PROTEIN POLYMORPHISM IN NORTHERN POPULATIONS OF FIELD MICE (*APODEMUS SYLVATICUS* L.)

Vol. 2. : Illustrations

A THESIS

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by

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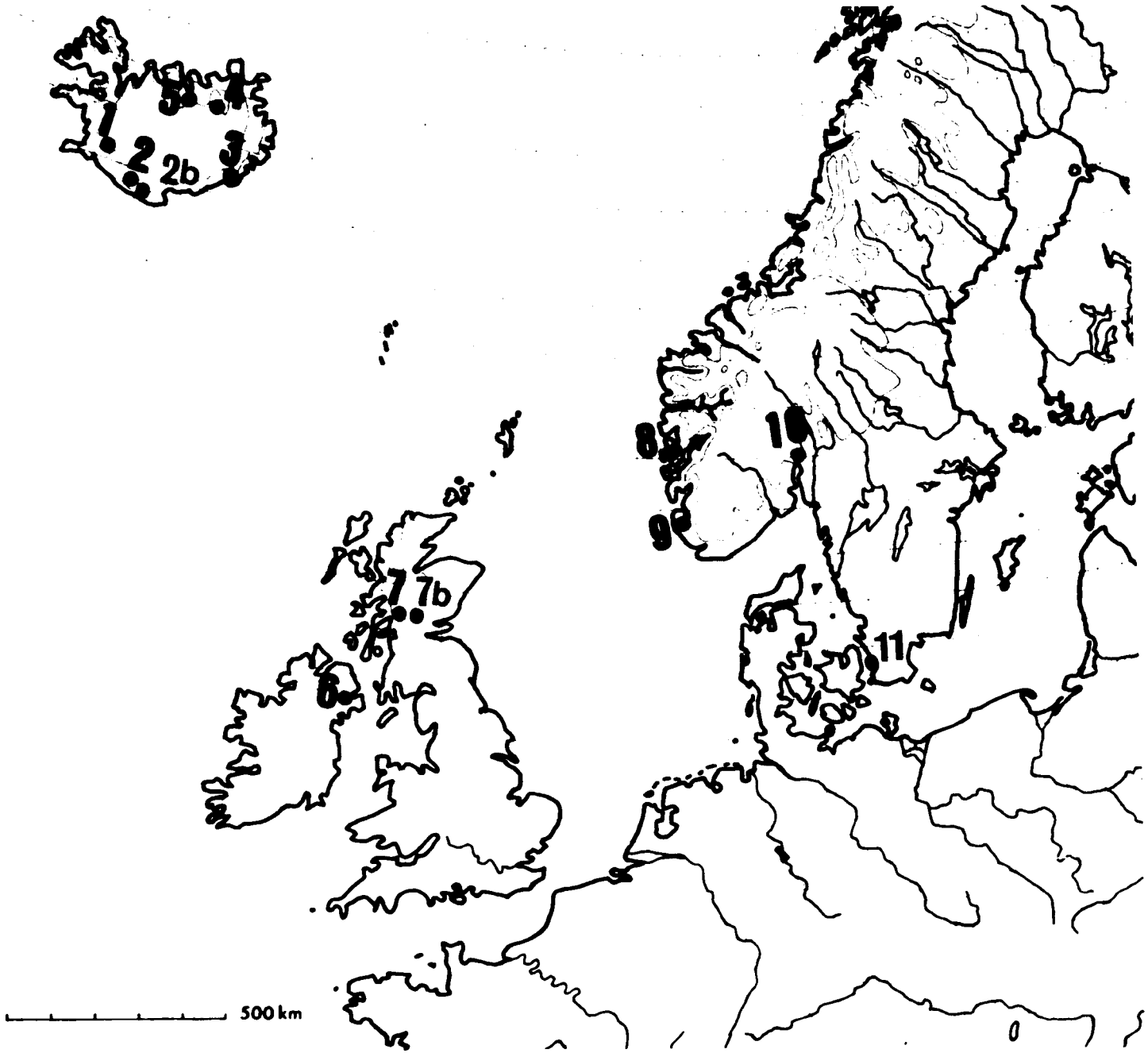
LIST OF ILLUSTRATIONS



Map 1

MAP 1.

Places where Apodemus sylvaticus were caught.

Iceland:

1. Laugarvatn area

2. Stóra-Mörk area

2b. Skálakot area

3. Hornafjörður

4. Mývatn area

5. Eyjafjörður.

Ireland:

6. Belfast area

Scotland:

7. Glasgow area

7b. Cumbernauld area

Norway:

8. Bergen

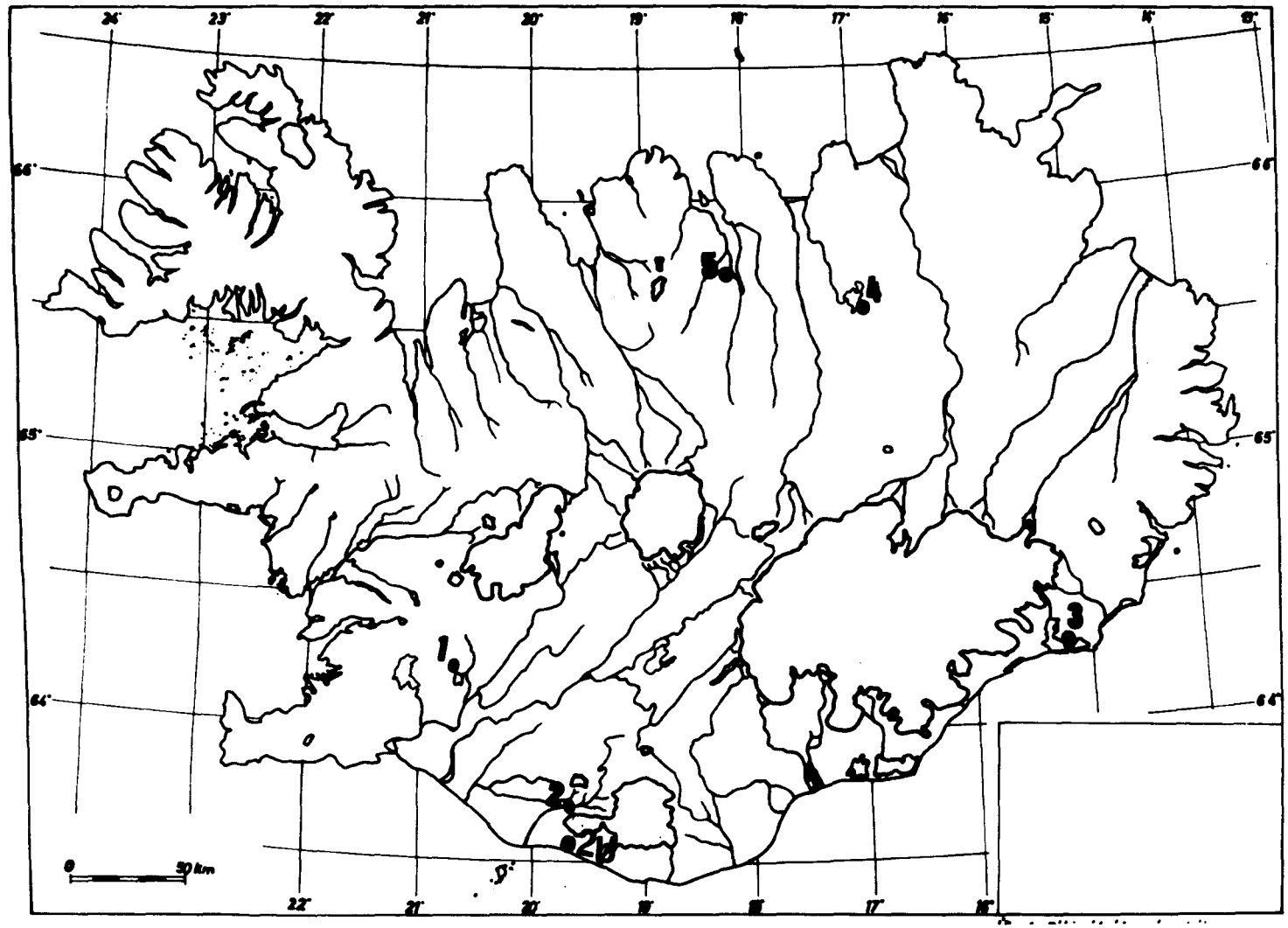
9. Jaeren

10. Oslo area

Sweden

11. Lund.

Map 2



MAP 2.

A bigger map of Iceland to show the same places as in Map 1. The places of greatest interest are 1 and 2.

Fig. 1

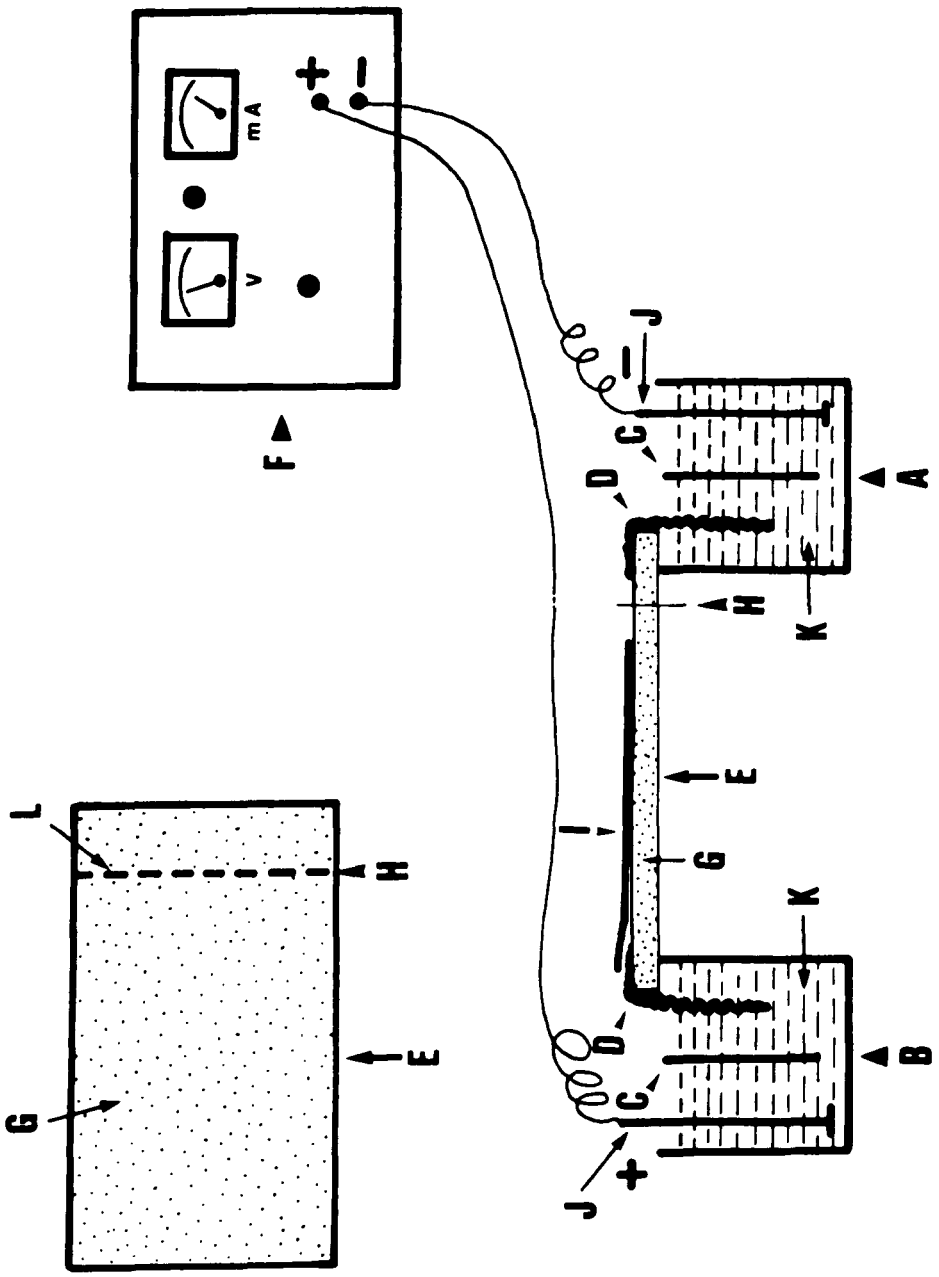


FIG. 1

A drawing to show outlay of electrophoretic apparatus.

A and B	Vessels containing buffer
C	Partition in Vessel
D	Lint Wicks
E	Perspex Tray
F	Power Unit
G	Starch Gel
H	Slit of Application
I	"Handiwrap" Cover
J	Electrodes (platinum)
K	Buffer
L	Sample Holder, made of filter paper.

THE FOLLOWING ABBREVIATIONS APPLY TO THE FIGURES.

E	epididymis homogenates
H	heart muscle homogenates
K	kidney homogenates
L	liver homogenates
M	thigh muscle homogenates
R	red cell lysates
S	serum
Sp	spleen homogenates
T	testis homogenates.

Fig.2

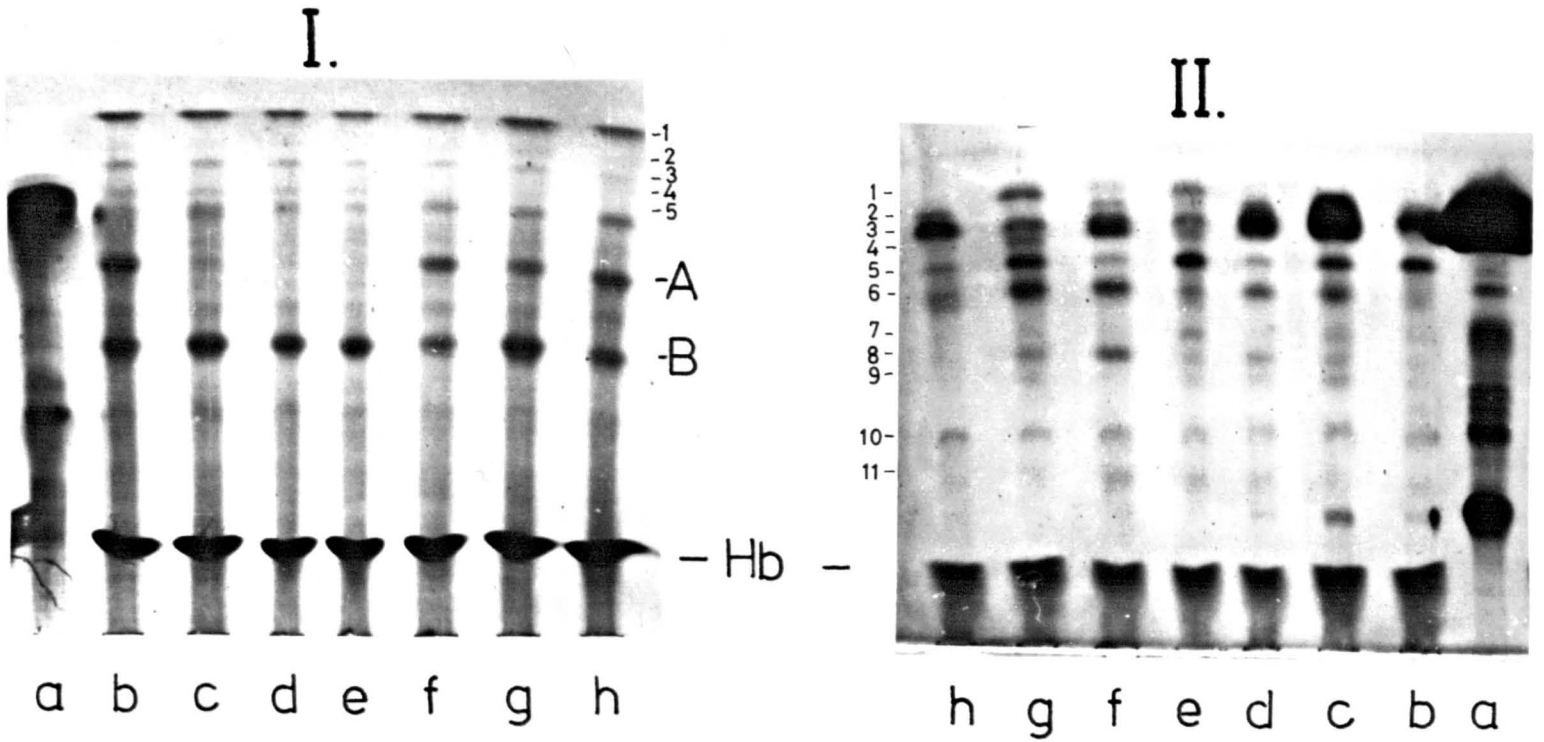


FIG. 2

I. Showing proteins of red-cell lysates from Apodemus sylvaticus. Up to 14 protein bands can be seen, but A and B are of a special interest (see Tables).

II. Same as I. except stained for esterases. Polymorphisms can be seen in fractions 1-4, and fractions 7-8.
Note the polymorphisms in esterases, but the uniformity in the protein pattern except for A and B.

a, serum of Apodemus sylvaticus

b - h, red-cell lysates.

Fig.3

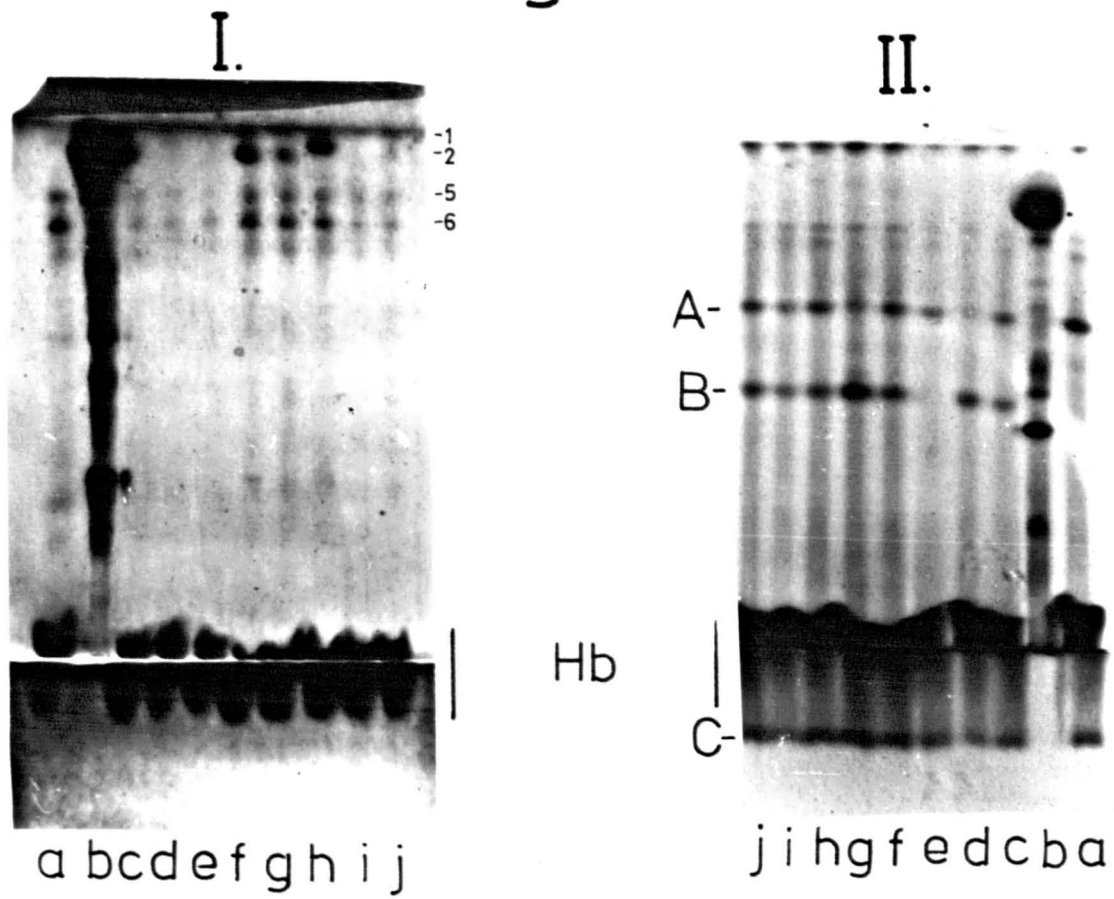


FIG. 3.

Shows comparison of the esterase pattern (I) and the protein pattern (II) of red-cell lysates of Apodemus sylvaticus. Note that there are no esterase fractions overlapping proteins A and B.

a, c - j : red-cell lysates from Icelandic Apodemus sylvaticus

b : serum sample.

I. One slice of the gel stained with a mixture of 1- and 2-naphthyl acetate as substrate.

II. The other half of I stained with nigrosine-amidoblack.

Tris-citrate pH 7.6.

Fig.4

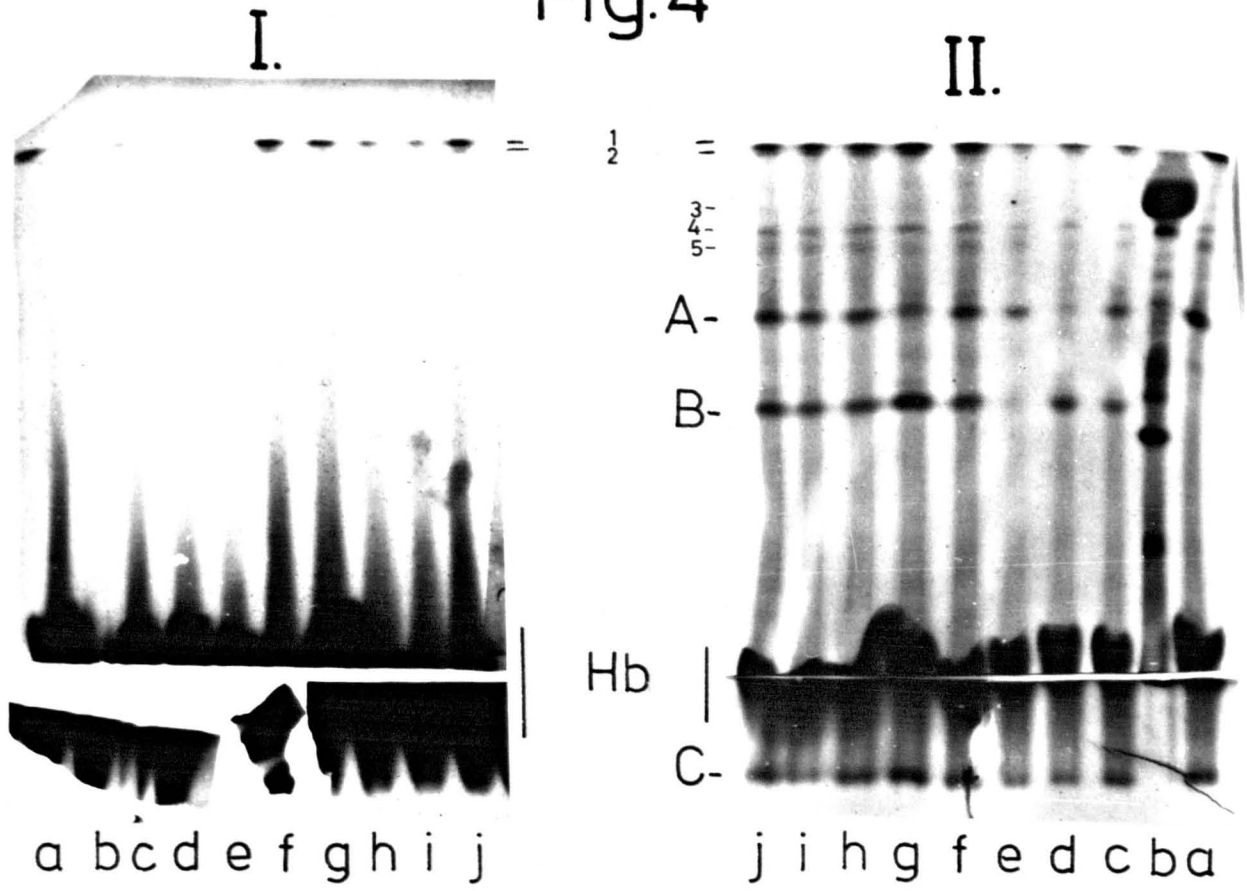


FIG. 4.

The same as fig. 3, except I is stained for peroxidase-activity. Note that fractions 1 and 2 as well as the Hb are stained. Fractions A and B show no reaction on I.

I. Stained for peroxidase-activity with O-dianisidine.

II. The other half of I stained with nigrosine-amidoblack.

Fig. 5

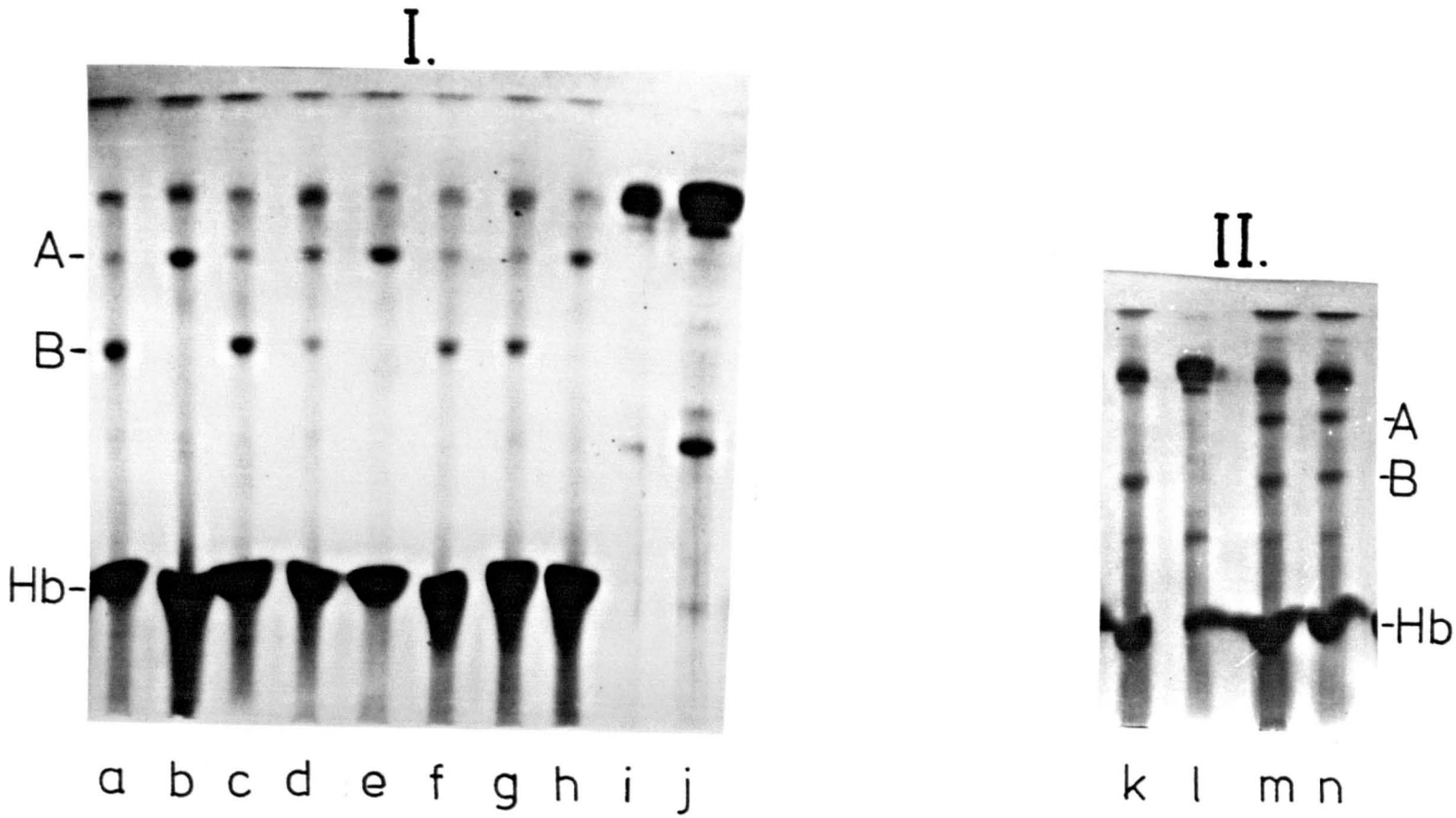


FIG. 5

To show protein bands of red-cell lysates, A and B.

I. a - h : red-cell lysates of Icelandic Apodemus sylvaticus
   i - j : serum samples.

II. k and m : Norwegian red-cell lysates.
   l : Serum sample
   n : Irish red-cell lysates.

Tris-citrate pH 7.6.

Fig. 6

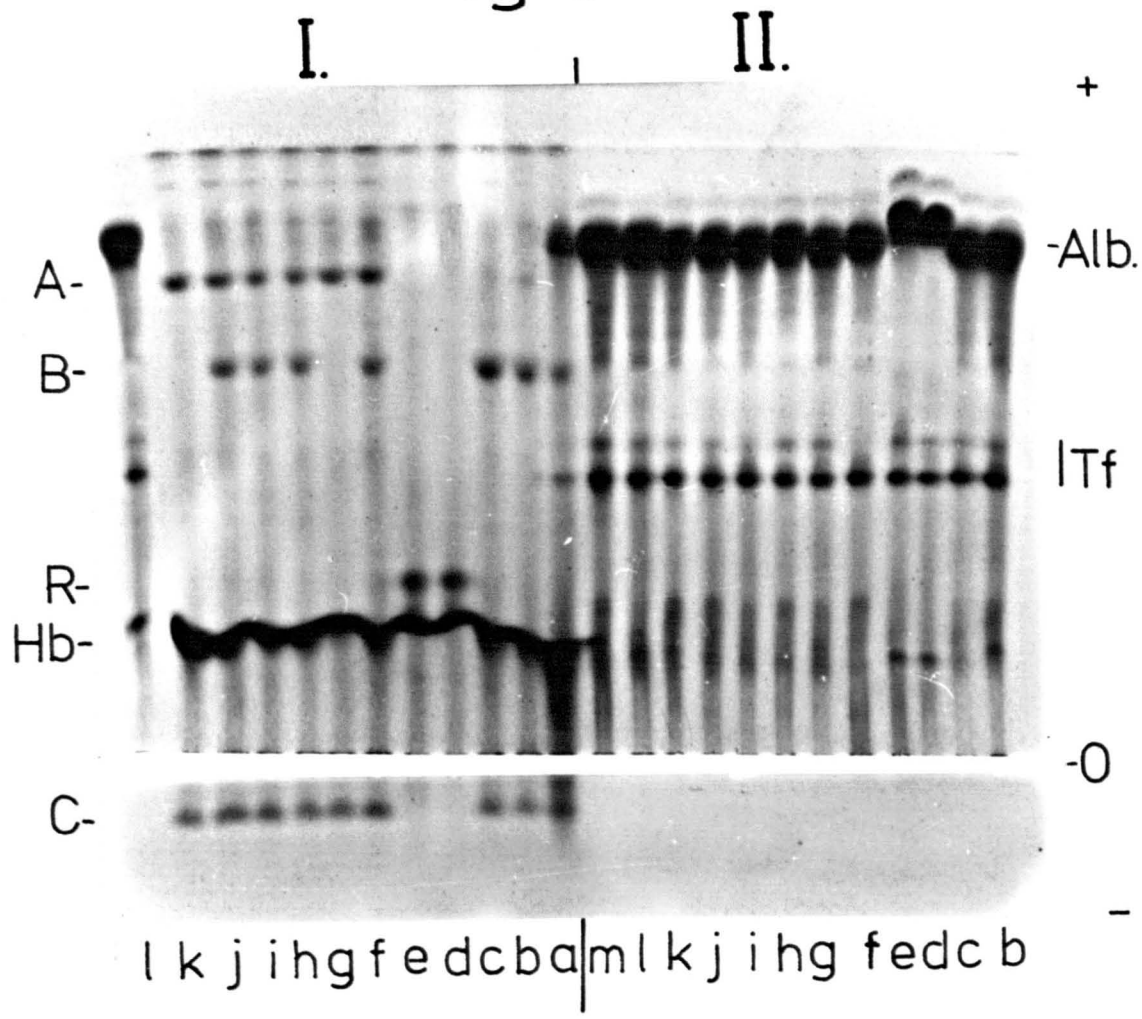


FIG. 6.

To show red-cell proteins A, B, and C in *Apodemus sylvaticus* and R in wild *Mus musculus*.

I. Red-cell lysates.

- a : Norwegian red-cell lysates, unwashed cells
- b, c and f : Scottish red-cell lysates
- d and e : red-cell lysates from wild *Mus musculus*
- g,h,i,j and k: Icelandic samples
- l and m : 1 year old sera from Icelandic *Apodemus sylvaticus*

II. Serum samples.

Same as I but sera instead of red-cell lysates.

Fig.7

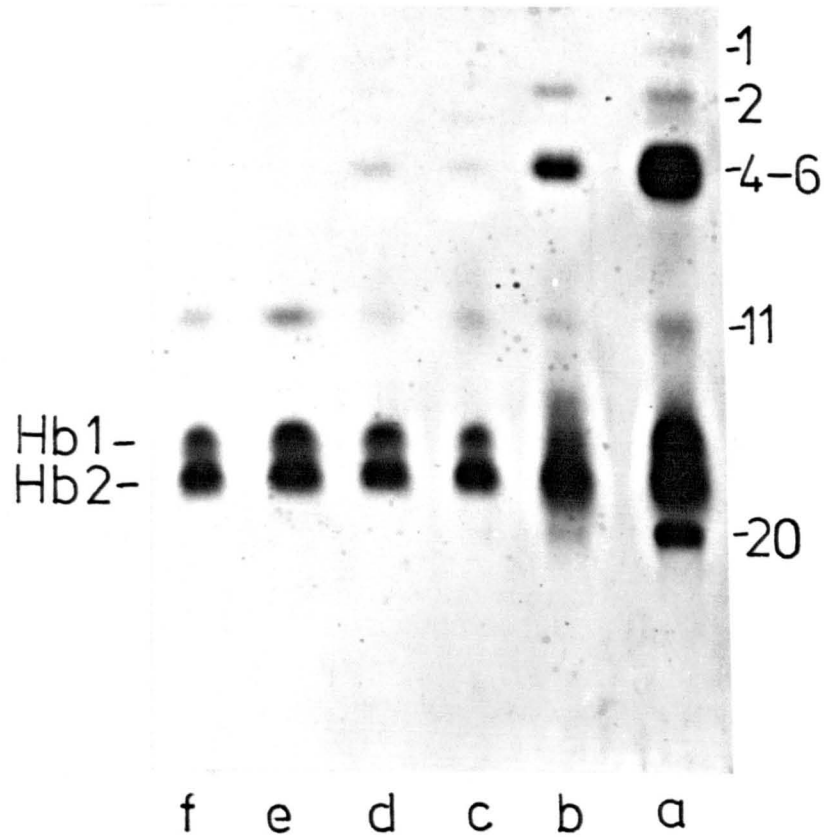


FIG. 7.

