CHAPTER THREE

R E S U L T S

C H A P T E R - 3

R E S U L T S

1) Blood Glucose Level :

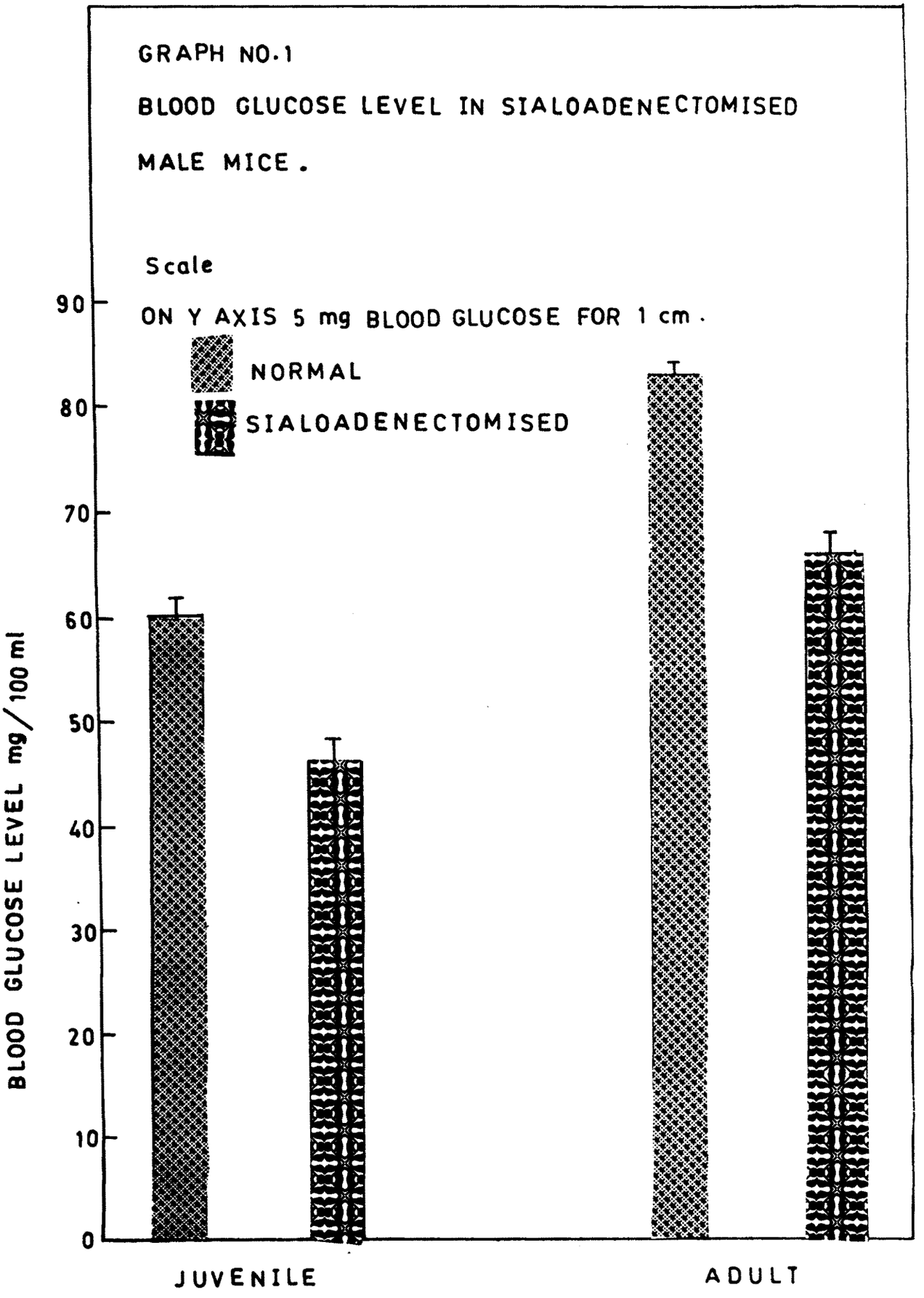
Blood glucose level in blood is estimated in juvenile adult (Age 8 weeks) and sialoadenectomised juvenile adult is described in Graph No.1 and Table No.1. Blood glucose estimated in adult and sialoadenectomised adult (Age 12 weeks) is also described in Graph No.1 and Table No.1. There was significant difference between blood glucose levels of normal and sialo-adenectomised mice from both the age groups. The initial glucose/100 ml of the blood of juvenile mice was 60.83 ± 1.541, reduced significantly in the sialoadenectomised mice to 46.665 § 1.541, reduced significantly in the sialoadenectomised mice to 46.665 ± 2.057 ($P < 0.001$). In the adult male of 12 weeks old, the blood glucose level was 78.30 ± 1.242, which was decreased to 66.02 ± 1.814 in operated mice of the same age group. The decrease was significant ($P < 0.001$).

TABLE No.1 - BLOOD GLUCOSE LEVEL IN SIALOADENECTOMISED MALE MICE

(Blood glucose mg/100 ml of Blood)

Values are Mean ± S.E.

Animals	No. of Animals	Age in Weeks	Control	Sialoadenectomised	t value	P value
Juvenile	5	8	60.83 ± 1.541	46.665 ± 2.057	19.81	P < 0.001
Adult	5	12	78.30 ± 1.242	66.02 ± 1.814	8.3253	P < 0.001


GRAPH NO.1
BLOOD GLUCOSE LEVEL IN SIALOADENECTOMISED MALE MICE.
Scale
ON Y AXIS 5 mg BLOOD GLUCOSE FOR 1 cm.
NORMAL
SIALOADENECTOMISED
BLOOD GLUCOSE LEVEL mg/100 ml
90
80
70
60
50
40
30
20
10
0
JUVENILE
ADULT

2) Glycogen Content in the Muscles :

Glycogen content is expressed in terms of glucose mg/100 mg of the wet weight of the tissue from the muscles of juvenile and adult sialoadenectomised mice. The glycogen content of rectus abdominis is shown in Table No.2 and Graph No.2. The glycogen content of rectus abdominis muscle of control juvenile adult was 1.003 ± 0.1053 and in sialoadenectomised mice of the same age it was increased to 1.8178 ± 0.314. The increase was significant ($P < 0.04$). In the adult control rectus abdominis the glycogen content was 1.524 ± 0.6815 and in sialoadenectomised mice it was significantly increased to 2.18 ± 0.9749 ($P < 0.02$).

The glycogen content of the gastrocnemius muscle of control and sialoadenectomised mice is described in Table No.3 and Graph No.2. The glycogen content of juvenile adult control gastrocnemius was 0.984 ± 0.0555 and in the operated animal it was significantly increased to 1.431 ± 0.1635 ($P < 0.04$). In the adult control gastrocnemius muscle glycogen content was 1.29 ± 0.5769 and in operated animal it was increased to 2.19 ± 0.2486. The increase was significant ($P < 0.01$).

Graph No.3 and Table No.4 show glycogen content of the soleus muscle of control and sialoadenectomised mice. The glycogen content was 4.03 ± 0.3078 in juvenile adult and in the sialoadenectomised mice it was increased to 6.649 ± 0.4903.

TABLE No.2 - EFFECT OF SIALOADENECTOMY ON GLYCOGEN CONTENT OF RECTUS ABDOMINIS MUSCLE

(Glucose mg/100 mg of the muscles)

Values are mean ± S.E.

Animal	No.of Animals	Age in Weeks	Control	Sialoadenectomised	t value	P value
Juvenile	5	8	1.003 ± 0.1053	1.8178 ± 0.314	2.454	P < 0.04
Adult	5	12	1.524 ± 0.6815	2.18 ± 0.9749	2.956	P < 0.02

TABLE No. 3 - EFEECT OF SIALOADENECTOMY ON GLYCOGEN CONTENT OF GASTROCNEMIUS MUSCLE

(Glucose mg/100 mg of the muscles)

Values are mean ± S.E.

Animal	No.of Animals	Age in Weeks	Control	Sialoadenectomised	t value	P value
Juvenile	5	8	0.984 ± 0.0555	1.431 ± 0.1635	2.586	P < 0.04
Adult	5	12	1.29 ± 0.5769	2.19 ± 0.2486	3.42	P < 0.01

GRAPH NO. 2

EFFECT OF SIALOADENECTOMY ON GLYCOGEN CONTENT OF MUSCLES.

