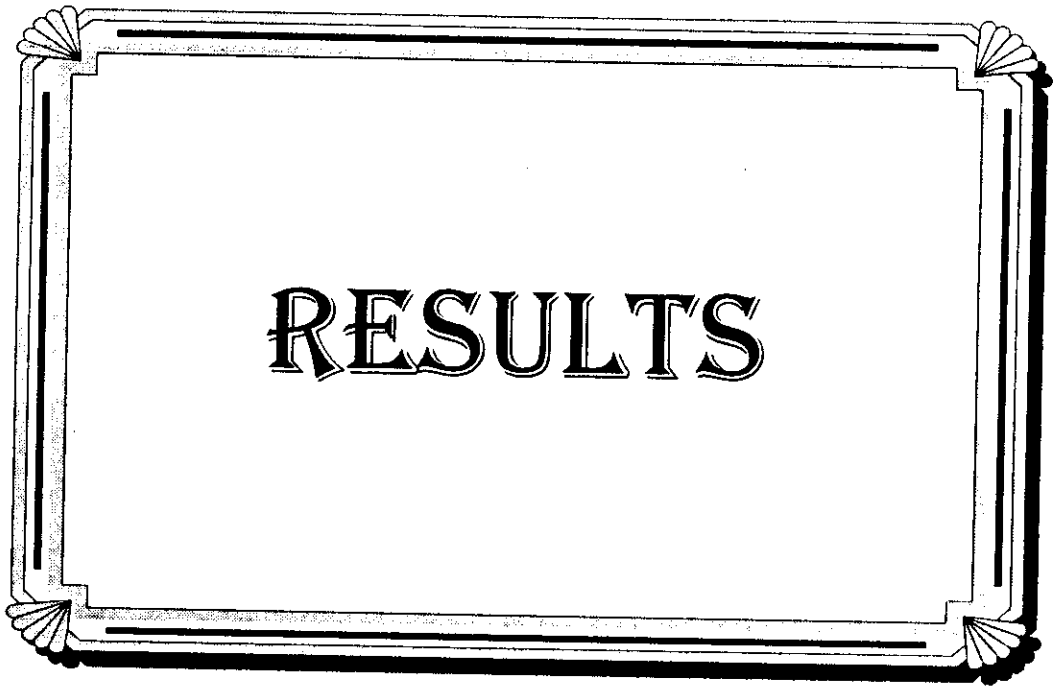

RESULTS

PART I

ISOLATION OF PLA ENZYMES FROM SISTRURUS MILIARUS BARBOURI CRUDE VENOM

Step (1): ESTIMATION OF THE PROTEIN CONTENT

Standard Curve of Protein

Fig (1) Show a standard cruve for protein (albumin from egg) according to Lawrey (1951).

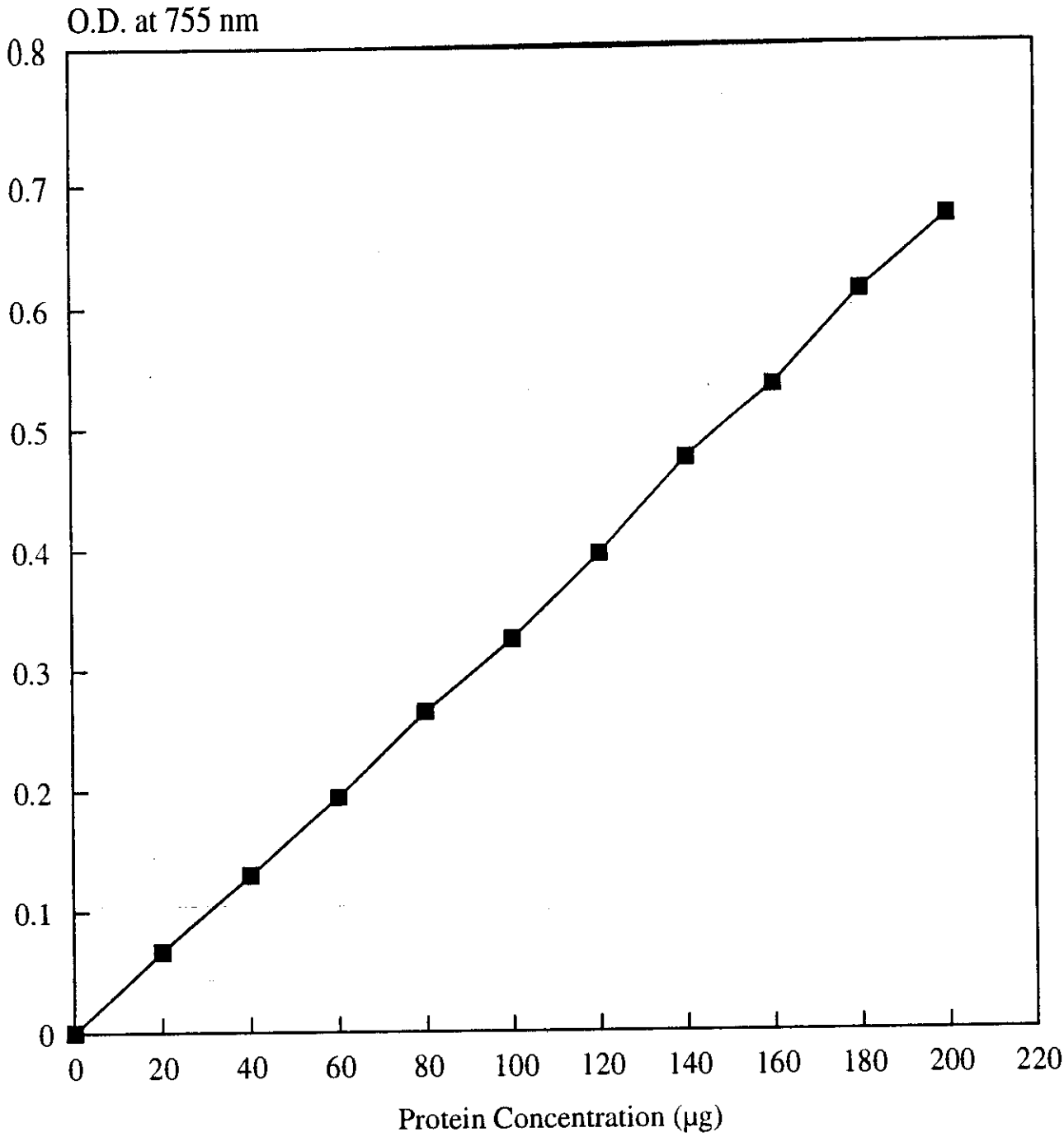
The curve was linear between concentrations (20-200 µg) and OD at 755 nm as shown in Table (1):

Table (1): The OD of different protein concentrations (albumin from egg) according to Lawrey (1951).

T.N.	Protein content, µg	O.D. at 755 nm
1	20	0.0668
2	40	0.1301
3	60	0.1945
4	80	0.2762
5	100	0.3248
6	120	0.3965
7	140	0.4773
8	160	0.5363
9	180	0.6145
10	200	0.6743

T.N. = tube number O.D. = optical density at 755 nm

Fig. (1): Standard curve for protein.



The Protein Content In The Crude Venom

Solution of crude venom (10 mg by weight of dry lyophilized venom, in 1 ml distilled water) prepared in aliquots of 0.01 ml were treated according to the method of Lawrey *et al.* (1951). The mean of readings were taken and the protein content was determined from the standard curve.

Table (2): Protein content in the crude venom.

Protein/0.01 ml crude venom	Protein content/ml
84 ± 1.2 µg	8.4 ± 0.12 mg

The results were the mean of six readings of protein content. The percentage of protein content, as determined by Lawrey method in the crude venom = 84%.

Step (2): ESTIMATION OF PHOSPHOLIPASE A2 ACTIVITY IN CRUDE VENOM

Standard Curve of Lecithin Concentration

Fig. (2) Shows the standard curve for lecithin concentration according to Augustyn and Elliott (1969).

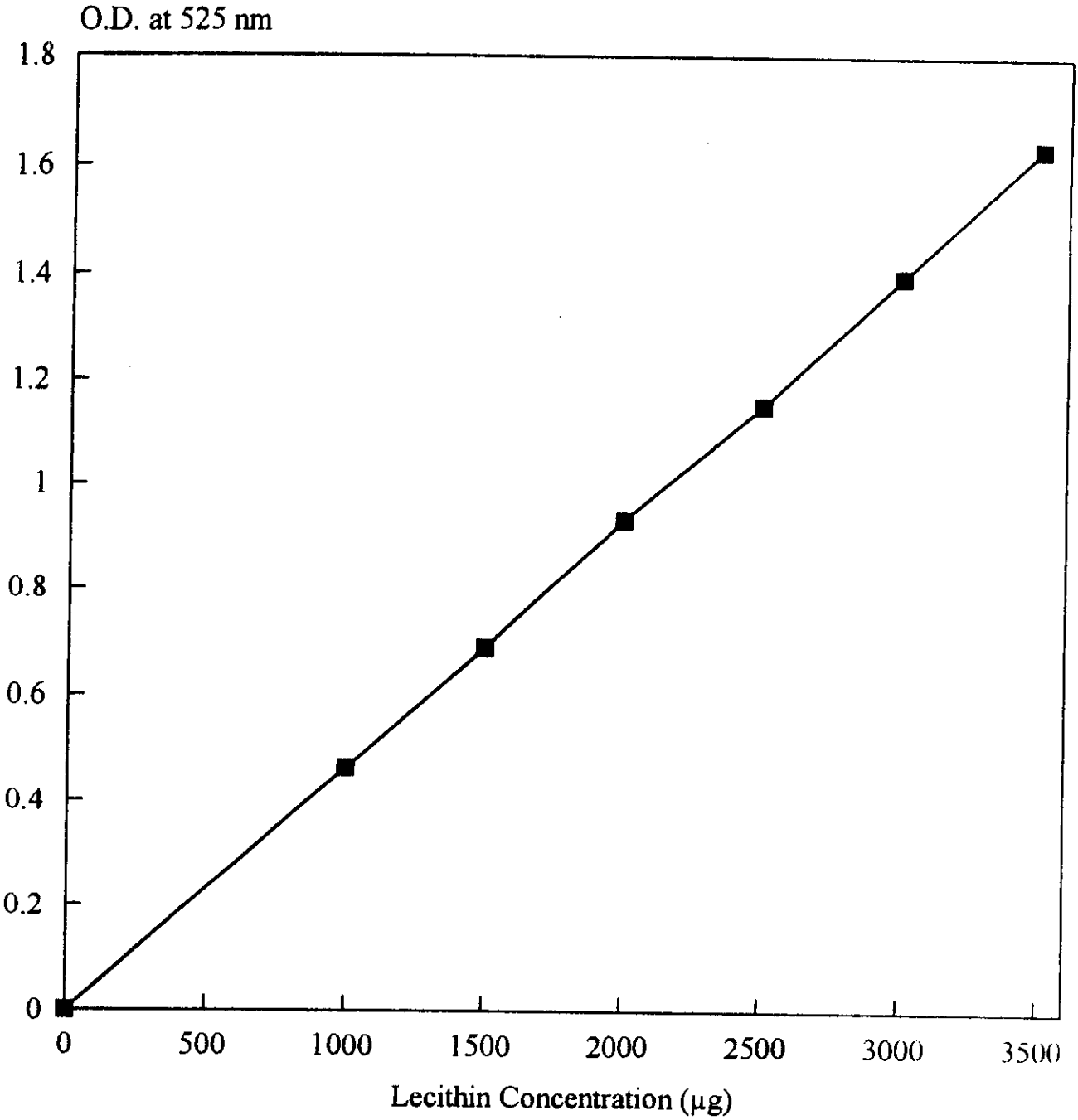
The relationship was linear between lecithin concentration (1000-3000 µg) and OD at 525 nm as shown in Table (3).

Table (3): The OD of different lecithin concentrations according to Augustyn and Elliott (1969).

T.N.	Lecithin content, µg	O.D. at 525 nm
1	1000	0.4631
2	1500	0.6892
3	2000	0.9337
4	2500	1.1538
5	3000	1.3977
6	3500	1.6360

T.N. = tube numberO.D. = optical density at 525 nm

Fig. (2): Standard curve for pure lecithin.



Estimation of Phospholipase A2 Specific Activity in Crude Venom

Table (4): Specific activity of phospholipase A2 in the crude venom of Sistrurus miliarius barbouri.

Lecithin consumed mg/ml/hour	Protein mg/ml	Specific Activity mg lecithin/hour/mg protein
1.2 ± 0.05	8.4 ± 0.12	0.143

This table show the specific activity of phospholipase A_2 in the crude venom of Sistrurus miliarius barbouri 0.143 mg lecithin/mg protein/hour and that the protein content of the crude venom was 8.4 mg per ml. The results were the mean of six readings.

Step (3): FRACTIONATION OF THE CRUDE VENOM

Trail (1)

Fractionation of Sistrurus miliairus barbouri crude venom (10 mg) on Sephadex G-75:

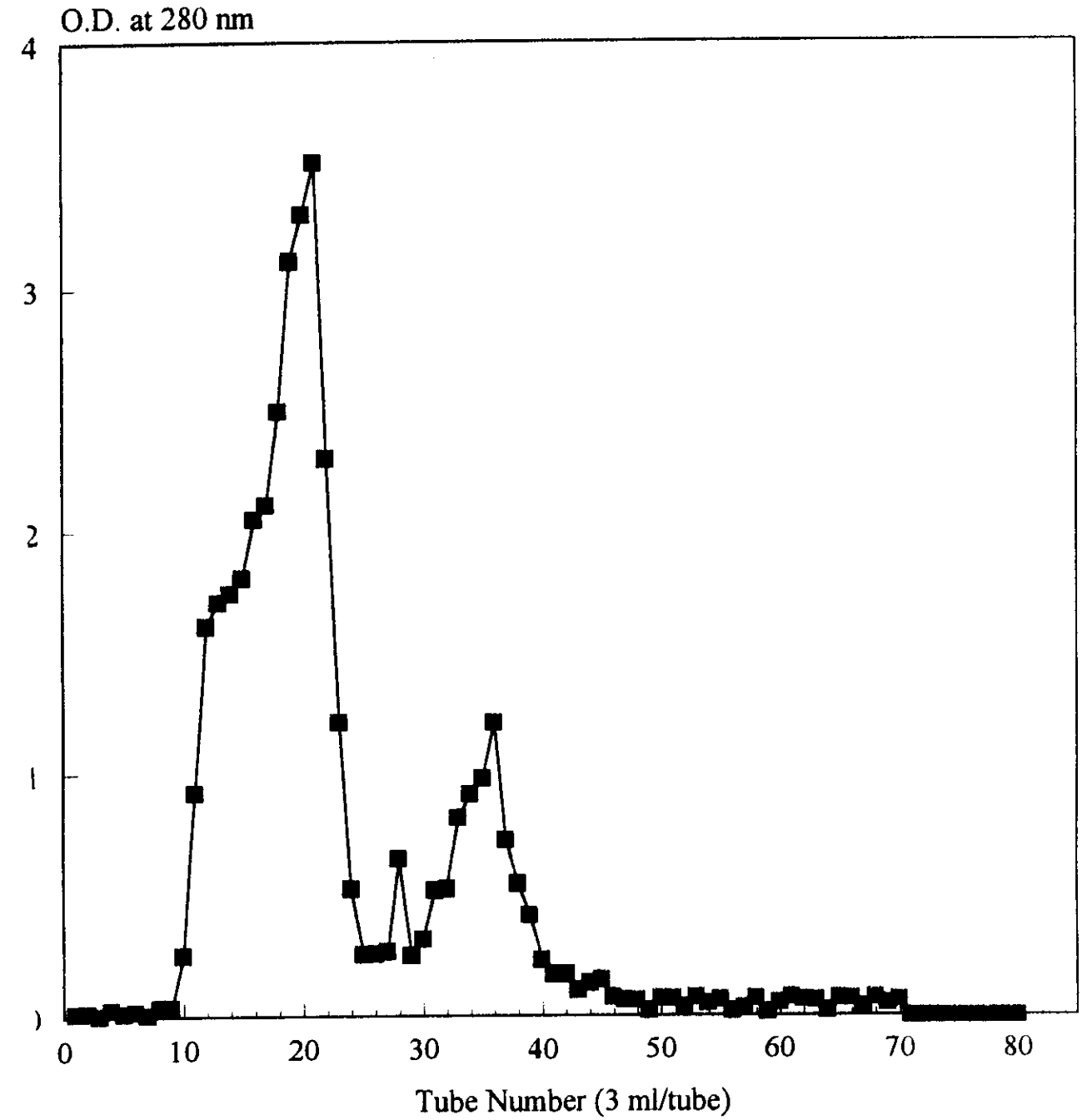
Using 0.02 M ammonium acetate buffer, pH 4.6 column size 1.7x70 cm, at a flow rate of 20 ml/hr, 3 ml/tube for 80 tubes. The protein concentration was estimated at OD 280 nm (Table 5). It was noticed that two peaks have been obtained. Fig. (3).

Table (5): Fractionation of S.M.B venom on Sephadex G-75.

T.N.	O.D.	T.N.	O.D.	T.N.	O.D.	T.N.	O.D.
1	0.0017	21	0.3519	41	0.0174	61	0.0080
2	0.0019	22	0.2308	42	0.0172	62	0.0070
3	0.0008	23	0.1219	43	0.0108	63	0.0068
4	0.0032	24	0.0525	44	0.0138	64	0.0022
5	0.0016	25	0.0255	45	0.0151	65	0.0075
6	0.0022	26	0.0256	46	0.0080	66	0.0074
7	0.0009	27	0.0265	47	0.0070	67	0.0038
8	0.0043	28	0.0645	48	0.0068	68	0.0079
9	0.0041	29	0.0250	49	0.0022	69	0.0055
10	0.0253	30	0.0315	50	0.0075	70	0.0070
11	0.0923	31	0.0519	51	0.0074	71	0.0002
12	0.1613	32	0.0524	52	0.0038	72	0.0001
13	0.1714	33	0.0818	53	0.0079	73	0.0000
14	0.1750	34	0.0917	54	0.0055	74	0.0000
15	0.1814	35	0.0982	55	0.0070	75	0.0000
16	0.2054	36	0.1218	56	0.0021	76	0.0000
17	0.2115	37	0.0724	57	0.0035	77	0.0000
18	0.2504	38	0.0544	58	0.0074	78	0.0000
19	0.3117	39	0.0417	59	0.0016	79	0.0000
20	0.3303	40	0.0229	60	0.0056	80	0.0000

T.N. = tube number O.D. = optical density at 280 nm

Fig. (3): Fractionation of Sistrurus miliarius barbouri crude venom (10 mg) on Sephadex G-75 column (1.7x70 cm), using 0.02 M ammonium acetate buffer pH 4.6.



Trial (2)

Fractionation of Sistrurus miliarius barbouri crude venom on Sephadex G-75:

Using 0.03 M sodium phosphate buffer pH 7.4, column size 1.7x70 cm, at a flow rate of 20 ml/hr, 3 ml/tube for 80 tubes. The protein concentration was estimated at OD 280 nm (Table 6). It was noticed that three peaks were obtained. Fig. (4).

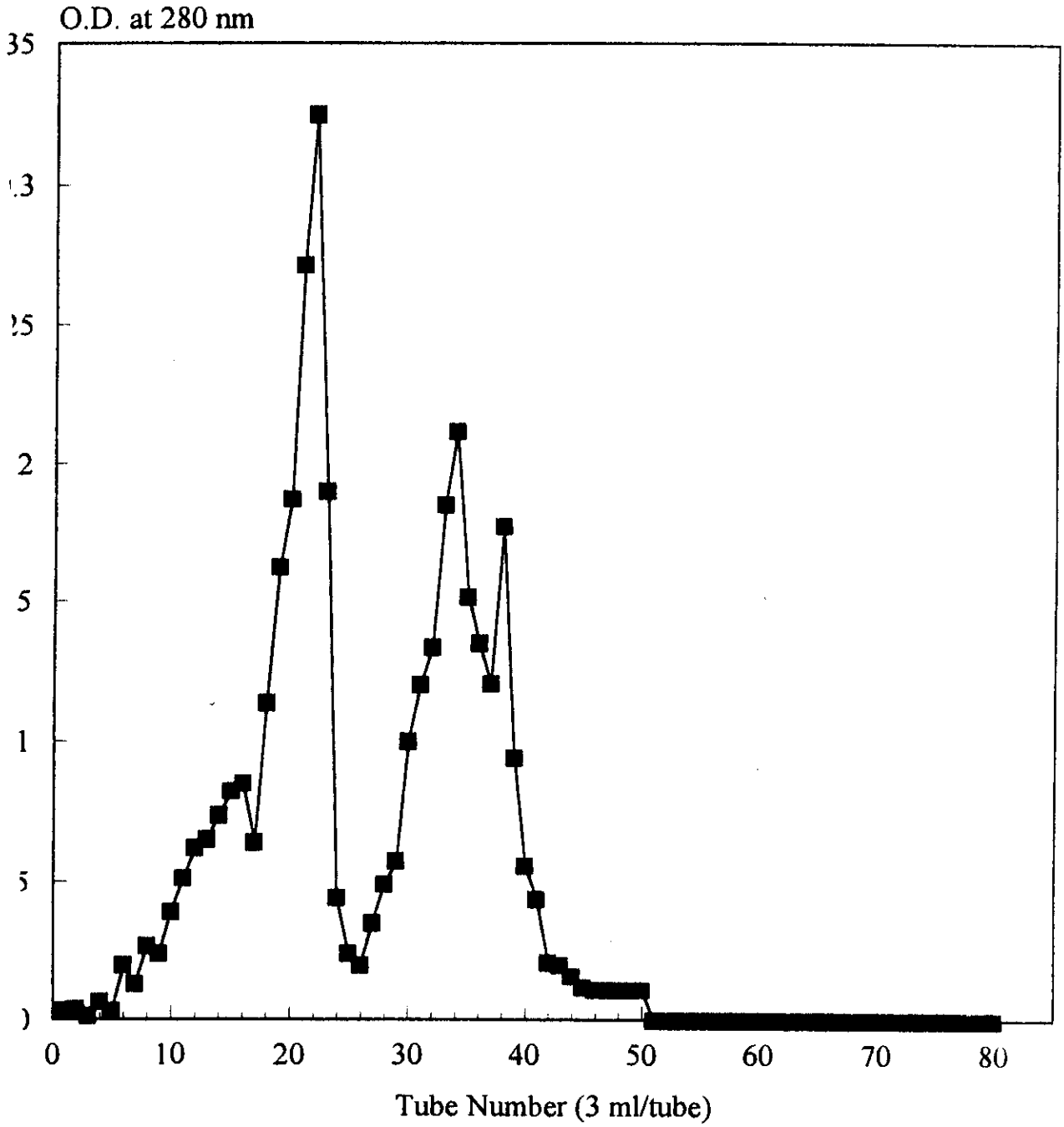
Table (6): Fractionation of S.M.B venom on Sephadex G-75.