To show Hb of Apodemus sylvaticus

a : frozen, unwashed red-cell lysate.

b : frozen, unwashed red-cell lysate.

c - f : red-cell lysates washed

c : Icelandic

d : Scottish

e : Norwegian

f : Irish

The Hbs were photographed unstained (being red), but the other bands were stained for esterases.

Tris-citrate borate pH 8.6.

Fig.8

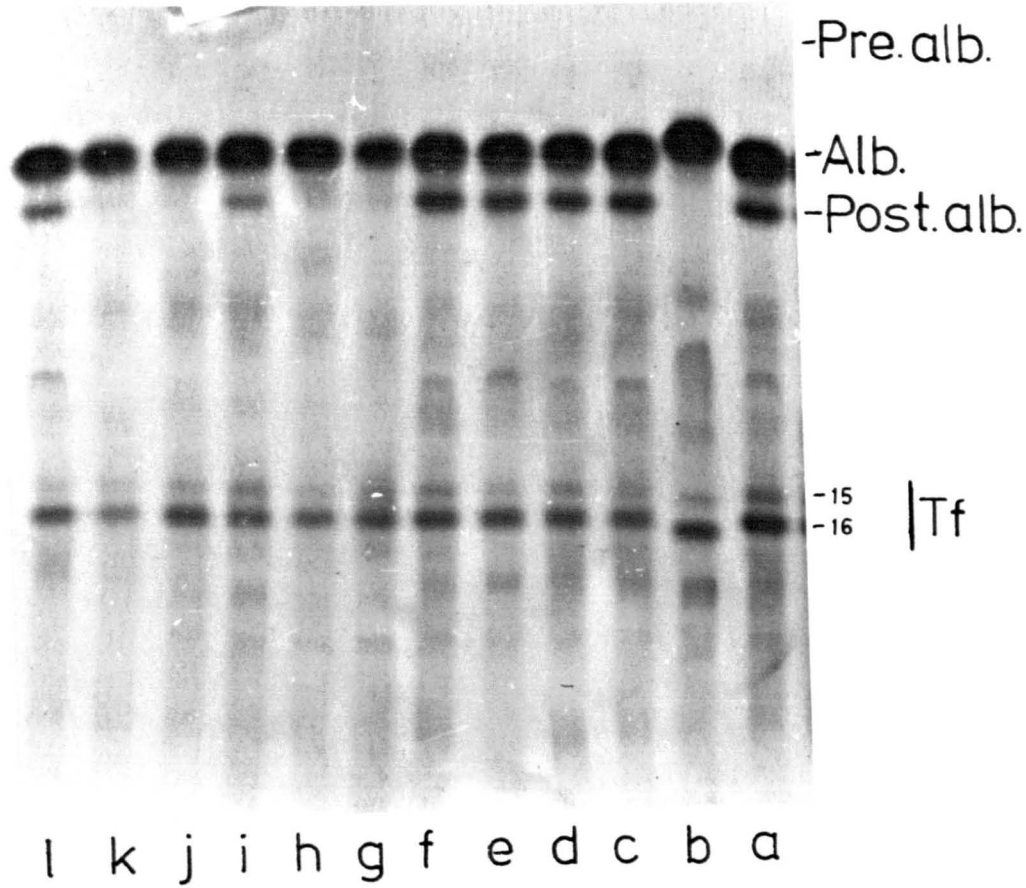


FIG. 8.

To show presence and absence of postalbumins and the uniformity of transferrins.

a and c - l: *Apodemus sylvaticus*

b : *Mus musculus*

Tris-citrate borate pH 8.6.

Fig. 8A

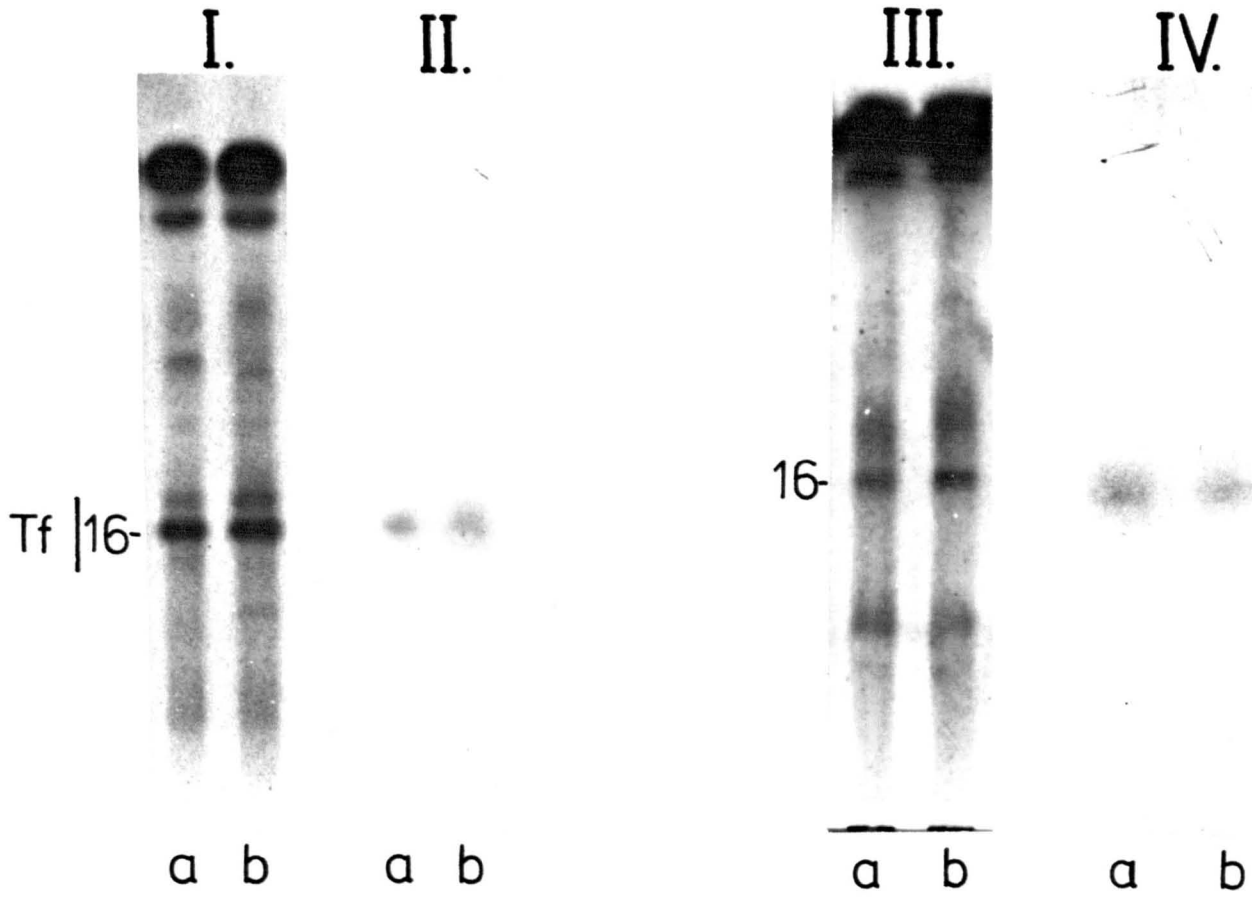


FIG. 8A

Demonstration of iron-binding proteins (Transferrins) in sera of Apodemus sylvaticus, using radioactive iron ^{59}Fe and auto radiography. The concentration of ^{59}Fe was ca. 5µc/ml serum.

I and III were stained for proteins in the usual manner.

II and IV are photographs of positive X-ray film, that was placed on the top of gels I and III and kept in dark for one week, and then developed

a and b: sera from Icelandic Apodemus sylvaticus.

I : Tris-citrate borate 8.6

III : Tris-citrate 7.6

Fig.9

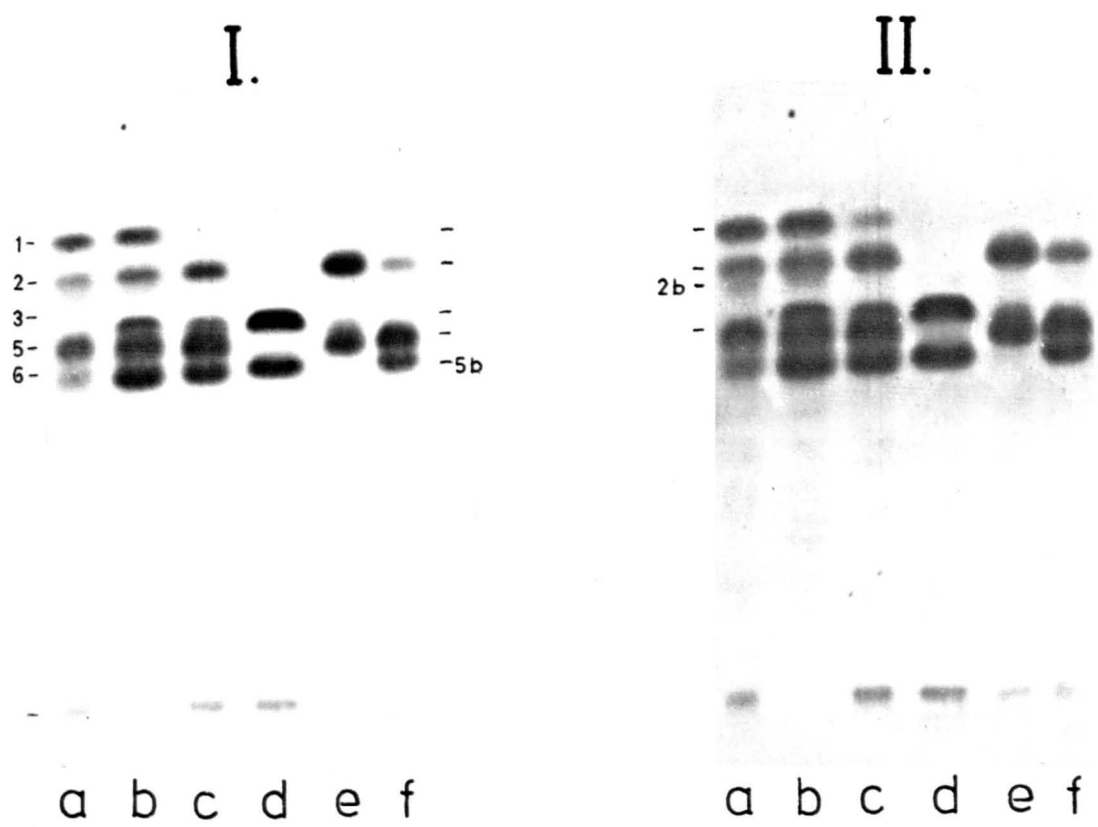


FIG 9.

Fast esterase-zone in Apodemus sylvaticus

Note different intensity of fractions 1, 2 and 3 and absence in some cases.

d, e and f	:	fraction 1 absent
d		fraction 2 absent
a, e and f	:	fraction 3 absent

The zymogram to the left (I) was stained for only 5 minutes.

a, b and c	: Icelandic specimens
d	: Scottish (rare type) specimen
e and f	: Norwegian (Oslo) specimens

Tris-citrate borate pH 8.6.

Fig.9A

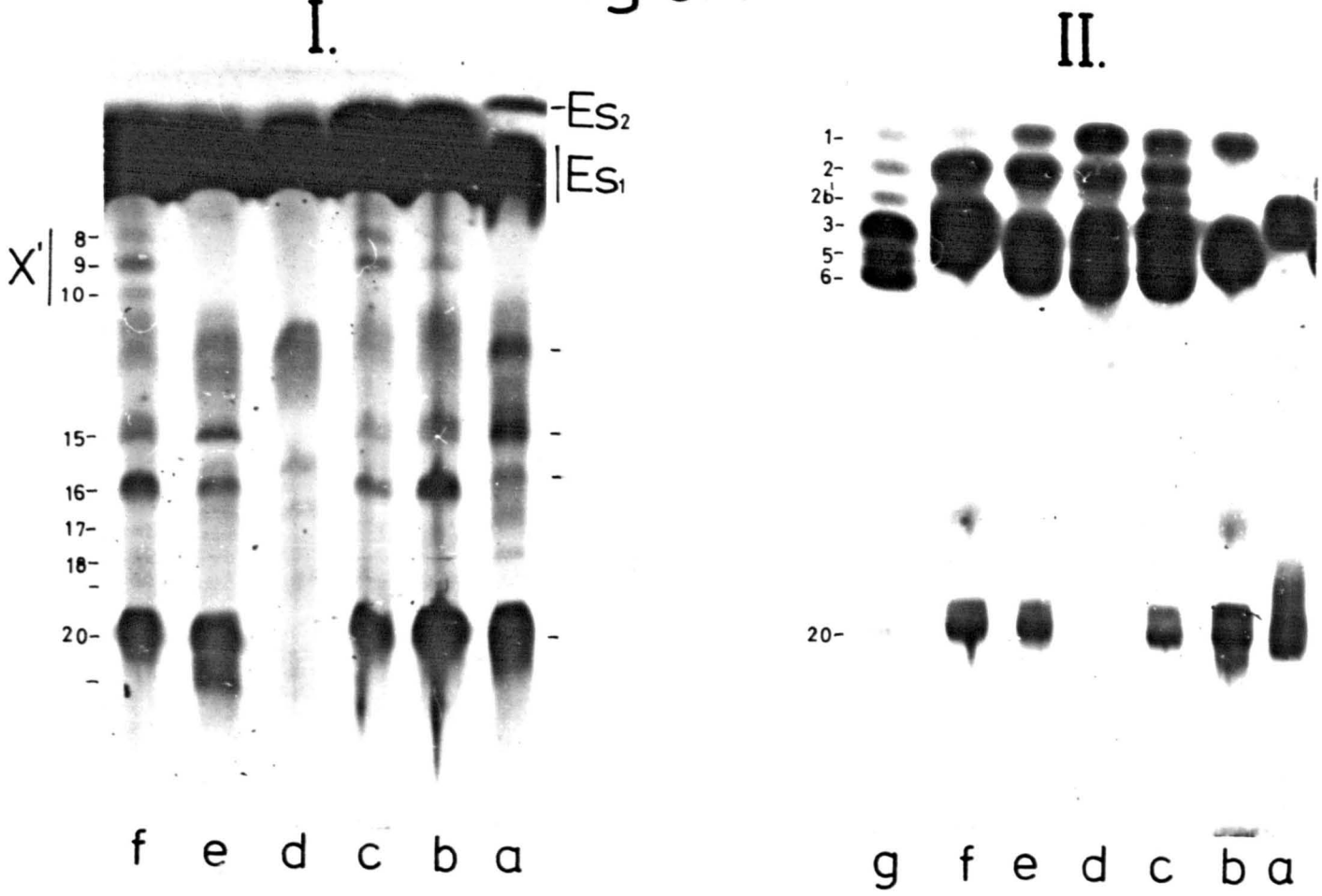


FIG. 9A

Esterase-zymograms demonstrating two rare variants. Firstly an Icelandic *Apodemus sylvaticus*, lacking fractions 15, 16 and 20 (I and I,d), secondly a Scottish *Apodemus sylvaticus* having a strong, fast moving fraction 2b' (II, c and g).

Note also absence of zone X' in the Icelandic specimens. All samples were sera.

I. Esterase zymogram at pH 7.6 (Tris-citrate)

II. Esterase zymogram at pH 8.6 (Tris-citrate borate)

a	: *Mus musculus* (wild)
b and c	: Scottish *Apodemus sylvaticus*
d	: Icelandic *Apodemus sylvaticus*, young female
e	: Pooled Icelandic *Apodemus sylvaticus* sera
f	: Norwegian (Jaeren) *Apodemus sylvaticus*
g	: the same as c at earlier stage in the staining.

Fig.10

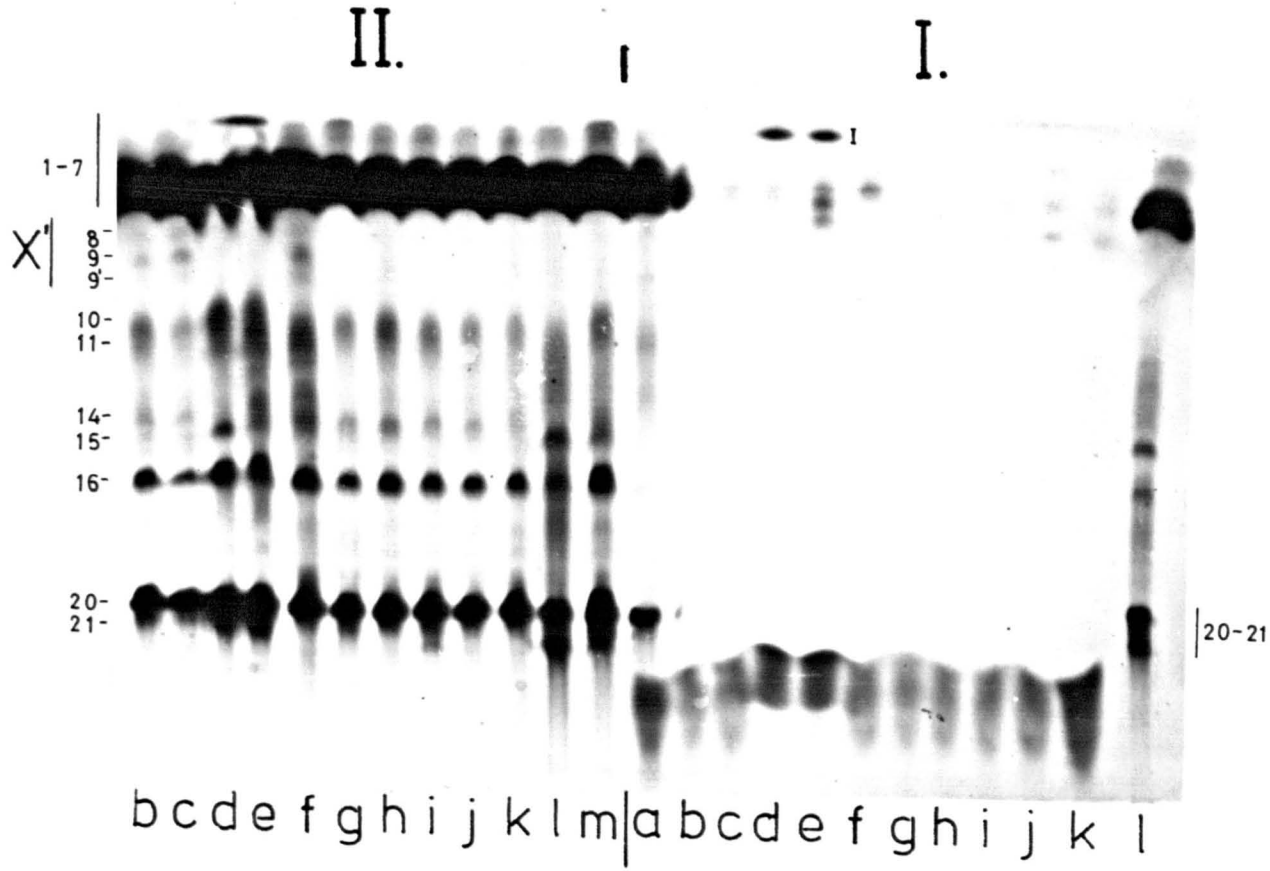

II.
I.
1-7
X'
8-
9-
9'-
10-
11-
14-
15-
16-
20-
21-
1
20-21
b c d e f g h i j k l m a b c d e f g h i j k l

FIG. 10

The same as Fig. 6, except stained for esterases.

Note the differences in zone X' between Scottish and the Icelandic field-mice. Note also fraction I in the house-mouse red-cell lysates. Samples l and m have developed storage bands overlapping fraction 21.

Fig.11

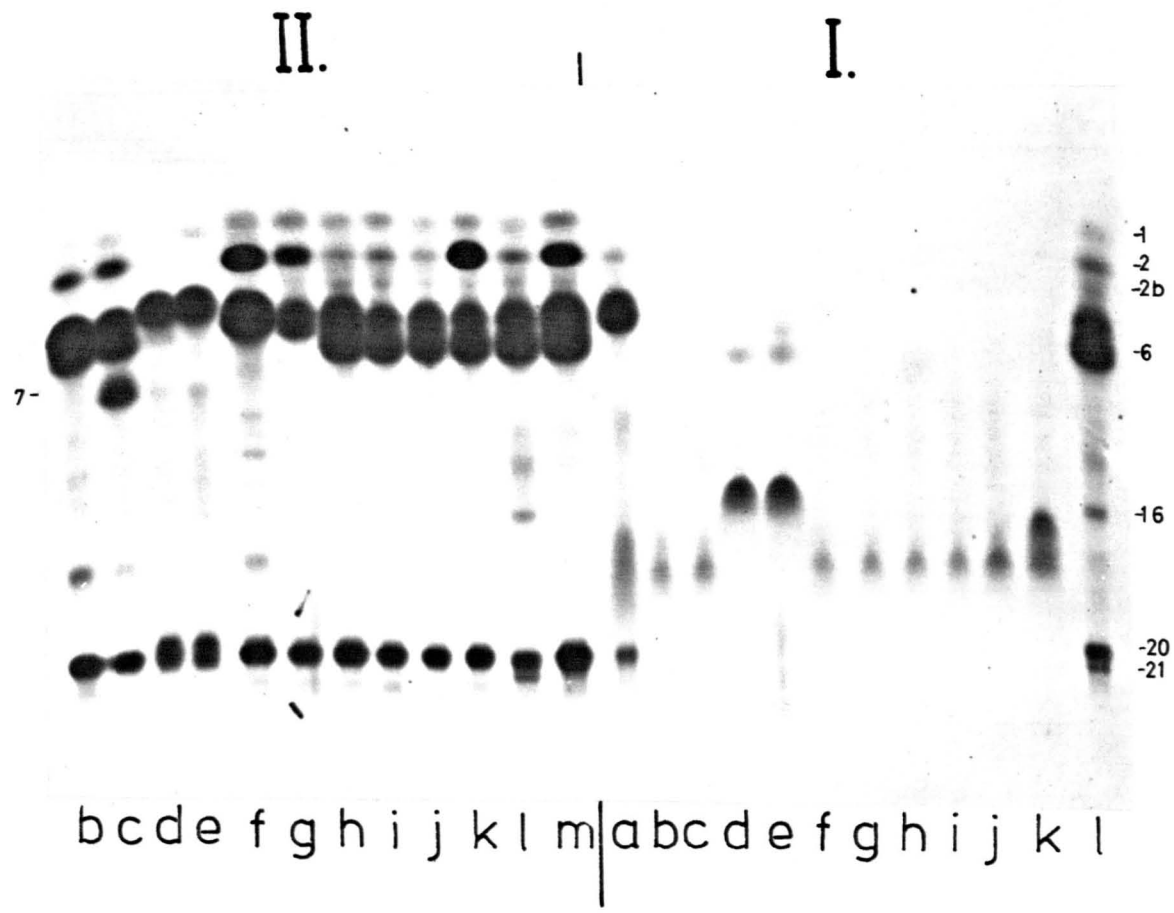


FIG. 11

Same as Fig. 6 and 10. Effect of freezing on Hb.

I.

a : Norwegian red-cell lysate unwashed frozen

b - j : frozen red-cell lysates

k : fresh red-cell lysate

II. sera

Tris-citrate borate pH 8.6

Stained for esterases.

Fig.12

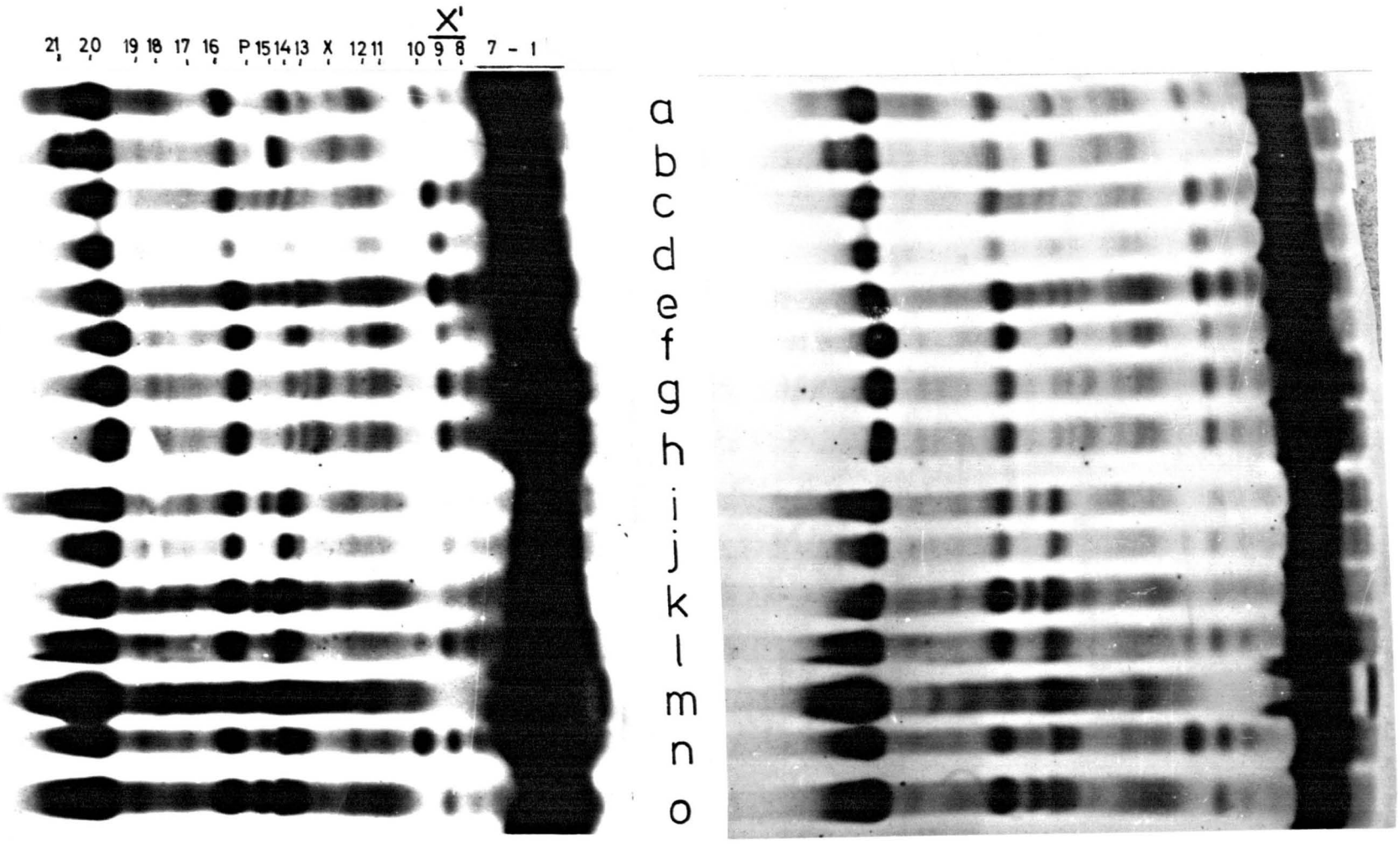


FIG. 12

Serum esterases in Apodemus sylvaticus from different countries, and also a wild Mus musculus from Norway (Jæren)
Also to show physiological changes in 13, 14, 15. Note zone X'.

a:	m	S.	(Scotland)	i:	f	N	(Norway) lactating
b:			(Icelandic) pooled	j:	m	N.	(mature)
c:	f	S.	had litter 5 days ago	k:	f	N.	pregnant
d:	m	S.	mature	l:	f	N.	(mature)
e:	f	S.	had litter 2 days ago	m:	m	N.	Mus musculus
f:	m	S.		n:	f	N.	lactating
g:	m	S.		o:	f	S.	pregnant
h:	m	(mature)					

Fig.13

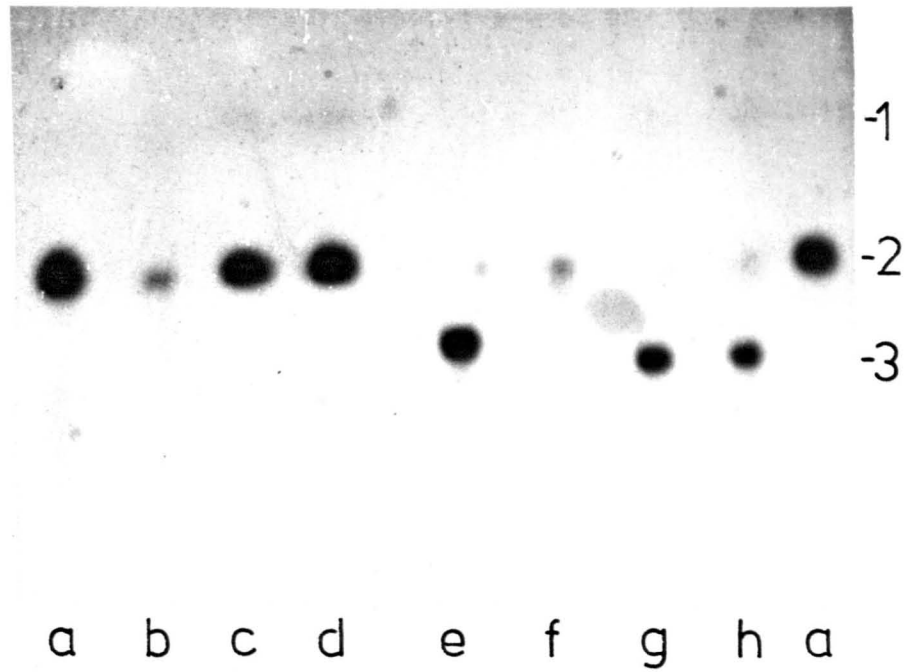


FIG. 13

Effects of ions on ceruloplasmin

a : fast moving ceruloplasmin, probably because of being heparinised.

b : a treated with Fe^{++}

c : a treated with Cu^{+}

d : a treated with Cu^{++}

e : slow moving ceruloplasmin, probably not treated with heparin

f : e treated with Fe^{++}

g : e treated with Cu^{+}

h : e treated with Cu^{++}

Tris-citrate pH 7.6.

Fig.14

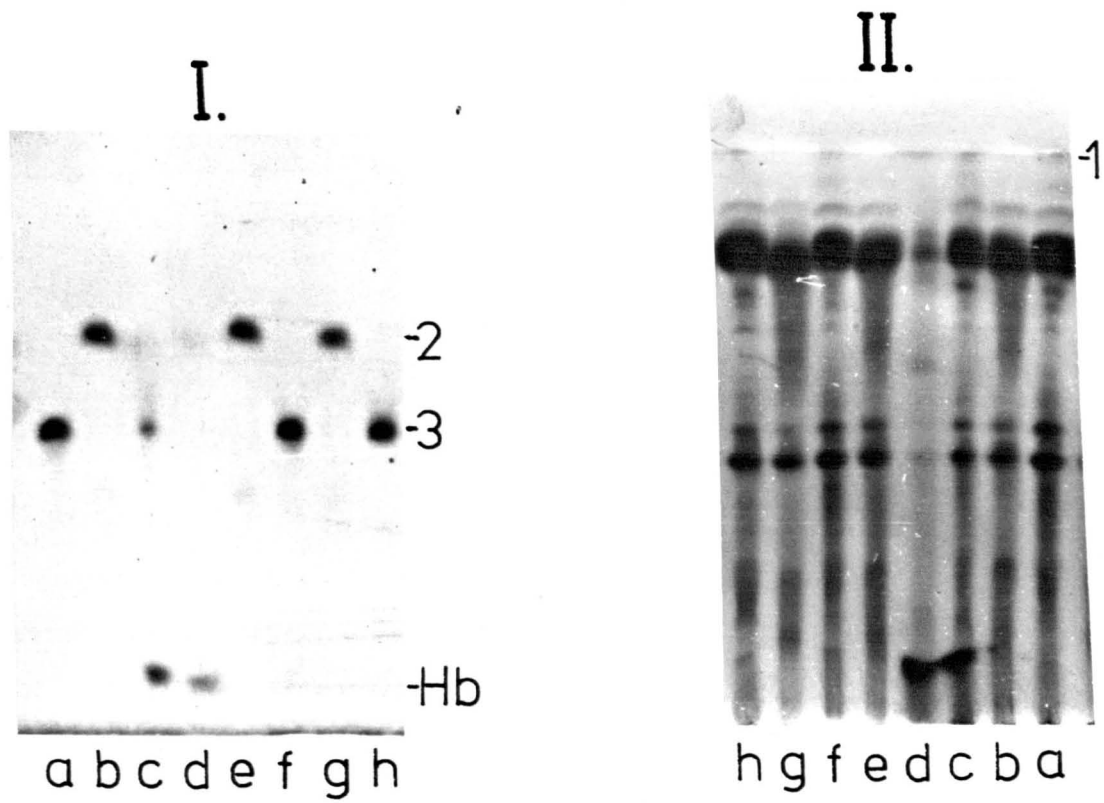


Fig. 14

Effects of heparin on the mobility of ceruloplasmin (I), and on the general protein-pattern (II).

I. Stained for ceruloplasmin

a : not treated with heparin

b : same serum as a: treated with heparin

c : same as a: not treated with heparin, but haemolysed serum. Note trailing of the ceruloplasmin

d : red-cell lysate, old and unwashed

e : treated with heparin

f : same as e: not treated with heparin

g : treated with heparin

h : g not treated with heparin

II. Same as I, stained with nigrosine. Note the smearing effects of heparin in the post-albumin region and also the effects in the pre-albumin region, and the decolourization of the background in the fraction 1 site.

Tris-citrate pH 7.6.

Fig.15

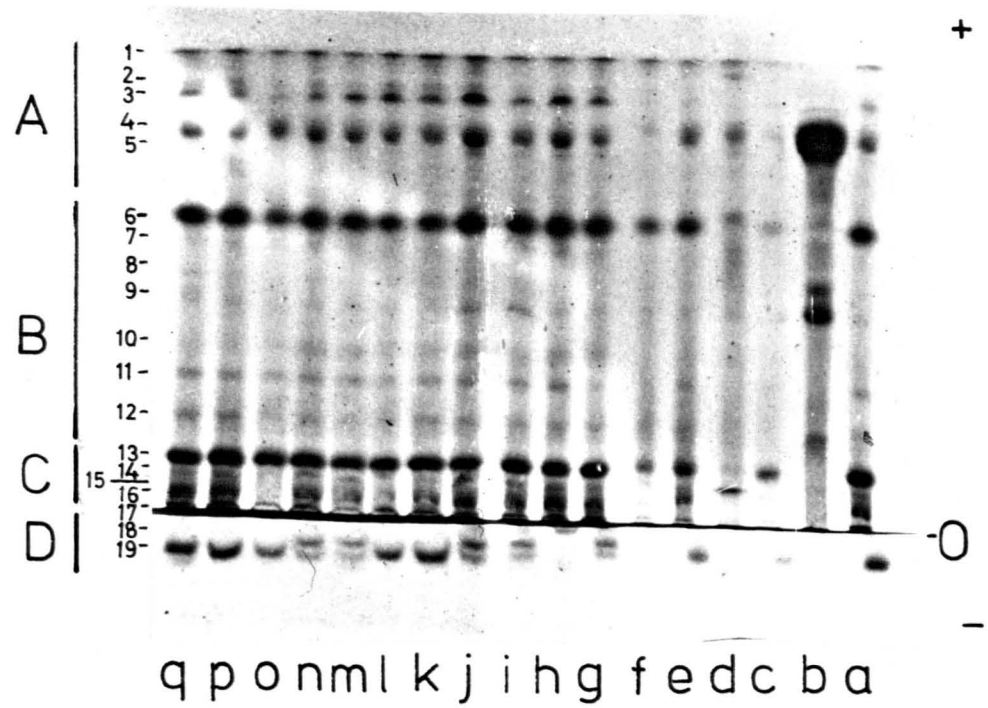

A
B
C
D
1-
2-
3-
4-
5-
6-
7-
8-
9-
10-
11-
12-
13-
14-
15
16-
17-
18-
19-
+
-0
-
q p o n m l k j i h g f e d c b a

FIG. 15

This figure demonstrates the protein bands (myogens) of the thigh muscle of Apodemus sylvaticus.

