CHAPTER FOUR

THE EFFECTS OF ALLOXAN DIABETES ON LIPASE ACTIVITIES IN THE RAT SALIVARY GLAND.

Several studies showed the consistent increase in Plasma and serum triglyceride concentrations in diabetic condition (Taskinen et al., 1982; Tayler, 1981; Walden et al., 1984). This may result from decreased use of Plasma triglyceride by tissues and they are not cleared by lipase (Robinson ,1970). It is known that heparin releases two kinds of lipolytic activities from tissues, hepatic triglyceride lipase (H-TGL) and extrahepatic lipoprotein lipase (LPL) (LaRosa et al., 1972; Krauss, 1973). Further investigation on the origin of these lipases have revealed that both LPL and H-TGL components are heterogenous, the former resides chiefly in adipose tissue, skeletal muscle, heart and mammary tissue (Nilsson-Ehle et al., 1980) and later on in ovary, adrenal gland and liver. The plasma free fatty acids are directed towards tissue for storage, where as in diabetic condition or in fasting state about 50 % are taken by the skeletal muscles and other tissues. Lipoprotein lipase is key enzyme in this process. It is synthesised in various cells from which it is secreted by energy requiring process (Cryer et al., 1975). The lipoprotein lipase molecules is bound to glucosaminoglycans (Olivercrona et al., 1977). The chylomicrons in the circulation are, therefore, efficiently hydrolyzed after the injection of heparin, as a result of release numerous lipoprotein lipase molecules from the tissues. Chylomicron consists of hydrophobic lipids (triglycerides and cholesterol esters) in the core of lipoprotein particles, the surface of which is coated with polypeptides (apolipoproteins)

and hydrophilic lipids (phospholipids and cholesterol). In adipose tissue the lipoprotein lipase activity has been found to be increased about 3 to 6 hrs after repeated meals (Lithell et al., 1978; Pagano MiraniOostdijk et al., 1983; Pykalisto et al., 1975) and insulin seems to be important hormonal regulator (Sadur and Eckel, 1982). In insulin treated diabetes, there is increase in Lipase activity in adipose tissue, Psoas minor, heart and diphragm; in fasting also there is decrease in lipase activity from liver, adipose tissue, Psoas minor, diaphragm but not from the heart (Nomura et al., 1984).

The above review indicates that lipase from some tissues secreted into plasma involved in triglyceride metabolism i.e. removal of triglyceride from plasma and the process is insulin-dependent. The aim of the present study was to investigate the effects of fasting and fasting fed alloxan diabetes on lipase activities of salivary glands, as to investigate the effect of insulin on lipase activity of salivary glands.

Material and Methods

Norwegian male rats (Rattus norvegicus) weighing about 200 to 225 gms were selected. About 80 rats were used for experimental purpose which were maintained on their normal diet. They were fasted overnight and diabetes was induced by intraperitoneal injection of alloxan monohydrate (40 mg/250 gm body weight in 1.00 ml of 0.9 % saline). Controls were

received 1.0 ml saline.

60 and 132 hrs after the alloxan administration six rats from each group were fasted for 12 hrs, three of them received food, after 12 hrs. of fasting for half an hour, then all the twelve rats from each group were killed by cervical dislocation along with their controls the salivary glands were removed, weighed and used for the estimation of Lipase. The Lipase was measured in milliunits/mg protein and milliunits/gm wet weight of the tissue by using 2-napthyl myristate (Arnold and Kramer, 1965) as a substrate, Blood was collected from sinus venosus by using heparin as an anticoagulant. Glucose was estimated by Folin Wu method (Hawk, 1965). Total proteins were estimated by using Lowry's method (Lowry et al., 1951).

Results

i) Submandibular Gland

Lipase specific activities/mg protein and Lipase activities/gm wet weight of the tissue from diabetic animals were described in Table No. 5 and their specific activities were depicted according to the concentration of glucose from blood in graph no. 16 and according to the time after alloxan administration in graph no. 17. The lipase activities/gm wet weight of the tissue were shown according to the blood glucose concentration in graph no. 18 and according to the time after alloxan administration in graph no. 19. As the blood glucose level increases there were decrease in the enzyme activities.

The decrease was significant (see graph no. 16 and 18). The specific enzyme activities of fasted controls were compared with 72 hrs diabetes fasted for 12 hrs, there was a significant difference ($P < 0.05$). But when control fasted fed was compared with alloxan fasting feeding, there was a highly significant difference ($P < 0.01$).

In enzymatic activities/gm also there was decrease as that of specific activities in both the cases, there was a highly significant difference ($P < 0.01$) between controls and diabetes.

In 72 hrs. fasted alloxan diabetes the specific activity was 20.5877 ± 0.4142 and in fasted controls it was 22.4273 ± 0.2341. In fasted but fed alloxan diabetes the specific activity was 19.8785 ± 0.2217 and it was 32.5348 ± 1.3813 in controls. The decrease was about two fold.

The lipase activity/gm of submandibular gland of 72 hrs alloxan diabetes fasted for 12 hrs showed a highly significant difference ($P < 0.01$) when compared with its respective controls. The lipase activity of fasted alloxan diabetes was 1041.66 ± 13.8112 and in its respective controls it was 1383.33 ± 1.3608. The highly significant difference between the lipase activities of submandibular gland of fasted but fed alloxan diabetes and controls was also noticed. It was $P < 0.01$. The activity in diabetic animals was 1087.50 ± 26.5167 and in controls it was 1966.66 ± 54.4347.

Table No. 5 : Effect of Alloxan Diabetes on the Specific Activities and Activities of Lipase in the Rat "Submandibular Gland".

Sr. No.	Time after Alloxan Administration	Animals	Glucose conc. ± SEM	Specific activities ± SEM	P	Mean ± SEM	P
1.	-	Control fasting 12 hr (CF)	41.05 ± 1.2695	22.4273 ± 0.2341 *3	P < 0.05 S	1383.33 ± 1.3608 *3	P < 0.01 HS
2.	72	Alloxan fasting 12 hr (AF)	105.55 ± 1.732	20.5877 ± 0.4142 *3		1041.66 ± 13.8112 *3	
3.	-	Control fasting + feeding $\frac{1}{2}$ hr (CFF)	42.10 ± 1.2914	32.5348 ± 1.3813 *3	P < 0.01 HS	1966.66 ± 54.4347 *3	P < 0.01 HS
4.	72	Alloxan fasting + feeding $\frac{1}{2}$ hr (AFF)	111.11 ± 1.2963	19.8785 ± 0.2217 * 3		1087.50 ± 26.5167 *3	
5.	-	CF	45.70 ± 0.3299	22.4273 ± 0.2341 *3	P > 0.05 NS	1383.33 ± 1.3608 *3	P < 0.01 HS
6.	144	AF	112.00 ± 1.2472	19.4963 ± 0.9904 *3		980.00 ± 9.4283 *3	
7.	-	CFF	46.88 ± 1.1511	32.5348 ± 1.3813 *3	P < 0.01 HS	1966.66 ± 54.4347 *3	P < 0.01 HS
8.	144	AFF	128.00 ± 2.0548	19.8258 ± 0.3698 *3		990.00 ± 5.4283 *3	