T.N.	O.D.	T.N.	O.D.	T.N.	O.D.	T.N.	O.D.
1	0.0034	21	0.2718	41	0.0435	61	0.0000
2	0.0038	22	0.3251	42	0.0210	62	0.0000
3	0.0016	23	0.1902	43	0.0200	63	0.0000
4	0.0065	24	0.0440	44	0.0160	64	0.0000
5	0.0032	25	0.0243	45	0.0120	65	0.0000
6	0.0200	26	0.0200	46	0.0110	66	0.0000
7	0.0130	27	0.0351	47	0.0110	67	0.0000
8	0.0270	28	0.0490	48	0.0110	68	0.0000
9	0.0242	29	0.0575	49	0.0110	69	0.0000
10	0.0391	30	0.1000	50	0.0110	70	0.0000
11	0.0513	31	0.1202	51	0.0000	71	0.0000
12	0.0621	32	0.1337	52	0.0000	72	0.0000
13	0.0652	33	0.1853	53	0.0000	73	0.0000
14	0.0734	34	0.2120	54	0.0000	74	0.0000
15	0.0821	35	0.1517	55	0.0000	75	0.0000
16	0.0851	36	0.1351	56	0.0000	76	0.0000
17	0.0641	37	0.1207	57	0.0000	77	0.0000
18	0.1136	38	0.1777	58	0.0000	78	0.0000
19	0.1625	39	0.0943	59	0.0000	79	0.0000
20	0.1872	40	0.0557	60	0.0000	80	0.0000

T.N. = tube number O.D. = optical density at 280 nm

Fig. (4): Fractionation of Sistrurus miliarius barbouri crude venom (10 mg) on Sephadex G-75 column (1.7x70 cm), using 0.03 M sodium phosphate buffer pH 7.4.

It was noticed that 3 peaks were obtained.



Trial (3)

Fractionation of Sistrurus miliarius barbouri crude venom (10 mg) on Sephadex G-75:

Using 0.2 M Tris - HCl buffer pH 8.0, column size 1.7x70 cm, at flow rate of 20 ml/hour, 3 cm/tube for 80 tubes. The protein concentration was estimated at OD of 280 nm (Table 7). It was noticed that two peaks were obtained. Fig. (5).

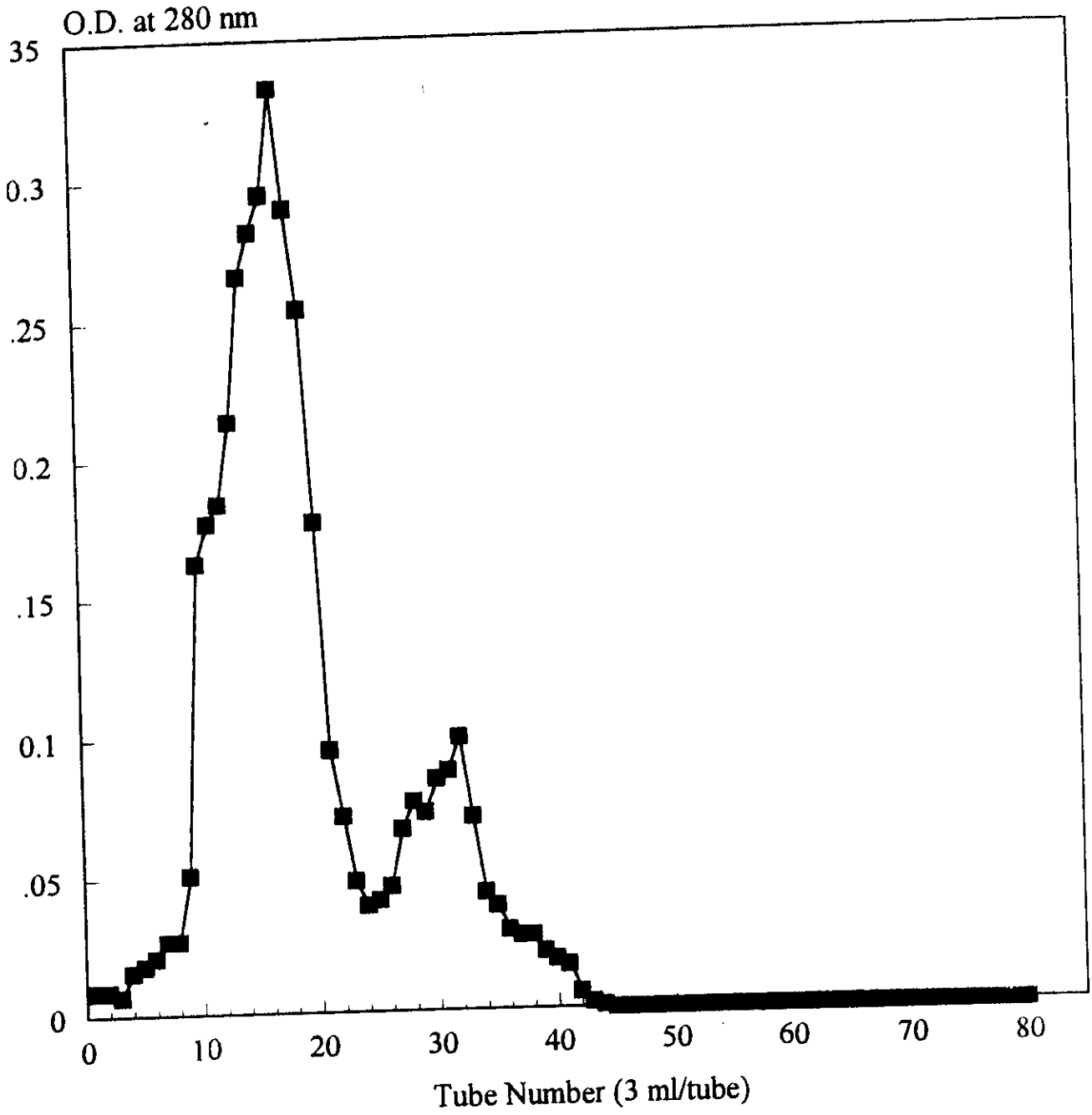
Table (7): Fractionation of S.M.B venom on Sephadex G-75.

T.N.	O.D.	T.N.	O.D.	T.N.	O.D.	T.N.	O.D.
1	0.0090	21	0.0950	41	0.0160	61	0.0000
2	0.0090	22	0.0710	42	0.0060	62	0.0000
3	0.0070	23	0.0480	43	0.0020	63	0.0000
4	0.0160	24	0.0390	44	0.0010	64	0.0000
5	0.0180	25	0.0410	45	0.0000	65	0.0000
6	0.0210	26	0.0460	46	0.0000	66	0.0000
7	0.0270	27	0.0660	47	0.0000	67	0.0000
8	0.0269	28	0.0760	48	0.0000	68	0.0000
9	0.0510	29	0.0720	49	0.0000	69	0.0000
10	0.1630	30	0.0840	50	0.0000	70	0.0000
11	0.1770	31	0.0870	51	0.0000	71	0.0000
12	0.1840	32	0.0990	52	0.0000	72	0.0000
13	0.2140	33	0.0700	53	0.0000	73	0.0000
14	0.2660	34	0.0430	54	0.0000	74	0.0000
15	0.2820	35	0.0380	55	0.0000	75	0.0000
16	0.2950	36	0.0290	56	0.0000	76	0.0000
17	0.3330	37	0.0270	57	0.0000	77	0.0000
18	0.2900	38	0.0270	58	0.0000	78	0.0000
19	0.2540	39	0.0210	59	0.0000	79	0.0000
20	0.1770	40	0.0180	60	0.0000	80	0.0000

T.N. = tube number O.D. = optical density at 280 nm

Fig. (5): Fractionation of Sistrurus miliarius barbouri crude venom (10 mg) on Sephadex G-75 column (1.7x70 cm), using 0.2 M Tris - HCl buffer pH 8.0.

It was noticed that two peaks were obtained.



Trial (4)

Fractionation of Sistrurus miliarius barbouri crude venom (10 mg) on Sephadex G-100:

Using 0.02 M ammonium acetate buffer pH 4.6, column size 1.7x70 cm, at a flow rate of 20 ml/hr, 3 ml/tube for 80 tubes. The protein concentration was estimated at OD 280 nm (Table 8). It was noticed that six peaks were obtained. Fig. (6).

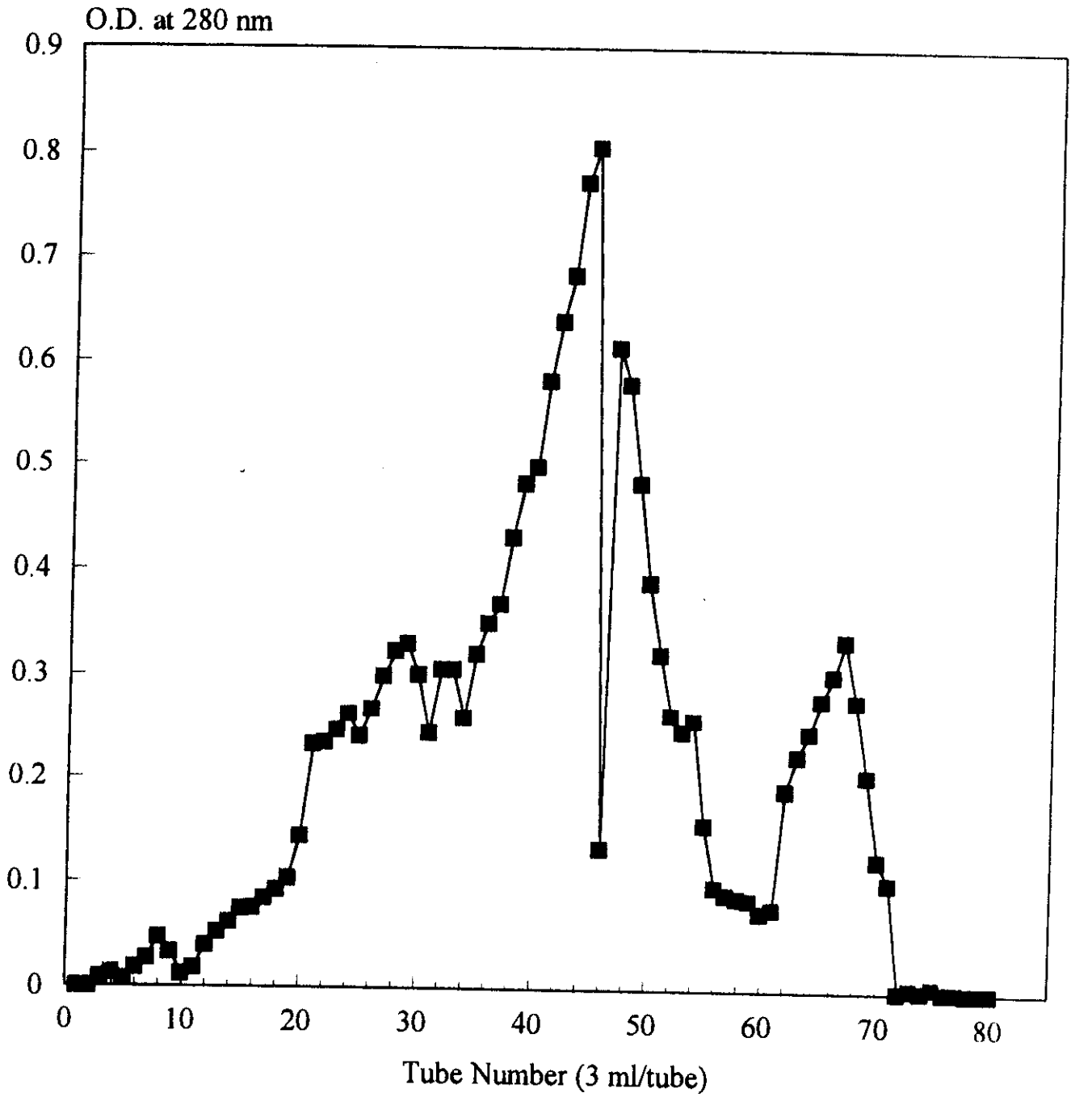
Table (8): Fractionation of S.M.B venom on Sephadex G-100.

T.N.	O.D.	T.N.	O.D.	T.N.	O.D.	T.N.	O.D.
1	0.000	21	0.223	41	0.584	61	0.079
2	0.000	22	0.235	42	0.641	62	0.192
3	0.009	23	0.247	43	0.685	63	0.226
4	0.013	24	0.263	44	0.774	64	0.248
5	0.007	25	0.241	45	0.807	65	0.280
6	0.018	26	0.268	46	0.134	66	0.304
7	0.027	27	0.300	47	0.616	67	0.337
8	0.047	28	0.324	48	0.581	68	0.279
9	0.033	29	0.331	49	0.485	69	0.207
10	0.012	30	0.302	50	0.391	70	0.126
11	0.018	31	0.245	51	0.323	71	0.104
12	0.039	32	0.307	52	0.264	72	0.001
13	0.052	33	0.307	53	0.248	73	0.004
14	0.062	34	0.260	54	0.259	74	0.002
15	0.075	35	0.322	55	0.158	75	0.006
16	0.076	36	0.351	56	0.099	76	0.001
17	0.085	37	0.370	57	0.092	77	0.001
18	0.093	38	0.432	58	0.089	78	0.000
19	0.104	39	0.484	59	0.087	79	0.000
20	0.144	40	0.500	60	0.075	80	0.000

T.N. = tube number O.D. = optical density at 280 nm

Fig. (6): Fractionation of Sistrurus miliarius barbouri crude venom (10 mg) on Sephadex G-100 column (1.7 x 70 cm), using 0.02 M ammonium acetate buffer pH4.6.

It was noticed that six peaks were obtained.



Trial (5)

Fractionation of Sistrurus miliaruis barbouri crude venom on Sephadex G-100:

Using 0.03 M sodium phosphate buffer pH 8.0, column size 1.7x70 cm, at a flow rate of 20 ml/hr, 3 ml/tube for 80 tubes. The protein concentration was estimated at OD 280 nm (Table 9). It was noticed that two peaks were obtained. Fig. (7).

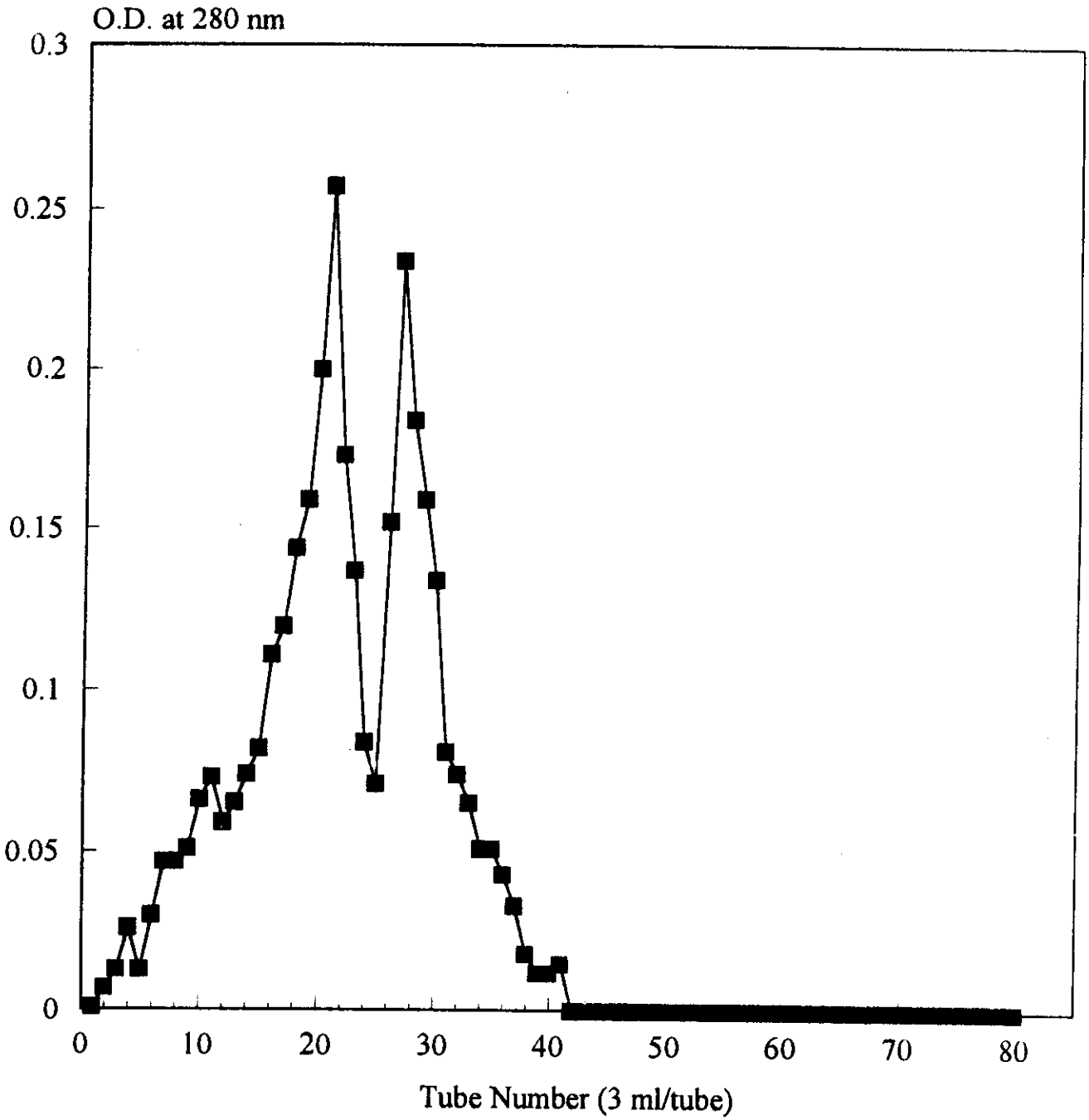
Table (9): Fractionation of S.M.B venom on Sephadex G-100.

T.N.	O.D.	T.N.	O.D.	T.N.	O.D.	T.N.	O.D.
1	0.001	21	0.257	41	0.015	61	0.000
2	0.007	22	0.173	42	0.000	62	0.000
3	0.013	23	0.137	43	0.000	63	0.000
4	0.026	24	0.084	44	0.000	64	0.000
5	0.013	25	0.071	45	0.000	65	0.000
6	0.030	26	0.152	46	0.000	66	0.000
7	0.047	27	0.234	47	0.000	67	0.000
8	0.047	28	0.184	48	0.000	68	0.000
9	0.051	29	0.159	49	0.000	69	0.000
10	0.066	30	0.134	50	0.000	70	0.000
11	0.073	31	0.081	51	0.000	71	0.000
12	0.059	32	0.074	52	0.000	72	0.000
13	0.065	33	0.065	53	0.000	73	0.000
14	0.074	34	0.051	54	0.000	74	0.000
15	0.082	35	0.051	55	0.000	75	0.000
16	0.111	36	0.043	56	0.000	76	0.000
17	0.120	37	0.033	57	0.000	77	0.000
18	0.144	38	0.018	58	0.000	78	0.000
19	0.159	39	0.012	59	0.000	79	0.000
20	0.200	40	0.012	60	0.000	80	0.000

T.N. = tube number O.D. = optical density at 280 nm

Fig. (7): Fractionation of Sistrurus miliarius barbouri crude venom (10 mg) on Sephadex G-100 column (1.7x70 cm), using 0.03 M sodium phosphate buffer pH 7.4.

It was noticed that two peaks were obtained.



Trial (6)

Fractionation of Sistrurus miliarius barbouri crude venom (10 mg) on Sephadex G-100

Using 0.2 M Tris - HCl buffer pH 8.0, column size 1.7x70 cm, at a flow rate of 20 ml/hour, 3 ml/tube for 80 tubes. The protein concentration was estimated at OD 280 nm (Table 10). It was noticed that two peaks were obtained. Fig. (8).

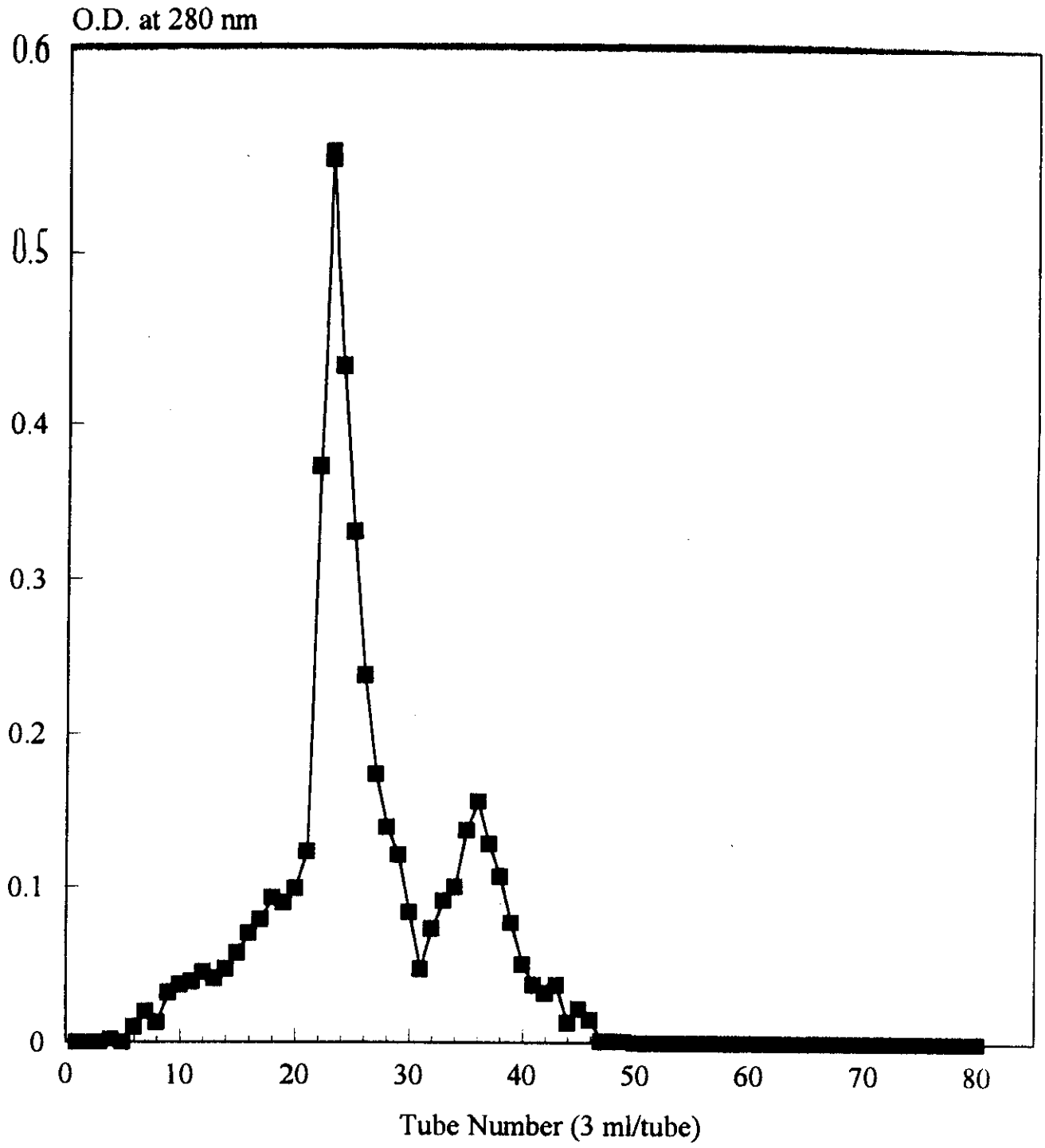
Table (10): Fractionation of S.M.B venom on Sephadex G-100.