Note strong band 6 in zone B, and 13 in C. Polymorphism is found in Zone D.

a	f:	(Iceland) muscle			
b	m:	(Iceland) serum			
c	f:	(Norway) muscle			
d	f:	(Iceland) heart (kept long frozen)			
e	f:	(Scotland) muscle			
f	f:	(Iceland) muscle (old sample)			
g	m:	(Iceland) muscle			
h	m:	(Iceland) muscle			
i	m:	(Iceland) muscle			
j	m:	(Iceland) muscle	n	f	:(Norway) muscle
k	m:	(Norway) muscle	o	f	:(Norway) muscle
l	m:	(Norway) muscle	p	m	:(Norway) muscle
m	f:	(Norway) muscle	q	f	:(Norway) muscle

Tris-citrate pH 7.6.

Fig.16


A
A2
B
C
D
1-
2-
3-
4-
5-
6-
7-
8-
9-
10-
11-
12-
13-
14-
15-
16-
17-
18-
19-
+
-1
-2-3
-4
-5
-6
-13
-14
-15
-16
-17
-19
A
B
C
D
−
g g f e d c b a k j i g h g g f e d c b a
M H M L R S M

FIG. 16

This figure demonstrates the general protein-pattern of heart- and thigh muscle of Apodemus sylvaticus. Compare the fractions of heart and skeletal muscle.

a,b,c, and g: Icelandic samples

d,e,f, : Scottish samples

h : red-cell lysate unwashed (Norwegian)

i, j and k : Norwegian (Oslo) samples

Fig.17

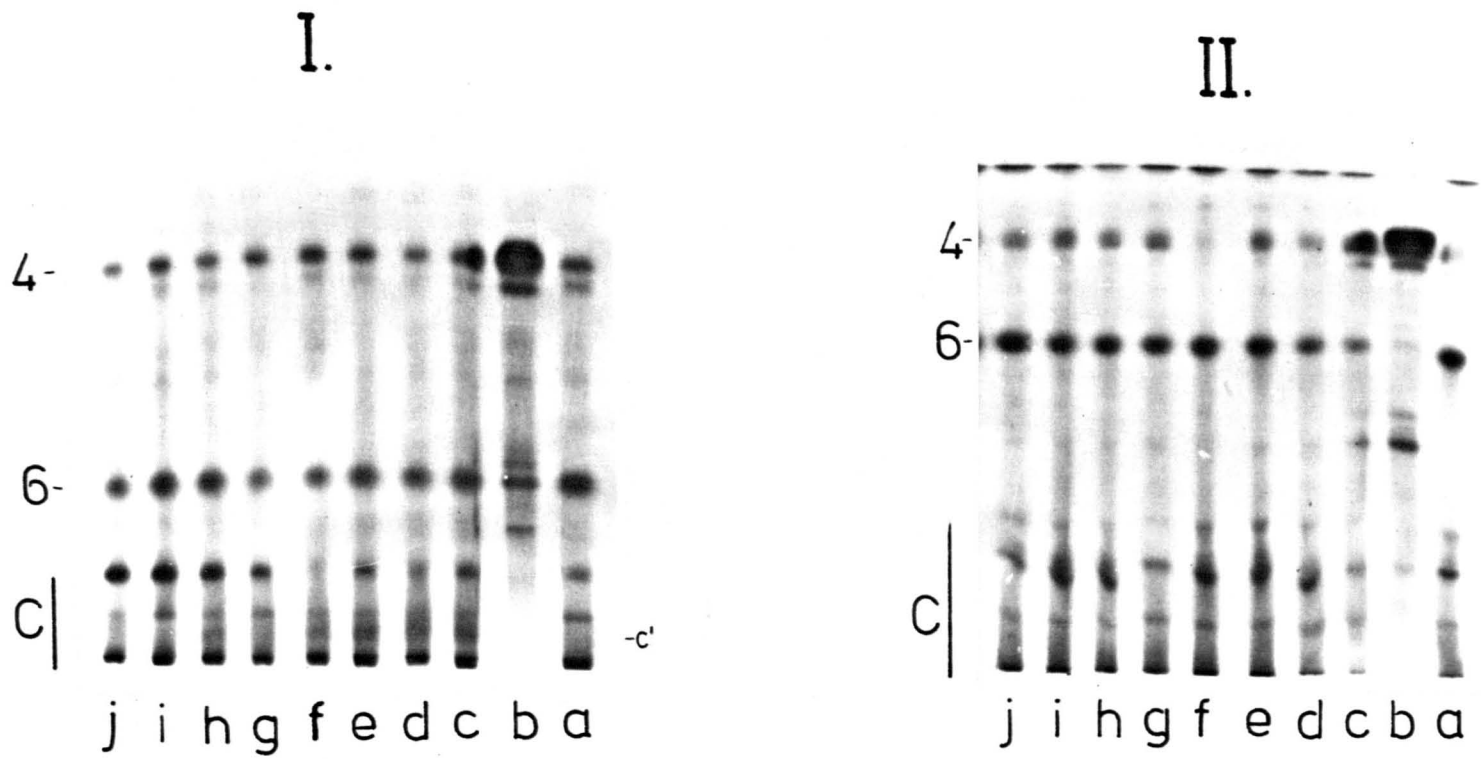


FIG. 17

Comparison of muscle-protein migration on two different gel-buffer systems:

I. Tris-citrate-borate pH 8.6

II. Tris-citrate pH 7.6

Note the difference in the positions of band 6 and the spreading of zone C on the pH 7.6 system.

On plate I note the band c' of Table 12

a, c - j : (Icelandic) thigh muscle

b : (Icelandic) serum.

Fig.18

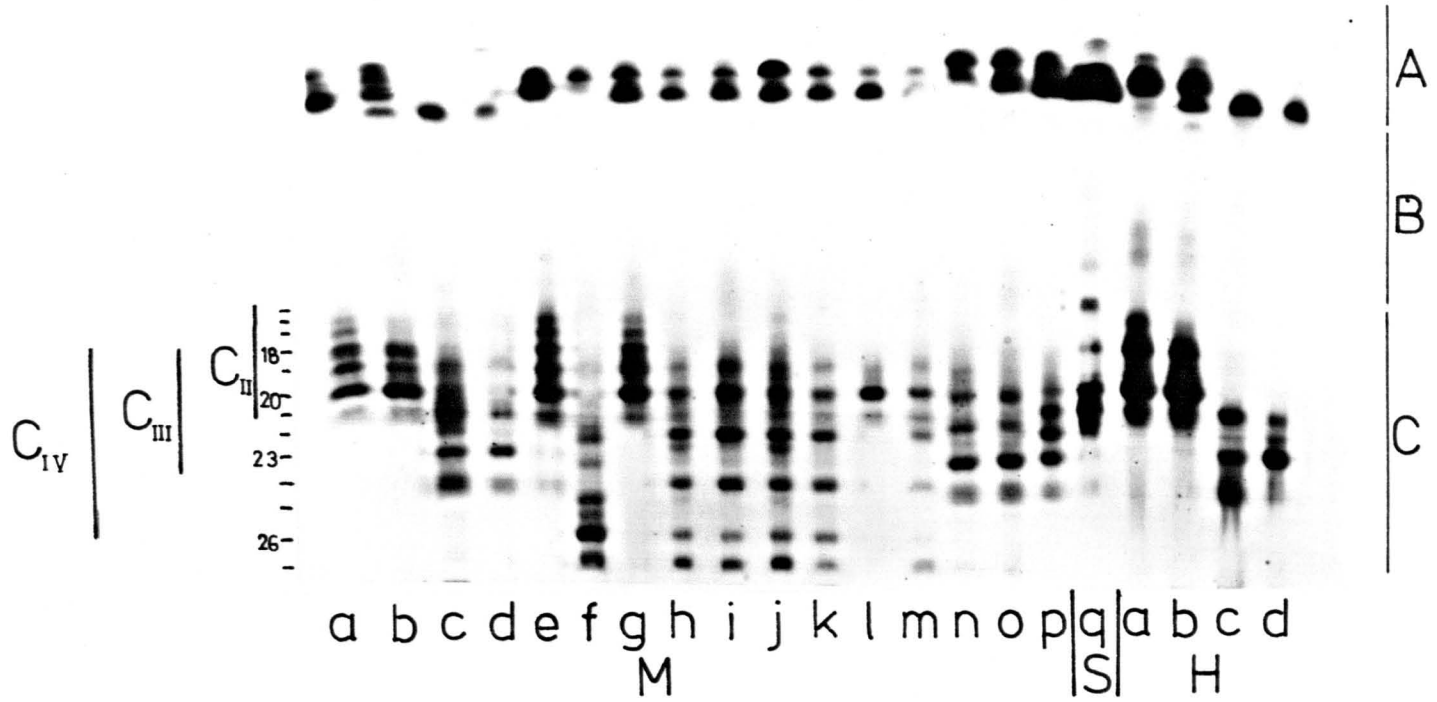


FIG. 18

Esterase zymogram demonstrating zones CII, CIII and CIV of heart and thigh muscle esterases:

a, b and e: Scottish samples demonstrating CII

c and d : Wild Mus species

f - m : Icelandic samples demonstrating CII and CIV

n - p : Norwegian samples demonstrating CIII

n and o : Jaeren

p : Oslo

Tris-citrate pH 7.6.

Fig.19

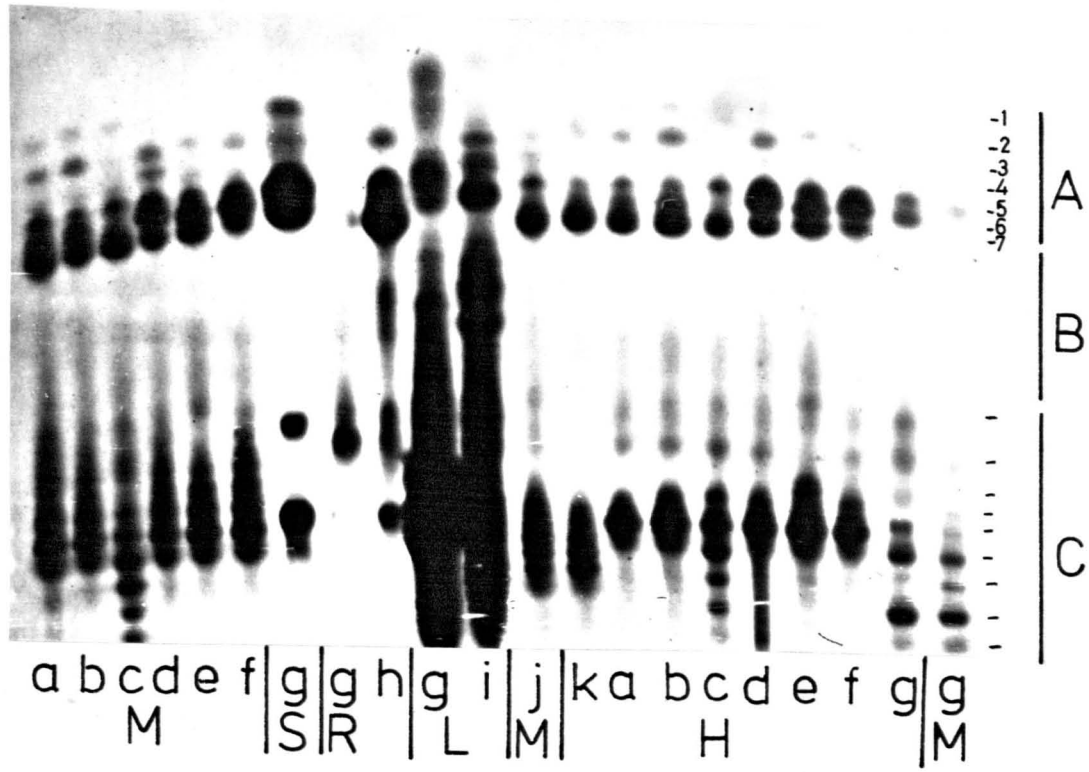


FIG. 19

Demonstrating the esterases of thigh muscle and heart of Apodemus sylvaticus. Note the similarities of the esterase pattern of these two tissues. The same samples as Fig. 16.

a, b, c and g, Icelandic specimens

d, e and f, Scottish specimens

h, i, j and k, Norwegian specimens

R, h, Norwegian red cell lysate, unwashed.

a, b, d, e and f show zone C_{II}

c and g show zone IV

j and K show zone CIII

Tris-citrate-borate pH 8.6

Fig. 20

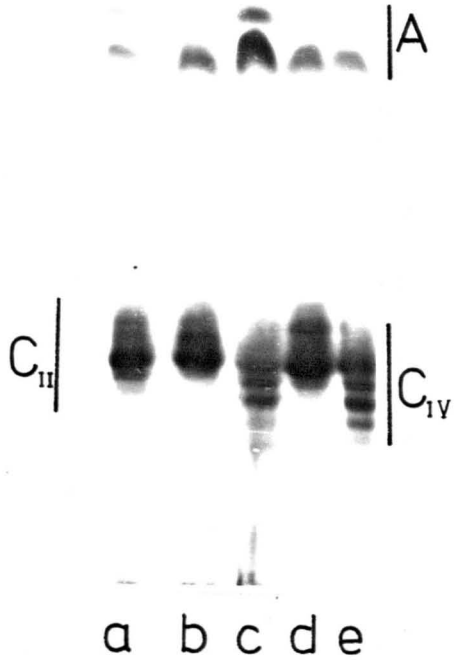


FIG. 20

Shows esterase zones C_{II} and C_{IV} of Irish and Icelandic field-mice.

The tissue used was muscle.

a,d. and e: Irish Apodemus sylvaticus

b and e : Icelandic Apodemus sylvaticus

Tris-citrate pH 7.6.

The concentration of starch is lower than in most of the other plates 10 g. starch/100 mls buffer.

Fig. 21

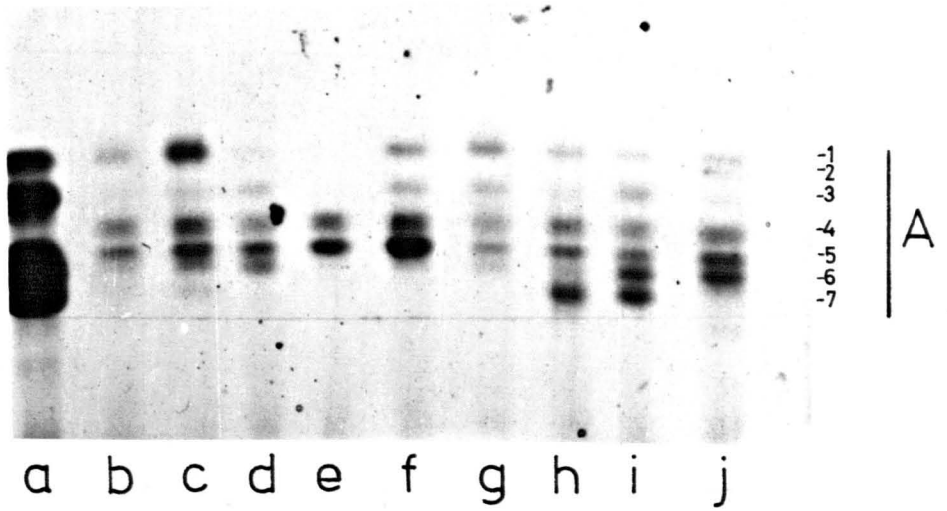


FIG. 21

Thigh muscle esterases in the zone-A. All but band 4 coincide with the serum esterases in this zone.

a : serum

b - j : Icelandic samples.

Tris-citrate-borate pH 8.6.

Fig.22

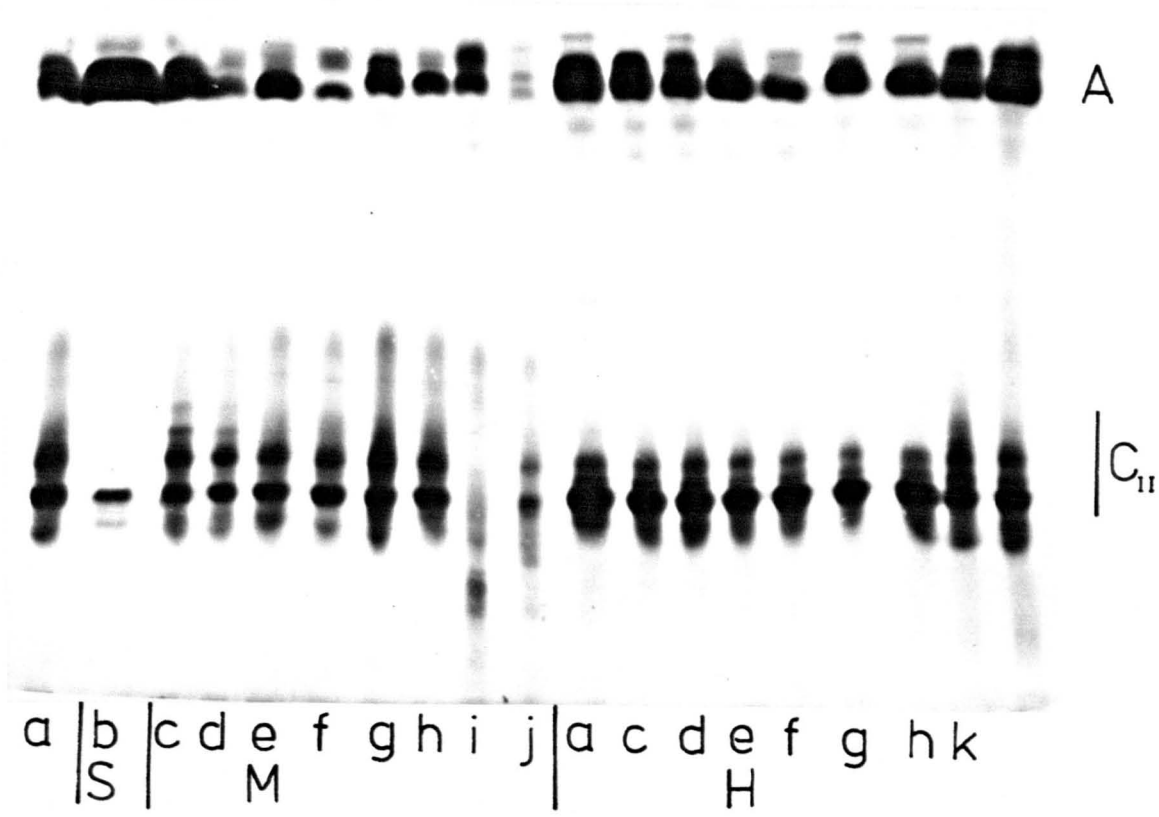


FIG. 22

Demonstrates the similarity of the esterase pattern of skeletal (M) and Heart (H) muscle of Apodemus sylvaticus.

Note the C_{II} zone.

a, c - k : skeletal and heart muscle homogenates of Icelandic Apodemus sylvaticus.

b : serum sample for comparison

i and j : samples frozen and thawn at least 10 times during three years, demonstrating C_{IV}.

Tris-citrate pH 7.6.

Fig.23

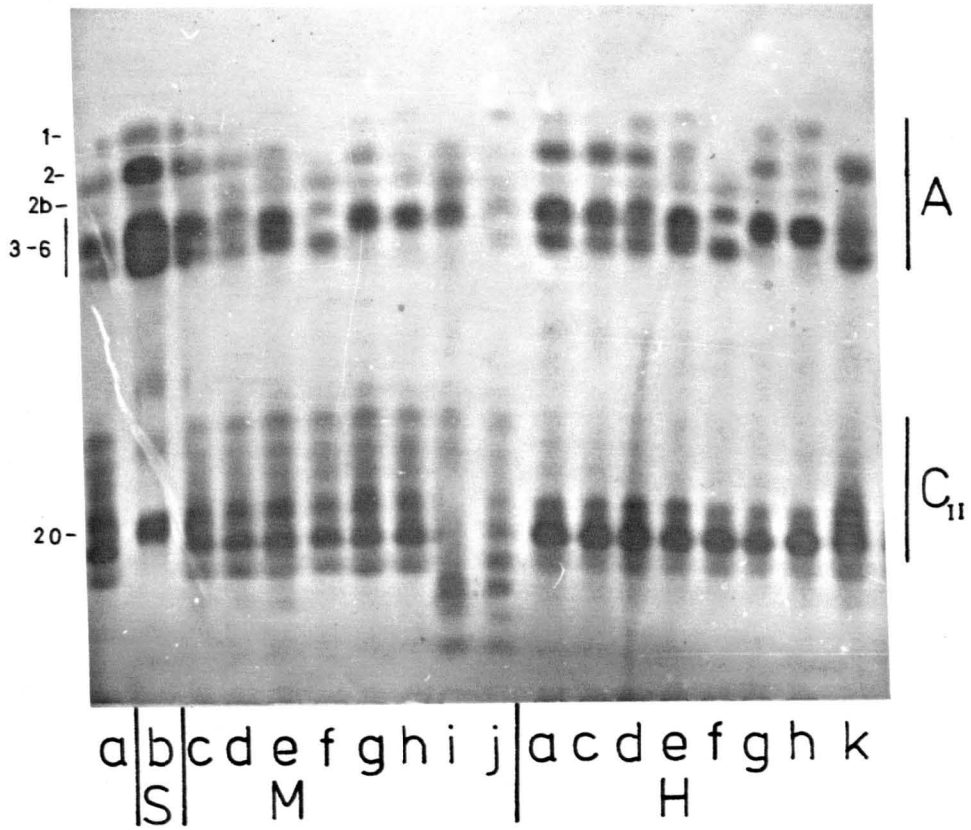


FIG. 23

Same as Fig. 22. Note the fractions in zone A and compare the fractions of skeletal (M) and heart muscle (H). In zone C_{II} note that the faster moving esterase-fractions are stronger in the heart than the skeletal muscle.

Tris-citrate-borate pH 8.6.

Fig.24

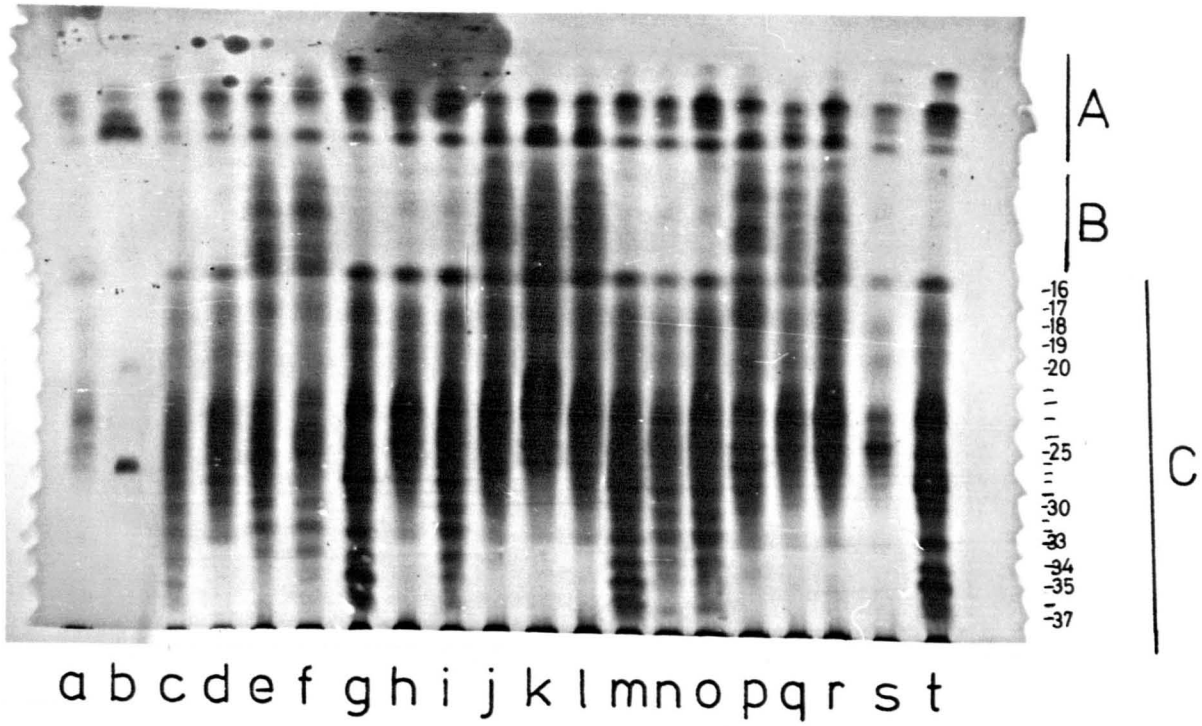


FIG. 24

This figure demonstrates the main zones of the esterase pattern of the liver homogenates of Apodemus sylvaticus.
Note the "weakness" or "absence" of fractions in zone B of the Icelandic samples. Note also the differences in zone C.

a,d,h,j,k,l,p,q,r and s	:	C_{II}
e and f	:	C_{III}
c,g,i,m,n,o,t	:	C_{IV}
a, c - t	:	liver homogenates
b	:	serum
a,b,c,d,g,h,i,m,n,o,s and t	:	Icelandic
e and f	:	Norwegian
j,k,l,p,q and r	:	Scottish
e and f	:	had been kept frozen for 285 days
c and d	:	" " " " " 55 "
s and t	:	" " " " " 2 "
the rest	:	" " " " " 5 "

Tris-citrate pH 7.6

Fig.25

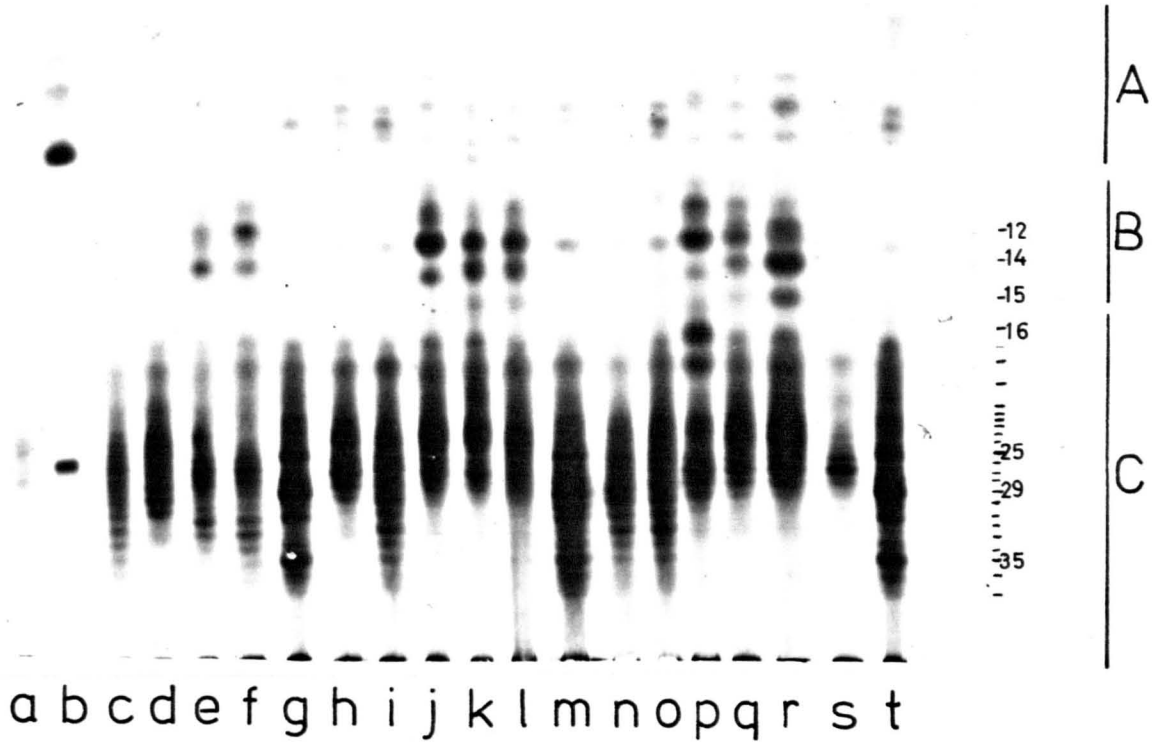


FIG. 25

The same esterase zymogram of liver esterases as in Fig. 24.

The picture was taken after 10 minutes staining.

Tris-citrate-borate pH 8.6.

Fig. 26

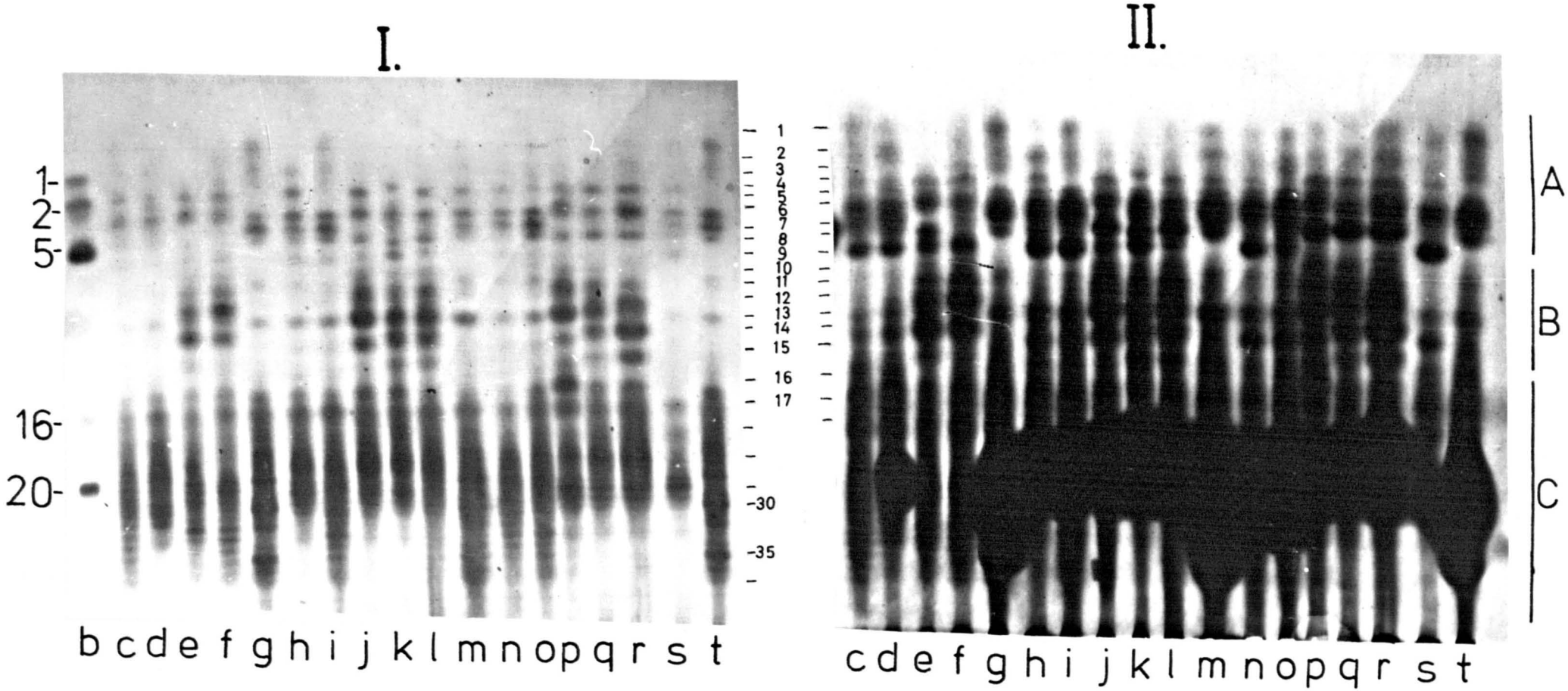

I.
II.
1-
2-
5-
16-
20-
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
-30
-35
A
B
C
b c d e f g h i j k l m n o p q r s t
c d e f g h i j k l m n o p q r s t

FIG. 26

The same as Fig. 25.

I. After 20-30 minutes in the stain

II. After 10 hours in the stain.

Note the differences in zones A and B between individual samples, and also between countries.

Tris-citrate-borate pH 8.6.

Fig. 27

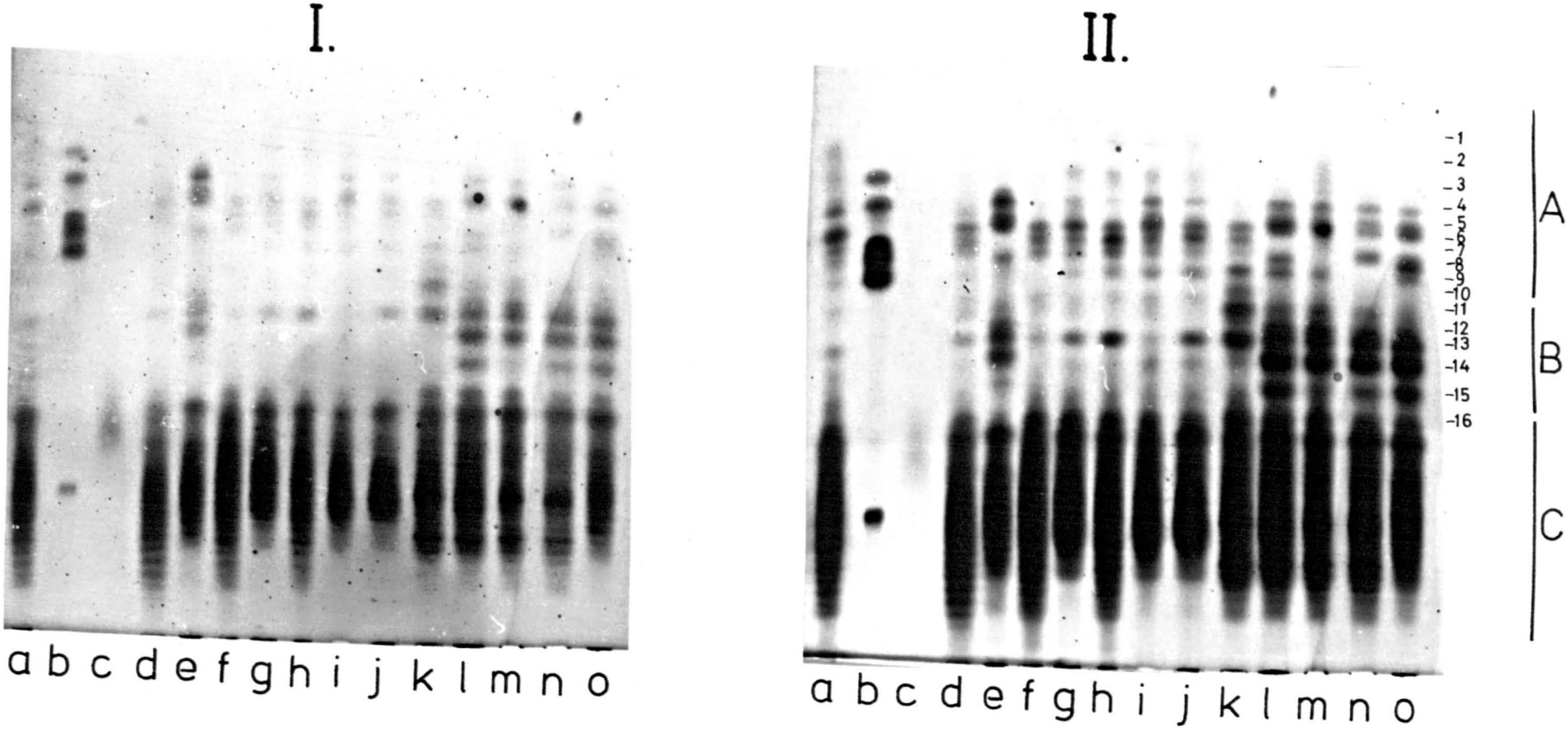

I.
II.
a b c d e f g h i j k l m n o
a b c d e f g h i j k l m n o
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
A
B
C

FIG. 27

Esterase zymogram of liver homogenates from Icelandic and Norwegian (Oslo) Apodemus sylvaticus.

