ +++	++++ +++	++++ ++++
Glucose-6-phosphatase	Central	++++	++	+	±~+	+~++	++ ≀ +++	++	++ ≀ +++	+++	++++	++ ≀ +++	++
	Peripheral	++++ ≀ ++++ +	+++	++	++	+++	++++	++++ +	++++ +	+++ ≀ ++++	++++ ≀ ++++ +	++++ +	++++ +

The number of plus signs can only be compared for the same enzyme, and represents relative activities.

in the senile case. However, the decrease was far less marked as compared with that of succinate dehydrogenase activity.

The activity was gradually restored after 30 hours of regeneration in the young adult and after 36 hours in the senile case. However, the age difference in changing pattern of the activity was not so distinctly observed.

5) Glucose-6-phosphatase:

In the young adult case, the activity decreased rapidly until 24 hours of regeneration and then gradually, reaching the lowest level at 36 hours after operation. The activity then started increasing, first gradually until 48 hours of regeneration, and then rapidly, reaching the normal level at 72 hours after partial hepatectomy.

The decrease in activity began in the centrilobular and mid-zonal areas and was extended into the periportal area, but the activity of some hepatic cells of larger size in the periportal area remained unchanged. And the increase in activity began in the periportal area and was extended into the centrilobular area, and at 72 hours of regeneration the activity still varied with cells.

On the contrary, the activity in the senile case increased gradually from the beginning especially in the centrilobular and mid-zonal areas, until 36th hour of regeneration, and then gradually decreased, and at 72 hours after hepatectomy the histochemical pattern of this enzyme activity within the hepatic lobule returned nearly to that in the resting stage. It was also notable that the giant-nuclear and binucleate hepatic cells exhibited a higher activity and that the activity varied with cells more markedly in the senile case than in the young adult.

DISCUSSION

1) Succinate dehydrogenase:

The action of succinate dehydrogenase is an important link in the oxidation of carbohydrate, protein and lipid in the Krebs' cycle.

It is also associated with cytochrome oxidase activity. Succinate dehydrogenase activity can often be considered to be an approximate measure of oxygen consumption and metabolic activity of tissues.

Bourne[11] reported that there was no histochemical evidence of decrease in the enzyme in the liver tissue of senile rats and that in fact there was some evidence of increase. Biochemical studies by Kitani *et al.*[38] revealed that succinate dehydrogenase activity level per dry weight of liver tissue of senile rats was markedly lower than that of young adult. Ross[63] observed biochemically a slight increase in succinate dehydrogenase activity in the liver tissue of the senile case, and Barrows[7)8)9)10)] reported that succinate dehydrogenase activity in the hepatic tissue of senile animals was not significantly lower than

that in the control.

The present study revealed that in the senile case, the activity was depressed in the centrilobular and mid-zonal areas and generally rather increased in the periportal area; so the hepatic cells with a high activity level were localized in relatively limited fields of periportal area. Masuko[39] had examined manometrically the same liver tissues which were examined histochemically by the present author and found no significant difference in the activity per wet weight and per nitrogen between the senile and the young adult cases.

These observations seemed to indicate that the senile decrease in the activity in the central and mid-zonal areas of the lobule was compensated by the increase in the activity in the periportal area and in the giant-nuclear and binucleate hepatic cells which were frequently found in the senile liver tissue.

There have been several investigations on the behaviour of succinate dehydrogenase during regenerating process after partial hepatectomy.

Perkinson[59] reported that succinate dehydrogenase activity was markedly depressed at 48 and 72 hours after partial hepatectomy and that the oxygen consumption was much more elevated than that of normal liver. Biochemical analysis by Novikoff[48], Hopsu[30], Kitani[38], and Tsuboi[82], as well as Pearsons' histochemical study[58], revealed that succinate dehydrogenase activity of the adult liver during regeneration after partial hepatectomy was significantly weaker than that in resting stage. Harkness[25][26] claimed that restoration of mitochondrial enzymes of the hepatic cells during regeneration after partial hepatectomy was generally slower than that of other enzymes. Allard *et al.*[2][3] showed that the number of mitochondria with which succinate dehydrogenase is closely associated markedly decreased in the average cell of regenerating liver, and thought the phenomenon to be characteristic for the process of stimulated growth after partial hepatectomy.

