EFFECT OF SOME PLANT PROTEASES INJECTED IN VIVO ON THE ENZYMATIC ACTIVITY OF LIVER TISSUE

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Our previous studies revealed that there were different changes in enzymatic activity of various subcellular liver fractions, treated in vitro with papain, ficin and bromelin, depending on protease concentration, time and temperature of incubation. An inhibition of liver enzymes to a different extent was established. An activation of succinic dehydrogenase and cytochrome oxidase activities was found under favourable conditions of treatment (1, 2, 3, 4, 5, 6). The present work constitutes a continuation of studies in that direction.

Papain application in vivo in minimum concentrations is known to lead to tenderization of liver and kidney tissue, without any effect on the muscles (7). For this reason, liver can be used as a model for morphological and enzymohistochemical studies of protease action. A determination of these changes will elucidate the process of meat aging after a treatment of the animals prior to slaughtering with proteolytic preparations.

The present study deals with the action of the plant proteases papain and ficin on the morphological pattern, activity and localization of some enzymes from the groups of oxidoreductases, hydrolases, and esterases in white rat liver.

MATERIAL AND METHODS

Investigations were carried out on liver of Wistar rats with a live weight of 150-200 g. Two doses - 5 and 25 mg - of both plant proteases papain (Wallerstein Lab. Comp.) and ficin (Calbiochem) were used. They were dissolved in 2 ml of physiological

solution and injected intraperitoneally. The control animals were treated with 2 ml of physiological solution. The animals were killed by decapitation at 2, 6 and 24 hours after the injection. The material needed for the study was immediately frozen in dry snow.

The following enzymohistochemical reactions were carried out on frozen sections 12 microns thick: succinic dehydrogenase (SDH) and NAD-diaphorase after Nachlas et al. (8), lactic dehydrogenase (LDH) and glucoso-6-phosphate dehydrogenase (G6P DH) after Hess et al. (9), As-esterase after Berston (1965). Two methods for alkaline phosphatase (AlP) and acid phosphatase (AcP) were applied: by Grogg and Pearse A.G.F. (after Barka /10/) and by Gomori (after Pearse A.G.F. /11/). Nitro BT was used as a final electrone acceptor in the oxidoreductases. Paraffin sections of material fixed in formol were stained with Hemalaun-eosine.

RESULTS AND DISCUSSION

A. P a p a i n

Two hours after injection of both doses of papain, the histological analyses of liver indicated a slight venose stagnation and a slightly expressed parenchymatous dystrophy in individual groups of hepatocytes, mainly in the centre of lobules.

On the 6-th hour the changes in animals injected with 5 mg were similar to those on the 2-nd hour. By using the dose of 25 mg, a well expressed parenchymatous dystrophy could be observed in the central part of liver lobules, as well as individual small hemorrhages and necrobiotic changes in the neighbouring hepatocytes - karyorhexis, karyolysis to a complete disappearance of nuclei.

The lesions observed 24 hours after the administration of the dose of 25 mg were the severest. Vast areas showing necrobi-

otic and necrotic changes in cells were found. These changes were chiefly in the centre of liver lobules, but in most cases they were connected with similar areas in the neighbouring lobules. In necrotic areas lymphoid and reticulo-endothelial cells could be seen. Young hepatocytes with strongly basofilic cytoplasm and dark nuclei were found around the necrotic foci. The dose of 5 mg resulted in much insignificant changes: only individual groups of necrotic cells were detected.

Oxidoreductases. Two hours after the treatment, an increase was observed in the activity of the oxidoreductases studied: LDH, G6P DH, SDH and NAD-diaphorase, in comparison with control animals. This increase in LDH and G6P DH was greater in the animals, treated with 25 mg, while in SDH and NAD-diaphorase such a difference could hardly be established. Furthermore, a particular change was observed in colouring and the nature of the granule of deposited formazane. In the demonstration of all oxidoreductases, in the central and intermediary part of liver lobule formazane showed an intensive blue colour and different size of granules, the bigger ones considerably predominating. The most intensive reaction was found in hepatocytes of intermediary part of the lobule. In the cells around the periportal space the enzymatic reaction was manifested by a normal blue-violet formazane granule, but here the activity was also higher than that in the control animals.

After 6 hours, at a dose of 5 mg, no significant difference was established in oxidoreductase activity when compared to that after 2 hours: there was an increase in the size of the area where the product of the reaction was the large blue formazane granule. At the dose of 25 mg, regardless of the fact that in nearly the whole liver lobule formazane had the blue colour mentioned above,

the activity was lower in comparison with that in animals treated with 5 mg and similar to that in the control ones. Furthermore, LDH and G6P DH activities were much lower, and those of SDH and NAD-diaphorase were less lower (Fig. 1 and 2).

