HISTOCHEMICAL STUDIES ON ALKALINE PHOSPHATASE IN LIVER, KIDNEY, AND THE OTHER ORGANS AFTER TOTAL PANCREATECTOMY.

by

HARUMI KISHIMURA

From the First Surgical Division, Kyoto University Medical School
(Director : Prof. Dr. CHISATO ARAKI)
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Our knowledge concerning the biochemical function of phosphatase is still limited, although it is presumed that it plays an exceedingly important part in the metabolism of carbohydrate, fat and nucleic protein in the living body. Ever since the proposal made by COLOWICK et al.[1] (in CORI's laboratory) that certain hormones (such as those of anterior pituitary gland, adrenal cortex, and pancreas) act on hexokinase, there have been an increasing number of studies on the relations between phosphatase, which is unseparably related to hexokinase, and carbohydrate metabolism. MARSH and DRABKIN[2] noted the increase in activities of renal acid and alkaline phosphatase in rats which were in a state of alimentary hyperglycemia. On the other hand, CANTOR et al.[3], DRABKIN and MARSH[4] noted that the increased activities of liver and plasma phosphatase in a rat with well established alloxan diabetes could be restored to normal by effective insulin therapy. Moreover, the possible connection of phosphatase to various hormones is indicated by some reports on the relations between tissue phosphatase and internal secretion[5~9]. As an effect of total pancreatectomy, the loss of internal secretion, especially of insulin, may well result in hyperglycemia and bring homeostatic changes of the other endocrine glands. This is suggested also by HASEGAWA's report[10] that the decrease in number of chromophile cells in anterior pituitary gland is noted after total pancreatectomy. The impairment of intestinal absorption of carbohydrate, fat and protein due to the lack of external pancreatic secretion also comes into the picture. What reactions, then, does phoshatase show after total pancreatectomy? The following is the results of my histochemical study on the changes of alkaline phosphatase in various organs of totally depancreatized dogs.

METHODS OF EXPERIMENT

Adult dogs weighing approximately 10 kg. were used in this study. Total pancreatectomy on dogs frequently leads to necrosis of duodenum and eventual death of the animal, not only because duodenum and pancreas are situated so closely to each other, but because the blood vessel system of these organs is highly interrelated. In order to avoid such complications, the necessity of total pancreaticoduodenectomy followed by gastrojejunostomy, and choledochojejunostomy has been pointed out by HONJO[11]. Even with minute care, however, stricture of common bile duct, infe-

ction of choledochus and liver were not infrequent.

Increase in alkaline phosphatase of liver following obstructive jaundice had been emphatically pointed out, and histochemically proved by many investigators [12~15]. In an attempt to eliminate these complications, I worked out the following method in collaboration with HASEGAWA. Brances of pancreaticoduodenal blood vessels to pancreas were carefully separated and cut off. This procedure allowed pancreaticoduodenal artery and vein to remain on the side of duodenum, and made it possible to carry out total pancreatectomy, not combined with duodenectomy, without causing necrosis of the duodenal wall. To confirm the feasibility of my operative procedure it was further studied macro- and microscopically at autopsy whether or not any pancreatic tissue remained.

In the case of subtotal resection of pancreas, about 1/10 of pancreas was left at the adjacent portion of the outlet of main pancreatic duct. These dogs were found after operations to be in a state of hyperglycemia.

Both totally and subtotally depancreatized dogs were treated with the regular insulin (5 to 15 I. U. a day), thus keeping a state of hyperglycemia from 200 to 500 mg/dl..

In order to ligate pancreatic duct, double ligations were performed at the site where pancreas was attached to duodenum. After severing pancreas from duodenum great omentum was inserted between both tissues to prevent the development of internal pancreatic fistula. Occasionally necrosis of the duodenal wall happened to occur after this operation, necessitating the resection of necrotic portion of duodenum. In this study, however, only the dogs which did not undergo the resection of any part of intestinal tract were used.

At appropriate times after operation, the animals operated on, together with the control group were anesthetized with 30 mg of Isomytal per 1 kg of body weight, and after death by bleeding the tissue sections were taken while they were not yet inactivated for alkaline phosphatase. The activity of alkaline phosphatase in tisssue sections was demonstrated by the method of SHIMIZU-ARIZONO[16]——a modification of KABAT-FURTH's method. Sections were cut at 6μ and were incubated for 3 hours. Incubation period of 3 hours was most fitted for practical purposes and it was used as a standard period of time for incubation. Sections incubated for 1 hour and 6 hours respectively were also used for examination. Nuclei were counterstained with haematoxylin in order to discriminate more accurately the microanatomical constituents of the tissue. Haematoxylineosin stain was also adopted for these tissue sections.

RESUITS

I) *ALKALINE PHOSPHASE IN LIVER.*

A) Normal Dogs :

a) Fasting Dogs (fasting from 18 to 20 hours)

Hepatic alkaline phosphatase activities in a state of fasting showed no abnormal findings worthy of mention. Difference of activities according to sex was little.