I. Note differences in Zone C.

a,d,f, and h are of type C_{IV}

e,g,i,j and o are of type C_{II}

k, l, m and n are of type C_{III}

Staining time 15 minutes.

II. The same as I, after 30 minutes staining.

Note fractions in zones A and B

a, d - o liver homogenates

b serum from same animal as h

c red blood cell lysate of Apodemus sylvaticus.

a,b,c,d,f,g,h,i and j: Icelandic Apodemus sylvaticus

e,k,l,m,n and o : Norwegian (Oslo) Apodemus sylvaticus.

Tris-citrate-borate pH 8.6.

Fig.28

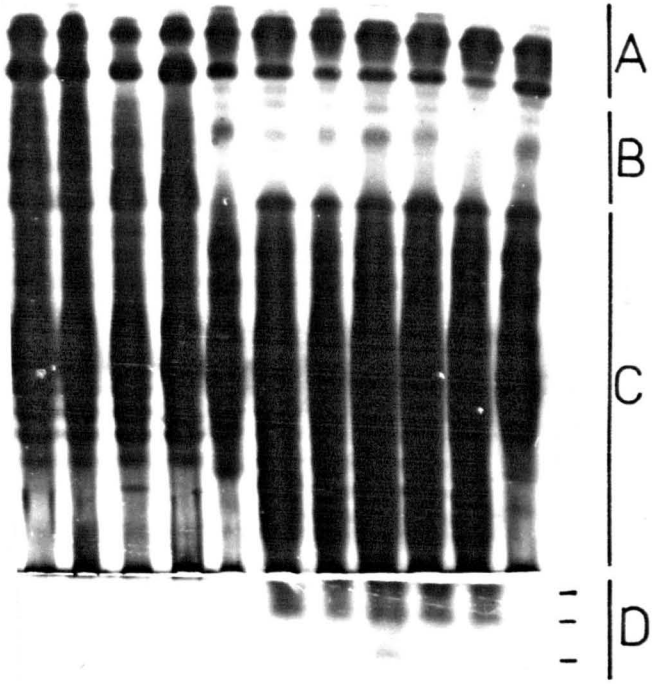


FIG. 28

Liver esterase zymogram showing the cathodally migrating zone D

a - k : liver homogenates

a - d : Norwegian (Oslo) Apodemus sylvaticus

e - k : Icelandic Apodemus sylvaticus

f,g,h,i and j show all C_{IV} type of their skeletal and heart homogenates

Tris-citrate pH 7.6.

Fig.29

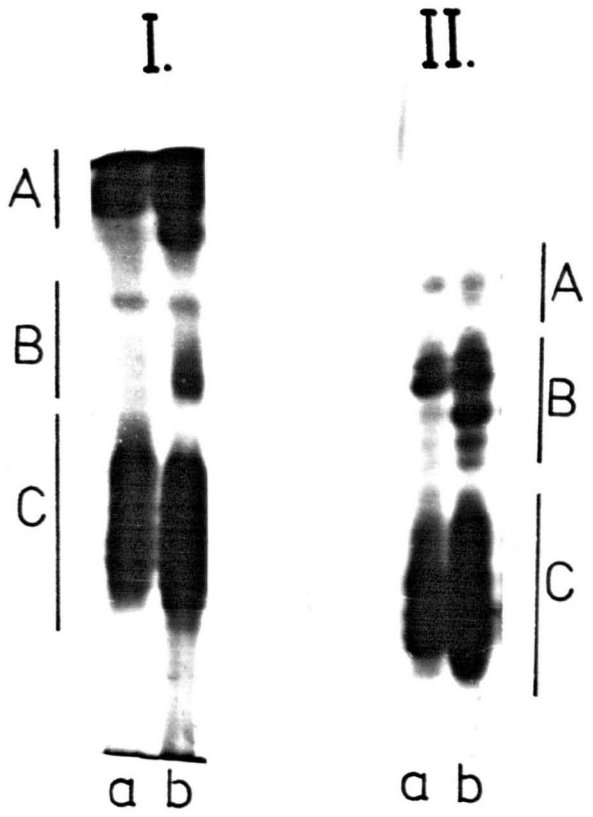


FIG. 29

This shows esterase zymograms from Irish Apodemus sylvaticus.

Note differences in zone B.

a and b : liver homogenates for Irish Apodemus sylvaticus

I. Tris-citrate pH 7.6.
10 g starch/100 mls buffer.

II. Tris-citrate-borate pH 8.6.

Fig.30

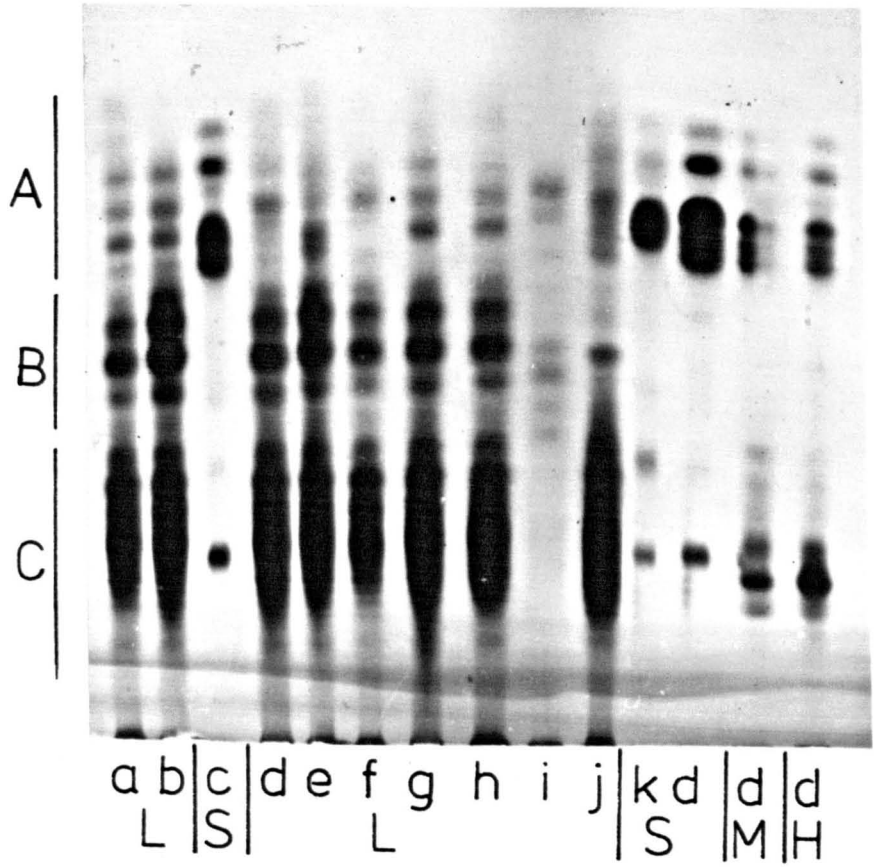


FIG. 30

This shows some ontogenetic comparison of liver esterases of Apodemus sylvaticus, as well as comparison of esterases of mice from different localities.

Note absence of slower fractions in zone A and all fractions in zone C of the foetal sample.

a,b,d,e,f,g,h : Scottish Apodemus sylvaticus

c and j : Icelandic Apodemus sylvaticus

i and k : Norwegian Apodemus sylvaticus

i : liver homogenates from ca. 15 day foetuses

k : serum from the mother of i.

Tris-citrate borate pH 8.6.

Fig.31

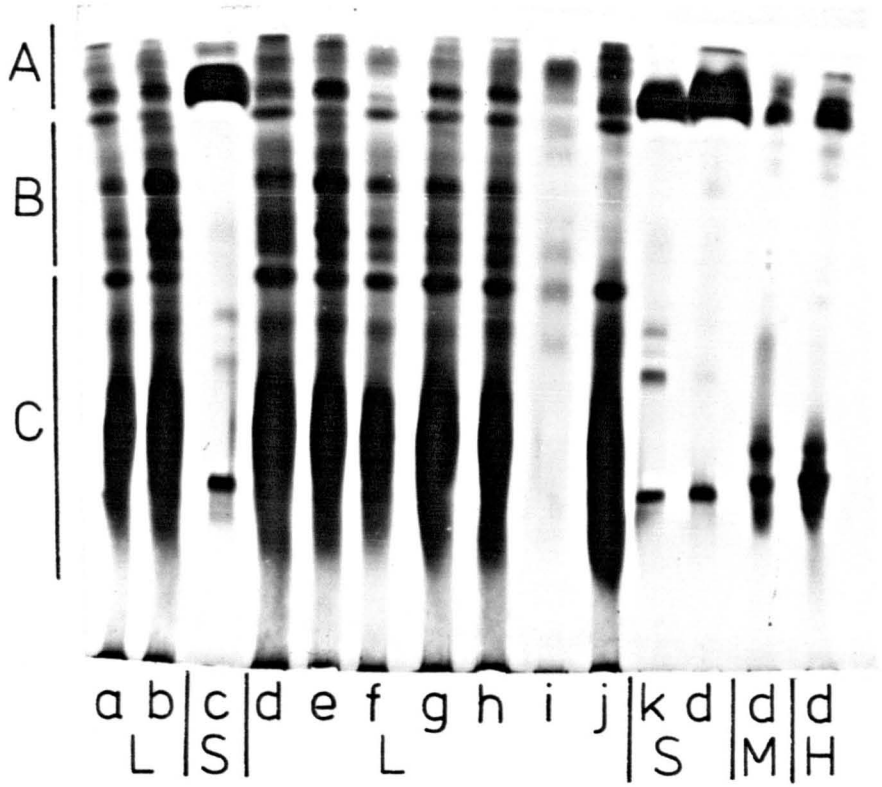


FIG 31

The same as Fig. 30, but the system used was Tris-citrate pH 7.6.

Note the absence of fractions from zones B and C in the foetal sample.

Fig.32

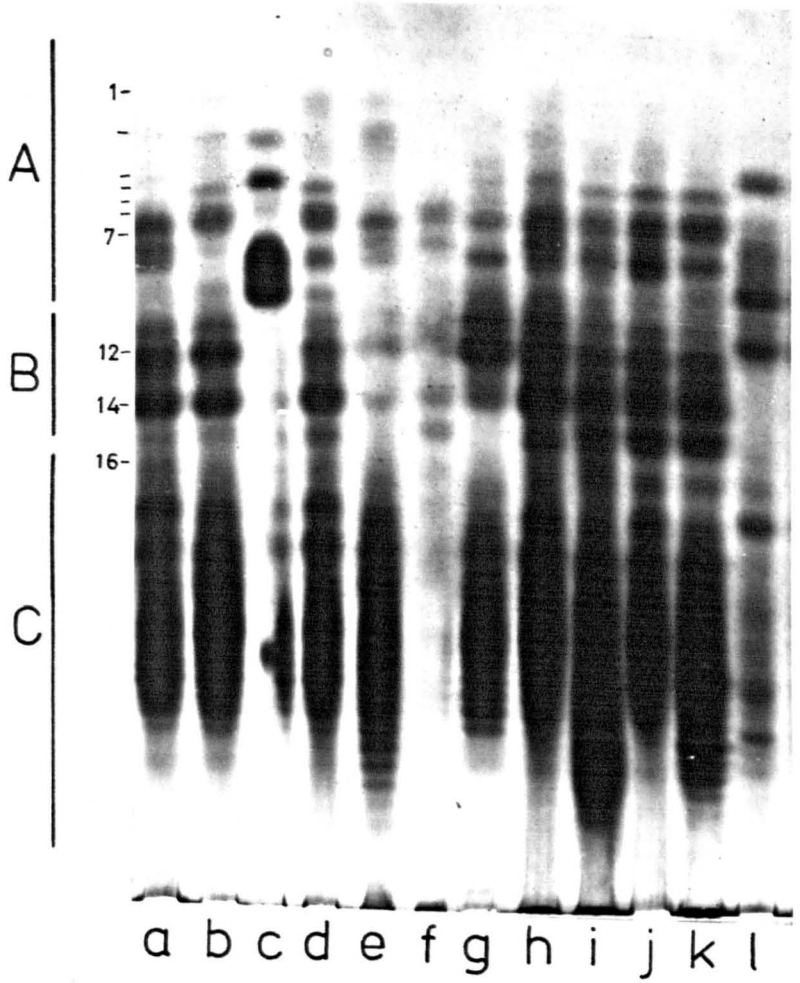


FIG. 32

Liver esterase-zymogram of *Apodemus sylvaticus*, adults and a foetus, and of a wild *Mus musculus*.

a,b,d - k : Liver homogenates of *Apodemus sylvaticus*

c : serum from Icelandic *Apodemus sylvaticus*

l : liver homogenates from wild *Mus musculus* (Norway).

a,b,d and g : Scottish *Apodemus sylvaticus*

e : Icelandic *Apodemus sylvaticus*

f,h,i,j and k : Nofwegian (Jaeren) *Apodemus sylvaticus*

e : Norwegian (Jaeren) *Mus musculus* (wild)

f : 15 day foetus of *Apodemus sylvaticus*

j : mother of f

Tris-citrate-borate pH 8.6.

Fig.33

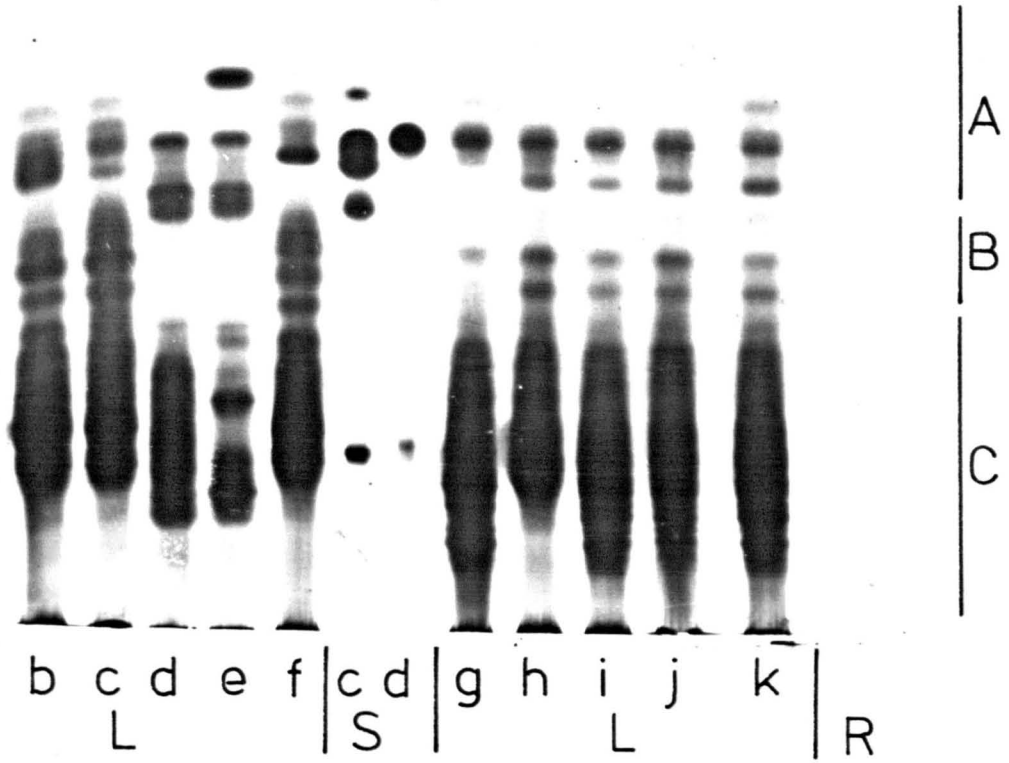


FIG. 33

Liver esterase zymogram illustrating differences between different populations of *Apodemus sylvaticus* and individuals of wild *Mus musculus*. All animals used in this experiment were killed the day before the electrophoretic run.

b, c and f : Scottish *Apodemus sylvaticus*

d and e : Scottish *Mus musculus* (wild)

g and k : Icelandic *Apodemus sylvaticus*

Tris-citrate-borate pH 8.6.

Fig.34

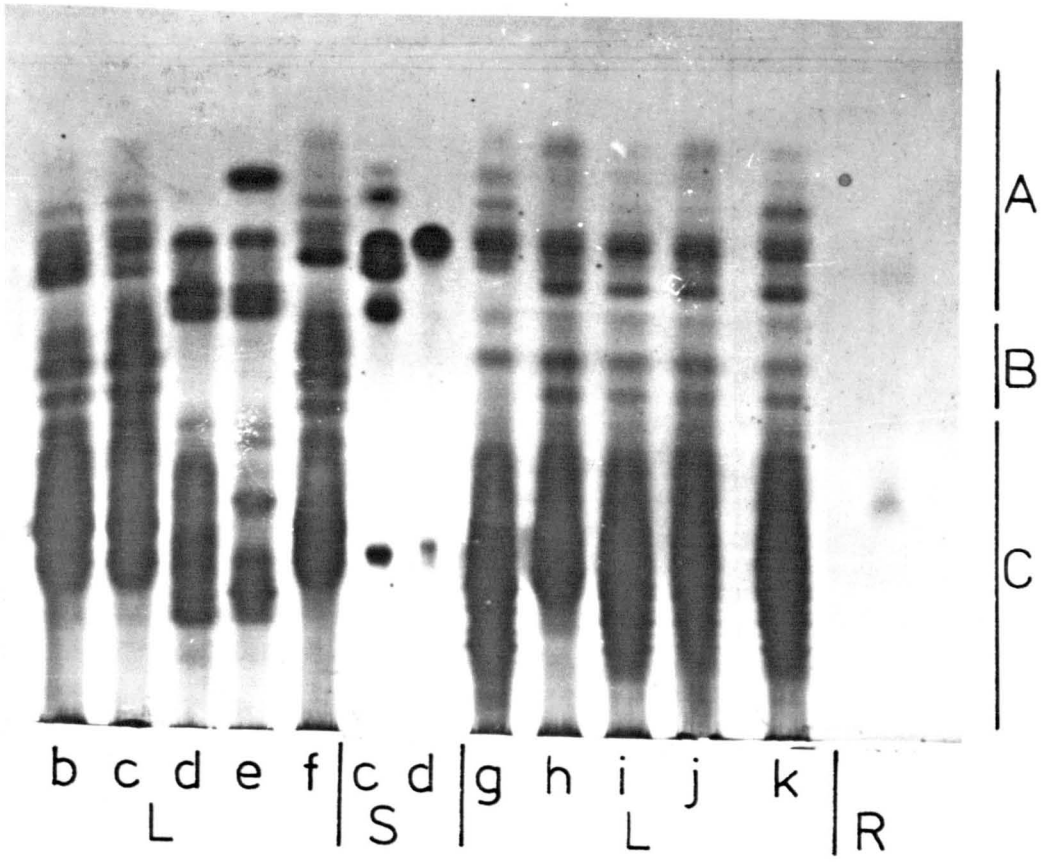


FIG. 34

Liveresterase zymogram, the same as Fig. 33 but at a later stage in the staining process.

Fig.35

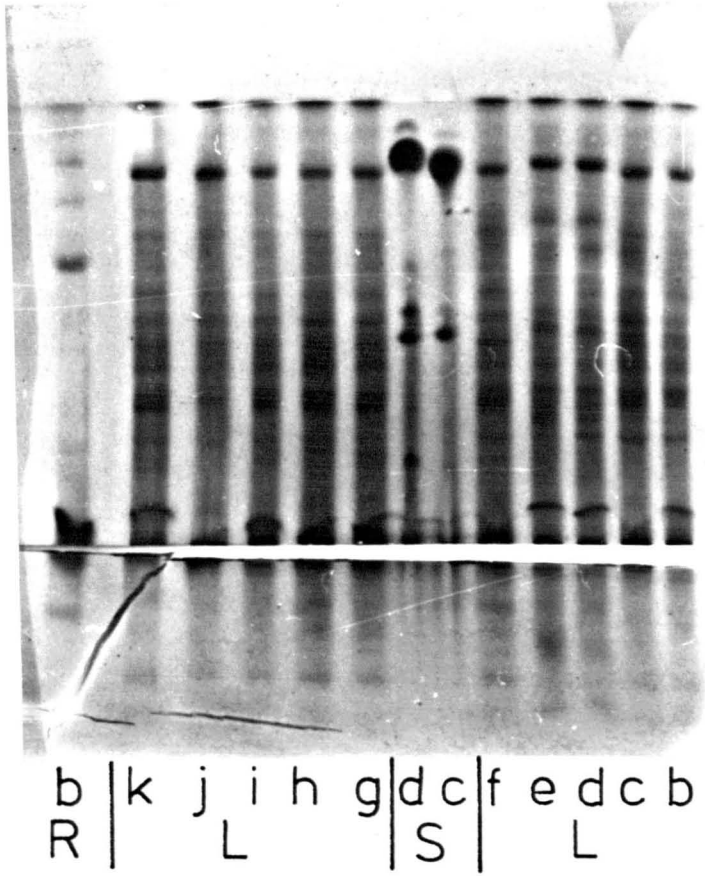


FIG. 35

Nigrosine stained slice of same plate at Fig. 34

Note the difference between the Apodemus sylvaticus and Mus musculus

Compare this protein pattern with Fig. 34. Figures 6, 10 and 11 show samples from the same animals.

Tris-citrate pH 7.6.

Fig.36

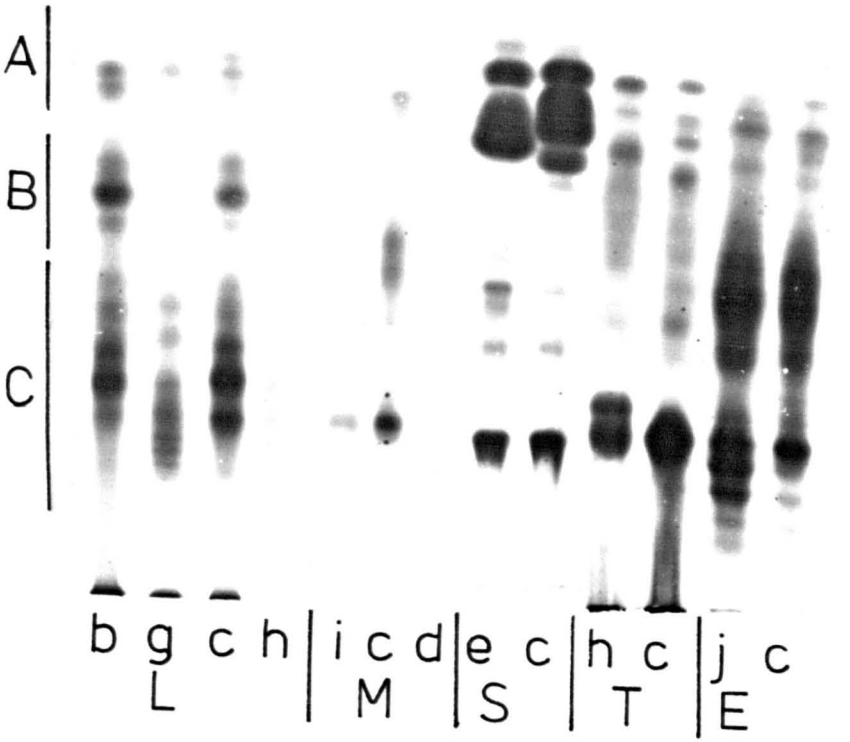


FIG. 36

Comparison of esterases of different tissues from Irish and Icelandic *Apodemus sylvaticus* staining time 60 minutes. All tissues, except liver, were homogenized in aliquots of water. The liver was homogenized in the ratio tissue: water; 5:1.

b and c : Irish *Apodemus sylvaticus*

d,e,h,i and j: Icelandic *Apodemus sylvaticus*

Tris-citrate-borate pH 8.6

Fig.37

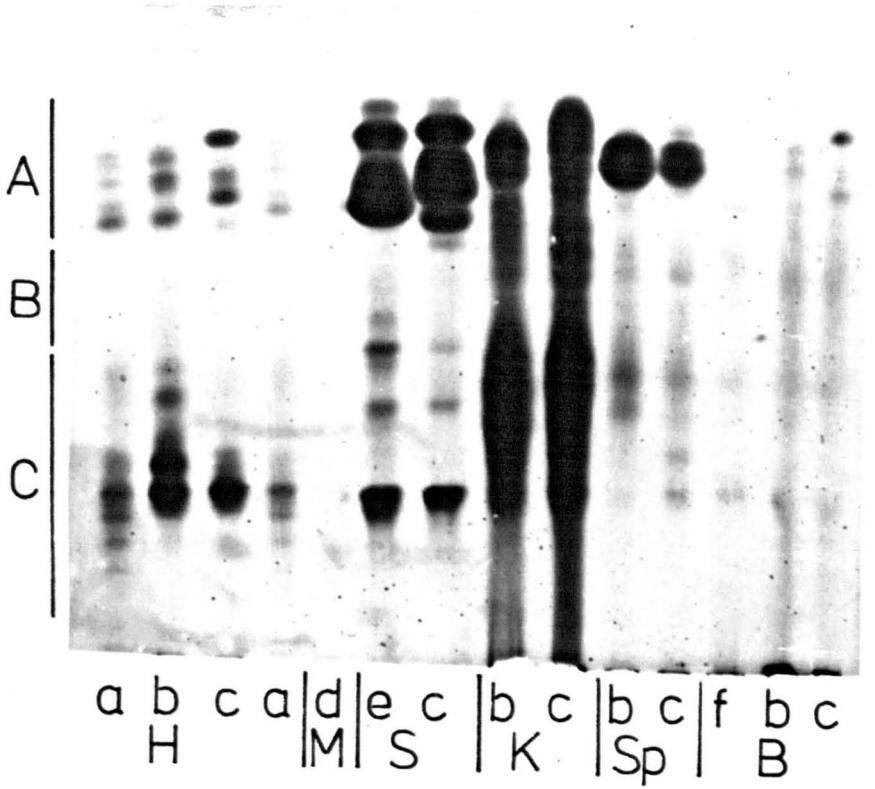


FIG. 37

Comparison of esterases of different tissues from Irish and Icelandic *Apodemus sylvaticus*. Staining time 2 hrs.

Note zone C in the heart homogenates: a: CIV, b: CI, c: CII

a,d,e and f : Icelandic *Apodemus sylvaticus*

b and c : Irish *Apodemus sylvaticus*

Tris-citrate-borate pH 8.6.

Fig. 38

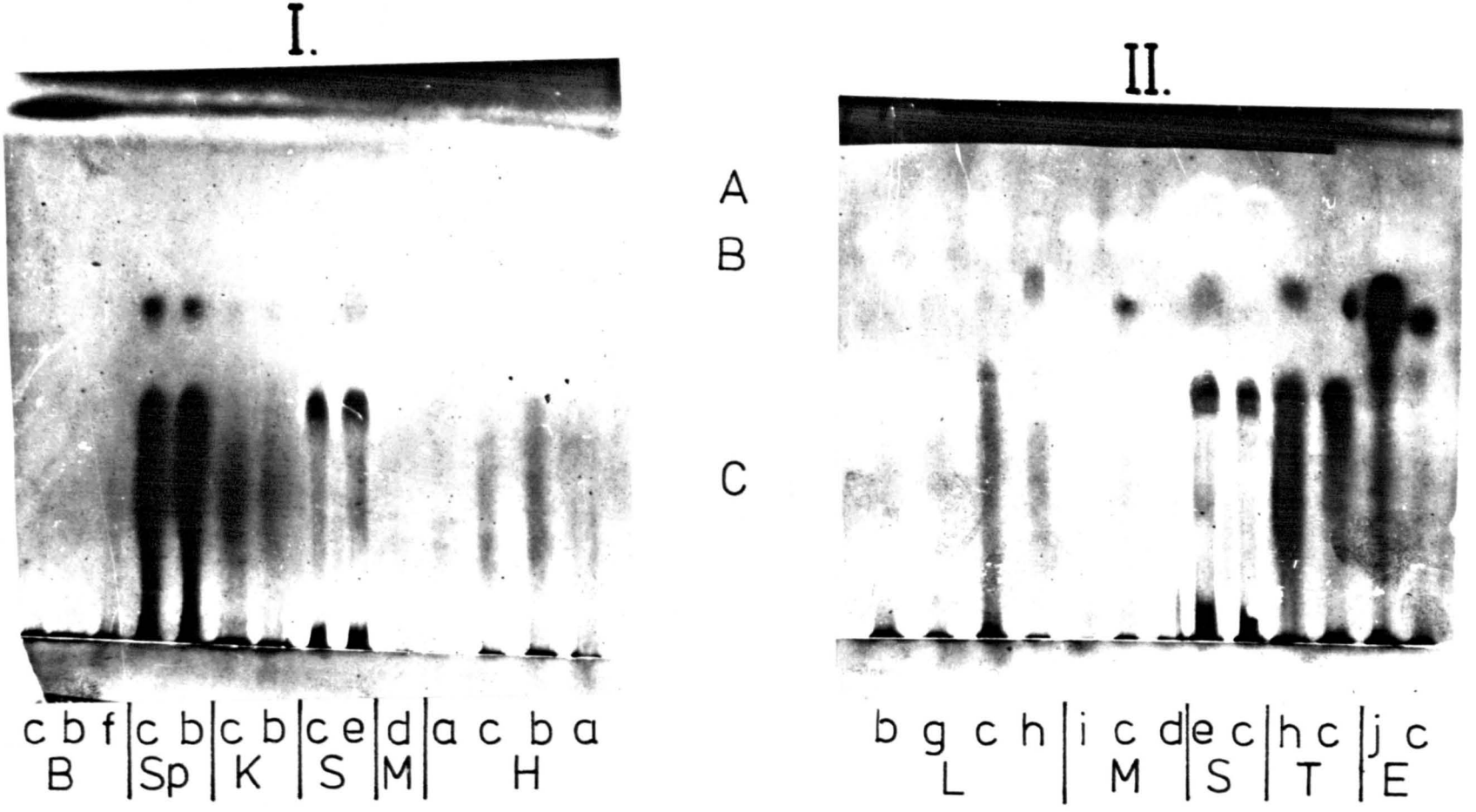


FIG. 38

Acid phosphatase-zymograms, demonstrating different reactions of different tissues to a-naphthyl phosphate Na salt as substrate and Fast Garnet as coupler dye.

Note:

I corresponds to Fig. 37

II corresponds to Fig. 36

Tris-citrate pH 7.6.

Fig.39

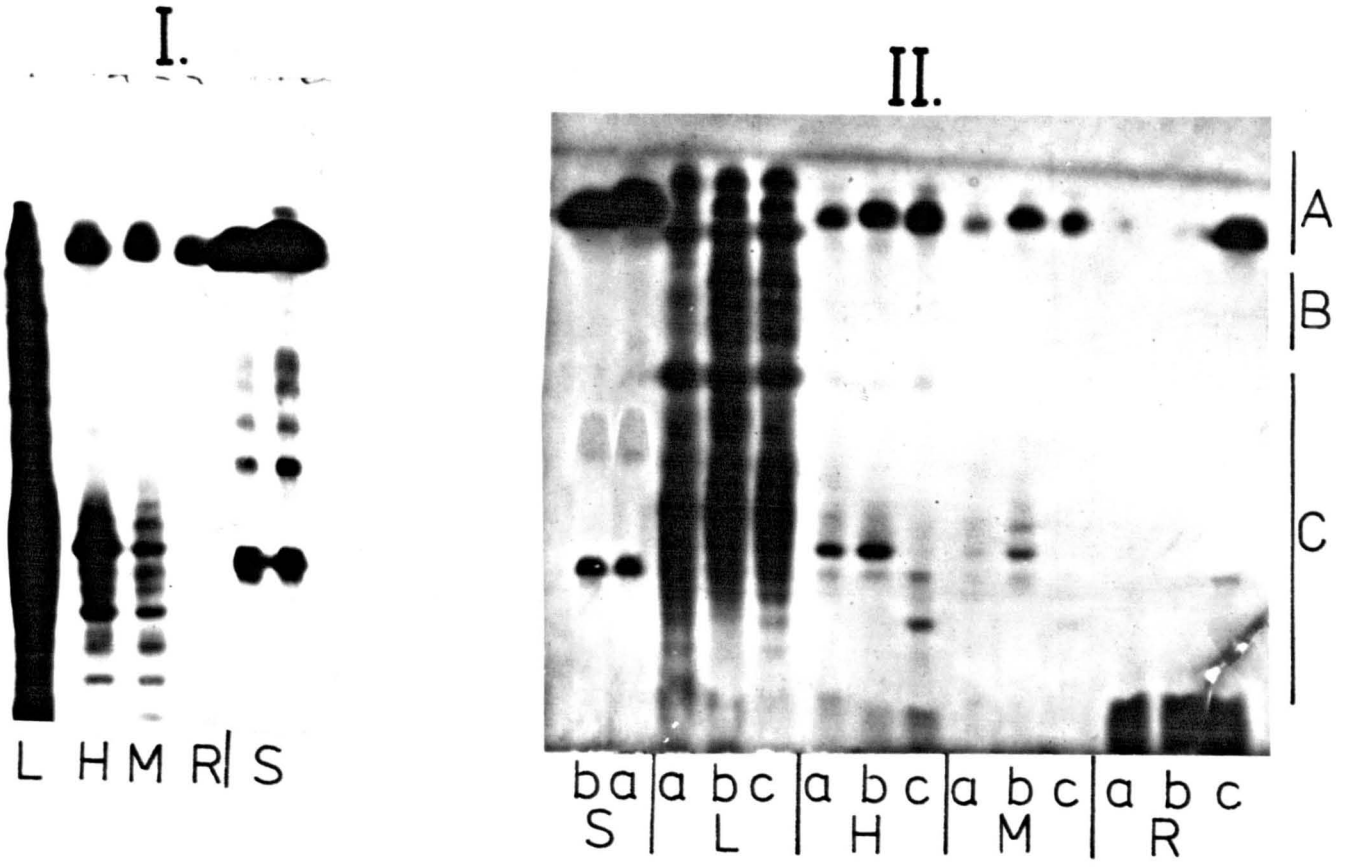


FIG. 39

Demonstrates how different tissue-homogenates of Apodemus sylvaticus react to 1-naphthyl acetate (I) and Naphthol-AS-acetate (II) as substrates.