T.N.	O.D.	T.N.	O.D.	T.N.	O.D.	T.N.	O.D.
1	0.000	21	0.123	41	0.037	61	0.000
2	0.000	22	0.373	42	0.032	62	0.000
3	0.000	23	0.542	43	0.037	63	0.000
4	0.002	24	0.437	44	0.013	64	0.000
5	0.000	25	0.331	45	0.022	65	0.000
6	0.010	26	0.238	46	0.015	66	0.000
7	0.020	27	0.174	47	0.001	67	0.000
8	0.013	28	0.139	48	0.001	68	0.000
9	0.032	29	0.121	49	0.001	69	0.000
10	0.037	30	0.084	50	0.000	70	0.000
11	0.039	31	0.047	51	0.000	71	0.000
12	0.045	32	0.073	52	0.000	72	0.000
13	0.041	33	0.091	53	0.000	73	0.000
14	0.047	34	0.100	54	0.000	74	0.000
15	0.057	35	0.137	55	0.000	75	0.000
16	0.070	36	0.156	56	0.000	76	0.000
17	0.079	37	0.128	57	0.000	77	0.000
18	0.093	38	0.107	58	0.000	78	0.000
19	0.090	39	0.077	59	0.000	79	0.000
20	0.099	40	0.050	60	0.000	80	0.000

T.N. = tube number O.D. = optical density at 280 nm

Fig. (8): Fractionation of Sistrurus miliarius barbouri crude venom (10 mg) on Sephadex G-100 column (1.7x70 cm), using 0.2 M Tris - HCl buffer pH 8.0.

It was noticed that two peaks were obtained.



Estimation of PLA2 Enzymatic Activity

PLA2 enzymatic activity in the fractions of trial (4) (six peaks) have been estimated .

Table (11): Show PLA2 enzymatic activity estimated according to Augastyn and Elliott (1969).

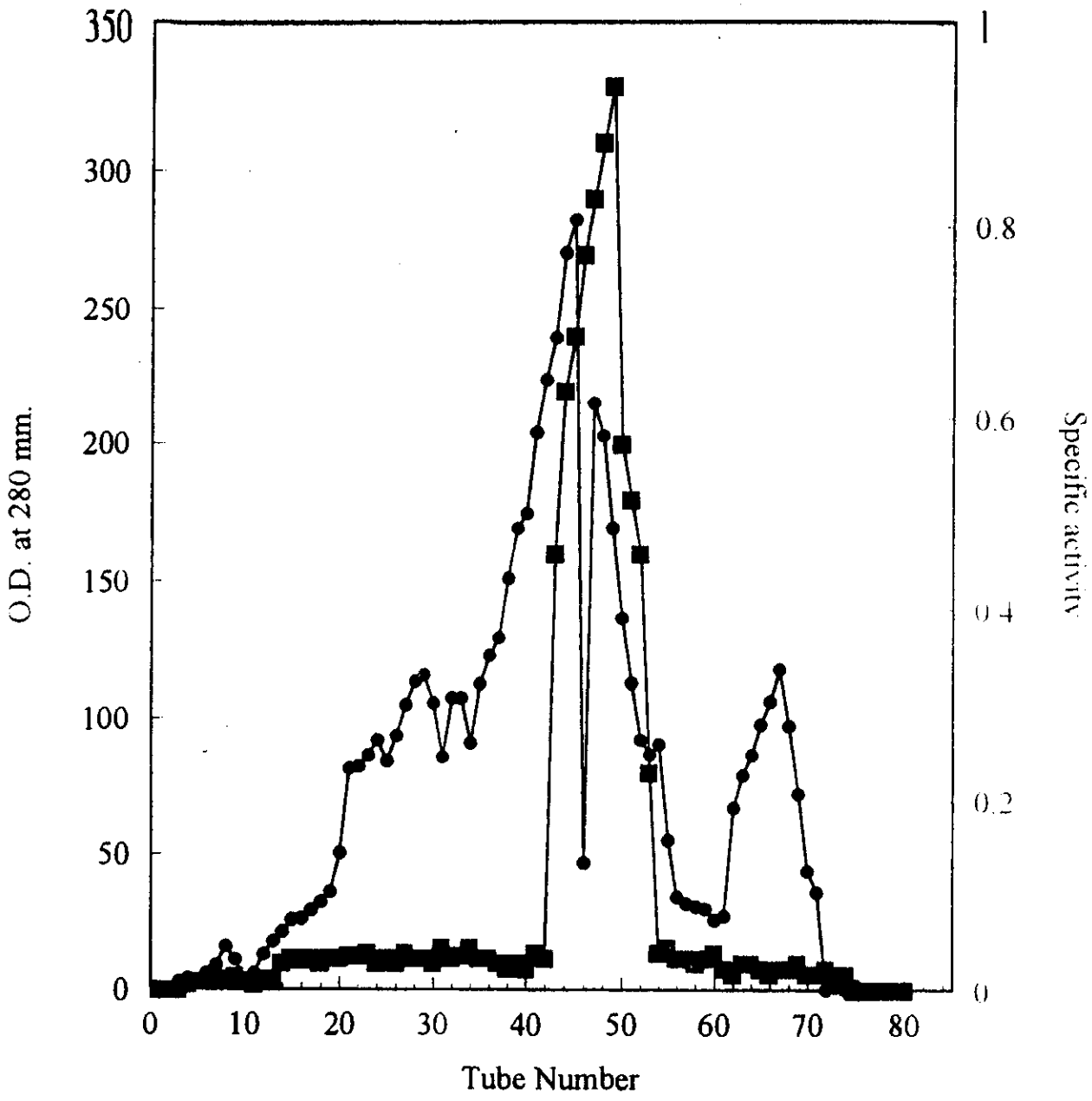
It was noticed that one phospholipase A2 enzyme has been obtained from fraction 4 and fraction 5 **Fig. (9).**

T.N.	E.A	T.N.	E.A	T.N.	E.A	T.N.	E.A
1	0	21	13	41	14	61	8
2	0	22	13	42	12	62	6
3	0	23	14	43	160	63	10
4	2	24	10	44	220	64	10
5	3	25	12	45	240	65	8
6	3	26	10	46	270	66	6
7	4	27	14	47	290	67	8
8	3	28	12	48	310	68	8
9	5	29	12	49	330	69	10
10	3	30	10	50	200	70	6
11	2	31	16	51	180	71	6
12	4	32	12	52	160	72	8
13	4	33	13	53	80	73	6
14	10	34	16	54	14	74	6
15	12	35	12	55	16	75	0
16	11	36	12	56	12	76	0
17	12	37	10	57	12	77	0
18	10	38	8	58	10	78	0
19	12	39	10	59	12	79	0
20	12	40	8	60	14	80	0

T.N. = tube number EA = PLA2 enzymatic activity in µg/hr

Fig. (9): PLA2 Enzymatic activity of Sistrurus miliarius barbouri.

PLA2 enzymatic activity in the fractions of trial (4) (six peaks) have been estimated.



In this figure the protein was resolved into six peaks. The phospholipase A2 activity was found in the plateau between fraction 4 and 5.

Refractionation on DEAE-Cellulose

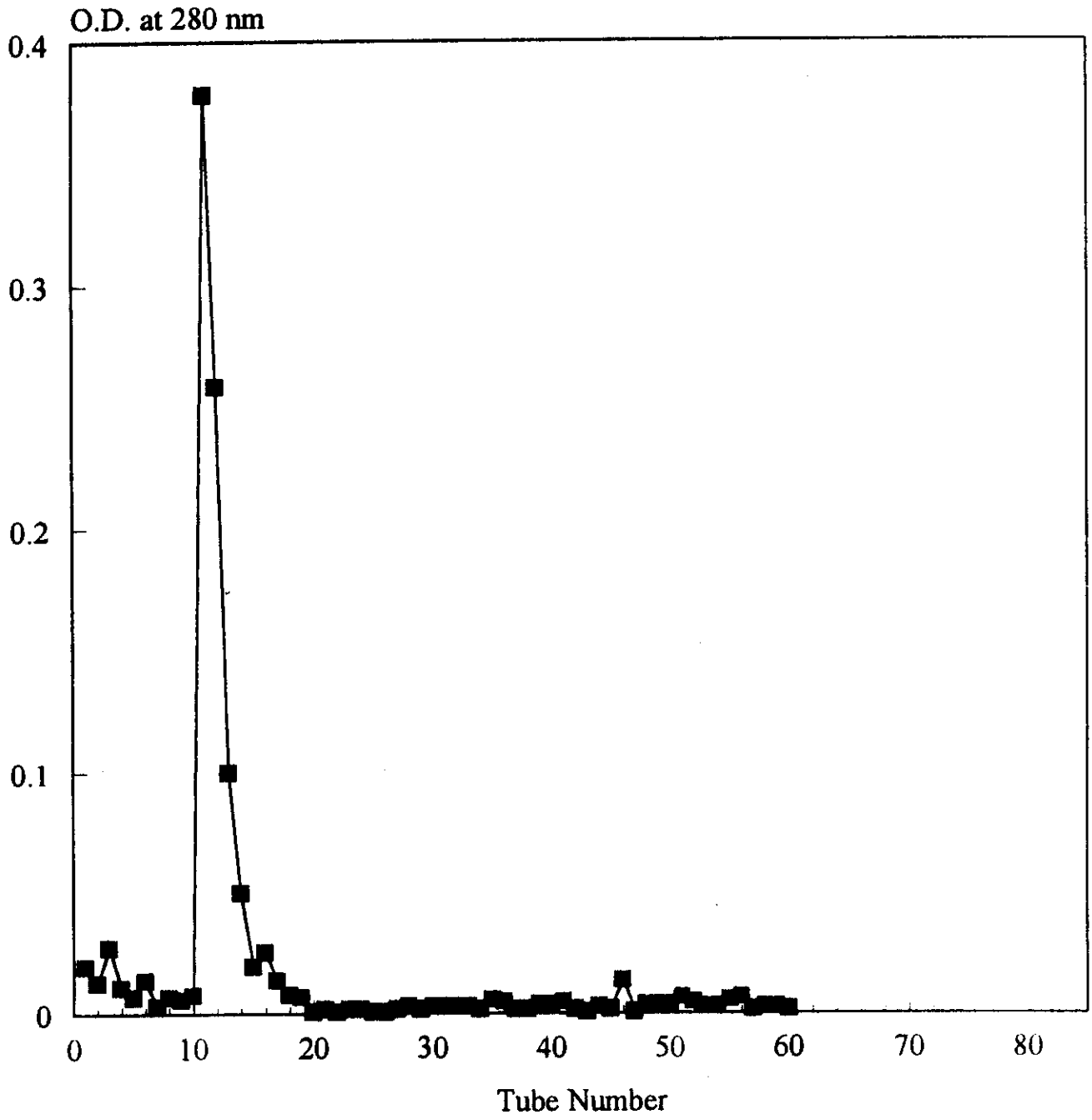
Figure 10 show refractionation of isolated phospholipase A2 from fraction 4 and 5 by Ion-exchange chromatography on DEAE-Cellulose column (1.7x30 cm), eluted at a rate of 30 ml/hr, 5 ml/tube for 60 tubes, using ammonium acetate buffer of increasing gradient molarity (0.05 M, 0.1 M, 0.2 M, 0.4 M, 0.6 M, 0.8 M and 1.0 M; 50 ml each) at a fixed pH 7.0. The protein content was estimated by measuring the OD at 280 nm. One fraction was obtained.

Table (12): Refractionation of isolated phospholipase A2 on DEAE-Cellulose.

T.N.	O.D.	T.N.	O.D.	T.N.	O.D.
1	0.020	21	0.002	41	0.005
2	0.013	22	0.001	42	0.002
3	0.028	23	0.002	43	0.001
4	0.011	24	0.002	44	0.003
5	0.007	25	0.001	45	0.002
6	0.014	26	0.001	46	0.014
7	0.003	27	0.002	47	0.001
8	0.007	28	0.003	48	0.004
9	0.006	29	0.002	49	0.004
10	0.008	30	0.003	50	0.004
11	0.379	31	0.003	51	0.007
12	0.259	32	0.003	52	0.005
13	0.100	33	0.003	53	0.003
14	0.050	34	0.002	54	0.003
15	0.020	35	0.006	55	0.006
16	0.026	36	0.005	56	0.007
17	0.014	37	0.002	57	0.002
18	0.008	38	0.002	58	0.003
19	0.007	39	0.004	59	0.003
20	0.001	40	0.004	60	0.002

T.N. = tube number O.D. = optical density at 280 nm

Fig. (10): Refractionation of isolated phospholipase A2 on DEAE-Cellulose.



Estimation of PLA2 enzymatic activity after purification on DEAE-Cellulose

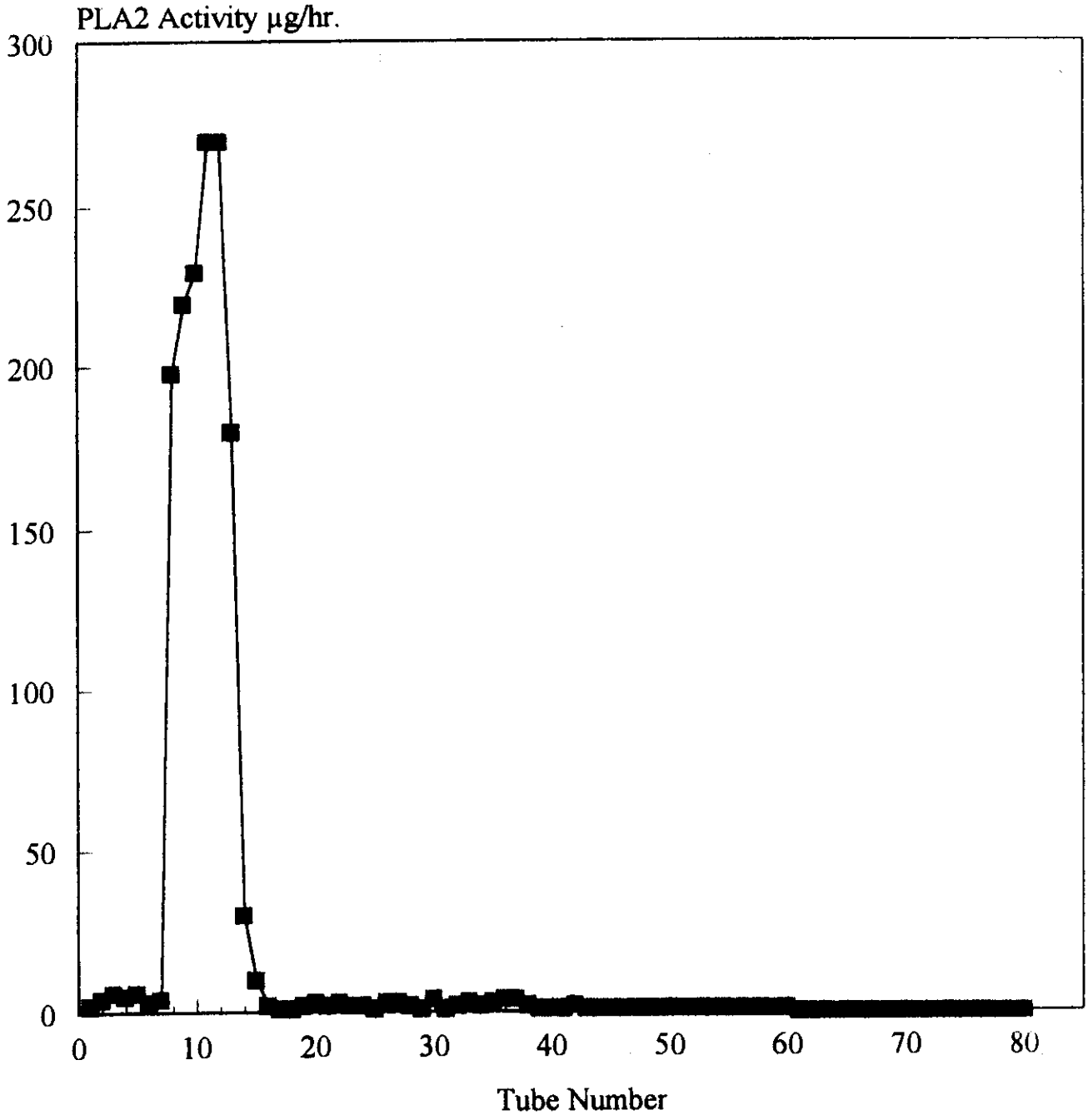
Fig. (11) Show PLA2 enzymatic activity estimated according to Augastyn and Elliott (1969). It was noticed that one PLA2 enzyme has been obtained which was named PL-I.

Table (13): PLA2 enzymatic activity after purification on DEAE-Cellulose

T.N.	E.A	T.N.	E.A	T.N.	E.A
1	2	21	2	41	1
2	4	22	3	42	2
3	6	23	2	43	1
4	5	24	2	44	1
5	6	25	1	45	1
6	3	26	3	46	1
7	4	27	3	47	1
8	198	28	2	48	1
9	220	29	1	49	1
10	230	30	4	50	1
11	270	31	1	51	1
12	270	32	2	52	1
13	180	33	3	53	1
14	30	34	2	54	1
15	10	35	3	55	1
16	2	36	4	56	1
17	1	37	4	57	1
18	1	38	2	58	1
19	2	39	1	59	1
20	3	40	1	60	1

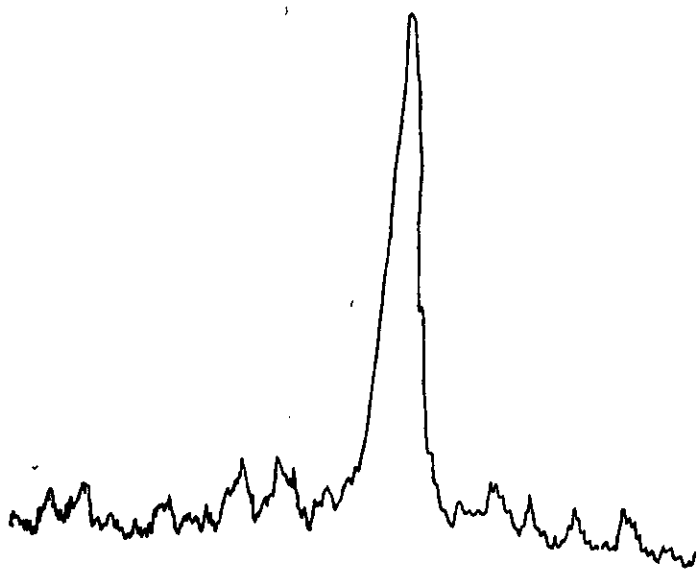
T.N. = tube number E.A = PLA2 enzymatic activity in µg/hr

Fig. (11): PLA2 Enzymatic activity after purification on DEAE-Cellulose



Checking the purity of PL-I obtained from DEAE-Cellulose column

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS - PAGE) was carried out for the isolated PL-I using slab gel method as shown in Fig. (12B). The resulted gel has been scanned at a rate of 20 cm/min. and voltage of 0.5 volt as shown in Fig. (12A). It was noticed that phospholipase A2 electrophoresis pattern gave one sharp band and its scanning gave one peak indicating its purity.



A

B

Fig. (12): Electrophortic pattern of PL-I:

A) Scanning of SDS-PAGE of PL-I showing one sharp peak.

B) Photography of SDS-PAGE of PL-I showing one homogenous band.

