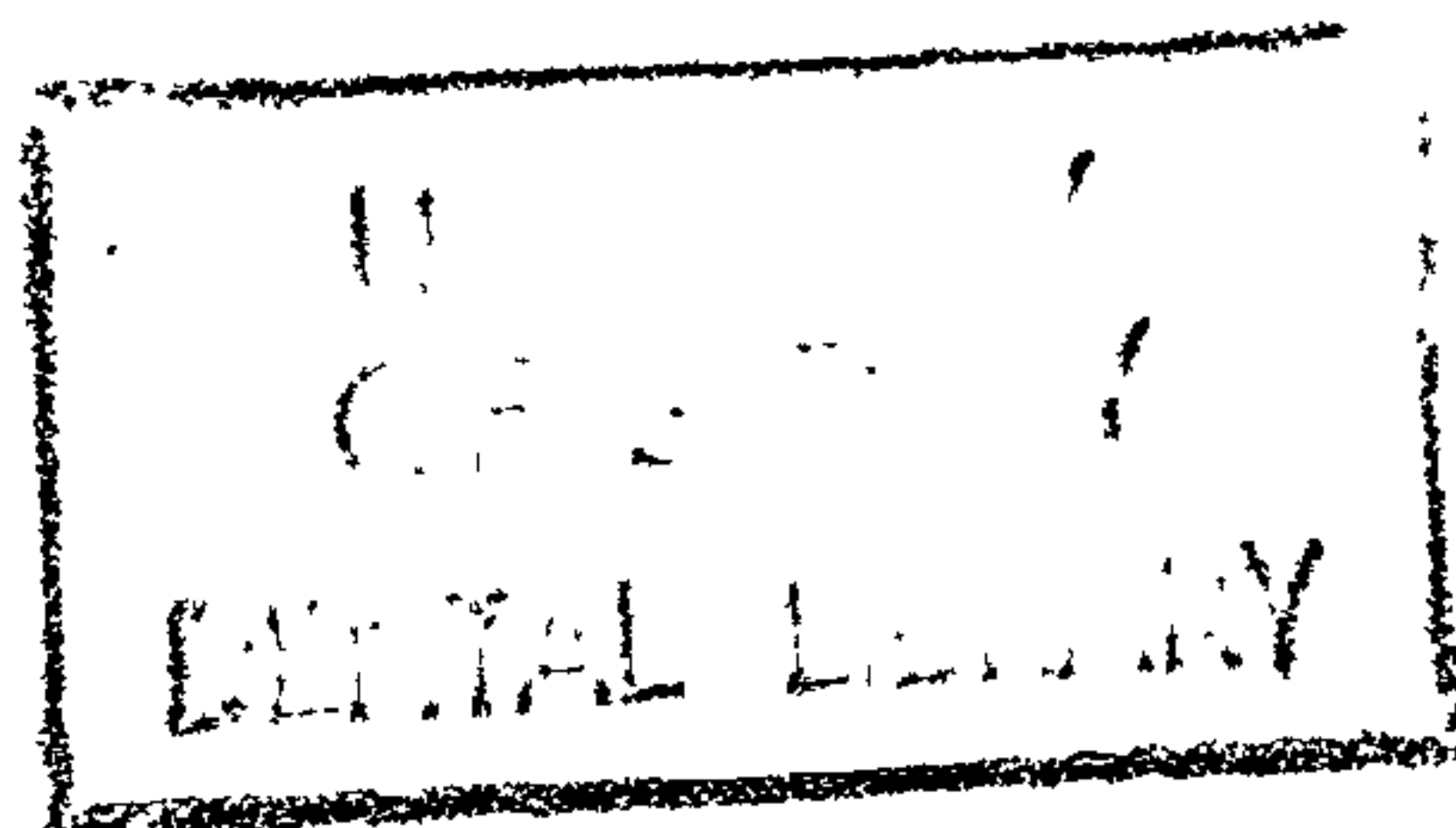


A LABORATORY ASSESSMENT
OF THE CARIOSTATIC POTENTIAL
OF IRON (II) AND IRON (III)
SALTS



Graham G. Craig, M.D.S.

A thesis submitted for the
degree of Doctor of Philosophy
in the University of Sydney

Department of Preventive Dentistry,
Faculty of Dentistry,
University of Sydney.

1977

ACKNOWLEDGEMENTS

The author would like to express his sincere appreciation to:
Professor N.D. Martin, Head of the Department of Preventive Dentistry
and Dean of the Faculty of Dentistry, for his support, advice and
constructive criticism during this study.

Professor K.W. Knox, Director of the Institute of Dental Research,
Sydney, for making research facilities available and for his valuable
advice on many occasions.

Dr. R.G. Schamschula, Deputy Director, Institute of Dental
Research, Sydney, for his constructive criticism of the final
manuscript and for his generous help whenever asked.