Results are expressed as mean ± SEM

*represents the number of animals

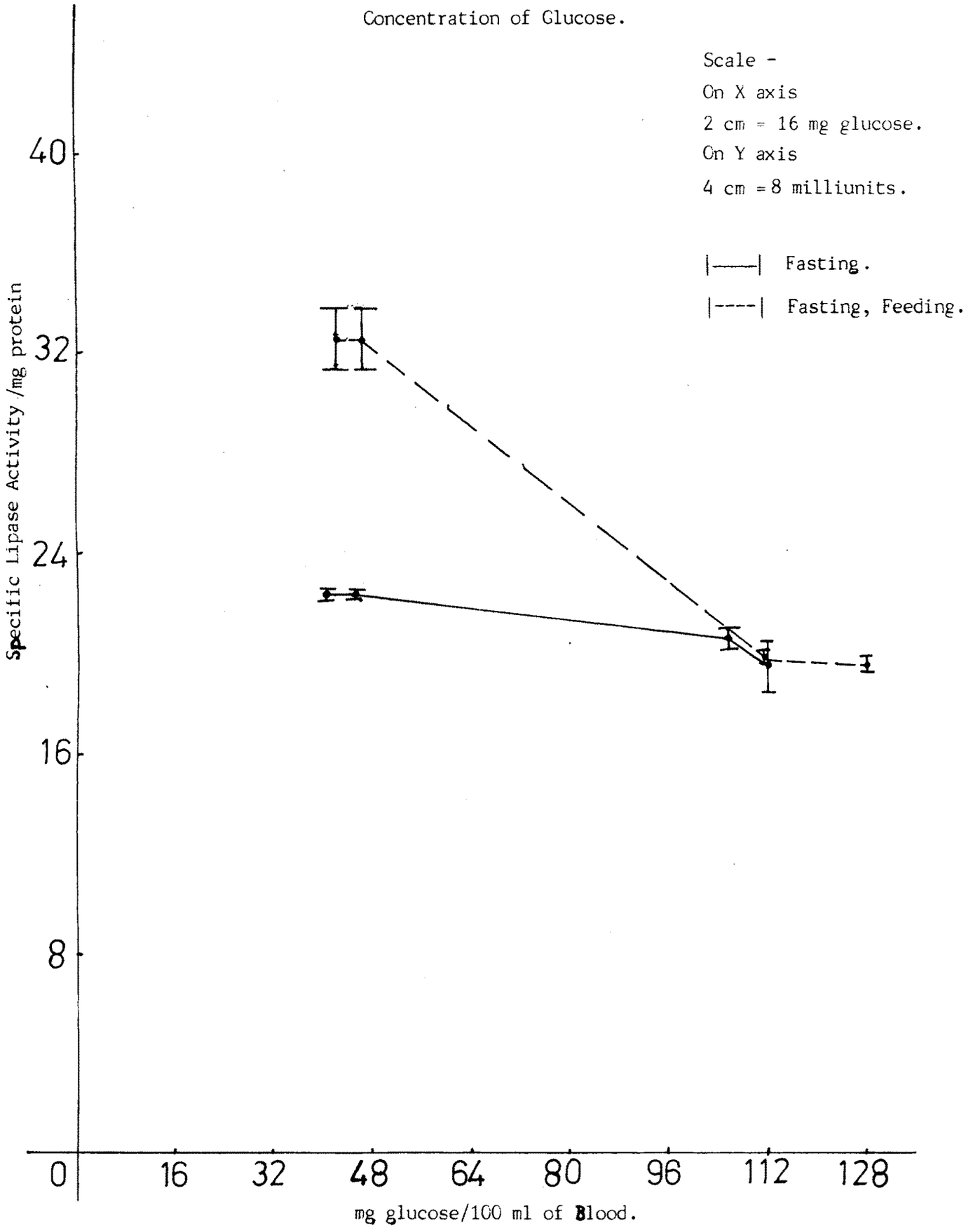
HS : Highly significant

S : Significant

NS : Nonsignificant

GRAPH NO.16

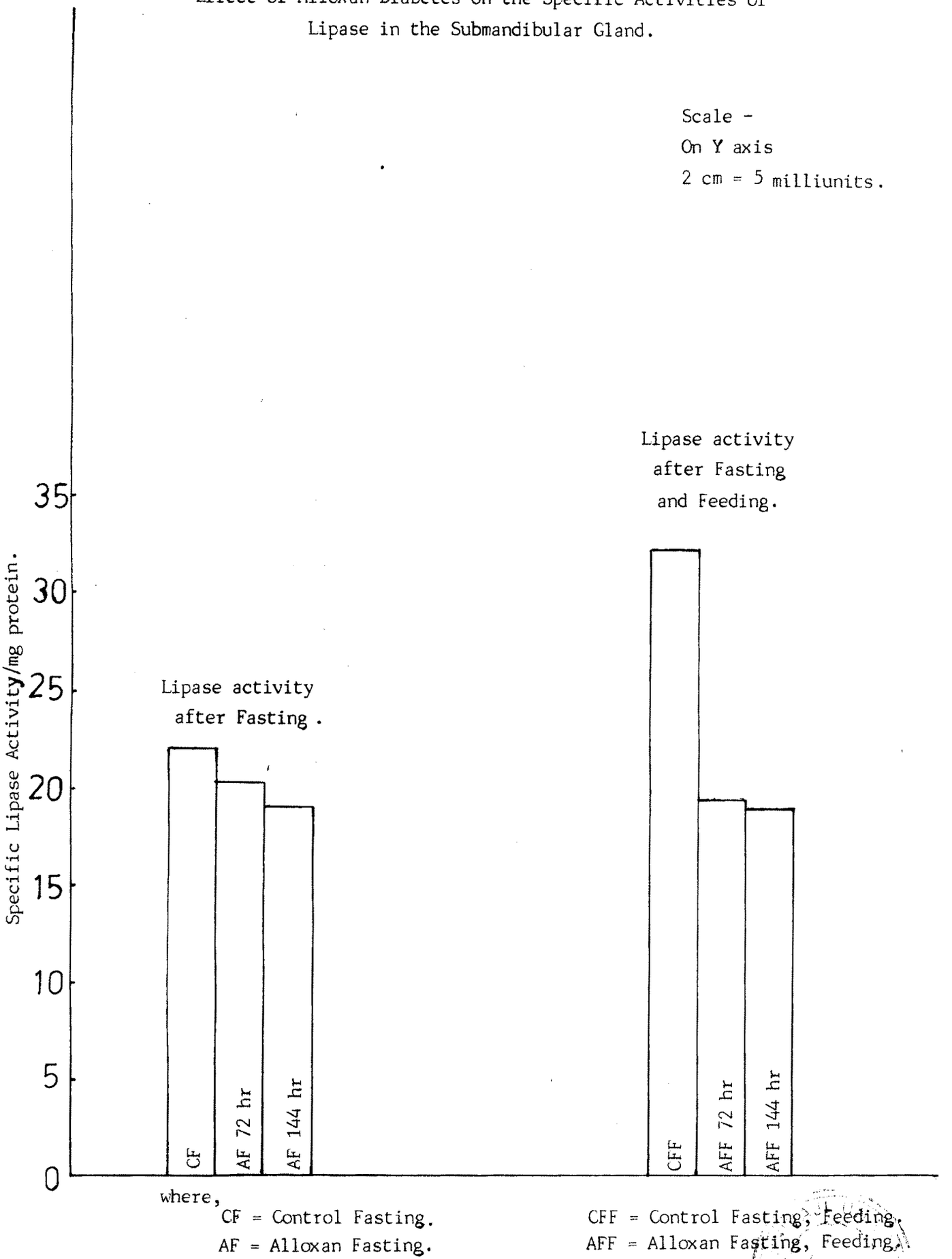
Specific Activity of Lipase in the Submandibular Gland against the Concentration of Glucose.



Each point represents a mean of 3 animals. Vertical bar denotes ± SEM.

GRAPH NO.17

Effect of Alloxan Diabetes on the Specific Activities of Lipase in the Submandibular Gland.