Generally speaking, the intensity of stain was most remarkable in the peripheral part of lobules, less remarkable in the central part, and least in the intermediate part. The stained bile capillaries in the peripheral part presented fine black lines, which formed an intricate and intercellular network with twists and branchlike shoots. Often both nuclei and cytoplasm of sinusoidal endothelium reacted in black. The cytoplasm of liver cells usually reserved faint phosphatase activity, while in most of the nuclei only nuclear membranes and nucleoli were stained. On the contrary, in the central zone of lobules, enzymic reaction of bile capillaries was less pronounced, and the number of capillaries stained was much smaller, and occasionally complete absence of any reaction was seen. The cytoplasm of liver cell reacted only slightly, and was recognizable in faint grayish color. Cells adjoining the central vein, however, displayed more marked activity than the other cells in the central portion. Nuclei of liver cells were hardly recognized. Central veins and cells in intermediate portion of lobules showed no reaction. KUPFFER cells contained usually no enzyme, but sometimes the active form of KUPFFER cells was stained in black. In the periportal field, only an epithelial lining of the inside of interlobular bile ducts reacted remarkably, but interlobular blood vessels and connective tissue showed no reaction (Fig. 1). Above picture of alkaline phosphatase activity in liver is almost similar to the results reported by WACHSTEIN and ZACK [12].

b) Anesthetized Dogs Following Intraabdominal Injection of Isomytal (30 -50 mg per 1 kg of body weight).

In my experiment totally depancreatized dogs were brought to death by blee ding under anesthetisation of Isomytal. In order to exclude the effects of injection of Isomytal upon the organism, I examined the specimens taken at 30, 60 and 120 minutes respectively after injection of Isomytal and none of them showed any significant difference from those of normal dogs.

c) One hepatic tissue section was taken from a dog, and 2 hours later another one was taken from the same liver. Between these two tissue sections no significant difference in phosphatase activity was observed.

d) Dogs after Feeding.

Alkaline phosphatase activities were examined 2, 2.5, 3 and 5 hours respectively after feeding, but they were almost the same as those of fasting dogs or slightly less in grade.

e) Dogs Injected with 20% Glucose Solution 40cc.

1) 10 minutes after injection.

2) After 6 times injections at intervals of 20 minutes.

These dogs revealed no significant difference from each other showing almost the same attitude in phosphatase activity as fasting ones. Namely, during the period of hyperglycemia which was induced by feeding or injection of glucose, alkaline phosphatase activity in liver tissue was almost equal to or rather less than that in a state of fasting.

f) When the dog underwent continued injection of regular insulin (1 I. U. per kg of body weight daily) for 2 weeks, alkaline phosphatase activity of

liver tissue was the same as in the case of normal dogs.

g) Postmortem Changes.

In fasting dogs, liver tissue sections were taken prior to, 4, and 16 hours after their death by bleeding respectively.

1) Biopsy.

Not different from the results obtained in other fasting dogs.

2) 4 hours after death (shade temperature about 21°C).

Atrophy of liver cells and "pyknosis" of their nuclei were observed. All bile capillaries, in peripheral zone of lobules, were stained black and cytoplasms of liver cells showed increased enzymic activity. On the other hand, in the central portion of lobule phosphatase activity was rather lowered.

3) 16 hours after death (shade temperature about 21°C).

In these cases atrophy of liver cells and "pyknosis" of their nuclei were more marked than in cases of 4 hours after death, and at some places melting nuclei were seen. In spite of the increase in phosphatase capillaries could not be recognized in the peripheral field.

B) Starved Dogs :

In starvation anterior pituitary gland, adrenal cortex, and spleen etc. tend to check the utilization of glycogen in liver and muscles[17a, 18, 19, 20]. The effects of starvaion on phosphatase activity are summarized in Table 1. Except S_5, the phosphatase activity in a state of starvation increased to a more marked degree than in normal dogs (Fig. 2).

C) Subtotally Depancreatized Dogs :

In the dogs examined 87 days (the level of blood sugar measured at the last time was 454 mg/dl.) and 292 days (the level of blood sugar measured at the last time 356 mg/dl.) respectively after operation, alkaline phosphatase activities in liver showed the reaction similar to that in normal dogs.

D) Dogs with Ligation of Pancreatic Duct :

All the dogs 42, 54 and 71 days respectively after operation indicated the enzymic reactions in peripheral portion similar to those in normal dogs, while in central portion markedly increased phosphatase activities (in cytoplasms of liver cells and bile capillaries) were observed. On the other hand, sinusoidal endothelium in both peripheral and central portion showed no enzymic reaction (Fig. 3).

E) Totally Depancreatized Dogs (Fig. 4) :

As seen Table 2, except T85, totally depancreatized dogs showed somewhat increased enzymic activities. In only T85, alkaline phosphatase activities showed no significant difference from those in normal dogs. It seems that the degree of increase in enzymic activities is related rather to blood sugar level than to the number of days after operation. T50, T58 and T85 with relatively low blood sugar level tended to show lower phosphatase activity than the other dogs. If hyperglycemia was continuously kept in spite of the injection of insulin, no significant difference in alkaline phosphatase activities of liver was seen between totally depancreatized dogs injected with a large quantity of insulin (10-15 I.U./day) and

those with a relatively small quantity of insulin (2-5 I.U./day). When 12 I. U. of insulin was injected in T83, the blood sugar went down from 397 mg/dl. to 313 mg/dl. within 2 hours after injection. Although, in a liver preparation which was incubated for 3 hours, the alkaline phosphatase activities hardly showed any difference between before and after the injection of insulin, enzymic activities decreased a little in a preparation incubated for 1 hour after injection. Following the injection of 10 I. U. of insulin in case of T84, the blood sugar fell from 353 mg/dl. to 210 mg/dl.. In this case the alkaline phosphatase activities in liver decreased considerably in a preparation incubated for either 1 or 3 hours.