Dr. T. Mori, Research Assistant, Department of Prosthetic
Dentistry, for the preparation of the scanning electron micrographs.

Miss Z. Benjamin, Mrs. J. Newton, Mrs. H. Agus, Mr. H. Fong and
Miss B. Bishof for their valuable assistance with various aspects of
the work.

My wife, Margaret, and my family for their support and patient
understanding throughout this study.

TABLE OF CONTENTS

	Page
Title Page	i
Acknowledgements	ii
Table of Contents	iii
List of Tables	vii
List of Figures	viii
<u>INTRODUCTION</u>	1
<u>REVIEW OF LITERATURE</u>	5
<u>ASSESSMENTS OF THE CARIOSTATIC POTENTIAL OF TOPICALLY APPLIED IRON SALT SOLUTIONS</u>	6
LABORATORY STUDIES	6
<u>Effect on Fluoride Uptake</u>	6
<u>Effect of Enamel Dissolution Rate</u>	8
<u>Effect on Bacteria</u>	12
ANIMAL STUDIES	14
<u>Effect of Iron Salts on Experimental Caries</u>	14
<u>Effect on Plaque</u>	15
CLINICAL TRIALS	15
<u>SECTION 1: THE EFFECT OF IRON (II) AND IRON (III) SALTS ON THE UPTAKE OF FLUORIDE BY ENAMEL FROM FLUORIDE SOLUTIONS (TEST 1)</u>	17
<u>MATERIALS AND METHODS</u>	17
MATERIALS	17
<u>Teeth</u>	17
<u>Chemicals</u>	18
<u>Apparatus</u>	18
METHODS	19
<u>Preparation of Enamel</u>	19
<u>Treatment of Enamel</u>	21
<u>Fluoride Determination</u>	24
<u>Calculations</u>	25
Fluoride Concentration	25
Depth of Etched Layer	26
Enamel Surface Area	26

	iv.
	Page
<u>Scanning Electron Microscopy</u>	27
<u>RESULTS</u>	28
<i>FLUORIDE UPTAKE</i>	28
<i>SCANNING ELECTRON MICROGRAPHS</i>	30
<u>DISCUSSION</u>	38
<u>SECTION 2: THE EFFECT OF IRON (II) AND IRON (III) SALTS ON THE DISSOLUTION RATE OF ENAMEL (TEST 2)</u>	44
<u>MATERIALS AND METHODS</u>	45
<i>MATERIALS</i>	45
<u>Teeth</u>	45
<u>Chemicals</u>	45
<u>Apparatus</u>	46
<i>Experiment 1</i>	46
<i>Experiment 2</i>	46
<i>METHODS</i>	47
<u>Preparation of Enamel</u>	47
<i>Experiment 1</i>	47
<i>Experiment 2</i>	47
<u>Treatment of Enamel (Experiment 1)</u>	49
<u>Dissolution of Enamel</u>	52
<i>Experiment 1</i>	52
<i>Experiment 2</i>	52
<u>Calcium Analysis</u>	53
<u>Phosphorus Analysis</u>	55
<u>Calculation of Enamel Surface Area</u>	55
<u>RESULTS</u>	56
<i>EXPERIMENT 1 - THE EFFECT OF TOPICAL TREATMENTS ON ENAMEL DISSOLUTION RATE</i>	56
<u>One Stage Treatments (Single application of test compounds)</u>	56
<u>Application of Iron Salts Followed by Topical Fluoride Treatment</u>	67
<i>EXPERIMENT 2 - THE EFFECT OF ADDITION OF TEST COMPOUNDS TO LACTATE BUFFER ON ENAMEL DISSOLUTION RATE</i>	70
<u>DISCUSSION</u>	79

SECTION 3: THE EFFECT OF IRON (II) AND IRON (III) SALTS ON *IN VITRO*
PLAQUE FORMATION BY *S. MUTANS*, *S. SANGUIS* AND *A. VISCOSUS* (TEST 3) 87

MATERIALS AND METHODS 88

MATERIALS 88

Teeth 88

Organisms 88

Media 88

Chemicals 88

METHODS 89

Preparation of Specimens 89

Treatment of Specimens 90

Assessment of Plaque Formation 92

RESULTS 93

IN VITRO PLAQUE FORMATION ON SURFACES EXPOSED TO
S. MUTANS STRAIN GS5 93

IN VITRO PLAQUE FORMATION ON SURFACES EXPOSED TO
S. SANGUIS STRAIN 804 96

IN VITRO PLAQUE FORMATION ON SURFACES EXPOSED TO
A. VISCOSUS STRAIN NY1 99

DISCUSSION 101

SECTION 4: THE EFFECT OF IRON (II) AND IRON (III) SALTS ON ACID
PRODUCTION BY *S. MUTANS*, *A. VISCOSUS* AND *L. CASEI* 106