PART II

BIOCHEMICAL CHARACTERIZATION OF ISOLATED ENZYME

DETERMINATION OF MOLECULAR WEIGHT

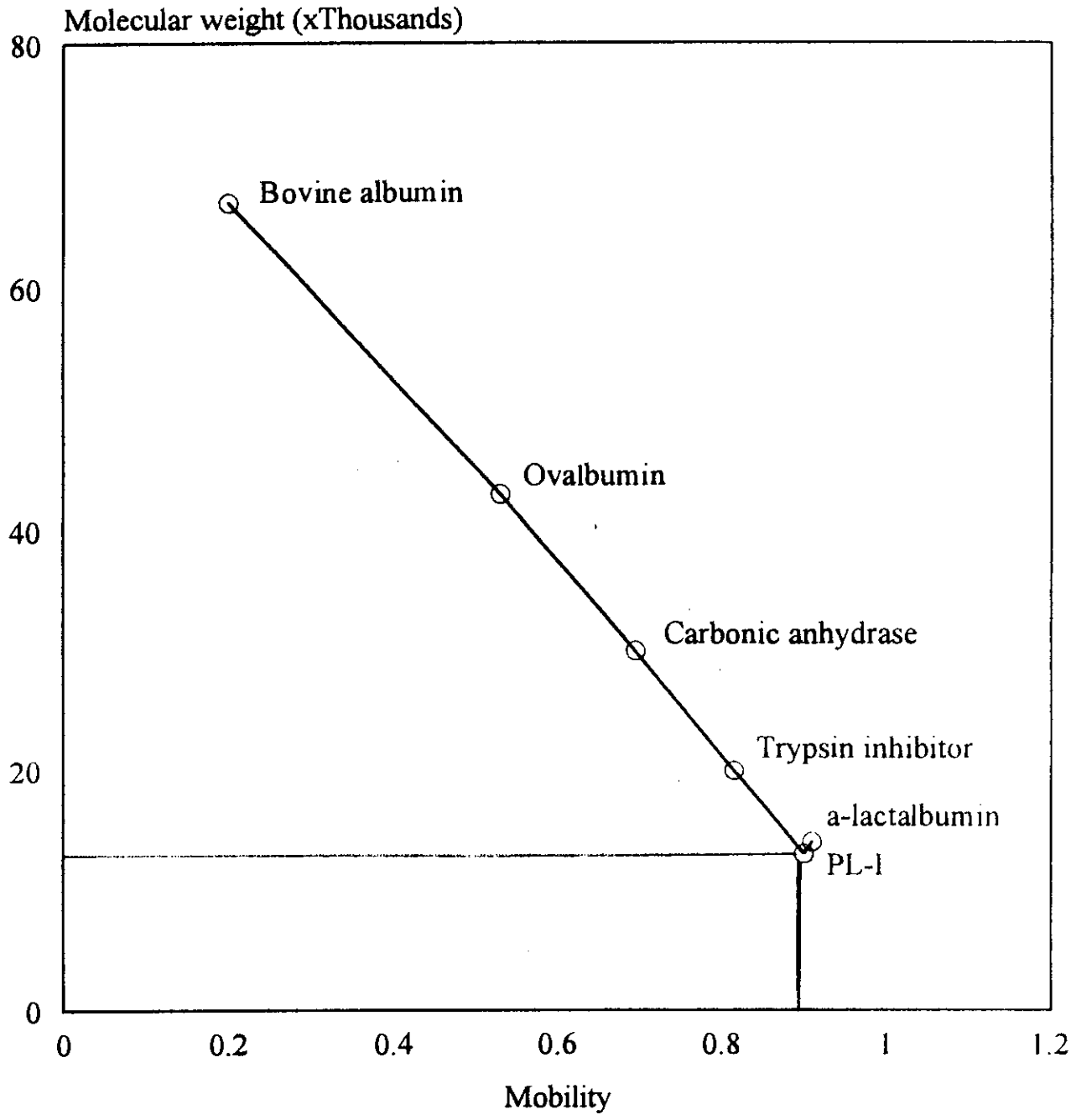
Determination of Molecular Weight of PL-I

The molecular weight of PL-I was determined using SDS-PAGE vertical slab gel according to the method described by Weber and Osborn (1969). Standard protein, bovine albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,000) and a-lactalbumin (14,000) were used.

From the electrophoresis mobility of PL-I compared with known standard molecular weight proteins; the molecular weight was found to be 13,000 Fig (13).

Fig. (13): Determination of the molecular weight of PL-I on SDS-PAGE.

The standard proteins used were: bovine albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,000) and a-lactalbumin (14,000).



EFFECT OF TEMPERATURE ON PL-I ENZYME ACTIVITY

Table (14) & Fig. (14) show that the activity of the enzyme was increased gradually with the rise in temperature with maximal activity at 45°C, then declined with further rise in temperature.

Table (14): Effect of temperature on PL-I activity.

Temp. °C	P.C. µg/ml	E.A. µg/hr	S.A µg/hr
10	62.5	170	2.7
20	62.5	220	3.5
30	62.5	230	3.7
37	62.5	290	3.0
45	62.5	300	4.8
55	62.5	220	3.5
65	62.5	200	3.2
75	62.5	90	1.4
85	62.5	60	1.0
95	62.5	20	0.3

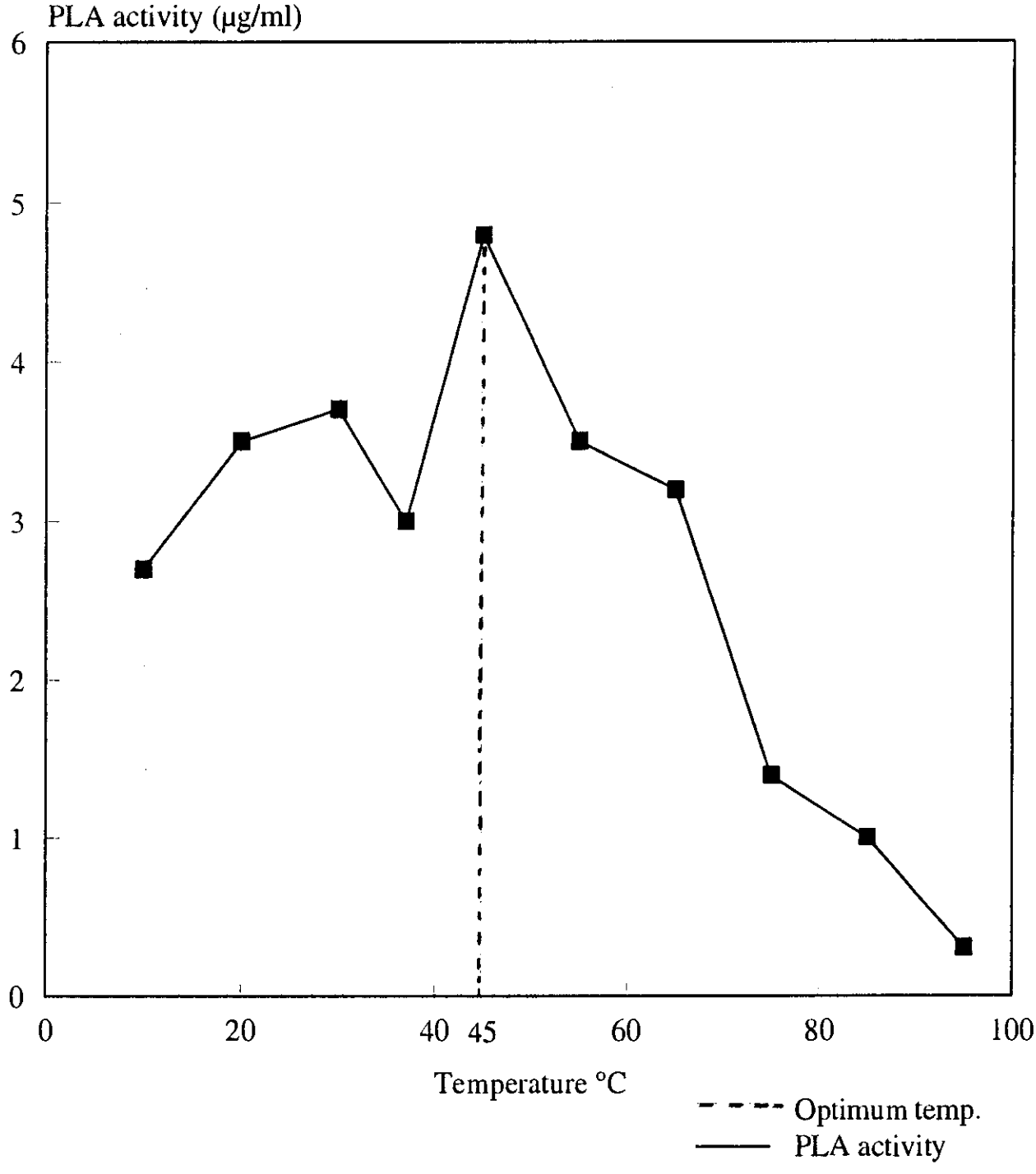
P.C = Protein concentration.

E.A = PLA enzyme activity.

S.A = PLA specific activity.

Fig. (14): Effect of temperature changes on PL-I enzyme activity.

(optimum temperature = 45°C)



EFFECT OF pH ON PL-I ENZYME ACTIVITY

The phospholipase A2 activity was estimated according to Augastyn and Elliot (1969) at different pH values. The maximal activity was at pH 7.0 as illustrated in Fig. (15) & Table (15).

Table (15): The effect of pH on PL-I activity.

pH	P.C µg/ml	E.A µg/hr	S.A µg/hr
4.5	125	180	1.44
5.0	125	260	2.08
5.5	125	300	2.40
6.0	125	325	2.60
7.0	125	385	3.68
7.5	125	340	2.72
8.0	125	390	2.32
8.5	125	395	2.56
9.0	125	95	0.76
9.5	125	75	0.60
10.0	125	60	0.48
10.5	125	40	0.32

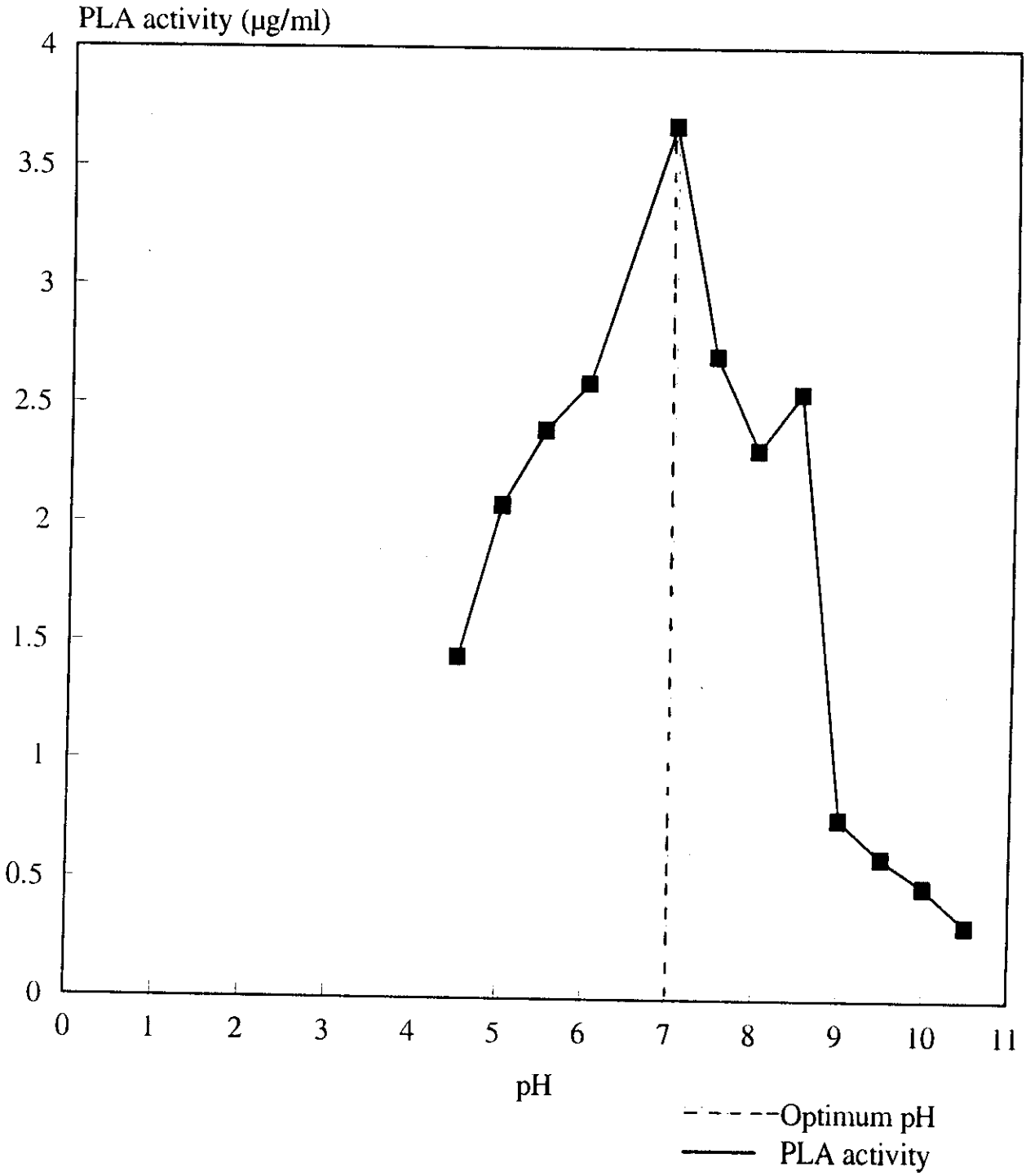
P.C = Protein concentration.

E.A = PLA enzyme activity.

S.A = PLA specific activity.

Fig. (15): Effect of pH changes on PL-I enzyme activity.

(optimum pH = 7.0)



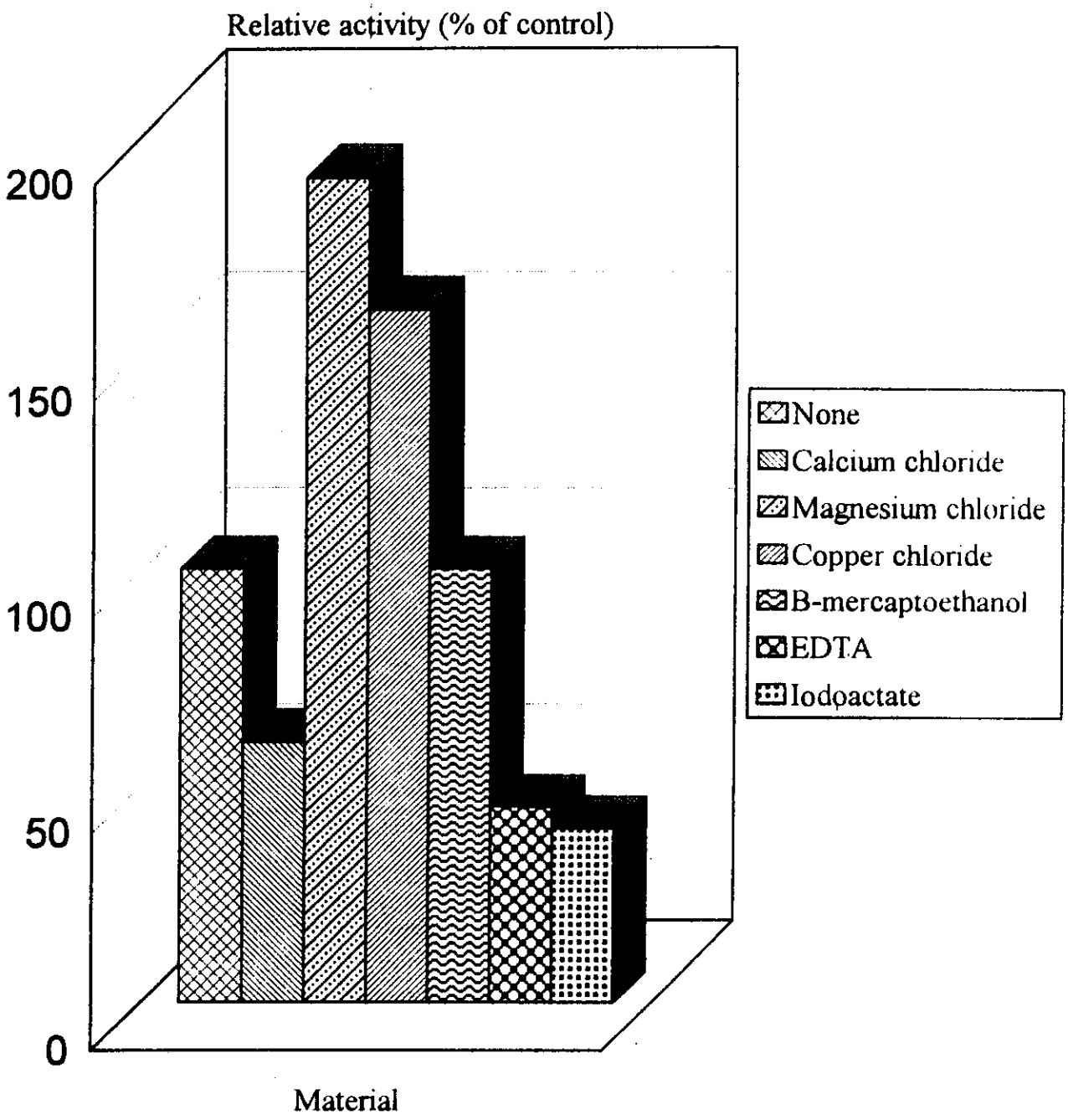
EFFECT OF SOME METAL IONS AND INHIBITORS ON PL-I ENZYME ACTIVITY

The enzyme activity was assayed at temperature 37°C. The activity was estimated according to the method of Augastyn and Elliot (1969). Table (16) & Fig. (16) represent the effect of some metal ions and inhibitors on the enzyme activity at 37°C showing that copper and magnesium chloride enhanced the activity while calcium chloride reduced it. On the other hand Iodoacetate and EDTA inhibited the activity while ß-mercaptoethanol showed no effect.

Table (16): The effect of some metal ions and inhibitors on PL-I enzyme activity.

Material	Concentration (M)	Relative activity (%)
None	---	100%
Calcium chloride	$8x10^{-4}$ M	60%
Magnesium chloride	$8x10^{-4}$ M	190%
Copper chloride	$8x10^{-4}$ M	160%
ß-meracptoethanol	10^{-3} M	100%
EDTA	10^{-3} M	45%
Iodoacetate	10^{-3} M	40%

Fig. (16): Effect of some metal ions and inhibitors on PL-I enzyme activity.



EFFECT OF SUBSTRATE CONCENTRATION ON PL-I ENZYME ACTIVITY

Fig. (17) & table (17) Show the effect of substrate concentration (egg yolks lecithin) on PLA enzymatic activity estimated by Augastyn and Elliott (1969) of PL-I. It was noticed that there was a linear relationship between the velocity of enzymatic activity (V) of PL-I measured in mg/ml at 37°C and the substrate concentrations in mg/test.

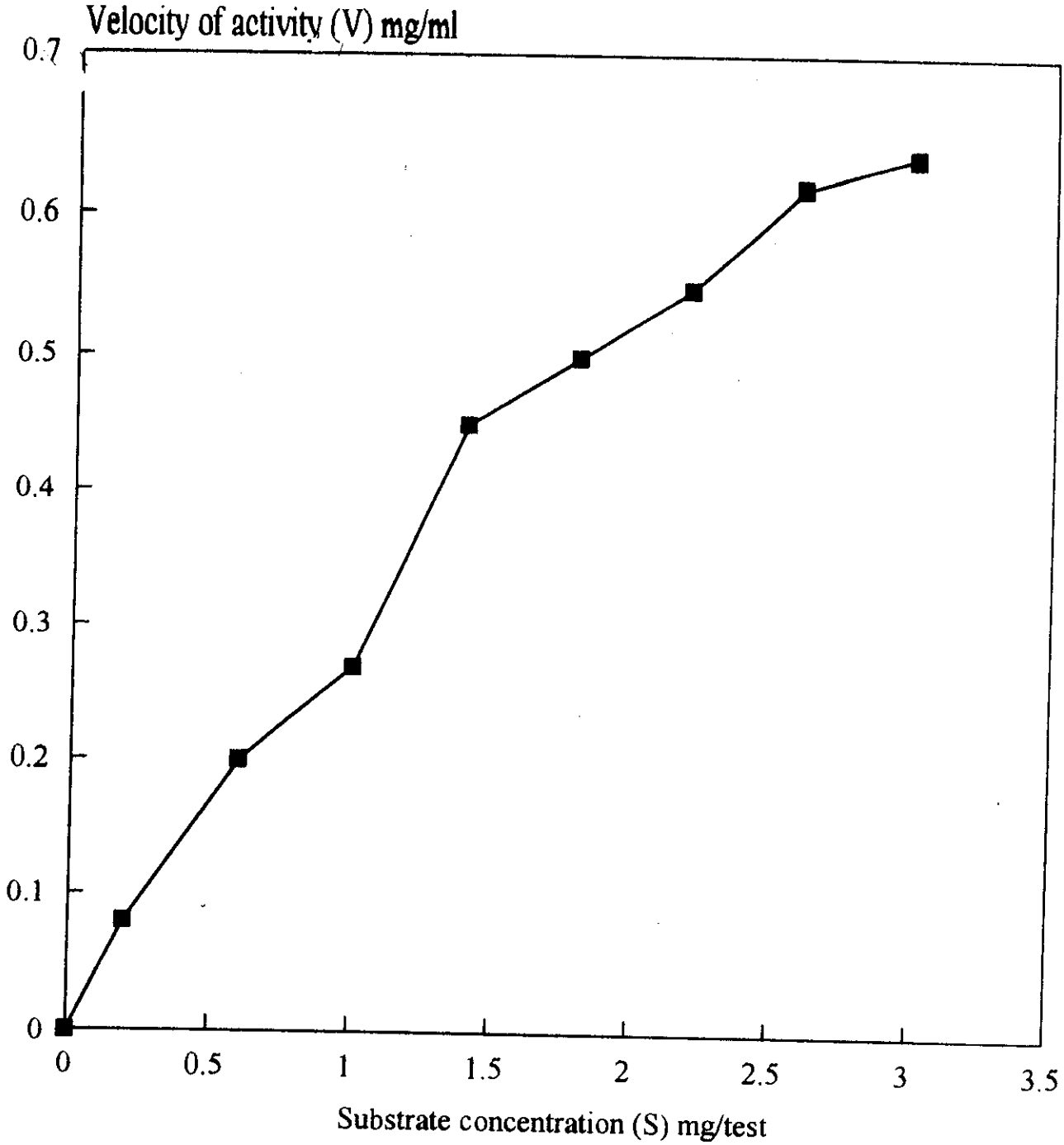
Table (17): Relationship between S and V of PL-I

Substrate concentrations mg/test (S)	Velocity of E.A mg/ml (V)
0.20	0.08
0.60	0.20
1.00	0.27
1.40	0.45
1.80	0.50
2.20	0.55
2.60	0.62
3.00	0.64

E.A = PLA enzymatic activity.

Fig. (17): Effect of substrate concentration on PL-I enzyme activity.

As shown in Fig. (17) the activity increased with the increase in substrate concentration.



DETERMINATION OF K_M OF PL-I

K_m (Michaelis Constant) of PL-I was determined from the double - reciprocal or lineweaver - Burk plot, by plotting 1/V versus 1/S (Rodwell 1983). Where V is the velocity of enzymatic activity in mg/ml at 37°C and S is the substrate concentration in mg/test as shown in fig. (18) and table (18).

Table (18): Relationship between 1/S and 1/V of PL-I.

1/S	1/V
5	12.5
1.7	5.0
1	3.7
0.7	2.2
0.6	2.0
0.5	1.8
0.4	1.6
0.3	1.5

Calculation:

From the plotting (Fig.18) it was found that:

* $1/v_{max} = 0.7$

$V_{max} = 1/0.7 = 1.43$ mg/ml at 37°C.

