CHAPTER THREE

THE EFFECT OF ALLOXAN DIABETES ON BODY WEIGHT SALIVARY GLANDS WEIGHT AND SALIVARY ESTERASE ACTIVITY.

Anderson and Johnson (1981) described that in alloxan diabetes, despite of the increased food consumption, final mean body weight of the diabetic rat was significantly less than that of non diabetic animals. Anderson (1983) also showed the difference in the body weight of the control and diabetic animals, they showed that there was 45 % increase in the body weight of normal animals over a period of 28 days, but in diabetic animals, the increase in the mean body weight was only 7 %. Pillai et al. (1989) showed significantly less body weight of the diabetic animals than the normal one. Liu and Lin (1969 a); Anderson and Shapiro (1980); showed the requirement of insulin for the growth of submandibular gland. Anderson and Johnson (1981) and Anderson (1983) showed the role of insulin over the parotid gland. They showed decrease in the weight of the parotid gland; but it was nonsignificant. Pillai et al. (1989) showed the effect of insulin on submandibular and sublingual salivary glands, they showed non-significant decrease in the salivary gland weight with respect to 100 gm. body weight. Pillai and Nadar (1987) showed the increase in the Lysosomal enzymes like acid phosphatase and β-glucuronidase. According to them the increase in the lysosomal enzymes may be due to Ketone bodies formed in diabetic condition. The relation between increase in the lysosomal enzymes activity and Ketone bodies has also described by Chari et al. (1983).

Barrett (1973) reviewed the reports of various workers about the existance of esterase enzyme activity in lysosomes.

The study on enzyme esterase in salivary glands during diabetic condition was selected for the present investigation keeping two views in mind.

i) An esterase is a lysosomal enzyme study of lysosomal enzymes during diabetes in various tissues is essential because in diabetes, there is elevation of lipid peroxidation (Karpen et al., 1982; Wada et al., 1983; Pritchard et al., 1986) and in lipid peroxidation there was increase in lysosomal enzyme activity (Menzel, 1976); leading to the further formation of Lipofuschin granules (Mead,1976).

ii) Esterase is involved in the metabolism of low molecular weight fatty acids (Bier, 1955). Abnormal metabolism of fatty acid and Lipoprotein in the diabetic condition were described by several workers in other organs (Epstein, 1967; Gracia, et al., 1974; Peter Nilsson-Ehle; 1980; Lithell et al., 1981; Nomura et al., 1982; Lithell et al., 1985; Cawthorne, 1986; Pritchard et al., 1986; Lithell, 1987).

Material and Methods

Norwegian male rats (Rattus norvegicus) weighing about 200 to 225 gms were selected. They were fasted overnight and diabetes was induced by intraperitoneal injection of alloxan monohydrate (40 mg/250 gm body weight in 1.0 ml of 0.9 % saline). Controls were received 1.0 ml saline. Every time their body weights and glucose levels in blood were recorded.

12 hrs, 36 hrs, 60 hrs and 132 hrs after the alloxan administration, from each group six rats were fasted for 12 hrs, three of them received food for half an hour; and they were killed by cervical dislocation along with their controls. Salivary glands were pulled, weighed and used for the estimation of esterase. Easterase was measured in μ moles/mg protein and μ moles/gm wet weight of the tissue by using p-nitrophenol acetate as a substrate (Stotz, 1955). 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, 1951).

RESULTS

a) Glucose level

Table and Graph No. 1 showed the increase in the blood glucose concentration in alloxan diabetes in course of time, as the time was increased after the alloxan administration, there was increase in the blood glucose level.

b) Body weight

As shown in the table no. 1 and Graph No. 2 there was no difference in the % loss in body weight of 12 hr. fasted controls and fasted but fed controls. But there was difference in the % loss in body weight of alloxan diabetes compared to the controls from both the groups i.e. fasted and fasted fed. In alloxan diabetes, there was gradual and steady decrease in

the % loss in body weight in course of time, but the difference was not significant. In control fasted animals % loss in body weight was about 2.2230 and in fasted but fed controls, it was 2.2698.

24 hrs alloxan diabetes fasted for 12 hrs the % loss in body weight was 7.1984 and in the fasted but fed, it was 5.8442. In a 48 hrs diabetic animals, there was increase in % loss in body weight in fasted rats. It was 9.07 but in fed, it was 7.0732. 72 hrs diabetes, fasted for 12 hrs the % loss in body was 9.0610 but in fasted but fed animals the loss was 8.8765. In 144 hrs alloxan diabetes there was slight increase in % loss in body weight as compared to earlier groups. So that the % loss in body weight of fasted animals was 6.6666 and in fasted but fed animals it was 7.7625. This showed that in the diabetic animals there was a loss in body weight, but it was not steady.

Graphically also (Graph No. 2) it was shown, that the range of % loss in body weight was less in low % of blood glucose, but at higher concentration of glucose there was increase in the % loss in body weight.

i) Submandibular Gland Weight

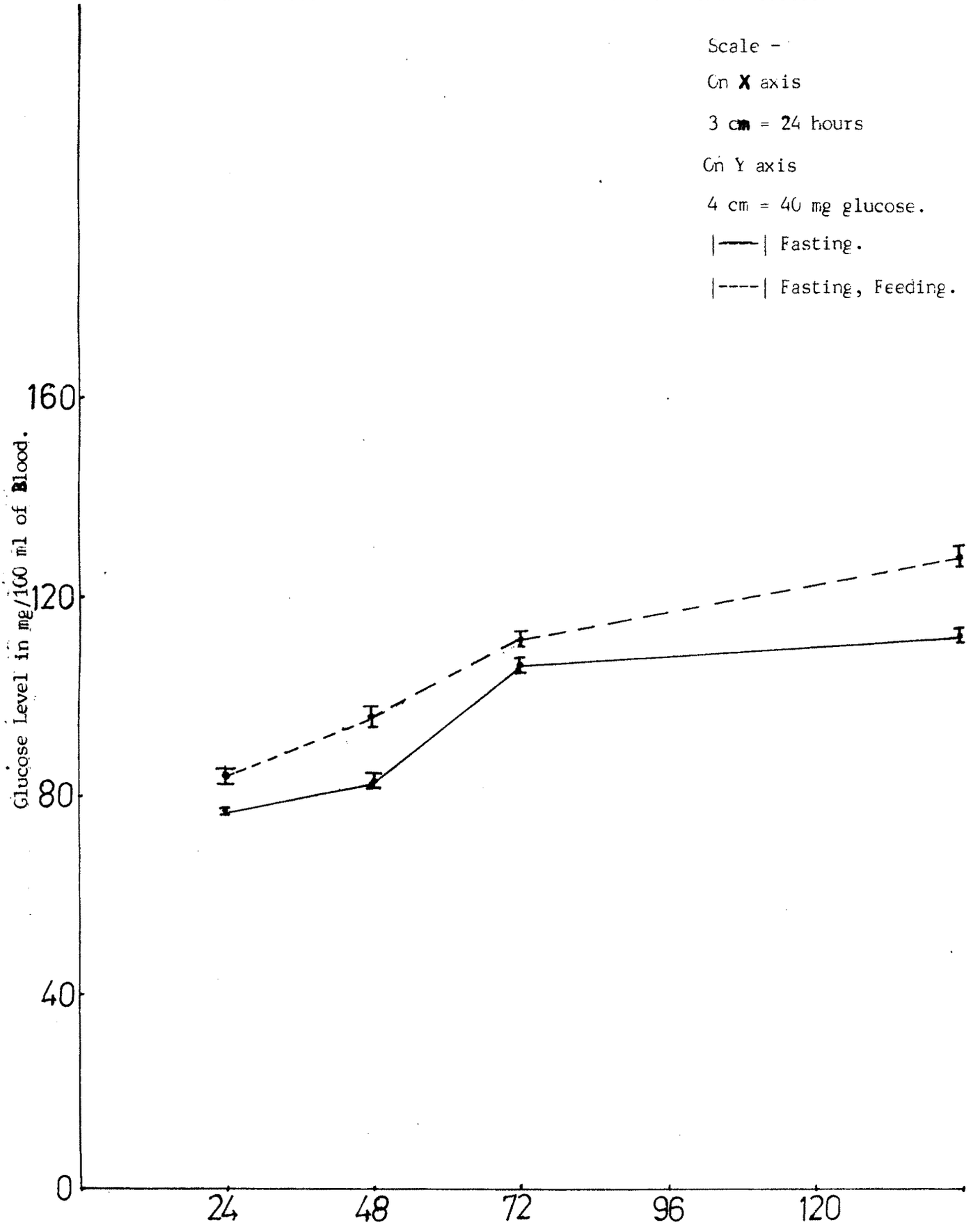
The % weight of the submandibular gland with respect to body weight of controls and alloxan diabetes were depicted in the table no.1 and graph no. 2. In all experimental animals there was decrease in the % weight of the submandibular

Table No. 1 : Effect of Alloxan Diabetes on Blood Glucose, Body Weight and Salivary Gland Weight

Sr. No.	Time after alloxan Administration	Animals	mg glucose/100 ml of blood Mean ± SEM	Body Weight				Salivary Gland Weight					
				Initial	Final	Loss in body weight (gm)	(% loss)	Submandibular		Sublingual		Parotid	
								Wt. of Gland in gms.	% Wt. of Gland with respect to body wt.	Wt. of Gland in gms.	% Wt. of Gland with respect to body wt.	Wt. of Gland in gms.	% Wt. of Gland with respect to body wt.
1.	-	Control fasting 12 hr (CF)	44.70 ± 0.3299	209.08	204.41	2.2230	(4.67)	0.202	0.101	0.02633	0.01314	0.1003	0.0500
2.	24	Alloxan fasting 12 hr (AF)	77.36 ± 0.6411	205.33	193.33	5.8442	(12)	0.1454	0.0778	0.024	0.01285	0.093	0.0498
3.	-	Control fasting + feeding $\frac{1}{2}$ hr (CFF)	45.88 ± 1.1939	204.66	199.91	2.2698	(4.75)	0.1926	0.0951	0.027	0.01334	0.0943	0.0466
4.	24	Alloxan fasting + feeding $\frac{1}{2}$ hr (AFF)	84.21 ± 1.7194	196.66	186.66	5.0849	(10)	0.1626	0.0841	0.025	0.01293	0.0893	0.0461
5.	-	CF	38.88 ± 1.6448	209.08	204.41	2.2230	(4.67)	0.1933	0.0946	0.0303	0.01482	0.101	0.0494
6.	48	AF	83.33 ± 1.3730	205.66	187	9.0732	(18.66)	0.175	0.0892	0.028	0.01428	0.094	0.0479
7.	-	CFF	42.22 ± 1.3456	204.66	199.91	2.2698	(4.75)	0.1936	0.0991	0.030	0.01535	0.1136	0.0581
8.	48	AFF	95.55 ± 2.2951	213.00	196	7.9812	(17)	0.1626	0.0869	0.0273	0.01459	0.108	0.0577
9.	-	CF	41.05 ± 1.2695	209.08	204.41	2.2230	(4.67)	0.203	0.0980	0.027	0.01304	0.106	0.0512
10.	72	AF	105.55 ± 1.732	213.33	194	9.0610	(19.33)	0.185	0.0953	0.025	0.01288	0.095	0.0489
11.	-	CFF	42.10 ± 1.2914	204.66	199.91	2.2698	(4.75)	0.2003	0.0996	0.026	0.01293	0.1043	0.0518
12.	72	AFF	111.11 ± 1.2963	210	191.66	8.8765	(18.67)	0.1696	0.0884	0.020	0.01043	0.095	0.0495
13.	-	CF	45.70 ± 0.3299	209.08	204.41	2.2230	(4.67)	0.190	0.0922	0.030	0.01456	0.120	0.0582
14.	144	AF	112.00 ± 1.2472	219	202	7.7625	(17)	0.179	0.0852	0.026	0.01238	0.109	0.0519
15.	-	CFF	46.88 ± 1.1511	204.66	199.91	2.2698	(4.75)	0.2003	0.0996	0.026	0.01293	0.1543	0.07676
16.	144	AFF	128.00 ± 2.0548	225	210	6.6666	(15)	0.185	0.0915	0.025	0.01237	0.122	0.06039