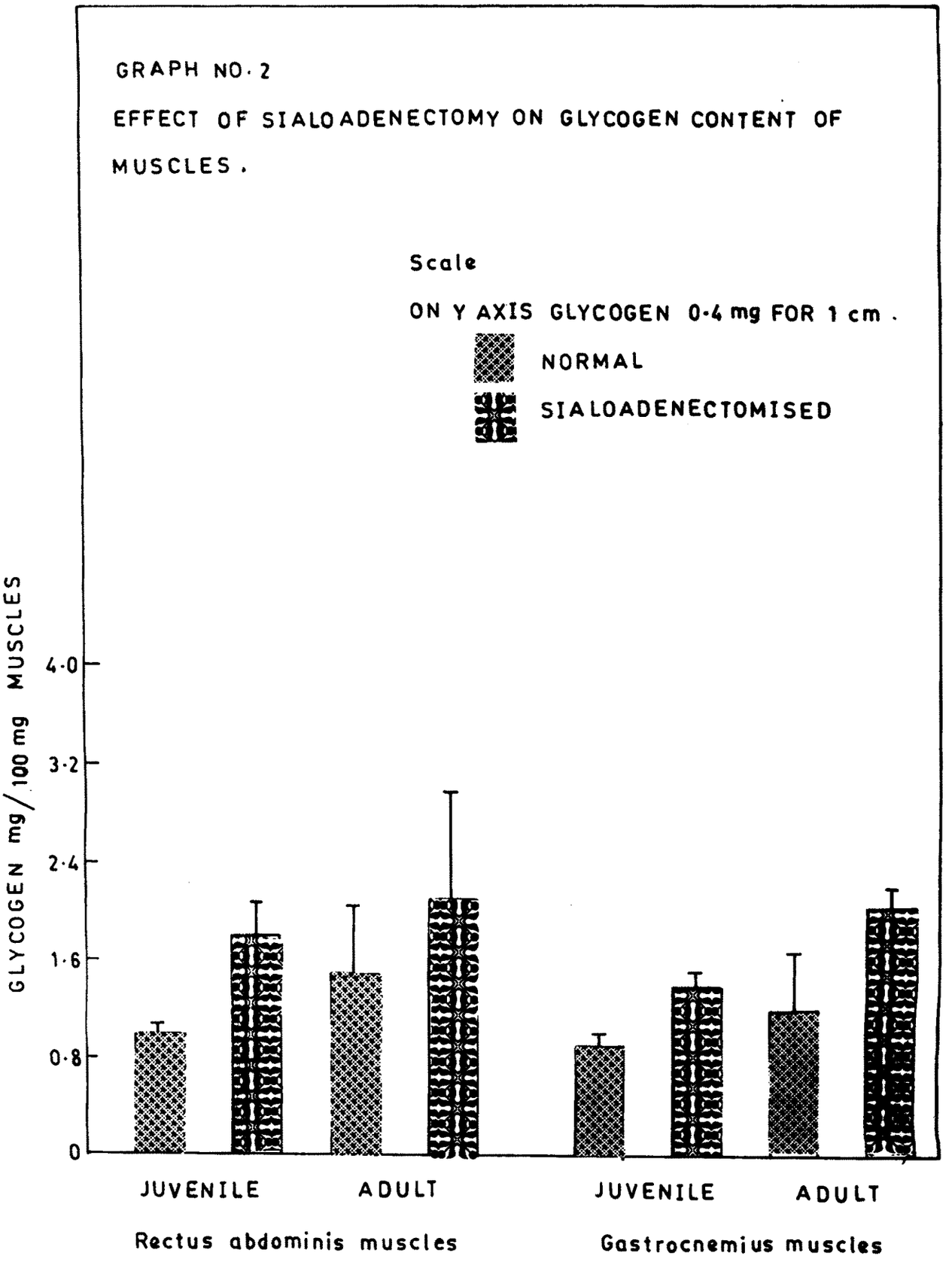
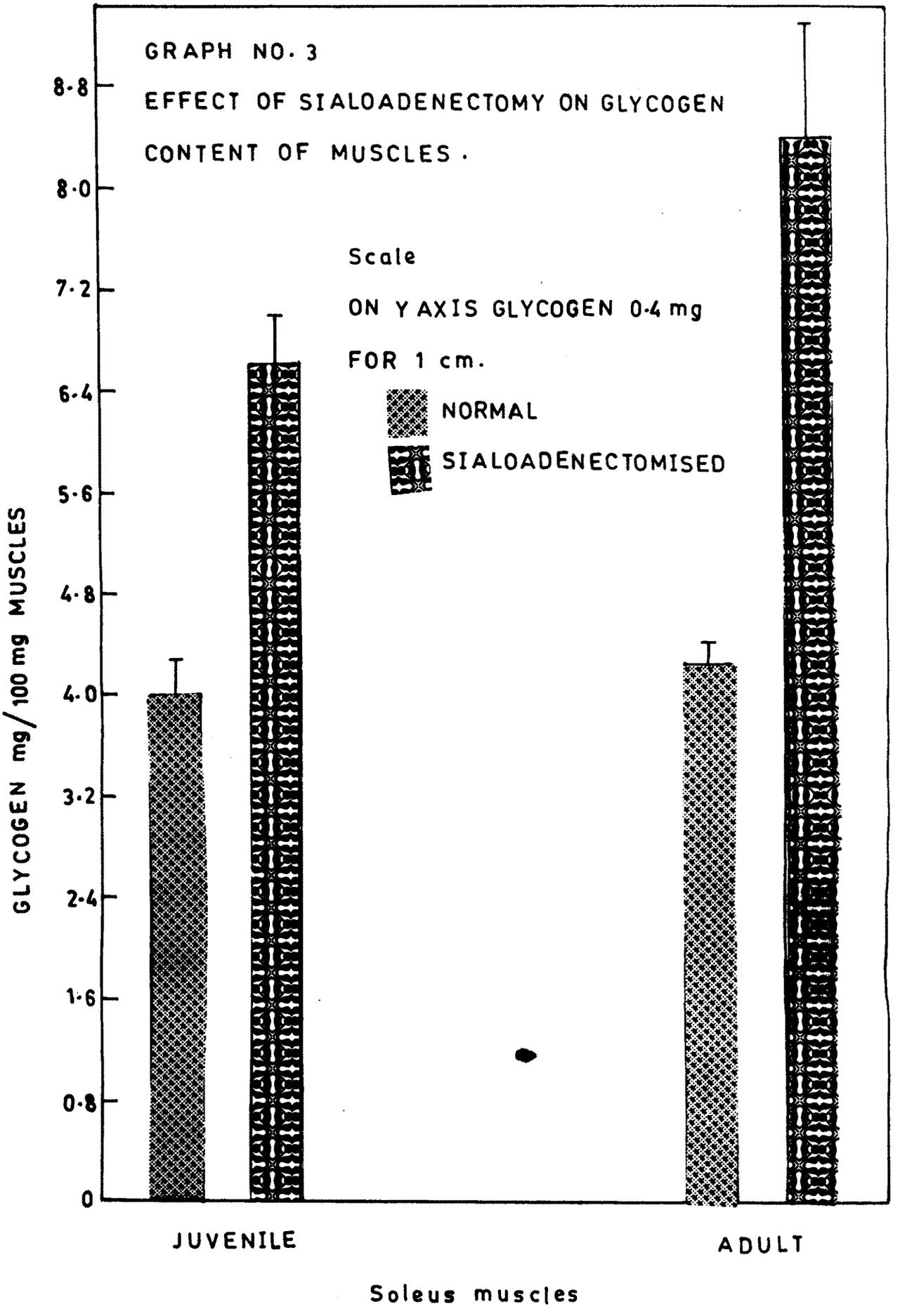


TABLE No.4 - EFFECT OF SIALOADENECTOMY ON GLYCOGEN CONTENT OF SOLEUS MUSCLE

(Glucose mg/100 mg of the muscles)

Values are mean ± S.E.

Animal	No.of Animals	Age in Weeks	Control	Sialoadenectomised	t value	P value
Juvenile	5	8	4.03 ± 0.3078	6.649 ± 0.4903	2.9702	P < 0.02
Adult	5	12	4.354 ± 0.6489	8.494 ± 2.1761	4.0763	P < 0.005


GRAPH NO. 3
EFFECT OF SIALOADENECTOMY ON GLYCOGEN CONTENT OF MUSCLES.
Scale
ON Y AXIS GLYCOGEN 0·4 mg
FOR 1 cm.
NORMAL
SIALOADENECTOMISED
GLYCOGEN mg/100 mg MUSCLES
8·8
8·0
7·2
6·4
5·6
4·8
4·0
3·2
2·4
1·6
0·8
0
JUVENILE
ADULT
Soleus muscles

The increase was significant ($P < 0.02$). The remarkable increase was observed in the soleus muscles of adult sialoadenectomised mice. In the control mice the glycogen content was 4.354 ± 0.6489 which was increased to 8.494 ± 2.1761 ($P < 0.005$) after the removal of submandibular gland.

3) Protein Content in Muscles :

Protein content from three muscles is described in Table No.5, 6, 7 and Graph No.4 and 5. In rectus abdominis it was observed that there was significant increase in the protein content of this muscles after sialoadenectomy. The protein content of juvenile adult rectus abdominis was 7.84 ± 0.4181 and in the sialoadenectomised mice ti was increased to 10.73 ± 0.4537. The increase was significant ($P < 0.002$). In the adult rectus abdominis protein content was 11.61 ± 0.981 and it was increased to 13.77 ± 0.3733 in the sialoadenectomised mice. The increase was significant ($P < 0.001$).

In sialoadenectomised mice, in gastrocnemius muscle there was significant increase in both juvenile and adult. In juvenile adult control gastrocnemius muscle the protein content was 6.13 ± 0.0857 and in operated mice it was increased to 9.33 ± 0.1469 ($P < 0.001$). In adult the protein content of gastrocnemius muscle was 9.53 ± 0.3444 and in sialoadenectomised mice it was increased to 12.62 ± 0.3564 ($P < 0.001$).

The increase in protein content was also observed in

TABLE No.5 - EFFECT OF SIALOADENECTOMY ON PROTEIN CONTENT OF RECTUS ABDOMINIS MUSCLE

(Protein mg/100 mg of muscles)

Values are Mean ± S.E.

Animal	No.of Animals	Age in Weeks	Control	Sialoadenectomised	t value	P value
Juvenile	5	8	7.84 ± 0.4181	10.73 ± 0.4537	4.686	P < 0.002
Adult	5	12	11.61 ± 0.981	13.77 ± 0.3733	5.1168	P < 0.001

TABLE No.6 - EFFECT OF SIALOADENECTOMY ON PROTEIN CONTENT OF GASTROCNEMIUS MUSCLE

(Protein mg/100 mg of muscles)

Values are Mean ± S.E.