Ⅱ) *ALKALINE PHOSPHATASE ACTIVITY IN KIDNEY.*

A) Normal Dogs (Fig. 5) :

a) Fasting Dogs.

The brush border and the apical portion of the proximal convoluted tubules showed most intense activity and were stained in the nuclei deeply. The closer to the exterior stratum (renal capsule), the stronger and more numerous the reactions became and, on the other hand, the nearer to the interior stratum (medullary substance), the weaker and fewer in number they became. The alkaline phosphatase revealed little activity in glomerulus. Both pars radiata in cortical substance and medullary substance showed no enzymic activities, but occasionally the trace of staining of nuclei of renal tubles was recognizable. These results roughly agreed with the records of DEAN and DEMPSEY [21] and ARIZONO [22].

b) Dogs 2 and 3 Hours after Feeding (Fig. 6).

Not only the brush border but also the nucleus and cytoplasma of proximal convoluted tubules were stained in black. The renal corpuscles also showed slightly more marked phosphatase activities than normal. Endothelial cells of blood capillaries both in pars radiata of cortical substance and in medulally substance and sometimes endothelial cells of large veins and arteries showed marked activities.

c) Dogs after Being Injected 6 Times with 20% Glucose Solution 40cc at Intervals of 20 Minutes.

In these cases alkaline phosphatase activities of proximal convoluted tubules increased more than in normal dogs. The glomerulus showed no enzymic activity, but capillaries in pars radiata revealed faint phosphatase staining and capillaries in medullary substance showed more pronounced activity than those in pars radiata.

B) Starved Dogs (Fig. 7) :

As shown in Table 3, in proximal convoluted tubules and glomerulus, phosphatase activities increased, and endothelial cells of capillaries in medullary substance and in pars radiata in cortical substance showed marked increase in phosphatase activities.

C) Subtotally Depancreatized Dogs :

Phosphatase activities of kindney in subtotally depancreatized dogs were similar to those in dogs with alimentary hyperglycemia.

D) Dogs Whose Pancreatic Duct Were Ligated :

Phosphatase activity in pars radiata showed greater increase than in normal

dogs, but no significant increase in phosphatase activity in capillaries could be found.

E) Totally Depancreatized Dogs (Fig. 8) :

Irrespective of the level of blood sugar, quantity of injected insulin, and the length of period after operation, kidney phosphatase activities in all the dogs with hyperglycemia increased as showed in Table 4. Although the morphological appearance of phosphatase activities in these dogs was qualitatively similar to that in dogs with alimentary hyperglycemia, the activity of enzyme in the former was more marked than in the latter showing almost the same result as that in starved dogs.

Ⅲ) *ALKALINE PHOSPHATASE IN ADRENAL CORTEX.*

A) Fasting Dogs :

In endothelial cells of the cortical substance the alkaline phosphatase was present and its activity was most prominent in zona glomerulosa. The enzymic activities in cytoplasm of cortical cells showed little reaction, while in nuclei the nuclear membranes and nucleoli were stained only slightly. Phosphatase activity of medullary substance could hardly be recognized, but occasionally phosphatase of endothelial cells of blood capillaries was stained in black.

B) Totally Depancreatized Dogs :

No difference of enzymic activities existed between normal dogs and those which were operated on. Occasionally, stained endothelia of blood capillaries were found to be more abundant in the latter.

Ⅵ) *ALKALINE PHOSPHATASE IN THYROID GLAND.*

A) Fasting Dogs :

Enzymic activities of cytoplasma and nucleus in thyroid gland were little. Sometimes alkaline phosphatase of colloid was stained markedly. In endothelia of blood capillaries adjoining the follicles pronounced concentration of enzyme was observed but the number of stained endotheliums was few as shown in the report of KABAT and FURTH [23].

B) Totally Depancreatized Dogs :

Phosphatase activities of totally depancreatized dogs were similar to those of normal dogs. Occasionally, the number of reacting blood capillaries was much larger in the former.

Ⅴ) *ALKALINE PHOSPHATASE IN PAROTID AND SUBMAXILLARY GLAND.*

A) Normal Dogs :

In submaxillary and parotid glands, except interlobular ducts, cytoplasms of cells of intercalated and intralobular ducts and those of salivary secretory alveoli contained nearly no enzyme, but only nucleoli were stained in traces. In interlobular ducts, occasionally, nuclei were stained in black and cytoplasms showed the activity of phosphatase a little. Endothelial cells of blood capillaries of terminal secreting alveoli showed marked enzymic reactions. Above findings were in accordance with the results obtained by DEAN [24].

B) Totally Depancreatized Dogs :

In interlobular ducts of totally depancreatized dogs the enzymic activities were lower than those in normal ones.