GRAPH NO.1

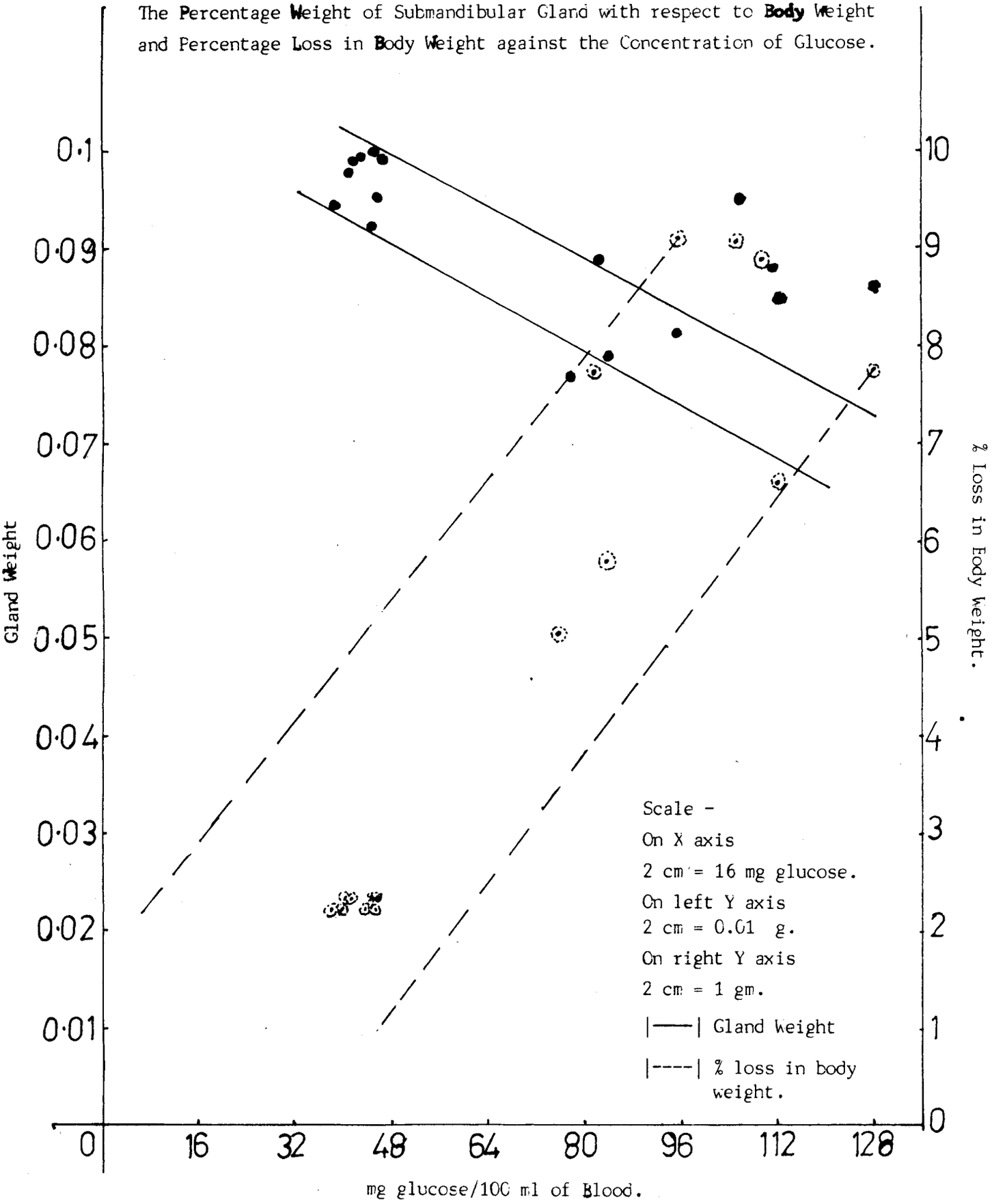
Glucose level in 100 ml of Blood after Alloxan Administration.



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

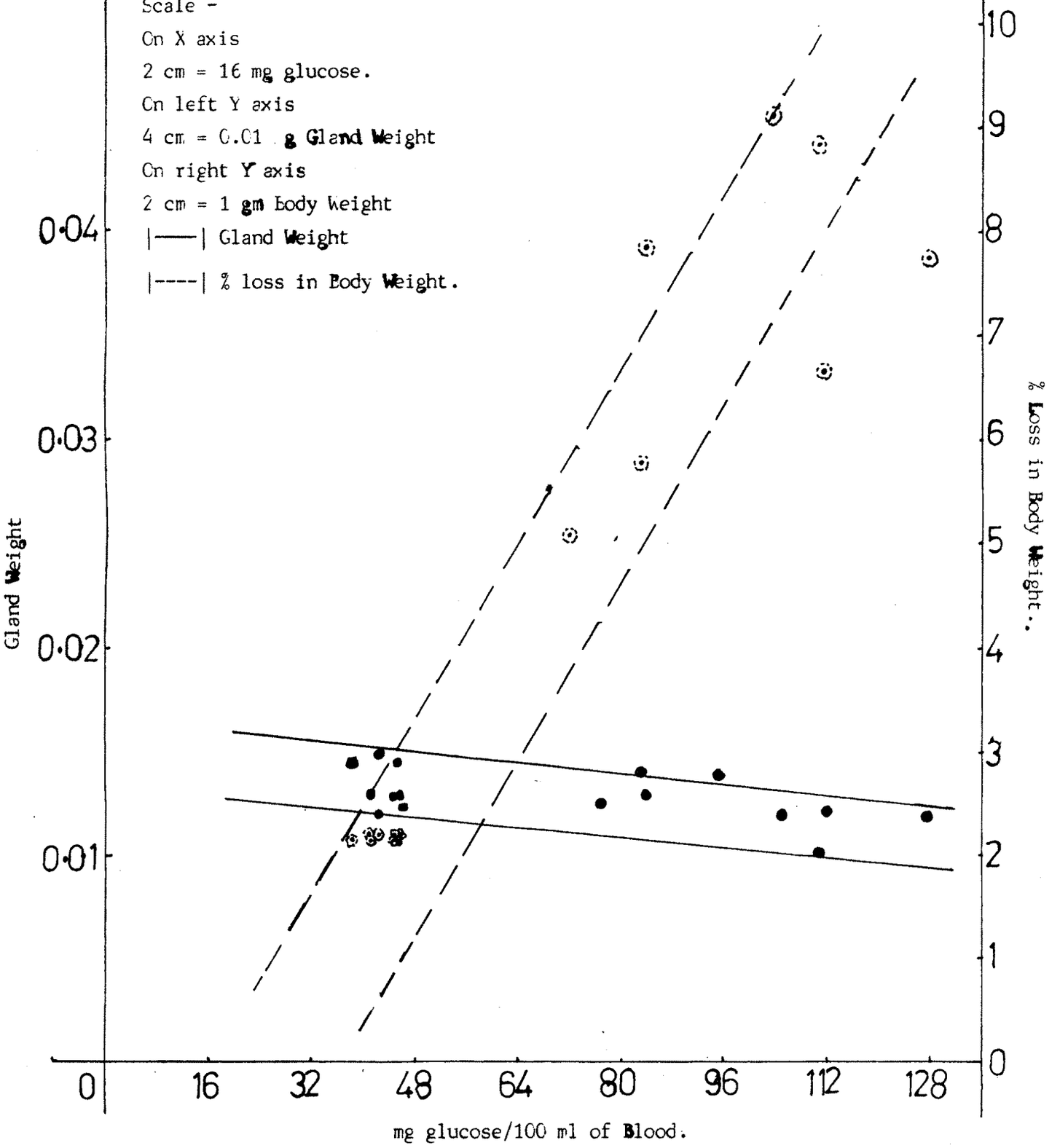
GRAPH NO.2

The Percentage Weight of Submandibular Gland with respect to Body Weight and Percentage Loss in Body Weight against the Concentration of Glucose.



GRAPH NO.3

The Percentage Weight of the Sublingual Gland with respect to Body Weight and Percentage Loss in Body Weight against the Concentration of Glucose.



gland. In both the controls, fasted and fasted but fed. % weight of submandibular gland with respect to body weight was about 0.09. In diabetic animals either from fasted or fasted but fed. The % weight of the gland with respect to body weight was nearly 0.08. When the % gland weight with respect to glucose level in the blood was compared, the range of gland weight was decreased as the blood glucose level increased, but the decrease was not gradual.

ii) Sublingual Gland

The % weight of the sublingual glands with respect to body weight during induced diabetes and according to the glucose concentration was shown in table no. 1 and graph no. 3. In all animals there was decrease in % weight of the sublingual gland with respect to body weight but no significant difference could be noted in controls and diabetic animals. The values were inbetween 0.01285 to 0.013 gm./100 gm. body weight in fasted and fasted but fed controls. The diabetic gland weights were inbetween 0.012 to 0.015 gm/100 gm body weight. When blood glucose levels and % weight of the gland with respect to body weights were compared, there was slight decrease in gland weight according to the concentration of glucose. There is correlation in the decrease in gland weight and body weight.

c) Esterase

i) Submandibular Gland

Esterase specific activities/mg protein and mean

activities/gm wet weight of the tissue from diabetic animals were described in Table No. 2, and the specific activities were depicted according to glucose concentration from blood in graph no. 4; according to the time after alloxan injection in graph no. 5. The esterase activity/gm wet weight of the tissue according to the blood glucose was shown in graph no. 6 and according to the time after alloxan administration was shown in graph no. 7. As the blood glucose level increase, there was increase in the enzyme activity, the increase was significant (see graph no. 4 and 6). When specific enzyme activities of fasted controls were compared with 24 hrs. and 48 hrs. of alloxan diabetes fasted for 12 hrs. showed non - significant difference, there was decrease in enzyme activity. The specific activities of the enzyme esterase from submandibular gland of fasted controls and fasted alloxan diabetes from 24 hrs and 48 hrs. were 0.1590 $\pm$ 0.0725, 0.0851 $\pm$ 0.0036 and 0.1247 $\pm$ 0.0037 respectively. When enzyme activities of control fasted were compared with alloxan fasted at 24 hrs. and 48 hrs. there was a highly significant difference ($P < 0.01$), there was decrease in the enzyme activity. The specific activities of the esterase of fed control and of fed diabetes at 24 hrs. and 48 hrs. were 0.1931 $\pm$ 0.003, 0.0868 $\pm$ 0.004 and 0.1725 $\pm$ 0.0026 respectively. The enzyme activities/mg protein were further decreased in diabetes during 72 and 144 hrs (Table No.2 and Graph No.4). The enzyme activities/gm wet weight of the tissue also showed difference in their values. After the induction of alloxan diabetes there were decrease in

enzyme activities in the submandibular gland ~~after~~ the decrease was significant at 24 hrs, but at 48 hrs the decrease was non significant.

The esterase activities/gm. submandibular gland weight of fasted controls and 24 hrs alloxan fasted were 10.625 ± 1.033 and 6.25 ± 0.3848 respectively. In fed animals activities were increased compared to fasted animals in fed controls and 24 hrs diabetes the activities were 14.0625 ± 0.5147 and 8.75 ± 0.1178 respectively. At 48 hrs fasted and fasted fed diabetes the activities were 7.50 ± 0.3117 and 12.5 ± 0.2045 respectively.

