COO-1630-26

TECHNICAL PROGRESS REPORT

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THE METABOLISM OF RARE EARTHS FOLLOWING INHALATION: PATHOLOGIC AND BIOCHEMICAL RESPONSE IN THE LUNG AND OTHER ORGANS

To

The Division of Biology and Medicine
Atomic Energy Commission
Washington, D. C.

From

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TECHNICAL PROGRESS REPORT

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A. Inhalation and Metabolism of Some Selected Rare Earths.

Studies of the inhalation parameters and the distribution and excretion following exposure to mixtures of the stable and radioactive forms of the rare earths were completed. The data has already been presented in Annual Reports COO-1630-2 (europium and scandium), COO-1630-3 (yttrium) and COO-1630-11 (cerium and ytterbium).

All of the inhalation exposures were comparable in terms of particle size distribution following aerosolization from stable chloride solutions spiked with the appropriate tracers (^{46}Sc, ^{88}Y, ^{144}Ce, $^{152-154}Eu$ and ^{169}Yb) for purposes of assay.

These data are to be collated and compared for differences in metabolism, excretion, etc. The data are voluminous and require extended calculation which it is anticipated will be accomplished in the near future.

B. Repetitive Inhalation Exposures to Mixtures of Stable and Radioactive $EuCl_3$.

The data for the distribution and lung burdens of Sprague-Dawley rats following a ten day consecutive exposure (5 hours per day) are given both in terms of organ and tissue activity and specific activity in the following table.

Tissue Activity During Repetitive Exposure to $^{154}EuCl_3$ Aerosols
(μ Ci x 10^{-3}/tissue wet weight)

Exposure Day	Necropsy Time	Animals/ Group	Carcass Mean	Carcass (S. D.)	Lung Mean	Lung (S. D.)	Trachea Mean	Trachea (S. D.)	Liver Mean	Liver (S. D.)
1	B	0	----	----	----	----	----	----	----	----
	A	5	55.2	(9.1)	2.24	(0.98)	N.S.		Sig.	
2	B	4	30.5	(4.8)	2.40	(1.20)	N.S.		0.15	(----)
	A	4	113.0	(26.4)	11.0	(5.70)	0.12	(----)	0.18	(----)
3	B	4	71.8	(61.4)	14.8	(5.0)	Sig.		0.37	(----)
	A	4	167.0	(24.0)	29.3	(4.6)	0.46	(----)	1.01	(0.46)
4	B	4	195.0	(108.0)	37.0	(5.4)	0.47	(----)	0.95	(0.27)
	A	4	146.0	(11.5)	37.8	(9.6)	0.27	(0.06)	1.33	(0.57)
5	B	4	82.7	(17.7)	57.7	(14.0)	0.18	(----)	1.17	(0.47)
	A	4	169.0	(54.0)	51.8	(13.7)	0.27	(----)	1.38	(0.53)
6	B	4	93.8	(20.6)	49.3	(16.8)	0.37	(----)	2.13	(0.99)
	A	4	158.0	(67.0)	68.0	(27.0)	0.43	(----)	1.58	(0.59)
7	B	4	112.0	(67.0)	90.5	(32.0)	0.80	(0.43)	1.36	(0.6)
	A	4	133.0	(38.0)	57.0	(18.0)	0.51	(----)	1.34	(0.12)
8	B	3	128.0	(24.0)	43.0	(11.8)	0.21	(----)	1.92	(0.43)
	A	3	242.0	(51.0)	94.3	(7.6)	Sig.		3.31	(0.43)
9	B	3	174.0	(36.0)	74.0	(7.5)	0.17	(----)	2.50	(0.76)
	A	3	216.0	(55.0)	104.0	(12.4)	0.62	(----)	2.42	(0.86)
10	B	3	131.0	(23.0)	94.7	(16.0)	Sig.		3.03	(1.20)
	A	3	220.0	(7.0)	116.0	(18.0)	0.65	(----)	2.19	(0.27)

(Continued)

Tissue Activity During Repetitive Exposure to $^{154}EuCl_3$ Aerosols
(μ Ci x 10^{-3}/tissue wet weight)

Exposure Day	Necropsy Time	Animals/ Group	Kidney Mean	Kidney (S. D.)	Spleen Mean	Spleen (S. D.)	Femur Mean	Femur (S. D.)	Blood Mean	Blood (S. D.)
1	B	0	----	----	----	----	----	----	----	----
	A	5	N. S.		N. S.		N. S.		N. S.	
2	B	4	N. S.		N. S.		N. S.		N. S.	
	A	4	N. S.		Sig.		N. S.		N. S.	
3	B	4	Sig.		N. S.		Sig.		N. S.	
	A	4	0. 23	(----)	N. S.		Sig.		N. S.	
4	B	4	Sig.		N. S.		Sig.		N. S.	
	A	4	Sig.		Sig.		0. 16	(----)	N. S.	
5	B	4	Sig.		N. S.		Sig.		N. S.	
	A	4	0. 17	(----)	N. S.		Sig.		N. S.	
6	B	4	0. 50	(----)	Sig.		0. 30	(----)	N. S.	
	A	4	0. 19	(----)	Sig.		0. 16	(----)	N. S.	
7	B	4	0. 15	(----)	N. S.		0. 31	(----)	N. S.	
	A	4	0. 73	(----)	Sig.		0. 32	(----)	N. S.	
8	B	3	0. 21	(----)	N. S.		0. 43	(----)	N. S.	
	A	3	Sig.		Sig.		0. 32	(----)	N. S.	
9	B	3	0. 91	(0. 41)	N. S.		0. 80	(0. 34)	N. S.	
	A	3	0. 14	(----)	N. S.		0. 89	(0. 31)	N. S.	
10	B	3	Sig.		Sig.		0. 82	(0. 37)	N. S.	
	A	3	0. 40	(----)	N. S.		0. 84	(0. 09)	N. S.	

Specific Tissue Activity During Repetitive Exposure to $^{154}EuCl_3$ Aerosols
(μ Ci x 10^{-3}/gram wet weight)

Exposure Day	Necropsy Time*	Animals/ Group	Carcass Mean	Carcass (S. D.)	Lung Mean	Lung (S. D.)	Trachea Mean	Trachea (S. D.)	Liver Mean	Liver (S. D.)
1	B	-	----	-----	----	-----	----	----	----	----
	A	5	0.48	(0.08)	2.30	(1.10)	N. S.		N. S.	
2	B	4	0.26	(0.05)	2.30	(1.40)	N. S.		0.15	(0.03)
	A	4	0.96	(0.26)	7.8	(4.8)	0.06	(0.03)	0.03	(----)
3	B	4	0.59	(0.48)	13.0	(4.10)	Sig.		0.09	(----)
	A	4	1.57	(0.28)	29.0	(7.7)	3.7	(----)	0.25	(0.11)
4	B	4	1.79	(1.10)	22.0	(8.6)	2.4	(----)	0.22	(0.05)
	A	4	1.31	(0.06)	32.0	(13.0)	1.4	(0.35)	0.35	(0.17)
5	B	4	0.66	(0.14)	41.0	(5.8)	0.9	(----)	0.22	(0.06)
	A	4	1.50	(0.55)	48.0	(7.2)	1.3	(----)	0.30	(0.11)
6	B	4	0.83	(0.24)	44.0	(11.8)	2.6	(----)	0.48	(0.27)
	A	4	1.36	(0.44)	49.8	(6.5)	4.3	(----)	0.38	(0.06)
7	B	4	1.00	(0.80)	69.0	(28.0)	4.5	(2.4)	0.33	(0.15)
	A	4	1.34	(0.71)	66.0	(26.0)	4.4	(----)	0.38	(0.15)
8	B	3	1.08	(0.31)	53.0	(25.3)	1.10	(----)	0.53	(0.15)
	A	3	2.06	(0.53)	69.0	(12.0)	Sig.		0.73	(0.08)
9	B	3	1.68	(0.31)	67.0	(0.18)	0.60	(----)	0.67	(0.21)
	A	3	1.81	(0.26)	91.0	(15.3)	3.10	(----)	0.63	(0.21)
10	B	3	1.19	(0.51)	66.0	(25.0)	Sig.		0.65	(0.31)
	A	3	1.61	(0.16)	97.0	(14.0)	6.4	(----)	0.47	(0.10)