Animal	No.of Animals	Age in Weeks	Control	Sialoadenectomised	t value	P value
Juvenile	5	8	6.13 ± 0.0857	9.33 ± 0.1469	7.05	P < 0.001
Adult	5	12	9.53 ± 0.3444	12.62 ± 0.3564	6.232	P < 0.001

GRAPH NO. 4

EFFECT OF SIALOADENECTOMY ON PROTEIN CONTENT OF MUSCLES.

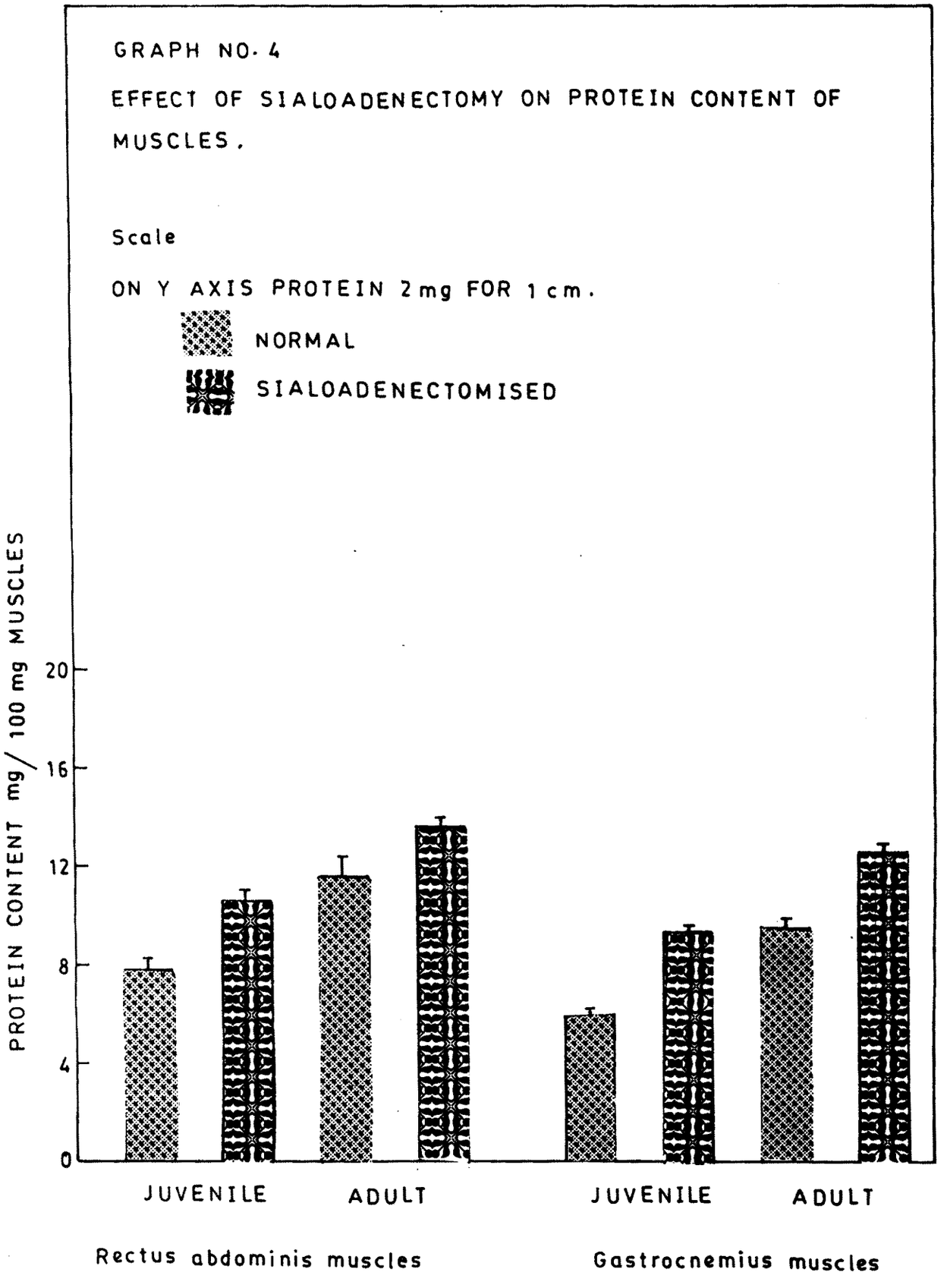


TABLE No. 7 - EFFECT OF SIALOADENECTOMY ON PROTEIN CONTENT OF SOLEUS MUSCLE

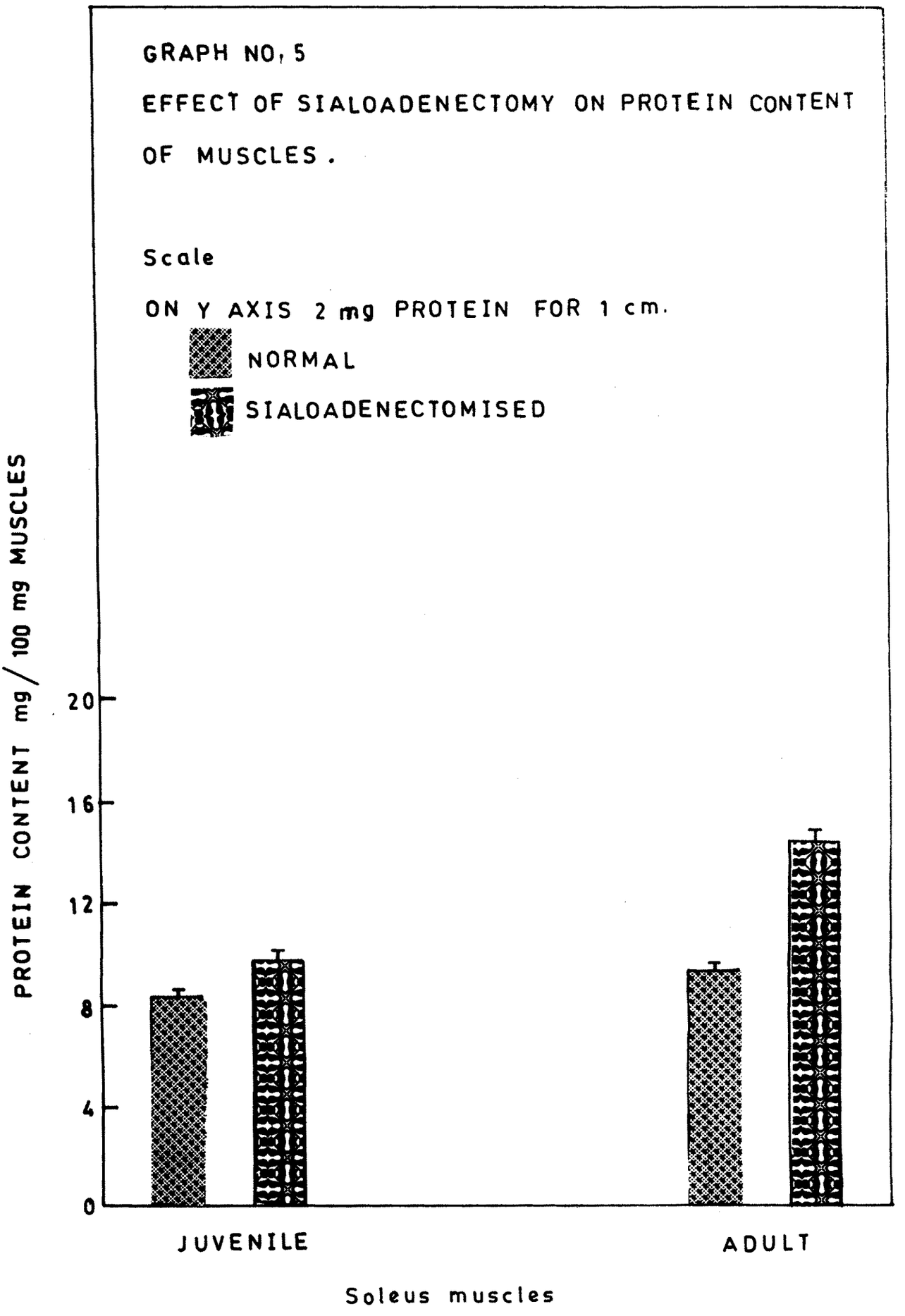
(Protein mg/100 mg of muscles)

Values are Mean ± S.E.

Animal	No.of Animals	Age in Weeks	Control	Sialoadenectomised	t value	P value
Juvenile	5	8	8.29 ± 0.2421	9.84 ± 0.5071	2.7565	P < 0.04
Adult	5	12	9.54 ± 0.3784	14.52 ± 0.5272	7.673	P < 0.001

GRAPH NO, 5

EFFECT OF SIALOADENECTOMY ON PROTEIN CONTENT OF MUSCLES.



soleus muscles of juvenile/adult and adult, the increase was significant. It was more than one and half fold in adult. The protein content of the juvenile adult soleus was 8.29 ± 0.2421 which was increased to 9.84 ± 0.5071 in operated animals ($P < 0.04$). The protein content of control adult soleus muscle was 9.54 ± 0.3784 which was increased to 14.52 ± 0.5272 in sialoadenectomised mice of the same age ($P < 0.001$).