I. Acts as control, stained by using 1-naphthyl acetate as substrate:

L : pooled liver homogenates

H : pooled heartmuscle homogenates

M : pooled thighmuscle homogenates

S : 2 serum samples.

II Stained with Naphthol-AS-acetate as substrate, staining time 10 hrs.

a : pooled samples from Icelandic Apodemus sylvaticus Type CII

b : pooled samples from Scottish Apodemus sylvaticus Type CII

c : pooled samples from Norwegian (Oslo) Apodemus sylvaticus. Type CIII

Note in the red-cell lysates of c, the cells had not been washed.

Tris-citrate pH 7.6.

Fig.40

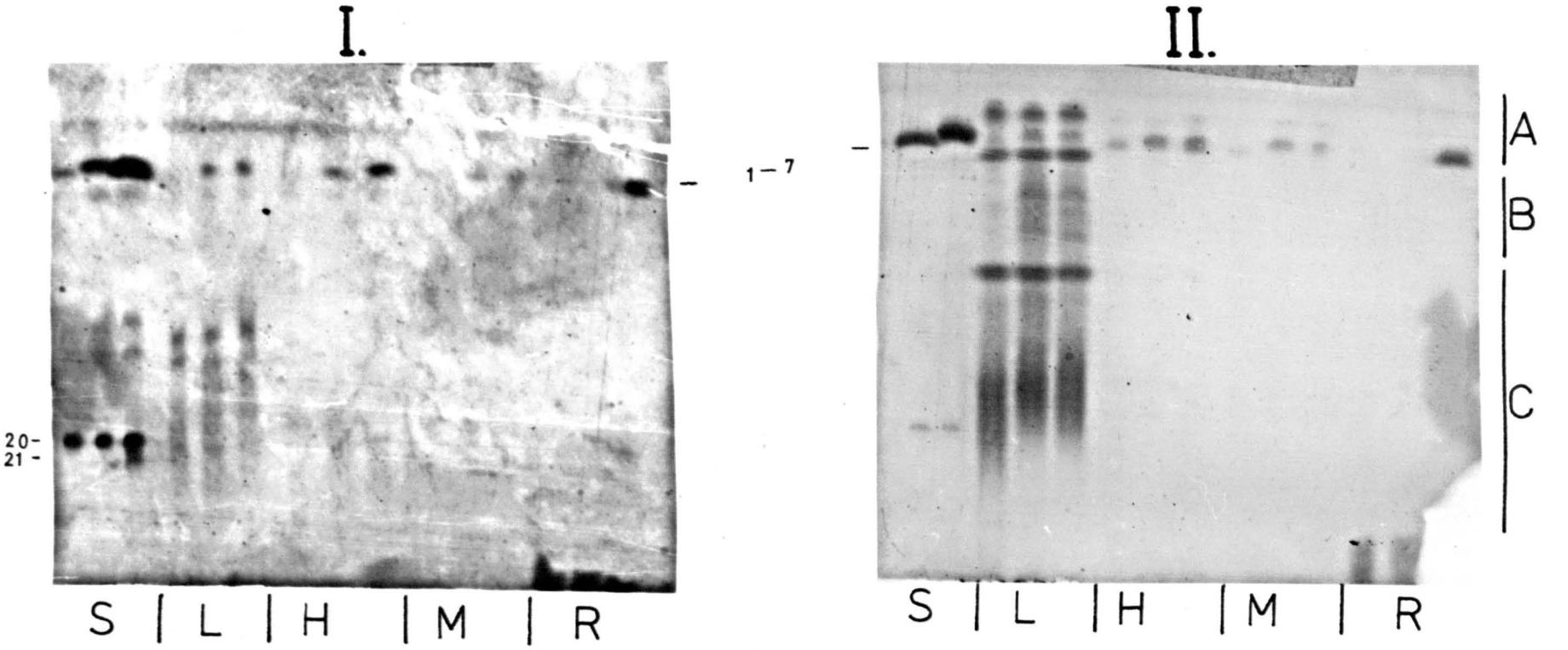


FIG. 40

Demonstrates substrate specificity.

I. Substrate : 6-bromo-2-carbo-naphthoxy choline iodide.

II. Substrate: Naphthol-AS-acetate, the same plate as 39, II, after 15 minutes staining

Note: I is the other half of II.

Tris-citrate pH 7.6.

Fig. 41

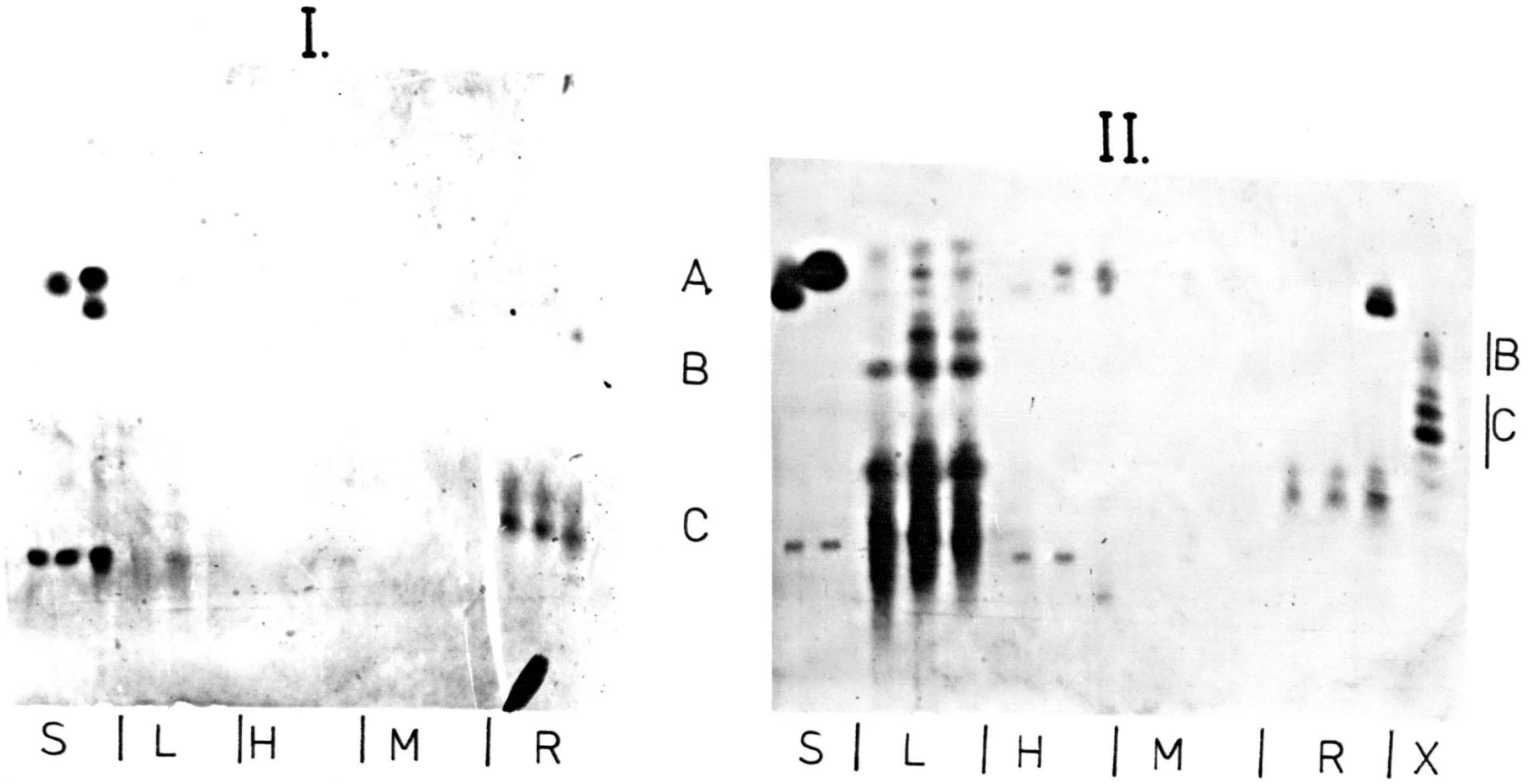


FIG. 41

Demonstrating substrate specificity.

The same as Fig. 40 I and II; and Fig. 39 II, except the gel buffer was Tris-citrate-borate pH 8.6.

I. Substrate: 6-bromo-2-carbonaphthoxy choline iodide.

Note how little reaction the liver homogenates show.

II. Substrate: Naphthol-AS-acetate

X : liver homogenate from Lagopus mutus islandicorum

tris-citrate-borate pH 8.6.

Fig.42

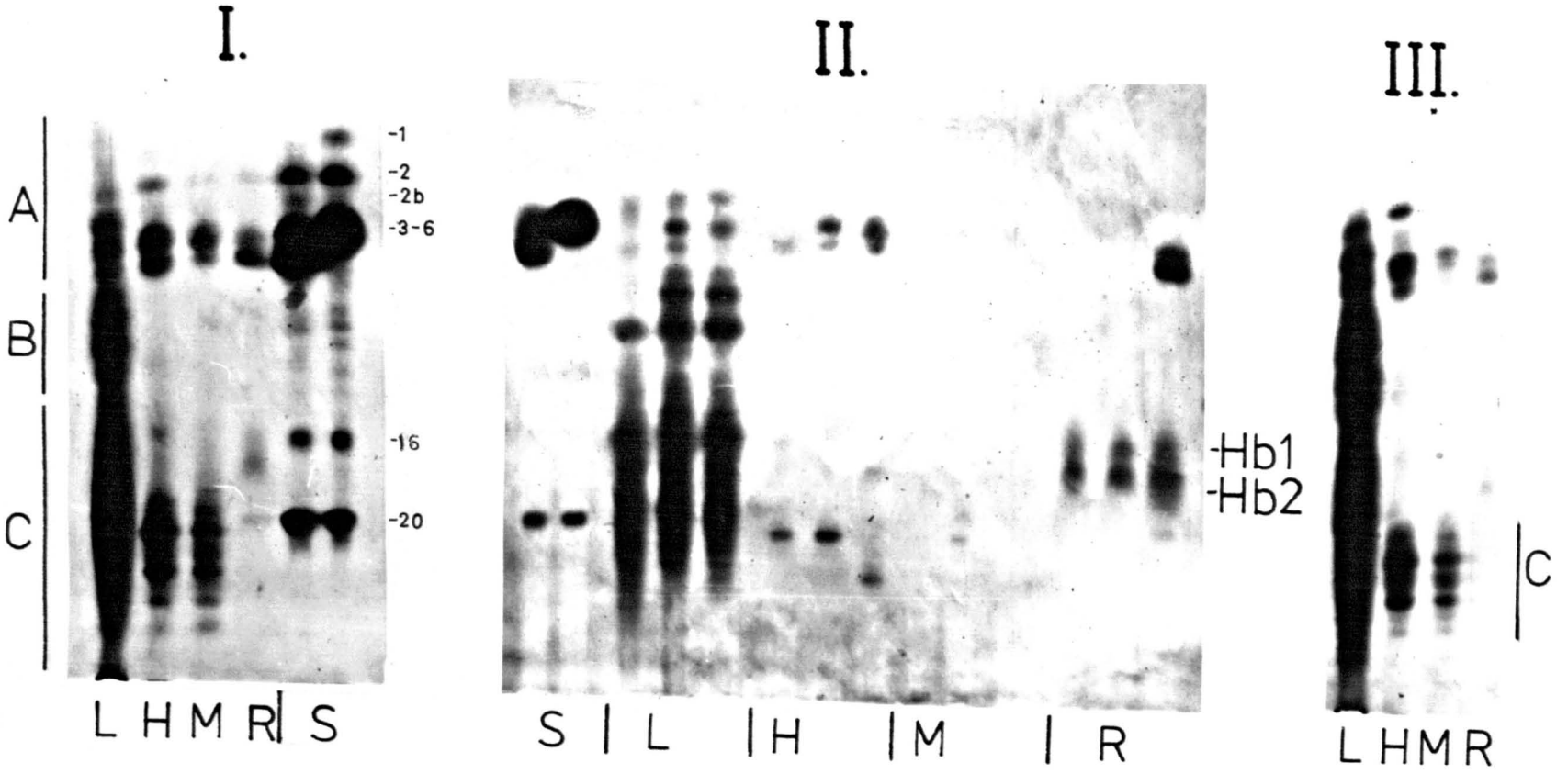


FIG. 42

The same as Fig. 39.

I. Substrate: 1-naphthyl acetate

II. Substrate: Naphthol-AS-acetate

The same as Fig. 41, II, at a later stage of staining.

III. 2-naphthyl acetate.

Tris-citrate-borate pH 8.6.

Fig. 43

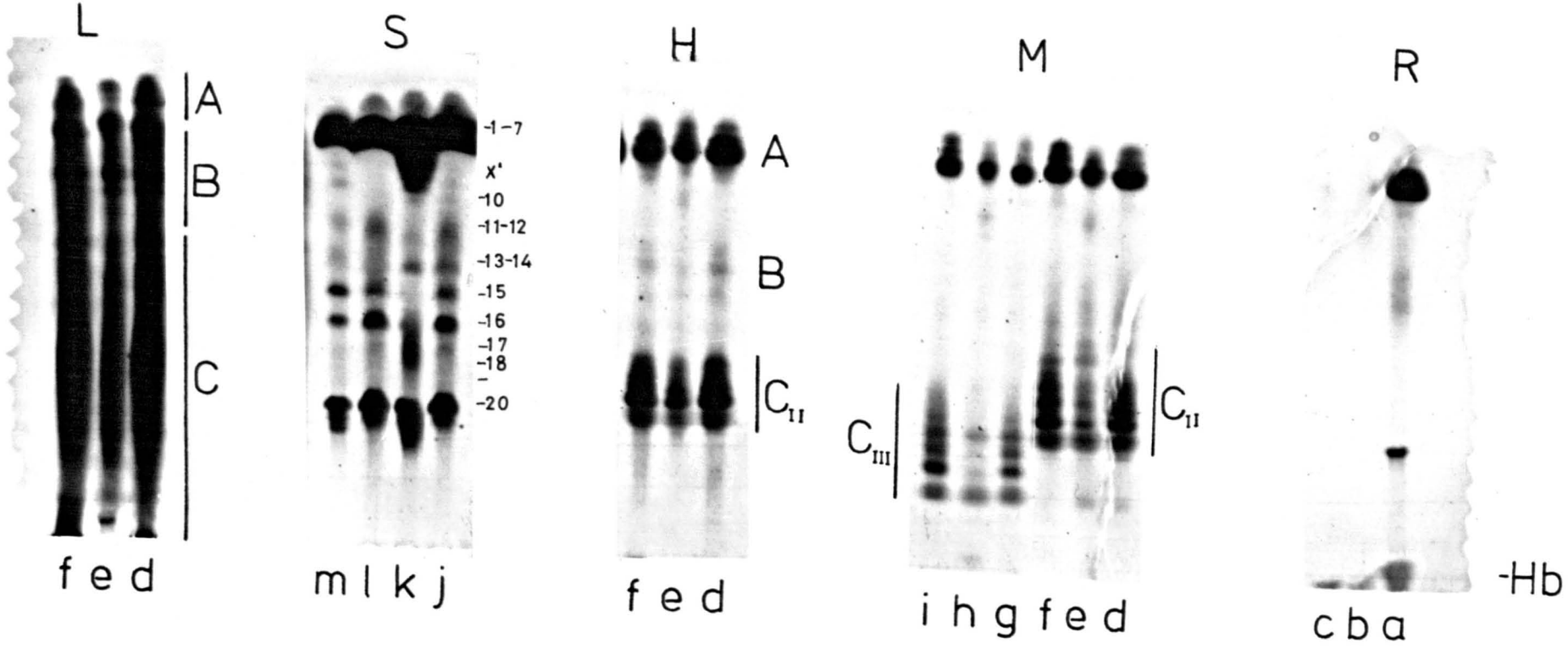

L
A
B
C
f e d
S
-1-7
x'
-10
-11-12
-13-14
-15
-16
-17
-18
-20
m l k j
H
A
B
C_{II}
f e d
M
C_{III}
C_{II}
i h g f e d
R
-Hb
c b a

FIG. 43

Effects of neuraminidase on the esterase-pattern of different tissues from Apodemus sylvaticus. The treated samples were incubated with neuraminidase at 37°C for three days. One control was incubated for three days at 37°C, the other was kept at -20°C for the same period.

a : Norwegian (Jaeren) red-cell lysate, not washed and not treated with neuraminidase

b : Icelandic red-cell lysate, washed and treated with neuraminidase.

c : control for b, kept at 37°C

d : samples from Scottish Apodemus sylvaticus, a control kept at 37°C

e : the same sample as d, but treated with neuraminidase

f : the same as d and e, kept at -20°C

g : sample from Norwegian (Oslo) Apodemus sylvaticus, a control kept at 37°C

h : the same as g, but treated with neuraminidase

i : the same sample as g and h, except kept at -20°C

j : pooled Icelandic and Scottish samples, a control for k

k : the same as j, treated with neuraminidase

l : an Icelandic sample

m : a Norwegian (Oslo) sample

Tris-citrate pH 7.6.

Fig. 44

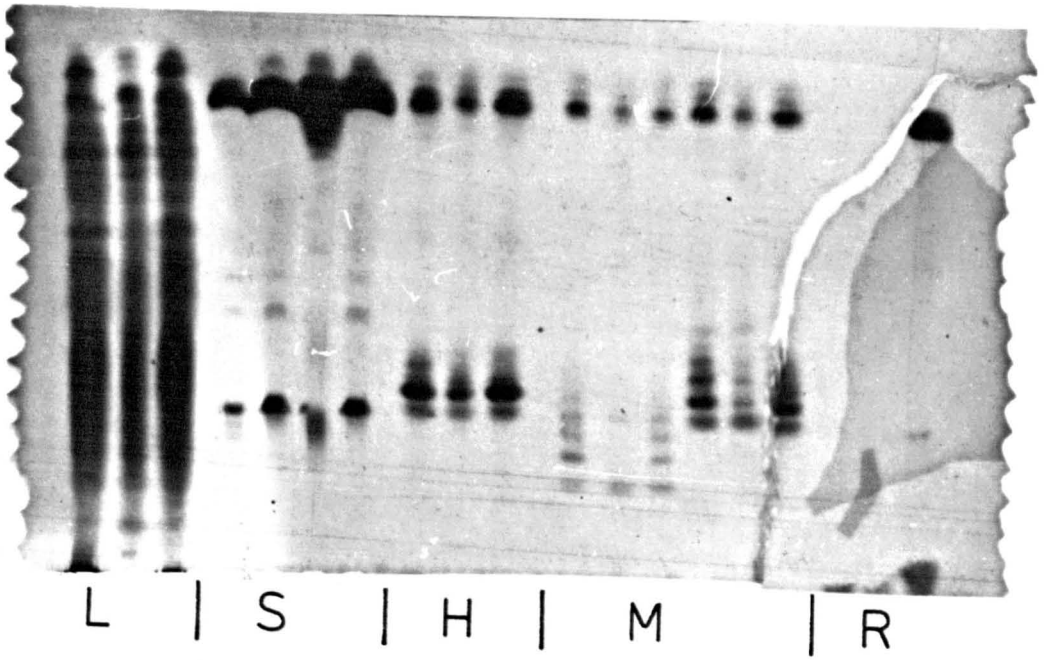


FIG. 44

The same as Fig. 43 at an earlier stage of staining to demonstrate the effect of neuraminidase on the liver homogenates. In this figure the plate is shown as a whole. For labelling see Fig. 43.

Tris-citrate pH 7.6.

Fig.45

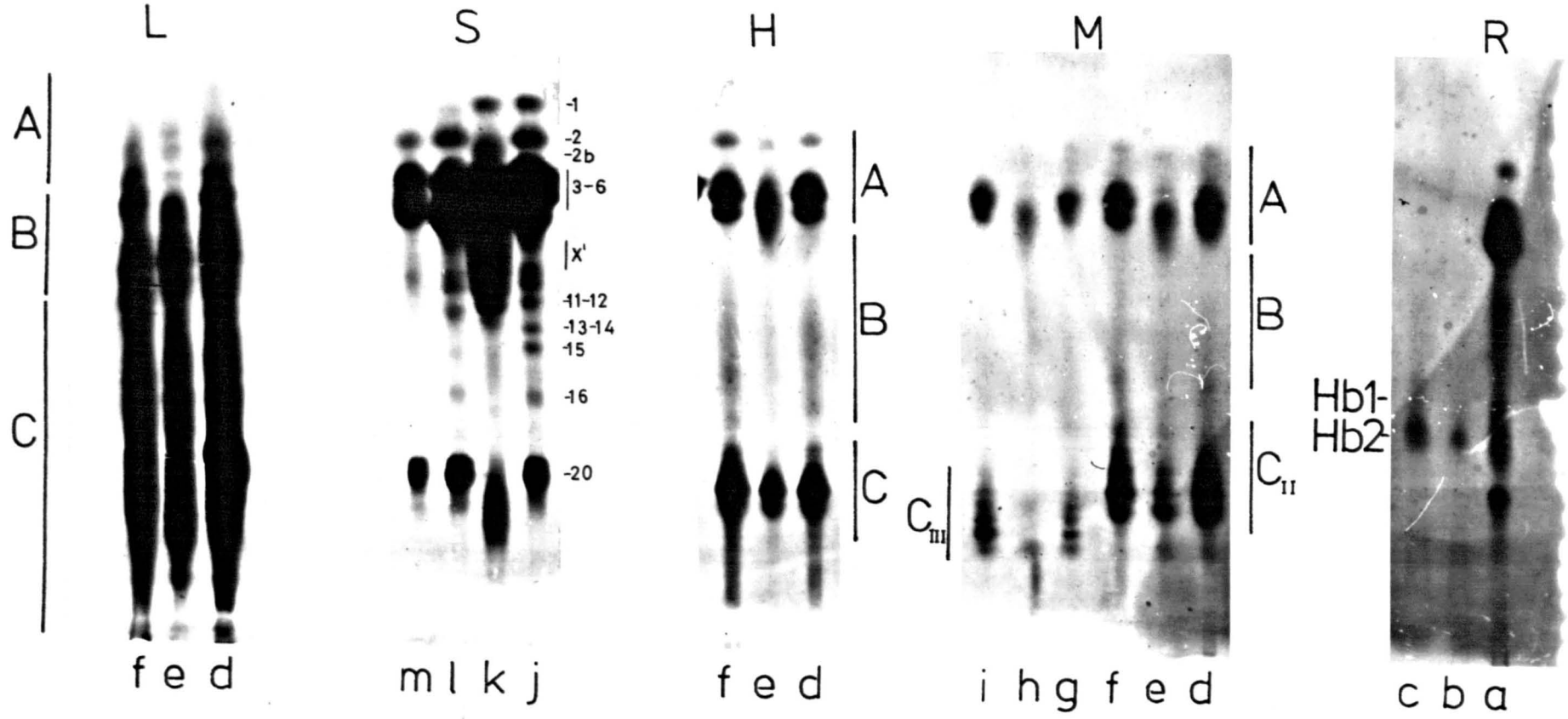

L
S
H
M
R
A
B
C
-1
-2
-2b
3-6
X'
-11-12
-13-14
-15
-16
-20
A
B
C
A
B
C
C_III
C_II
Hb1-
Hb2-
f e d
m l k j
f e d
i h g f e d
c b a

FIG. 45

The same as Fig. 43, showing the effects of neuraminidase on the esterases, as separated at pH 8.6.

Note especially how the neuraminidase changes the migration rate in zones A and B.

For labelling see Fig. 43.

Tris-citrate-borate pH 8.6.

Fig.46

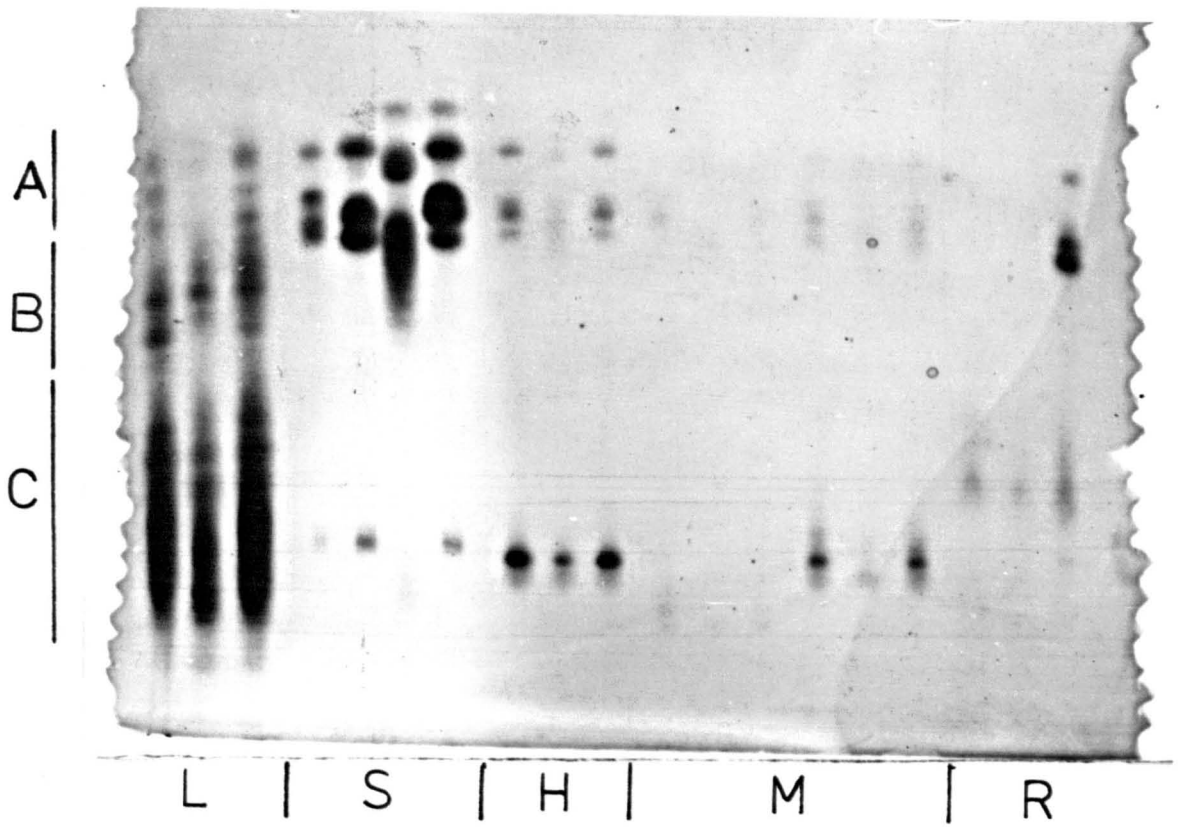


FIG. 46

The same plate as Fig. 45 at an earlier stage of staining. Note the effects of neuraminidase on the fastmoving liver-esterase fractions, as well as on zone C.

Tris-citrate-borate pH 8.6.

Fig.47

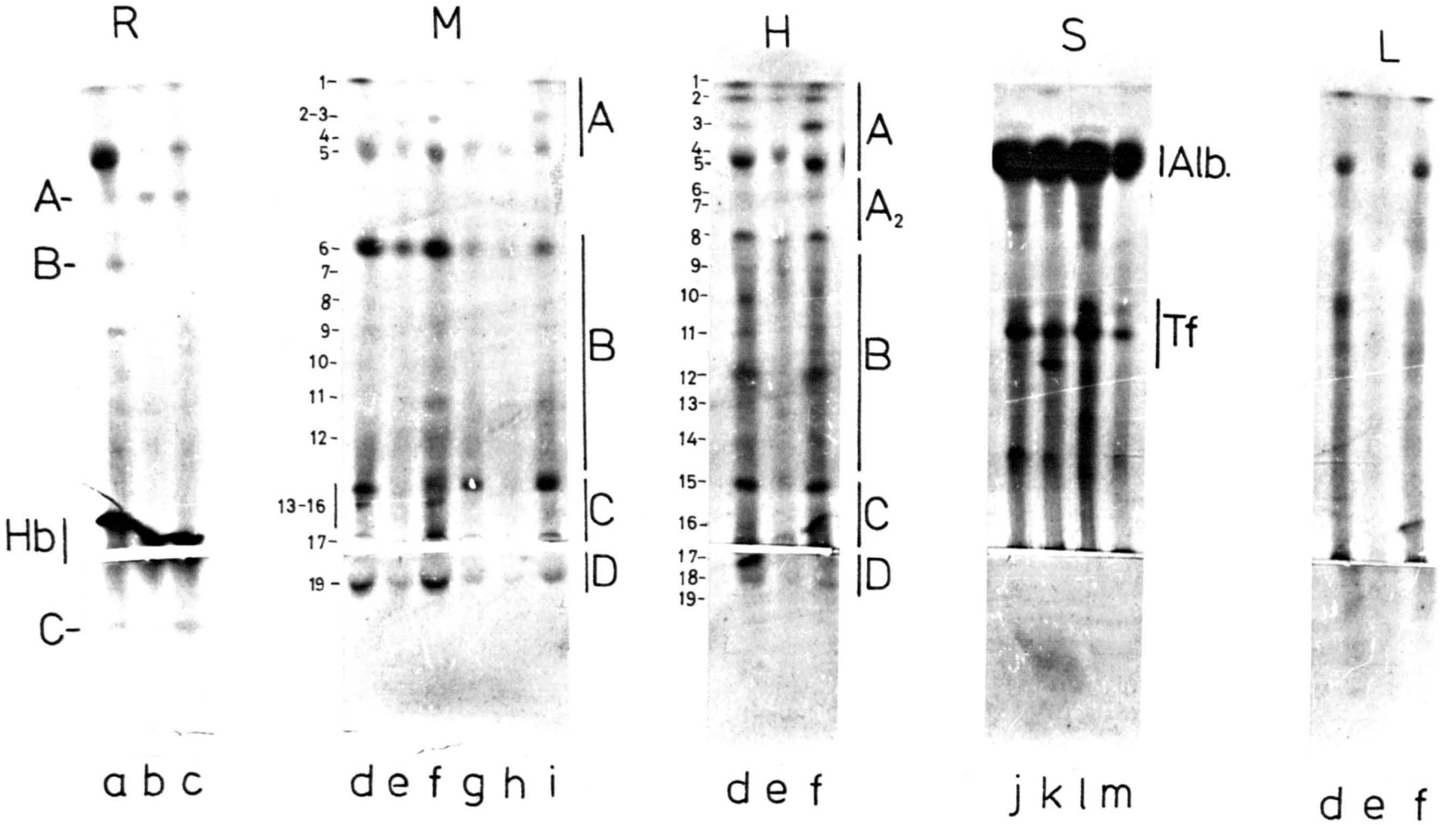

R
M
H
S
L
A-
B-
Hb
C-
1-
2-3-
4-
5-
6-
7-
8-
9-
10-
11-
12-
13-16
17-
19-
A
B
C
D
1-
2-
3-
4-
5-
6-
7-
8-
9-
10-
11-
12-
13-
14-
15-
16-
17-
18-
19-
A
A2
B
C
D
IAlb.
Tf
a b c
d e f g h i
d e f
j k l m
d e f

FIG. 47

The effects of neuraminidase on the protein pattern of 5 tissues of Apodemus sylvaticus. The other half of the plate in Fig. 43 and 44. The same labelling applies here as in Fig. 43, regarding samples.

Tris-citrate pH 7.6.

Stain: Nigrosine-amidoblack.

Fig.48

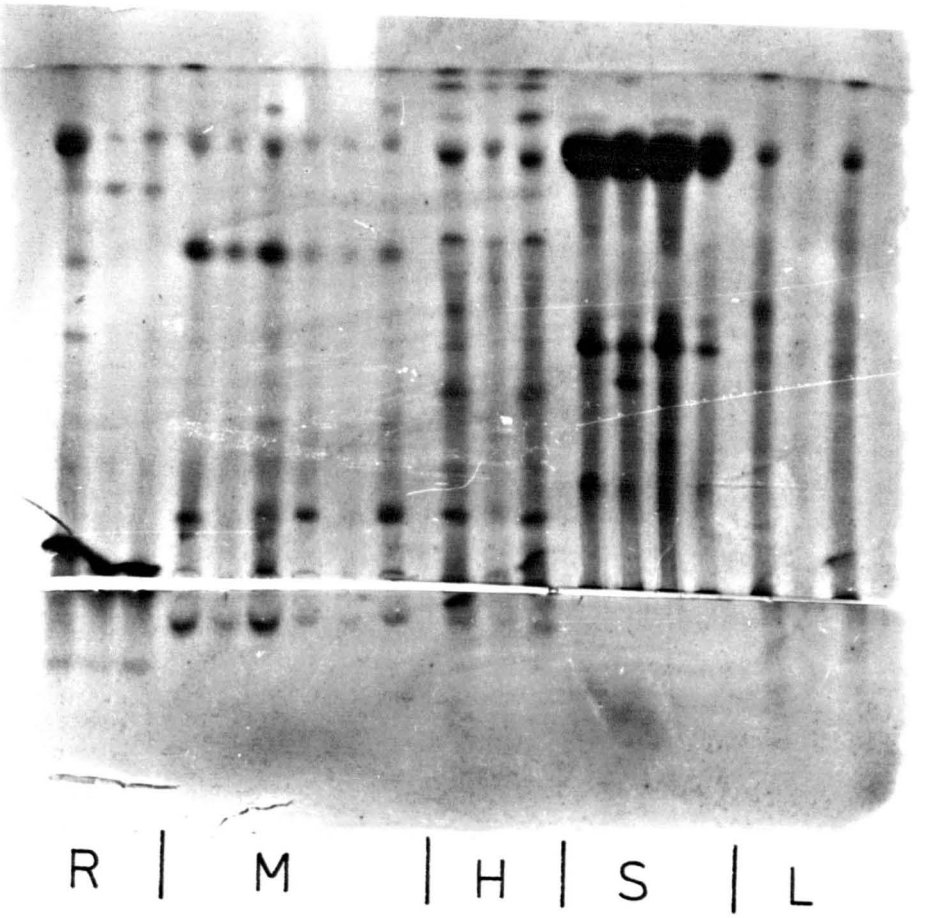


FIG. 48

The same as Fig. 47, but showing the whole plate for a better comparison of tissues.

Tris-citrate pH 7.6.