* $1/K_m = -0.3$

$K_m = 1/0.3 = 3.3$ mg / L

Fig. (18): Lineweaver - Burk Plot of PL-I

(for determination of K_m value)

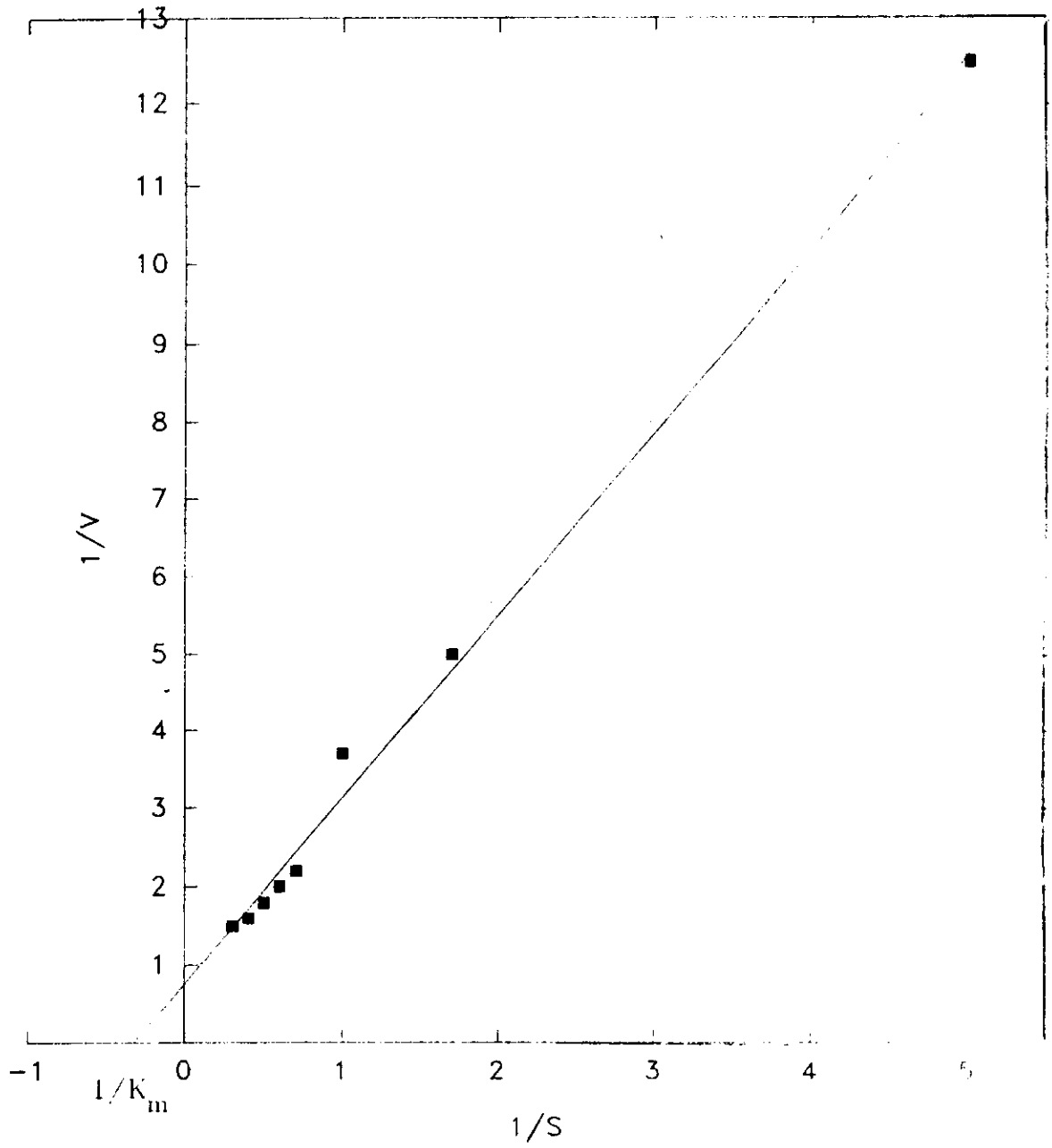


Table (19): Some biochemical characterization of PL-I.

Molecular weight	Optimum temp.	Optimum pH	V_{max}	K_m
13,000	45°C	7	1.43	3.3

This table shows some biochemical characterization of the isolated enzyme. The molecular weight of PL-I estimated by SDS-PAGE was found to be 13,000 with a k_m of 3.3 mg/L and their V_{max} was 1.43 mg/ml at 37°C. The optimum temperature was 45°C, while the optimum pH 7.0.

PART III

IN VITRO STUDIES OF SOME BIOLOGICAL ACTIVITIES OF THE ISOLATED ENZYME

The Anticoagulant Activity of PL-I

The effect of PL-I on blood coagulation was tested in comparison with that of the crude venom. This was accomplished by assaying the plasma recalcification time (PRT) of each according to the method described by Hamilton *et al.* (1974) as shown in table (20). It was noticed that PRT for control was 230 seconds and PL-I was 200 seconds wherease no clotting occured with regard to that mixed with crude venom even after several hours.

Table (20): Anticoagulant activities of CV and pL-I assayed by measuring PRT of each.

Substance	Control	C.V	PL-I
PRT minutes	230 seconds**	No clotting	200 seconds*

* : Denote the experiment was repeated 3 time.

** : Denote mean value.

CV: Sistrurus miliarius barbouri crude venom.

PRT: Plasma recalcification time.

Hemolytic Activities of PL-I

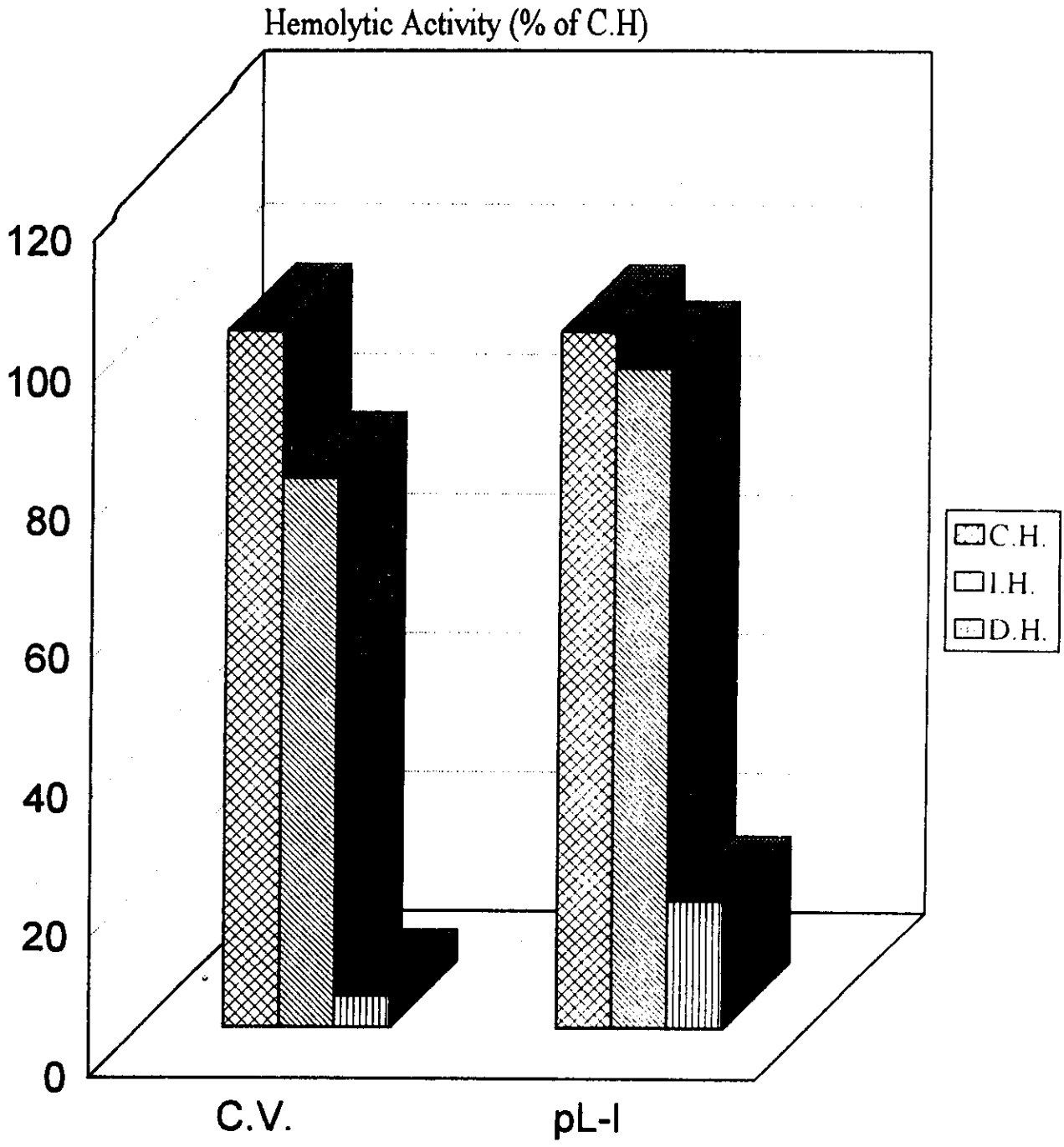
The hemolytic activity of PL-I and crude venom was tested by assaying the direct and indirect effects of each on the washed packed human R.B.C. according to the method described by Boman and Katella (1957) as shown in table (21) and fig. (19) the activity was expressed as change in OD at 540 nm. It was noticed that CV and PL-I showed slight direct haemolysis (4.4%) (18.3%) respectively. On the other hand, the CV and PL-I showed indirect hemolytic activities but PL-I was more active than CV.

Table (21): Direct and indirect hemolytic activities of PL-I in comparison with CV.

Test	O.D at 540 nm of PL-I	% of complete hemolysis	O.D at 540 nm of curde venom	% of complete hemolysis
Complete hemolysis	1.15*	100.0%	1.15*	100.0%
Indirect hemolysis	1.09*	94.9%	1.91*	79.0%
Direct hemolysis	0.21*	18.3%	0.05*	4.4%

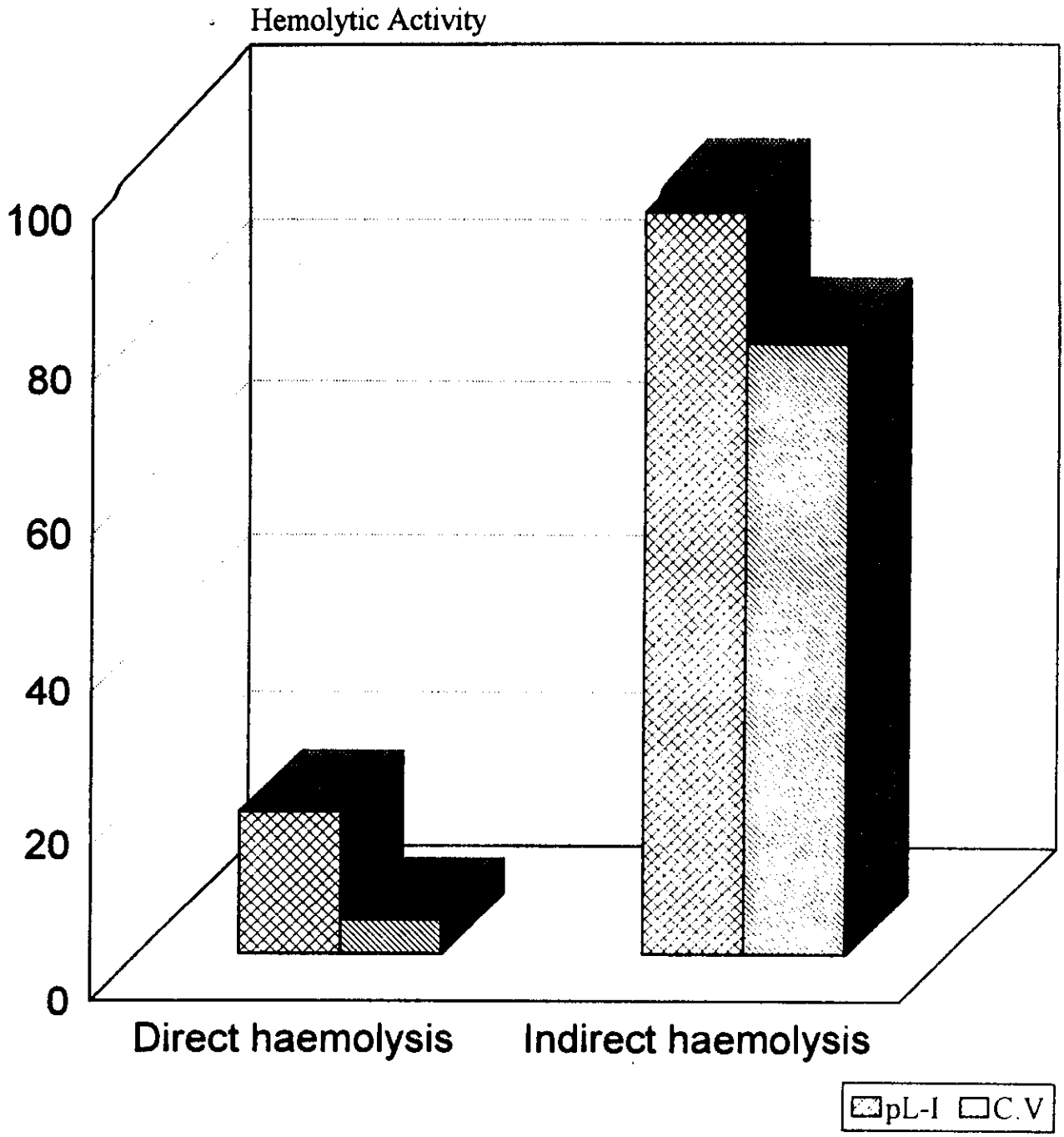
* : Denote mean value.

Fig. (19): Hemolytic activities of C.V and PL-I.



(IH, DH and CH = Indirect, direct and complete hemolysis)

Fig. (20): Direct and indirect hemolytic activities of C.V and PL-I.

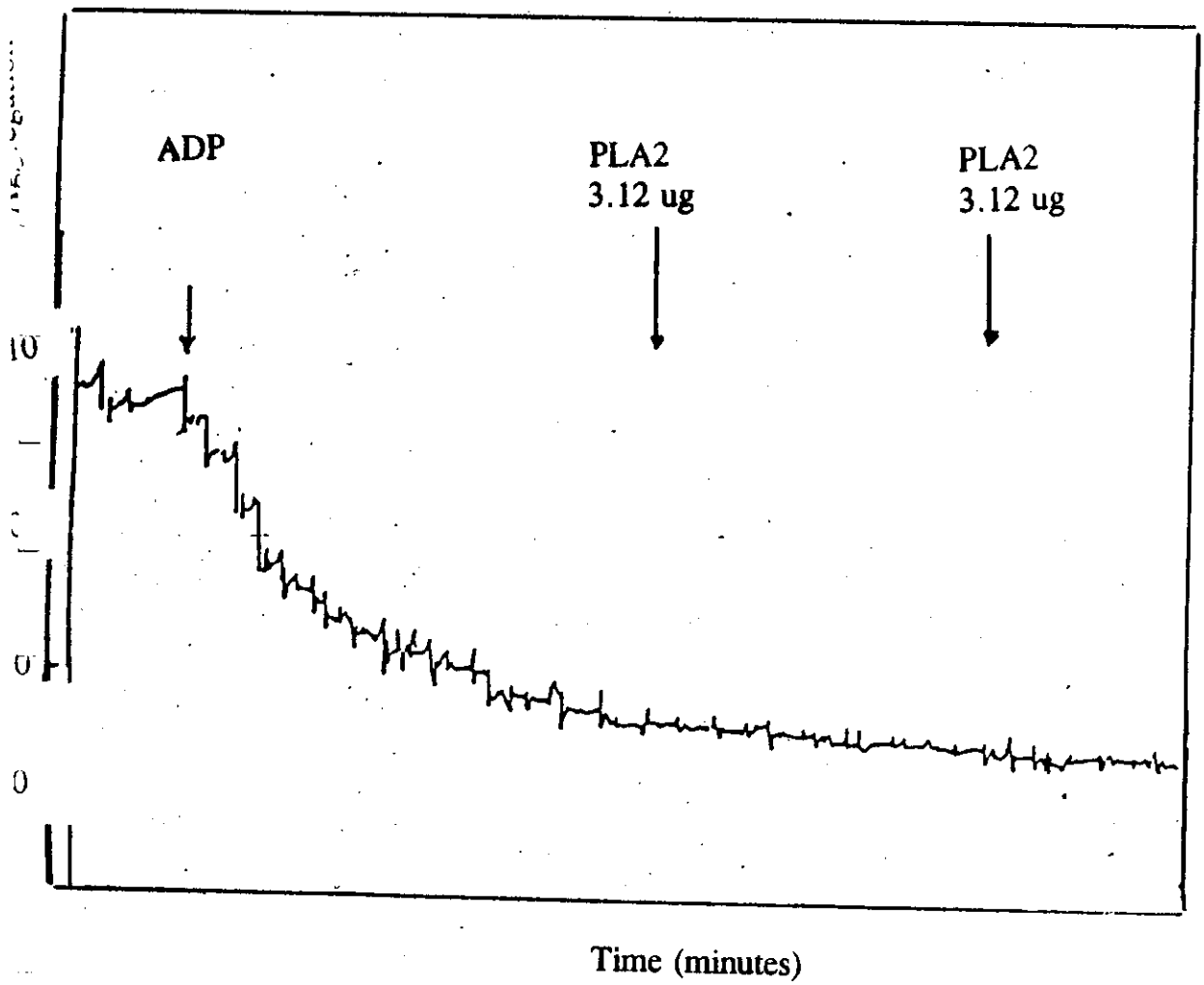


PLATELET AGGREGATION

A. Addition of 50 µl ADP to human rich plasma produced an increase in aggregation equal to 40% (maximal aggregation) and on addition of PLA2 (3.12 µg/50 µl) in two separates times to platelet rich plasma exposed to ADP for 2-5 minutes produced minimal effect. Fig. (21).

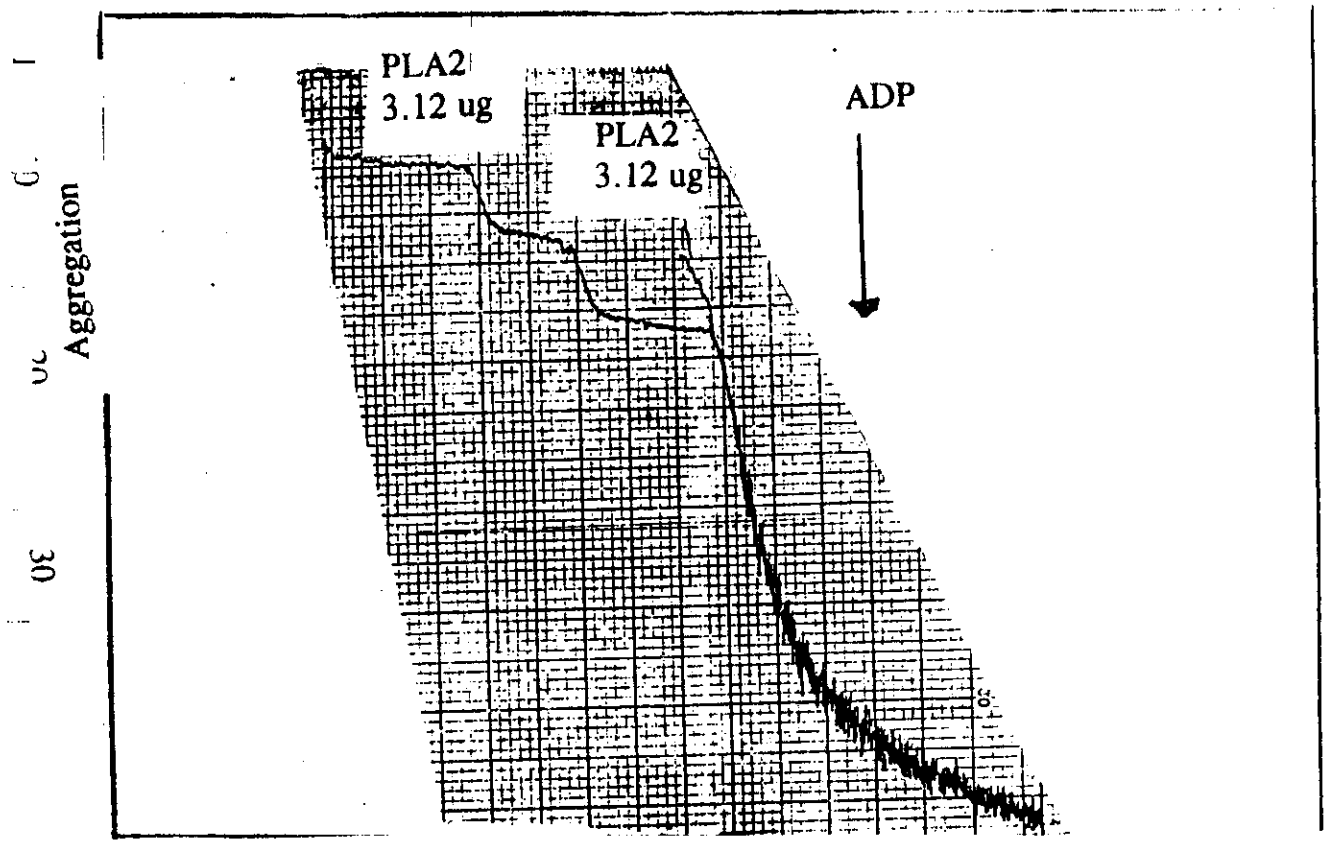
B. Addition of PLA2 (3.12 µg/50 µl) to human rich plasma caused an increase in light transmission corresponding to 15% which was less than the maximal Fig. (22a) while with another equal dose of PLA2 (3.12 µg/50 µl) added to human rich plasma produced an increase in aggregation equal to 15% and on addition of 50 µl ADP to platelet rich plasma exposed to PLA2 produced aggregation equal to 40% (maximal aggregation). Fig. (22a).

C. Addition of crude venom (3.12 µg/50 µl) to human rich plasma produced an increase in aggregation equal to 10% and on addition of another equal dose of crude venom, an increase in aggregation equal to 10% was obtained also, on addition of 50 µl ADP to P.R.P. exposed to crude venom for 2-5 minutes produced 20% aggregation. Fig. (22b).