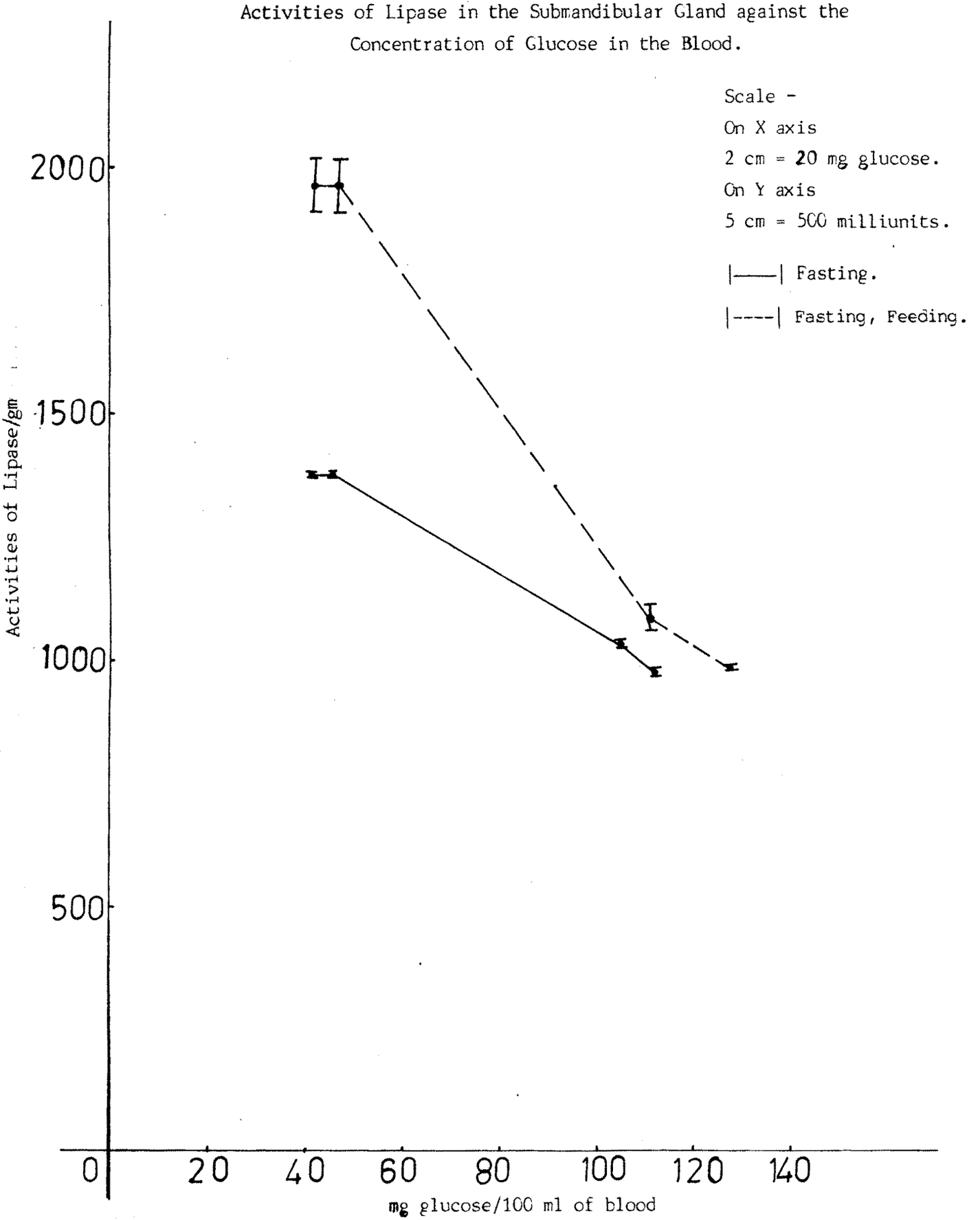
where,

CF = Control Fasting.
AF = Alloxan Fasting.

CFF = Control Fasting, Feeding.
AFF = Alloxan Fasting, Feeding.

GRAPH NO.18

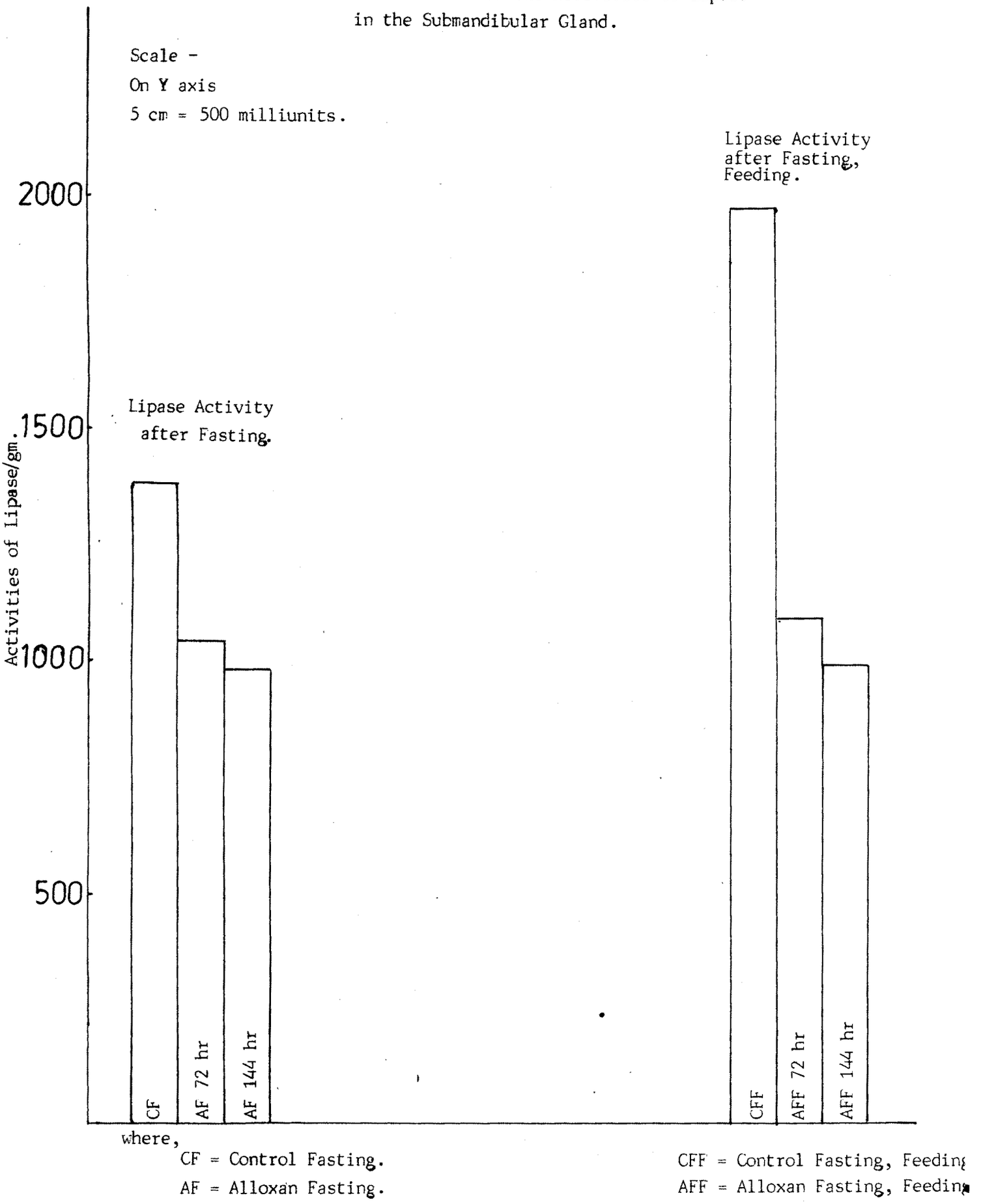
Activities of Lipase in the Submandibular Gland against the Concentration of Glucose in the Blood.



Each point represents a mean of 3 animals. Vertical bar denotes ± SEM.

GRAPH NO.19

Effect of Alloxan Diabetes on the Activities of Lipase in the Submandibular Gland.



where,

CF = Control Fasting.

AF = Alloxan Fasting.

CFF = Control Fasting, Feeding

AFF = Alloxan Fasting, Feeding

In 144 hrs alloxan diabetes fasted for 12 hrs the specific activity was 19.4963 ± 0.9904 and in controls it was 22.4273 ± 0.2341, the difference was non significant ($P > 0.05$) but in fasted, fed alloxan diabetes the specific activity was 19.8258 ± 0.3698 and in controls it was 32.5348 ± 1.3813. The difference was very significant ($P < 0.01$).

When the lipase activity/gm of the tissue of 144 hrs. alloxan diabetes fasted for 12 hrs. was compared with their controls, there was a highly significant difference ($P < 0.01$). In submandibular gland of diabetes it was 980.00 ± 9.4283 and in controls it was 1383.33 ± 1.3608.

The very significant difference was revealed in lipase activities between 144 hrs fasted but fed alloxan diabetes and controls ($P < 0.01$). In alloxan diabetes it was 990.00 ± 5.4283 and in controls it was 1966.66 ± 54.4347.

The specific activities as well as lipase activities went on decreasing according to the time and glucose concentration as glucose concentration went on increasing after the alloxan administration.

ii) Sublingual Gland

The lipase specific activities/mg protein and lipase activities/gm wet weight of the sublingual gland from diabetic animals were described in Table No. 6 and their specific activities and activities were plotted according to the blood

glucose concentration in graph no. 20 and 22 respectively and according to the time after alloxan administration in graph No. 21, 23 respectively. As the blood glucose level increased there were decrease in the enzyme activities. The decrease was significant (see graph No. 20 and 22).

The specific enzyme activities of fasted controls were compared with 72 hrs diabetes fasted for 12 hrs, but difference was non significant ($P > 0.05$). But when fasted fed controls were compared with fasted fed diabetic animals, there was a significant difference ($P < 0.01$).

In enzymatic activities/gm tissue also there was decrease like that of specific activities in both the cases, there was a very significant difference ($P < 0.01$) between controls and diabetes.

In 72 hrs fasted alloxan diabetes the specific activity of the sublingual gland lipase was 46.555 ± 2.9791 and in fasted controls it was 59.0563 ± 2.5811. In fasted but fed alloxan diabetes the specific activity was 35.3881 ± 3.4575 and it was 66.8572 ± 1.6881 in controls. The difference was highly significant at the level of $P < 0.01$.

The lipase activity per gm sublingual gland of 72 hrs alloxan diabetes fasted for 12 hrs showed a highly significant difference ($P < 0.01$), when compared with its respective control. The lipase activity of fasted alloxan diabetes was 1350.00 ± 26.2474 and in controls it was 2591.66 ± 17.0516.

The still more significant difference between the lipase activities of sublingual gland of fasted but fed alloxan diabetes and controls was noticed. It was at the level of $P < 0.01$. The enzyme activity in the sublinguals of diabetic rats was 1375.00 ± 53.0335 and in controls it was 3083.33 ± 14.4020.