DISCUSSION

Since TAKAMATSU [25] and GOMORI [26] published, almost simultaneously, their own methods for histochemical demonstration of alkaline phosphatase, there have been a number of reports concerning the distribution of the enzyme in normal and morbid conditions. On the other hand the biochemical studies on the function of phosphatase have been much promoted and now it is understood that phosphatase acts as a catalyst in the process of dephosphorylation of certain phosphate esters. SWANSON [27] demonstrated recently that chemical property of glucose-6-phosphatase is not the same as that of phosphatase described by GOMORI-TAKAMATSU. But histochemistry of phosphatase, even at the present time, is yet unknown. From ARIZONO' s report [28] that electrical stimulation of autonomic centers causes the changes in acid and alkaline phosphatase activities in liver and kidney and also from other studies concerning hormonal regulations of enzyme, it is readily supposed that the enzymes in the living body are functioning under extremely complicated situations. It must be, therefore, rather too premature to expound any biological theory on phosphatase from only the results of experimental histochemical studies. My attempt will not go any farther than comparing the results of my study with those reported already by the other authors.

I) *ALKALINE PHOSPHATASE IN LIVER.*

There are few reports concerning the postmortem changes of alkaline phosphatase. It is generally accepted that the enzyme in the living body will make a change after death. WACHSTEIN [29] has emphasized that uncertainty in histochemical finding of alkaline phosphatase activity in human body is due to postmortem changes of the enzyme. I also obtained the evidences indicating an increased phosphatase activity in peripheral portion of the liver from which specimens were taken 4 and 16 hours respectively after death. Whether the cause is autolysis or stasis of bile or some other factor is not yet determined. At any rate, it may be said that WACHSTEIN' s hypothesis was confirmed by the findings in my study.

Little is known also of the effect of food ingestion on liver alkaline phosphatase. BODANSKY [30] stated that the alimentary hyperglycemia was accompanied by an increase in serum alkaline phosphatase activity, and BINET and PAURATE [31] reported that in diabetes induced by pancreatectomy, increases in serum alkaline phosphatase activity were almost proportional to those in blood sugar levels. CANTOR [32] found that in rats, in a well-established alloxan diabetic state, serum phosphatase became three times as much as that in normal state and DRABKIN [4] showed that in an established alloxan diabetic rat alkaline phosphatase activity in liver increased by 38%, but according to those investigators, the effective insulin therapy applied to rats with well established alloxan diabetes was found to restore increased serum and liver phosphatase activities to normal. COLOWICK [32] and SHIMIZU [33]

reported also that they could recognize an increase in alkaline phosphatase activity in livers of alloxan diabetic rats and rabbits. These reports serve to suggest the existence of parallelism between the blood sugar level and the concentration of blood serum alkaline phosphatase, and also of close relationship between the blood sugar level and the concentration of liver alkaline phosphatase. It is, therefore, natural to assume that alimentary hyperglycemia is accompanied by an increase in liver phosphatase activity. In my experiment on the animals, contrary to what was expected, liver alkaline phosphatase activity following venous injection of glucose solution and feeding of diet was similar to or even less than normal. DRABKIN [4] reported that no significant quantitative difference was recognized in the levels of liver alkaline phosphatase between the rats which were fed and those fasting for 24 hours. Although in his cases no mention is made as to the time which elasped after feeding, his results can be considered as in line with my findings. Judging from the concentration of serum alkaline phosphatase after feeding, JACKSON [34] concluded that most of serum alkaline phosphatase in rats did not originate from liver as was reported previously by many other investigators [35 a c b], but had its origin in the intestine.

The results obtained in my experiments on the totally depancreatized dogs coincide with the above-mentioned findings in alloxan diabetic animals. In T83, in which decrease in blood sugar concentration is not marked, decrease in liver alkaline phosphatase activity was hardly recognized; but, on the other hand, in T84, in which blood sugar levels decreased rather markedly, decrease in liver alkaline phosphatase activity was somewhat pronounced. This seems to be in accordance with the finding of DRABKIN that effective insulin therapy applied to rats with well established alloxan diabetes was found to restore alkaline liver phosphatase activity to normal.

Many investigators reported an elevation of serum alkaline phosphatase in dogs following pancreatic duct ligation, and recently SHAY et al. [36] ascribed the increase in serum alkaline phosphtase to fatty degeneration of liver and also to pericholangitis following pancreatitis. I noted an increase in alkaline phosphatase activity in the central portion of liver lobules after pancreatic duct ligation, but its mechanism is not yet clarified. Such enzymic changes of liver may be explained partly by the study of SHAY et al. as mentioned above, but a role played by the changes of metabolism after the loss of pancreatic juice should also be taken into consideration. WACHSEIN [37] reported that an increase in liver enzymic activity was observed in mice and rats after starvtion. ARIZONO [22] described an elevation of liver alkaline phosphatase activities in rats which were kept in a state of fasting for 24 or 48 hours, and also the disappearance of phosphatase within 72 hours after the beginning of starvation. In my experiment all the dogs, except dog S_5 which was kept fasting for comparatively short periods, showed an increase in liver phosphatase activity. Because totally depancreatized dogs are, more or less, in a state of chronic starvation, those factors like starvation or ligation of pancreatic duct should also be taken into consideration in explaining the mechanisms of increased liver

phosphatase activities after total pancreatectomy.