In 72 hrs alloxan diabetes fasted animals the specific activity was 0.4481 ± 0.0071 and in fasted controls it was 0.1590 ± 0.0725. In fasted but fed diabetic animals there was increase in the specific activity to 0.5211 ± 0.0110 and in fasted but fed controls it was 0.1931 ± 0.0030.

In 144 hrs alloxan diabetic fasted animals the specific activity was 0.5545 ± 0.0122 and in fasted controls it was 0.1590 ± 0.0725 and in fasted but fed alloxan animals it was raised to 0.7955 ± 0.0604 whereas in fasted but fed controls it was 0.1931 ± 0.0030. This shows that after alloxan administration the specific enzyme activity was increased according to the time as well as glucose concentrations. The increase was about 5 time in fasted alloxan diabetes and 7 times in fasted but fed alloxan diabetes at 144 hrs after alloxan

Table No. 2 : Effect of Alloxan Diabetes on the Specific activities and the activities of Esterase 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)	44.70 ± 0.3299	0.1590 ± 0.0725 *3	P > 0.05 NS	10.625 ± 1.033 *3	P < 0.05 S
2.	24	Alloxan fasting 12 hr (AF)	77.36 ± 0.6411	0.0851 ± 0.0036 *3		6.25 ± 0.3848 *3	
3.	-	Control fasting + feeding $\frac{1}{2}$ hr (CFF)	45.88 ± 1.1939	0.1931 ± 0.0030 *3	P < 0.01 HS	14.0625 ± 0.5147 *3	P < 0.01 HS
4.	24	Alloxan fasting + feeding $\frac{1}{2}$ hr (AFF)	84.21 ± 1.7194	0.0868 ± 0.0040 *3		8.75 ± 0.1178 *3	
5.	--	CF	38.88 ± 1.6448	0.1590 ± 0.0725 *3	P > 0.05 NS	10.625 ± 1.033 *3	P > 0.05 NS
6.	48	AF	83.33 ± 1.3730	0.1247 ± 0.0037 *3		7.50 ± 0.3117 *3	
7.	-	CFF	42.22 ± 1.3456	0.1931 ± 0.0030 *3	P < 0.05 S	14.0625 ± 0.5147 *3	P > 0.05 NS
8.	48	AFF	95.55 ± 2.2951	0.1725 ± 0.0026 *3		12.5 ± 0.2045 *3	
9.	-	CF	41.05 ± 1.2695	0.1590 ± 0.0725 *3	P < 0.05 S	10.625 ± 1.033 *3	P < 0.01 HS
10.	72	AF	105.55 ± 1.732	0.4481 ± 0.0071 *3		24.00 ± 0.3118 *3	
11.	-	CFF	42.10 ± 1.2914	0.1931 ± 0.0030 *3	P < 0.01 HS	14.0625 ± 0.5147 *3	P < 0.01 HS
12.	72	AFF	111.11 ± 1.2963	0.5211 ± 0.0110 *3		28.75 ± 0.1178 *3	
13.	--	CF	45.70 ± 0.3299	0.1590 ± 0.0725 *3	P < 0.01 HS	10.625 ± 1.033 *3	P < 0.01 HS
14.	144	AF	112.00 ± 1.2472	0.5545 ± 0.0122 *3		26.66 ± 1.3608 *3	
15.	-	CFF	46.88 ± 1.1511	0.1931 ± 0.0030 *3	P < 0.01 HS	14.0625 ± 0.5147 *3	P < 0.01 HS
16.	144	AFF	128.00 ± 2.0548	0.7955 ± 0.0604 *3		40.00 ± 4.0826 *3	

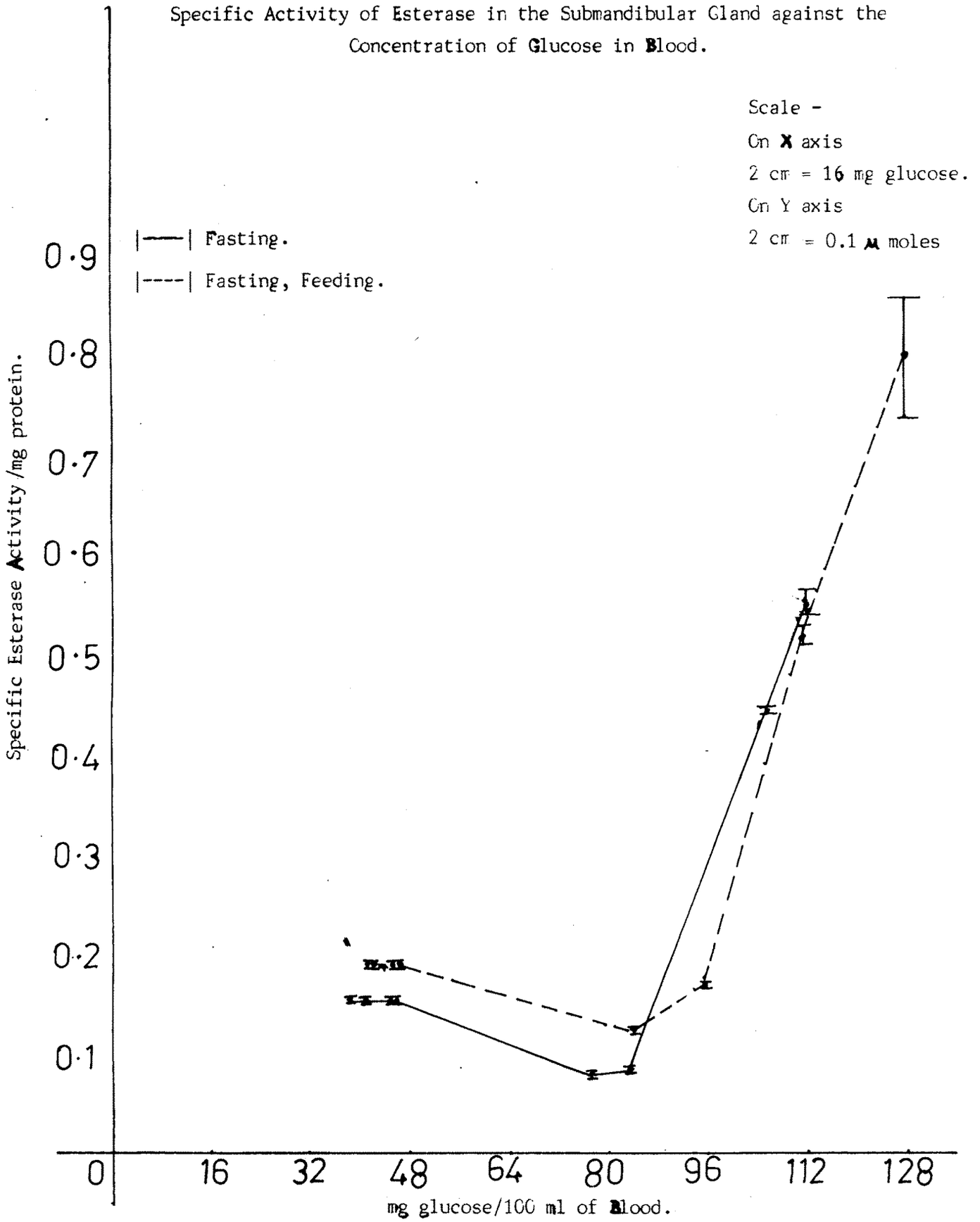
Results are expressed as mean + SEM

*represents the number of animals

HS : Highly significant
S : Significant
NS : Non significant

GRAPH NO.4

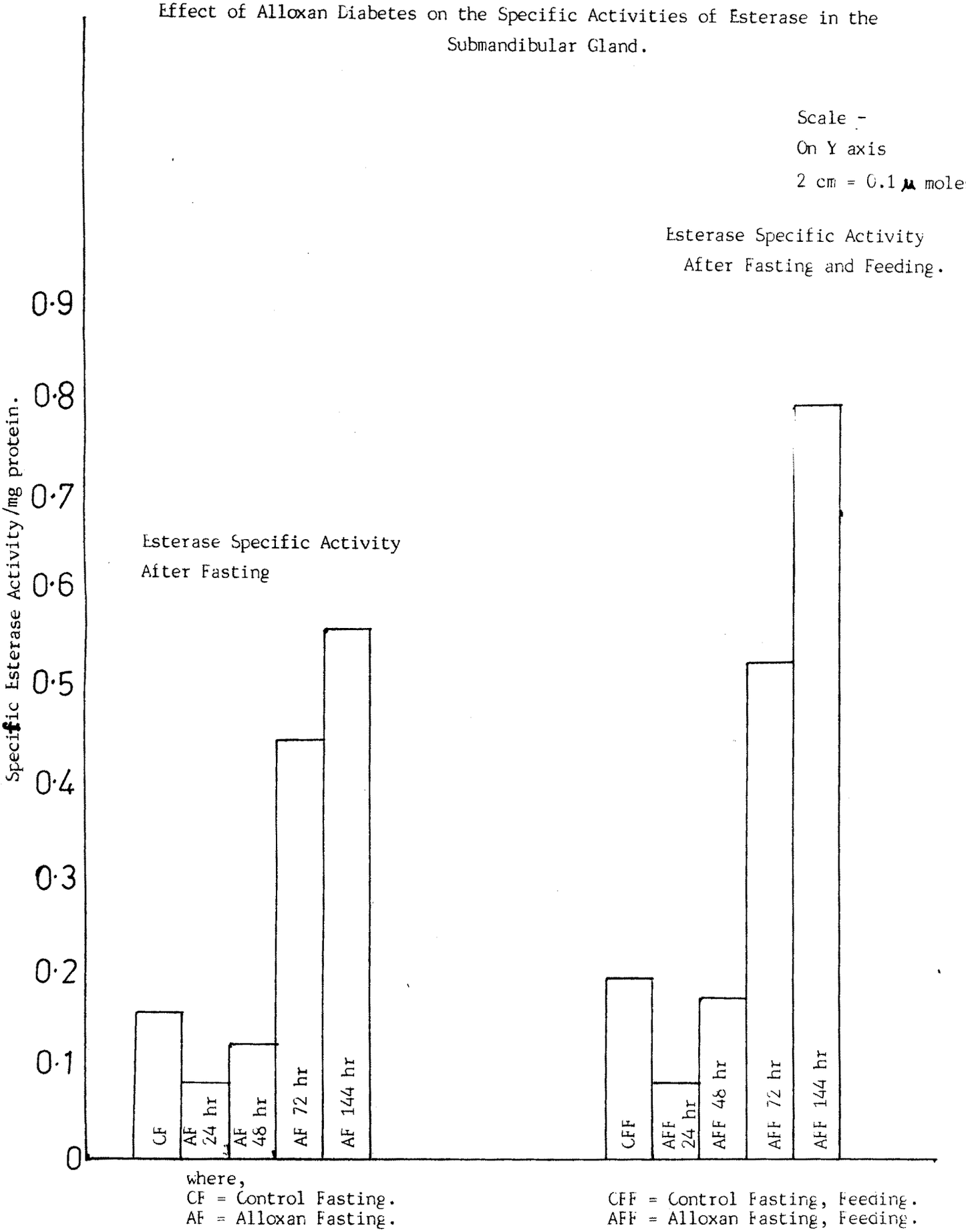
Specific Activity of Esterase in the Submandibular Gland against the Concentration of Glucose in Blood.