MATERIALS AND METHODS 108

MATERIALS 108

Organisms 108

Media 109

Chemicals 109

Apparatus 109

Fermenter 109

Holder for Organisms 110

METHODS 113

Growth of Organisms 113

Treatment of Bacterial Pellets 114

Exposure of Organisms to Treatment Solutions and
Measurement of Acid Production 115

<u>RESULTS</u>	118
ACID PRODUCTION BY <u>S. MUTANS</u> STRAIN G85	119
ACID PRODUCTION BY <u>A. VISCOSUS</u> STRAIN NY1	125
ACID PRODUCTION BY <u>L. CASEI</u> VAR. CASEI	131
 <u>DISCUSSION</u>	 137
 <u>GENERAL DISCUSSION</u>	 142
 <u>SUMMARY AND CONCLUSIONS</u>	 150
 <u>BIBLIOGRAPHY</u>	 160
 <u>APPENDIX</u>	 181

LIST OF TABLES

<u>Table</u>	<u>Designation</u>	Page
1	Treatment solutions and treatment times used in fluoride uptake study	22
2	Fluoride uptake by enamel from topical treatments	29
3	Treatment solutions and treatment times used in enamel dissolution rate study (Experiment 1)	50
4	Cations and anions added to pH 4.5 lactate buffer in enamel dissolution rate study (Experiment 2)	54
5	Calcium and phosphorus loss from treated enamel after three hour immersion period in pH 4.5 lactate buffer	60
6	Calcium loss from enamel during topical treatments	61
7	Dissolution rate of enamel immersed in pH 4.5 lactate buffer containing added cations or anions	72
8	Treatment solutions and treatment times used in <i>in vitro</i> plaque formation study	91
9	Treatment solutions and treatment times used in acid production study	116
10	Acid production by <i>S. mutans</i> strain GS5 after treatment with test compounds	120
11	Acid production by <i>A. viscosus</i> strain NY1 after treatment with test compounds	126
12	Acid production by <i>L. casei</i> var. <i>casei</i> after treatment with test compounds	132

LIST OF FIGURES

		Page
<u>Figure</u>	<u>Abbreviated Designation</u>	
1	Apparatus used for the treatment of enamel specimens	20
2	Enamel specimen mounted on an acrylic rod	20
3	Flow diagram of procedures followed in fluoride uptake study	23
4	Scanning electron micrographs of untreated and treated enamel	31
5	Scanning electron micrographs of enamel treated with iron (II) chloride followed by APF	32
6	Scanning electron micrographs of enamel treated with iron (III) chloride followed by APF	33
7	Scanning electron micrographs of enamel treated with iron (II) chloride followed by 476 mM sodium fluoride	34
8	Scanning electron micrographs of enamel treated with iron (III) chloride followed by 476 mM sodium fluoride	35
9	Scanning electron micrographs of enamel treated with fluoride solutions containing iron	36
10	Scanning electron micrographs of enamel treated with tin (II) fluoride or sodium fluoride	37
11	Acrylic rod supporting enamel specimens in place in a plastic vial containing lactate buffer	48
12	Flow diagram of procedures followed in enamel dissolution rate study	51
13	Dissolution rates of enamel in pH 4.5 lactate buffer following one stage topical treatments with non-fluoride iron salts	57
14	Dissolution rates of enamel in pH 4.5 lactate buffer following one stage topical treatments with test compounds containing fluoride	58
15	Scanning electron micrographs of enamel treated with iron (II) chloride	63

<u>Figure</u>	<u>Abbreviated Designation</u>	
16	Scanning electron micrographs of enamel treated with 1 mM iron (III) chloride	64
17	Scanning electron micrographs of enamel treated with 10 mM iron (III) chloride	65
18	Scanning electron micrographs of enamel treated with 100 mM iron (III) chloride	66
19	Dissolution rates of enamel in pH 4.5 lactate buffer following two stage topical treatments with iron (II) chloride or iron (III) chloride and APF	68
20	Dissolution rates of enamel in pH 4.5 lactate buffer following two stage topical treatments with iron (II) chloride or iron (III) chloride and 476 mM sodium fluoride	69
21	Ca/P (molar) ratios in pH 4.5 lactate buffer following the immersion of enamel subjected to two stage topical treatments	71
22	Scanning electron micrographs of enamel immersed in pH 4.5 lactate buffer containing added Na^+ or Ca^{2+} cations	74
23	Scanning electron micrographs of enamel immersed in pH 4.5 lactate buffer containing added PO_4^{3-} anions or Fe^{2+} cations	75
24	Scanning electron micrographs of enamel immersed in pH 4.5 lactate buffer containing added Fe^{3+} or Sn^{2+} cations	76
25	Scanning electron micrographs of enamel immersed in pH 4.5 lactate buffer containing added F^- anions	77
26	Scanning electron micrographs of enamel immersed in pH 4.5 lactate buffer containing added F^- anions	78
27	Three stages in the preparation of specimens	90
28	<i>In vitro</i> plaque formation on test surfaces exposed to <i>S. mutans</i> strain GS5 following treatment with test compounds	94
29	Photographs showing an untreated specimen and <i>in vitro</i> plaque formation on two specimens exposed to <i>S. mutans</i> strain GS5	95