4) Lactate Dehydrogenase :

The lactate dehydrogenase activity was decreased in sialoadenectomised mice compared to control. Lactate dehydrogenase activity m mol h^{-1}, L^{-1} min was estimated from rectus abdominis muscle by using sodium lactate as a substrate and NAD^{+} as coenzyme is described in Table No.8 and Graph No.6. In the rectus abdominis muscle of juvenile adult the enzyme activity was 1.87 ± 0.1518 /m mols h^{-1} , L^{-1} min, and in opereated mice it was decreased to 1.165 ± 0.1449. The decrease was significant ($P < 0.01$). In the adult rectus abdominis muscle of control the enzyme activity was 1.1158 ± 0.1223 and in sialoadenectomised mice of the same age it was decreased remarkably to 0.4939 ± 0.0148. The decrease was significant ($P < 0.001$). Similarly in the gastrocnemius muscle also there was remarkable decrease.

In the gastrocnemius muscle of control juvenile the enzyme activity was 2.425 ± 0.2502 which was decreased to 1.643 ± 0.06135 in sialoadenectomised animal. The decrease was significant ($P < 0.02$) shown in Table No.9 and depicted in

TABLE No.8 - EFFECT OF SIALOADENECTOMY ON LACTATE DEHYDROGENASE ACTIVITY / m mol h^{-1}, L^{-1} min OF RECTUS ABDOMINIS MUSCLE

Values are Mean ± S.E.

Animal	No.of Animals	Age in weeks	Control	Sialoadenectomised	t value	P value
Juvenile	5	8	1.87 ± 0.1518	1.165 ± 0.1449	3.358	P < 0.01
Adult	5	12	1.1158 ± 0.1223	0.4939 ± 0.0148	5.0473	P < 0.001

TABLE No.9 - EFFECT OF SIALOADENECTOMY OF LACTATE DEHYDROGENASE ACTIVITY / m mol h^{-1}, L^{-1} min OF GASTROCNEMIUS MUSCLE

Values are Mean ± S.E.

Animals	No.of Animals	Age in Weeks	Control	Sialoadenectomised	t value	P value
Juvenile	5	8	2.425 ± 0.2502	1.643 ± 0.06135	3.0347	P < 0.02
Adult	5	12	1.1466 ± 0.115	0.638 ± 0.1069	3.242	P < 0.02

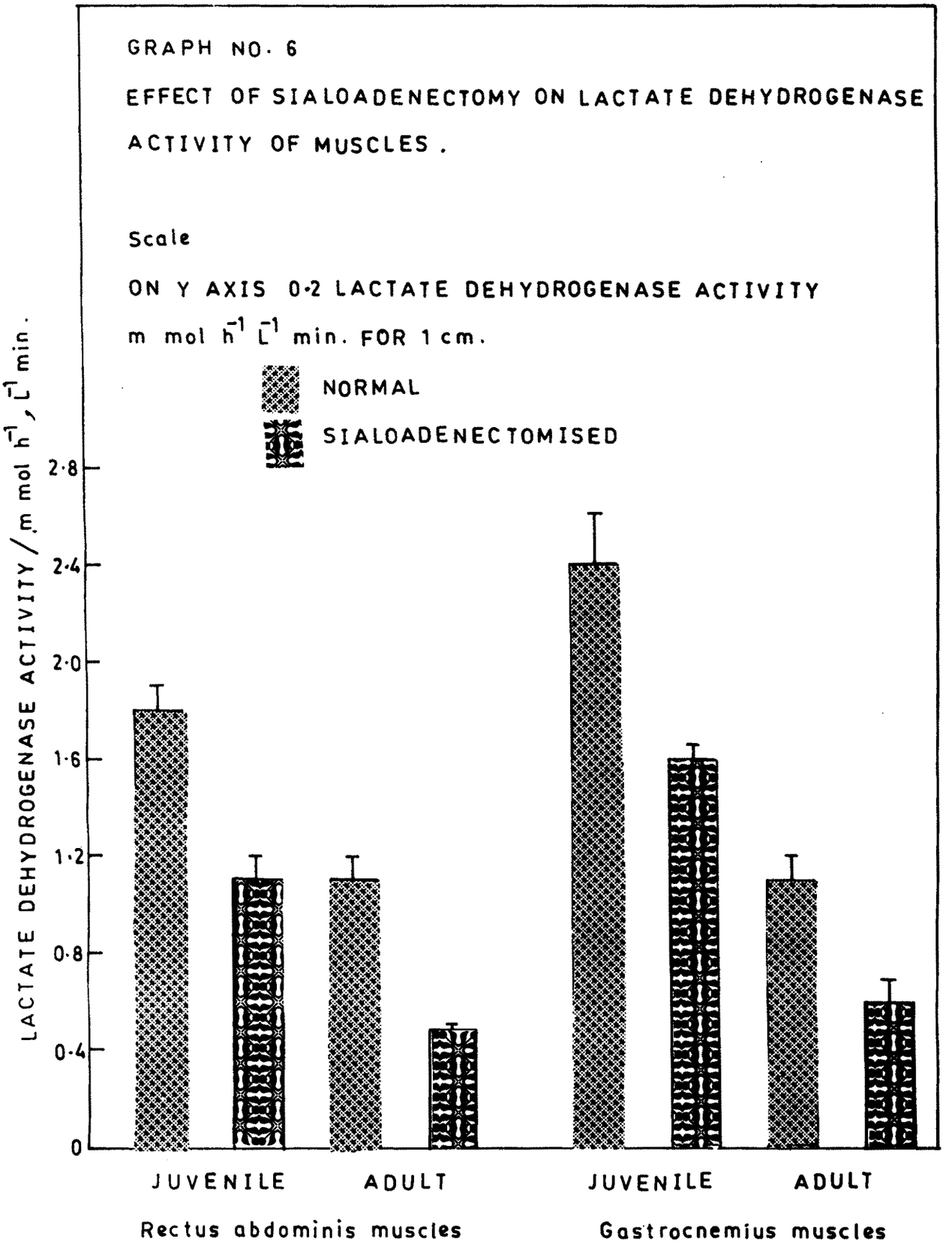
GRAPH NO. 6

EFFECT OF SIALOADENECTOMY ON LACTATE DEHYDROGENASE ACTIVITY OF MUSCLES.

Scale

ON Y AXIS 0·2 LACTATE DEHYDROGENASE ACTIVITY

m mol h^{-1} L^{-1} min. FOR 1 cm.



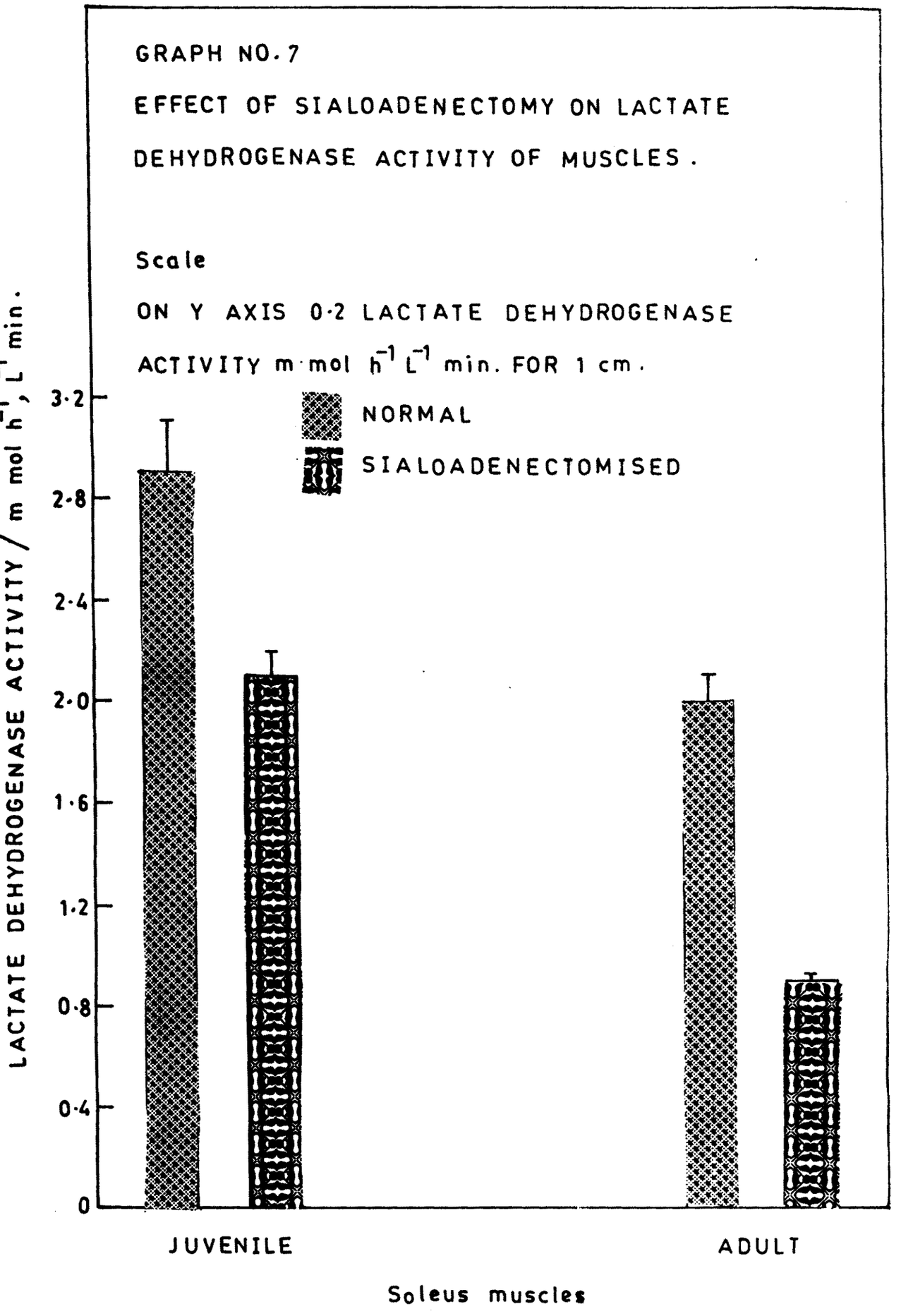
Graph No.6. The enzyme activity in adult control gastrocnemius muscle was 1.1466 ± 0.115, which was significantly decreased to 0.638 ± 0.1069 in the submandibular gland removed mice of the same age group ($P < 0.02$).