Sänchez *et al.*[65] reported that succinate dehydrogenase activity slightly increased at 12 hours of regeneration, markedly decreased after that stage, and then showed a gradual restoration.

However, there are only a few papers on the behaviour of succinate dehydrogenase of the regenerating liver of senile rats.

Kitani *et al.*[38] reported that the activity after partial hepatectomy in senile rats more markedly decreased and its restoration was delayed as compared with young rats.

According to the present enzyme histochemical study on the regenerating liver after partial hepatectomy, the activity sharply decreased until 24 hours after partial hepatectomy and gradually increased thereafter in the young adult, while in the senile ones restoration of this enzyme activity was generally slower. An increase in number of giant-nuclear and binucleate hepatic cells with an elevated activity during regenerating process was more marked in the senile case than in the young adult.

2) TPN-linked glucose-6-phosphate dehydrogenase:

This enzyme is a member of the dehydrogenase system in the hexose-monophosphate pathway which plays an important role in ribose synthesis, especially in RNA-synthesis[1)20)21)28)39)57)67)68)69)].

In regenerating hepatic cells after partial hepatectomy Schneider *et al.*[69)] demonstrated a remarkable increase in incorporating rate of glucose-1-C^{14}.

The glucose-6-phosphate dehydrogenase activity was reported to increase in the proliferating hepatic cells[1)5)60)73)]. This enzyme activity also increased in tumors[41)56)92)94)95 97)] and the activity markedly increased in rapidly growing hepatomas and less in slow-growing ones[96)].

In the present study, this enzyme activity of the resting liver in senile case showed a relatively high level as compared with that in the young adult. On the other hand, the decrease in activity of this enzyme in the earlier stage after partial hepatectomy was more marked, and the increase in the later stage (after 24 hours of regeneration) was less marked in the senile case than in the young adult.

These results may suggest that the proliferation of the hepatic cell in the senile case after partial hepatectomy is less active and senile hepatic cells in resting stage have more active behaviour than young adult cells.

3) DPN-linked lactate dehydrogenase:

This enzyme belongs to the enzymes which play an important role in glycolytic pathway.

Biochemical study by Ross[63)] revealed that the activity of this enzyme increased with increasing age, and Boxer *et al.*[12)] showed biochemically that until 54 hours after partial hepatectomy the activity was increased progressively. And also there have been several reports[46)73)85)] on the high activity level of this enzyme in tumor tissues.

The present histochemical study revealed that this enzyme activity of the hepatic cells in the resting stage was high in the periphery of the lobule in the senile case, and that in the regenerating process after partial hepatectomy the activity increased in both senile and young cases until 36th hour and then decreased, but these changes in activity were less marked in the senile case.

It may be therefore inferred from these facts that proliferative activity of residual liver after partial hepatectomy is higher in the young adult than in the senile, while the resting senile hepatic cells have a higher potentiality for proliferation than the resting young ones.

4) DPN diaphorase:

DPN diaphorase belongs to the flavoprotein group of enzymes. It is of vital importance because of its role in the transfer of hydrogen within the Krebs' tricarboxylic acid cycle. In animal tissues it is responsible for aerobic oxidaton in the Krebs' cycle.

Numerous publications[5)6)44)53)85)86)] have shown that this enzyme activity in the tumor tissue was relatively high, and the evidence[42)] indicated that it was closely associated with process of cell division and multiplication. Seno[71)] observed histochemically a high activity in hypertrophic parenchymal cells of animals fed 3′-Me DAB, and Wachstein[85)] reported that the activity was relatively high in hepatic cells during regenerating process after partial hepatectomy.

The author did not observe any marked difference between the young adult and senile cases, either in resting or regenerating liver.