Fig.49

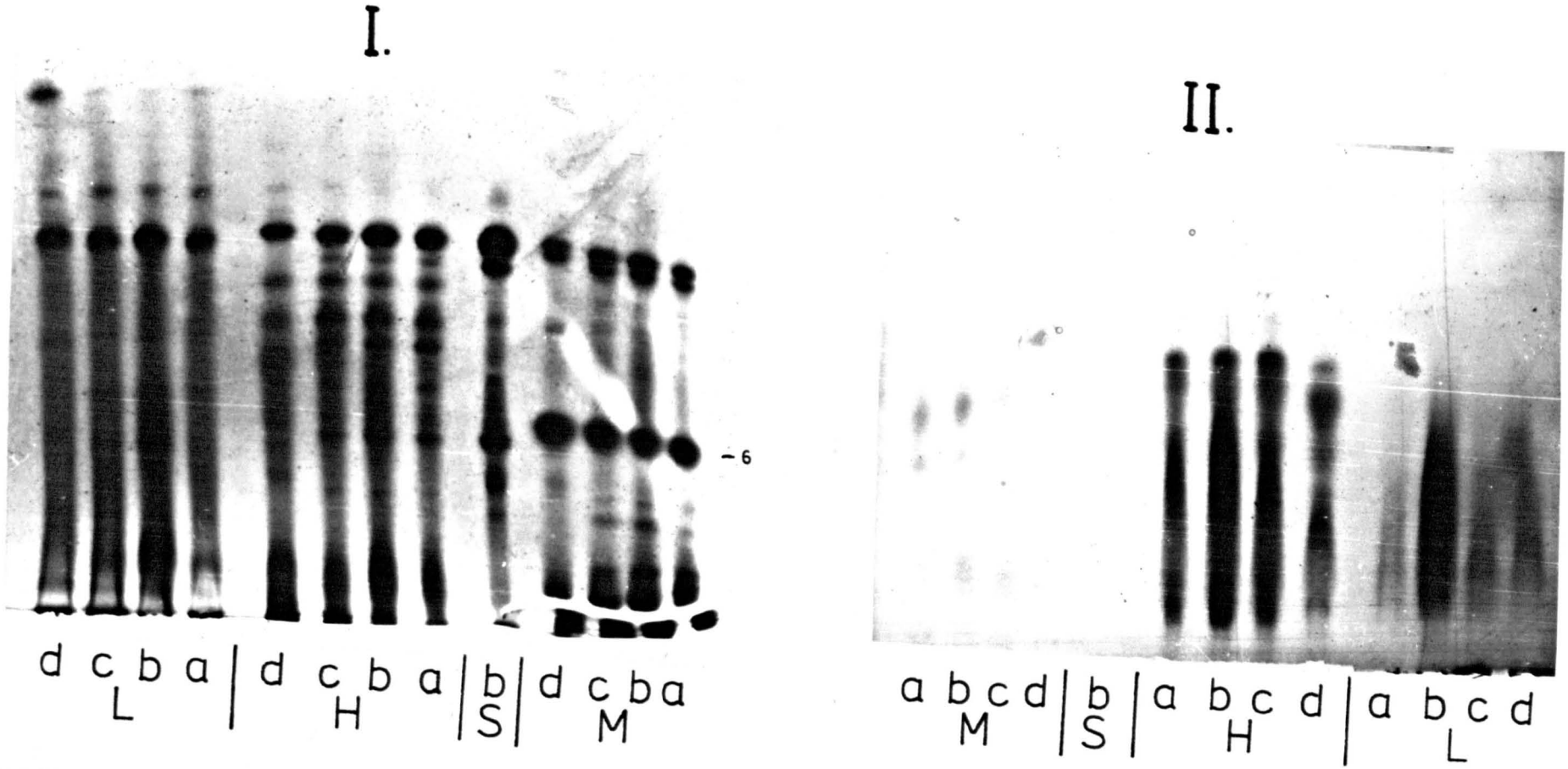


FIG. 49

Demonstrating peroxidase activity of liver , skeletal-, and heartmuscle homogenates of Apodemus sylvaticus and Mus musculus.

I. The nigrosine stained control slice.

II. Stained for peroxidase activity by using o-dianisidine and H_2O_2. Note how strong the peroxidase activity is in the heart, compared to the striated muscle. Note as well how much faster the peroxidase active proteins migrate in this system (pH 8.6) compared to Fig. 50.

a, b and c : Samples from Icelandic Apodemus sylvaticus

d : Samples from a laboratory Mus musculus

Tris-citrate-borate pH 8.6.

Fig.50

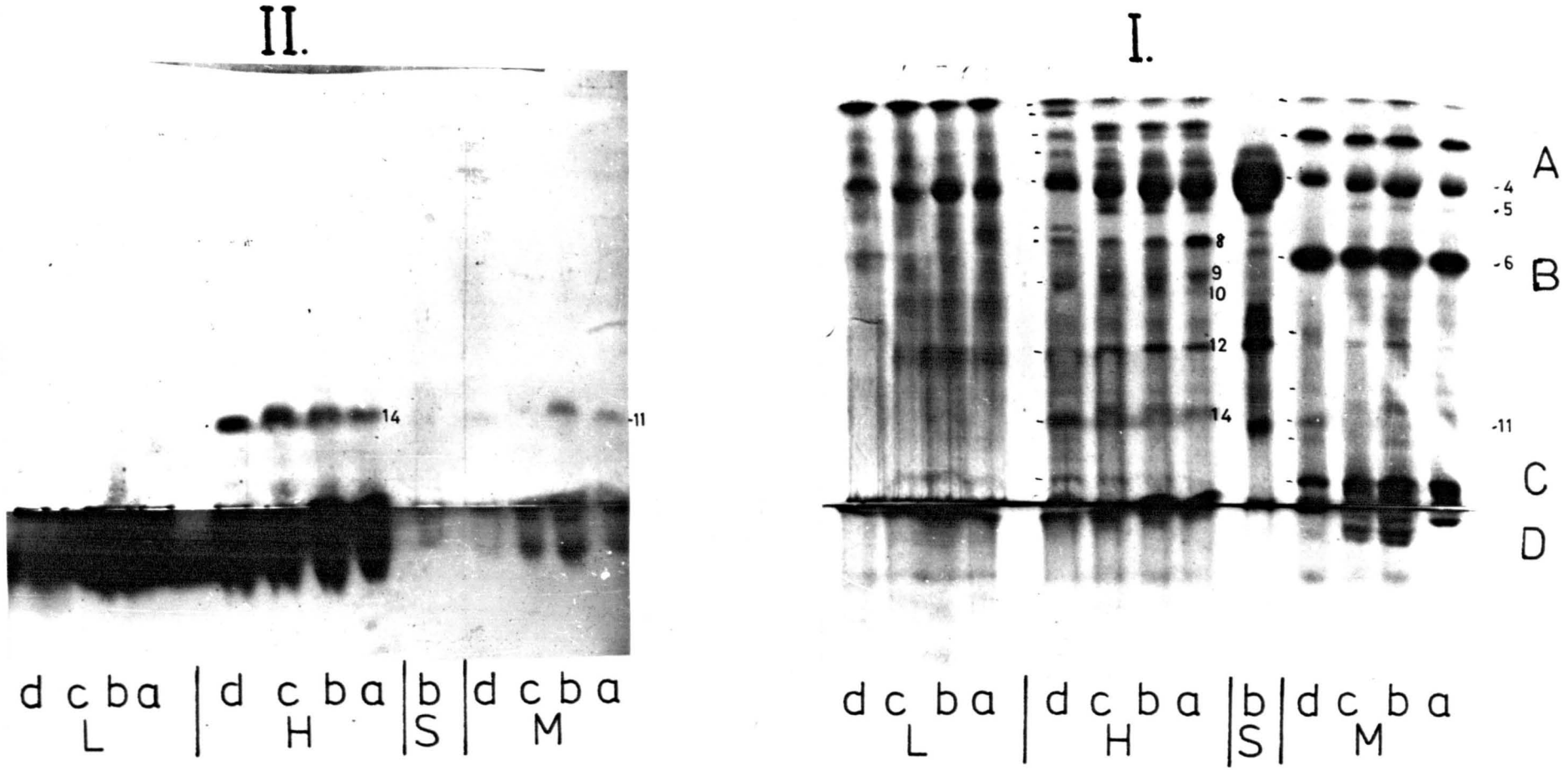

II.
14
11
d c b a
L
d c b a
H
b
S
d c b a
M
I.
A
4
5
B
6
8
9
10
12
14
11
C
D
d c b a
L
d c b a
H
b
S
d c b a
M

FIG. 50

Comparison of a nigrosine stained half and of a peroxidase stained half of a starch plate, in which liver, heart-, skeletal- muscle homogenates as well as serum proteins had been distributed by electrophoresis. The same samples as in Fig. 49.

Note the overlapping of peroxidase active fraction with protein fraction 11 of the skeletal muscle and fraction 14 of the heart muscle.

Note also strong activity in zone D, not overlapping with nigrosine stained fractions. The similarity between the two species is apparent.

Tris-citrate pH 7.6.

Fig. 51

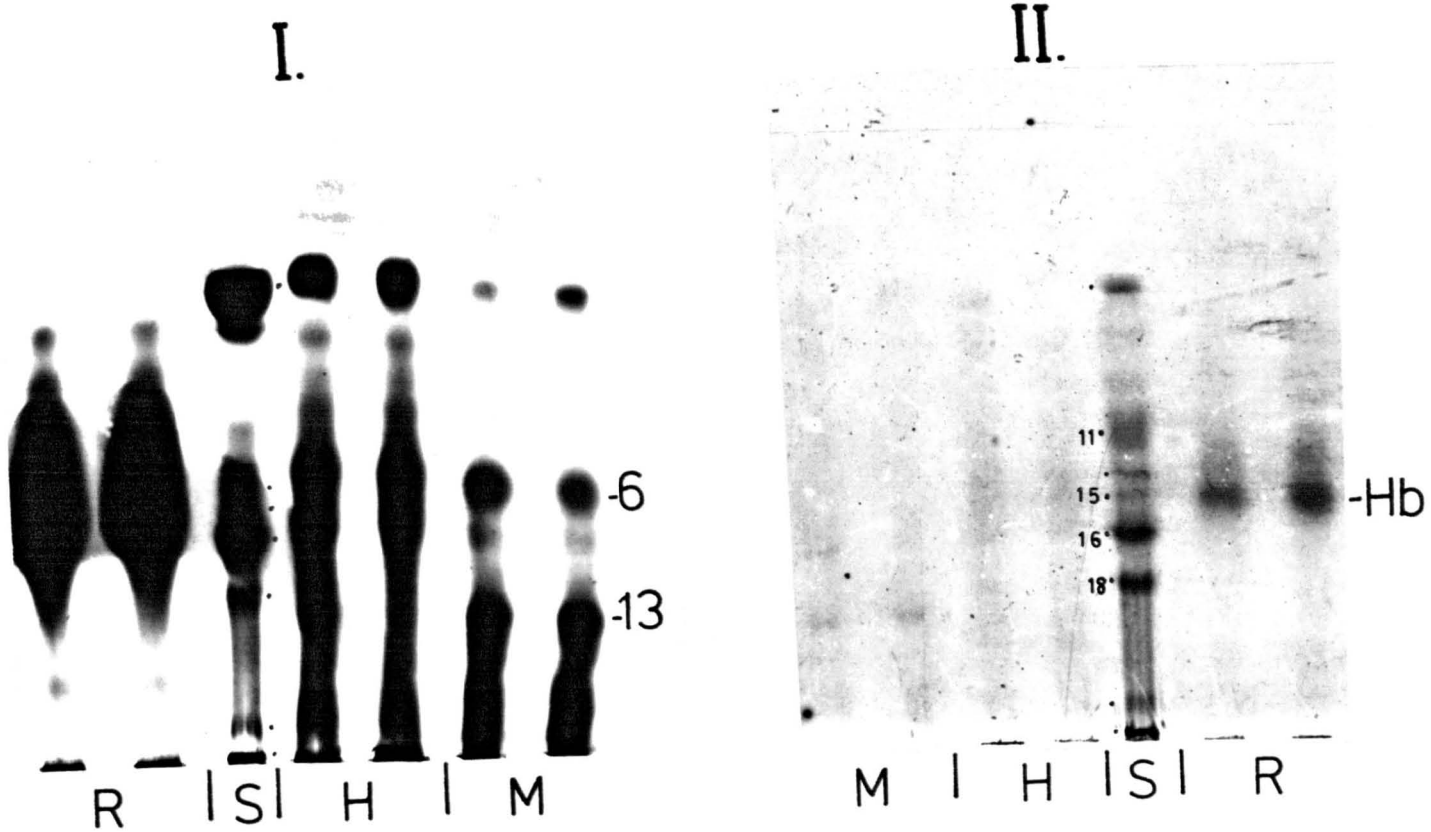


FIG. 51

Comparison of two halves of polyacrylamide gels, stained for proteins (amidoblack) and glyco-proteins (P.A.S.) respectively.

Note that the serum is the only "tissue" that really reacts with P.A.S. the Hb did not react, but is shown because of its red colour.

I. Amidoblack stained half

II. P.A.S. stained half

Tris-citrate borate pH 8.6

7.5% poly acrylamide gel.

Fig. 52

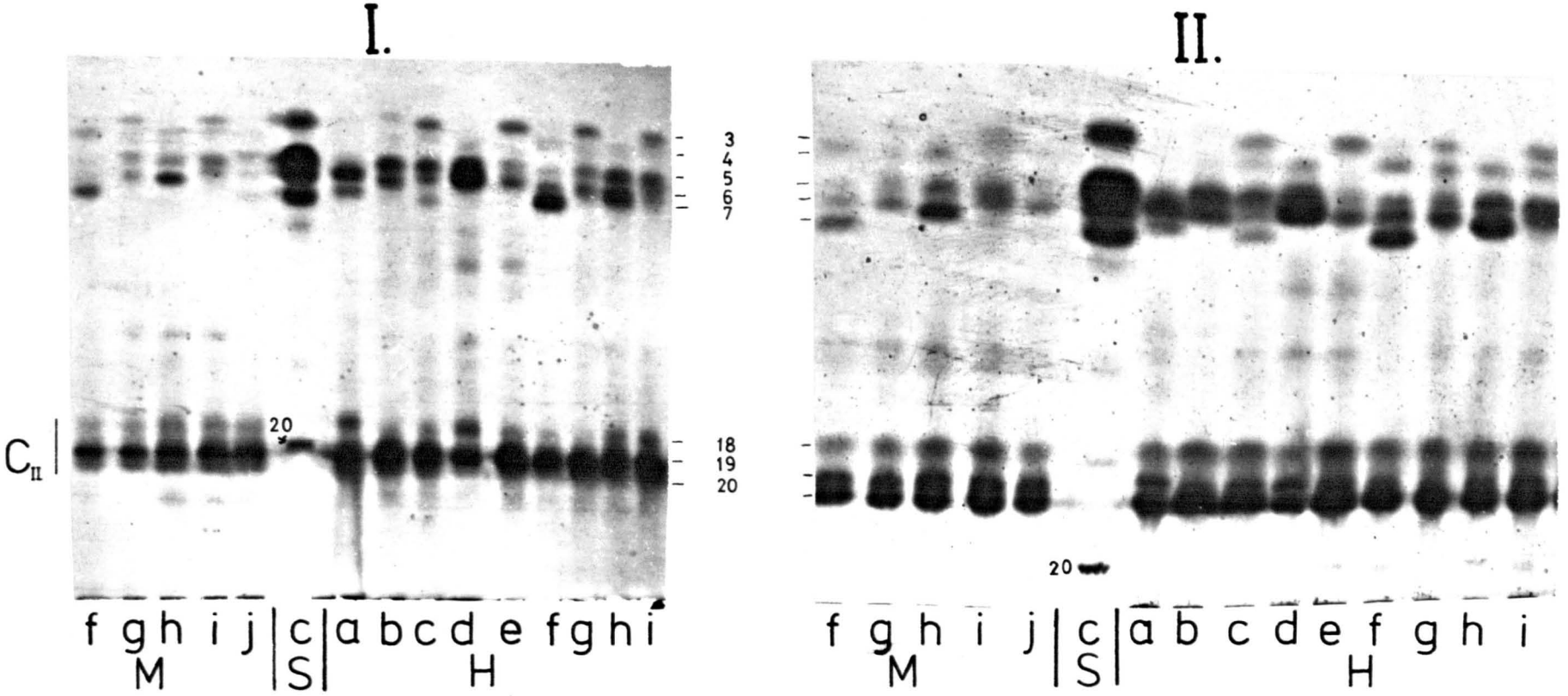

I.
II.
3
4
5
6
7
18
19
20
20
C_{II}
f g h i j c a b c d e f g h i
M S H
f g h i j c a b c d e f g h i
M S H

FIG. 52

Comparison of esterase distribution in starch (I) and polyacrylamide (II) gels respectively

Note: (1) Presence and absence of fast moving esterases.

(2) Difference in the relative position of serum esterase fraction 20 in the two systems, as compared to zone C II of heart and striated muscle homogenates

I. H, M, and S run in starch gel.

II. Same as I run in polyacrylamidegel

a - j : Scottish (Cumbernauld) Apodemus sylvaticus

a, c, e, f, g and j are males

b, d, h, and i are females

Tris-citrate borate pH 8.6

Fig. 53

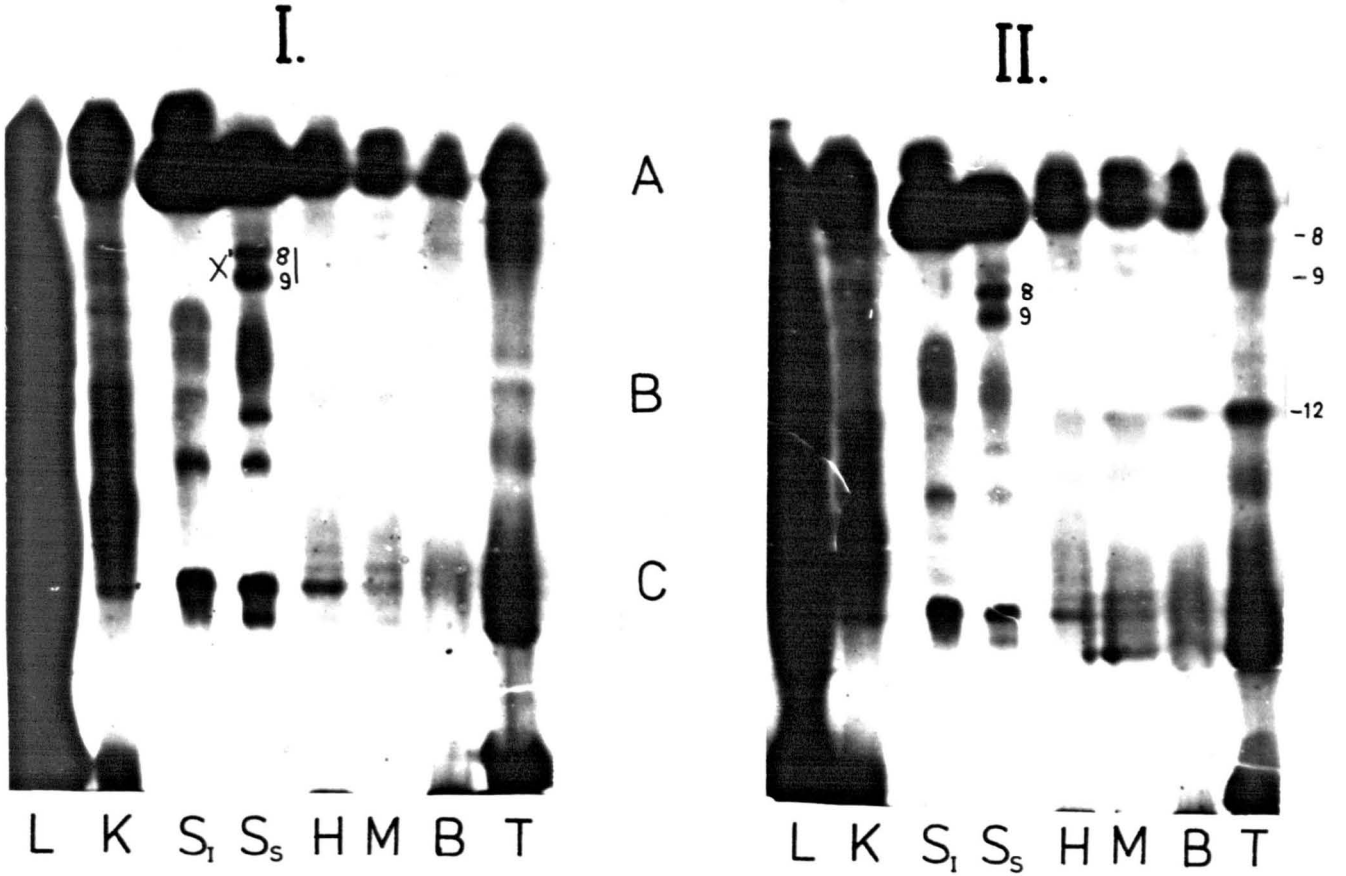


FIG. 53

Comparison of different tissues from Apodemus sylvaticus, with regard to substrate specificity. All samples are pooled. Note the strong fraction 12 of the testis on II.

I. substrate: 1-naphthyl acetate

II. substrate: 1-naphthyl propionate

S_I : pooled sera from Swedish-Icelandic hybrids of Apodemus sylvaticus

S_S : pooled sera from Scottish Apodemus sylvaticus

Tris-citrate pH 7.6

Fig. 54

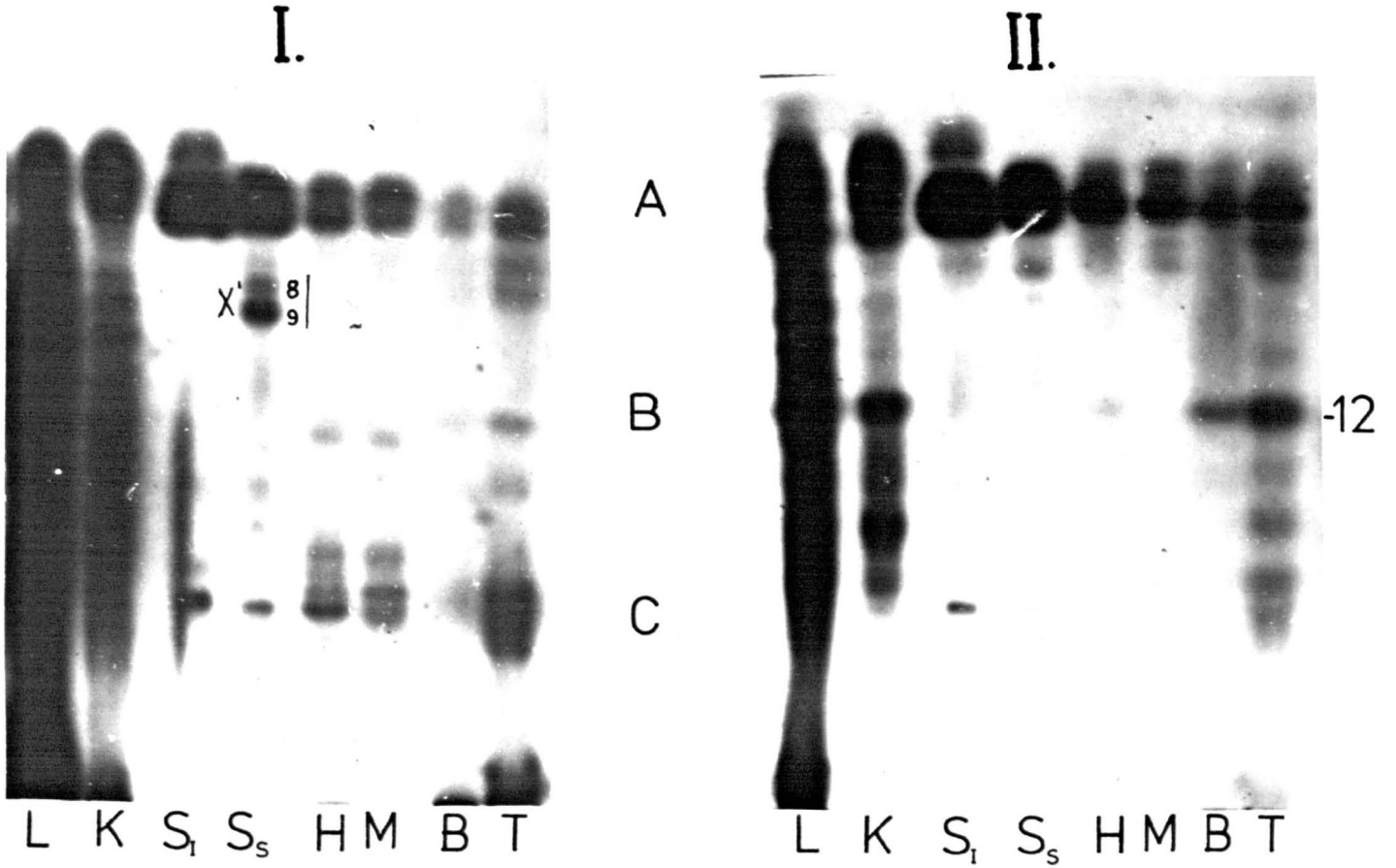


FIG. 54

Substrate specificity of esterases of different tissues from Apodemus sylvaticus.

The same samples as Fig. 53.

Note: (1) strong fraction 12 of the testis and a corresponding fraction in the kidney (II)

(2) Slow reaction of zone C to naphthol -AS- acetate (II)

I. substrate 2-naphthyl acetate

II. substrate naphthol -AS- acetate

Fig. 55

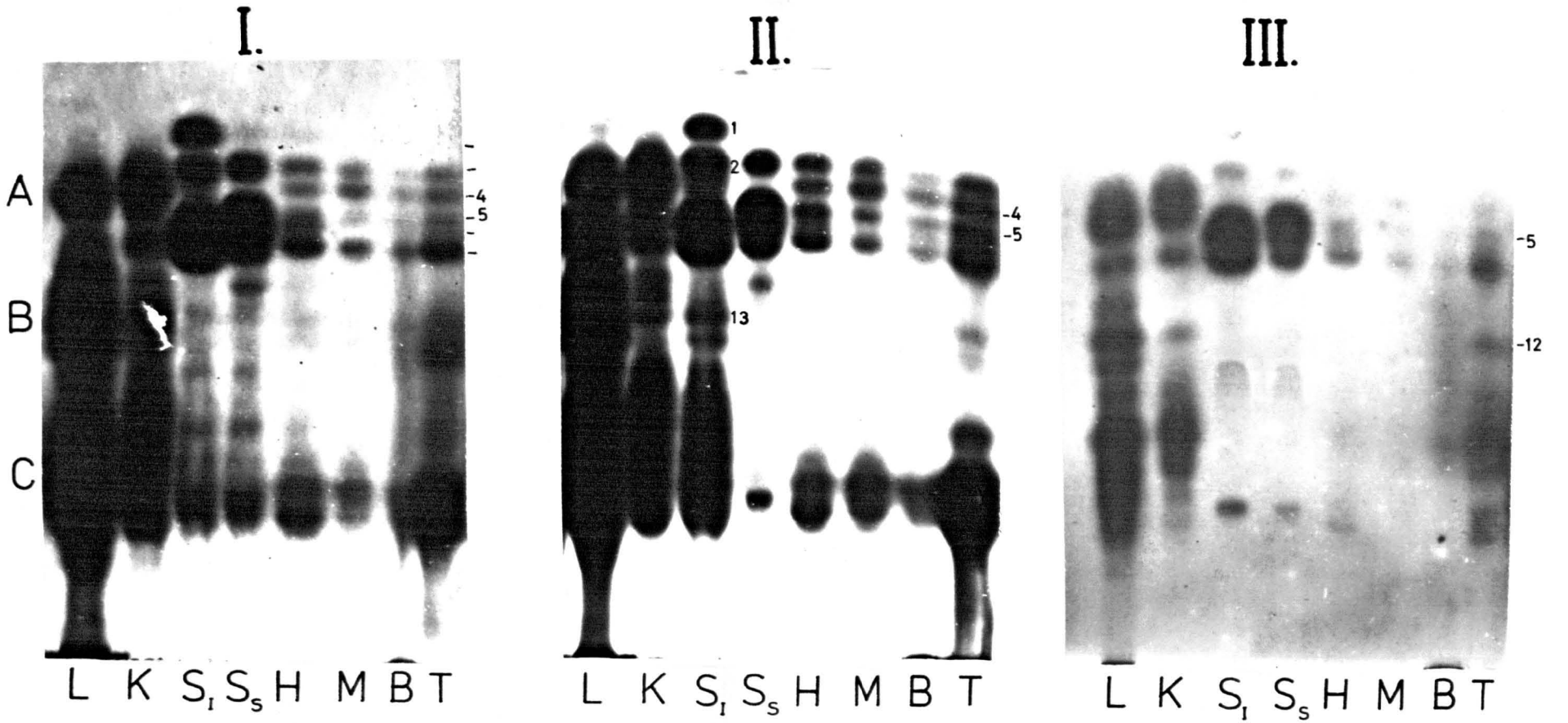

I.
II.
III.
A
B
C
-4
-5
1
2
13
-4
-5
-5
-12
L K S$_I$ S$_s$ H M B T
L K S$_I$ S$_s$ H M B T
L K S$_I$ S$_s$ H M B T

FIG. 55

Demonstration of substrate specificity and tissue homogenates comparison. Same samples as in Figs. 53 and 54.

I. substrate: 1-naphthyl acetate

II. substrate: 1-naphthyl propionate

Note that S_I has been contaminated by kidney homogenate

III. substrate: naphthol-AS-OL acetate
(Koch and Light)

Compare this zymogram carefully with the other zymograms.

Tris-citrate borate pH 8.6

Fig. 56

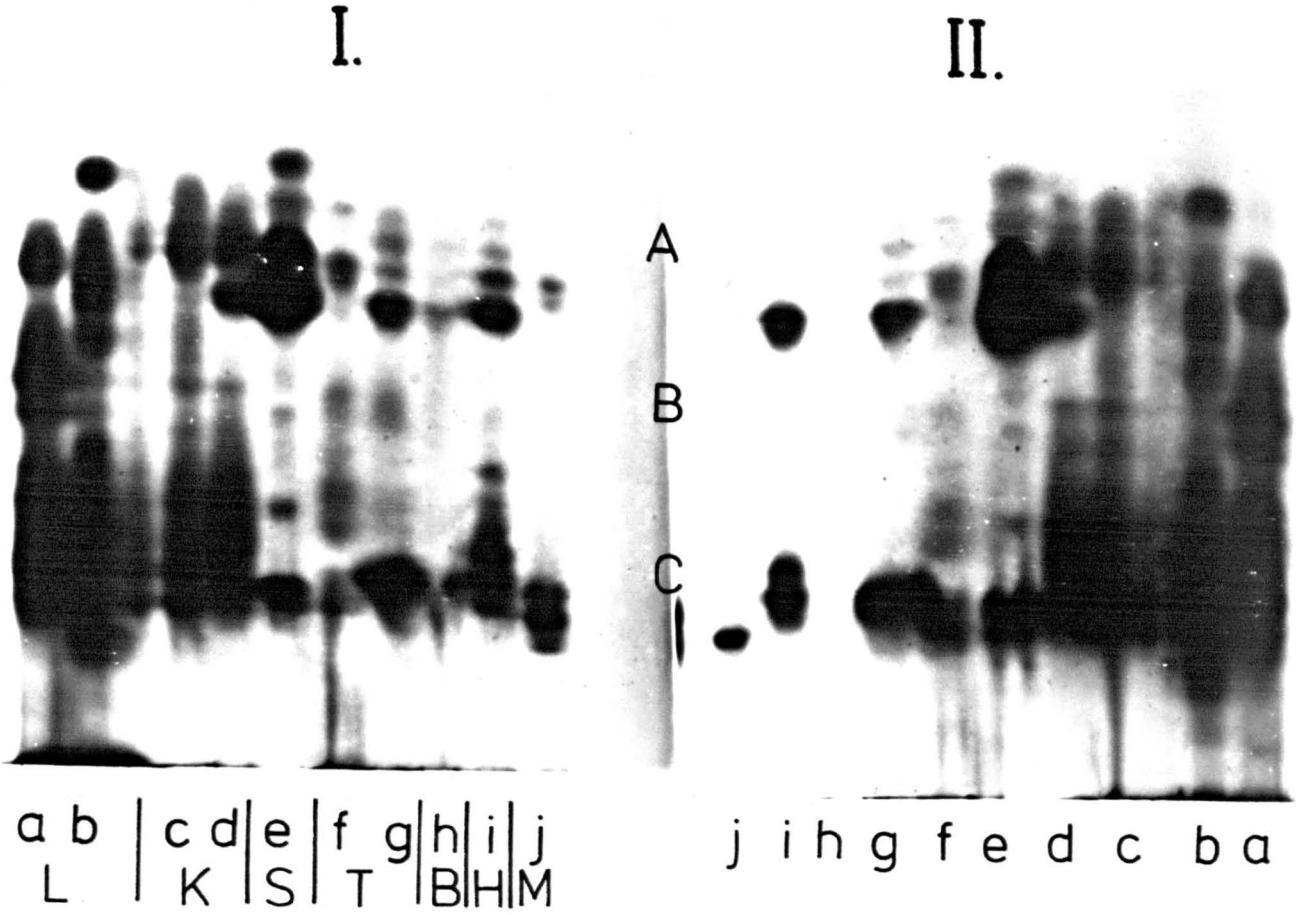


FIG. 56

The effect of physostigmine sulphate (eserine) (10^{-4} M) on esterase patterns of different tissues of Apodemus sylvaticus.

I. Control plate; substrate: 1-naphthyl acetate; coupler dye: Fast Garnet

II. This plate was incubated in 10^{-4} M eserine for 30 minutes before the substrate was added. Note the effect on the middle fractions of the serum.

a, c, f and j: Samples from Scottish Apodemus sylvaticus

b : Liver homogenate from Mus musculus (CB57)

d and e : samples from Swedish - Icelandic hybrids of Apodemus sylvaticus

g, h and i : Samples from Swedish Apodemus sylvaticus

Tris-citrate borate pH 8.6.