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

GRAPH NO.5

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

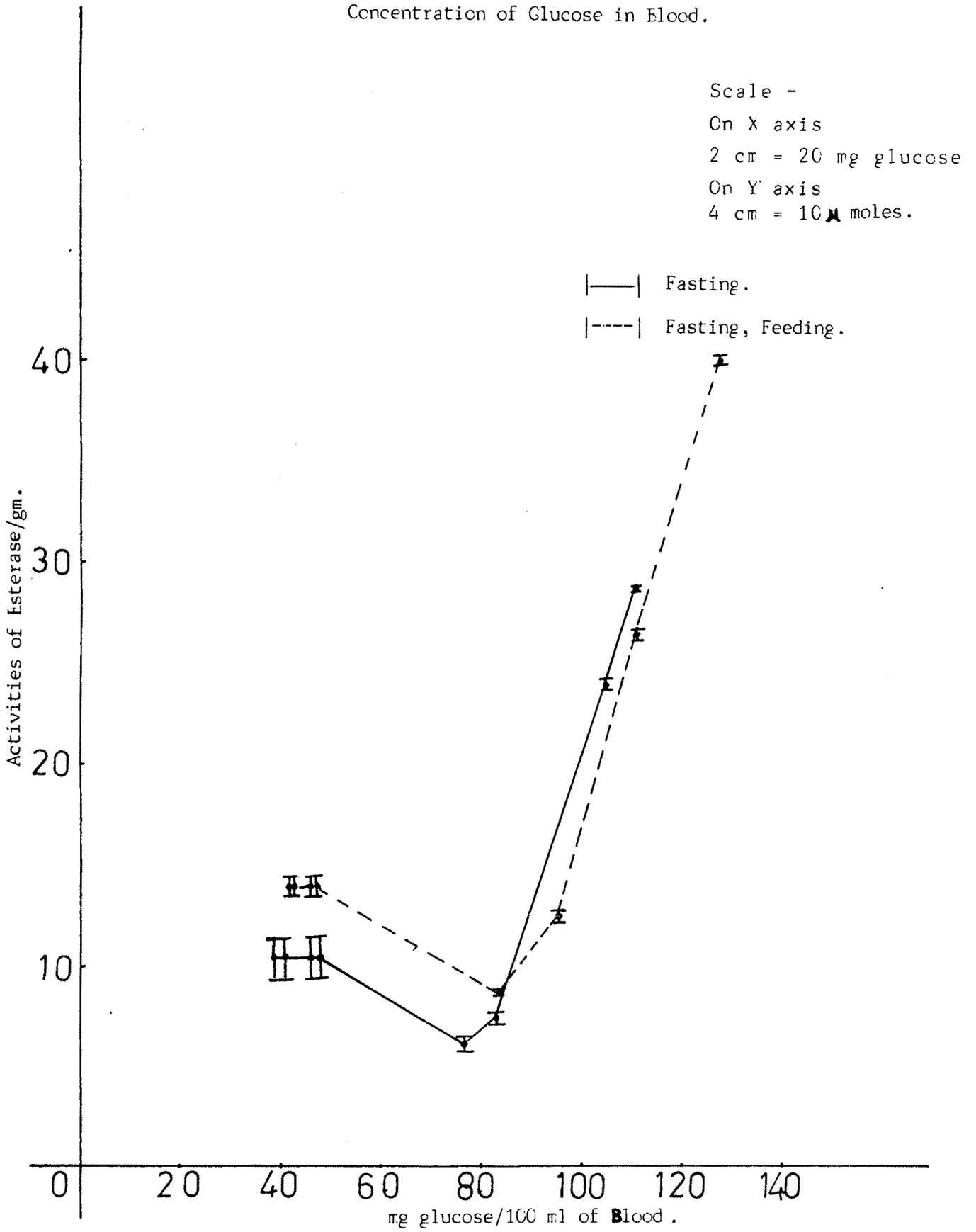


where,
CF = Control Fasting.
AF = Alloxan Fasting.

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

GRAPH NO.6

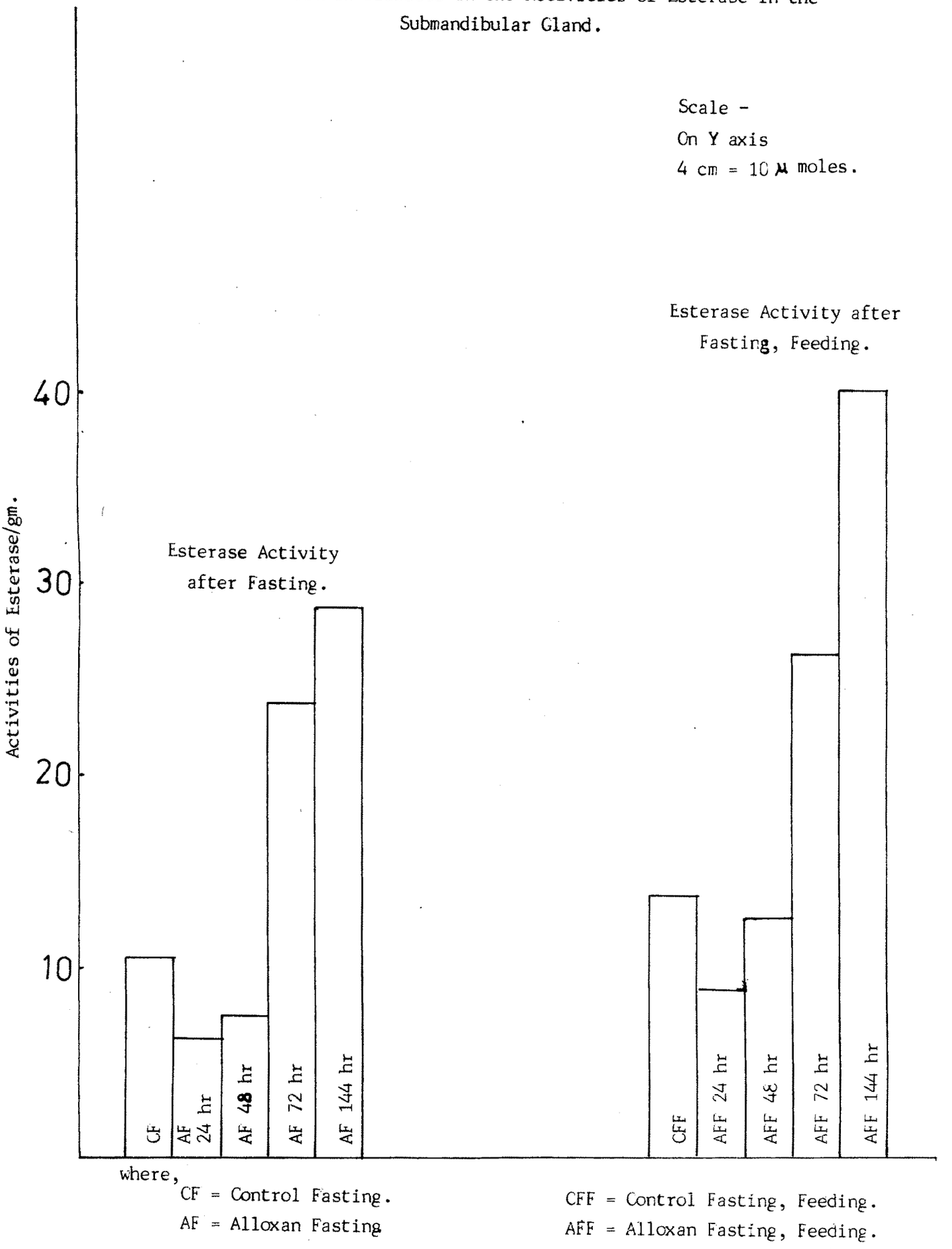
Activities of Esterase in the Submandibular Gland against the Concentration of Glucose in Blood.



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

GRAPH NO.7

Effect of Alloxan Liabetes in the Activities of Esterase in the Submandibular Gland.



where,

CF = Control Fasting.

AF = Alloxan Fasting

CFF = Control Fasting, Feeding.

AFF = Alloxan Fasting, Feeding.

administration. When enzyme activities/gm of the tissues were compared with fasted controls; there was highly significant difference ($P < 0.01$) at 72 hrs. In fasted alloxan diabetes the activity was 24.00 ± 0.3118 and in respective controls it was 10.625 ± 1.033. In fasted but fed alloxan diabetes it was 28.75 ± 0.1178 and in fasted but fed controls it was 14.0625 ± 0.5147.

In fasted 144 hrs alloxan diabetes the mean activity was 26.66 ± 1.3608 and in controls it was 10.625 ± 1.033, this difference was highly significant at the level of $P < 0.01$. In fasted but fed alloxan diabetes the mean activity was 40 ± 4.0826 and in fasted but fed controls it was 14.0625 ± 0.5147. There was highly significant difference between these two groups ($P < 0.01$). All these observations showed that there was significant changes during diabetic conditions.

ii) Sublingual Gland

The esterase activities/mg protein and per gm wet weight of the sublingual gland of diabetic animals were described in table no. 3 and these were plotted according to blood glucose concentration in graph no. 8 and 10 respectively. The activities were plotted according to the time after alloxan administration in graph no. 9 and 11.

Enzyme activity/mg protein of fasted controls was 0.2899 ± 0.0066 and in 24 hrs diabetic rats fasted for 12 hrs

it was 0.0512 ± 0.0039. In 24 hrs of diabetes the activity was decreased. In fed animals the activity was increased to 0.3348 ± 0.0036 in controls, and 0.1031 ± 0.0009 in diabetes. Compared to fasting animals here the enzyme activity was increased. 48 hrs onwards the enzyme activities were increased in diabetic rats. The difference in the activities was at the level of $P < 0.01$. In fasted, fed animals the enzyme activities were increased. The difference in diabetic salivary gland lipase and respective controls was too high and significant at 144 hrs after the alloxan administration, the enzyme activity was 9 to 10 times more than controls. The esterase activity per gm of sublingual gland of control fasted for 12 hrs. was compared with fasted alloxan diabetes, the difference was significant ($P < 0.01$). The fasted fed controls were compared with fasted fed alloxan diabetes, there was also a significant difference ($P < 0.01$). The esterase activities of fasted control and 24 hrs diabetes were 13.4375 ± 0.7804 and 2.500 ± 0.2045 respectively. In fed controls the enzyme activity was 16.875 ± 0.9375 and in diabetic rats it was 6.25 ± 0.0623, compared to controls in 24 hrs of diabetic animals the enzyme activity was low, the increase was noticed in fed animals from both the groups.

In 48, 72 and 144 hrs of alloxan diabetes the enzyme activity/mg protein was increased considerably both in fasted as well as fasted but fed animals. The increase was significant at the level of $P < 0.01$. The activities were too high compared

Table No. 3 : Effect of Alloxan Diabetes on the Specific Activities and Activities of Esterase in the Rat "Sublingual Gland".