5) Cytochrome oxidase:

Cytochrome oxidase contains iron and plays an important role in aerobic cell respiration. This enzyme is entirely intramitochondrial and closely bound to the structure of the mitochondria.

Katsunuma[33)], Burstone[13)], and Novikoff *et al.*[50)] reported in their histochemical studies that the activity was more pronounced in the periportal area of the hepatic lobule than in the central portion, while Hannibal *et al.*[24)] showed that the liver had an abundant amount of this enzyme throughout its parenchymal cells, without any difference between the central and periportal zones.

In the present study, the activity was pronounced in the periportal area in young adult rats. There has been no histochemical study on this enzyme activity of the senile liver; the present study revealed that the behaviour of this enzyme characteristic in senile case paralleled that of succinate dehydrogenase.

6) Glucose-6-phosphatase:

Glucose-6-phosphatase plays an important role in the regulation of hepatic glucose metabolism.

This enzyme activity was demonstrated by many authors in the hepatic parenchymal cells more abundantly in the peripheral area of the hepatic lobule than in the central portion[16)17)83)], but Reynold[62)] did not observe any significant difference within the hepatic lobule. No significant change in enzyme distribution was observed in relation to its diurnal cycle[17)].

Zorzoli[100)] examined biochemically the mice liver and reported that the activity rose after birth and reached the peak for the life span on the third postnatal day, and declined from the fifth day until 9 months of age rapidly at first and then gradually, and from 9 through 27 months no significant change occurred.

The present study revealed that the activity in the young adult liver was somewhat more pronounced in the peripheral area, and that in the liver of old rats it was markedly depressed in the central area and rather increased in the periphery, especially in the giant-nuclear and binucleate hepatic cells.

Since many investigators reported the increase in this enzyme activity in case of fasting[4)40)87)91)93)], senile changes of hepatic cells seemed to differ from

changes due to fasting.

The behaviour of glucose-6-phosphatase in regenerating liver has recently been described. In regenerating liver after partial hepatectomy, restoration of the activity ran paralleled with the regeneration of liver parenchyma[90], and the glucose-6-phosphatase activity in the microsomal fractions slightly decreased simultaneously with the relative increase of the microsomal nucleoprotein[19].

Recently the activity of glucose-6-phosphatase was studied also in homogenates. The activity was remarkably weak or absent in hepatoma which was derived from parenchymal cells, which normally contained much glucose-6-phosphatase[5,61,88,89,92,94,96,97]. And, in contrast with glucose-6-phosphate dehydrogenase activity, the glucose-6-phosphatase activity was absent in rapidly growing hepatomas and was maintained at relatively high value in slow-growing ones[96]. It was also confirmed that hepatic glucose-6-phosphatase activity decreased remarkably after feeding with the carcinogenic dye[16,72,99]. Perske *et al.*[5,60] studied on carbohydrate enzyme of culture strain of human liver and reported that homogenates prepared from these cultures showed no glucose-6-phosphatase activity, although glucose-6-phosphate dehydrogenase activity was present.

The present histochemical study revealed that the activity in the young adult liver started decreasing soon after partial hepatectomy and restored after the peak of proliferation. On the contrary, the activity in the senile liver rather increased from the beginning, in the centrilobular and mid-zonal areas in the earlier stage of regenerating process.

The senile liver cells seemed to show a different behaviour from the young adult cells.

7) *Acid phosphatase:*

Acid phosphatase is a monoesterase which splits phosphate ester at an acid pH, and is considered to be located chiefly in lysosomes in the cell[23,50,52].

The activity was histochemically observed to be relatively stronger in the peripheral area[23,49,50,84], with an exception of Changs' report in which the reaction was usually distributed evenly throughout the lobule[16].

Zorzoli[99] reported using biochemical method that acid phosphatase activity relatively decreased in the senile livers.

The present study revealed that this enzyme activity was somewhat higher in the periportal area, and that in the senile case it was slightly depressed without any alteration of the distribution pattern in the hepatic lobules.