In 144 hrs alloxan diabetes fasted for 12 hrs the specific activity was 44.7008 ± 1.8652 and in controls it was 59.0563 ± 2.5811, the difference was significant ($P < 0.05$) but in fasted, fed alloxan diabetes there was more decrease in the enzyme activity, the specific activity was 32.1308 ± 1.7053 and in fasted fed controls it was 66.8572 ± 1.6681. The difference was highly significant at the level of $P < 0.01$. In between 144 hrs alloxan diabetes fasted for 12 hrs and controls there was a highly significant difference ($P < 0.01$). In diabetes sublingual glands, the activity was 1200.00 ± 47141 and in controls 2591.66 ± 17.0516. The highly significant difference was revealed in lipase activities between fed 144 hrs alloxan diabetes and controls ($P < 0.01$). In alloxan diabetes it was 1210.00 ± 4.7141 and in controls it was 3083.33 ± 14.4020.

The specific activities/mg protein as well as lipase activities/gm tissue were decreased according to the time after alloxan administration and glucose concentration as it also increased after alloxan injection.

Table No. 6 : Effect of Alloxan Diabetes on the Specific Activities and Activities of Lipase in the Rat "Sublingual Gland"

Sr. No.	Time after Alloxan Administration	Animals	Glucose conc. ± SEM	Specific Activities ± SEM	P	Mean ± SEM	P
1.	-	Control fasting 12 hr (CF)	41.05 ± 1.2695	59.0563 ± 2.5811 *3		2591.66 ± 17.0516 *3	
					P > 0.05 NS		P < 0.01 HS
2.	72	Alloxan fasting 12 hr (AF)	105.55 ± 1.732	46.5551 ± 2.9791 *3		1350.00 ± 26.2474 *3	
3.	-	Control fasting + feeding $\frac{1}{2}$ hr (CFF	42.10 ± 1.2914	66.8572 ± 1.6881 *3		3083.33 ± 14.4020 *3	
					P < 0.01 HS		P < 0.01 HS
4.	72	Alloxan fasting + feeding $\frac{1}{2}$ hr (AFF)	111.11 ± 1.2963	35.3881 ± 3.4575 *3		1375.00 ± 53.0335 *3	
5.	-	CF	45.70 ± 0.3299	59.0563 ± 2.5811 *3		2591.66 ± 17.0516 *3	
					P < 0.05 S		P < 0.01 HS
6.	144	AF	112.00 ± 1.2472	44.7008 ± 1.8652 *3		1200.00 ± 4.7141 *3	
7.	-	CFF	46.88 ± 1.1511	66.8572 ± 1.6681 *3		3083.33 ± 14.4020 *3	
					P < 0.01 HS		P < 0.01 HS
8.	144	AFF	128.00 ± 2.0548	32.1308 ± 1.7053 *3		1210.00 ± 4.7141 *3	

Results are expressed as mean ± SEM

*represents the number of animals.

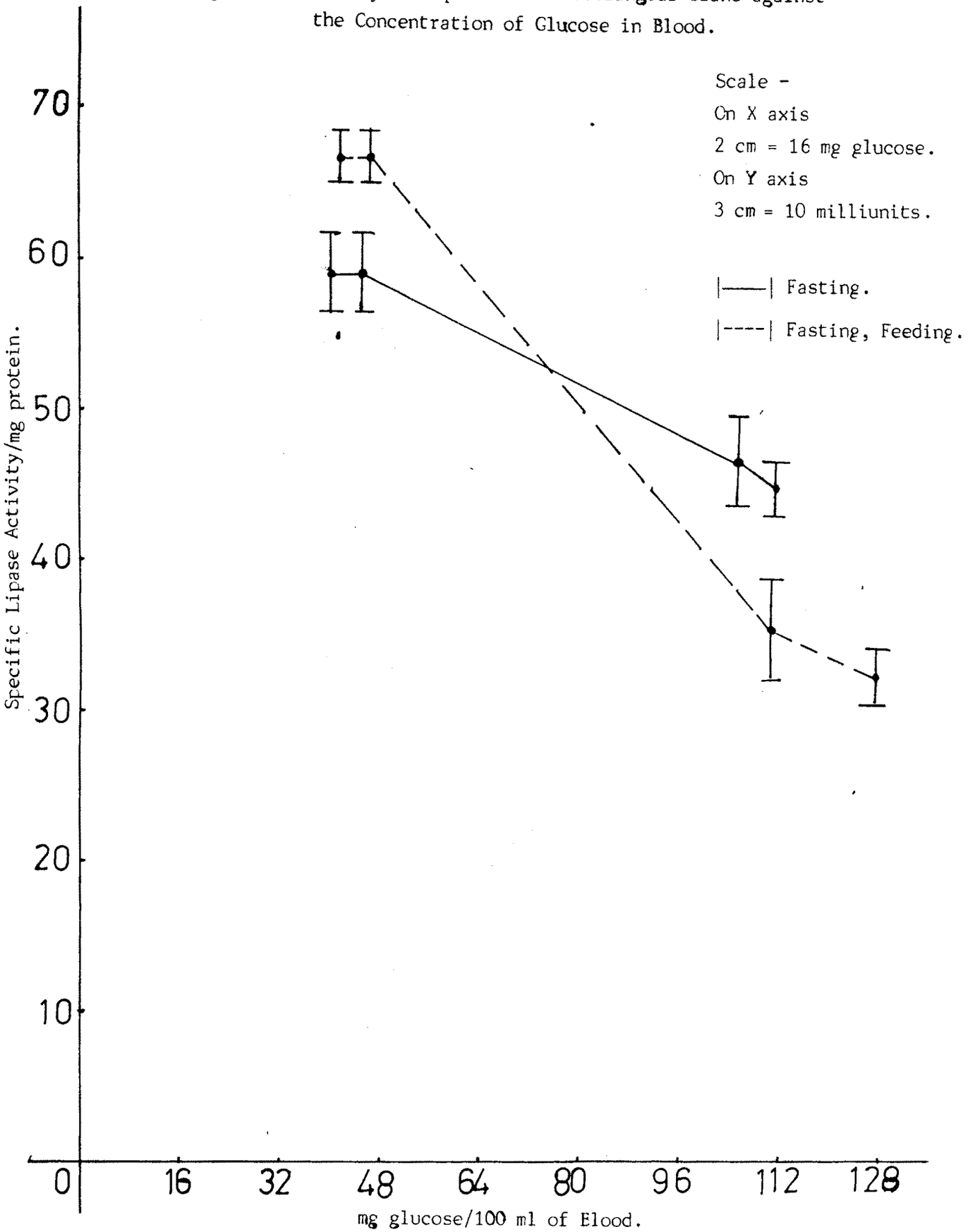
HS : Highly Significant

S : Significant

NS : Non-Significant

GRAPH NO.20

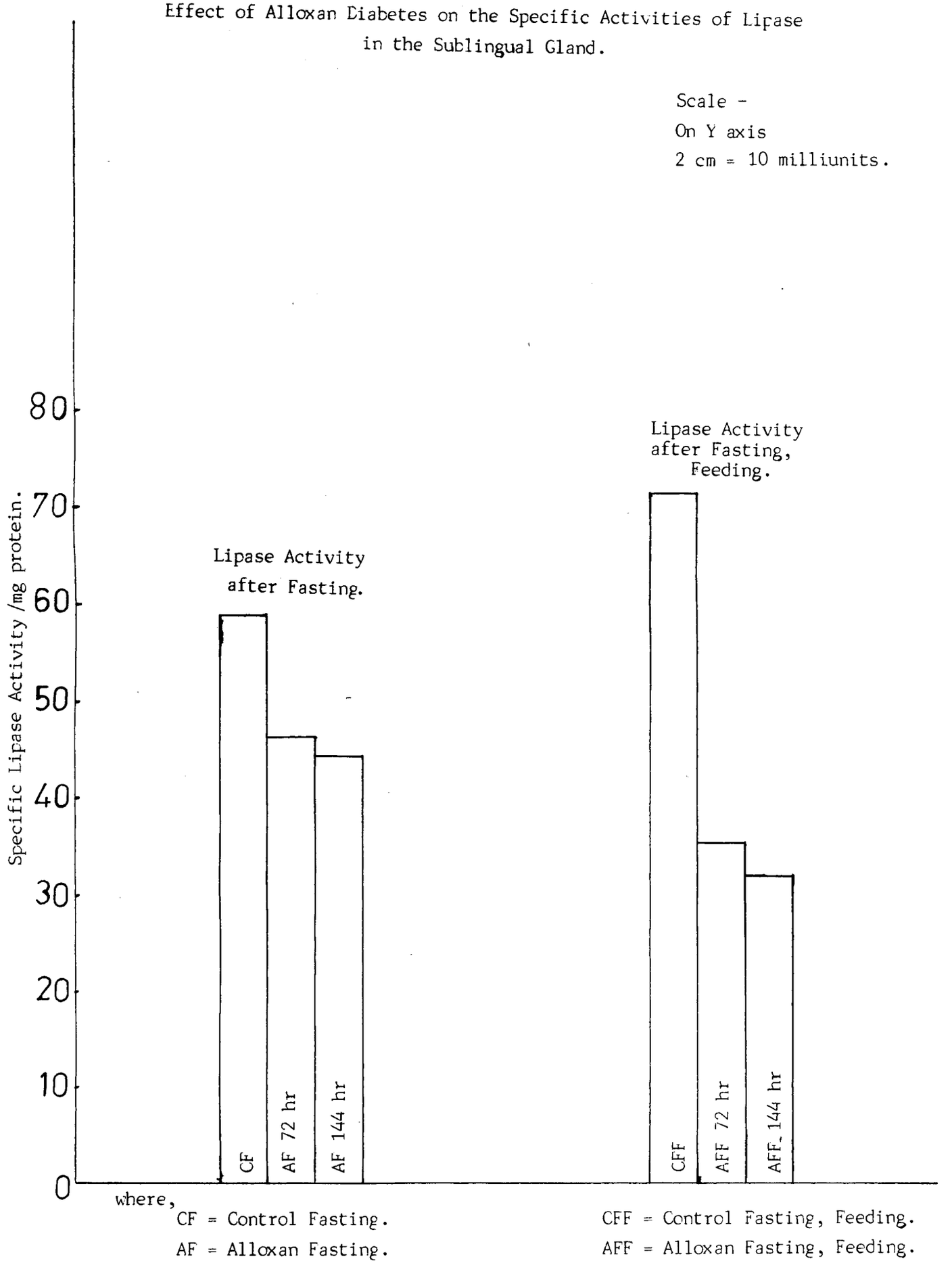
Specific Activity of Lipase in the Sublingual Gland against the Concentration of Glucose in Blood.