* B - Sacrifice immediately before exposure (5 hours/day)
A - Sacrifice immediately after exposure (5 hours/day)

(Continued)

Specific Tissue Activity During Repetitive Exposure to $^{154}EuCl_3$ Aerosols
(μ Ci x 10^{-3}/gram wet weight)

Exposure Day	Necropsy Time*	Animals/ Group	Kidney Mean	Kidney (S. D.)	Spleen Mean	Spleen (S. D.)	Femur Mean	Femur (S. D.)	Blood Mean	Blood (S. D.)
1	B	-	----	----	----	----	----	----	----	----
	A	5	N. S.		N. S.		N. S.		N. S.	
2	B	4	N. S.		N. S.		N. S.		N. S.	
	A	4	N. S.		Sig.		N. S.		N. S.	
3	B	4	Sig.		N. S.		Sig.		N. S.	
	A	4	0. 20	(----)	N. S.		Sig.		N. S.	
4	B	4	Sig.		N. S.		Sig.		N. S.	
	A	4	Sig.		Sig.		0. 23	(----)	N. S.	
5	B	4	Sig.		N. S.		Sig.		N. S.	
	A	4	0. 13	(----)	N. S.		Sig.		N. S.	
6	B	4	0. 35	(----)	Sig.		0. 37	(----)	N. S.	
	A	4	0. 13	(----)	Sig.		0. 24	(----)	N. S.	
7	B	4	0. 12	(----)	N. S.		0. 42	(----)	N. S.	
	A	4	0. 48	(----)	Sig.		0. 45	(----)	N. S.	
8	B	3	0. 14	(----)	N. S.		0. 66	(----)	N. S.	
	A	3	Sig.		Sig.		0. 40	(----)	N. S.	
9	B	3	0. 57	(0. 16)	N. S.		1. 10	(0. 47)	N. S.	
	A	3	0. 09	(----)	N. S.		1. 20	(0. 23)	N. S.	
10	B	3	Sig.		Sig.		1. 20	(0. 53)	N. S.	
	A	3	0. 19	(----)	N. S.		1. 30	(0. 30)	N. S.	

* B - Sacrifice immediately before exposure (5 hours/day)
A - Sacrifice immediately after exposure (5 hours/day)

The aerosol was generated from a 5% (W/V) of europium chloride containing a tracer of $^{152-154}$europium chloride. The mean concentration over the entire 10 day period was 4.17 ± 1.69 $\mu Ci \times 10^{-3}$ per liter of air, with a standard error of the mean of ± 0.05. The particle size distribution previously determined was Mg = 0.145 microns $\div$ 1.83 (s.d.) (COO-1630-11).

The data although disappointingly erratic in terms of pulmonary deposition and relative inter-exposure clearance will be analyzed to determine whether clearance mechanisms change with pulmonary burden levels.

C. Pathological Response in the Dog Lung Related to Intratracheally Administered Lung Levels of Stable Europium Chloride as Determined by Neutron Activation.

This study (COO-1630-15) was presented at the IVth International Congress on Radiation Research July 1970 and the abstract given in Annual Report of 1970 (COO-1630-22). The study, briefly, was designed to determine in the beagle dog the comparative pathological effect of intratracheally administered stable europium alone and in combination with the radio nuclide, both in chloride form. The assumption was made that if the material were

similarly placed anatomically to ensure equivalent deposition, initially at least, that the pathology associated with the easily assayed radioisotope could be compared to similar tissue samples where assay was not easily feasible viz the stable isotope, europium chloride. Since radio assay however, indicated that the assumption was not completely valid in terms of retention areas in the lung where radio assay was used, the problem of stable nuclide analysis arose in order to further show the dose-response parameter and to confirm the pathological data for the stable plus radio nuclide effect. The latter nevertheless was consistent within itself and indicated that the granulomatous response could be attributed to the stable isotope and not the radionuclide per se (see COO-1630-15).

In order to relate pathology to the amounts of stable europium, paraffin-embedded control tissue blocks were neutron activated to indicate the feasibility of this analytical procedure. Then the paraffin-embedded tissue blocks which had been used to prepare the slides for histopathological examination were assayed in like manner. These data are shown in the following table and again indicate the close relationship between the degree of severity of the granuloma and the amount of retained stable isotope.

Pathological Response to Stable Europium Chloride

Lung Tissue*		Dog #1		Dog #3		Dog #7		Dog #8		Dog #9	
		Path.**	µg Eu***	Path.	µg Eu	Path.	µg Eu	Path.	µg Eu	Path.	µg Eu
Left Apical	A	0	Sig.	0	Sig.	3	160	0	Sig.	0	Sig.
	M	0	31	5	79	2	250	0	Sig.	0	Sig.
	P	0	Sig.	1	Sig.	0	Sig.	0	Sig.	0	Sig.
Left Cardiac	A	0	57	0	Sig.	0	Sig.	0	Sig.	1	66
	M	0	720	2	Sig.	0	Sig.	0	Sig.	0	Sig.
	P	0	540	0	Sig.	0	Sig.	0	Sig.	0	Sig.
Left Diaphragm.	A	1	Sig.	2	Sig.	5	2300	0	51	4	2700
	M	0	Sig.	0	Sig.	5	410	3	300	4	1400
	P	0	Sig.	0	130	2	--	5	1900	4	1300
Right Apical	A	1	Sig.	2	Sig.	0	Sig.	0	Sig.	0	Sig.
	M	0	Sig.	2	Sig.	0	--	0	Sig.	0	Sig.
	P	0	--	1	Sig.	0	Sig.	0	Sig.	0	Sig.
Right Cardiac	A	0	Sig.	0	88	0	Sig.	0	Sig.	0	Sig.
	M	1	Sig.	2	30	0	--	0	Sig.	0	Sig.
	P	0	Sig.	1	49	0	Sig.	0	Sig.	0	Sig.
Right Diaphragm.	A	1	Sig.	2	Sig.	0	Sig.	0	Sig.	0	Sig.
	M	0	Sig.	0	63	0	Sig.	0	Sig.	0	Sig.
	P	1	Sig.	2	2300	0	Sig.	0	Sig.	0	Sig.
Right Intermediate	A	--	Sig.	3	600	--	Sig.	0	Sig.	0	Sig.
	M	--	Sig.	3	2400	--	Sig.	0	Sig.	0	Sig.
	P	--	Sig.	4	3000	--	Sig.	0	Sig.	0	Sig.

*A, M, P - Anterior, medial and posterior lung lobe sample.
**Degree of increasing severity of granuloma from 0 to 5.
***Neutron activation assay of paraffin-embedded tissue blocks. All tissue had detectable activity. Only those above 20 µg indicated in table.

These data will be integrated with those obtained previously and reported as soon as available.

D. Mucociliary Activity in the Sprague-Dawley Rat.

A preprint of the paper was presented in Annual Report COO-1630-22 as COO-1630-6. The paper has been published as "The Roentgenographic Determination of Tracheal Microciliary Transport in the Rat" in AIHA Jour. 32, 174-178 (1971). A reprint will be appended to this report if received in time.

E. Lymphocyte Ratio and Uranyl Nitrate.

The data for this study was presented briefly in Annual Report COO-1630-22, as an abstract and given at the AIHA Conference in Detroit in 1970. A fuller treatment of this study is appended in this report as COO-1630-23 and will be shortly revised and submitted for publication in the future.