Ⅱ) *ALKALINE PHOSPHATASE IN KIDNEY*

LUNDSGAARD et al.[38], and KALCKAR[39] postulated the participation of the phosphorylation in the mechanism of reabsorption of glucose, and BECK[40] confirmed it, though his experiments were limited to acid phosphatase. Then it was evidenced histochemically by GOMORI[41] that the proximal tubule was one of the sites where the renal phosphatase was contained in high concentration. Recently MARSH and DRABKIN[2] confirmed the view expressed by LUNDSGAARD and BECK and stated as follows : the alimentary hyperglycemia was found to be accompanied by increased activities in renal alkaline phosphatase by about 70% and under the administration of phloridzin the activity of renal alkaline phosphatase was demonstrated to be lowered in vivo as well as in vitro. From those findings phloridzin glycosuria may be ascribed to insufficiency of kidney phosphatase activity. They, therefore, concluded that the renal threshold for glucose indicated, at least in part, the limit to which phosphatase activity could be raised. In my work, too, in the alimentary hyperglycemia and in the hyperglycemia following injection of glucose solution the renal phosphatase activities increased markedly not only in the proximal convoluted tubules and glomerulus but in the endothelial cells of blood capilaries in pars radiata of cortical substance and in medullary substance, showing black stain in those areas. Such increase in phosphatase activities in kidney seems to indicate the renal hyperfunction in reabsorbing glucose during hyperglycemia. I have not found any description of such black staining of endothelium of blood capillaries in the literature and the cause of such staining is to be sought for. As the black stains were recognized in the endothelial cells of blood capillaries at the time of increased intestinal absorption it seems probable that blood capillaries not only allow the fluid to pass through their walls but perform some other function assisted by alkaline phosphatase. MATHIES[9] and KOCHAKIAN[42] emphatically pointed out that the alkaline phosphatase activity in kidney decreased corresponding to the lowering of renal function. Judging from the results of my experiment on totally and subtotally depancreatized dogs, and also on dogs fed with diet and on those injected with glucose solution, alkaline phosphatase activity of kidney, contrary to that of liver, seems to increase in proportion to the blood sugar level. The increase in the phosphatase activity was higher in totally depancreatized dogs than in dogs fed with diets. Injection of insulin in totally depancreatized dogs failed to exert any significant effect on the enzymic activity of kidney perhaps because of the failure to give the proper dose. The renal alkaline phosphatase activity of starved dogs was similar to that of totally depancreatized dogs. This may be explained as a sign of accelerated function of kidney in retaining the blood sugar in the body. The increase in activity of alkaline phosphatase was observed also in the dogs whose pancreatic duct was ligated, although in these cases such reaction was limited only to proximal convoluted tubules. It seems reasonable, therefore, to attribute an increase in renal phosphatase activity in totally depancreatized dogs not only to postoperative hyperglycemia but partly to the effects of starvation

and ligation of the pancreatic duct.

Ⅲ) *ALKALINE PHOSPHATASE IN THE OTHER ORGANS.*

As the metabolism of carbohydrate has a very close rlationship with the function of adrenal cortex, thyroid gland, and salivary glands, I have studied the alkaline phosphatase activities in these organs. SHIMIZU [33] reported an increase in activity of alkaline phosphatase in zona reticularis of adrenal cortex in alloxan diabetic rabbit. I, however, could not recongnize any significant difference of alkaline phosphatase activity between totally depancreatized dogs and normal ones. Also concerning the phosphatase of thyroid gland, there was no difference worthy of mention between the two groups of animals.

MOBACK and MONTAGNA [43] reported that no change of alkaline phosphatase activity was observed in a state of hyperfunction of parotid gland, but that in serozymogenic cells of submaxillary gland an increase in alkaline phosphatase activity was demonstrated. In my study on totally depancreatized dogs, a decrease in phosphatase activities both in interlobular ducts of parotid and in submaxillary gland was recognized, although its significance has not yet been clarified.

SUMMARY

(1) 2, 2.5, 3, and 5 hours respectively after feeding of diet, 10 minutes after injection of 40cc of 20% glucose solution and after 6 times injection of the same amount of glucose at intervals of 20 minutes, normal dogs showed no significant difference in the enzymic activity of liver from fasting animals. But the renal alkaline phosphatase activity increased generally after feeding and after venous injection of glucose solution. The increase in renal enzymic activity was remarkable in the proximal convoluted tubules and glomerulus and endothelial cells of blood capillaries in pars radiata of cortical substance and in medullary substance.

(2) Regular insulin injection continued for 2 weeks (1 I. U. per kg of body weight daily) did not exert any particular effect on the alkaline phosphatase activity of liver tissue, the result being much the same with that in normal dogs.

(3) Dogs whose pancreatic duct was ligated showed the marked increase in the enzymic reation in the central portion of lobules of liver. The renal alkaline phosphatase activity in these dogs also showed an increase though only in pars radiata.

(4) In spite of the continued hyperglycemia, the hepantic alkaline phosphatase activity in subtotally depancreatized dogs did not show any increase, but the renal enzymic activity showed the similar increase to that in normal dogs with hyperglycemia induced by feeding of diet.

(5) In starved dogs, alkaline phosphatase activities both of liver and of kidney showed a marked increase.