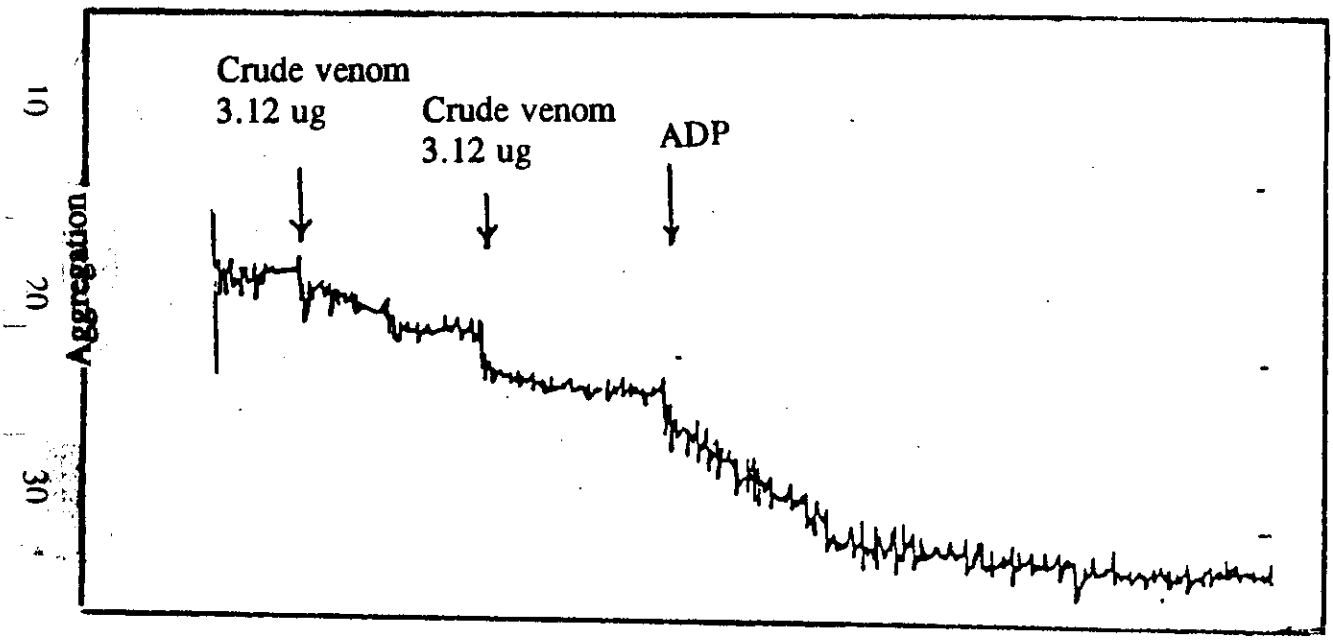
Base line (10-90), ADP: 40% aggregation; PLA2: zero aggregation.

Fig. (21): The effect of ADP followed by 3.12 µg/50 µl PL-I on platelets aggregation of human, followed by addition of 3.12 µg/50 µl PL-I on platelet aggregation of human.



Base line (10-90), PLA2: 15% , ADP: 40% aggregation

Fig. (22a): The effect of addition of 3.12 μg/50 μl PL-I followed by addition of 3.12 μg of PL-I, followed by addition of ADP on platelets aggregation of human RBCs.



Base line (10-90), crude venom (3.12 μg): 10% aggregation, ADP: 20% aggregation

Fig. (22b): The effect of addition of 3.12 μg/50 μl followed by addition of 3.12 μg of crude venom, followed by addition of ADP on platelets aggregation of human RBCs.

PART IV

IN VIVO STUDIES OF SOME BIOLOGICAL AND TOXICOLOGICAL PROPERTIES OF THE ISOLATED ENZYME

STUDY OF LETHALITY

Determination of The Approximate LD_{50} of The Crude Venom

The approximate intraperitoneal LD_{50} of the Sistrurus miliarius barbouri crude venom had been determined using the method described by Reed and Muench (1938). Table (22) shows different doses of the Sistrurus miliarius barbouri (S.M.B.) venom and the corresponding mortality mice using a constant factor (1.2) for determination of the median lethal dose (LD_{50}) of the venom.

$$\text{Proportionate distance} = \frac{50\% - \%\text{ mortality just below } 50\%}{\%\text{ mortality just above } 50\% - \%\text{ mortality below } 50\%}$$

$$= \frac{50 - 25}{54.54 - 25} = \frac{25}{29.54} = 0.8463$$

Log LD_{50} = Log LD just below 50% + (log increasing factor x proportionate distance).

= 0.9206 + (0.0792 x 0.8463)

= 0.9206 + 0.0670

= 0.9876

LD_{50} of S.M.b. venom = 9.72 μg/gm/Mice

Table (22): Shows different doses of the Sistrurus miliarius barbouri venom using a constant factor (1.2) and the corresponding mortality mice for determination of the median lethal dose (LD_{50}).

Dose of venom μg/gm/mice	No. of animals	Survivals	Deaths	Total		Mortality %
				S	D	
5.78	6	6	0	20	0	0.00%
6.94	6	5	1	14	1	6.67%
8.33	6	4	2	9	3	25.00%
10.00	6	3	3	5	6	54.54%
12.00	6	2	4	2	10	83.33%
14.40	6	0	6	0	16	100.00%

Total S = Total survivals.

Total D = Total deaths.

Determination of The Approximate LD_{50} of Isolated PL-I

LD_{50} of PL-I was not known, apilot experiment was carried out by I.P. injection of a group of 6 mice with serial of PL-I doses of 10, 15, 20 and 30 µg/gm body weight, but the animals did not die even after several weeks (4 weeks) from injection.

We studied the biochemical and histopathological changes induced by I.P. injection of sublethal doses (1/10 LD_{50}) of C.V. and isolated PL-I at different doses from injection.

BIOCHEMICAL EFFECTS OF CRUDE VENOM (C.V) ON LIVER AND KIDNEY FUNCTION TESTS

A: Liver Function Tests

1- Serum Total Protein (T.P.):

Table (23) & Fig. (23) Show the effect of intraperitoneal crude venom injection of sublethal dose of crude venom (1/10 LD_{50}) (1/10 9.72 mg/kg B.W.) on serum total protein after 4 hours from injection compared with the normal control group.

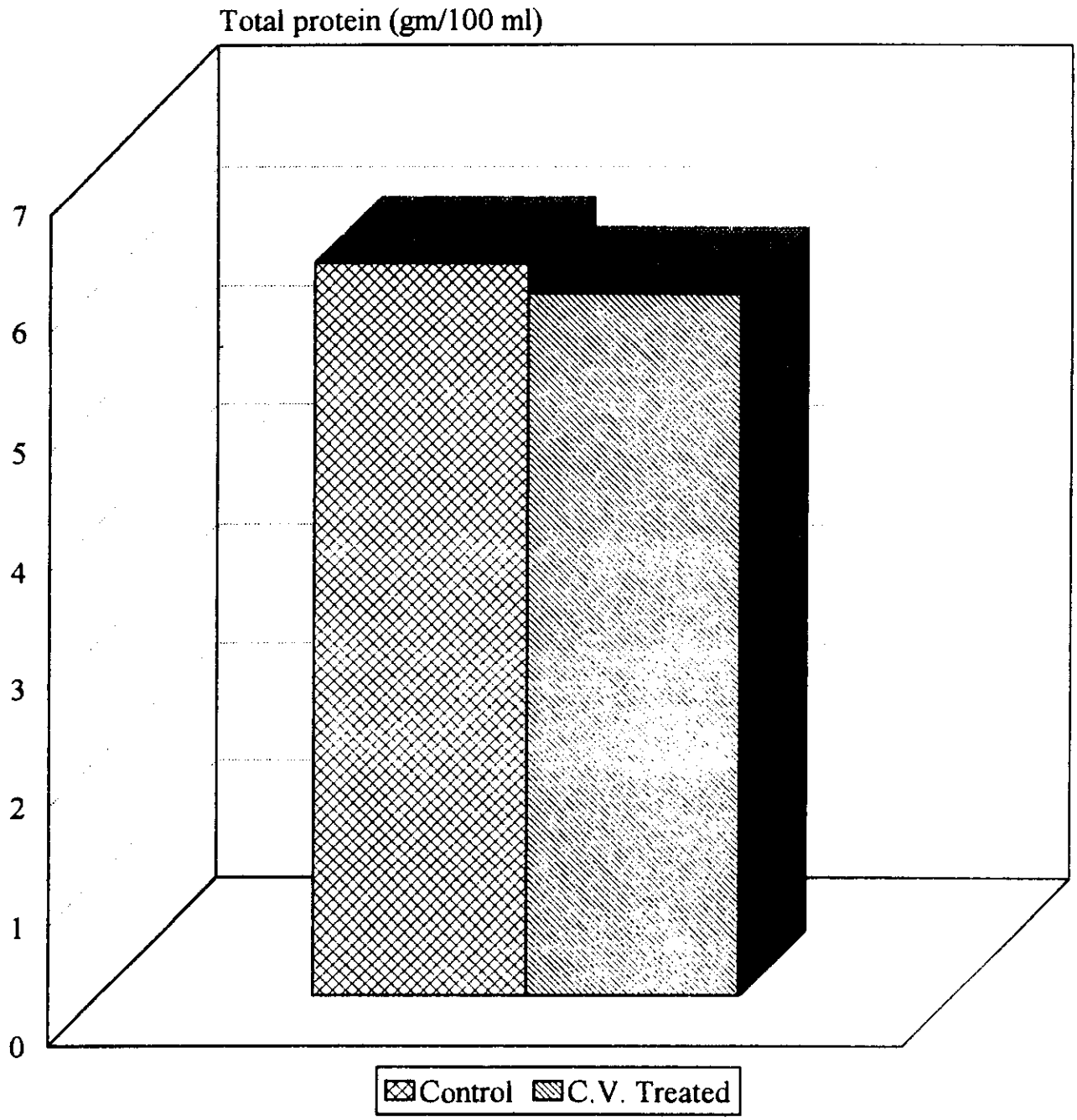
- The mean ± SD values of the control group was 6.17 ± 0.27 gm/100 ml.
- The mean ± SD values of the treated mice was 5.92 ± 0.32 with P value P> 0.05.
- The mice injected with crude venom showed a non significant decrease P> 0.05 from the corresponding control.

Table (23): Effect of I.P. injection of sublethal dose of C.V (1/0 LD_{50}) on serum total protein (TP) after 4 hours from injection.

Number of cases	Serum total protein (gm/100 ml)	
	Control	Treated mice
1	6.00	5.5
2	5.89	5.7
3	6.04	6.0
4	6.46	6.1
5	6.48	6.3
Mean ± SD	6.17 ± 0.27	5.92 ± 0.32
"t"	1.33	
P	P> 0.05	
	N.S	

N.S.: Non significant difference from the corresponding control values at P> 0.05

Fig. (23): Effect of I.P injection of sublethal dose of C.V. (1/10 LD_{50}) on total serum protein (TP) after 4 hours from injection.



2- Serum Alkaline Phosphatase (A.P):

Table (24) & Fig. (24) Show the effect of intraperitoneal injection of sublethal dose (1.10 LD_{50}) of crude venom on serum alkaline phosphatase after 4 hours from injection compared with normal control group.

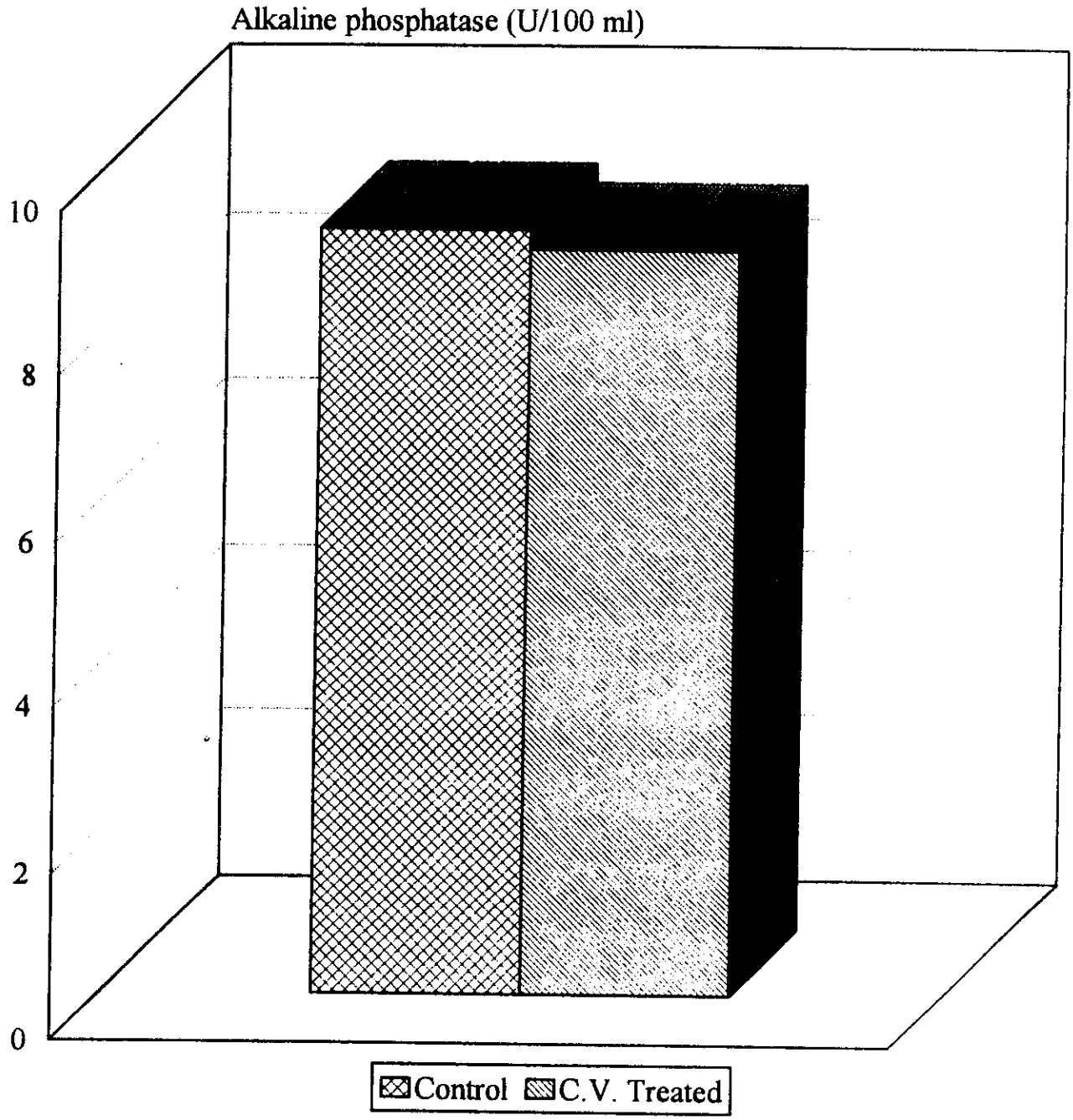
- The mean ± SD value of the control group was 9.23 ± 1.27 U/100 mL.
- The mean ± SD value of the treated mice was 9.0 ± 0.98 with P values P> 0.05.
- The injected mice with sublethal dose of curde venom (0.972 µg.gm B.W.) showed a non significant decrease P> 0.05 from the corresponding control.

Table (24): Effect of I.P. injection of a sublethal dose of C.V (1/10 LD_{50}) on serum alkaline Phosphatase (AP) after 4 hours from injection.

Number of cases	Serum alkaline Phosphatase (U/100 mL)	
	Control	Treated mice
1	8.88	8.0
2	9.53	9.6
3	9.33	10.0
4	7.44	7.9
5	10.96	9.5
Mean ± SD	9.23 ± 1.27	9.0 ± 0.98
"t"	0.319	
P	P> 0.05	
	N.S	

N.S: Non significant difference from the corresponding control at P> 0.05.

Fig. (24): Effect of I.P injection of sublethal dose of C.V. (1/10 LD_{50}) on serum alkaline phosphatase (AP) after 4 hours from injection.



3- Serum Aspartate Aminotransferase (AST)

Table (25) & Fig. (25) Show the effect of intraperitoneal injection of sublethal dose (1/10 LD_{50}) of crude venom on serum Aspartate Aminotransferase after 4 hours of the injected mice compared with normal control group.

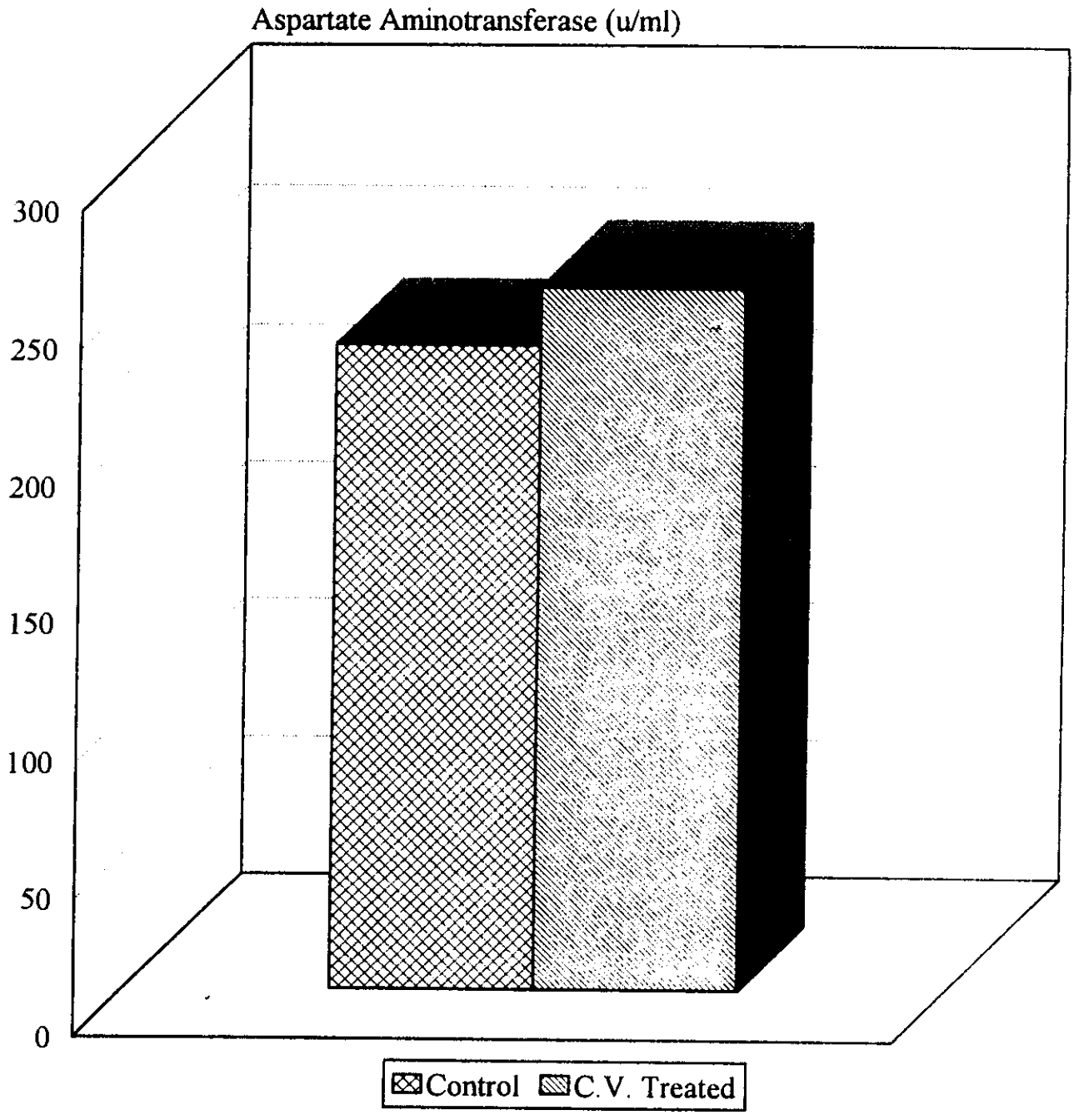
- The mean ± SD value of the control group was 234.72 ± 26.39 U/mL.
- The mean ± SD value of the treated group was 256 ± 24.85 with p Values P> 0.05.
- The injected mice with sulbethal dose of crude venom showed a non significant increased P> 0.05 from the corresponding control.

Table (25): Effect of I.P. injection of a sublethal dose of C.V (1/10 LD_{50}) on Aspartate Aminotransferase (AST) after 4 hours from injection mice.

Number of cases	Serum Aspartate Aminotransferase	
	Control	Treated mice
1	260.5	255
2	198.4	270
3	235.0	212
4	220.5	260
5	259.2	280
Mean ± SD	234.72 ± 26.39	256 ± 24.85
"t"	1.31	
P	P> 0.05	
	N.S	

N.S.: Non significant difference from the corresponding control values at P> 0.05.

Fig. (25): Effect of I.P injection of sublethal dose of C.V (1/10 LD_{50}) on serum aspartate aminotransferase (AST) after 4 hours from injection.



4- Serum Alanine Aminotransferase (ALT)

Table (26) & Fig. (26) Show the effect of intraperitoneal injection of sublethal dose (1/10 LD_{50}) of crude venom on serum Alanine Aminotransferase after 4 hours from injected mice compared with normal control group.