Lactate Dehydrogenase activity decreased significantly in the above muscle but that much decrease was not observed in soleus muscle of sialoadenectomised juvenile but it was significant. The enzyme activity in control juvenile adult soleus muscle was 2.937 ± 0.258 and it was decreased to 2.1377 ± 0.1440 in the sialoadenectomised mice. The decrease was significant ($P < 0.04$) shown in Table No.10 and depicted in Graph No.7 whereas the enzyme activity in control adult soleus muscle was 2.063 ± 0.1472 which was significantly decreased to 0.9184 ± 0.0261 ($P < 0.001$) in sialoadenectomised adult mice.

TABLE No.10 - EFFECT OF SIALOADENECTOMY ON LACTATE DEHYDROGENASE ACTIVITY/ m mol h^{-1}, L^{-1} min OF SOLEUS MUSCLE

Values are Mean ± S.E.

Animal	No.of Animals	Age in Weeks	Control	Sialoadenectomised	t value	P value
Juvenile	5	8	2.937 ± 0..258	2.1377 ± 0.1440	2.7030	P < 0.04
Adult	5	12	2.063 ± 0.1472	0.9184 ± 0.0261	7.6548	P < 0.001


GRAPH NO. 7
EFFECT OF SIALOADENECTOMY ON LACTATE DEHYDROGENASE ACTIVITY OF MUSCLES.
Scale
ON Y AXIS 0·2 LACTATE DEHYDROGENASE ACTIVITY m mol h⁻¹ L⁻¹ min. FOR 1 cm.
NORMAL
SIALOADENECTOMISED
LACTATE DEHYDROGENASE ACTIVITY / m mol h⁻¹, L⁻¹ min.
3·2
2·8
2·4
2·0
1·6
1·2
0·8
0·4
0
JUVENILE
ADULT
Soleus muscles

5) Alkaline Phosphatase :

The alkaline phosphatase activity was estimated by using p-nitrophenyl phosphate as a substrate. The enzyme activity was expressed as µ mols of p-nitrophenol /mg of the protein. the highest enzyme activity was observed in soleus muscles. In juvenile adult sialoadenectomised mice there was increase in the enzyme activity of all muscles but it was not significant in gastrocnemius muscles. The increase was remarkable in the activity of sialoadenectomised adult muscles. In the rectus abdominis muscle of juvenile the enzyme activity was 2.69 ± 0.00816 and in the operated animal it was increased to 3.69 ± 0.0339. The increase was highly significant ($P < 0.001$) shown in Table No.11 and Graph No.8. In the rectus abdominis muscle of adult the enzyme activity was 4.37 ± 0.258 and it was increased to 5.94 ± 0.0326 in the operated animals of same age. The increase was significant ($P < 0.001$).

In gastrocnemius muscle of the juvenile adult the enzyme activity was 4.66 ± 0.0286 and in sialoadenectomised mice it was increased to 4.67 ± 0.06944. The increase was non-significant shown in Table No.12. In the adult gastrocnemius muscle the enzyme activity was 4.84 ± 0.03265. It was increased to 9.38 ± 0.02054 in the sialoadenectomised mice of the same age group. The increase was highly significant ($P < 0.0005$).

TABLE No.11 - EFFECT OF SIALOADENECTOMY ON ALKALINE PHOSPHATASE ACTIVITY µmole OF p-NITROPHENOL/mg OF PROTEIN OF RECTUS ABDOMINIS MUSCLE.

(Values are Mean ± S.E.)

Animals	No.of Animals	Age in Weeks	Control	Sialoadenectomised	t value	P value
Juvenile	5	8	2.69 ± 0.00816	3.69 ± 0.0339	42.72	P < 0.001
Adult	5	12	4.37 ± 0.258	5.94 ± 0.0326	8.9966	P < 0.001

TABLE No.12 - EFFECT OF SIALOADENECTOMY ON ALKALINE PHOSPHATASE ACTIVITY µ mole OF p-NITROPHOENOL/mg OF PROTEIN OF GASTROCNEMIUS MUSCLE

Values are Mean ± S.E.

Animal	No.of Animals	Age in Weeks	Control	Sialoadenectomised	t value	P value
Juvenile	5	8	4.66 ± 0.0286	4.67 ± 0.06944	0.1986	Non-Significant
Adult	5	12	4.84 ± 0.03265	9.38 ± 0.02054	175.40	P 0.0005

GRAPH NO. 8

EFFECT OF SIALOADENECTOMY ON ALKALINE PHOSPHATASE ACTIVITY OF MUSCLES.

Scale

ON Y AXIS ALKALINE PHOSPHATASE ACTIVITY

μ MOLE p-NITROPHENYL PHOSPHATE / mg PROTEIN.

(1) NORMAL

(2) SIALOADENECTOMISED

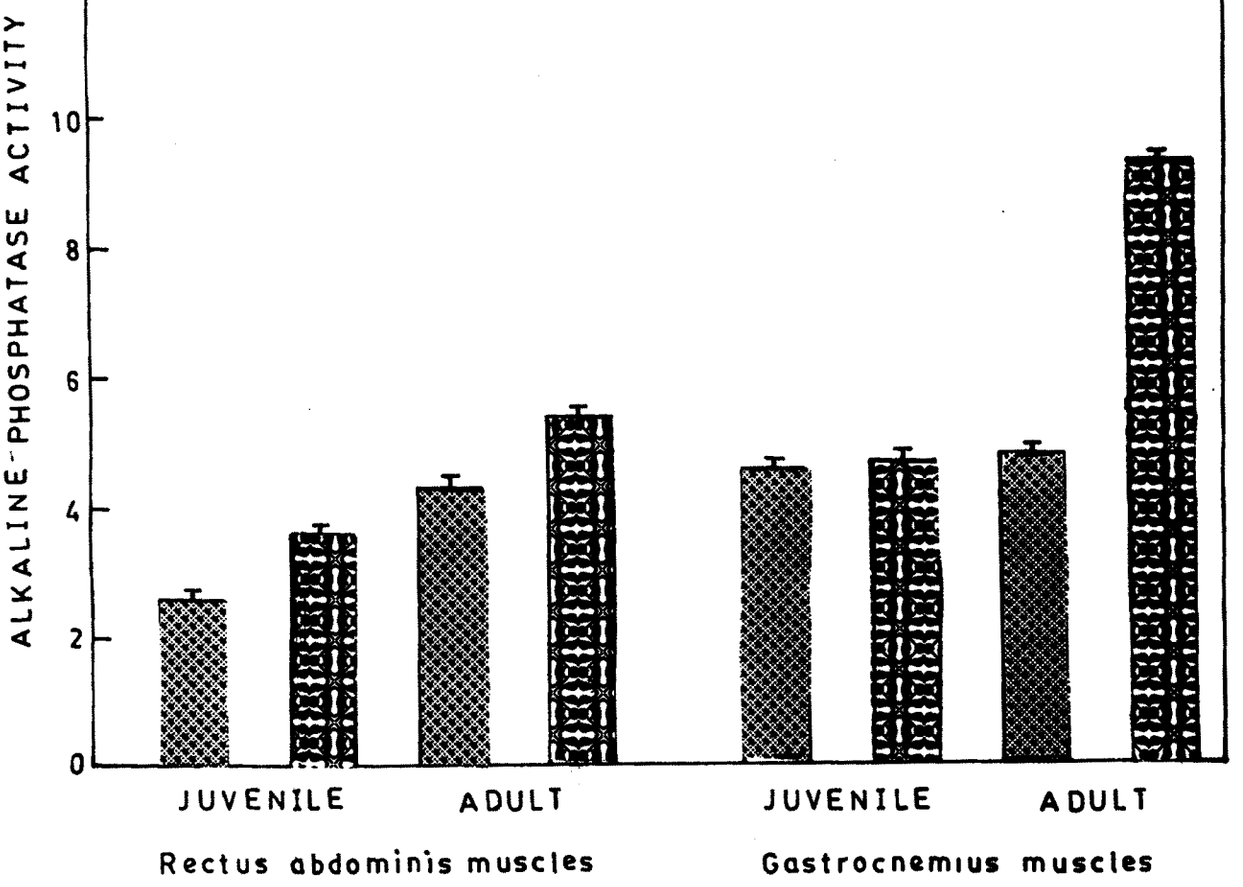
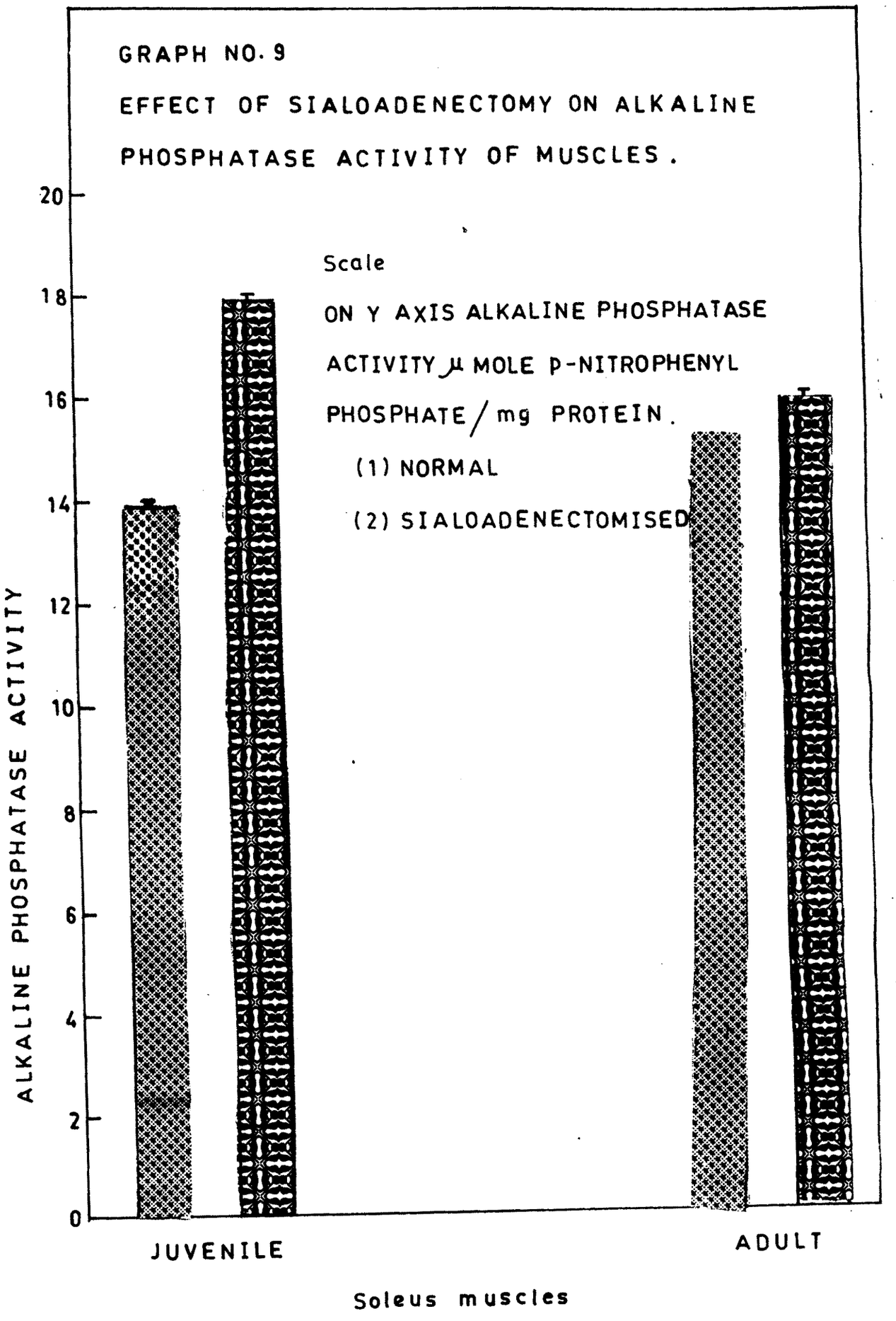