Since acid phosphatase activity has been demonstrated to increase in tissue with degenerative process[22,25] and also during starvation[3], senile changes of liver tissues should be considered to differ from the process of degeneraton or starvation.

8) Alkaline phosphatase:

In the liver, the alkaline phosphatase activity was usually revealed histochemically to be strong in the periportal zone and localized chiefly at the bile canaliculi, in Kupffer and endothelial cells, and in the wall of the sinusoid[16) 84) 94)].

It was confirmed by many investigators[49) 53) 56)] that in the regenerating liver the alkaline phosphatase activity of the liver remnant after partial hepatectomy strikingly increased, and that this increase seemed to coincide with the time of maximum rate of cell division. It was also found[3)] that alkaline phosphatase activity was stronger in primary hepatic tumors than in the normal liver.

Zorzoli[99)] demonstrated biochemically that alkaline phosphatase activity in the liver tissue was higher in the senile mice than in the young.

The author also demonstrated histochemically that alkaline phosphatase activity was pronounced in the periportal area of the rat liver and it was stronger in the senile case than the young adult.

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EXPLANATION OF FIGURES

Histochemical demonstration of the enzymatic activities in the rat liver.

Key to abbreviations:

P: Portal triad, C: Central vein

FIG. 1. Succinate dehydrogenase in the resting liver of the young adult rat (×142).

FIG. 2. Succinate dehydrogenase in the resting liver of the senile rat. The activity is decreased in the central and mid-zonal areas, and increased in the periphery of the lobule, compared with that in the young adult (Fig. 1) (×142).

FIG. 3. Same tissue as in Figure 2 at a higher magnification. The activity is markedly noticed in the giant-nuclear and binucleate hepatic cells (×300).

FIG. 4. Succinate dehydrogenase in the regenerating liver at 72 hours after partial hepatectomy in the senile rat. The activity is marked especially in the giant-nuclear and binucleate hepatic cells (×300).

FIG. 5. Glucose-6-phosphatase in the resting liver of the young adult rat (×142).

FIG. 6. Glucose-6-phosphatase in the resting liver of the senile rat. The activity is markedly lowered in the central area and moderately lowered in the periphery of the lobule, compared with that in the young adult (Fig. 5) (×142).

FIG. 7. Glucose-6-phosphatase in the regenerating liver at 36 hours after partial hepatectomy in the young adult rat. The activity is depressed in the central and mid-zonal areas, compared with that in the resting liver (Fig. 5) (×142).

FIG. 8. Gluose-6-phosphatase in the regenerating liver at 36 hours after partial hepatectomy in the senile rat. The activity is increased in the central and mid-zonal areas, compared with that in the resting stage (Fig. 6) (×142).

FIG. 9. Glucose-6-phosphatase of the hepatic cells in the peripheral area of the lobule of the senile rat. The activity is markedly especially in the giant-nuclear and binucleate hepatic cells (×300).

FIG. 10. TPN-linked gluose-6-phosphate dehydrogenase in the resting liver of the young adult rat (×142).

FIG. 11. TPN-linked glucose-6-phosphate dehydrogenase in the resting liver of the senile rat. The activity is more marked, compared with that in the young adult (Fig. 9) (×142).

FIG. 12. TPN-linked glucose-6-phosphate dehydrogenase in the regenerating liver at 48 hours after partial hepatectomy in the senile rat (×142).