Each point represents a mean of 3 animals. Vertical bar denotes ± SEM.

GRAPH NO.21

Effect of Alloxan Diabetes on the Specific Activities of Lipase in the Sublingual Gland.



where,

CF = Control Fasting.

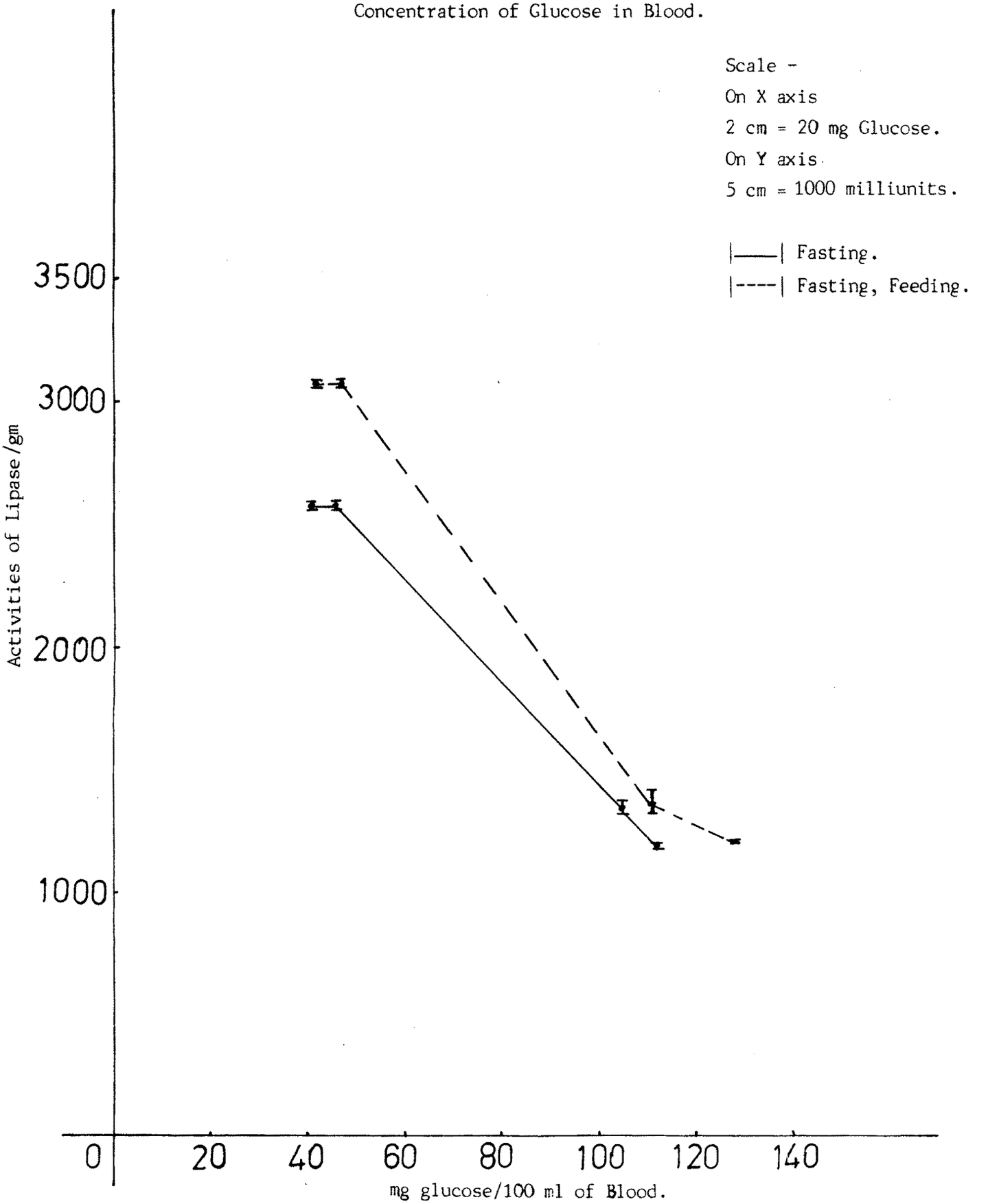
AF = Alloxan Fasting.

CFF = Control Fasting, Feeding.

AFF = Alloxan Fasting, Feeding.

GRAPH NO.22

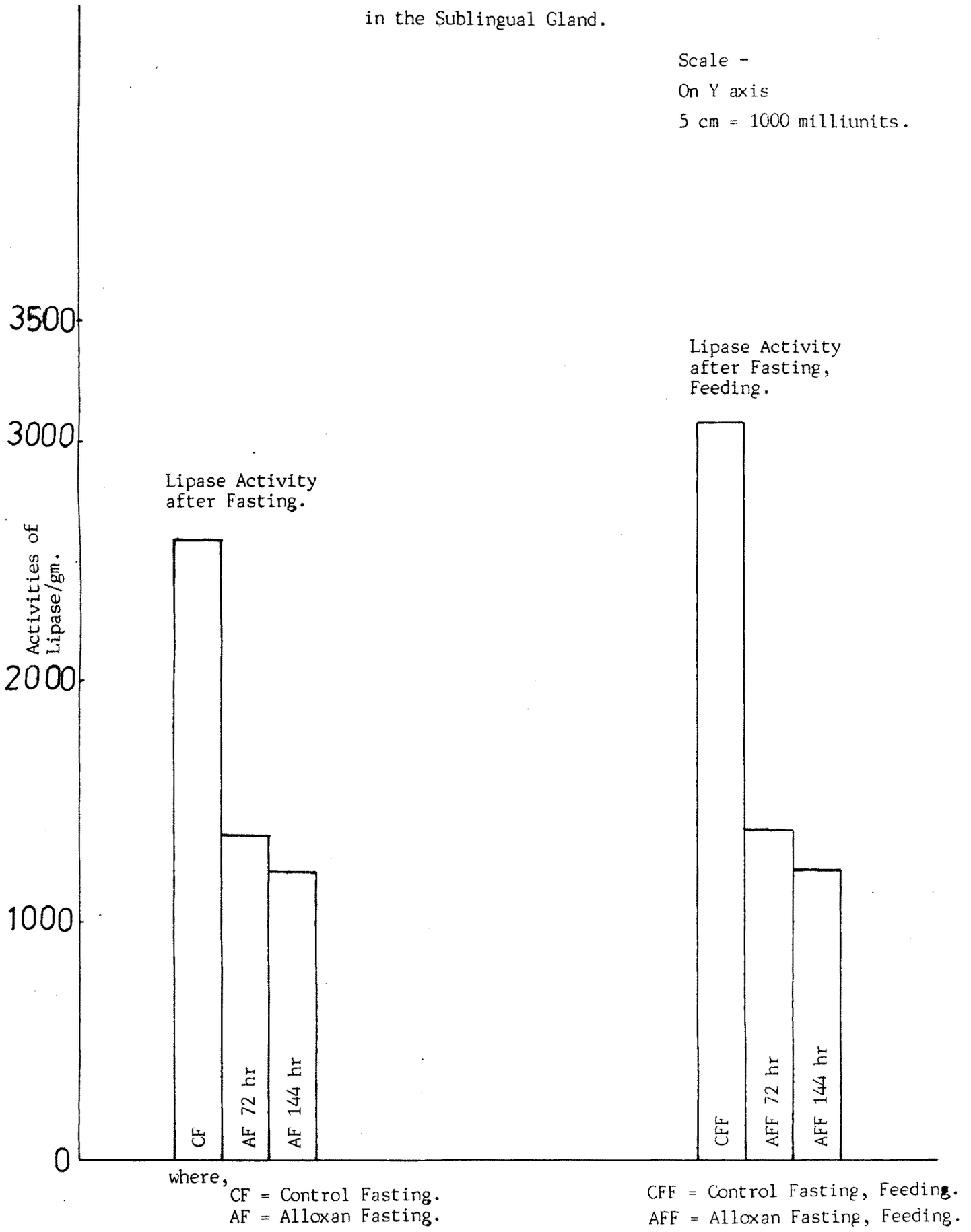
Activities of Lipase in the Sublingual Gland against the Concentration of Glucose in Blood.