F. Occupational Effects of X-Irradiation in the Female Radiographic Technician.

A very short abstract of that study appended in Annual Report COO-1630-22. The study is now in more complete form

and is appended in this report as COO-1630-24. It will require some revision and will be submitted when ready for publication.

G. In vitro Binding Studies of Rare Earths with Blood Serum of the Sprague-Dawley Rat Using Starch Electrophoresis.

A study of the effect of some selected rare earths on the starch electrophoretic patterns of rat serum in vitro has been briefly reported in Annual Report COO-1630-11. Since most of the circulating blood activity following intake of the rare earths following exposure appears to be extracellular, the precise binding to blood proteins as shown by the electropherogram is of interest. Changes in starch electrophoretic patterns were shown previously (COO-1630-11). At present, using a densitometric method, a quantitative appraisal of dye intensity fluctuations at varying molarities of the rare earths (scandium, yttrium, cerium, europium and ytterbium) added to rat serum has been determined. The tested serum moieties tested were serum protein, esterase, lactic acid dehydrogenase and alkaline phosphatase.

The data appears in the following tables and densitometric

curves. In addition, a record of the immediate changes following addition of the rare earth to serum compared to those after refrigerator storage for a week and centrifugation are also indicated (see table). The changes in pH in the clear solutions to be electrophoresed is also given as a matter of record.

The tabular data for each serum protein was constructed by comparing as closely as possible the peak positions and relative dye intensity with the control in each series of runs. Line 2 of each table (total as % of control) is the total dye intensity compared to that of the control. While color value (line 3) refers to recovery of total intensity as the summation of each electropherogram peak. Correction for loss or gain of color intensity over controls were not made because the relationship is shown much more clearly in the dye intensity curves which follow.

Briefly:

1. Protein patterns

    a) There appears to be an increase in dye intensity at the starting point (slot) at and beyond a concentration of 10 millimoles in all cases and a change in peak location.

2. Esterase patterns

   a) Scandium - at 10 millimoles and beyond peaks begin to disappear and are practically gone at 20 to 50 millimole.

   b) Yttrium - slight changes.

   c) Ce, Eu, Yb - changing patterns at > 10 mM.

3. Lactic acid dehydrogenase

   a) Scandium - slot color intensity enhanced beyond 1 millimolar. All peaks greatly depressed beyond 10 millimole.

   b) Yttrium - generalized color intensity decrease with little change in peak position up to 10 mM. Peak disappearance beyond 20 mM.

   c) Cerium - slight peak position changes up to 4 mM with intensified color intensity.
   Smear at 10 mM and beyond with practically no dye color development.

   d) Europium - color intensity decreases at 4 mM with little change in peak positions at 5 mM and beyond color intensity approaches zero.

e) Ytterbium - color intensity decreases at 5 mM and peak positions greatly altered above 10 mM.

4. Acid phosphatase - color development very poor in all cases

a) Scandium - dye intensity in slot increases above 10 mM, with little change in peak position below 5 mM.

b) Yttrium - dye intensification in slot up to 5 mM with little change in peak position.

c) Cerium - little effect.

d) Europium - little effect except at slot position where intensity increases above 10 mM.

e) Ytterbium - little effect.

There is little doubt that these metals interfere in one way or another with the dye intensity and position of peaks with increasing concentrations despite fairly level sample pH' s (see table). The changes in acidity are not considered important since the starch gel is also buffered strongly and would tend to equalize these pH values.

CHANGES IN SERUM AFTER MIXING WITH RARE EARTHS*

Conc. m M	YCl_3		$ScCl_3$		$CeCl_3$		$EuCl_3$		$YbCl_3$	
	1st day	after centrifuging	1st day	after centrifuging	1st day	after centrifuging	1st day	after centrifuging	1st day	after centrifuging
0	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear
0.5	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear
1.0	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear
2.0	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear
4.0	very slightly turbid	clear, no ppt.	clear	clear	slightly turbid	clear, trace ppt.	slightly turbid	clear, trace ppt.	clear	clear, trace ppt.
5.0	slightly turbid	clear, trace ppt.	clear	trace ppt.	slightly turbid	clear, trace ppt.	slightly turbid	clear, trace ppt.	slightly turbid	clear, trace ppt.
10.0	heavy ppt. 20%	clear ppt. 5%	heavy ppt.	clear 15-20% ppt.	slightly turbid	clear ppt. 5%	turbid	clear ppt. 10%	slightly turbid	clear ppt. 5%
20.0	medium ppt.	clear ppt. 5%	slight ppt.	clear	slightly turbid	trace turbid	slightly turbid	clear, trace ppt.	slightly turbid	clear ppt. 5%
50.0	slightly turbid	clear ppt. 5%	medium ppt.	clear ppt. 5%	slightly turbid	trace turbid	slightly turbid	clear trace ppt.	clear	clear ppt. 10%
100.0	slightly turbid	clear trace ppt.	clear	clear slight ppt. on top	slightly turbid	trace turbid	slightly turbid	clear trace ppt.	clear	turbid trace ppt.

*Centrifugation for 20 minutes at 8000 rpm or RCF X G = 10,400

pH of Rare Earth-Rat Serum Mixtures in Vitro

Conc. m M	$ScCl_3$	YCl_3	$CeCl_3$	$EuCl_3$	$YbCl_3$
0	7.4	7.4	7.4	7.4	7.4
0.5	7.4	7.4	7.4	7.2	7.2
1.0	7.4	7.4	7.4	7.2	7.2
2.0	7.2	7.4	7.4	6.8	7.2
4.0	7.0	6.8	6.8	6.6	6.8
5.0	6.8	6.8	6.6	6.6	6.8
10.0	6.0	5.0	6.0	6.0	6.0
20.0	6.0	5.3	6.0	6.0	6.0
50.0	6.0	5.5	5.5	5.8	5.8
100.0	6.0	5.0	5.5	5.8	5.8

ch Electrophoretic Patterns of Rat Serum as Modified by Graded Concentrations of Scandium Chl e

Serum Protein

peak area	milli moles	0.00	0.5	1.0	2.0	4.0	5.0	10.0	20.0	50.0
	total as % of control*	100.	89.1	88.4	96.9	85.0	89.1	89.1	83.3	96.9
	color value **	97.1	97.7	97.9	97.9	100.0	96.9	98.2	99.2	97.7
	serum pH	7.4	7.4	7.4	7.2	7.0	6.8	6.0	6.0	6.0
1		3.4	1.9	1.9	2.1	1.2	1.5	5.0	4.5	6.7
2		29.3	29.8	30.0	28.8	29.2	29.8	14.9	23.3	15.8
3		12.6	11.8	11.2	11.2	11.2	11.8	8.0		17.2
4		7.1	6.5	6.2	6.0	5.6	7.6	10.7	11.0	9.5
5		5.8	6.5	5.4	5.6	6.4	5.7	6.9	11.8	----
6		9.2	10.7	11.2	9.1	12.4	10.3	15.3	11.8	8.8
7		10.2	9.9	10.8	12.3	11.2	10.7	13.7	10.2	15.8
8		4.8	6.1	6.2	5.6	6.0	6.1	7.6	13.1	11.2
9		12.2	13.0	13.8	14.4	14.8	12.6	13.4	13.5	9.5
10		3.1	1.5	1.2	2.8	2.0	0.8	2.7		3.2

Serum Esterase

peak area	total as % of control	100.0	100.0	104.5	106.1	101.0	108.6	26.7	8.6	13.2
	color value	96.2	95.8	96.3	96.3	97.2	98.4	95.6	90.5	84.5
1		4.9	7.0	7.1	7.4	4.9	4.9	7.8	----	----
2		65.8	63.5	58.3	57.4	59.8	58.7	63.1	4.8	9.4
3		8.2	8.6	11.0	9.7	9.8	9.5	----	66.7	59.4
4		11.5	13.9	14.2	14.0	15.4	15.9	13.8	9.5	6.3
5		2.9	1.6	3.1	3.9	4.5	6.4	7.8		6.3
6		2.9	1.2	2.6	3.9	2.8	3.0	3.1	9.5	3.1