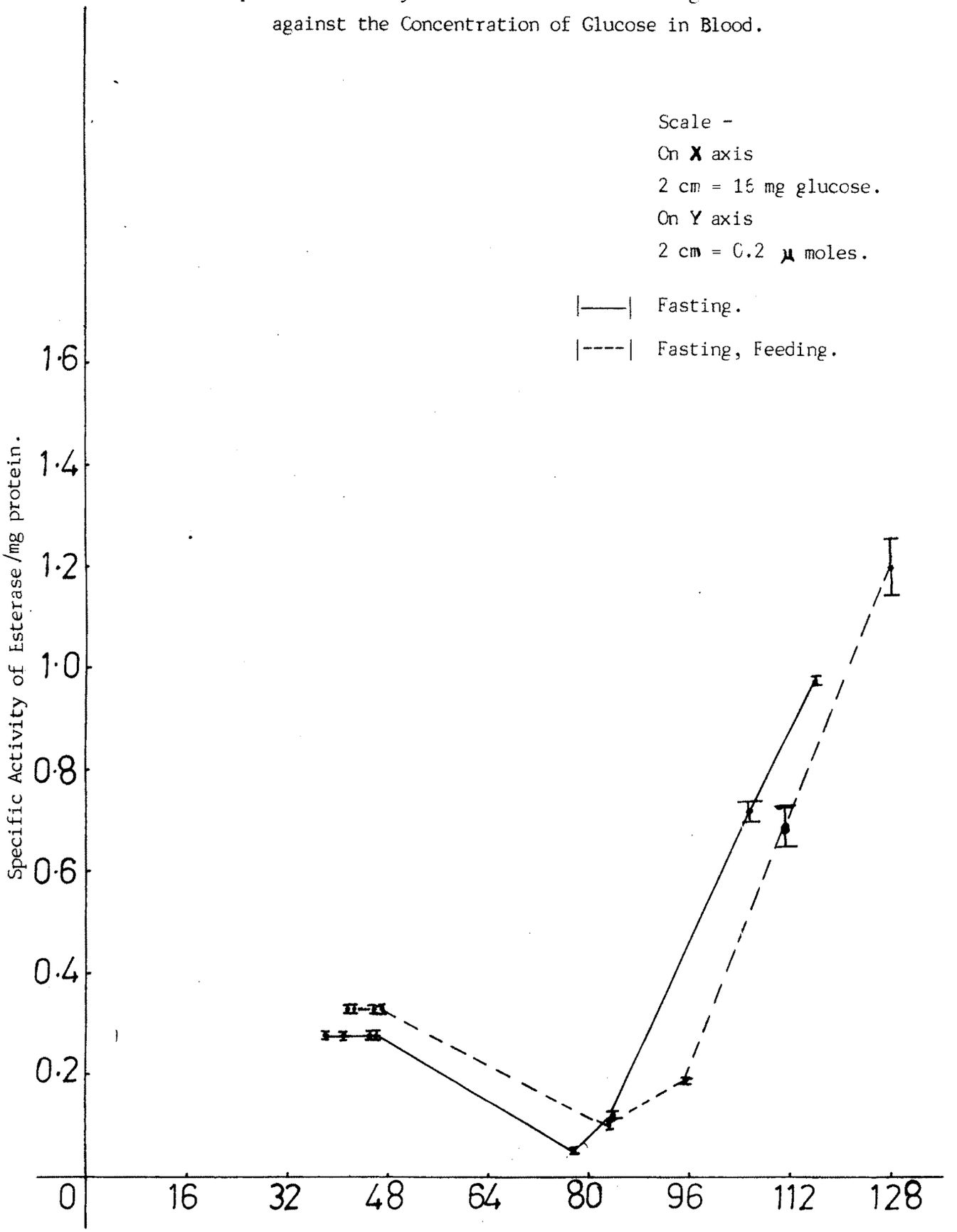
Sr. NO.	Time after Alloxan Administration	Animals	Glucose Conc. ± SEM	Specific activities ± SEM	P	Mean ± SEM	P
1.	-	Control fasting 12 hr (CF)	44.70 ± 0.3299	0.2899 ± 0.0066 *3	P < 0.01 HS	13.4375 ± 0.7804 *3	P < 0.01 HS
2.	24	Alloxan fasting 12 hr (AF)	77.36 ± 0.6411	0.0512 ± 0.0039 *3		2.500 ± 0.2045 *3	
3.	-	Control fasting + feeding $\frac{1}{2}$ hr (CFF)	45.88 ± 1.1939	0.3348 ± 0.0036 *3	P < 0.01 HS	16.875 ± 0.9375 *3	P < 0.01 HS
4.	24	Alloxan fasting + feeding $\frac{1}{2}$ hr (AFF)	84.21 ± 1.7194	0.1031 ± 0.0009 *3		6.25 ± 0.0623 *3	
5.	-	CF	38.88 ± 1.6448	0.2899 ± 0.0066 *3	P < 0.01 HS	13.4375 ± 0.7804 *3	P < 0.01 HS
6.	48	AF	83.33 ± 1.3730	0.1216 ± 0.0031 *3		5.75 ± 0.3535 *3	
7.	-	CFF	42.22 ± 1.3456	0.3348 ± 0.0036 *3	P < 0.01 HS	16.875 ± 0.9375 *3	P < 0.05 S
8.	48	AFF	95.55 ± 2.2951	0.1880 ± 0.0055 *3		11.00 ± 0.4082 *3	
9.	-	CF	41.05 ± 1.2695	0.2899 ± 0.0066 *3	P < 0.01 HS	13.4375 ± 0.7804 *3	P < 0.01 HS
10.	72	AF	105.55 ± 1.732	0.7206 ± 0.0198 *3		21.00 ± 0.4713 *3	
11.	-	CFF	42.10 ± 1.2914	0.3348 ± 0.0036 *3	P < 0.01 HS	16.875 ± 0.9375 *3	P < 0.01 HS
12.	72	AFF	111.11 ± 0.2963	0.6811 ± 0.0303 *3		27.500 ± 0.1699 *3	
13.	-	CF	45.70 ± 0.3299	0.2899 ± 0.0066 *3	P < 0.01 HS	13.4375 ± 0.7804 *3	P < 0.01 HS
14.	144	AF	112.00 ± 1.2472	0.9757 ± 0.0099 *3		26.33 ± 1.0836 *3	
15.	-	CFF	46.88 ± 1.1511	0.3348 ± 0.0036 *3	P < 0.01 HS	16.875 ± 0.9375 *3	P < 0.01 HS
16.	144	AFF	128.00 ± 2.0548	1.3514 ± 0.0560 *3		51.00 ± 0.8165 *3	

Results are expressed as mean ± SEM
*represents the number of animals.

HS = Highly significant
S = Significant.

GRAPH NO.8

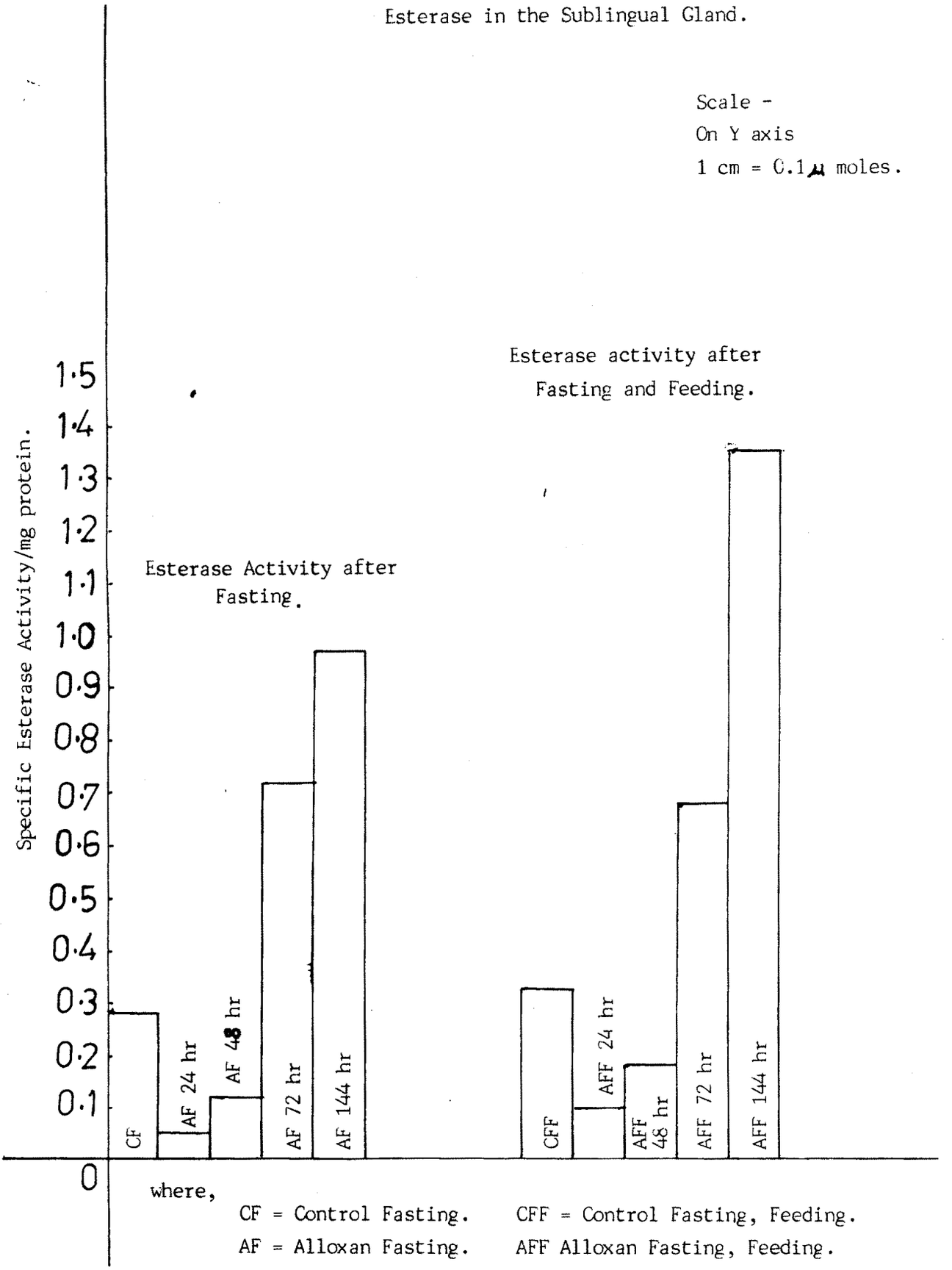
Specific Activity of Esterase in the Sublingual Gland against the Concentration of Glucose in Blood.



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

GRAPH NO.9

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



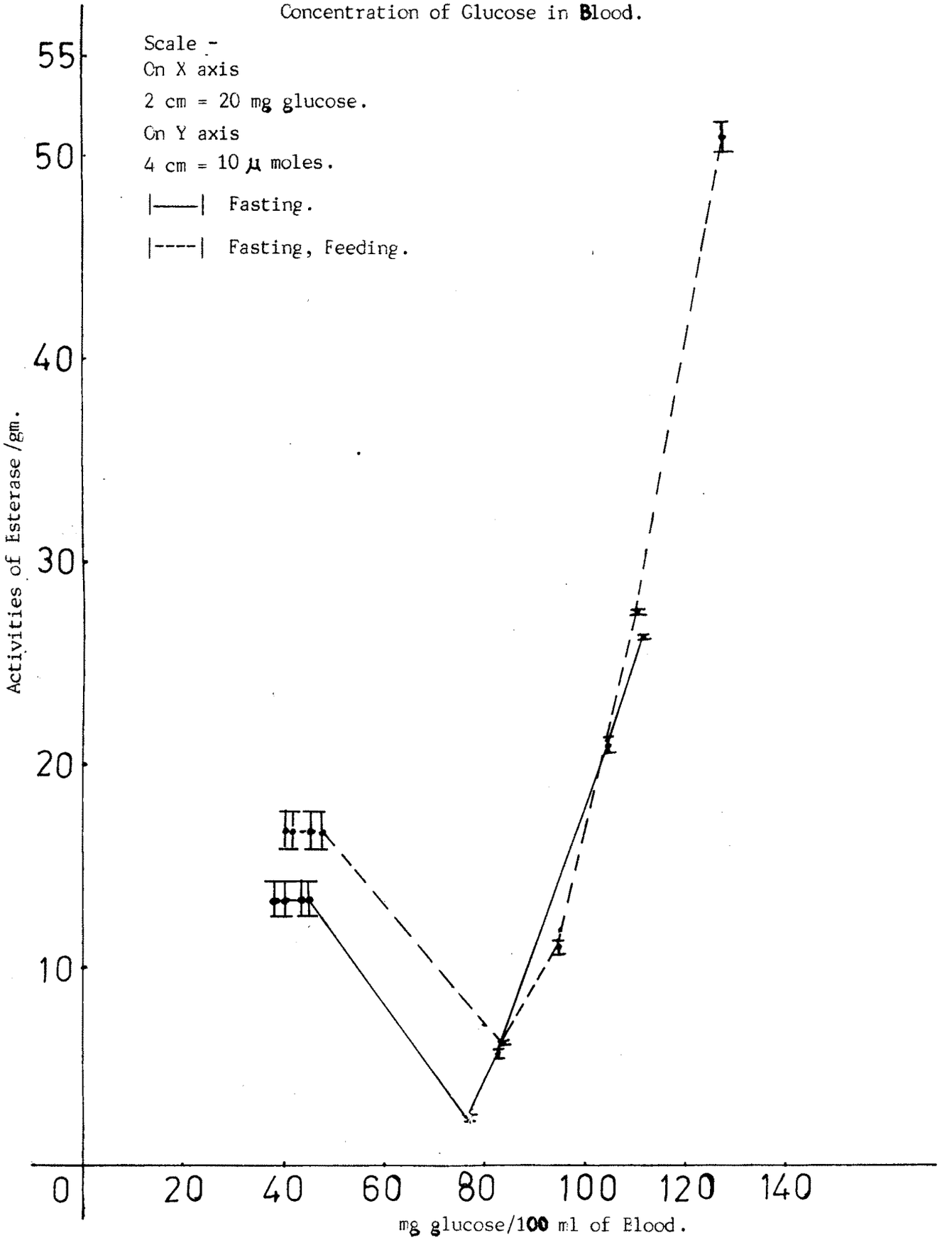
where,

CF = Control Fasting. CFF = Control Fasting, Feeding.