(6) In totally depancreatized dogs, an increase in enzymic activities was observed, and the increase like this seems to be derived rather from the blood sugar level than from the length of time after operation. In totally depancreatized dogs, if the blood sugar level was lowered effectively by injection of insulin, revealed a tendency to diminish. The renal alkaline phosphatase activity in totally depancre-

atized dogs showed the similar increase to that in dogs with hyperglycemia appearing after feeding of diet.

(7) The enzymic activity of adrenal cortex and of thyroid gland in totally depancreatized dogs showed no particular difference from that in normal ones.

(8) The enzymic activity in submaxillary and parotid glands in totally depancreatized dogs did not differ much from that in normal dogs except in interlobular ducts where its decrease was observed.

(9) In normal subjects, 4 and 16 hours after death, the increased alkaline phosphatase activity was recorded in liver sections, especially in the peripheral fields of lobules.

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和文抄録

膵全剔犬諸臓器，特に肝，腎のアルカリ性フォスファターゼの組織化學的研究

京都大学医学部外科学教室 第一講座（荒木千里 教授指導）
岸 村 治 美

正常犬，膵部分切除犬，膵管結紮犬，飢餓犬を対照とし，膵全剔犬の肝，腎を主とし，更に甲状腺，副腎，耳下腺，顎下腺のアルカリ性「フオ」を清水，有薗法を用いて組織化学的に検索し，次の成績を得た．

1) 正常犬の食後 2, 2.5, 3, 5 時間の肝アルカリ性「フオ」及び20%葡萄糖液 40cc 毎20分静脈内注射後 10, 120分の肝アルカリ性「フオ」反応は正常犬空腹時のものと有意の差はない．腎アルカリ性「フオ」反応は全体として増強する．即ち細尿管主部，腎小体に増加し，且髄質，皮質髄放線部の毛細血管内被細胞が黒染する．

2) 正常犬にインスリン体重Per kg 1 I.U.を6週間連続注射した例の肝アルカリ性「フオ」反応は正常の範囲にあるか稍々減少している．

3) 膵管結紮犬の肝アルカリ性「フオ」反応は小葉部中心で増強する．腎アルカリ性「フオ」も細尿管主部のみに増強する．

4) 高血糖を持続するに拘らず，膵部分切除犬の肝アルカリ性「フオ」は増加しないが，腎「フオ」は正常犬の食餌性高血糖時の所見に類似した反応増加を見る．

5) 飢餓犬の肝及び腎アルカリ性「フオ」は共に反応増強を認めた．

6) 膵全剔犬の肝アルカリ性「フオ」反応は増強する．其の程度は術後日数の長短に関係なく，寧ろ血糖値に関係があるらしい．又術後のインスリン投与が比較的少量でも又多量でも血糖値が余り下らないと両者に肝及び腎「フオ」の差を認め難い．

同一全剔犬にインスリンを投与し，その前後の肝アルカリ性「フオ」を検したが，インスリンが比較的効果的に働くと肝「フオ」も減少する傾向がある．腎アルカリ性「フオ」は増加する．その所見は食餌性高血糖時の所見と類似する．

7) 膵全剔犬の副腎，甲状腺のアルカリ性「フオ」反応は正常犬との間に有意の差は認めない．

8) 膵全剔犬の耳下腺，顎下腺のアルカリ性「フオ」反応は共に排泄管に減少を認めたが，他の部にはなかつた．

9) 同一正常犬で死後 4, 16 時間の肝週辺部特に毛細胆管，肝細胞に反応の増加を認めた．

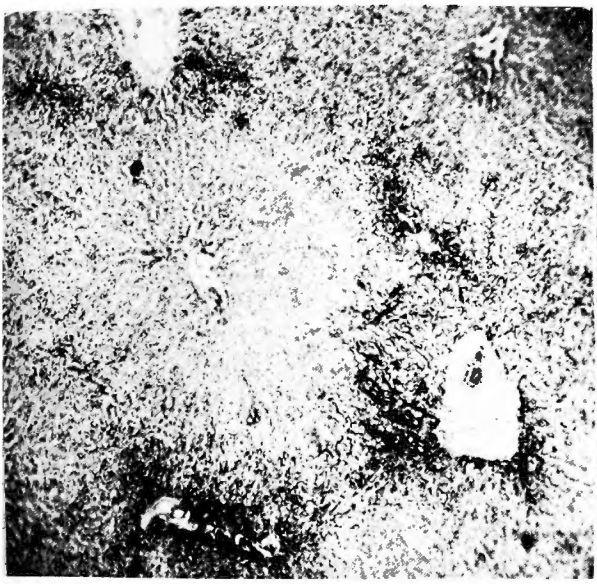

Fig. 1) Alkaline phosphatase activity in liver of a fasting normal dog. (×80)