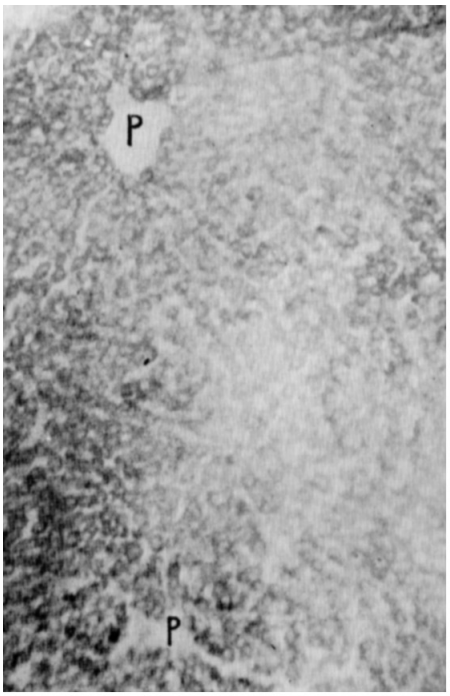


FIG. 1

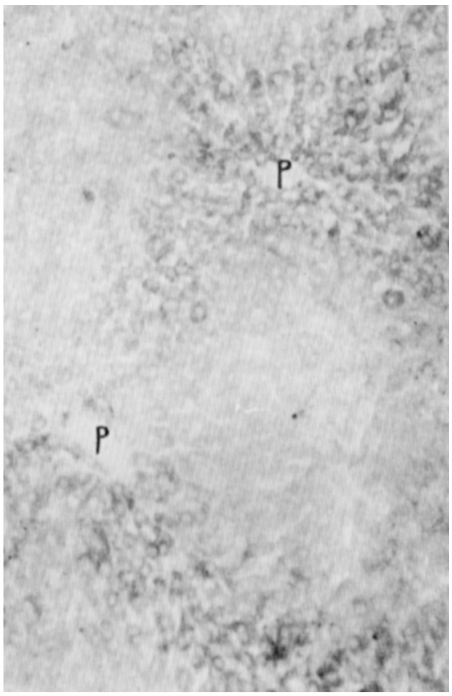


FIG. 2

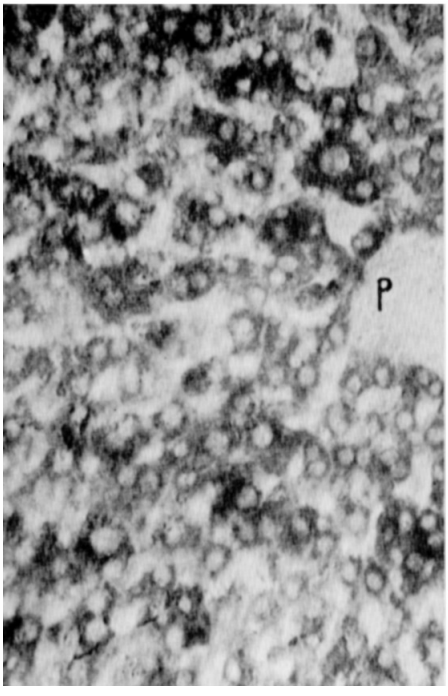


FIG. 3

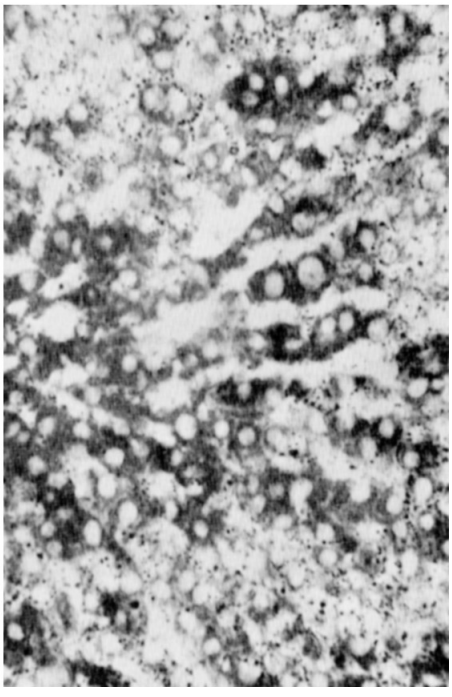