Each point represents a mean of 3 animals. Vertical bar denotes ± SEM.

GRAPH NO.23

Effect of Alloxan Diabetes on the Activities of Lipase in the Sublingual Gland.



where,

CF = Control Fasting.
AF = Alloxan Fasting.

CFF = Control Fasting, Feeding.
AFF = Alloxan Fasting, Feeding.

iii) Parotid Gland

Lipase specific activities/mg protein and Lipase activities/gm wet weight of the parotid gland diabetic animals were shown in Table No. 7 and their specific activities were depicted according to the glucose concentration from blood in graph no. 24 and according to the time after alloxan administration in graph No. 25. The lipase activities/gm wet weight of the tissue were plotted against the blood glucose concentration in graph No. 26. and according to the time after alloxan administration in graph No. 27. As the blood glucose levels increased there were decrease in the enzymatic activities (See graph No. 24 & 26). The specific enzyme activities of 12 hrs fasted controls compared with fasted 72 hrs alloxan diabetes, there was a very significant difference ($P < 0.01$). But when control fasted, fed was compared with fasted, fed alloxan diabetes, there was no significant difference ($P > 0.05$).

In enzymatic activities also there was decrease as that of specific activities in both the cases, there were highly significant differences ($P < 0.01$).

The lipase activity of parotid gland of 72 hrs alloxan diabetes fasted for 12 hrs showed a highly significant difference ($P < 0.01$) when compared with its respective control. The lipase activity of fasted alloxan diabetes was 1133.33 $\pm$ 33.1133 and in control it was 2133.33 $\pm$ 14.4020. The highly significant difference between the lipase activities

of parotid gland of fasted but fed alloxan diabetes and controls was shown. It was highly significant ($P < 0.01$). The activity in diabetic animal was 1300.00 ± 35.3566 and in control it was 2716.66 ± 14.4020.

In 144 hrs alloxan diabetes fasted for 12 hrs. the specific activity was 22.1095 ± 0.6147 and in fasted controls it was 34.4063 ± 0.9437, the difference was highly significant ($P < 0.01$). In fasted, fed alloxan diabetes the specific activity was 24.6335 ± 0.4539 and in controls it was 41.1568 ± 1.6483. The difference was very significant ($P < 0.01$).

When the lipase activity of 144 hrs alloxan diabetes fasted for 12 hrs was compared with fasted controls, there was a very significant difference at the level of $P < 0.01$. In parotid gland of diabetes it was 1103.33 ± 7.2010 and in controls it was 2133.33 ± 14.4020. The highly significant difference was revealed in lipase activities between 12 hrs fasted fed 144 hrs alloxan diabetes and controls ($P < 0.01$). In alloxan diabetes it was 1230.00 ± 9.4283 and in controls it was 2716.66 ± 14.4020.

The specific activities as well as lipase activities went on decreasing according to the time and concentration of glucose. The glucose concentration was increased gradually after the alloxan administration.

Table No. 7 : Effect of Alloxan Diabetes on the Specific Activities and Activities of Lipase in the Rat "Parotid Gland".

Sr. No.	Time after Alloxan Administration	Animals	Glucose conc. ± SEM	Specific activities ± SEM	P	Mean ± SEM	P
1.	-	Control fasting 12 hr (CF)	41.05 ± 1.2695	34.4063 ± 0.9427 *3	P < 0.01 HS	2133.33 ± 14.4020 *3	P < 0.01 HS
2.	72	Alloxan fasting 12 hr (AF)	105.55 ± 1.732	23.5133 ± 0.4875 *3		1133.33 ± 33.1133 *3	
3.	-	Control fasting + feeding ½ hr (CFF)	42.10 ± 1.2914	41.1568 ± 1.6483 *3	P > 0.05 NS	2716.66 ± 14.4020 *3	P < 0.01 HS
4.	72	Alloxan fasting + feeding ½ hr (AFF)	111.11 ± 1.2963	35.8130 ± 0.5129 *3		1300.00 ± 35.3566 *3	
5.	-	CF	45.70 ± 0.3299	34.4063 ± 0.9437 *3	P < 0.01 HS	2133.33 ± 14.4020 *3	P < 0.01 HS
6.	144	AF	112.00 ± 1.2472	22.1095 ± 0.6147 *3		1103.33 ± 7.2010 *3	
7.	-	CFF	46.88 ± 1.1511	41.1568 ± 1.6483 *3	P < 0.01 HS	2716.66 ± 14.4020 *3	P < 0.01 HS
8.	144	AFF	128.00 ± 2.0548	24.6335 ± 0.4539 *3		1230.00 ± 9.4283 *3	

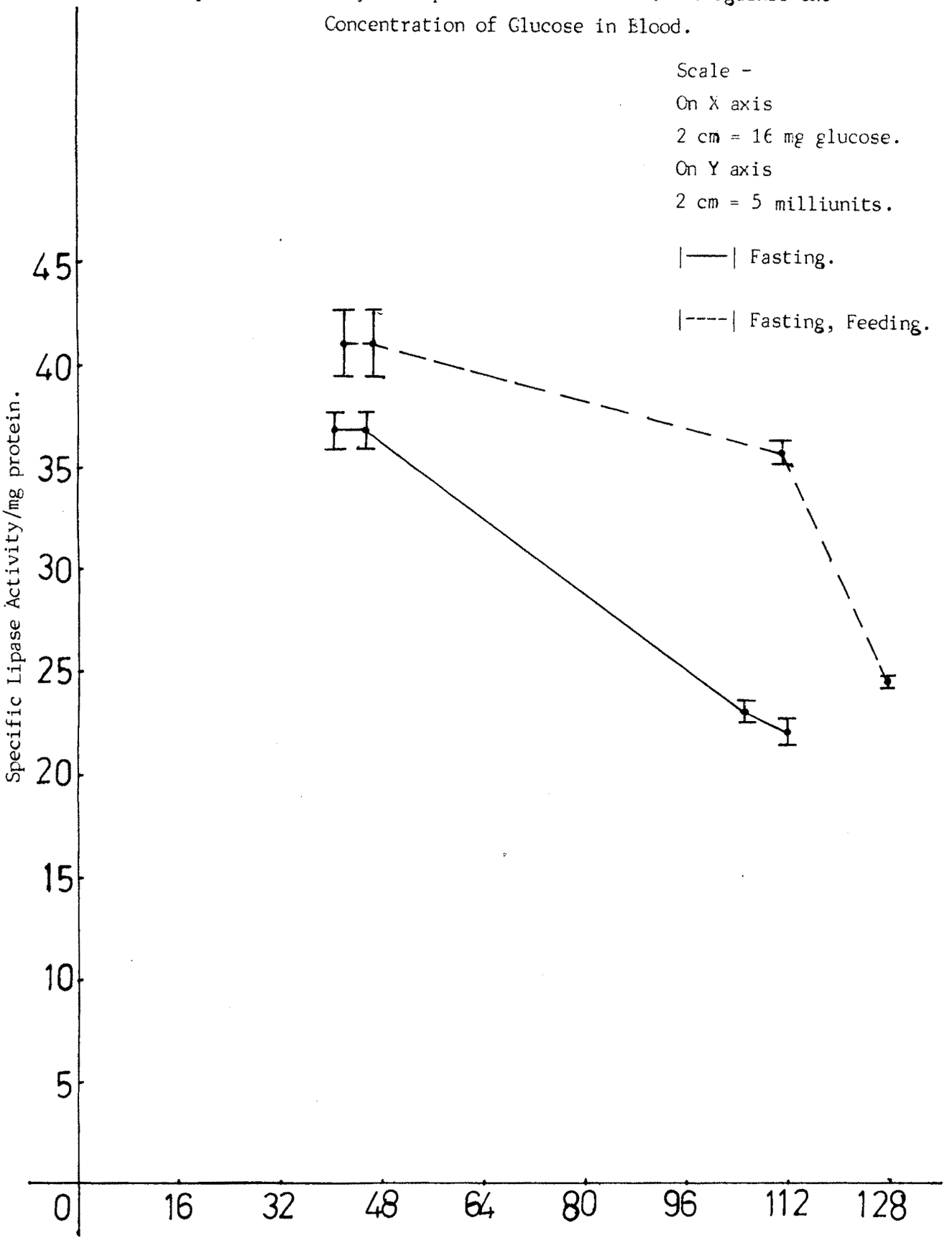
Results are expressed as mean ± SEM

* represents the number of animals

HS : Highly significant
S : Significant
NS : Non-significant.