TABLE No. 13 - EFFECT OF SIALOADENECTOMY ON ALKALINE PHOSPHATASE ACTIVITY μ mole of p-NITROPHENOL/mg OF PROTEIN OF SOLEUS MUSCLE

(Values are Mean ± S.E.)

Animal	No.of Animals	Age in Weeks	Control	Sialoadenectomised	t value	P value
Juvenile	5	8	13.99 ± 0.00471	17.98 ± 0.009427	564.23	P < 0.0005
Adult	5	12	15.46 ± 0.05716	15.95 ± 0.02054	281.98	P < 0.0005


GRAPH NO. 9
EFFECT OF SIALOADENECTOMY ON ALKALINE PHOSPHATASE ACTIVITY OF MUSCLES.
Scale
ON Y AXIS ALKALINE PHOSPHATASE ACTIVITY μ MOLE P-NITROPHENYL PHOSPHATE / mg PROTEIN.
(1) NORMAL
(2) SIALOADENECTOMISED
ALKALINE PHOSPHATASE ACTIVITY
0
2
4
6
8
10
12
14
16
18
20
JUVENILE
ADULT
Soleus muscles

The enzyme activity in the control juvenile adult soleus muscle was 13.99 ± 0.00471 and in the operated mice of the same age group it was increased to 17.98 ± 0.009427. The increase as highly significant ($P < 0.0005$). In the adult the enzyme activity was 15.46 ± 0.05716 it was increased to 15.95 ± 0.02054 in the sialoadenectomised mice. The increase was highly significant $P < 0.0005$) which is described in Table No.113 and Graph No.9.

6) Electrophoretic Separation of Lactate Dehydrogenase :

The separation of lactate dehydrogenase isoenzyme in skeletal muscle was revealed by polyacrylamide gel electrophoresis and histochemical staining, from three skeletal muscles (Rectus abdominis, Gastrocnemius, Soleus) of juvenile adult, (i.e. 2 months of age) adult (3 months) sialoadenectomised juvenile adult and adult mice. The flow of migration was from cathode to anode. Lactate dehydrogenase was separated into five or four bands. Bands were labelled from cathode to anode. The band at origin is labelled as 5 th band, which was intense and broad.

A) Rectus Abdominis Muscle :

1) Juvenile adult : Fig. and Scan 1 and 2 show the distribution of lactate dehydrogenease in the rectus abdominis of juvenile adult mice of 2 months old. In control mice (2 months old) lactate dehydrogenase was separated into four clear bands