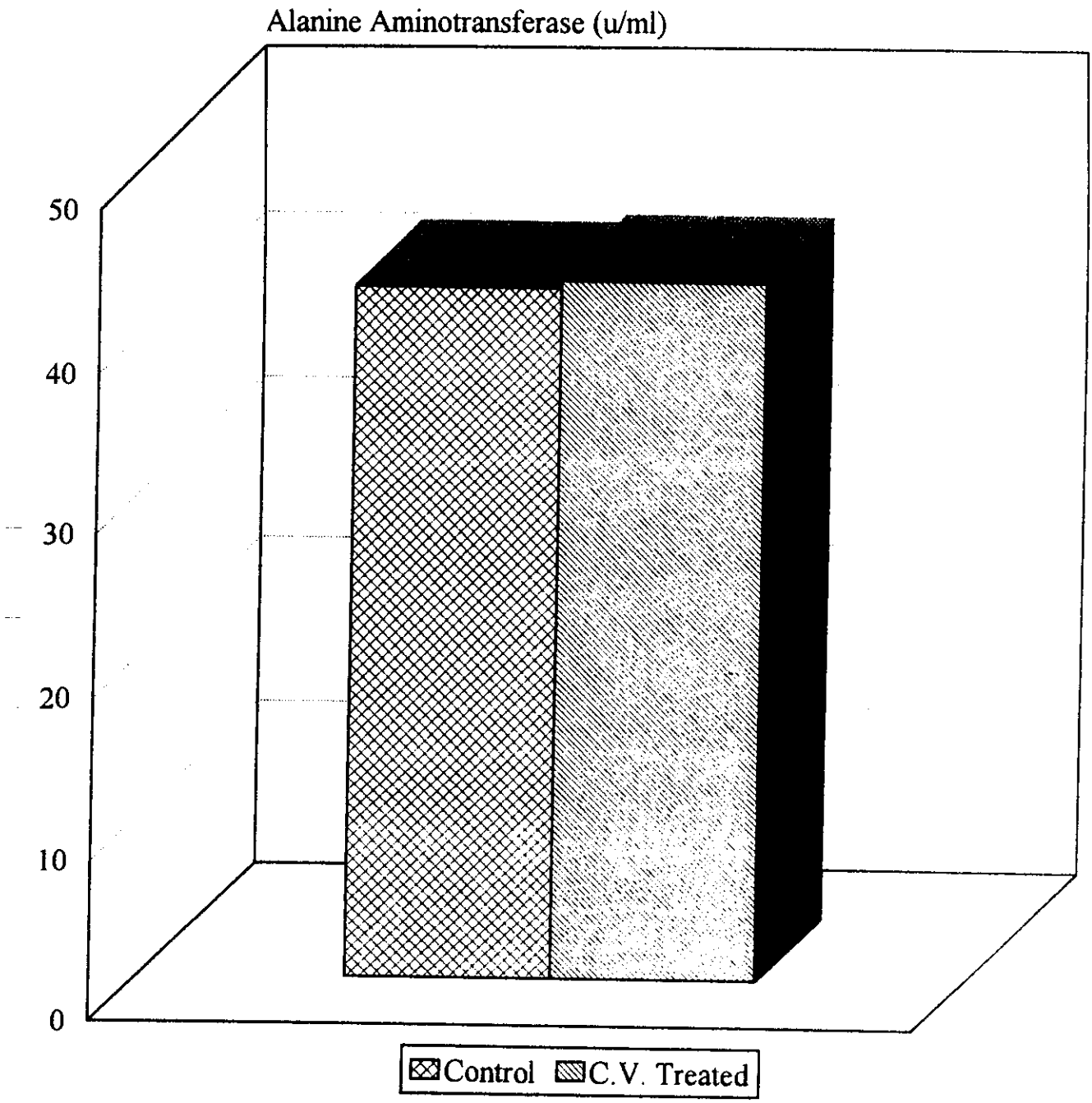
- The mean ± SD values of control group was 42.45 ± 5.9 U/mL.
- The mean ± SD values of the treated mice was 43 ± 4.36 with P values P> 0.05.
- The injected mice with sublethal dose of crude venom showed a non significant increased P> 0.05 from the corresponding control.

Table (26): Effect of I.P. injection of a sublethal dose of C.V (1/10 LD_{50}) on serum alanine aminotransferase (ALT) after 4 hours from injection.

Number of cases	Serum Alanine Aminotransferase (μ/ml)	
	Control	Treated mice
1	46.85	44
2	38.20	39
3	49.75	48
4	42.00	38
5	35.46	46
Mean ± SD	42.45 ± 5.9	43 ± 4.36
"t" P	0.166 P> 0.05 N.S	

N.S.: Non significant difference from the corresponding control values at P> 0.05.

Fig. (26): Effect of I.P injection of a sublethal dose of C.V. (1/10 LD_{50}) on serum alanine aminotransferase (ALT) after 4 hours from injection.



B- Kidney Function Test

1- Blood Urea Nitrogen (BUN)

Table (27) & Fig. (27) Show the effect of intraperitoneal injection of sublethal dose (1/10 LD_{50}) (1/10 9.72 mg/kg B.W.) of crude venom on Blood Urea Nitrogen after 4 hours from injected mice compared with the normal control group.

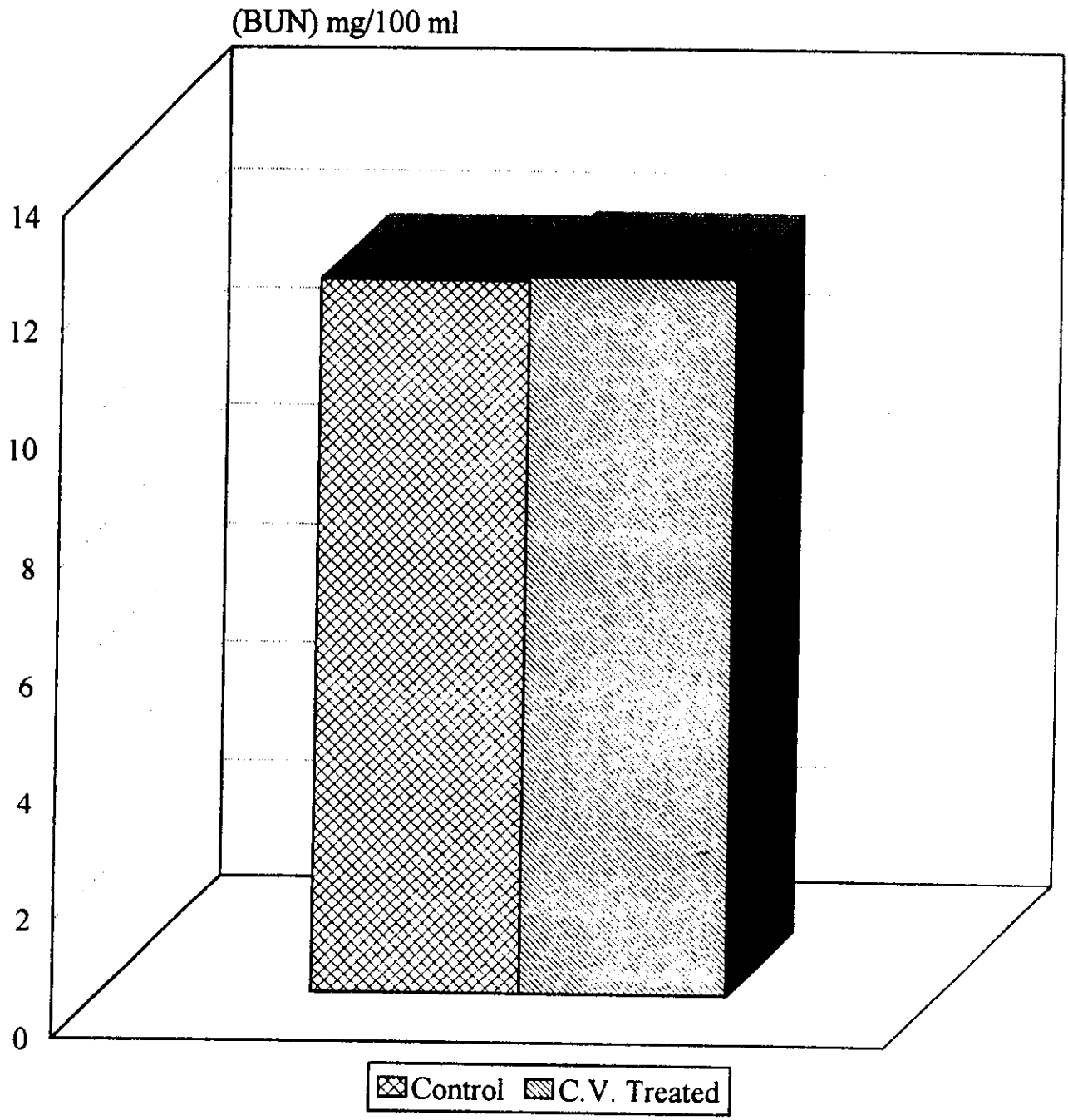
- The mean ± SD values of control group was 12.12 ± 0.7 mg/dl.
- The mean ± SD values of the treated mice was 12.2 ± 0.67 with P values P> 0.05.
- The injected mice with sublethal dose of crude venom showed a non significant increased P> 0.05 from corresponding control.

Table (27): Effect of I.P. injection of a sublethal dose of C.V on blood urea nitrogen (BUN) after 4 hours from injected mice.

Number of cases	Blood Urea Nitrogen mg/dl	
	Control	Treated mice
1	12.63	13.00
2	11.75	12.80
3	11.20	11.50
4	12.77	11.70
5	12.70	12.00
Mean ± SD	12.12 ± 0.70	12.20 ± 0.67
"t"	0.023	
P	P> 0.05	
	N.S.	

N.S.: Non significant difference from the corresponding control values at P> 0.05.

Fig. (27): Effect of I.P injection of sublethal dose of C.V (1/10 LD_{50}) on blood urea nitrogen (BUN) after 4 hours from injection.



BIOCHEMICAL EFFECTS OF ISOLATED PL-I ON LIVER AND KIDNEY FUNCTION TESTS

A: Liver Function Test

1- Serum Total Protein (TP)

Table (28) & Fig. (28) Show the effect of intraperitoneal injection of different doses of isolated PL-I (10 μg, 15 μg, 20 μg and 30 μg) per gm body weight on serum total protein after 4 hours from injected mice compared with normal control group.

- The mean $\pm$ SD value of control group was 6.17 $\pm$ 0.27.
- The mean $\pm$ SD value of injected mice with 10 μg PL-I/gm body weight was 6.0 $\pm$ 0.25 with P values P> 0.05.
- The mean $\pm$ SD value of injected mice with 15 μg PL-I/gm body weight was 6.06 $\pm$ 0.24 with P values P> 0.05.
- The mean $\pm$ SD value of injected mice with 20 μg PL-I/gm body weight was 6.14 $\pm$ 0.27 with P> 0.05.
- The mean $\pm$ SD value of injected mice with 30 μg PL-I/gm body weight was 6.26 $\pm$ 0.30 with P> 0.05.
- The injected mice with 10 μg, 15 μg and 20 μg PL-I showed a non significant decrease in total serum protein whereas the injected mice with 30 μg PL-I showed a non significant increase from the corresponding control mice.

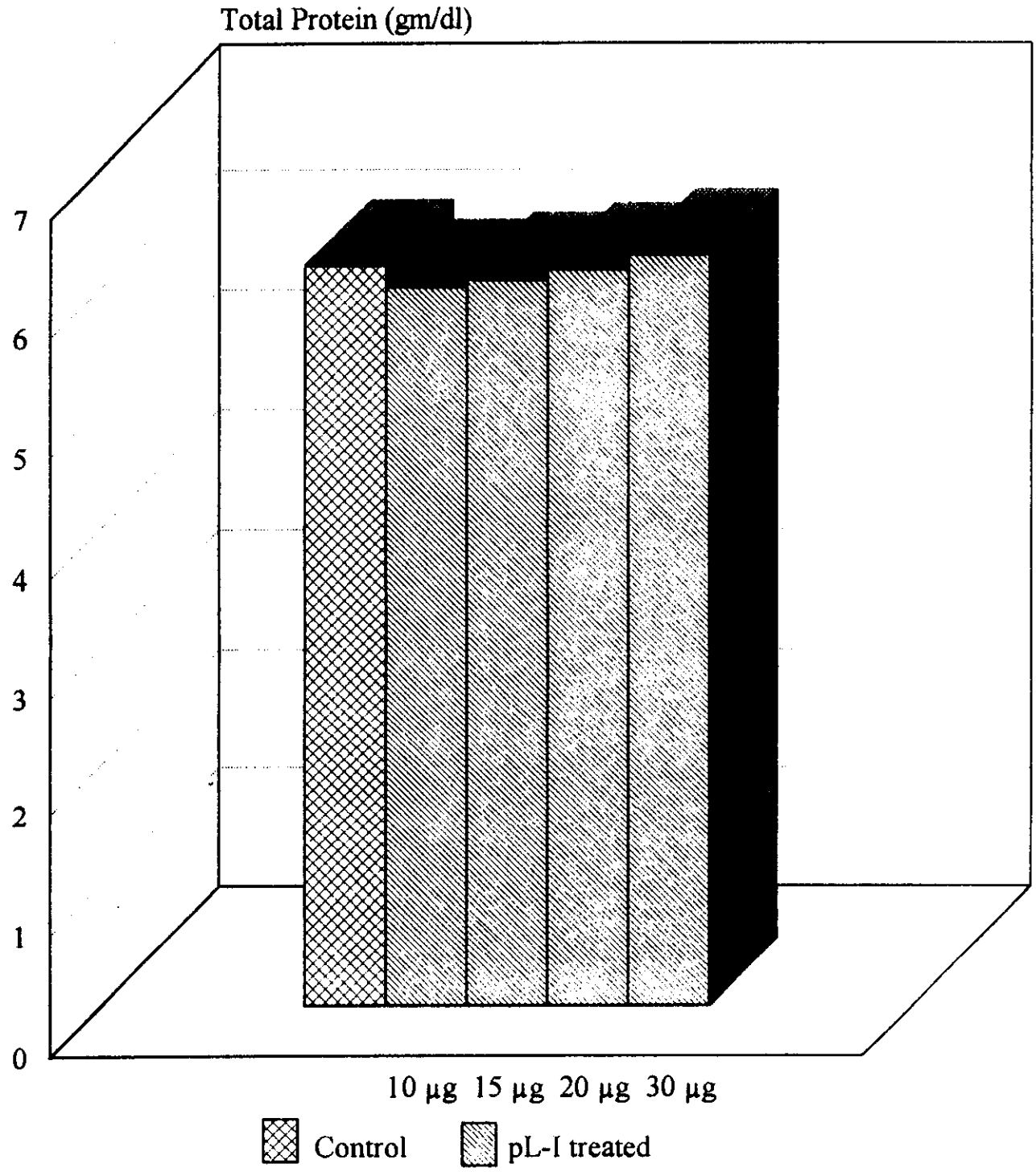
Table (28): Effect of I.P injection of different doses of PL-I on serum total protein after 4 hours from injection.

Serum Total Protein (gm/dl)

Number of cases	Control	Treated mice with 10 µg pL-I	Treated mice with 15 µg pL-I	Treated mice with 20 µg pL-I	Treated mice with 30 µg pL-I
1	6.00	6.00	6.00	6.50	5.90
2	5.89	6.30	6.40	5.80	6.00
3	6.04	6.10	6.20	6.30	6.60
4	6.46	6.00	5.90	6.10	6.50
5	6.48	5.60	5.80	6.00	6.30
Mean ± SD	6.17±0.27	6.00±0.25	6.06±0.24	6.14±0.27	6.26±0.30
"t"		1.04	0.68	0.17	0.5
P		P> 0.05 N.S	P> 0.05 N.S	P> 0.05 N.S	P> 0.05 N.S

N.S.: Non significant difference from corresponding control values at P> 0.05

Fig. (28): Effect of I.P injection of Different Doses of PL-I on serum total protein (TP).



2- Serum Alkaline Phosphatase (AP):

Table (29) & Fig. (29) Show the effect of intraperitoneal injection of different doses of isolated PL-I (10 μg, 15 μg, 20 μg and 30 μg)/gm body weight on serum alkaline phosphatase after 4 hours from injected mice, compared with normal control group.

- The mean ± SD values of control group was 9.23 ± 1.27.
- The mean ± SD values of injected mice with 10 μg PL-I/gm mouse was 8.78 ± 1.45 with P values $P > 0.05$.
- The mean ± SD values of injected mice with 15 μg PL-I/gm mouse was 8.98 ± 1.5 with P values $P > 0.05$.
- The mean ± SD values of injected mice with 20 μg PL-I/gm mouse was 8.94 ± 1.55 with P values $P > 0.05$.
- The mean ± SD values of injected mice with 30 μg PL-I/gm mouse was 9.3 ± 1.63 with P values $P > 0.05$.
- The injected mice, with 10 μg, 15 μg and 20 μg PL-I showed a non significant decrease in serum alkaline phosphatase, but a non significant increase with 30 μg PL-I was obtained from the corresponding control mice.

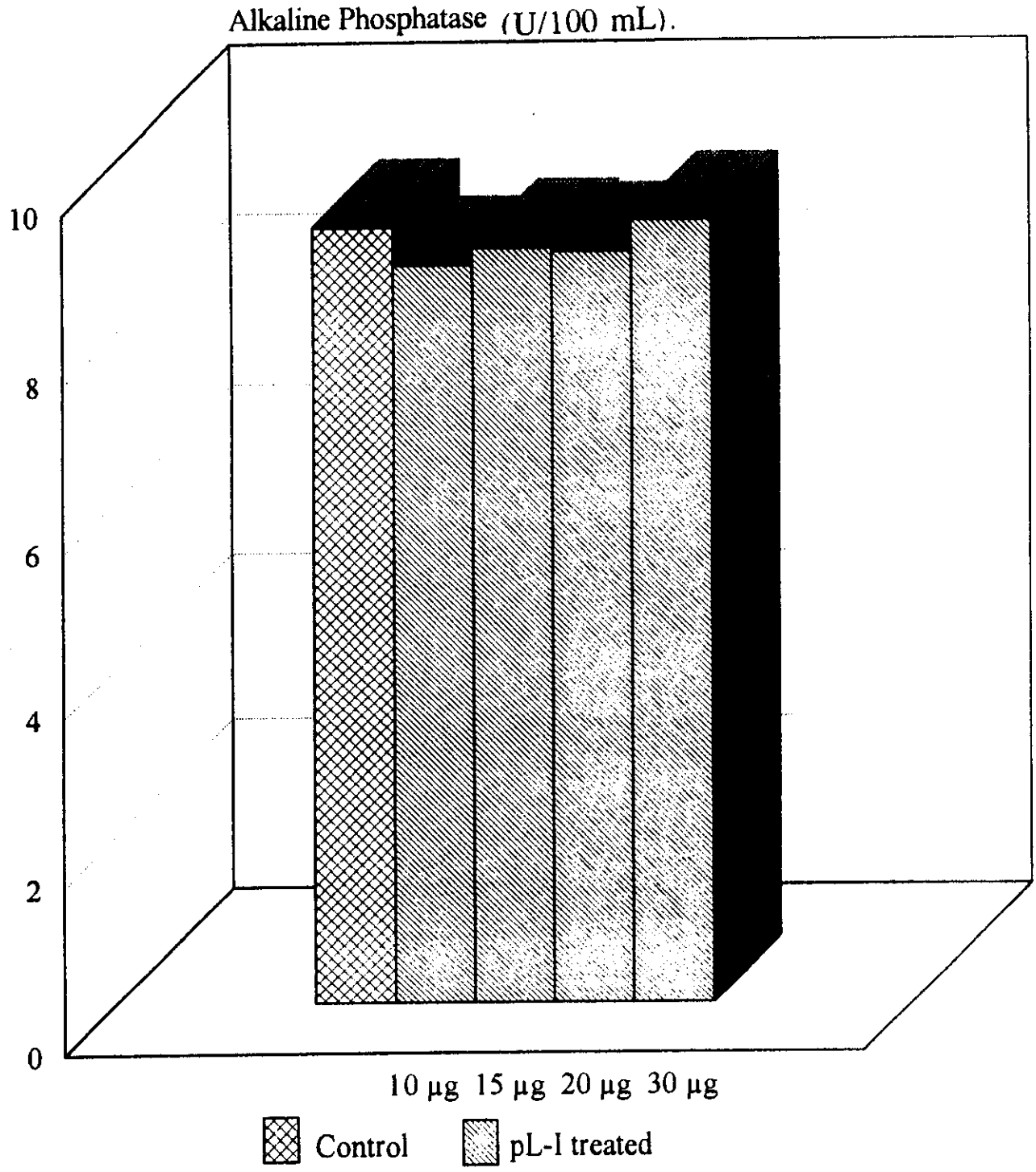
Table (29): Effect of I.P injection of different doses of PL-I on serum alkaline phosphatase (AP) after 4 hours from injection.

Serum Alkaline Phosphatase (U/100 mL)

Number of cases	Control	Treated mice with 10 μg pL-I	Treated mice with 15 μg pL-I	Treated mice with 20 μg pL-I	Treated mice with 30 μg pL-I
1	8.88	7.9	8.0	9.0	12.0
2	9.53	11.0	9.7	8.7	9.5
3	9.33	9.5	11.3	7.5	7.8
4	7.44	7.5	7.7	8.0	8.2
5	10.96	8.0	8.2	11.5	8.7
Mean ± SD	9.23±1.27	8.78±1.45	8.98±1.5	8.94±1.55	9.3±1.63
"t"		0.52	0.32	0.32	0.08
P		P> 0.05 N.S	P> 0.05 N.S	P> 0.05 N.S	P> 0.05 N.S

N.S.: Non significant difference from corresponding control values at P> 0.05.

Fig. (29): Effect of I.P. injection of different Doses of PL-I on alkaline phosphatase (AP) after 4 hours from injection (U/100 mL).



3- Serum Aspartate Aminotransferase (AST):

Table (30) & Fig. (30) Show the effect of intraperitoneal injection of the different doses of the isolated PL-I (10 μg, 15 μg, 20 μg and 30 μg)/gm mice on serum Aspartate Aminotransferase after 4 hours from injected mice, compared with normal control group.

- The mean ± SD values of control group was 234.72 ± 26.39.
- The mean ± SD values of injected mice with 10 μg PL-I was 233 ± 25.88 with P value P> 0.05.
- The mean ± SD values of injected mice with 15 μg PL-I was 231 ± 27.47 with P value P> 0.05.
- The mean ± SD values of injected mice with 20 μg PL-I was 233.4 ± 28.42 with P value P> 0.05.
- The mean ± SD values of injected mice with 30 μg PL-I was 242 ± 30.33 with P value P> 0.05.
- The injected mice, with 10 μg, 15 μg and 20 μg PL-I showed a non significant decrease in serum Aspartate Aminotransferase, but a non significant increase with 30 μg PL-I was obtained from the corresponding control mice.