GRAPH NO.24

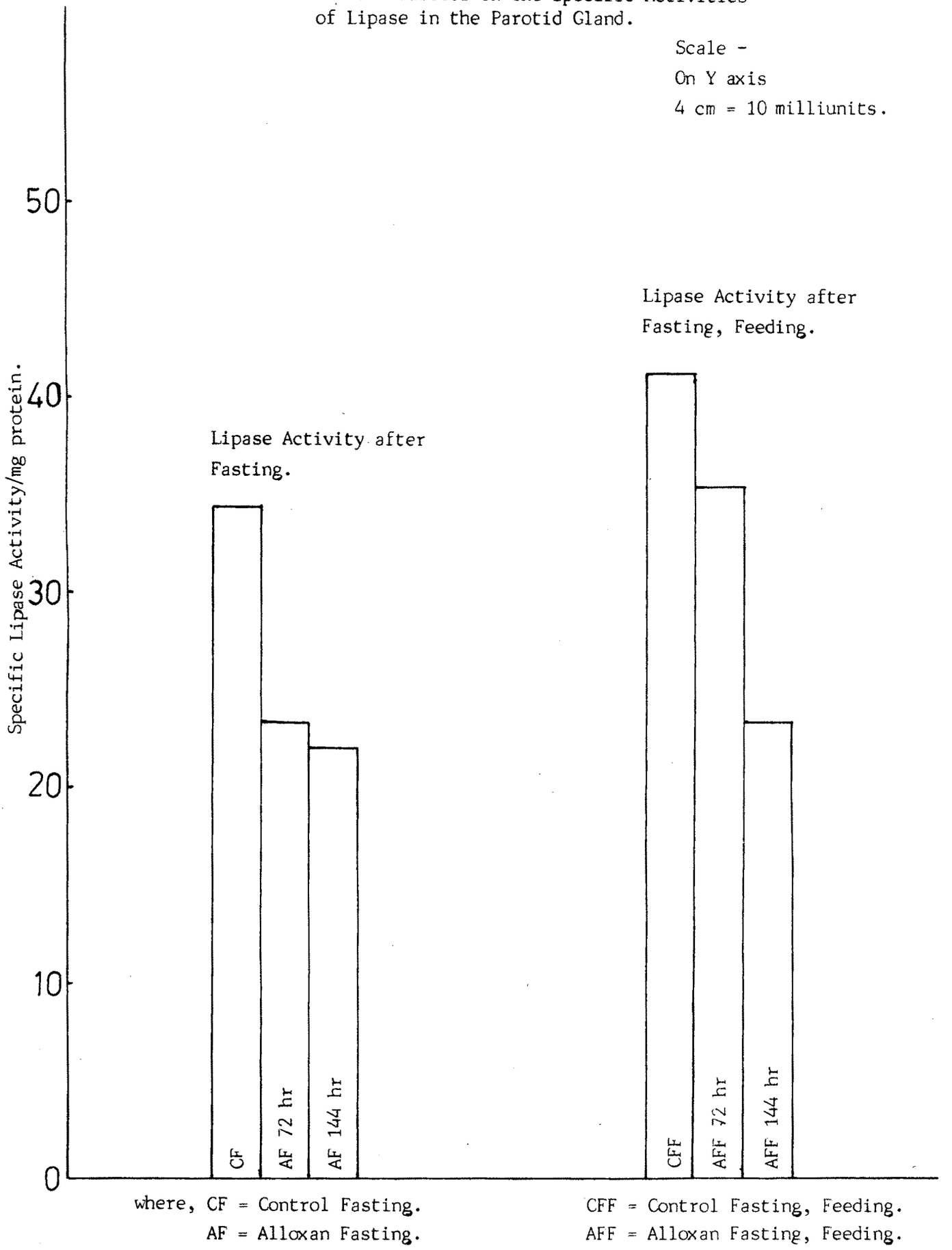
Specific Activity of Lipase in the Parotid Gland against the Concentration of Glucose in Blood.



Each point represents a mean of 3 animals. Vertical bar denotes ± SEM.

GRAPH NO.25

Effect of Alloxan Diabetes on the Specific Activities of Lipase in the Parotid Gland.

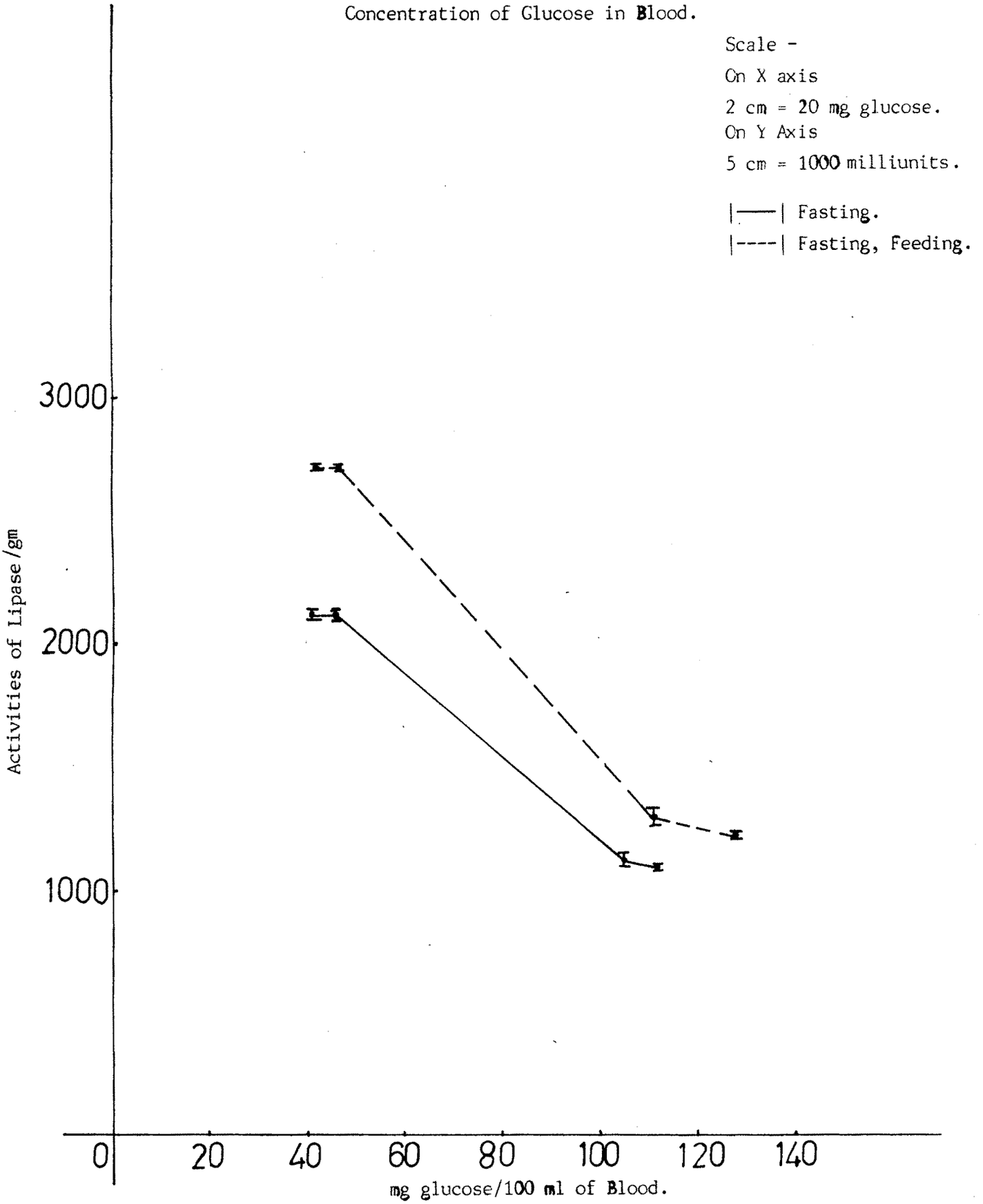


where, CF = Control Fasting.
AF = Alloxan Fasting.