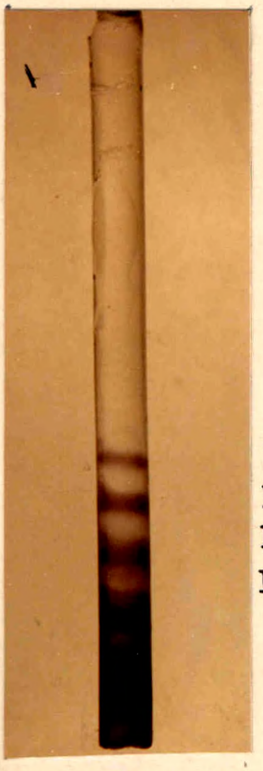


Fig. No. 1

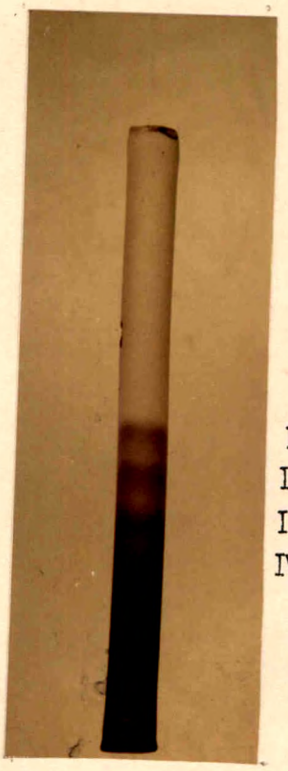


Fig. No. 2

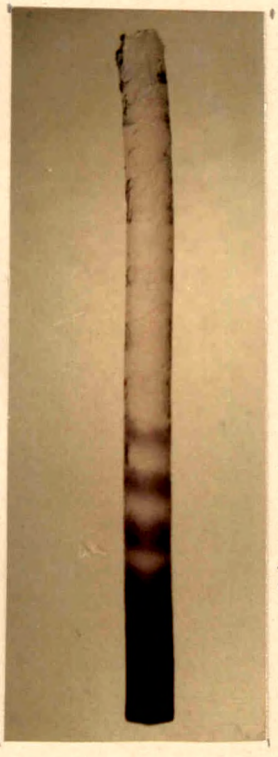


Fig. No. 3

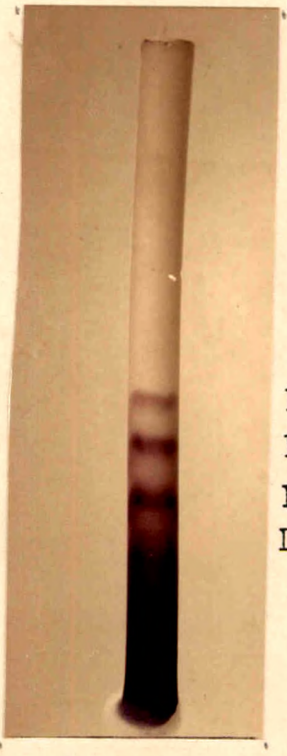
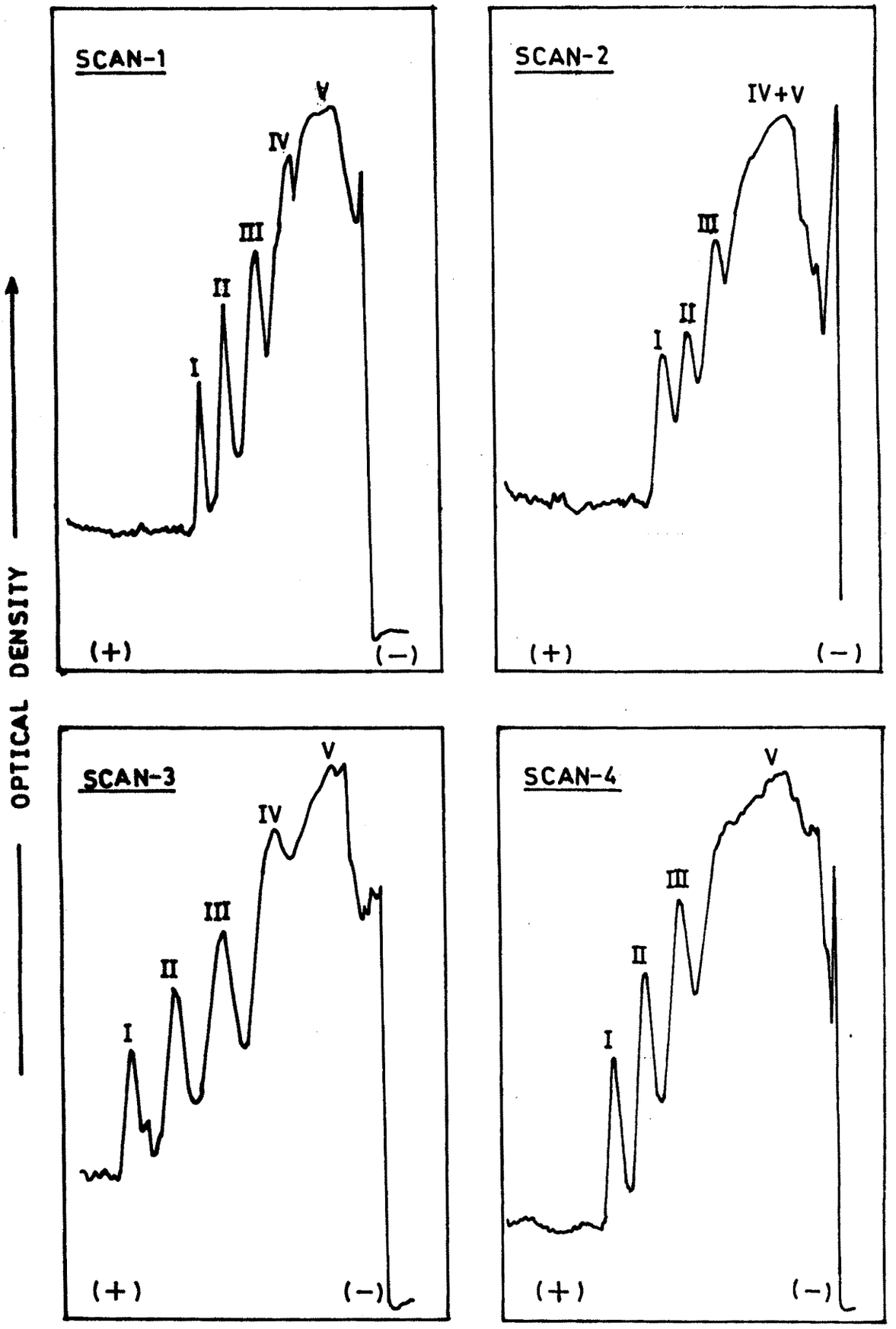


Fig. No. 4


SCAN-1
I
II
III
IV
V
(+)
(−)
SCAN-2
I
II
III
IV+V
(+)
(−)
SCAN-3
I
II
III
IV
V
(+)
(−)
SCAN-4
I
II
III
V
(+)
(−)
OPTICAL DENSITY

i.e. 1, 2, 3, 5. The band 4th was just appearing, but not yet clearly separated from the 5th band. In sialoadenectomised mice there was change in the separation of the enzyme where band 1,2 and 5 were clearly separated and band 3 was just appear and not shifted from the 5th band,

2) Adult (3 months old) : Fig and Scan No.3 and 4 show the separation of lactate dehydrogenase of the rectus abdominis of adult and sialoadenectomised adult mice. In adult LDH was separated into five bands all the bands were intensely stained. Fig.3, Scan No.3. But in operated mice LDH was separated into four bands (Fig.4, Scan 4), band No.4 was absent. Intensity of the enzyme which was revealed histochemically also showed less enzyme activity at band 1 to 3, but it was intense at band No.5.

B) Gastrocnemius Muscle :

1) Juvenile Adult : Fig. and Scan 5 and 6 show the separation of lactate dehydrogenase in the gastrocnemius muscle of juvenile adult mice of 2 months old, lactate dehydrogenase was separated into 1, 2, 3 clear bands and 4 and 5 bands are mixed. In sialoadenectomised mice there was alteration in the separation of LDH, where 1 and 2 bands were very clearly separated, 3rd band was also separated but yet in complex with 4th and 5th band; Demarkation could not be possible in between 4th and 5th bands. The gel scanning clearly showed absence

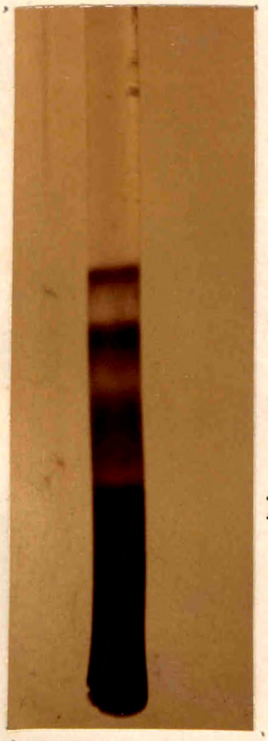


Fig. No. 5

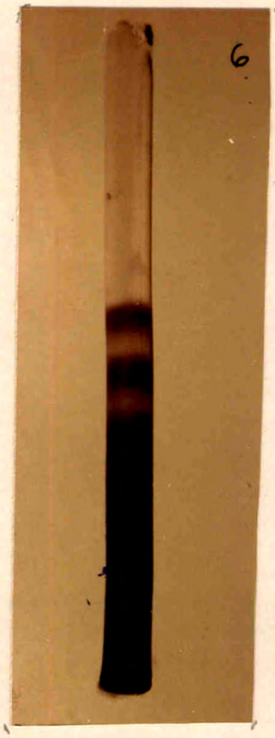


Fig. No. 6

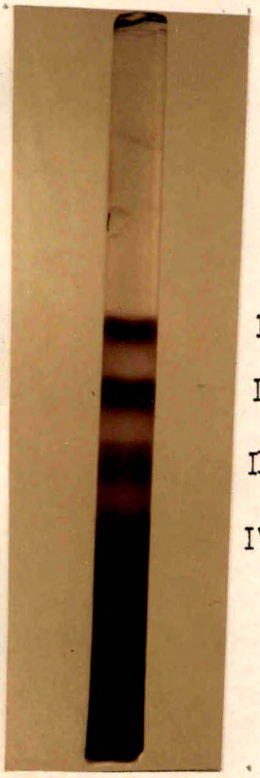


Fig. No. 7

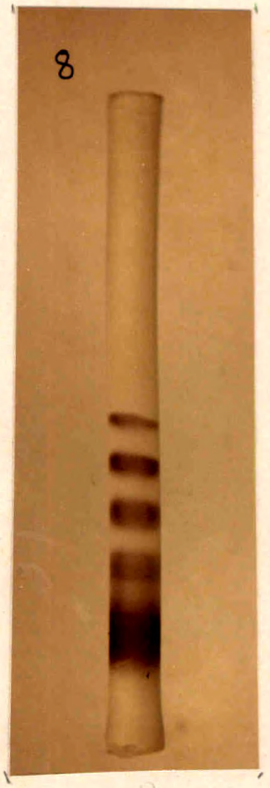
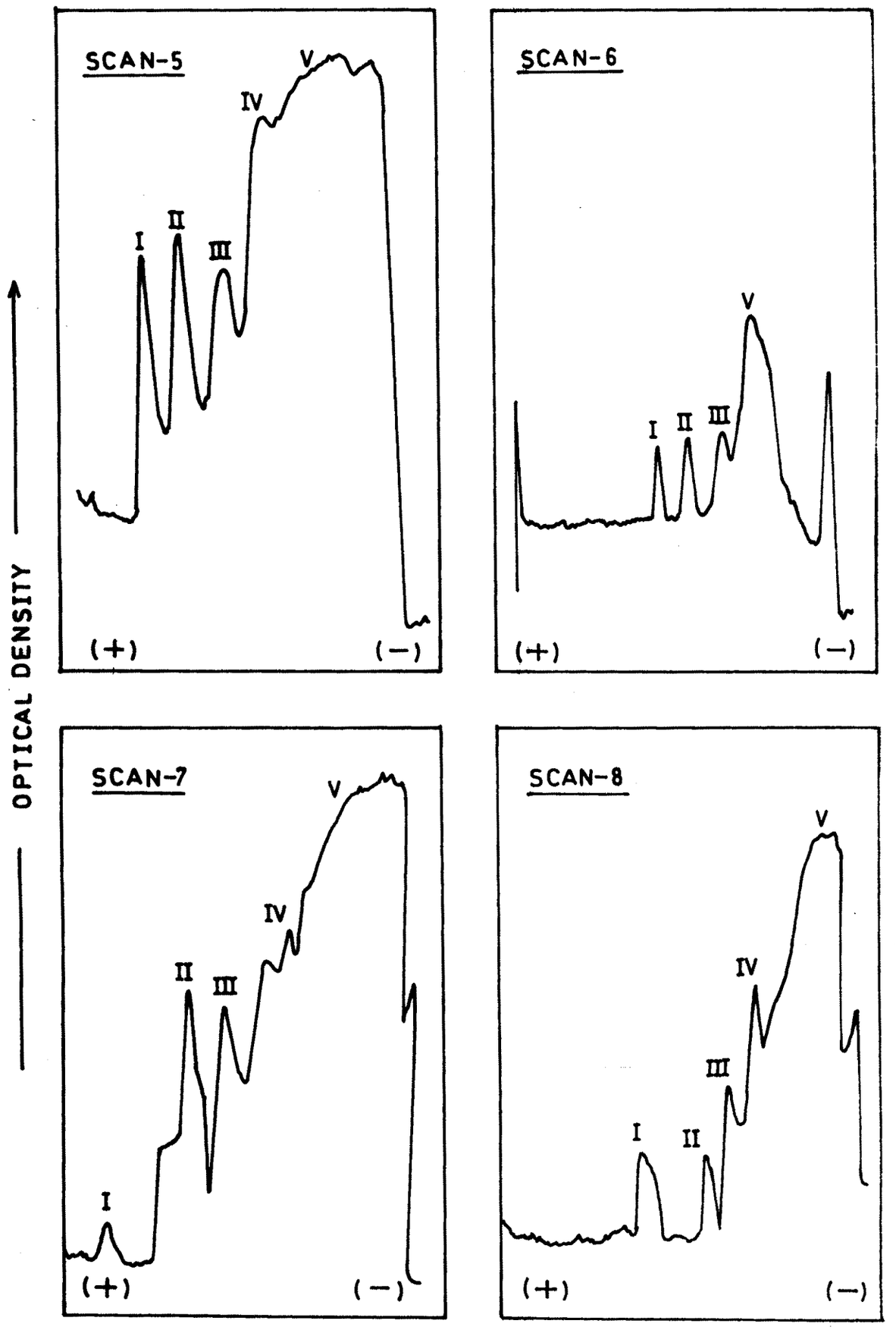