FIG. 4

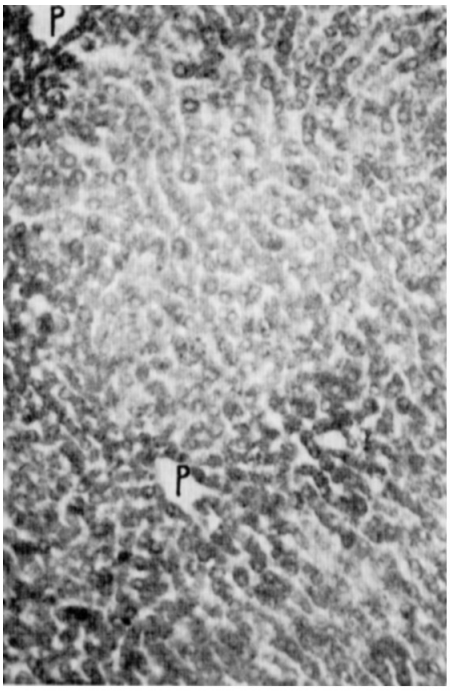
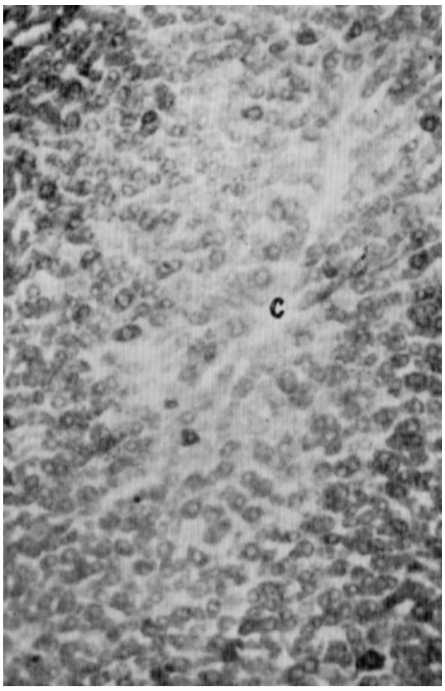


FIG. 5



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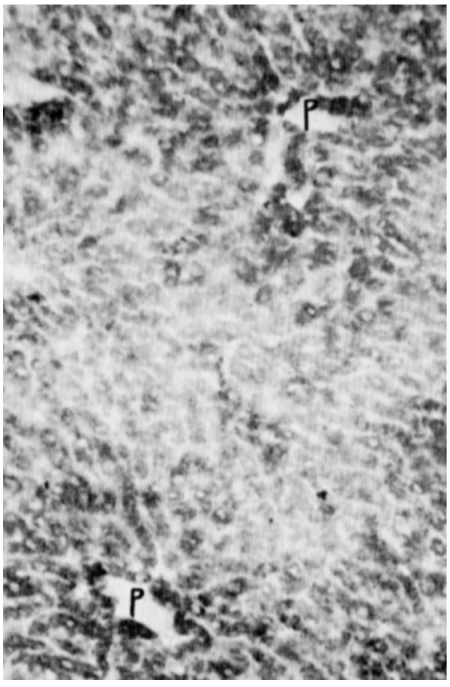