AF = Alloxan Fasting. AFF Alloxan Fasting, Feeding.

GRAPH NO.10

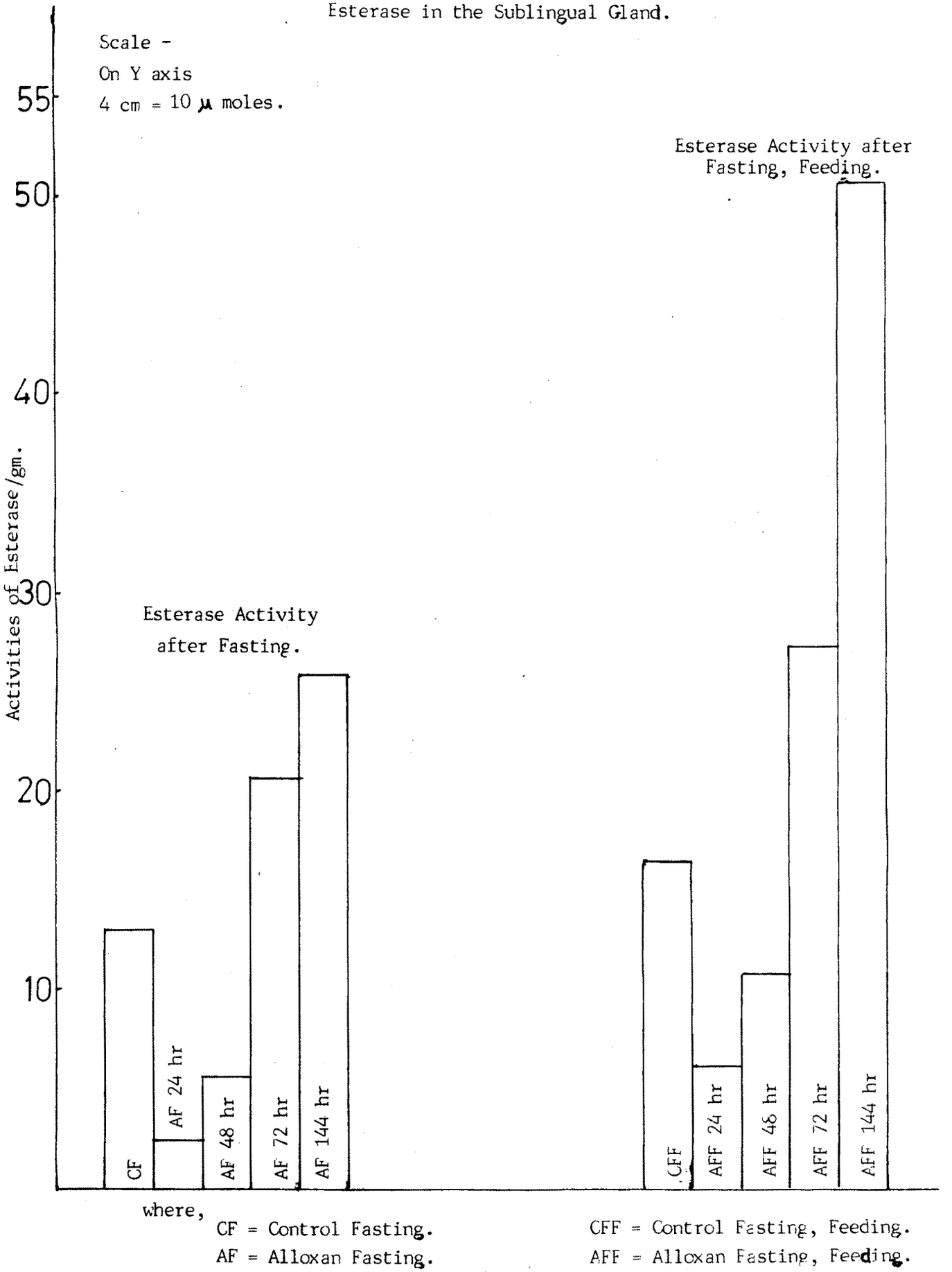
Activities of Esterase in the Sublingual Gland against the Concentration of Glucose in Blood.



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

GRAPH NO.11

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



where,

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

to controls they were increased gradually after the alloxan injection as well as they are closely related with glucose concentration in blood, specially at high blood glucose levels. The enzyme activities were also high. In 48, 72 and 144 hrs diabetes fasted for 12 hrs the enzyme activities were 0.1216 ± 0.0031, 0.7206 ± 0.01986, and 0.9797 ± 0.0099 and in fed they were 0.1880 ± 0.0055, 0.6811 ± 0.0303 and 1.3514 ± 0.0560 respectively. The behaviour of enzyme activity/gm tissue weight was also like that of specific activities, but 48 hrs after the alloxan injection there was increase in enzyme activities, the increase was significant at the level of $P < 0.05$ when compared with their respective controls. In 48, 72 and 144 hrs of fasted alloxan diabetes the enzyme activities were 5.75 ± 0.03535, 21.00 ± 0.4713 and 26.33 ± 1.0836 respectively. In fasted fed rats the activities were 11.00 ± 0.4082, 27.500 ± 0.1699 and 51.00 ± 0.8165. The difference in respective controls and diabetic salivary gland lipase was significant, at 144 hrs. the increase was about 2 times in fasted and 3 times in fasted but fed animals.

iii) Parotid Gland.

The esterase specific activities/mg protein and esterase activities/gm wet weight of the parotid gland from diabetic animals was described in Table No.4 and its specific activities and activities were depicted according to the blood glucose concentration in graph no. 12 and 14 respectively and according to the time after alloxan administration in graph no. 13 and 15.

As the blood glucose level increased, there were increase in the enzyme activities. The increase was significant (see graph no. 12 and 14).

The specific esterase activities of fasted and fed controls were compared with 24 hrs diabetes fasted for 12 hrs and fed for half hrs, the difference was significant ($P < 0.01$).

In 24 hrs fasted alloxan diabetes the specific esterase activity of the parotid gland was 0.0801 ± 0.0004 and in fasted controls it was 0.2113 ± 0.0032. In fasted but fed alloxan diabetes and controls the specific activities were 0.1083 ± 0.0069 and 0.24 ± 0.0037. In both fasted and fasted fed alloxan diabetes the specific esterase activities were decreased as compared to their respective controls. The esterase activity/gm of parotid gland of 24 hrs alloxan diabetes fasted for 12 hrs showed a highly significant difference ($P < 0.01$) when compared to its controls. In control it was 13.75 ± 0.0625, and in alloxan treated animal it was 6.25 ± 0.7178, in fasted but fed alloxan diabetes the esterase activity was 8.75 ± 0.3118 and in controls it was 16.875 ± 0.9375. The difference was highly significant at the level of $P < 0.01$. In both fasted and fasted, fed alloxan diabetes there were decrease in esterase activities as compared to their controls.

The specific activities of fasted controls were compared with 48, 72 and 144 hrs alloxan diabetes fasted for 12 hrs.

The differences were highly significant at the level of $P < 0.01$. Similarly when fasted fed controls were compared with 48, 72 and 144 hrs fasted fed alloxan diabetes, there were highly significant differences at the level of $P < 0.01$. In fasted 48, 72 and 144 hrs alloxan diabetes the specific esterase activities of the parotid gland were 0.1191 ± 0.00178, 0.3616 ± 0.0015 and 0.7656 ± 0.0106, and in fasted fed alloxan diabetes, the esterase activities were 0.1281 ± 0.0021, 0.5163 ± 0.0240 and 0.92 ± 0.0155.

In both fasted and fasted fed alloxan diabetes at 48 hrs the specific activities were decreased as compared to their controls.

But in both fasted and fed alloxan diabetes of 72 and 144 hrs, the specific esterase activities were increased as compared to their controls, the increase was about four fold compared to control and ten fold compared to 24 hrs, alloxan diabetes. ~~in 144 hrs diabetic rats~~.

The esterase activities of fasted and fasted but fed controls were compared with 48, 72 and 144 hrs alloxan diabetes fasted for 12 hrs and fasted but fed for one half hr. The differences were highly significant at the level of $P < 0.01$.

In 48, 72 and 144 hrs fasted alloxan the esterase activities were 7.75 ± 0.3118, 18.75 ± 0.3535 and 36.00 ± 1.2472, where as in fasted fed alloxan diabetes the activities were 10.00 ± 0.3118, 28.25 ± 0.2041 and 46.00 ± 1.2472 respectively.

Table No. 4 : Effect of Alloxan Diabetes on the Specific Activities and Activities of Esterase in the Rat "Parotid Gland".