* Total dye intensity relative to control

** Color value refers to recovery as sum of all color peaks

Starch Electrophoretic Patterns of Rat Serum as Modified by Graded Concentrations of Scandium Chloride

Serum LDH

peak area	milli moles	0.0	0.5	1.0	2.0	4.0	5.0	10.0	20.0	50.0
	total as % of control	100.0	---	114.1	100.0	119.2	96.2	57.7	76.9	61.5
	color value	94.9	---	101.1	95.5	96.7	98.6	95.6	95.5	104.2
1		5.1	---	5.1	1.3	8.6	1.3 20.0	5.0 48.9	16.7 38.3	12.5 41.7
2		30.8	---	19.1	6.4 19.2	30.8	21.3	22.2	21.7	25.0
3		23.1	---	31.5	26.9	29.0	32.0	6.7	8.3	10.4
4		35.9	---	42.7	23.1 19.2	12.9 29.0	24.0	6.7	10.0	14.6
5			---							
6			---							

Serum Acid Phosphatase

peak area	total as % of control	100.0	174.0	146.0	141.0	148.7	115.4	46.2	48.7	48.7
	color value	94.8	88.1	89.5	98.2	101.7	88.9	94.4	100.0	100.0
1		38.5	52.9	63.2	67.3	60.3	28 9	33.3	26.3	26.3
2		17.9	17.6	7.0	9.1	12.1	20.0			
3		20.5	13.2	12.3	12.7	15.5	28.9	27.8	52.6	57.9
4		17.9	4.4	7.0	9.1	13.8	11.1	33.3	21.1	15.8

Starch Electrophoretic Patterns of Rat Serum as Modified by Graded Concentrations of Yttrium Chloride

Serum Protein

peak area	milli moles	0.00	0.50	1.0	2.0	4.0	5.0	10.0	20.0	50.0
	total as % of control*	100.0	100.9	89.4	104.2	94.0	97.3	94.0	102.1	91.8
	color value **	97.6	97.7	96.3	98.6	97.7	96.1	97.0	97.3	97.5
	serum pH	7.4	7.4	7.4	7.4	6.8	6.8	5.0	5.3	5.5
1		5.7	6.3	4.4	7.0	5.1	4.3	3.9	4.7	3.6
2		29.0	25.7	29.1	27.0	27.3	28.6	28.9	30.2	30.6
3		13.9	12.6	11.5	13.3	12.5	13.0	10.6	9.8	9.9
4		7.6	8.4	8.1	7.8	8.0	8.1	9.6	9.2	9.2
5		5.4	4.8	5.4	4.9	5.8	5.9	5.8	5.9	6.3
6		10.3	12.0	10.8	11.3	10.0	9.9	10.9	12.4	11.2
7		7.6	8.4	8.4	8.7	8.7	8.4	7.7	4.7	6.3
8		4.8	4.8	5.1	5.2	5.8	4.3	4.2	6.5	5.9
9		11.8	13.2	12.5	12.5	13.5	12.4	13.5	11.5	13.2
10		1.5	1.5	1.0	0.9	1.0	1.2	1.9	2.4	1.3

Serum Esterase

peak area	total as % of control	100.0	90.9	99.6	86.6	100.0	100.7	91.3	90.6	81.5
	color value	98.1	97.2	97.3	97.9	96.7	96.4	96.1	95.2	98.7
1		2.5	1.6	1.5 1.9	4.2	5.4	4.0	5.2	2.4	4.0
2		57.2	54.2	55.3	58.2	55.8	59.0	55.2	54.8	52.0
3		18.5	19.1	16.7	16.7	15.6	15.8	13.5	13.2	11.1
4		13.0	14.7	13.1	13.0	12.0 2.5	12.2	19.4	19.2	14.2
5		3.6	3.2	3.3	3.6	2.9	2.9			6.2
6		3.3	4.4	5.5	3.3	2.5	1.4 1.1	2.0 0.8	3.2 2.4	7.6 3.6

* Total dye intensity relative to control

** Color value refers to recovery as sum of all color peaks

Starch Electrophoretic Patterns of Rat Serum as Modified by Graded Concentrations of Yttrium Chloride

Serum LDH

peak area	milli moles	0.00	0.5	1.0	2.0	4.0	5.0	10.0	20.0	50.0
	total as % of control	100.0	54.7	57.0	62.8	50.0	58.1	62.8	14.0	14.0
	color value	93.1	95.7	93.9	98.2	93.1	96.0	92.5	----	----
1		10.5	6.4	4.1	5.6	2.3	4.0	3.7	low	low
2		22.1	14.9	18.4	22.2	7.0 16.3	30.0	14.8	low	low
3		14.0	25.5	28.6	20.4	23.3	20.0	25.9	low	low
4		11.6	10.6		11.1	11.6	8.0		low	low
5		2.3	2.1	2.0	1.9	0.0	2.0	11.1	low	low
6		32.6	36.2	40.8	37.0	32.6	32.0	37.0	low	low

Serum Acid Phosphatase

peak area	total as % of control	100.0	111.1	77.7	137.0	125.9	129.6	92.6	96.3	92.6
	color value	92.6	103.3	90.4	91.8	105.9	91.4	92.0	96.2	100.0
1		66.7	53.3	57.1	48.6	50.0	45.7	52.0	57.1	52.0
2		14.8	16.7	14.3	21.6	20.6	25.7	16.0	23.1	16.0
3		3.7	10.0	9.5	13.5	14.7	11.4	12.0		16.0
4		7.4	23.3	9.5	8.1	20.6	8.6	12.0	15.4	16.0

Starch Electrophoretic Patterns of Rat Serum as Modified by Graded Concentrations of Cerium Chloride

Serum Protein

peak area	milli moles	0.00	0.50	1.0	2.0	4.0	5.0	10.0	20.0	50.0
	total as % of control*	100.0	83.3	101.3	85.7	102.6	80.6	99.3	94.0	96.0
	color value **	97.0	97.2	98.7	95.4	96.1	98.4	96.2	97.6	97.4
	serum pH	7.4	7.4	7.4	7.4	6.8	6.6	6.0	6.0	5.5
1		3.7	2.0	3.9	2.3	3.6	1.7	2.7	3.2	3.1
2		31.7	31.6	31.3	33.1	31.5	32.2	27.5	28.4	27.4
3		11.7	13.2	12.8	12.1	11.7	12.4	10.1	8.2	8.9
4		6.3	6.8	7.6	6.6	6.8	7.0	9.7	9.2	7.6
5		6.0	5.6	5.3	4.3	5.8	6.2	5.0	6.4	4.9
6		10.3	11.6	12.5	10.9	10.1	10.7	12.4	10.6	13.2
7		9.0	8.8	7.6	7.8	7.8	8.7	8.7	6.4	5.9
8		4.3	4.8	4.9	5.8	5.5	5.8	4.7	9.2	9.4
9		12.0	10.8	11.2	11.3	11.4	11.6	13.4	14.9	16.0
10		2.0	2.0	1.6	1.2	1.9	2.1	2.0	1.1	1.0

Serum Esterase

peak area	total as % of control	100.0	97.8	104.4	86.1	82.5	91.4	100.7	87.3	75.4
	color value	97.4	95.2	95.4	98.4	98.2	96.7	98.1	97.0	98.0
1		5.6	7.3	9.6	7.4	6.8	6.5	3.3	7.3	8.4
2		57.1	56.5	54.3	58.9	59.3	56.7	46.7	20.9 18.8	19.8 26.2
3		13.4	11.5	9.3	11.7	13.1	13.5	19.3	14.1	12.4
4		14.9	15.3	15.4	15.6	15.8	16.3	14.8	21.4	18.8
5		3.4	2.7	4.3	2.2	2.3	2.9	9.6 3.3	9.8	8.4
6		3.0	1.9	2.5	2.6	0.9	0.8	1.1	4.7	4.0