After 24 hours a decrease in LDH and G6P DH activities was observed when both doses were used, in comparison with those after 6 hours, as well as with those in the control animals. This decrease was more markedly expressed in the case of the higher dose. In the animals treated with 5 mg the reaction showed a trend towards a normalization. At the dose of 25 mg, cells with a very intensive reaction were found in the periphery of necrotic sectors. A part of these cells gave an enzymatic reaction normal in character for oxidoreductases. Probably these cells were regenerating young hepatocytes and lymphoid and reticulo-endothelial cells. At the dose of 5 mg, these cells were much fewer and their activity was much weaker. In hepatocytes in sectors unaffected by necrosis, the enzymatic reactions proceeded with the large blue granules described above, which were fewer in number and unevenly dispersed in the cytoplasm.

From the 6-th to the 24-th hour, at the dose of 25 mg, SDH and NAD-diaphorase activities were not changed considerably, and at the dose of 5 mg, they decreased. Therefore, after 24 hours there was not any difference in the activities of these enzymes in the animals treated with the two doses of papain. Furthermore, after 24 hours SDH and NAD-diaphorase activities were higher than those of the other oxidoreductases and they were also higher than those in the control animals, both in the morphologically unaffected sectors and in cells in the periphery of necrotic foci.

Hydrolases. Out of all enzymes studied, alkaline phosphatase was the least affected. After 2 hours, no changes were established in comparison with the control animals. After 6 hours, a slight increase was observed in the number of cells around the blood vessels and around the periportal space, which reacted positively to AlP. After 24 hours, at a dose of 25 mg of papain, an intensive reaction to AlP was found in a comparatively greater number of cells, situated around the blood vessels and necrotic foci. Groups of cells with a high activity were observed in different places of the section. The cells, showing high AlP activity, were probably the great number of lymphoid and reticulo-endothelial cells, observed after 24 hours in those places.

Acid phosphatase increased greatly after the administration of both papain doses: two hours after the use of both doses the activity was nearly the same, while after 6 and 24 hours it was higher in the animals injected with 5 mg. AcP activity was the highest after 6 hours, when an intensive reaction in the hepatocytes of the whole lobule was observed. After 2 and 24 hours, the intensity of the reaction was nearly the same. On liver lobule level the activity was the highest in the cells around the periportal space. After 24 hours, at the dose of 25 mg, AcP activity was the highest around the necrotic sectors and blood vessels; there the reaction was particularly intensive in a part of the cells (Fig. 3).

Esterases. After the treatment of the animals with papain, certain changes were observed in the reaction to As-esterase. Instead of the fine product found in the control animals, in the experimental ones treated with 5 and 25 mg of papain there was a production of coarse granules deposited diffusely through the whole

cell. Activity of the enzyme was higher in cells situated immediately around v. centralis and around the periportal space. As-esterase activity was the highest 6 hours after injection; the reaction was more intensive when a dose of 25 mg was used. After 24 hours, cells with a high esterase activity were observed in and around necrotic foci. Cells in sectors unaffected by necrosis gave the reaction already described.

B. F i c i n

The histological changes in the liver after the injection of ficin were considerably slighter in comparison with those resulted from the use of papain. No changes were found after 2 hours. After 6 hours, a slight parenchymatous dystrophy was found in the cells of the lobule centres. After 24 hours, a venose stagnation, small hemorrhages with dystrophic and necrobiotic changes in neighbouring hepatocytes were observed. In the periphery of lobules many young hepatocytes were found. At the dose of 25 mg, the changes were more markedly pronounced than those at the dose of 5 mg of ficin.

Oxidoreductases. The dynamics of changes in the activity and the nature of reactions for oxidoreductases in the animals treated with ficin, were similar to those when papain was used, at both doses. Differences were observed in the degree of fluctuation of the activity with respect to the control animals. The increase in enzymatic activity 2 hours after injection, as well as the decrease in the cases of longer periods, were more slightly expressed.

After 6 and 24 hours, a gradual decrease in SDH and NAD-diaphorase activities was observed, thus reaching those of the control animals. However, the large blue granules, dispersed unevenly

in the cytoplasm and often agglomerating, were the constant product of the reaction.

Hydrolases. Data on alkaline phosphatase were similar to those obtained after the use of papain, with the exception that the amount of cells showing an intensive reaction 24 hours after ficin injection was considerably lower.