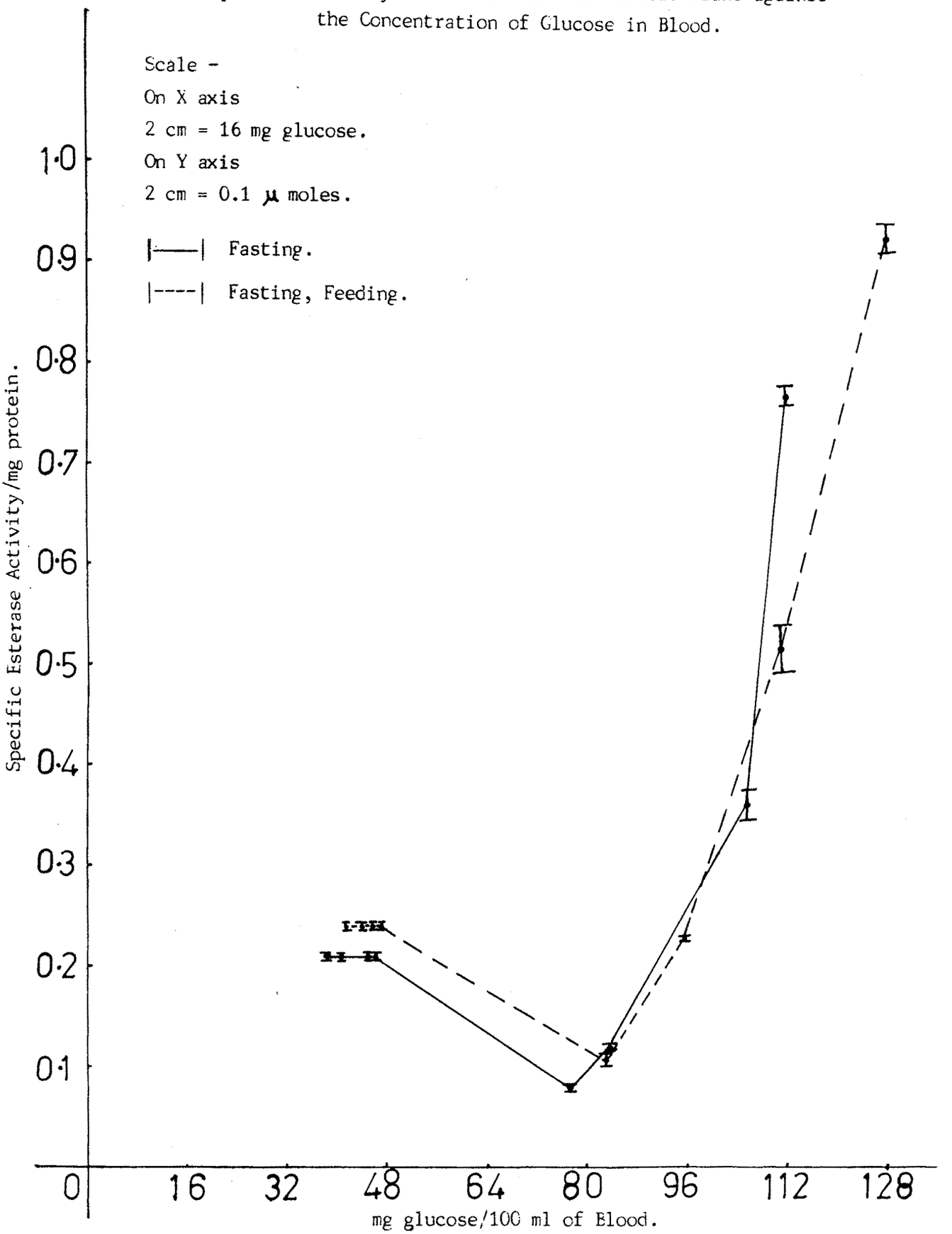
Sr. No.	Time after Alloxan Administration	Animals	Glucose conc. ± SEM	Specific activities ± SEM	P	Mean ± SEM	P
1.	-	Control fasting 12 hr (CF)	44.70 ± 0.3299	0.2113 ± 0.0032 *3	P < 0.01 HS	13.75 ± 0.625 *3	P < 0.01 HS
2.	24	Alloxan fasting 12 hr (AF)	77.36 ± 0.6411	0.0801 ± 0.0004 *3		6.25 ± 0.1178 *3	
3.	-	Control fasting + feeding $\frac{1}{2}$ hr (CFF)	45.88 ± 1.1939	0.2400 ± 0.0037 *3	P < 0.01 HS	16.875 ± 0.9375 * 3	P < 0.01 HS
4.	24	Alloxan fasting + feeding $\frac{1}{2}$ hr (AFF)	84.21 ± 1.7194	0.1083 ± 0.0069 *3		8.75 ± 0.3118 *3	
5.	-	CF	38.88 ± 1.6448	0.2113 ± 0.0032 *3	P < 0.01 HS	13.75 ± 0.625 *3	P < 0.01 HS
6.	48	AF	83.33 ± 1.3730	0.1191 ± 0.0017 *3		7.75 ± 0.3118 *3	
7.	-	CFF	42.22 ± 1.3456	0.2400 ± 0.0037 *3	P < 0.01 HS	16.875 ± 0.9375 *3	P < 0.01 HS
8.	48	AFF	95.55 ± 2.2951	0.1281 ± 0.0021 *3		10.00 ± 0.3118 *3	
9.	-	CF	41.05 ± 1.2695	0.2113 ± 0.0032 *3	P < 0.01 HS	13.75 ± 0.625 *3	P < 0.01 HS
10.	72	AF	105.55 ± 1.732	0.3616 ± 0.0015 *3		18.75 ± 0.3535 *3	
11.	-	CFF	42.10 ± 1.2914	0.2400 ± 0.0037 *3	P < 0.01 HS	16.875 ± 0.9375 *3	P < 0.01 HS
12.	72	AFF	111.11 ± 1.2963	0.5163 ± 0.0240 *3		28.25 ± 0.2041 *3	
13.	-	CF	45.70 ± 0.3299	0.2113 ± 0.0032 *3	P < 0.01 HS	13.75 ± 0.625 *3	P < 0.01 HS
14.	144	AF	112.00 ± 1.2472	0.7656 ± 0.0106 *3		36.00 ± 1.2472 *3	
15.	-	CFF	46.88 ± 1.1511	0.2400 ± 0.0037 *3	P < 0.01 HS	16.875 ± 0.9375 *3	P < 0.01 HS
16.	144	AFF	128.00 ± 2.0548	0.92 ± 0.0155 *3		46.00 ± 1.2472 *3	

Results are expressed as mean + SEM
*represents the number of animals

HS = Highly significant

GRAPH NO.12

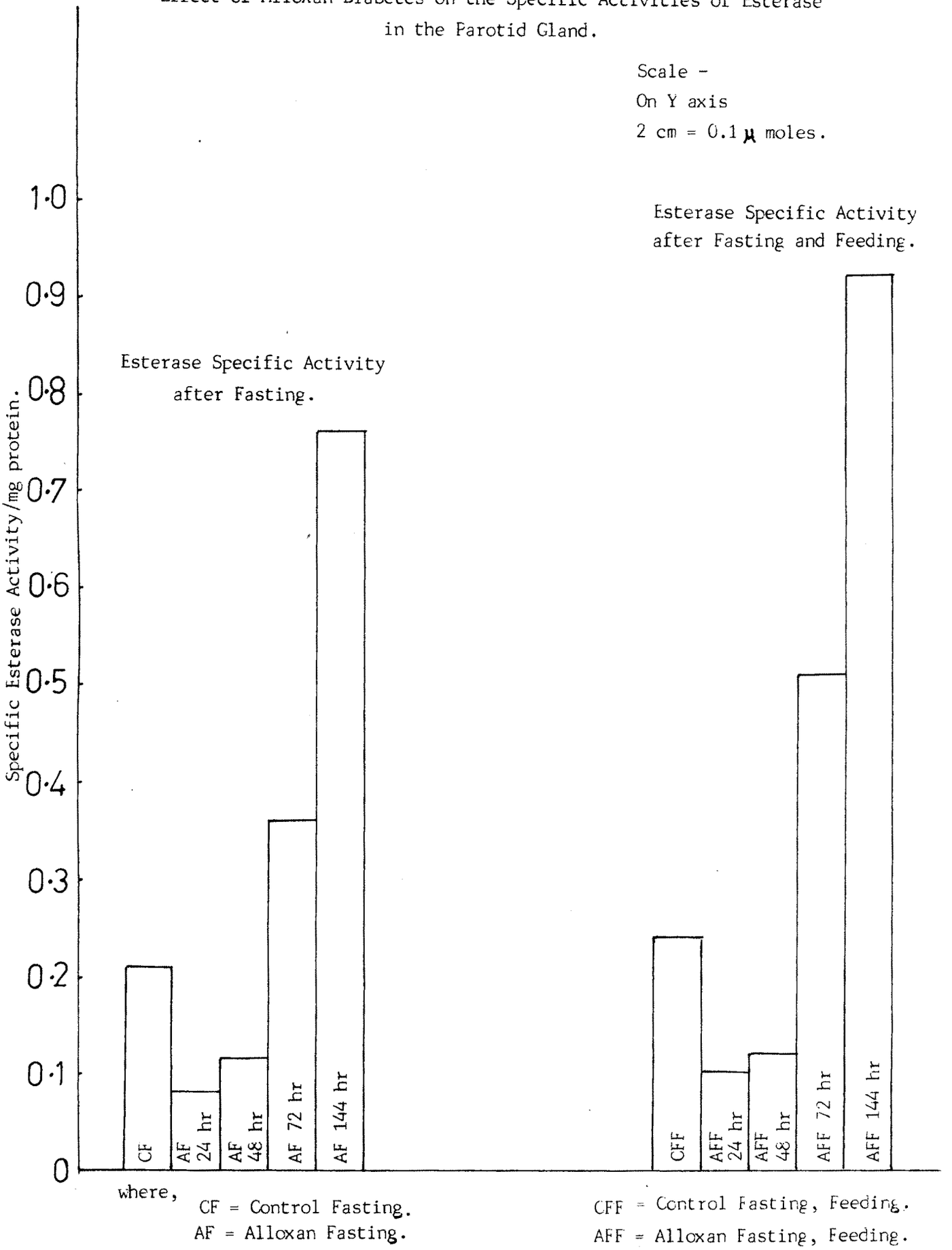
Specific Activity of Esterase in the Parotid Gland against the Concentration of Glucose in Blood.



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

GRAPH NO.13

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



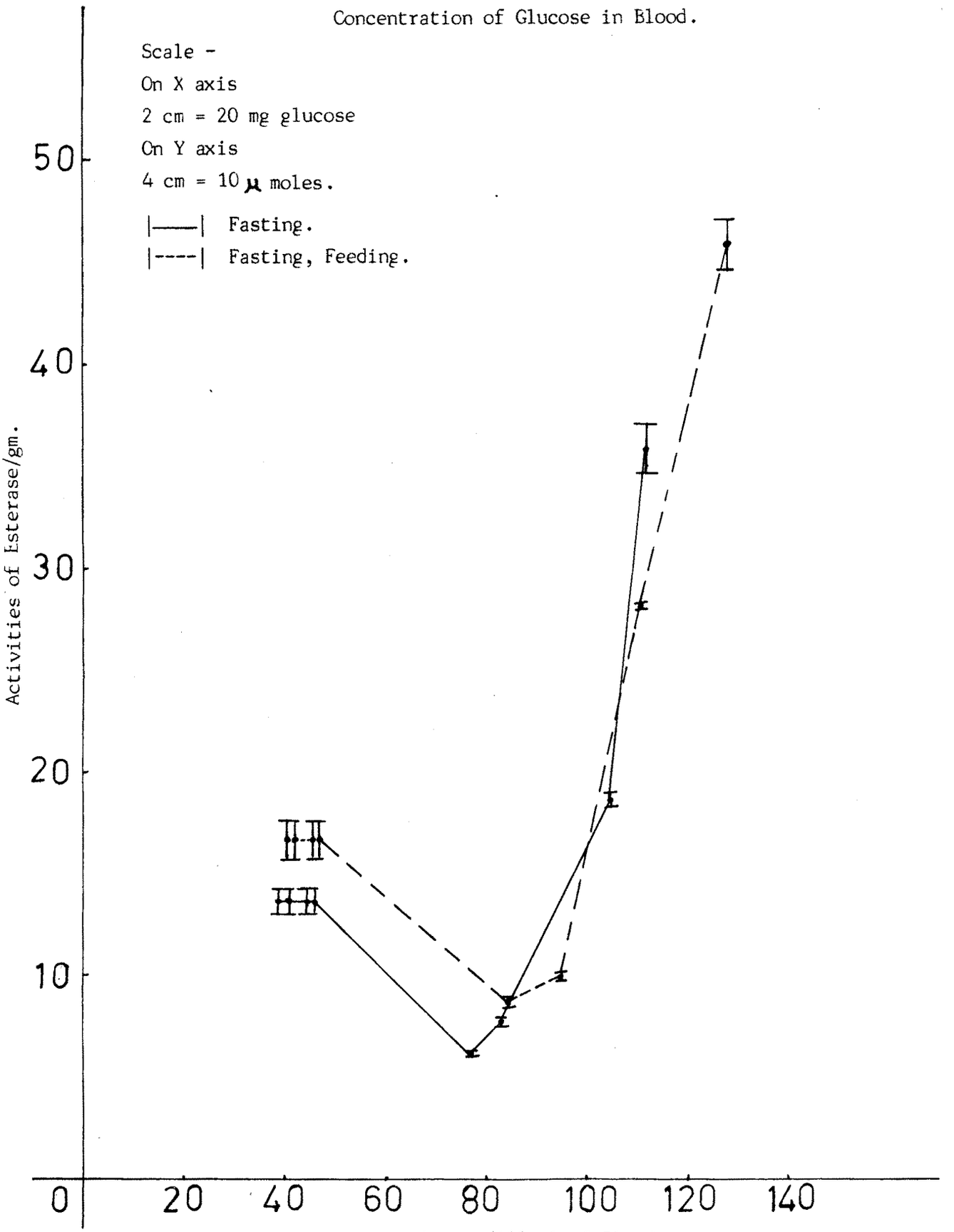
where,

CF = Control Fasting.
AF = Alloxan Fasting.