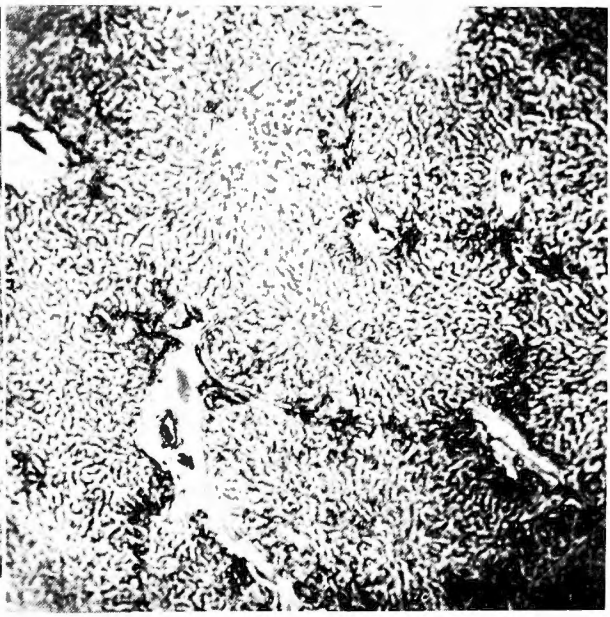

Fig. 2) .Alkaline phosphatase activity in liver of a starved dog (S_2) (×80)

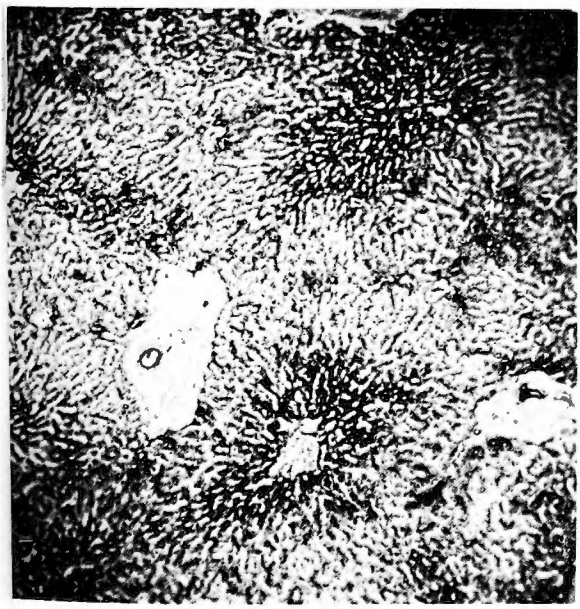

Fig. 3) Alkaline phosphatase activity in liver of a dog whose pancreatic duct was ligated. (42 days after opertion) (×80)

Fig. 4) Alkaline phosphatase activity in liver of a totally depancreatized dog. (T56) (×80)

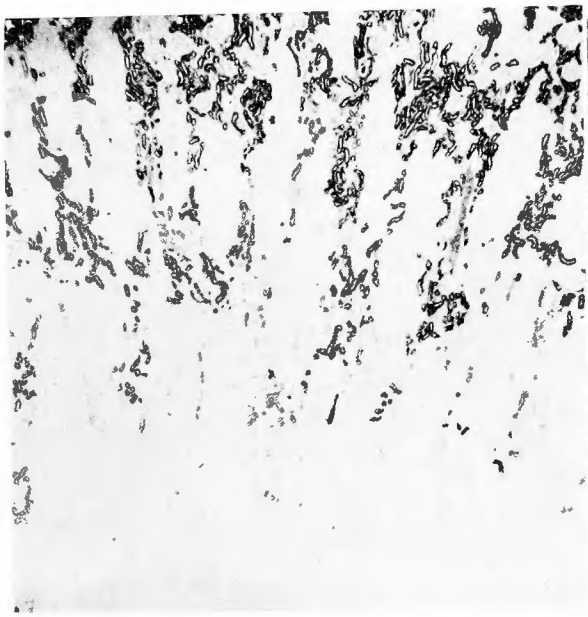

Fig. 5) Alkaline phosphatase activity in kidney of a fasting normal dog. (×80)

Fig. 6) Alkaline phosphatase activity in kidney of a normal dog, 2 hours after feeding. (×36)

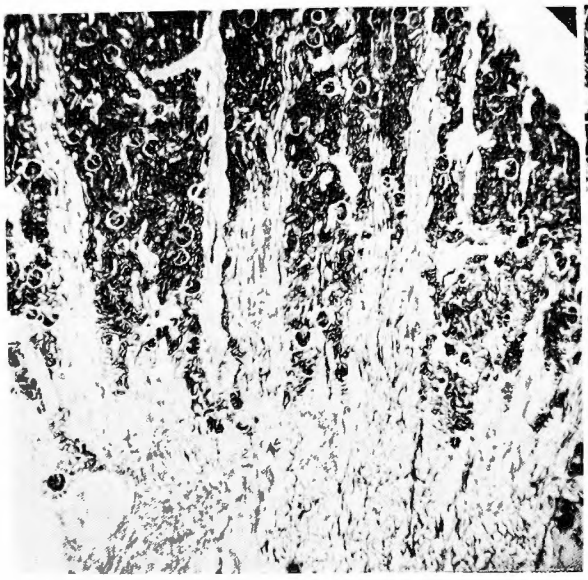

Fig. 7) Alkaline phosphatase activity in kidney of a starved dog. (S_4) (×36)