After 2 hours, no significant differences were recorded in the activity and localization of AcP. After 6 and 24 hours, the application of both doses resulted in an increase in the activity; the increase was more significant when 25 mg were administered. Reaction to AcP was the most intensive after 24 hours. At liver lobule level, activity was higher in the cells situated immediately around v. centralis and around the periportal space (Fig. 3).

Esterases. Already 2 hours after the ficin injection, an increase in As-esterase activity was established, the character of the reaction being like that described for papain. Between the 6-th and the 24-th hour no significant changes could be recognized in the activity of the enzyme. The reaction was the most intensive in the cells around the periportal space. After 24 hours, cells with a reaction close to the normal one could be seen in that place.

From the results stated so far it becomes clear that the plant proteases papain and ficin, introduced into the organism intraperitoneally in doses of 5 and 25 mg per 100-150 g of live weight, injure the structure and enzymatic activity of liver cells. The lesion is heavier when the higher concentration of proteolytic enzymes is used. While morphological changes in liver can be established by light microscopy 6 and 24 hours after the injection of

proteases, already after 2 hours a severe injury of cell metabolism is observed, manifested in enzymohistochemical reactions. Besides, the changes are more markedly pronounced both morphologically and enzymohistochemically in papain, than in ficin.

The increase in oxidoreductase activity, observed 2 hours after the treatment of the animals, could not be taken for an increase of the quantity of active enzyme in the hepatocytes. It is known that as a result of a lesion of the membrane of cells and mitochondria and a disturbance of their permeability, the rate of the enzymatic reaction increases sharply and a great amount of formazane is deposited per unit of time (12). Proceeding from these data, we could accept that the increase of oxidoreductase activity after 2 hours is due to an initial injury of cellular and mitochondrial membranes by proteases and to the changes that have come about in their physiological status. The data on the histological pattern of liver and the changes observed in the characteristics of the enzymatic reaction on cytological level come in support of this. Similar data on LDH and SDH have been reported also by Hecht (13).

With a more continuous action of the proteases, for 6 and 24 hours, a decrease in oxidoreductase activity occurs proportionally to the quantity introduced. This effect ought to be associated and explained with the proteolysis in progress, and the direct attack of enzyme molecules. The necrobiotic and necrotic changes observed in the hepatocytes support this and demonstrate the severe structural and functional injuries occuring in the tissue.

The differences in the extent of the influence of proteases on LDH and G6P DH on the one hand, and on SDH and NAD-diaphorase, on the other, seem to occur as a result of the difference in their

localization. LDH and G6P DH, being located in the hyaloplasm of the cell, are subject to a more rapid and severe influence in comparison with SDH and NAD-diaphorase, located within the mitochondria, and being to some extent "more protected".

The high AcP activity and the dynamics of its changes with the two proteases are connected with the lysis that has occured in the tissue components and the development of regeneration processes. It was established that the regeneration in rat liver is accompanied by a marked increase of acid phosphatase activity (14). The gradual increase observed in the activity of AcP under the influence of ficin, points to the latter's less destructive action and better expressed regeneration processes, in comparison with papain. This is supported by the data obtained for the other enzymes and the histological changes in the liver.

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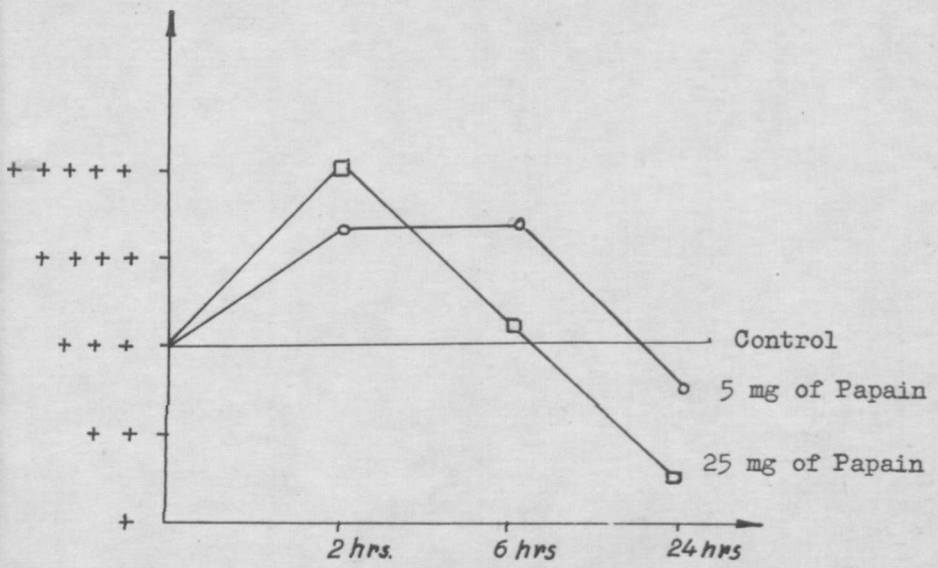


Fig. 1. Dynamics of changes in LDH and G6P DH activities under the influence of two different doses of papain.