Fig. 57

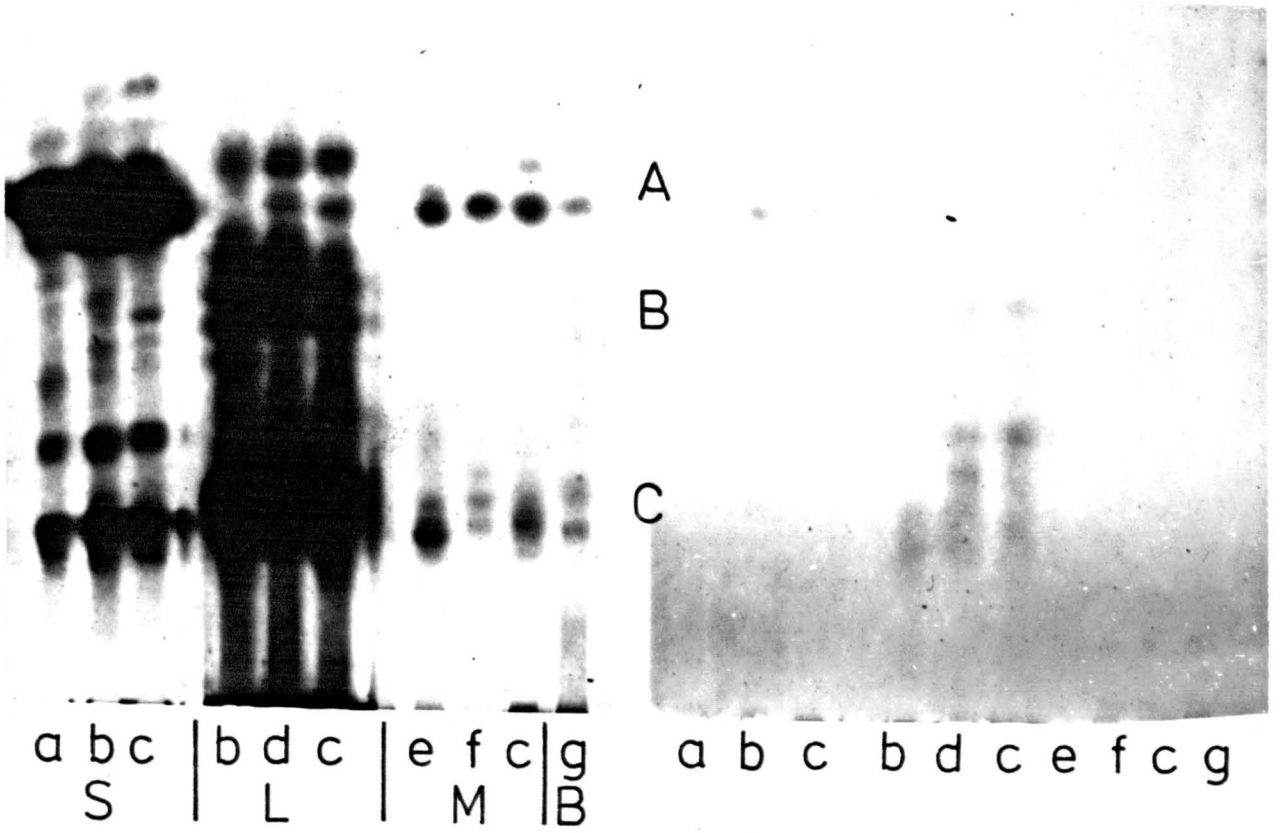


FIG. 57

Demonstration of inhibition by D.F.P. of esterases of various tissue homogenates of Apodemus sylvaticus.

I. Control plate stained for esterases using 1-naphthyl acetate

II. Same plate as I incubated in 10^{-4} M solution of D.F.P. for 30 min. prior to staining. Note some fractions showing up in sera and liver homogenates.

a, b, and e : Scottish Apodemus sylvaticus

b, d, f and g : Swedish Apodemus sylvaticus

c : Swedish-Icelandic hybrid of Apodemus sylvaticus

Tris-citrate borate pH 8.6

Fig. 58

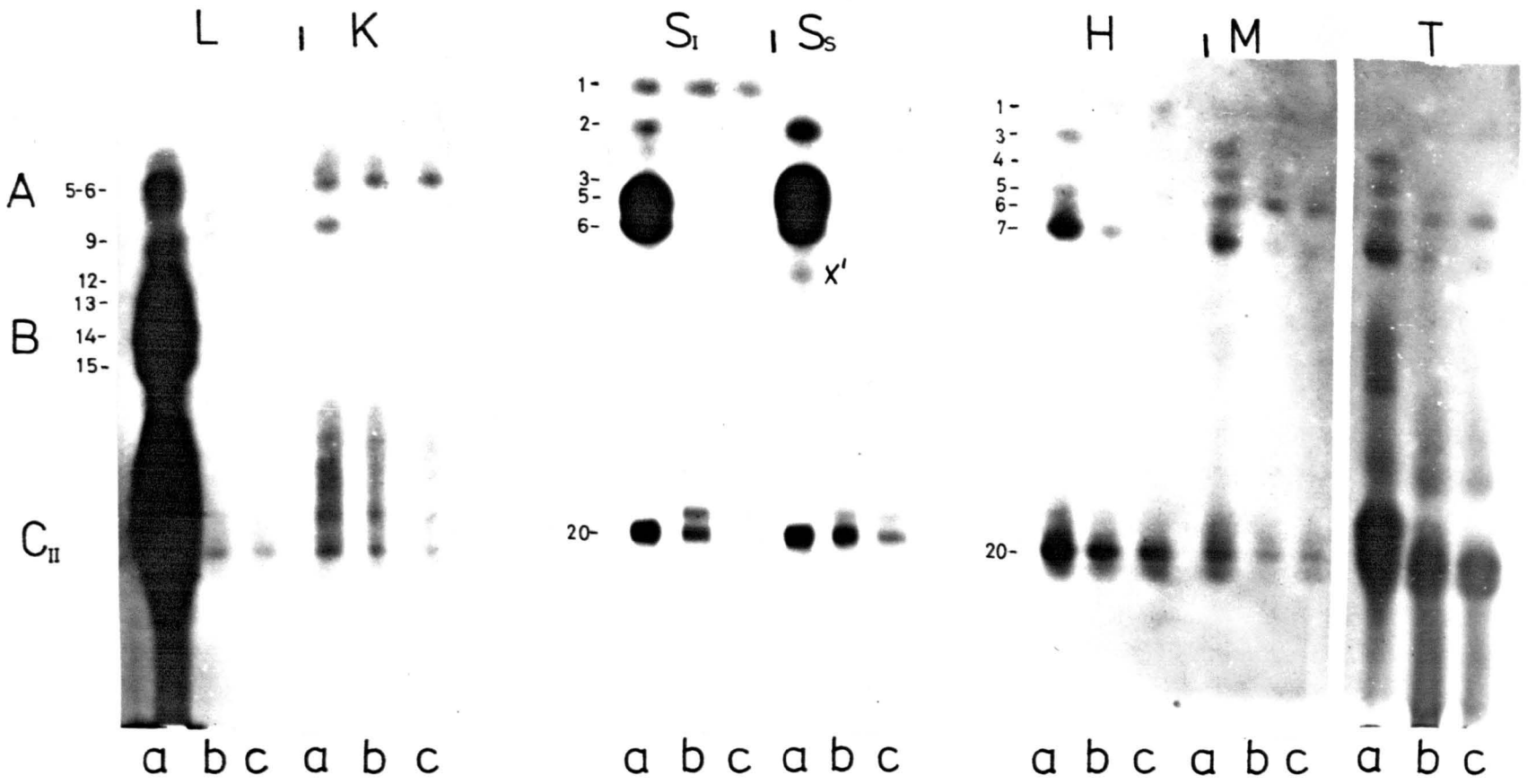

L
K
A
5-6-
9-
12-
13-
B
14-
15-
C II
a b c a b c
S I
S S
1-
2-
3-
5-
6-
X'
20-
a b c a b c
H
M
T
1-
3-
4-
5-
6-
7-
20-
a b c a b c a b c

FIG. 58

Effects of heat on esterase patterns of various tissues of Apodemus sylvaticus. The supernatants of tissue homogenates were drawn up into capillaries, which were then sealed and incubated at 56°C for different lengths of time.

Note difference in sensitivity towards this treatment in different fractions.

a : Control samples, not treated

b : Samples incubated for 5 minutes at 56°C.

c : Samples incubated for 10 minutes at 56°C.

S_I and S_S : serum from Icelandic and Scottish mice respectively

Tris-citrate borate pH 8.6

Fig.59

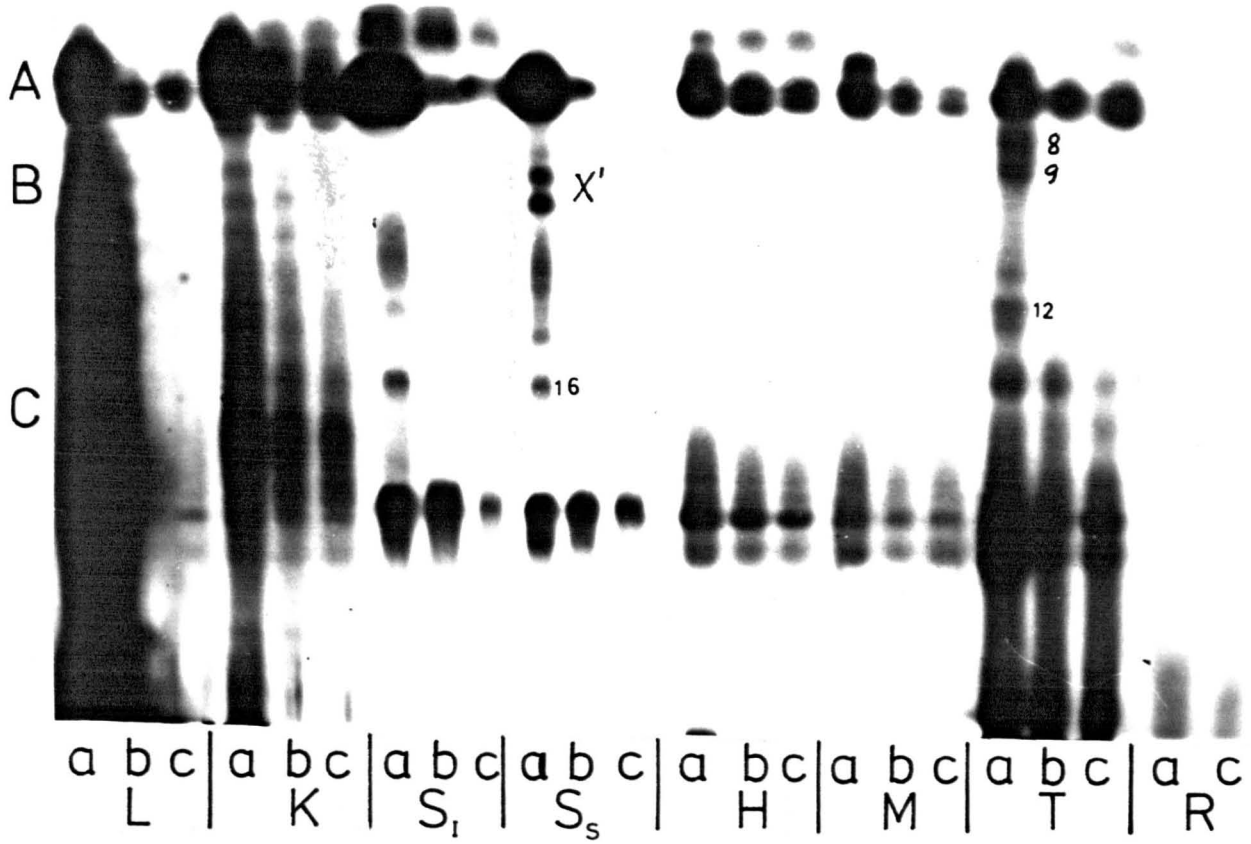


FIG. 59

Demonstration of heat effects on esterases of Apodemus sylvaticus. Same as Fig. 58, except different buffer system. Note smear in zone C of the testis - this is due to inadequate centrifugation.

Tris-citrate pH 7.6

Fig. 60

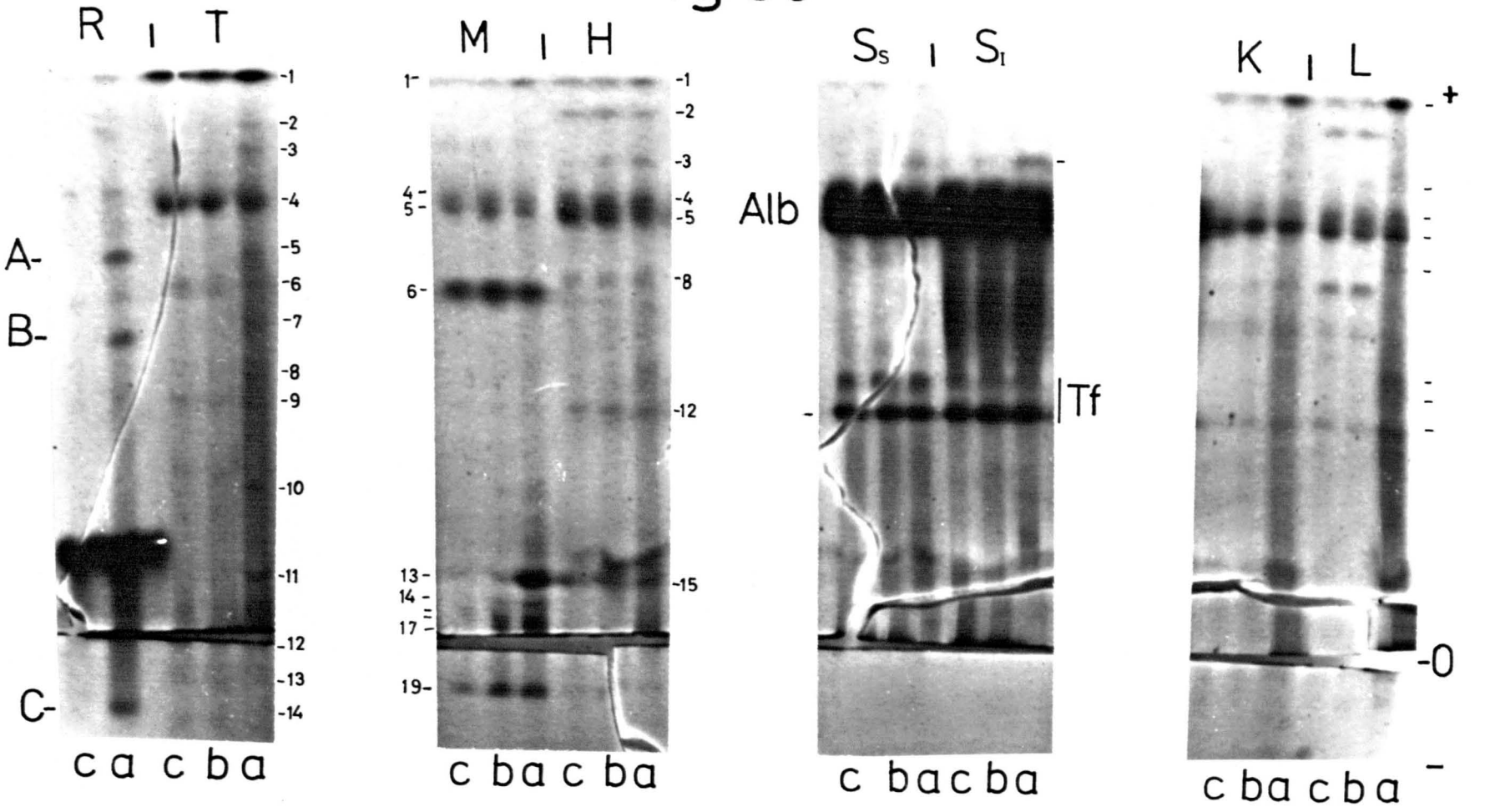


FIG. 60

Effect of heat treatment on protein patterns of Apodemus sylvaticus.
The top half of gel shown in Fig. 59

Tris-citrate pH 7.6
Nigrosine-amidoblack.

Fig.61

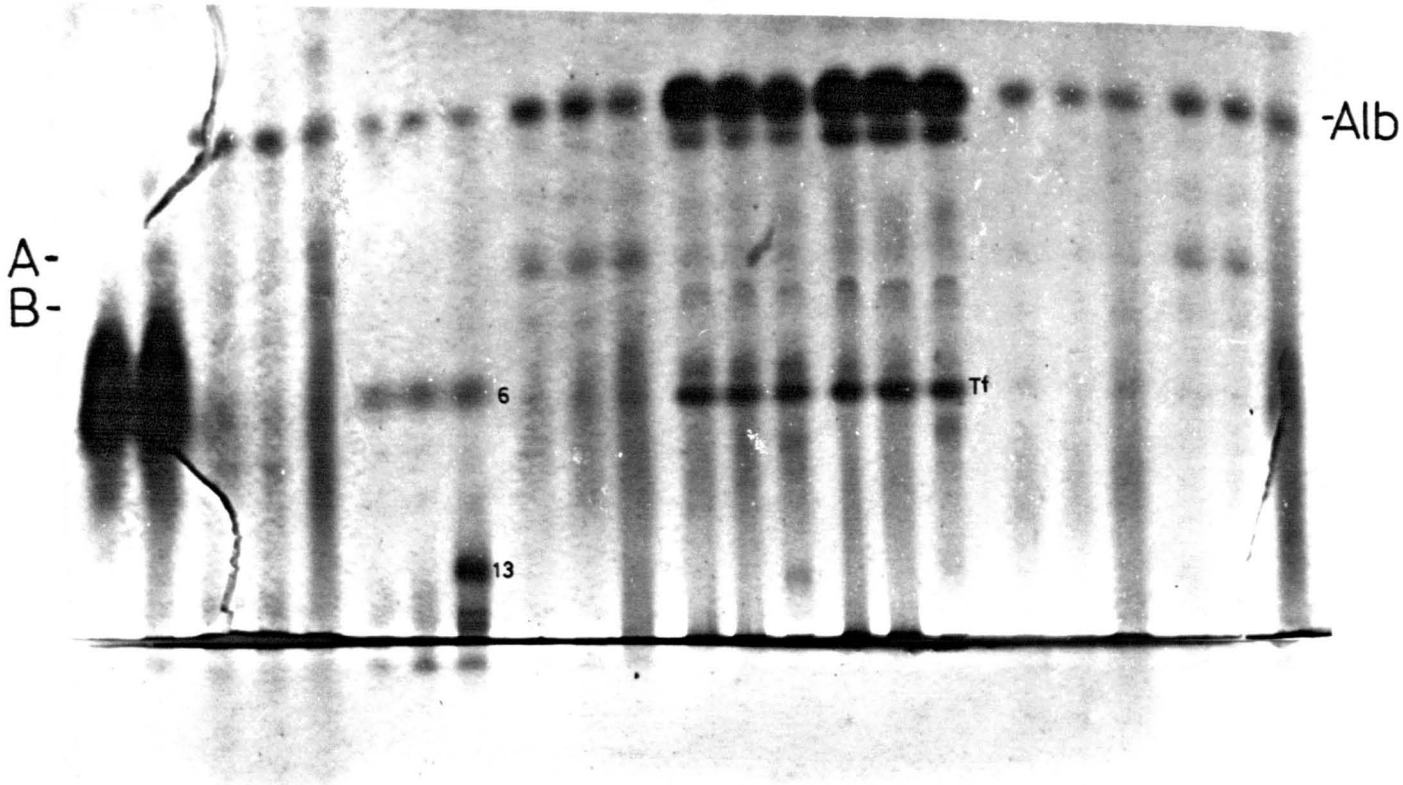


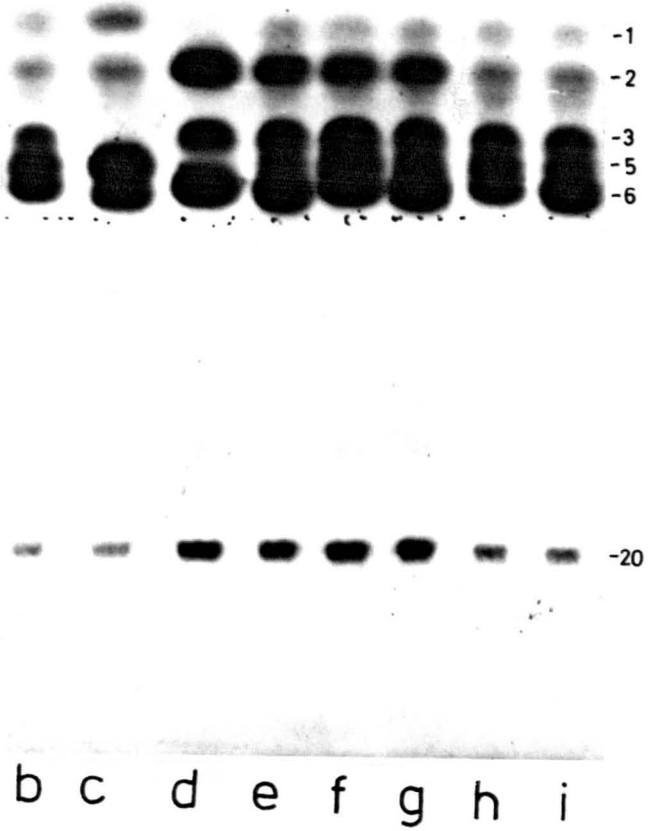
FIG. 61

Sensitivity of different proteins of Apodemus sylvaticus to heat incubation. The top half of gel shown in Fig. 58

Tris-citrate borate pH 8.6 Nigrosine-amidoblack.

Fig. 62

I.



II.

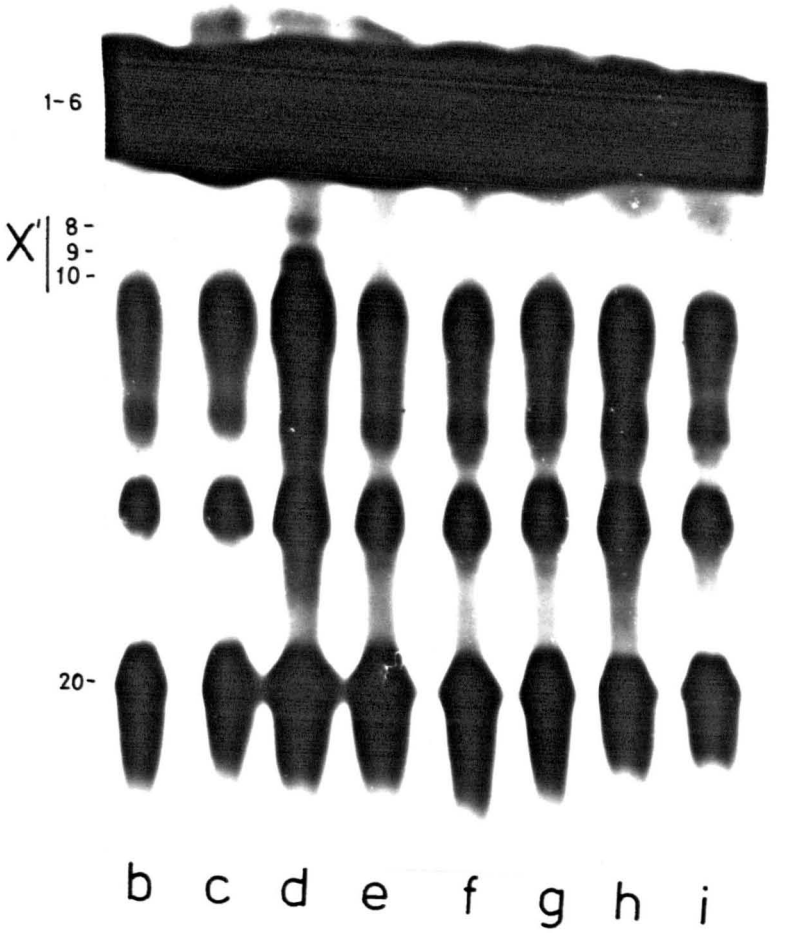


FIG. 62

Esterase zymograms demonstrating the mode of inheritance of certain serum esterases in Apodemus sylvaticus.

Note the fast moving esterases in zymogram I and zone X' in zymogram II.

I. Tris-citrate borate pH 8.6

Fraction 1:

c	: "strong" fraction 1 (homozygous)
d	: "lacking" fraction 1 (homozygous)
b, e - i	: "Intermediate" fraction 1 (heterozygous)

Fraction 2:

d, e, f and g: "strong" fraction 2 (homozygous)
b, c, h and i: "Intermediate" fraction 2 (heterozygous)

Fraction 3:

c	: "Lacking" fraction 3 (homozygous)
d	: "strong" fraction 3 (homozygous)
b, e - i	: "intermediate" fraction 3 (heterozygous)

II. Tris-citrate pH 7.6

Note the presence of X' in sample d, but absence in the others.

c	: Male from Iceland
d	: Female from Sweden
b, e - i	: Offsprings of c and d
b, f and i	: males
e, g and h	: females.

Fig.63

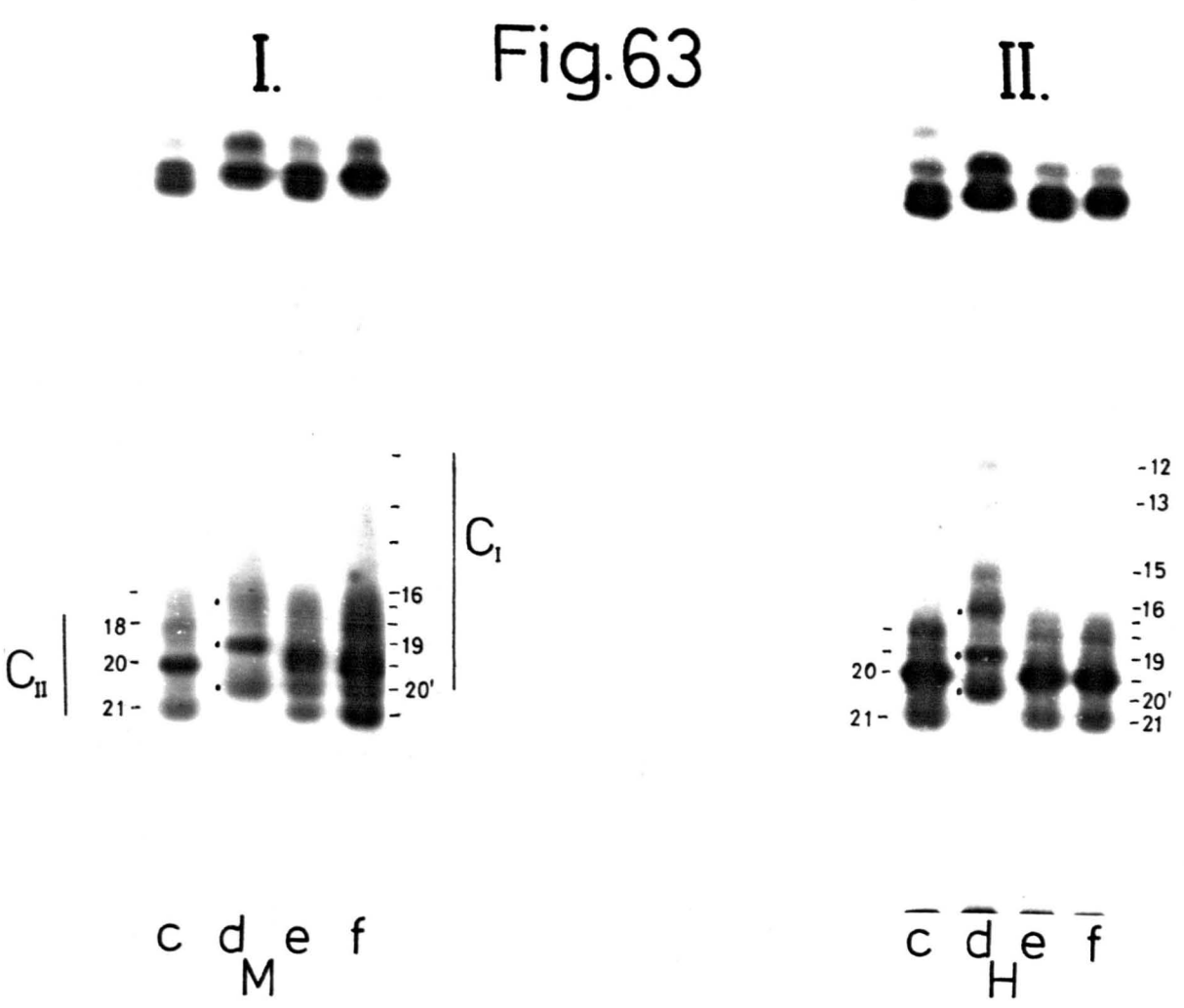


FIG. 63

A postulated inheritance of esterase fractions in zone C of heart and skeletal muscle homogenates of Apodemus sylvaticus.

I. Esterase pattern of striated muscle homogenates.

The father (c) is of type C_{II} and the mother (d) is of type C_I.

Note that the father (c) has strong fractions 20 and 21, the mother (d) has strong 19 and 20'. The offsprings (e and f) present fractions 20, 20' and 21 i.e. hybrid pattern

II. Esterase pattern of heart muscle homogenates.

Note that the offsprings have the same pattern as the father. The fraction 20' is not expressed in the offsprings as is the case with the skeletal muscle.

c : Male from Iceland, type C_{II}

d : Female from Sweden, type C_I

e and f : Offsprings of c and d.

Tris-citrate pH 7.6

Fig.64

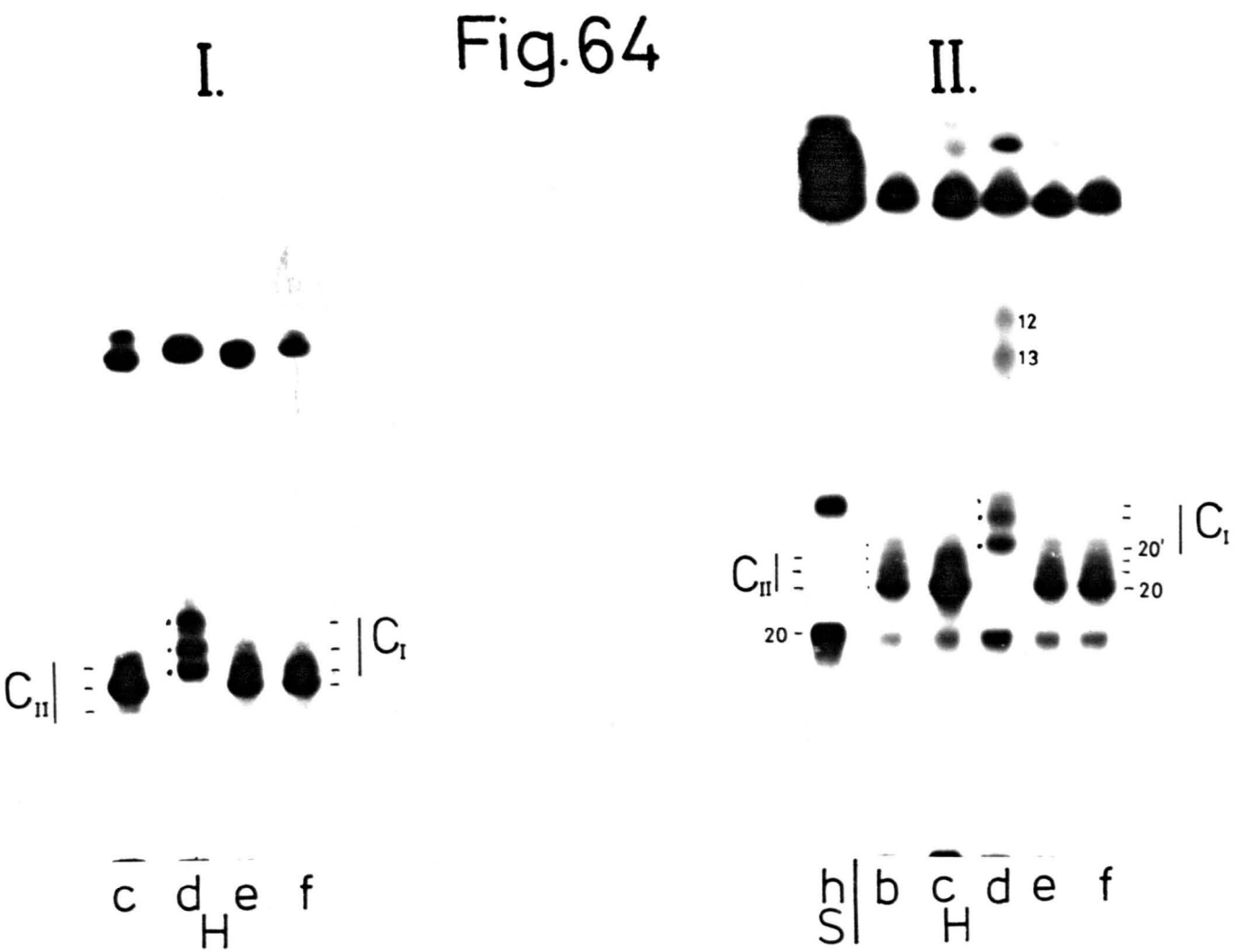


FIG. 64

A postulated inheritance of esterase fractions in zone C of heart homogenates of Apodemus sylvaticus.

I. Tris-citrate borate pH 8.6
Same samples as in Fig 63 I and II.
Note that the offsprings seem to have the same appearance of zone C as the father - the heterozygosity is hidden.