* Total dye intensity relative to control

** Color value refers to recovery as sum of all color peaks

Starch Electrophoretic Patterns of Rat Serum as Modified by Graded Concentrations of Cerium Chloride

Serum LDH

peak area	milli moles	0.00	0.50	1.0	2.0	4.0	5.0	10.0	20.0	50.0
	total as % of control	100.0	126.5	142.9	153.0	132.7	67.3	18.4	16.3	20.4
	color value		62.0	70.0	75.0	65.0	33.0	9.0	8.0	10.0
1		---	---	10.0	1.3	6.2	low	low	low	low
2		14.3	11.3	27.1	13.3	18.5	low	low	low	low
3		34.7	35.5	12.9	13.3	26.2	low	low	low	low
4		6.1	16.1	20.0	26.7	7.7	low	low	low	low
5		16.3	12.9	12.9	17.3	12.3	low	low	low	low
6		8.2	12.9	7.1	12.0	15.4	low	low	low	low
7		12.2	6.5	2.9	16.0	9.2	low	low	low	low

Serum Acid Phosphatase

peak area	total as % of control	100.0	62.7	119.0	62.7	77.9	84.7	66.1	49.1	55.9
	color value	94.9	89.1	91.5	97.2	95.6	86.0	94.8	93.0	96.9
1		42.4	29.7	32.9	29.7	23.9	26.0	25.6	34.5	33.3
2		22.0	29.7	32.9	35.1	34.8	30.0	33.3	31.0	33.3
3		22.0	24.3	17.1	24.3	23.9	22.0	17.9	17.2	24.2
4		8.5	5.4	8.6	8.1	13.0	8.0	7.7	10.3	6.1

Starch Electrophoretic Patterns of Rat Serum as Modified by Graded Concentrations of $EuCl_3$

Serum Protein

peak area	milli moles	0.00	0.5	1.0	2.0	4.0	5.0	10.0	20.0	50.0	100.0
	total as % of control*	100.0	104.9	75.7	78.0	91.9	86.7	98.4	101.0	92.6	
	color value**	97.9	98.1	96.9	98.7	97.8	96.7	96.7	98.1	97.5	
	serum pH	7.4	7.2	7.2	6.8	6.6	6.6	6.0	6.0	5.8	5.8
1		3.2	2.5	1.3	2.9	2.1	1.9	3.6	4.8	4.5	
2		28.5	30.2	30.3	30.7	32.7	32.8	29.3	31.4	28.0	
3		11.3	13.9	13.2	12.9	11.6	11.9	11.5	9.0	11.2	
4		8.1	7.4	8.1	8.3	8.1	8.2	9.2	9.3	10.1	
5		6.1	5.5	6.0	5.4	5.3	5.6	5.6	6.7	8.4	
6		11.3	11.1	11.5	12.4	11.6	11.6	11.2	11.2	10.5	
7		7.1	8.0	6.0	8.3	8.1	7.1	7.2	5.8	6.3	
8		5.5	4.0	8.5	6.2	6.3	3.4	4.6	6.1	6.3	
9		14.2	13.6	12.0	11.6	12.0	14.2	12.2	11.9	10.5	
10		2.6	1.9					2.3	1.9	1.7	

Serum Esterase

peak area	total as % of control	100.0	97.2	82.8	86.0	95.3	89.8	145.1	115.3	114.0	73.0
	color value	98.6	98.1	99.5	97.8	98.2	96.3	97.4	96.8	97.5	97.4
1		3.7	2.4	3.9	3.2	4.9	5.7	3.5	2.0	2.9	3.7
2		63.3	63.2	60.7	61.1	62.9	63.7	51.3	25.9	17.1	23.6
									19.4	25.7	14.0
3		10.2	10.5	10.1	8.6	8.8	8.3	16.7	16.1	15.5	17.8
4		15.8	18.7	16.9	17.3	17.6	17.1	22.1	16.1	17.1	24.2
5		1.4						---	8.5	9.0	14.6
6		1.9	1.9	2.8	2.2	2.0	0.5	3.2	8.1	7.3	
7		2.3	1.4	5.1	5.4	2.0	1.0	0.6	1.2	2.9	1.3

* Total dye intensity relative to control

** Color value refers to recovery as sum of all color peaks

Starch Electrophoretic Patterns of Rat Serum as Modified by Graded Concentrations of $EuCl_3$

Serum LDH

peak area	milli moles	0.00	0.50	1.0	2.0	4.0	5.0	10.0	20.0	50.0
	total as % of control	100.0	48.3	61.6	75.6	52.3	9.9	14.0	11.6	----
	color value	97.0	97.6	96.2	97.0	94.4	---	---	---	----
1		8.1	1.2	2.8	2.3	7.8	---	---	---	----
2		28.5	7.2 24.1	24.5	25.4	23.3	---	---	---	----
3		25.6	28.9	13.2 19.0	16.2 23.8	21.1	low	low	low	----
4		14.5	13.3	15.1	7.7 8.5	8.9 13.3	---	---	---	----
5		20.3	22.9	20.8	13.1	20.0	low	---	---	----

Serum Acid Phosphatase

peak area	total as % of control	100.0	68.7	112.5	96.8	103.1	53.7	126.6	97.5	107.3	100.0*
	color value	87.6	95.4	88.9	83.9	96.8	90.9	90.5	90.0	93.2	97.6
1		56.3	63.6	52.8	45.2	54.5	18.2	23.1	30.0	2.3 2.3 25.0	31.7
2		21.9	13.6	25.0	29.0	24.2	31.8	23.1	30.0	31.8	41.5
3		9.4	18.2	11.1	9.7	18.1	9.1	30.8	12.5 7.5	18.2	14.6
4							31.8	13.5	10.0	13.6	9.8

* Control for 5.0 to 50 mM $EuCl_3$

Starch Electrophoretic Patterns of Rat Serum as Modified by Graded Concentrations of Chloride Ytterbium

Serum Protein

peak area	milli moles	0.00	0.50	1.0	2.0	4.0	5.0	10.0	20.0	50.0	100.0
	Total as % of control*	100.0	85.8	93.4	101.3	92.7	111.8	86.1	95.4	95.3	
	color value**	98.4	96.5	96.8	96.2	97.1	96.2	97.7	98.3	97.6	
	serum pH	7.4	7.2	7.2	7.2	6.8	6.8	6.0	6.0	5.8	
1		2.3	0.4	1.4	2.3	1.8	4.1	1.5	2.4	2.1	
2		26.6	29.9	29.2	26.9	29.1	26.2	27.9	29.7	30.3	
3		15.8	14.9	14.4	14.6	13.8	14.7	14.1	11.0	16.2	
4		7.2	6.5	6.3	7.8	7.4	7.1	9.5	9.3	----	
5		6.3	5.7	6.7	5.8	5.3	5.6	6.1	5.2	-----	
6		13.2	12.3	12.3	9.1	11.3	10.3	11.5	11.4	19.0	
7		6.9	8.4	---	7.1	6.0	5.6	5.7	4.8	3.8	
8		5.3	6.9	13.4	6.5	9.6	7.6	8.4	11.0	11.4	
9		12.5	10.0	12.0	14.3	11.7	12.4	11.5	11.4	12.4	
10		2.3	1.5	1.1	1.3	1.1	2.6	1.5	2.1	2.4	

Serum Esterase

peak area	total as % of control	100.0	108.8	107.3	107.8	120.1	120.1	129.0	131.5	101.9	102.4
	color value	99.6	97.4	97.3	95.9	99.1	94.7	98.1	95.8	95.7	98.5
1		3.0	4.1	3.2	5.5	4.5	4.1	3.8	2.6	1.0	3.8
2		65.5	61.1	63.3	59.8	59.0	55.6	56.1	38.2	44.9	39.9
3		11.3	13.6	11.0	11.4	13.1	13.2	12.6	23.2	16.4	18.3
4		14.3	15.4	16.1	16.0	18.0	17.7	19.5	22.1	23.2	22.1
5		3.0	1.8	2.3	2.3	2.9	2.5	3.4	8.2	9.2	10.6
6		2.5	1.4	1.4	0.9	1.6	1.6	2.7	1.5	1.0	3.8