Fig. No. 8


SCAN-5
I
II
III
IV
V
(+)
(−)
SCAN-6
I
II
III
V
(+)
(−)
SCAN-7
I
II
III
IV
V
(+)
(−)
SCAN-8
I
II
III
IV
V
(+)
(−)
OPTICAL DENSITY

of 4th band.

2) Adult (3 months old) : Fig and Scan 7 and 8 show the separation of LDH of the gastrocnemius muscle of adult and sialo-adenectomised adult mice. In adult mice LDH was separated into five bands (Fig. and Scan 7). All bands were intensely stained. In sialoadenectomised adult mice bands were clearly separated into 3 bands 4th and 5th bands were mixed but even 4th band could show its separate activity.

C) Soleus Muscle :

1) Juvenile adult : Fig. and Scan No.9, 10 show separation of lactate dehydrogenase from soleus muscles of juvenile- adult, control and sialoadenectomised juvenile adult. In the soleus muscle of juvenile adult control all five bands were clear and sharp in operated mice 1, 2, 3 and 5 were clear and 4th was appeared as a sub band of 5th. The intensity of bands was less in sialoadenectomised mice.

2) Adult : Separation of lactate dehydrogenase in soleus muscle is described in Figs. and scan No.11 and 12. In soleus muscle of adult control and sialoadenectomised mice there were 5 bands. They were very well separated. In the operated mice 4th band was very short appear as a shoulder band of the 5th band.

***

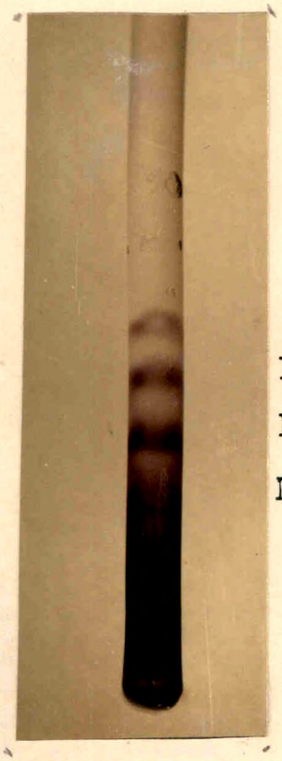


Fig. No. 9

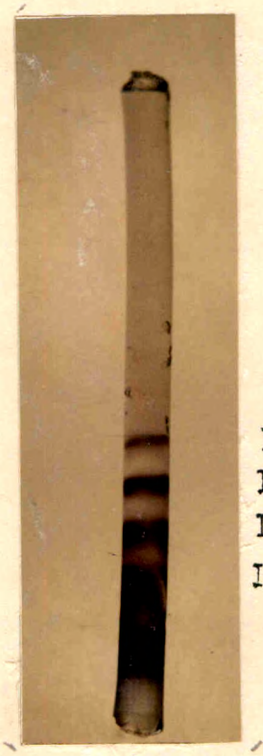


Fig. No. 10

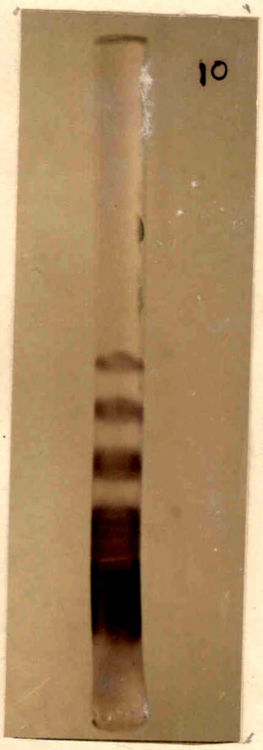


Fig. No. 11

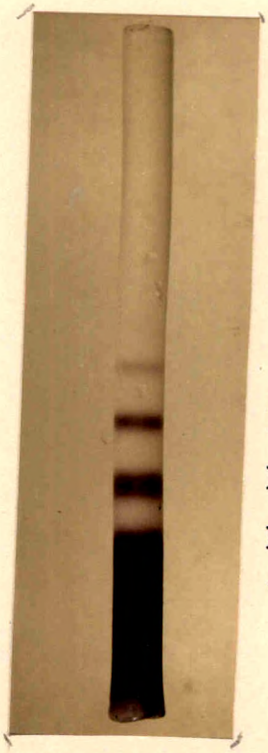
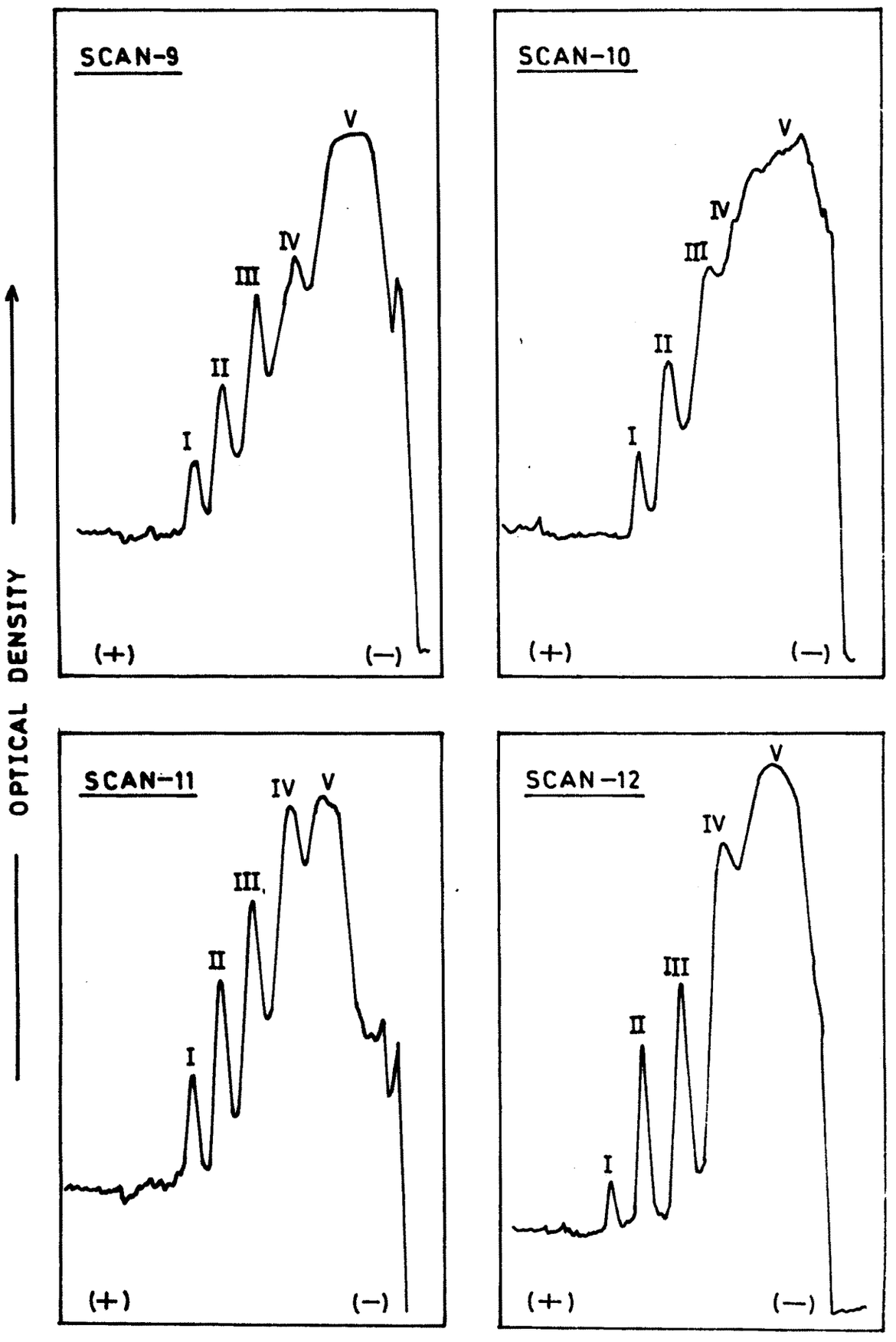


Fig. No. 12


SCAN-9
I
II
III
IV
V
(+)
(−)
SCAN-10
I
II
III
IV
V
(+)
(−)
SCAN-11
I
II
III
IV
V
(+)
(−)
SCAN-12
I
II
III
IV
V
(+)
(−)
OPTICAL DENSITY