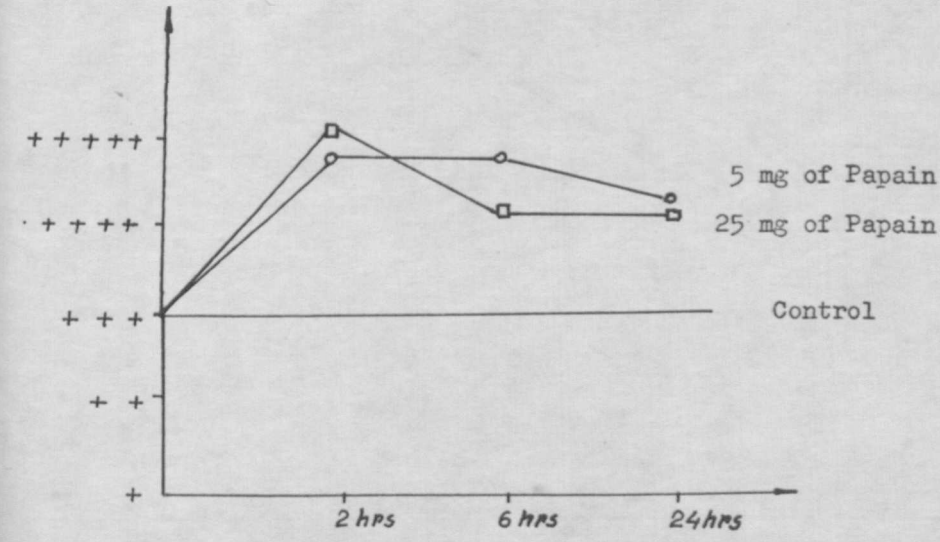


Fig. 2. Dynamics of changes in SDH and NAD-diaphorase activities under the influence of two different doses of papain.