FIG. 7

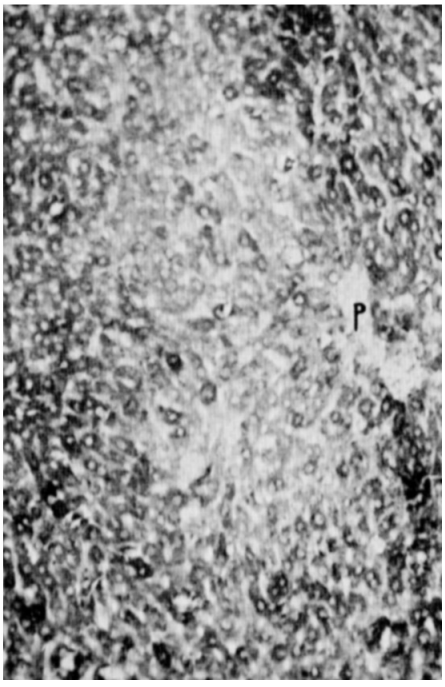

FIG. 8

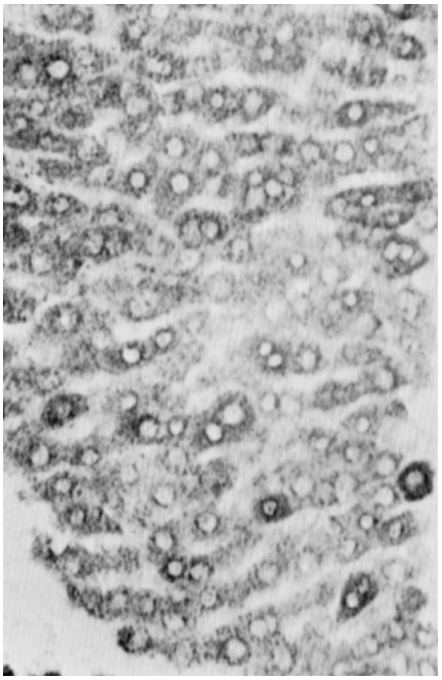

FIG. 9

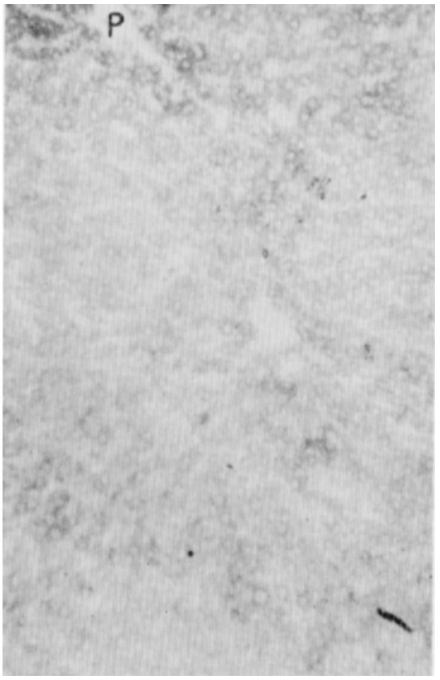

FIG. 10

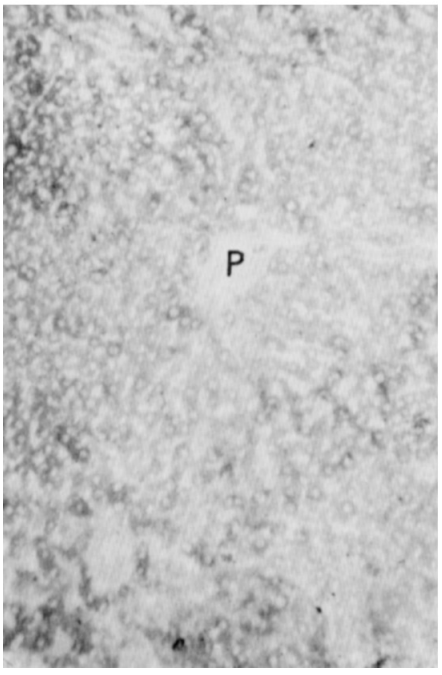

FIG. 11

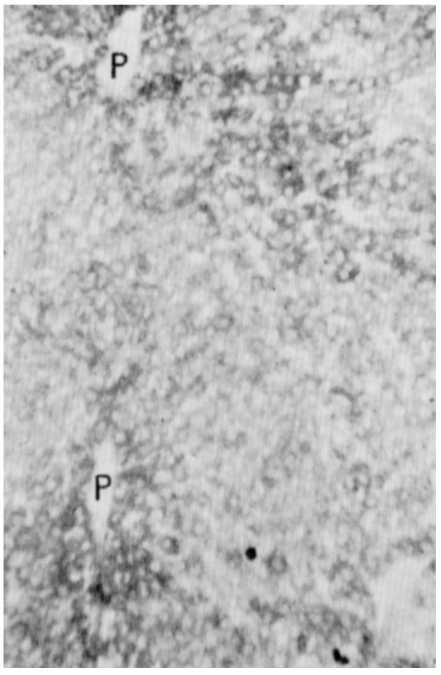

FIG. 12