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

GRAPH NO.14

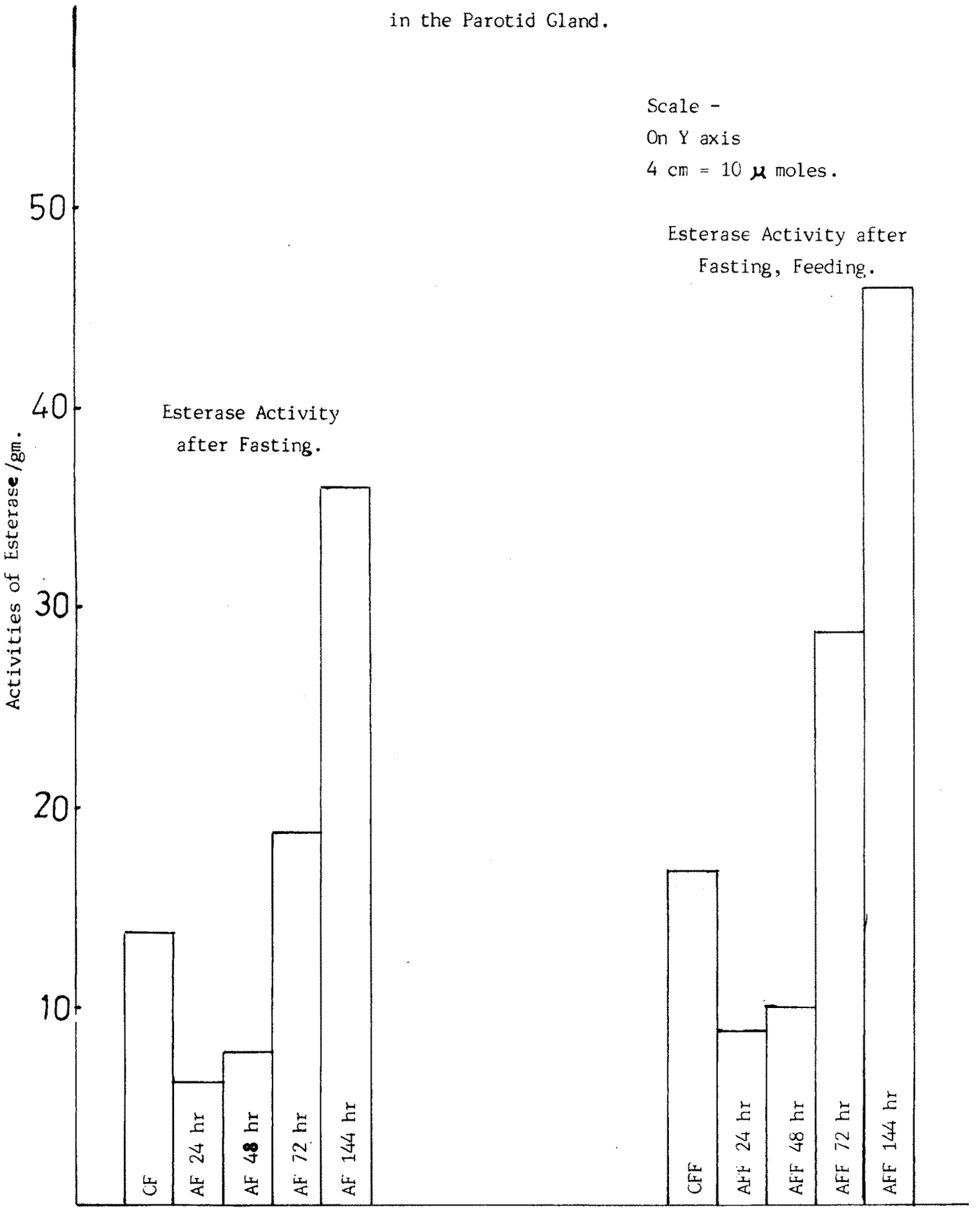
Activities of Esterase in the Parotid Gland against the Concentration of Glucose in Blood.



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

GRAPH NO.15

Effect of Alloxan Diabetes on the Activities of Esterase in the Parotid Gland.



where,

CF = Control Fasting.

AF = Alloxan Fasting.

CFF = Control Fasting, Feeding.

AFF = Alloxan Fasting, Feeding.

In both fasted and fasted fed alloxan diabetes of 48 hrs, the esterase activities were decreased as compared to their controls.

In both fasted and fasted fed alloxan diabetes of 72 hrs and 144 hrs the esterase activities were increased but in fed animals when compared with control rats the increase was about 3 fold.

As the time of alloxan administration and concentration of glucose increased, there were also increase in esterase activities/mg protein as well as esterase activities/gm of wet weight of the tissue.

Discussion

In diabetes there are several changes in a body, and it is generally considered that the most affected organs in diabetic conditions are liver, skeletal muscles, kidney and heart, which are supposed to be the most vital organs in our body. But after the investigation of twenty seven biologically active polypeptides in the submandibular salivary gland, this organ also became very important and elegant like kidney (Barka 1980; Hosoi, 1982). It has also shown that the synthesis and secretion of salivary glands is hormone dependent. The role of various hormones in the secretion of salivary glands has already been discussed in the introductory chapter. There are large number of studies described the role of insulin on salivary secretion (Pallar et al., 1967; Liu & Lin 1969 a,b;

Szymczyk et al., 1971; Murakami et al., 1974; Zebrowski and Brimmer, 1978; Anderson and Shapiro, 1979, 1980; Anderson, 1983, Pillai et al., 1989). Most of the work is carried out in Parotid and submandibular gland but no one except Pillai and Nadar (1987), and Pillai et al. (1989) included sublingual gland in their study, we think that the study of sublingual gland is also essential because only the sublingual gland secrete large amount of glycoprotein material and therefore more sugar metabolism may be taking place in this gland.

Much of the earlier work describes role of insulin and effects of diabetes mellitus on salivary gland; carbohydrate metabolism, RNA, DNA, protein and salivary secretory enzymes like trypsin and amylase. Anderson and Johnson (1981) in Parotid and Pillai and Nadar (1987) in all salivary gland studied peroxidase and lysosomal enzymes like β-glucuronidase and acid phosphatase respectively. In diabetes apart from the impairement of glucose metabolism, there are other two important metabolic disorders also take place i.e. change in lipid metabolism and formation of lipid peroxides. One of the earliest defects is abnormal Plasma free fatty acids. The oxidation of these free fatty acids takes place in the liver to molecules of Acetyl CoA which provides source of energy for neoglucogenesis, nonmetabolised acetyl CoA in converted into Ketone bodies. The greater availability of free fatty acids leads to synthesis of more ketone bodies by the liver which then can be utilized by the peripheral tissues (Mayes, 1988).

To study the effect of Ketone bodies on cell, the study of lysosomal enzymes is essential. Chari et al. (1983), Pillai and Nadar (1987) showed increase in lysosomal enzyme activities in induced diabetes. There was one more reason to study esterase in induced diabetics that is formation of lipid peroxides already discussed in the introduction.

In the persent investigation all the three major salivary glands were studied during induced diabetes; their % weights with respect to body weight were decreased in diabetic condition though not significantly. There was correlation in between the glucose concentration from blood and esterase activity both per mg protein and per gm wet weight of the tissues. Esterase activities studied both in fasting as well as fasting but fed conditions and always in fasting but fed conditions the enzyme activity was more. The increase in the esterase activity in fed animals salivary glands may be due to the increase in the salivary gland secretion after food stimulus. There is always an increase in lysosomal enzymes during secretion. Increase in lysosomal enzymes may be of two reasons. One is crinophagy - the mechanism by which secretory granules produced in excess of physiological needs may be removed. This is special case of autophagy, was first observed in the pitnitary gland, but it is likely that it is present in many types of exocrine and endocrine glands (De-Robertis,1980). The another reason of increase in lysosomal enzymes during secretion, is secretory granule membrane retrival (Nagasawa

et al., 1971; Heuser and Reese, 1973; De-Camilli et al., 1976; Masur et al., 1972; Abrahams and Holtzman, 1973; Geuze and Kramer, 1974; Kalina and Rabinovitch, 1975; Oliver and Hand, 1977). In early alloxan diabetes the enzyme activity was very low both specific and enzyme activity/gm of the tissue even lower than the respective controls but in 72 hr and 144 hrs after the alloxan administration enzyme activities were increased several folds. The increase was more in specific activities than the activities/gm. During these hours there was also increase in blood glucose level, 144 hrs after the alloxan injection blood glucose level was reached to 128 mg/ 100 ml of blood. During early hours of the diabetes though the blood glucose level was higher than those of respective controls, enzyme activities were low but in late hours and at high glucose levels, these were increased. Here possibility of activation of lysosomal enzymes due to toxicity of alloxan is ruled out because no immediate response was noticed. The later increase in the lysosomal enzymes in diabetic condition must be then due to various disorders which are developed in diabetic conditions. One of them may be formation of ketone bodies. There is involvement of ketone bodies in the elevation of cardiac cathepsin D activity, continuous and prolonged injection of ketone bodies brought about higher specific activity of hepatic acid phosphatase in rats, the significance increase in the activity of acid phosphatase is described by Chari et al. (1983). Fisher (1980) discussed with 13 other references that alterations of pancreatic islet cell function

by alloxan is through the generation of free radicals. Second disorder takes place in diabetic animals is an elevation of lipid peroxides which result from free radical reactions in the lipid biomembranes. Possible sources of these free radicals could be the NADPH oxidase system, Lipoxynase and Cyclooxygenase. The NADPH oxidase system generates O_2 (Fong et al., 1973) and H_2O_2 (Hildebrandt et al., 1973). Potent lipid peroxidizing agents lipoxygenase activity which produces hydroperoxy fatty acids from arachidonic acid is increased in diabetes (Karpen et al., 1985) cyclooxygenase activity, which produces endo-peroxides of arachidonic acid is also increased in platelets from diabetic rats (Karpen et al., 1982). Another consequence of increased lipid peroxides is an increase in phospholipase activity (Panganamala and Cornwell, 1982 and Pritehard et al., 1986). Studies on the phospholipid-hydrolysing activity of rat liver lysosomes established the presence of phospholipase in lysosomes (Stoffel and Greten, 1967; Fowler, 1967) and several histochemical and biochemical studies have showed the presence of esterase activity in lysosomes (Holt, 1963; Shibko and Tappel, 1964). Esterases, acting on higher fatty acid ester of P-nitrophenol were found to be concentrated in the lysosomes (Mahadevan and Tappel, 1968), we hypothesize that high lipid peroxides accumulate in salivary glands of diabetic rats and cause damage to membranes of mitochondria and microsomes, which further lead to increase in lysosomal enzyme activity.