* Total dye intensity relative to control

** Color value refers to recovery as sum of all color peaks

Starch Electrophoretic Patterns of Rat Serum as Modified by Graded Concentrations of Chloride Ytterbium

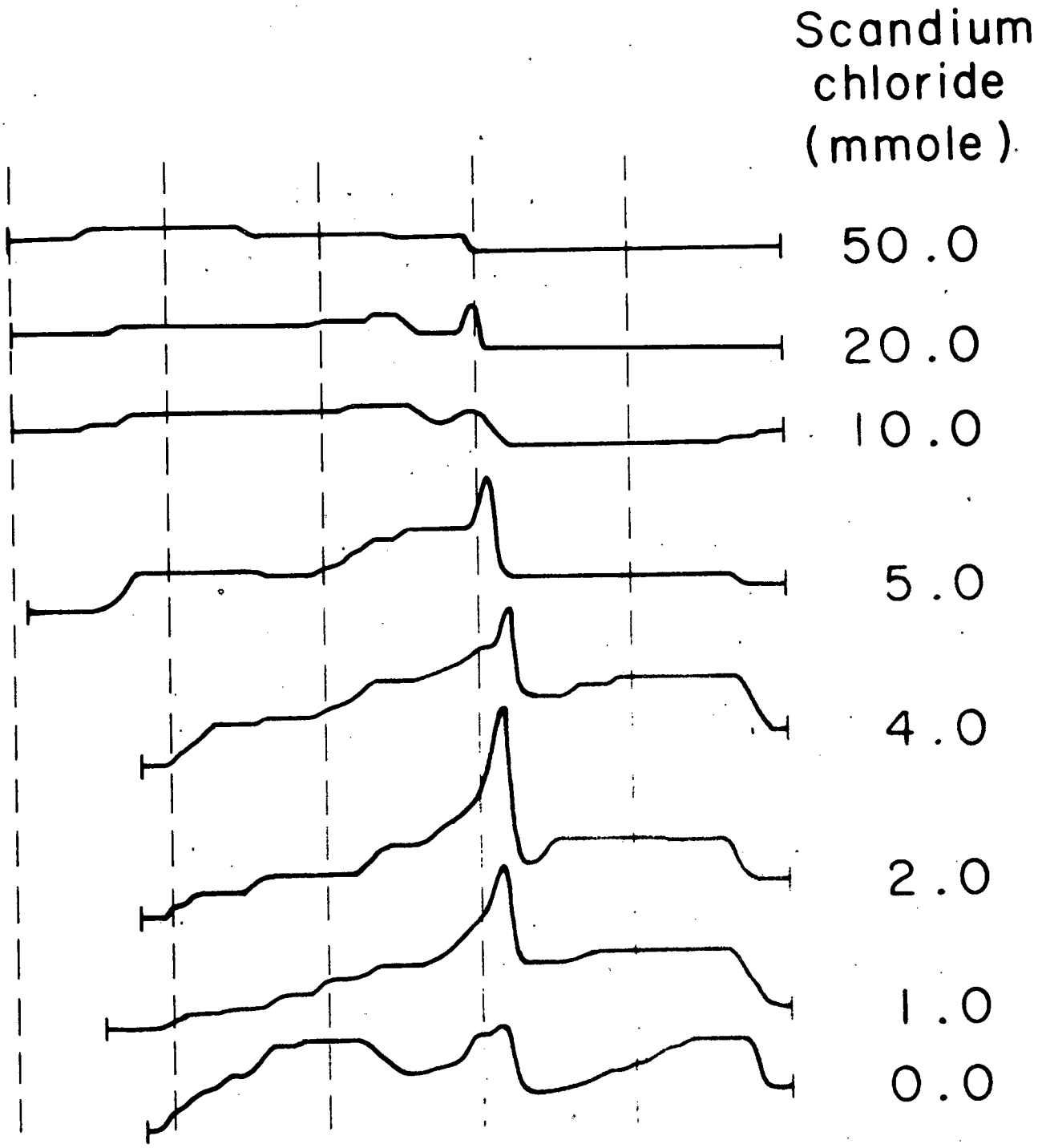
Serum LDH

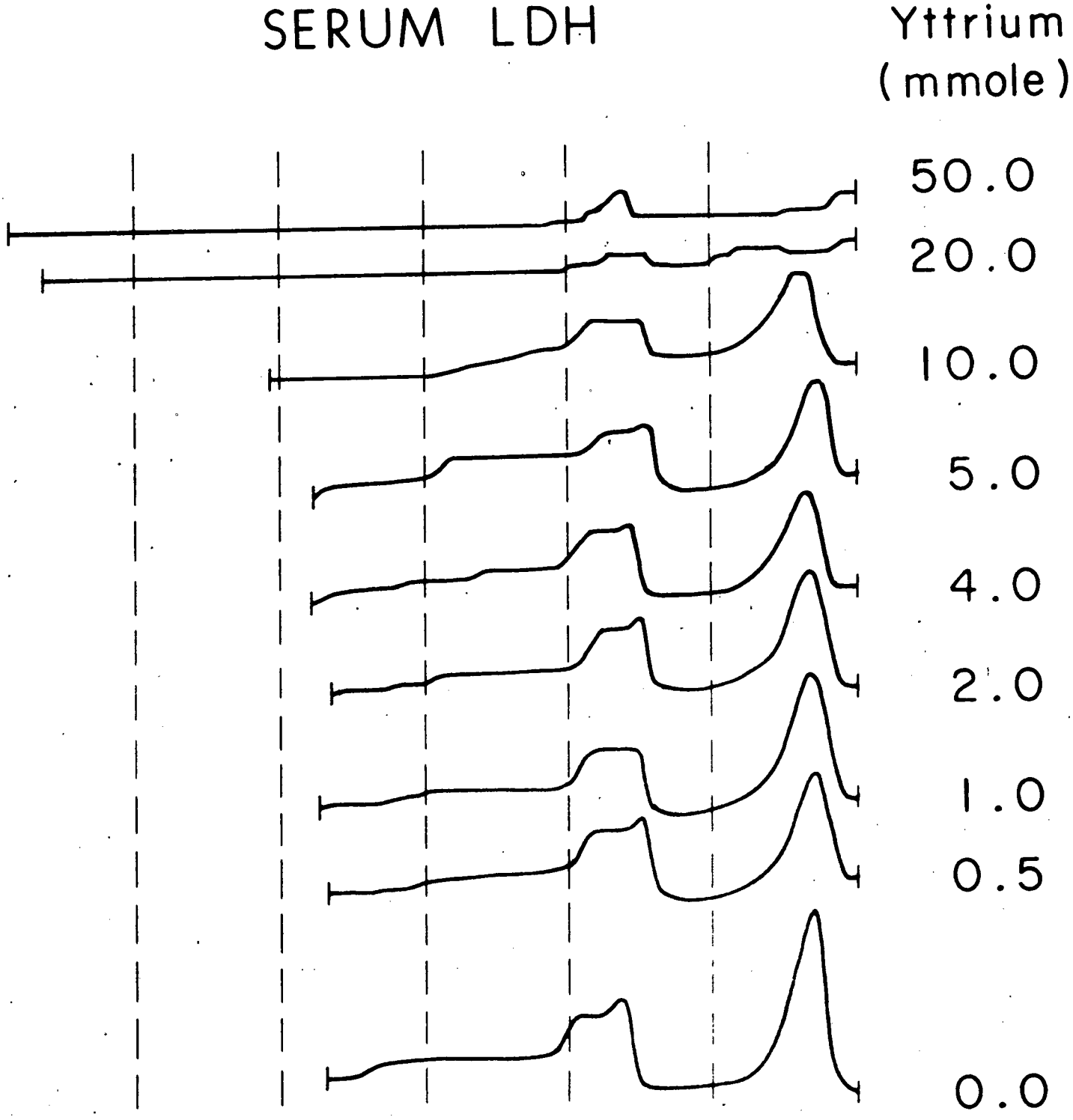
	milli moles	0.00	0.5	1.0	2.0	4.0	5.0	10.0	20.0	50.0
peak	total as % of control	100.0	59.5	87.0	54.1	50.0	24.0	55.5	54.8	62.3
area	color value	100.0	96.5	96.8	96.2	97.2	85.7	95.0	96.4	96.8
1		2.7	0.0	4.7	0.0	1.4	2.9	6.2	18.8	20.9
2		16.4	23.0	7.9	13.9	20.5	5.7	21.0	36.3	26.4
3		42.5	46.0	15.7	32.9	23.3	45.7	18.5	25.0	26.4
4		15.8		38.6	21.5			22.2		
5		7.5	11.5	12.6		15.1	14.3	11.1	10.0	12.1
6		6.2	8.0	6.3	8.9	16.4	5.7	4.9	6.3	11.0
7		8.9	8.0	11.0	19.0	20.5	11.4	11.1		