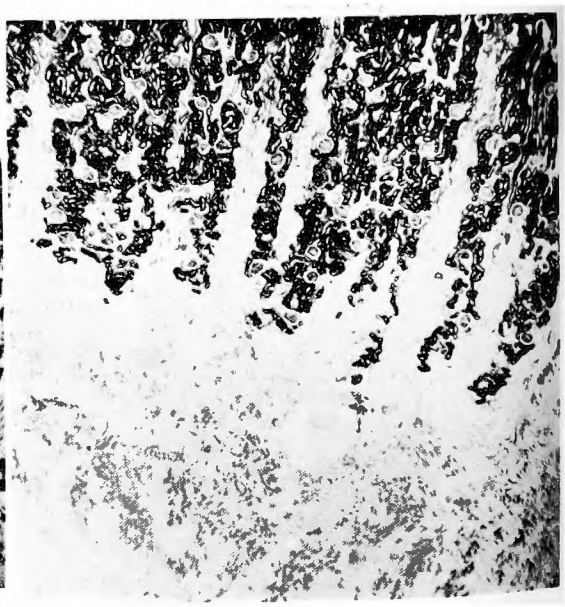

Fig. 8) Alkaline phosphatase activity in kidney of a totally depancreatized dog. (T56) (×36)

Table 1. Alkaline Phosphatase Activity in Liver of Starved Dogs.

No. of Dog	Sex	Starvaion (Day)	H-E Stain	Alkalline Phosphatase
S_2	♂	18	Haemostasis	Cytoplasms of liver cells and bile capillaries were stained markedly, but nuclei of liver cells showed no reaction. Peripheral portion of lobules reacted more distinctly than central portion.
S_4	♀	19	Ibid.	The reaction increased moderately in general.
S_5	♀	8	Infiltration of round cells here and there.	Almost the same as in normal dogs.
S_6	♀	14	Nothing unusual.	Increased reaction was seen as a whole.

Table 3. Alkaline Phosphatase Activity in Kidney of Starved Dogs.

No. of Dog	Sex	Starvation (Day)	H-E Stain	Alkaline Phosphatase
S_4	♀	19	Haemostasis. Atrophy of proximal convolution.	Pronounced increase in phosphatase in cytoplasms of cells in proximal convoluted tubules and in glomerulus. Endothelial cells of capillaries in medullary substance and pars radiata in cortical substance also showed a marked increase.
S_5	♀	8	Nothing unusual.	No reaction in glomeruli but increased reaction was recognizable proximal convolution and in endothelium of blood capillaries of medullary substance.
S_6	♀	14	Vacuoles in cells in proximal convoluted tubules.	Same as S_4.

Table 2. Alkaline Phosphatase Activity in Liver of Totally Depancreatized Dogs.

No. of Dog	Sex	Days after Operat.	Blood Sugar (mg/dl.)	Weight before Operat. (kg)	Weight after Operat. (kg)	Insulin Dosage a Day (I. U.)	H-E Stain	Alkaline Phosphatase
T 50	♂	32	231	6.5	5.5	3	Nothing unusual.	In general, reaction of cytoplasms of liver cells increased and peripheral part of lobules was stained more markedly than central part.
T 53	♀	41	346	8.0	5.5	5	A few intracellular vacuoles in peripheral part and atrophy of liver cells slightly recognizable.	As a whole increased activity recognizable (especially that of cytoplasms of liver cells)
T 56	♀	64	445	9.5	5.4	5	Ibid.	Somewhat increased generally, only bile capillaries were stained exceedingly.
T 58	♂	34	135	11.0	6.5	5	Ibid.	In central part of lobules, cytoplasms of liver cells and bile capillaries were stained somewhat more deeply than normal.
T 80	♂	25	456	10.0	7.5	15	Nothing unusual.	Cytoplasms of liver cells and bile capillaries reacted somewhat more heavily than normal.
T 83	♂	13	397	13.0	10.5	13	Ibid.	Definitely increased in general.
T 84	♂	7	353	14.0	13.5	15	Ibid.	Ibid.
T 82	♀	21	339	9.0	7.0	10	Vacuoles in cells in peripheral part	generally increased, only bile capillaries were normal.
T 85	♀	8	286	12.5	12.0	5	Haemostasis	Almost normal.

Table 4. Alkaline Phosphatase Activity in Kidney of Totally Depancreatized Dogs.

No. of Dog	Sey	Days after Operat.	Blood Sugar (mg/dl.)	Insulin Dosage (I.U./day)	H-E Stain	Alkaline Phosphatase
T 50	♂	32	231	3	Nothing unusual.	Markedly increased reaction in proximal convolution and blood capillaries in medulla and in pars radiata of cortical stratum were stained in black.
T 53	♀	41	346	5	Ibid.	Cytoplasms of cells in proximal convolution were stained in black. Glomerulus showed also increased reaction. Endothelial cells in medulla and in pars radiata were stained in black, especially in the boundary of medullary substance the reaction was marked.
T 56	♀	64	445	5	Vacuoles in cells in convolution.	Ibid.
T 58	♂	34	195	5	Nothing unusual.	Same as T53, but in glomerulus no enzymic reaction was seen.
T 80	♂	25	456	15	Ibid.	Same as T53.
T 82	♀	21	339	10	Ibid.	Ibid.
T 83	♂	13	397	13	Infiltration of round cells in some part of interstitial portion of cortical stratum.	Ibid. (2 hours after injection of insulin 12 I. U.)
T 84	♂	7	353	15	Simple swelling of cells in some part of proximal convolution.	Ibid. (3 hours after injection of insulin 10 I. U.)
T 85	♀	8	286		Same as T83	Ibid., but glomerulus was not stained.