II. Tris-citrate pH8.6 (no borate added)
Same samples as in I with addition of b and h. Note how relative position of zones and fractions has changed compared to I. Fraction 2U of the serum is now moving much slower than fraction 2U of the heart. Zones C_I and C_{II} are widely separated. The hybrid offsprings show multiple banding, which may fuse.

b, e, f and h : Offsprings of c and d

c : Male from Iceland

d : Female from Sweden

Fig.65

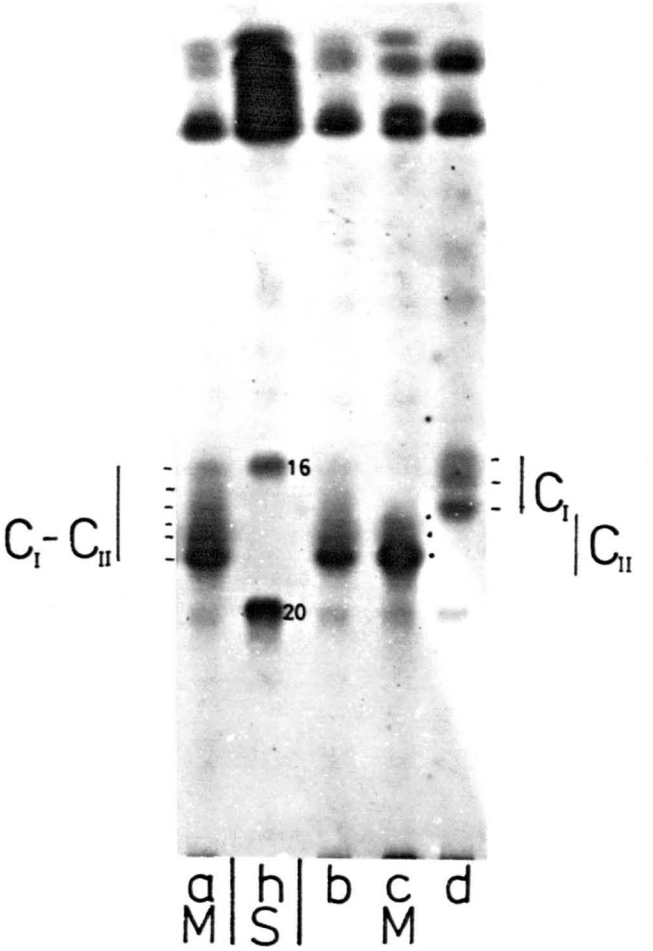


FIG. 65

A postulated mode of inheritance of skeletal muscle esterases of Apodemus sylvaticus. The buffer system used was Tris-citrate pH8.6 (no borate). Note that under these conditions (same as Fig. 64,II) zones C_I and C_{II} are well separated. The hybrid apparently possesses a mixture of zones C_I and C_{II}

c : Male from Iceland having zone C_{II} (homozygous)

d : Female from Sweden having zone CI (homozygous)

a and b : Offsprings of c and d having hybrid zone C_I - C_{II} (heterozygous)

Fig.66

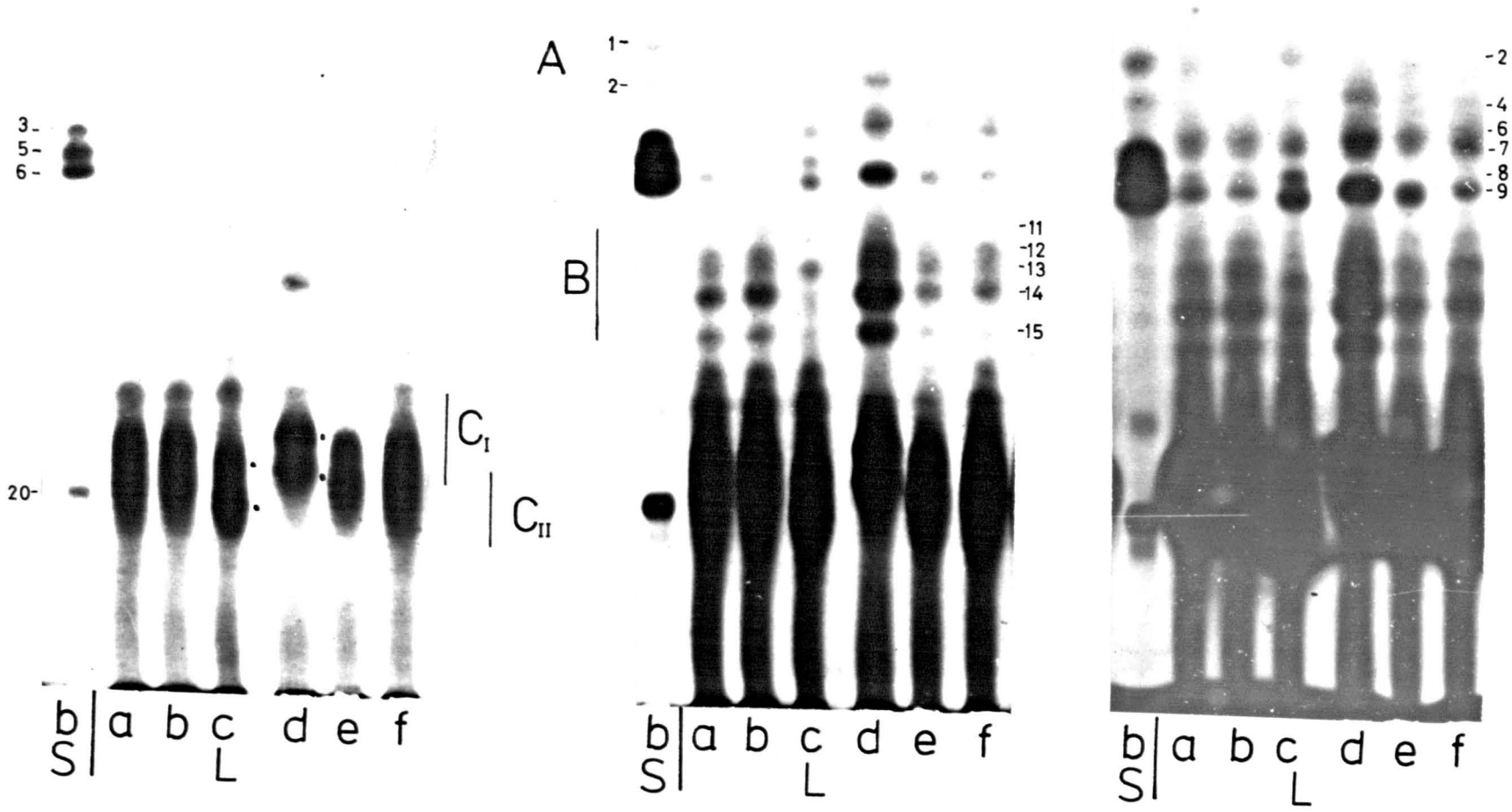


FIG. 66

Mode of inheritance of liveresterase fractions in Apodemus sylvaticus. Figs. I, II and III show the same gel after 10, 20 and 120 minutes staining respectively.

I. Zone C:

c	: Male from Iceland of C_{II} type (homozygous)
d	: Female from Sweden of C_{I} type (homozygous)
a, b, e and f	: Offsprings of c and d having zone C consisting of multiple fine fractions - a hybrid type (heterozygous)

II. Zone B:

c	: Male from Iceland having this zone "nearly silent" (homozygous)
d	: Female from Sweden having "strong" fractions in this zone (homozygous)
a, b, e and f	: Offsprings of c and d having "intermediate" fractions in this zone (heterozygous)

III. This shows the same gel after 120 mins. staining. Note that the faster moving fractions (zone A) are staining up. At this stage zone C cannot be read.

Tris-citrate borate pH 8.6

Fig.67

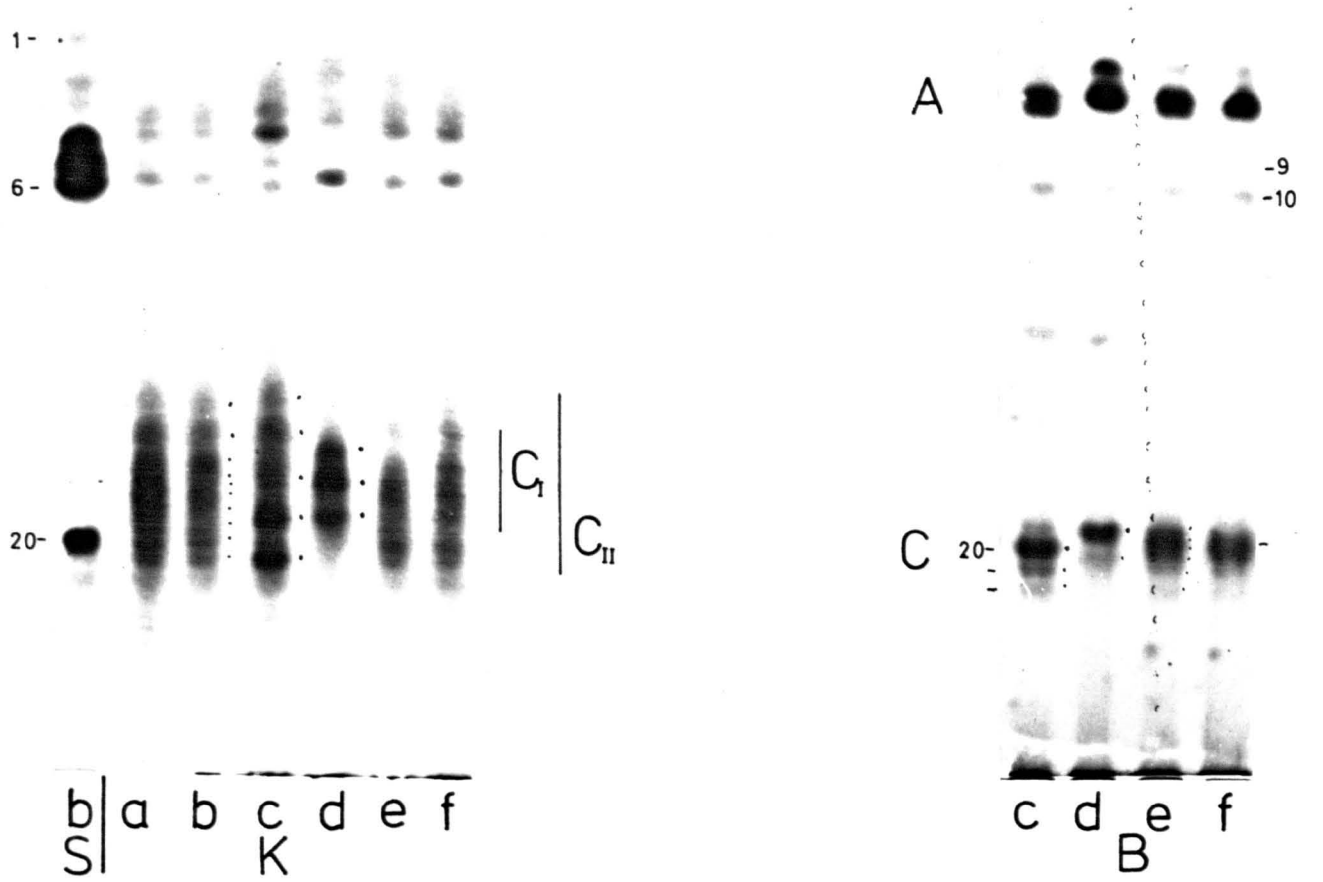


FIG. 67

Zymograms demonstrating the postulated mode of inheritance of esterase fractions of zone C of kidney (I) and brain (II) homogenates of Apodemus sylvaticus. Same animals as in previous photographs (Figs. 62-66)

I. Tris-citrate borate pH 8.6

A zymogram of kidney esterases

C	:	Male from Iceland having zone C_{II} (homozygous)
d	:	Female from Sweden having zone C_I (homozygous)
a, b, e and f	:	Offsprings of c and d having hybrid zone $C_I - C_{II}$ (heterozygous)

II. Tris-citrate pH 7.6

A zymogram of brain esterases

Note fractions 9 and 10, which are characteristic of brain and testis homogenates

Zone C:

c	:	Male from Iceland having slower fractions than d, i.e. C_{II} (homozygous)
d	:	Female from Sweden having zone C_I (homozygous)
e and f	:	Offsprings of c and d having hybrid C zone, consisting of multiple esterase fractions (heterzygous)

Fig. 68

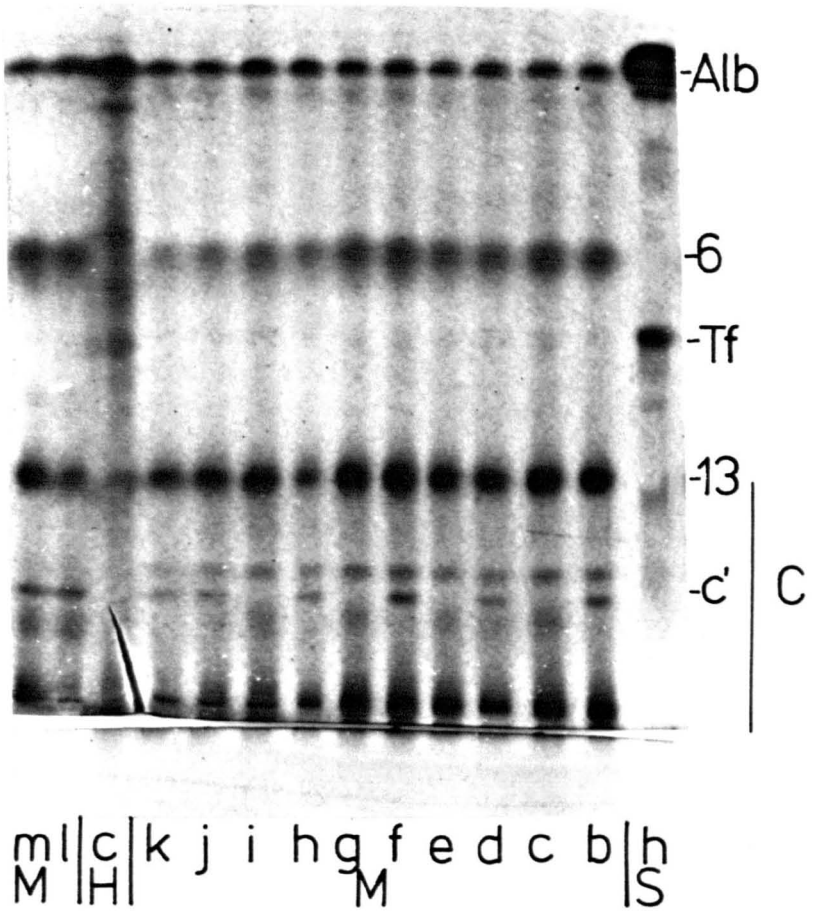


FIG. 68

An electropherogram illustrating the postulated mode of inheritance of skeletal muscle protein fraction c' in *Apodemus sylvaticus*.

c	:	Male from Iceland lacking c' (homozygous)
d	:	Female from Sweden having C' (heterozygous)
e, g and i:		Offsprings of c and d lacking C' (homozygous)
b, f, h, j and k:		Offsprings of c and d having C' (heterzygous)
b, f, i, j and k:		males
e, g and h:		females
l	:	*Mus musculus* (CBA)
m	:	*Mus musculus* (C57)

Tris-citrate pH 8.6

Fig.69

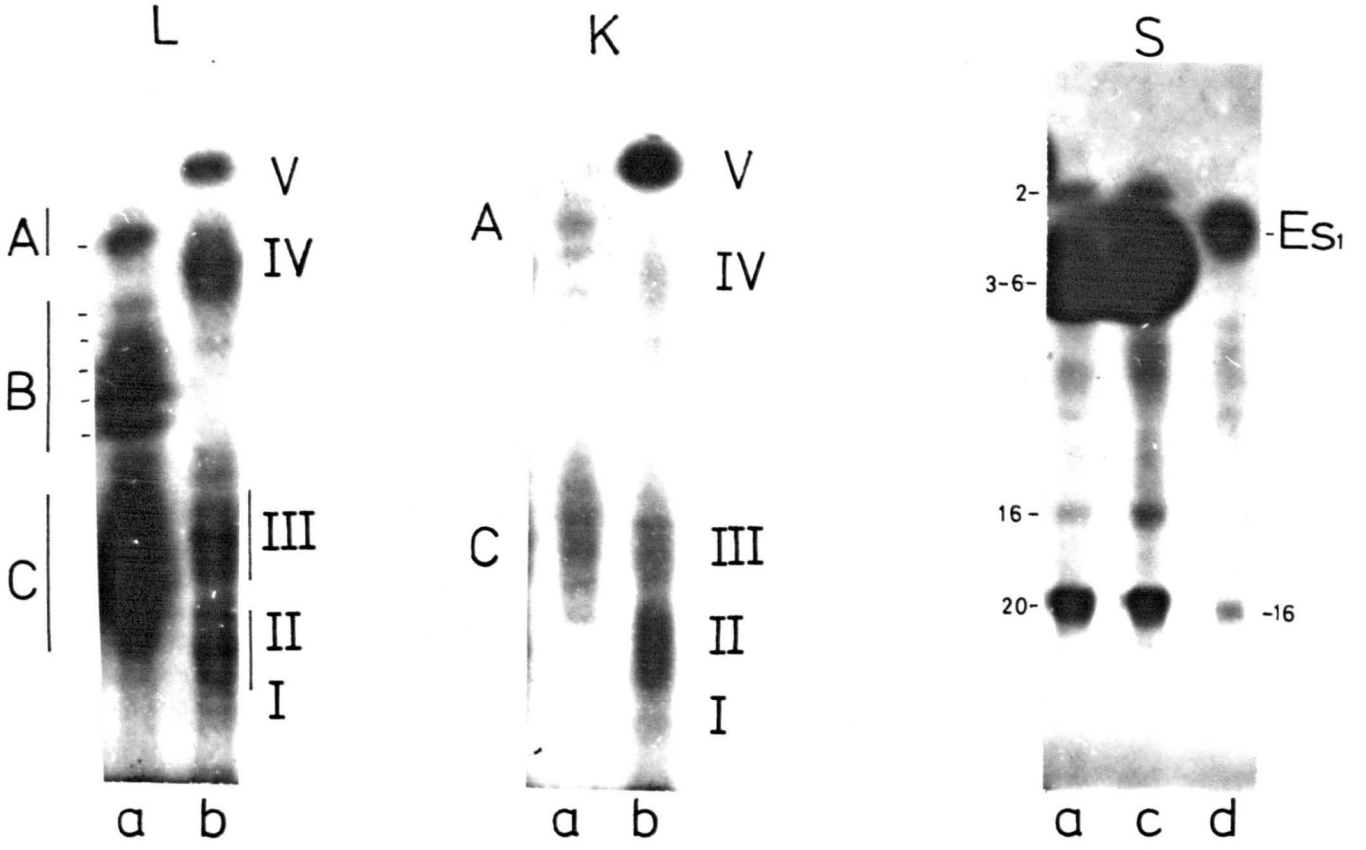


FIG. 69

Zymograms comparing esterase patterns of various tissue homogenates from Apodemus sylvaticus and Mus musculus.

Note that Mus musculus does not have an esterase zone overlapping with zone B of Apodemus sylvaticus.

a : Pooled samples from Apodemus sylvaticus

b : Pooled samples from Mus musculus, C 57

c : Pooled sera from Scottish Apodemus sylvaticus

d : Serum from two week old Mus musculus

Tris-citrate borate pH 8.6

Fig. 70

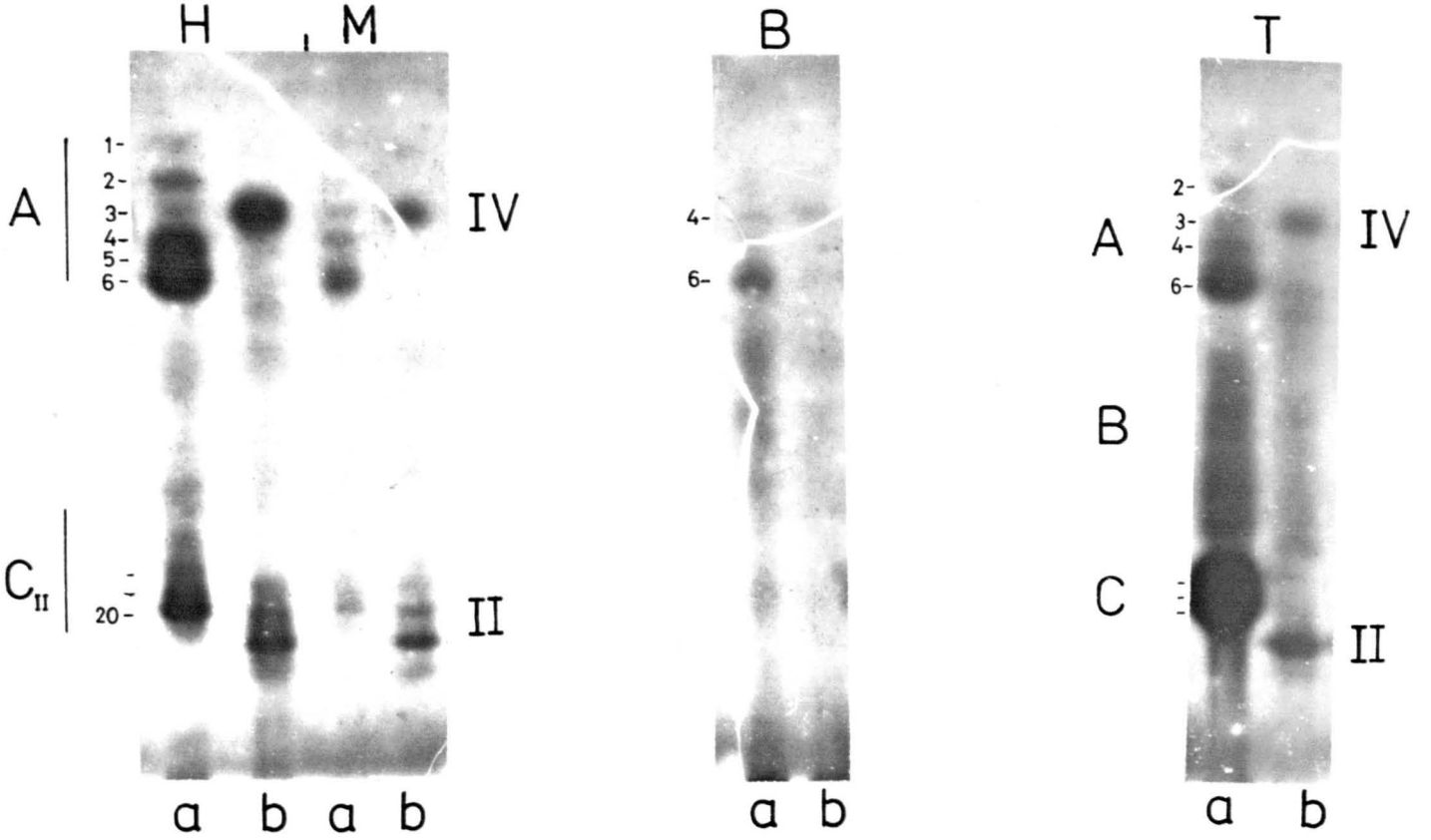


FIG. 70

Comparison of esterase patterns of various tissues from Apodemus sylvaticus and Mus musculus. Relative position of esterase activity is shown.

a. : Pooled homogenates from Apodemus sylvaticus

b. : Pooled homogenates from Mus musculus (C 57)

Tris-citrate borate pH 8.6

Fig.71

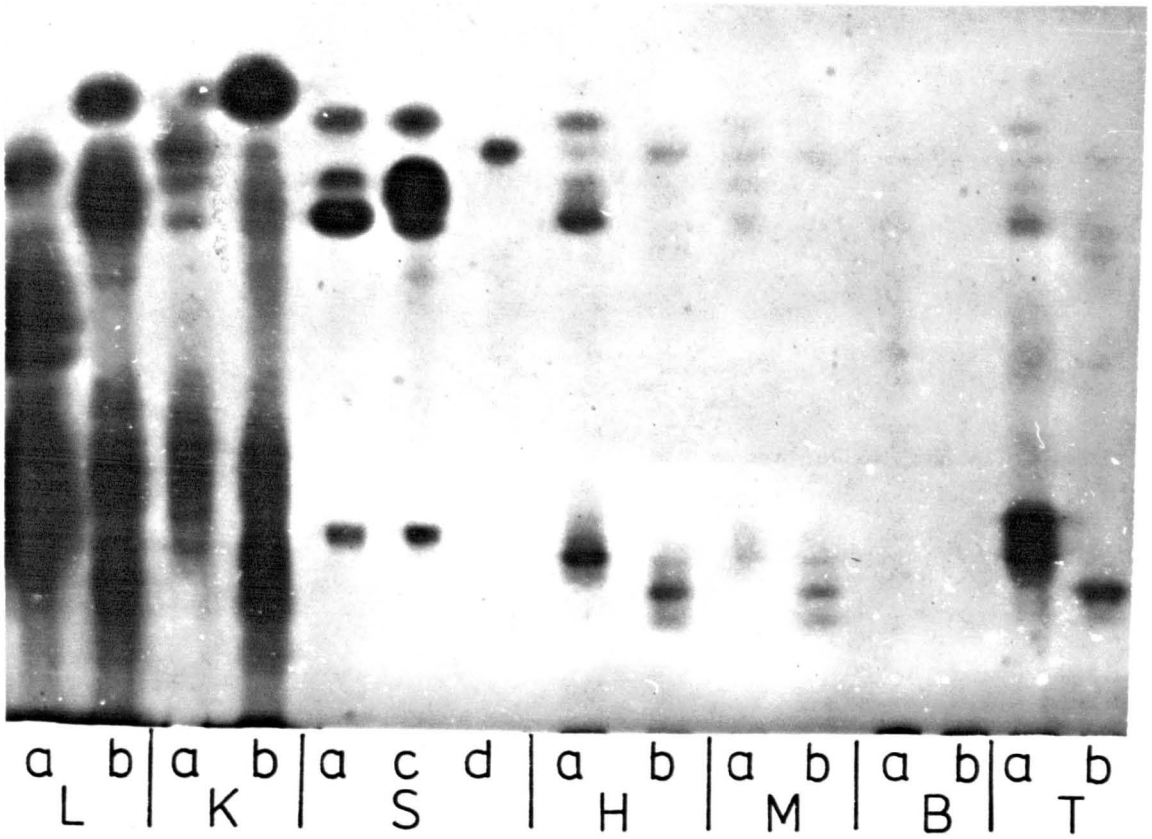


FIG. 71

The uncut zymogram of Figs. 69 and 70. For labelling see Figs. 69 and 70.

Tris-citrate borate pH 8.6

Fig. 72

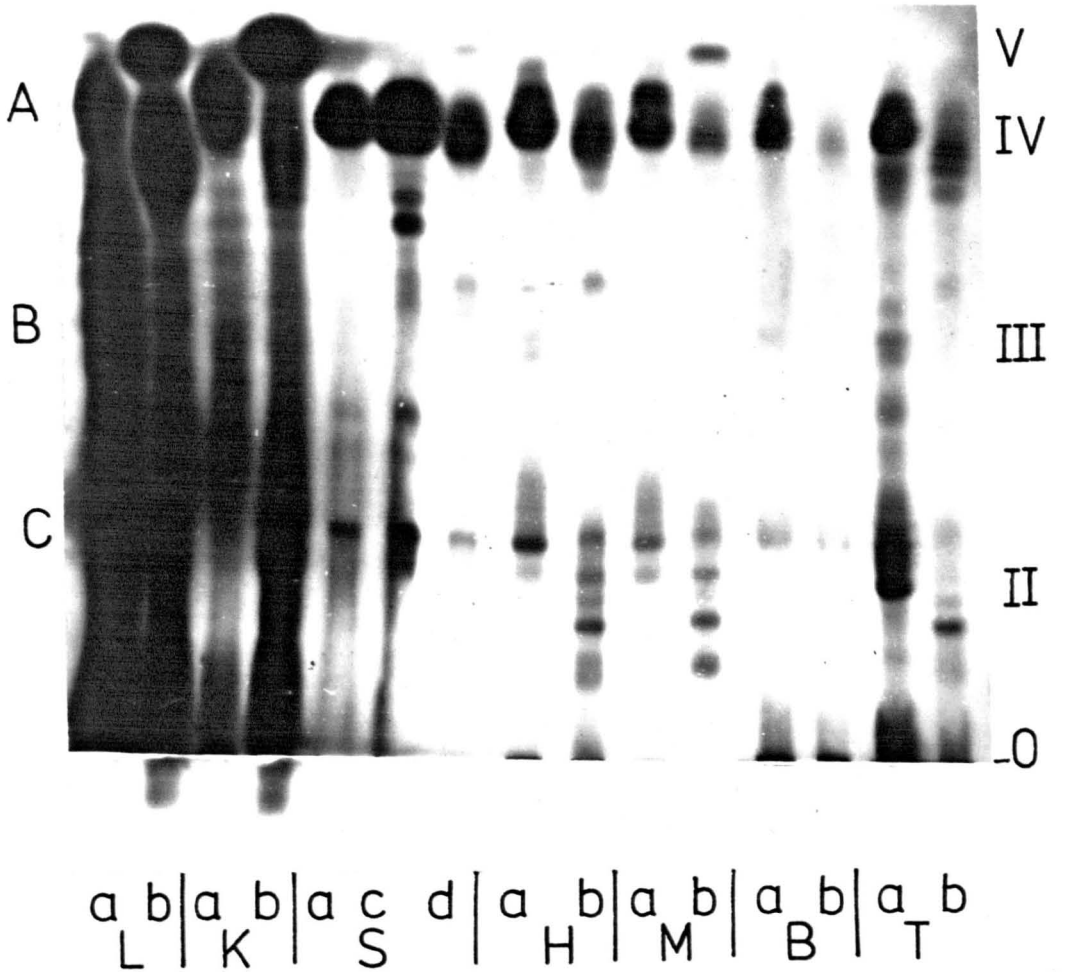


FIG. 72

Comparison of esterases of Apodemus sylvaticus and Mus musculus.

Same samples as Fig. 71.

Note improved separation of heart and striated muscle fractions in zone II of Mus musculus under this pH.

Tris-citrate pH 7.6

Fig.73

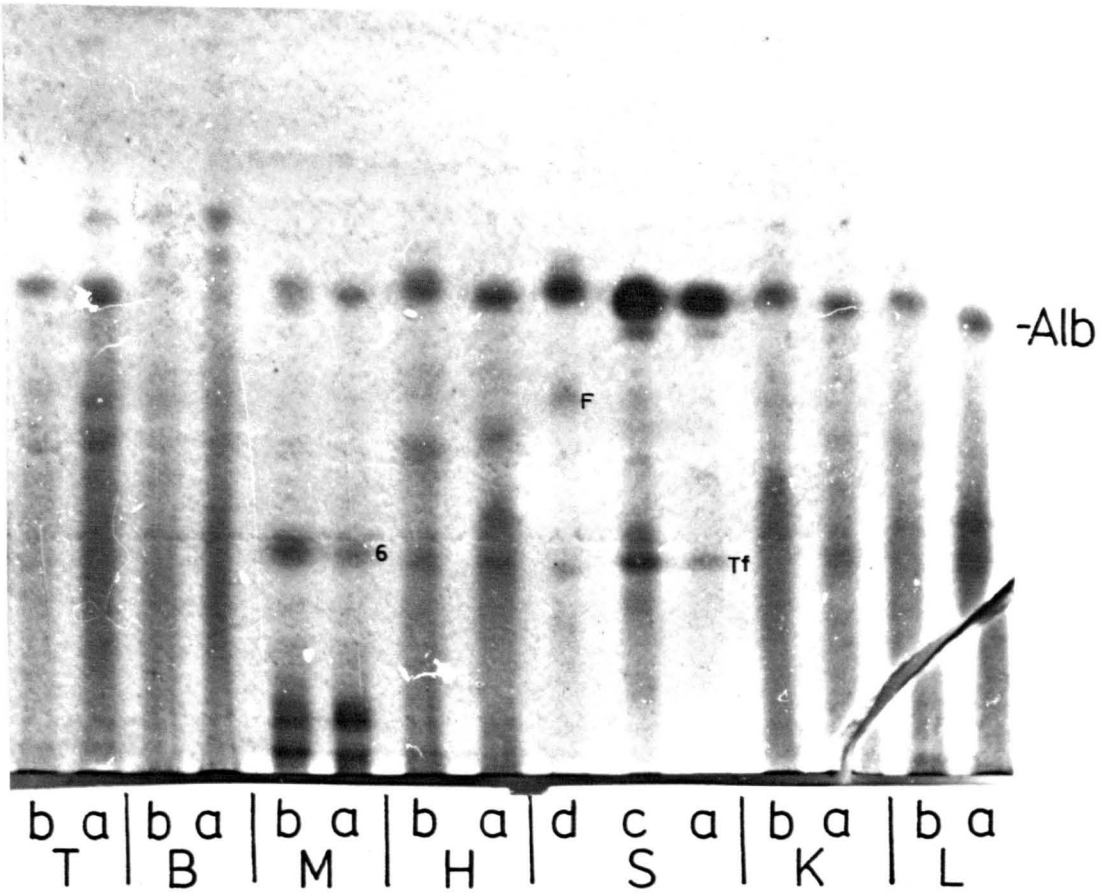


FIG. 73

The top half of plate shown in Fig. 71, stained for proteins.

Note the similarity in the general protein patterns of the two species in this system.

The opposite is the case for esterase patterns (Fig. 71)

Samples same as in Fig. 71 and 72

Tris-citrate borate pH 8.6

Fig.74

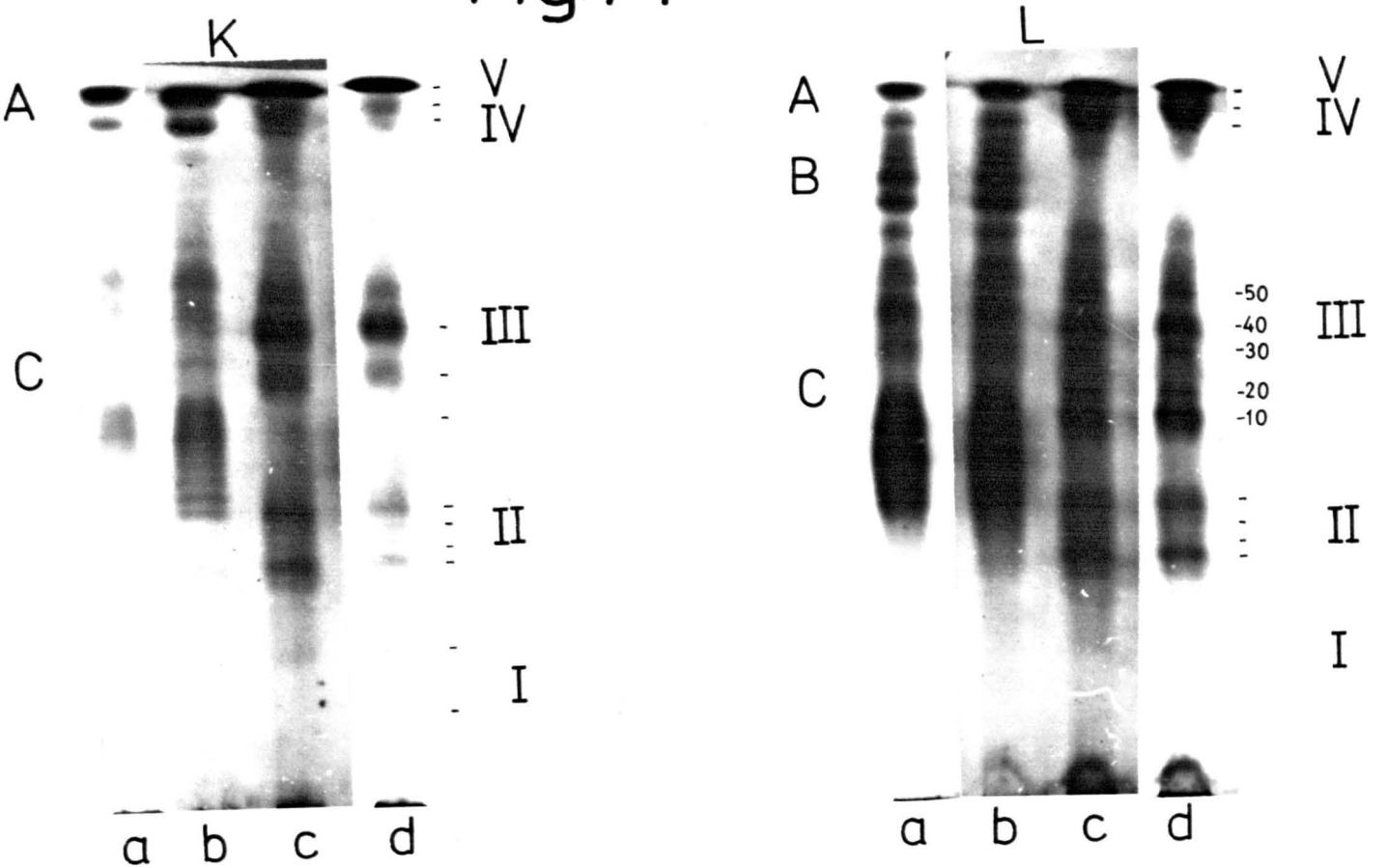


FIG. 74

Esterase zymograms of liver and kidney homogenates comparing Apodemus sylvaticus and Mus musculus. Tris-citrate pH 8.6. Note the increased separation in zone C of Apodemus sylvaticus. There is no zone in Mus musculus corresponding to B in Apodemus sylvaticus.

b : Pooled samples from Apodemus sylvaticus

c : Sample from Mus musculus (C57)

a : Same as b at an earlier stage of staining

d : Same as c at an earlier stage of staining.