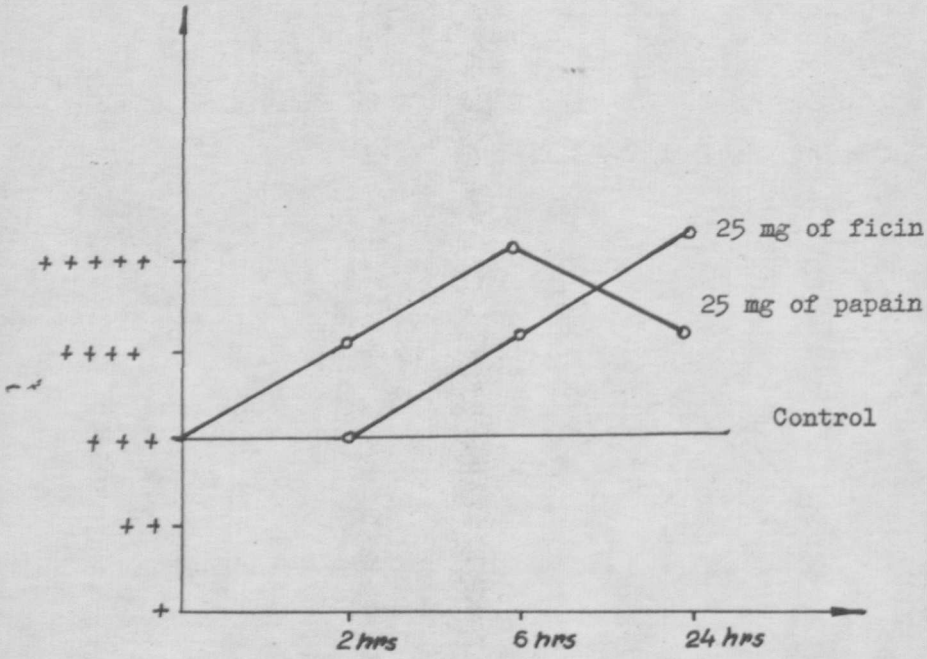
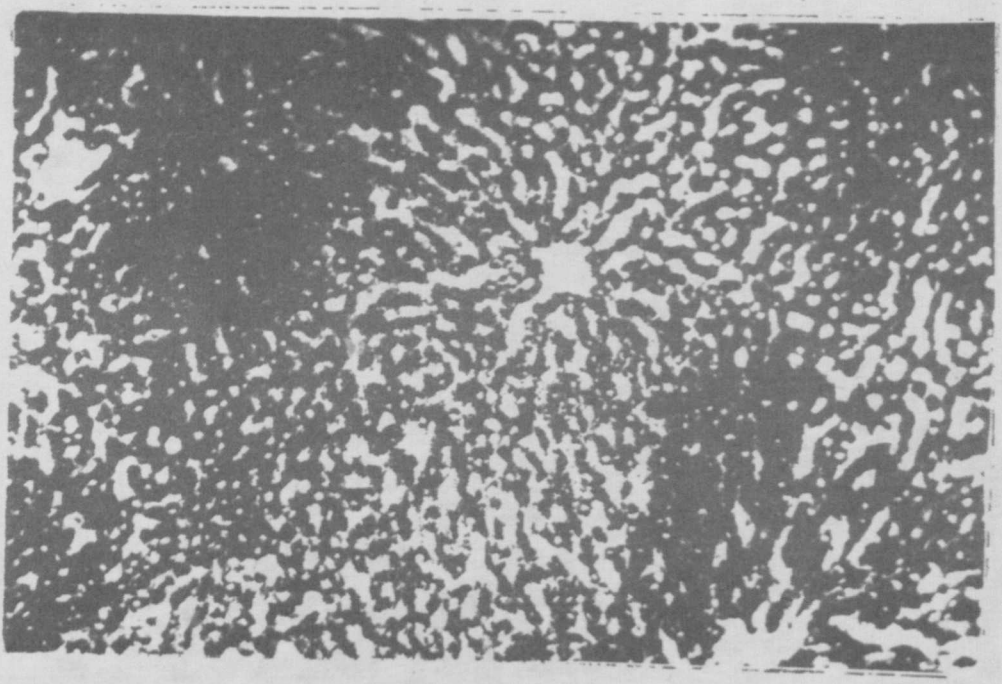
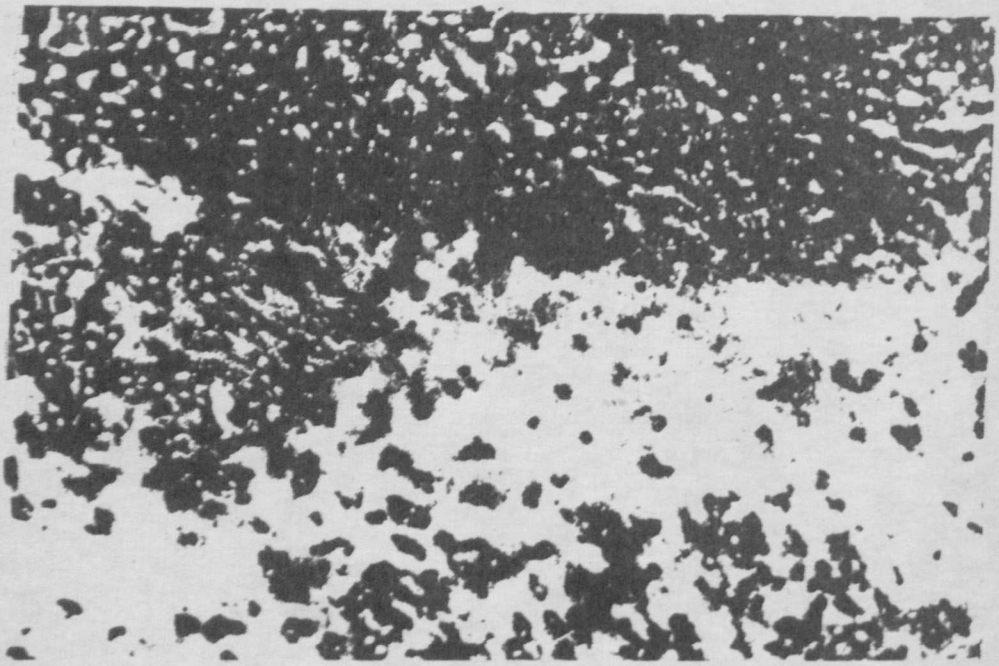


Fig. 3. Dynamics of changes in acid phosphatase activity under the influence of 25 mg of papain and 25 mg of ficin.



Lactatdehydrogenasae - 2 hrs
after injection with 25 mg Papain



Lactatdehydrogenasae
Above - 24 hrs after injection
with 25 mg Papain
Down - Control