Serum Acid Phosphatase

peak	total as % of control	100.0	122.0	125.9	100.0	103.7	85.1	122.2	166.6	77.7
area	color value	85.1	91.0	100.0	85.1	85.8	95.6	91.0	84.4	76.2
1		44.4	42.4	35.3	14.8	17.9	8.7	27.3	22.2	9.5
2			27.3	26.5	22.2	39.3	34.8	27.3	28.9	28.6
3		40.7	15.2	17.6	29.6	14.3	21.7	18.2	13.3	14.3
4			6.1	20.6	18.5	14.3	30.4	18.2	20.0	23.8

SERUM LDH

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Yttrium
(mmole)
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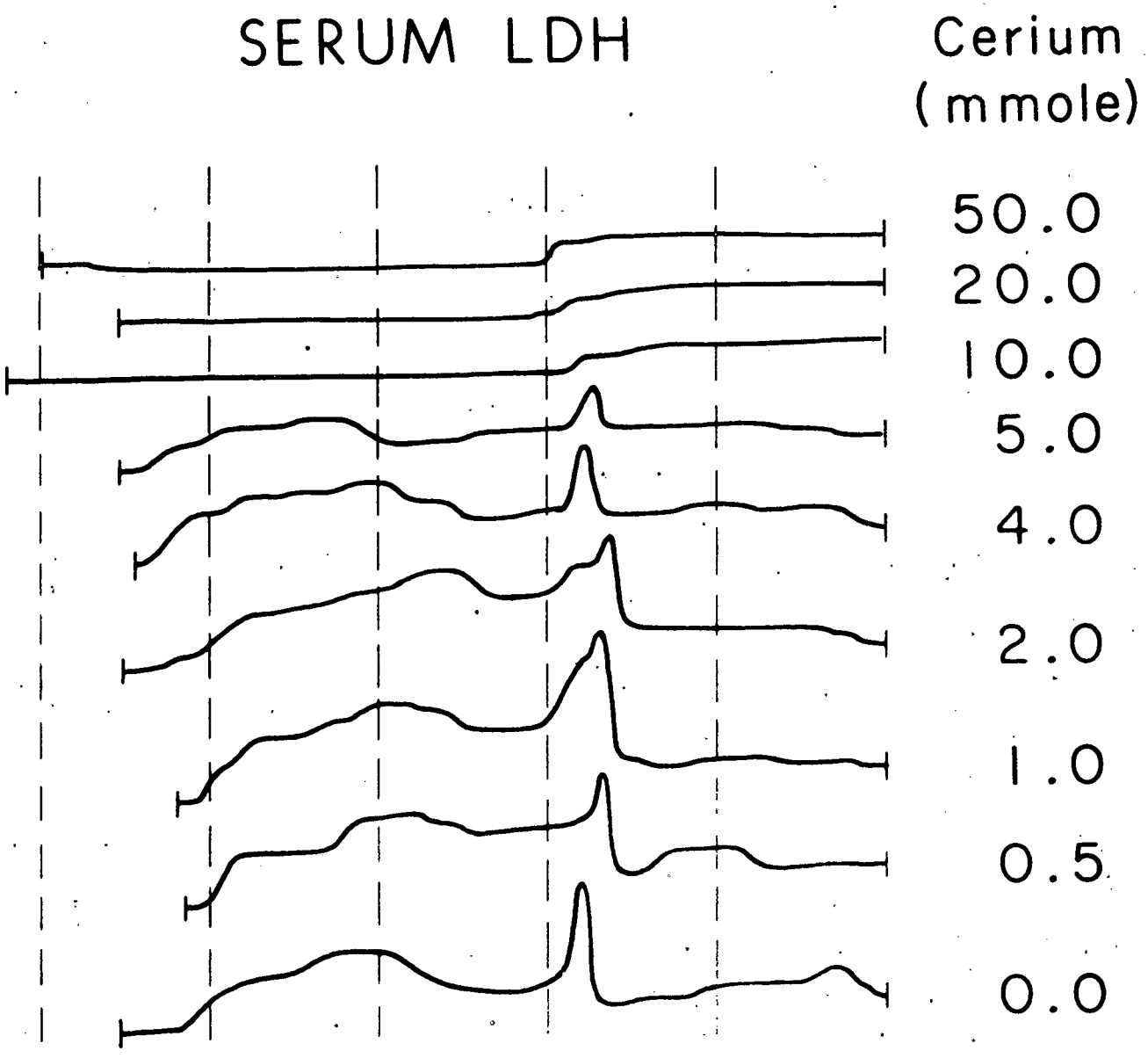
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Cerium
(mmole)
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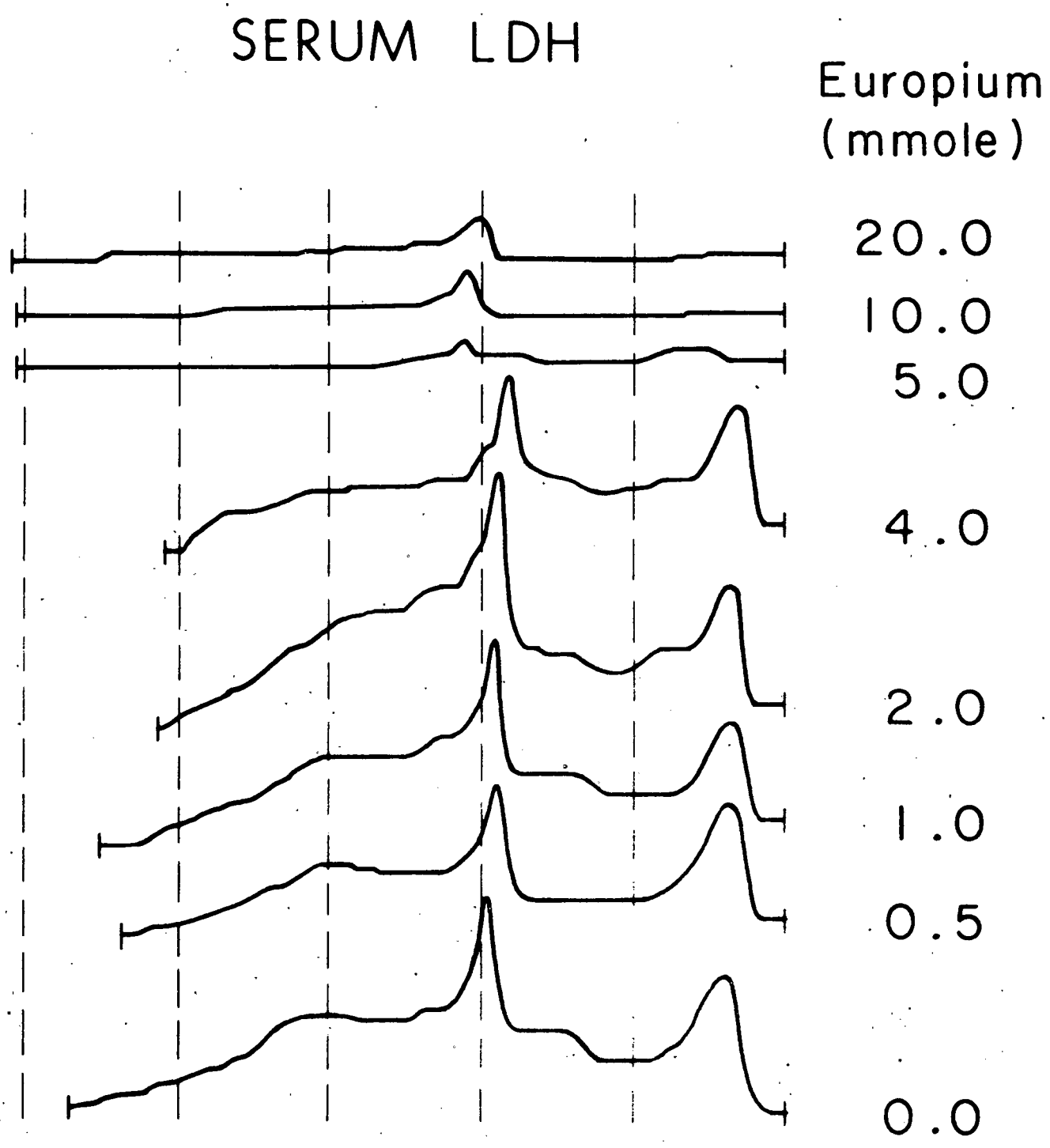
SERUM LDH
Europium
(mmole)
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5.0
4.0
2.0
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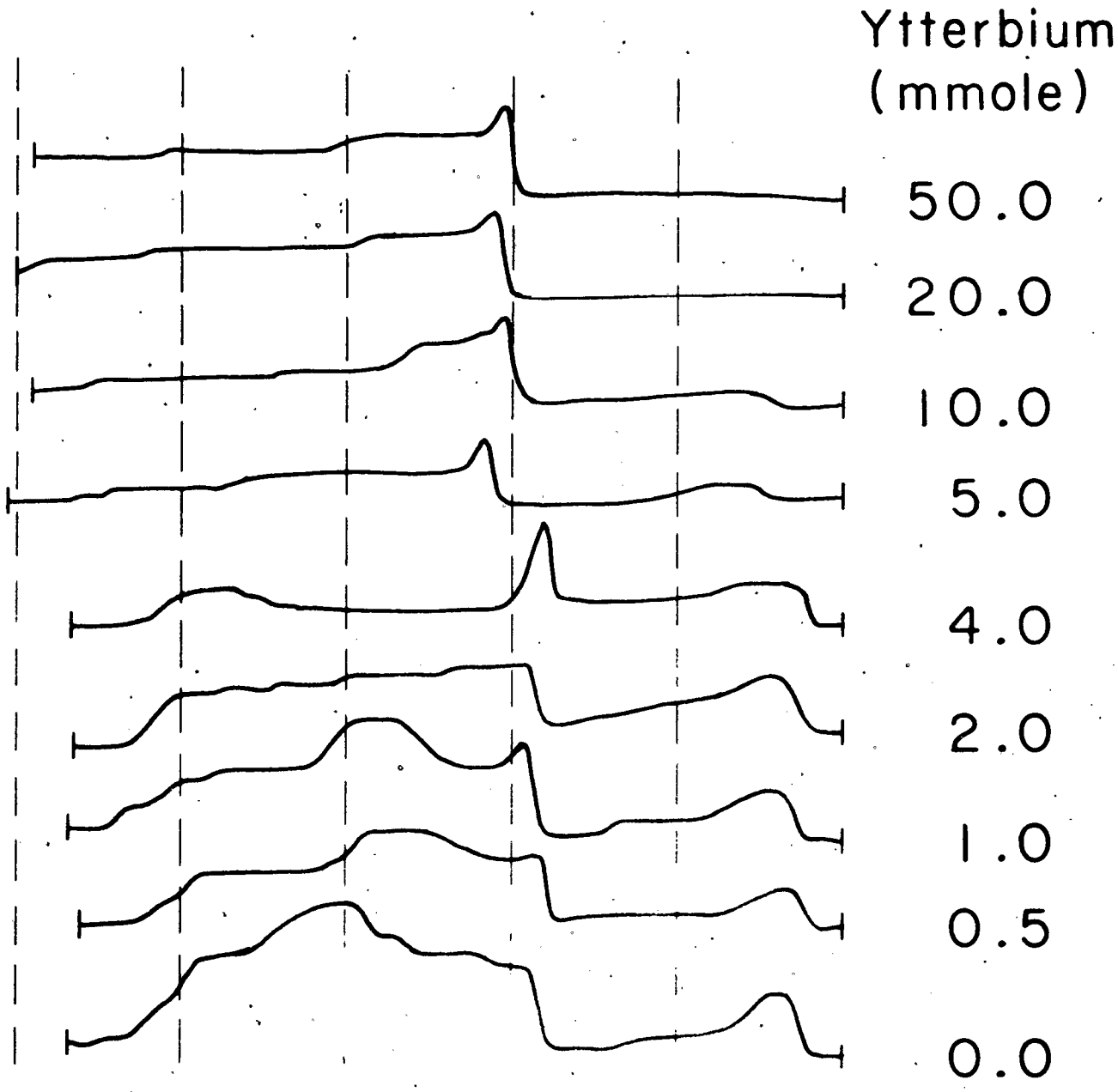
0.0

SERUM LDH



A cursory inspection of intensity curves clearly indicates the changes delineated above.

Further analysis of these patterns and their association with known protein positions or the patterns (gamma globulins, albumine, etc.) is necessary to define the various rare earth affinities. In any case the methodology should prove useful for our purposes.

H. Preliminary Findings in the Mercury Poisoned Sprague-Dawley Rat.

Recent reports in the literature (1, 2) have indicated that some therapeutic agents (ascorbate and an adrenal hormone containing a sulfhydryl moiety-spironolactone) are very effective in the prevention of renal pathology following the intake of mercuric