CFF = Control Fasting, Feeding.
AFF = Alloxan Fasting, Feeding.

GRAPH NO.26

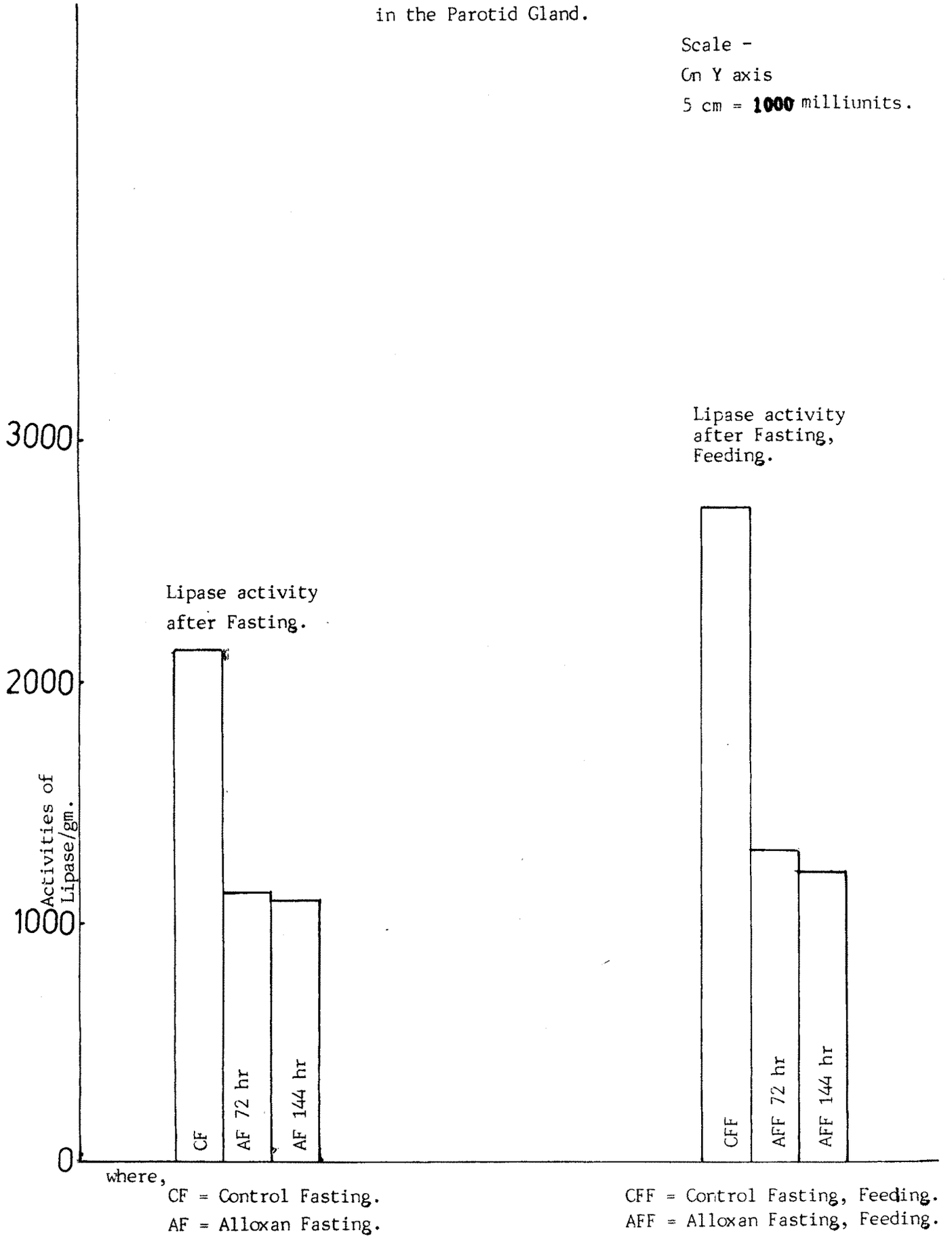
Activities of Lipase in the Parotid Gland against the Concentration of Glucose in Blood.



Each point represents a mean of 3 animals. Vertical bar denotes ± SEM.

GRAPH NO.27

Effect of Alloxan Diabetes on the activities of Lipase in the Parotid Gland.



where,

CF = Control Fasting.

AF = Alloxan Fasting.

CFF = Control Fasting, Feeding.

AFF = Alloxan Fasting, Feeding.

Discussion

The current study demonstrates that in alloxan diabetes lipase activities are affected, both specific/mg protein and activities/gm, wet weight of the salivary glands.

We have demonstrated reduction in the lipase activities due to starvation and after the induction of alloxan diabetes in rat salivary glands. The lipase activities studied during starvation and diabetic condition showed the decrease in the lipase activities in all major salivary glands. In submandibular gland the decrease can be corrolated with the glucose concentration in blood. At low glucose concentration of fasted but fed animals activities were very high but gradually and significantly they were reduced as glucose concentration increased after the alloxan administration. A very slowl and gradual decrease in lipase activity was noticed in fasted animals, but decrease was significant and can be corrolated with increase in glucose concentration. In sublingual gland, decrease in specific lipase activity was pronounced in fasting but fed animals at higher glucose concentration (Graph No. 20). The enzyme activities was very low in fed animals and it was even lower than the fasted ones. In parotid gland also lipase activities/mg protein and per gm tissue were high at low concentration of glucose but gradual and slowly decrease in lipase activities was noticed at higher concentrations, the decrease was there even in fed animals. The time after the alloxan administration also showed decrease in lipase activity during late diabetic condition when there

was increase in glucose concentration. This shows that in fasting but fed controls lipase activity was very high, but in fasting controls it was low, and in alloxan diabetes enzyme activities of fed animals was lower than the fasting ones.

In fasting, activity which was reduced could be restored by refeeding. Previous findings of others also showed that in fasting of rats there is decrease in lipase activity in liver (Nomura et al., 1982), adipose tissues (Elkeles and Hambley, 1977; Wings and Robinson, 1968; Tan et al., 1977), diaphragm (Boresztain et al., 1972; Linder et al., 1976), here in the present investigation after refeeding the activity of lipase did not restored but it further decreased. Graphs recording lipase activities against glucose concentration clearly showed that at high concentration of glucose, in fed animals there is steep decrease in lipase activities.

In diabetic animals also there were changes in salivary gland lipase activities similar to these of fasted and fasted but fed. When there was low blood glucose concentration and animals were fasted for 12 hrs lipase activities were high but at higher concentration of glucose in alloxan treated rats, there was decrease in lipase activity. Chen et al. (1979) indicated that the insulin deficient rats were incapable of increase in adipose tissue lipase activity. Wada et al. (1983) showed inhibition of lipoprotein lipase bound to coronary vessels of heart in diabetic condition. Nakai et al. (1979), reported decreased hepatic triglycerides lipase activity in

streptozotocin treated animals. These evidences strongly suggest that insulin is the hormone involved in the regulation of the tissue specific lipase system. Kessler (1963) also pointed out possible role of insulin in regulation of heart lipase. But why there is decrease in lipase activity in refeeding of animals from alloxan fasting feeding (AFF) than alloxan fasting both at 72 and 144 hrs. of diabetic animals? The exact cause of it can not be explained at this moment, the further work is essential.

The evidence that the tissue specific lipase activity is important determinant of the ability of the tissue to remove plasma triglycerides from the circulation has been reviewed by Robinson (1970) and Scow et al. (1976). Thus it is probable that the changes in the enzyme activities among the tissues under the hormonal and nutritional environment might determine the direction of plasma triglyceride to certain tissues. In the present investigation it was clearly indicated that the lipase activities of salivary glands are changed not only by starvation of normal rats but also by withdrawal of insulin by inducing alloxan diabetes. The decrease in the insulin dependant lipase may be then the possible cause of elevation of plasma-triglycerides in diabetic animals.

Nomura et al. (1982) in their articles "The Effects of fasting and streptazotocin Diabetes on Triglycericle Lipase Activity of Rat Liver Plasma Membrane" showed the decrease in lipase activity in diabetes may be then due to damage to the

plasma membrane, because susceptibility of microsomal membrane to lipid peroxidation is shown by Kornbrust and Maris (1979).

The presence of lipase in plasma membrane is shown by Assmann et al.(1973). We feel to conclude that high lipid peroxide accumulate in salivary gland of diabetic rats and causes damage to the membrane bound lipase, which result in low lipase activity and high plasma triglycerides. But with this conclusion, we are unable to explain decrease in lipase activity in control fasted and increase in enzyme activity in control fed animals; or high level of lipase during lower concentration of glucose and decrease in lipase activity at higher concentration of glucose, it must be then insulin that may be elevating lipoprotein lipase in fed animals, as in these animals, there is high insulin content and decrease in lipoprotein lipase in diabetic and fasted animals may be due to low levels of insulin in blood.