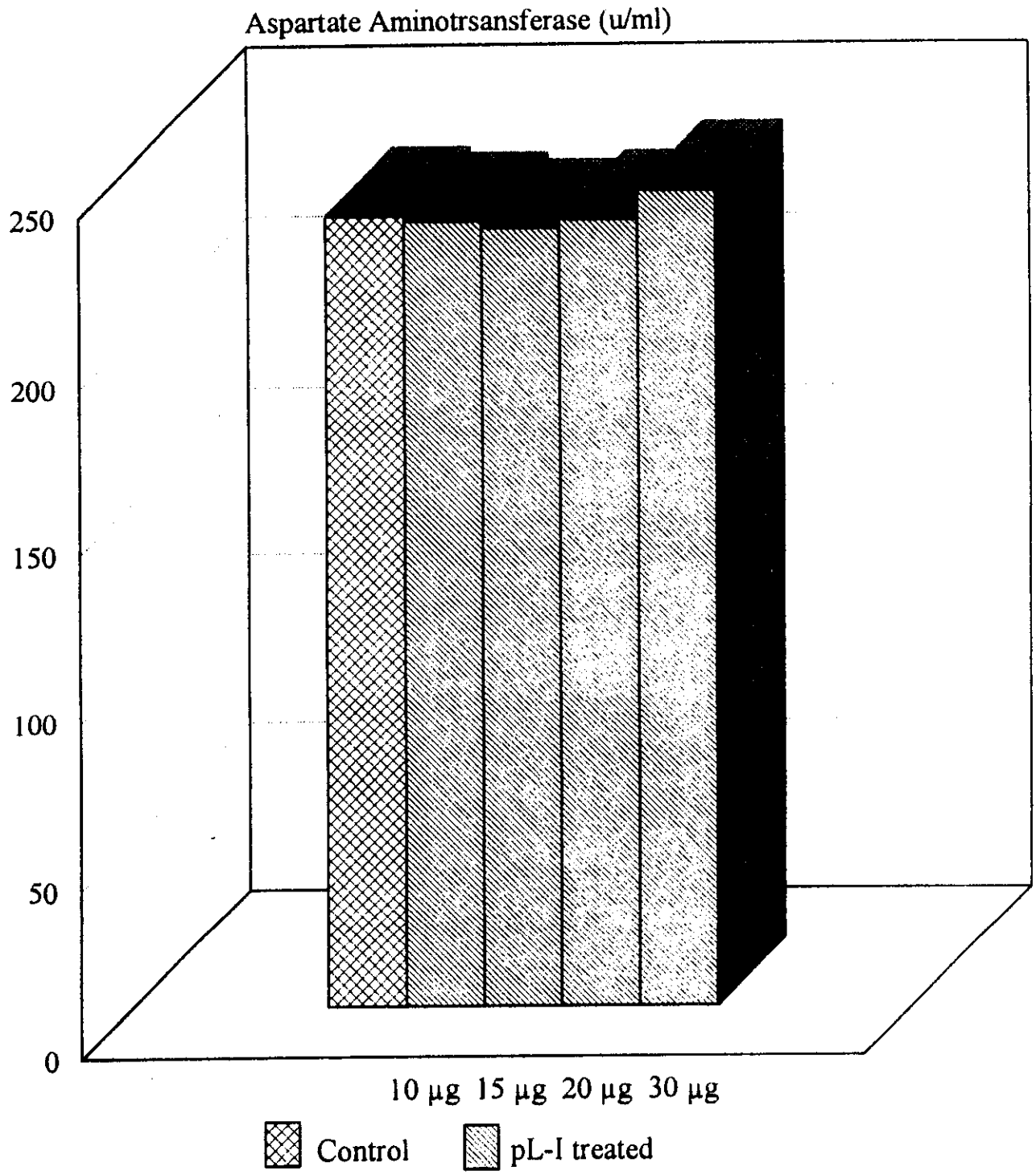
Table (30): Effect of I.P injection of different doses of PL-I on serum aspartate amino-transferase (AST) after 4 hours from injected mice.

Serum aspartate aminotransferase (u/ml)

Number of cases	Control	Injected mice with 10 μg pL-I	Injected mice with 15 μg pL-I	Injected mice with 20 μg pL-I	Injected mice with 30 μg pL-I
1	260.5	260	200	222	205
2	148.4	240	270	215	235
3	235.0	250	210	240	265
4	220.5	195	240	280	280
5	259.2	220	235	210	225
Mean±SD	234.72±26.39	233±25.88	231±27.47	233.4±28.42	242±30.33
"t"		0.1	0.21	0.08	0.4
P		P> 0.05	P> 0.05	P> 0.05	P> 0.05
		N.S	N.S	N.S	N.S

N.S.: Non significant difference from corresponding control values at P> 0.05.

Fig. (30): Effect of I.P. injection of different doses of PL-I on aspartate aminotransferase (AST) after 4 hours from injected mice.



4- Serum Alanine Aminotransferase (ALT):

Table (31) & Fig. (31) Show the effect of interperitoneal injection of different doses of isolated PL-I (10 μg, 15 μg, 20 μg and 30 μg)/ gm body weight on serum Alanine Aminotransferase after 4 hours from injected mice, compared with normal control group.

- The mean $\pm$ SD values of control group was 42.45 $\pm$ 5.9.
- The mean $\pm$ SD values of injected mice with 10 μg PL-I was 41.2 $\pm$ 5.54 with P value $P > 0.05$.
- The mean $\pm$ SD values of injected mice with 15 μg PL-I was 42.6 $\pm$ 5.86 with P value $P > 0.05$.
- The mean $\pm$ SD values of injected mice with 20 μg PL-I was 43.2 $\pm$ 6.4 with P value $P > 0.05$.
- The mean $\pm$ SD values of injected mice with 30 μg PL-I was 43.6 $\pm$ 5.18 with P value $P > 0.05$.
- The injected mice with 10 μg PL-I showed a non significantly decreased from the corresponding mice, while injection with 15 μg, 20 μg and 30 μg PL-I showed a non significant increase was obtained from corresponding control mice.

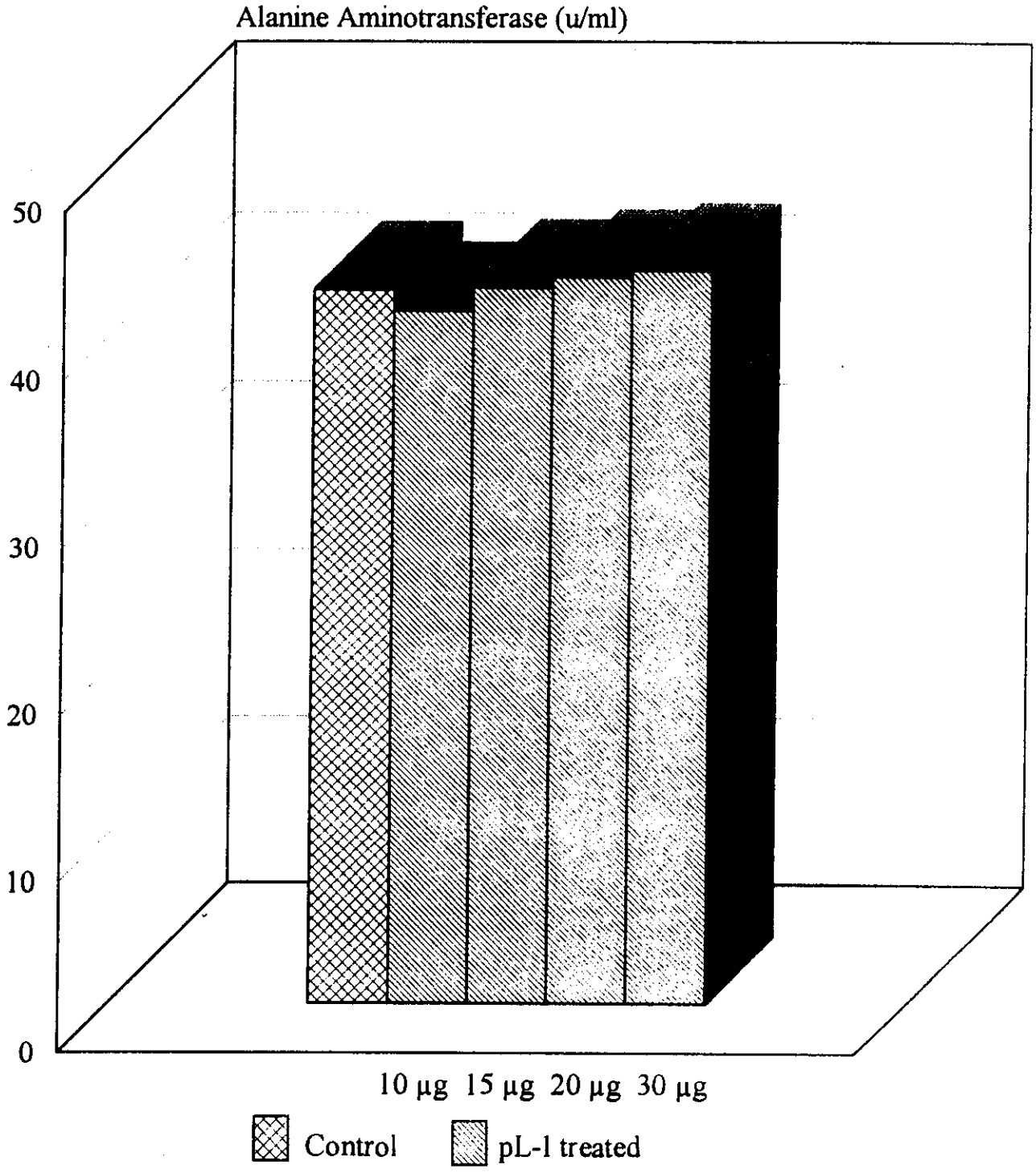
Table (31): Effect of I.P injection of different doses of PL-I on serum alanine amino-transferase(ALT) after 4 hours from injected mice.

Serum alanine Aminotransferase (u/ml)

Number of cases	Control	Injected mice with 10 μg pL-I	Injected mice with 15 μg pL-I	Injected mice with 20 μg pL-I	Injected mice with 30 μg pL-I
1	46.85	44	38	44	50
2	38.20	36	46	38	36
3	49.75	35	45	36	44
4	42.00	43	35	52	42
5	35.46	48	49	46	46
Mean±SD	42.45±5.9	41.2±5.54	42.6±5.86	43.2±6.4	43.6±5.18
"t"		0.34	0.04	0.19	0.33
P		P> 0.05	P> 0.05	P> 0.05	P> 0.05
		N.S	N.S	N.S	N.S

N.S.: Non significant difference from corresponding control values at P > 0.05

Fig. (31): Effect of I.P injection of different doses of PL-I on alanine aminotransferase (ALT) after 4 hours from injected mice.



B- Kidney Function Test

1- Blood Urea Nitrogen (BUN):

Table (32) & Fig. (32) Show the effect of intraperitoneal injection of different doses of isolated PL-I (10 μg, 15 μg, 20 μg and 30 μg) on Blood Urea Nitrogen after 4 hours from injected mice compared with normal control group.

- The mean $\pm$ SD values of control group was 12.21 $\pm$ 0.7.
- The mean $\pm$ SD values of injected mice with 10 μg PL-I/mg B.W. was 12.98 $\pm$ 1.4 with P value P> 0.05.
- The mean $\pm$ SD values of injected mice with 15 μg PL-I/mg B.W. was 13.16 $\pm$ 1.74 with P value P> 0.05.
- The mean $\pm$ SD values of injected mice with 20 μg PL-I/mg B.W. was 13.2 $\pm$ 1.48 with P value P> 0.05.
- The mean $\pm$ SD values of injected mice with 30 μg PL-I/mg B.W. was 13.8 $\pm$ 1.92 with P value P> 0.05.
- Non significant rise was found in Blood Urea Nitrogen after 4 hours from injected mice with 10 μg, 15 μg, 20 μg and 30 μg PL-I from the corresponding control mice.

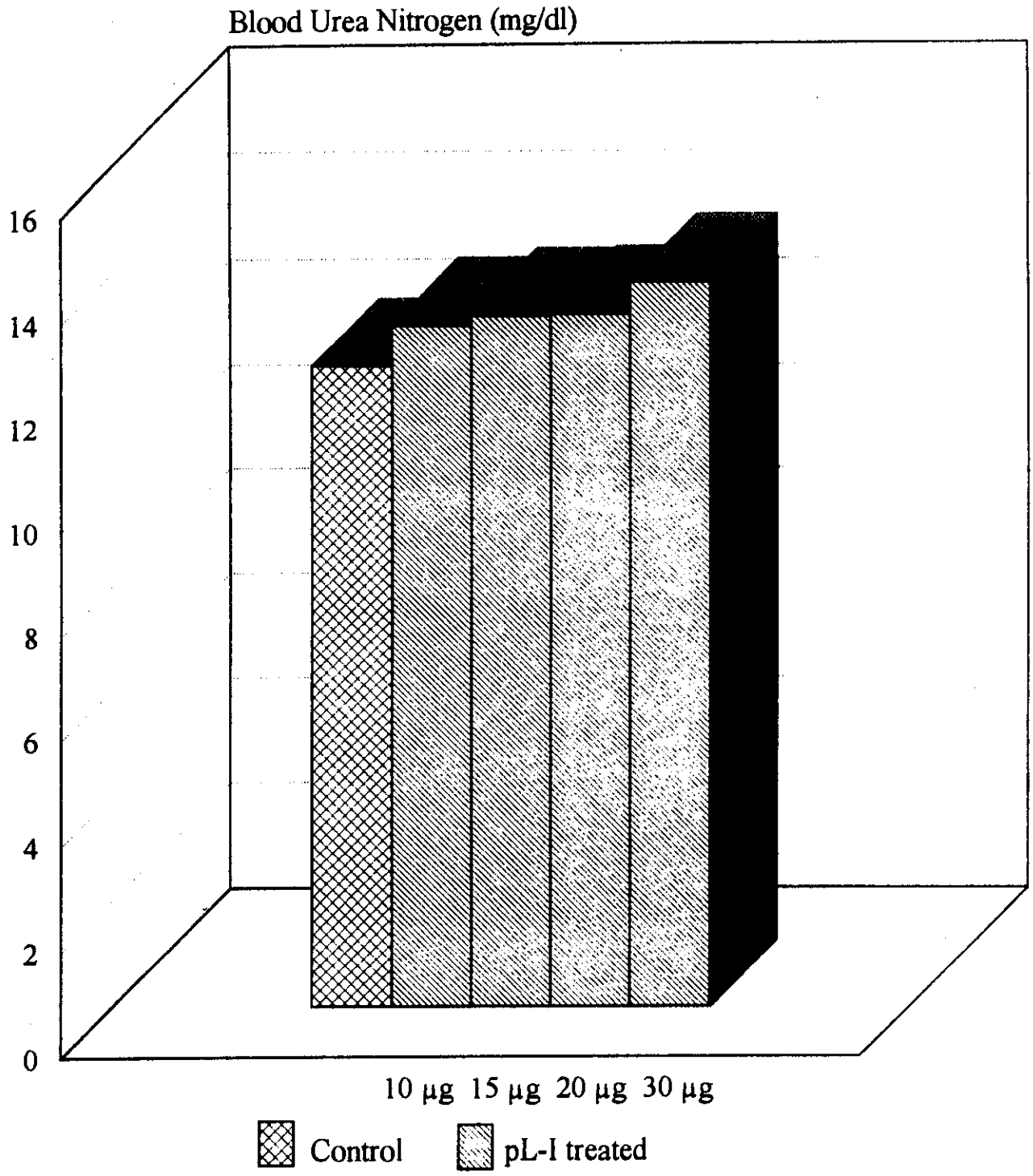
Table (32): Effect of I.P injection of different doses of PL-I on blood urea nitrogen (BUN) after 4 hours from injected mice.

Blood Urea Nitrogen (mg/dL)

Number of cases	Control	Injected mice with 10 μg pL-I	Injected mice with 15 μg pL-I	Injected mice with 20 μg pL-I	Injected mice with 30 μg pL-I
1	12.63	13.8	11.9	15	12
2	11.75	11.9	12.5	13	13
3	11.20	11.5	13.6	13	14
4	12.77	15.0	11.8	11	17
5	12.70	12.7	16.0	14	13
Mean±SD	12.21±0.7	12.98±1.4	13.16±1.74	13.2±1.48	13.8±1.92
"t"		1.1	1.12	1.85	1.74
P		P> 0.05 N.S	P> 0.05 N.S	P> 0.05 N.S	P> 0.05 N.S

N.S.: Non significant difference from corresponding control values at P> 0.05.

Fig. (32): Effect of I.P. injection of different doses of PL-I on Blood Urea Nitrogen (BUN) after 4 hours from injected mice.



STUDY OF THE HISTOPATHOLOGICAL CHANGES

The histopathological changes induced by I.P injection of a sublethal doses (1/10 LD_{50}) of C.V. or different doses of PL-I were studied.

Effects of Crude Venom (C.V.)

A: The liver:

All the liver samples show congestion with few lymphocyte.

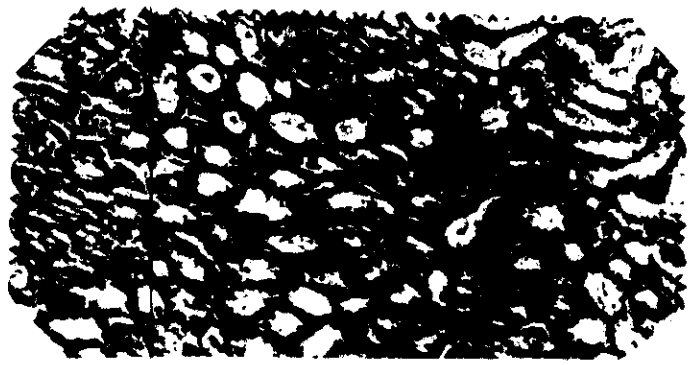


Fig. (33): Photomicrograph of sections in mice liver 4 hours after IP injection of C.V. showing congestions with few lymphocyte.

B: The kidney:

Mild inflammation in all parenchyma.

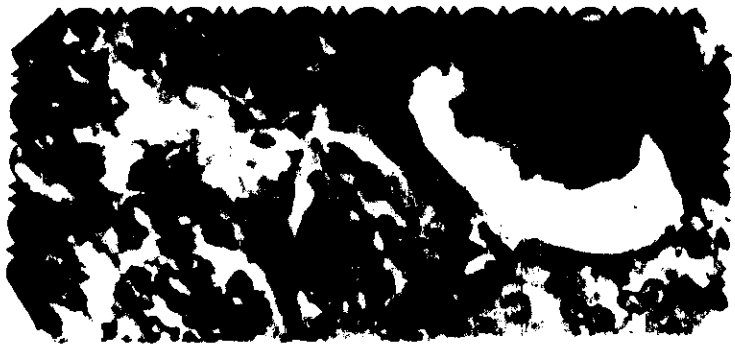


Fig. (34): Photomicrograph of sections in mice kidney, 4 hours after IP injection of C.V. showing mild inflammation in all parenchyma tissues.

C: The heart:

All the heart samples show normal heart tissue with congestion.

Fig. (35): Photomicrograph of sections in mice heart, 4 hours after IP injection of C.V. showing normal heart tissues.

Histopathological changes induced by I.P injection of 10 μg and 15 μg PL-I on liver, kidney and heart muscle show no changes in tissue samples, while a similar mild effects on tissue samples after IP injection of 20 μg and 30 μg PL-I was noticed.

A: The liver:

Hydropic degeneration (cloudy swelling) with few lymphocyte was found in all liver samples.

Fig. (36): Photomicrograph of sections in mice liver, 4 hours after I.P injection of 10 μg PL-I showing normal liver tissues.

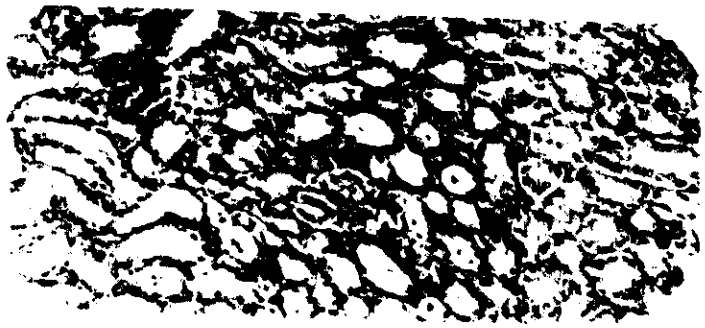


Fig. (37): Photomicrograph of sections in mice liver, 4 hours after I.P injection of 15 μg PL-I showing normal liver tissues.

Fig. (38): Photomicrograph of sections in mice liver, 4 hours after I.P injection of 20 μg PL-I showing hydropic degeneration with few lymphocyte.

Fig. (39): Photomicrograph of sections in mice liver, 4 hours after I.P injection of 30 μg PL-I showing hydropic degeneration with few lymphocytes.

B: The Kidney:

Normal kidney tissues with mild inflammation was found in all kidney samples.

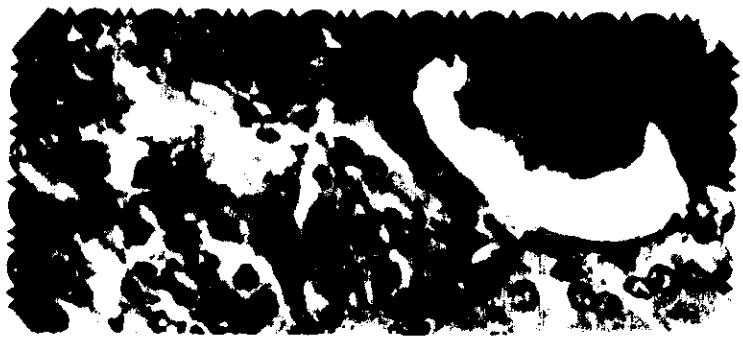


Fig. (40): Photomicrograph of sections in mice kidney, 4 hours after I.P injection of 10 μg PL-I showing normal kidney tissues.

Fig. (41): Photomicrograph of sections in mice kidney, 4 hours after I.P injection of 15 μg PL-I showing normal kidney tissues.

Fig. (42): Photomicrograph of sections in mice kidney, 4 hours after I.P injection of 20 μg PL-I showing mild inflammation in most parenchmatus tissues.

Fig. (43): Photomicrograph of sections in mice kidney, 4 hours after I.P injection of 30 μg PL-I showing mild inflammation in most parenchmatus tissues.

C: The heart:

All the samples showed normal heart tissues with congestion.

Fig. (44): Photomicrograph of sections in mice heart, 4 hours after I.P injection of 10 μg PL-I showing normal heart tissues.

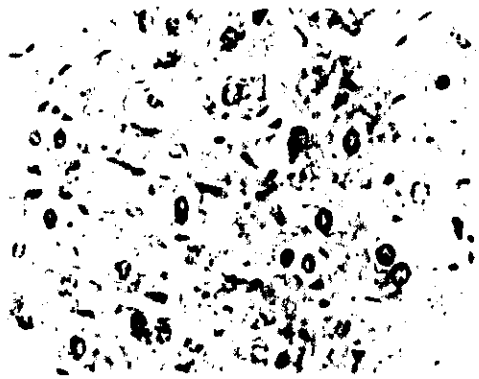


Fig. (47): Photomicrograph of sections in mice heart, 4 hours after I.P injection of 30 μg PL-I showing normal heart tissues with congestions.