chloride. Although chelation of the metal is probably the therapeutic rationale in the case of BAL (British anti lewisite) and other sulfhydryl agents, the action of the reducing material is not well defined. The conversion of the divalent to monovalent form of mercury which is very sparingly soluble in tissue fluids has been postulated but, at present, no further studies are available. In order to elucidate the mechanism of action of the reducing agent, Sprague-Dawley rats (24) were injected with 1.0 milligram of

$HgCl_2$ per 100 grams of body weight sub-cutaneously, a near lethal dose. Half of these had received intraperitoneally 180 mgm of sodium ascorbate per 100 grams. All of the ascorbate treated animals are alive and well after 2 months while nine of twelve receiving $HgCl_2$ alone died within 4 days and 3 are still alive. These results are consistent with those reported by Carroll et al (1).

Urine and feces during the early phases of the study were collected in all cases and are being analyzed using atomic absorption methods in an attempt to show changes in excretory patterns to account for the lethality differences observed. In addition histopathological examination and analysis for mercury in the brain and kidney are in progress.

Further reports on the study will be presented in the near future.

References:

1. Carrol, R. et al. "Protection Against Mercuric Chloride Poisoning of the Rat Kidney" Arzneimittel - Forsettung 15 1361 (1965).

2. Selye, H. "Mercury Poisoning: Prevention by Spironolactone" Science 169 775 (1970).