<u>Figure</u>	<u>Abbreviated Designation</u>	
30	<i>In vitro</i> plaque formation on test surfaces exposed to <i>S. sanguis</i> strain 804 following treatment with test compounds	97
31	Examples of <i>in vitro</i> plaque formation on specimens exposed to <i>S. sanguis</i> strain 804 or <i>A. viscosus</i> strain NY1	98
32	<i>In vitro</i> plaque formation on test surfaces exposed to <i>A. viscosus</i> strain NY1	100
33	Examples of precipitate formation in liquid culture media following addition of iron salts	107
34	Design of fermenter used in culture of test organisms	111
35	Apparatus which allowed organisms to be (i) treated with test compounds, (ii) washed and (iii) monitored for acid production, whilst they remained <i>in situ</i>	112
36	Reductions in acid production by <i>S. mutans</i> strain GS5 following treatment with test compounds	121
37	Rates of acid production by <i>S. mutans</i> strain GS5 following treatment with iron (II) chloride or iron (III) chloride	122
38	Rates of acid production by <i>S. mutans</i> strain GS5 following treatment with iron (II) fluoride, iron (III) fluoride, APF or a solution containing iron (III) chloride and sodium fluoride	123
39	Rates of acid production by <i>S. mutans</i> strain GS5 following treatment with tin (II) fluoride or sodium fluoride	124
40	Reductions in acid production by <i>A. viscosus</i> strain NY1 following treatment with test compounds	127
41	Rates of acid production by <i>A. viscosus</i> strain NY1 following treatment with iron (II) chloride or iron (III) chloride	128
42	Rates of acid production by <i>A. viscosus</i> strain NY1 following treatment with iron (II) fluoride, iron (III) fluoride, APF or a solution containing iron (III) chloride and sodium fluoride	129
43	Rates of acid production by <i>A. viscosus</i> strain NY1 following treatment with tin (II) fluoride or sodium fluoride	130

<u>Figure :</u>	<u>Abbreviated Designation</u>	
44	Reductions in acid production by <i>L. casei</i> var. casei following treatment with test compounds	133
45	Rates of acid production by <i>L. casei</i> var. casei following treatment with iron (II) chloride or iron (III) chloride	134
46	Rates of acid production by <i>L. casei</i> var. casei following treatment with iron (II) fluoride, iron (III) fluoride, APF or a solution containing iron (III) chloride and sodium fluoride	135
47	Rates of acid production by <i>L. casei</i> var. casei following treatment with tin (II) fluoride or sodium fluoride	136

INTRODUCTION

Iron is an essential element for man⁽⁷⁰⁾. It is involved in such vital processes as the transport of oxygen (haemoglobin and myoglobin), the activation of molecular oxygen (a number of oxygenases and oxidases) and the transport of electrons (cytochromes and iron-sulphur proteins)⁽¹⁹⁹⁾.

These processes utilize two important chemical properties of iron, namely its ability to be involved in oxidation-reduction reactions through the presence of two relatively stable valency states, and its ability, common to transition metals, to form a wide range of complexes⁽²⁴¹⁾.

The adult human body contains approximately 4 g of iron⁽³⁵⁾, of which an estimated 66 per cent is found in circulating haemoglobin, nine per cent in myoglobin and one per cent in haem-containing enzymes or in transport through plasma attached to transferrin⁽⁵⁾⁽¹⁷⁾. Most of the remainder is found in ferritin and haemosiderin in the liver, spleen and bone marrow⁽¹³⁸⁾.

Minor amounts of iron have been detected in stimulated whole saliva⁽⁵⁶⁾⁽²¹⁵⁾. However, no published information was found concerning the iron content of parotid, submaxillary or sublingual saliva.

Iron is also found in enamel⁽⁴⁸⁾⁽¹³³⁾⁽¹³⁴⁾⁽¹⁹⁷⁾⁽²²¹⁾. The reported mean concentrations for iron in whole enamel differ widely, ranging from 4.4 ppm⁽¹³⁴⁾ to 338 ppm⁽¹⁹⁷⁾. Although there is an absence of detailed information on the distribution of iron in enamel, the available evidence suggests that its concentration is higher in surface enamel than in subsurface enamel⁽²⁸⁾.

In view of the role of dental plaque in the initiation of dental caries and gingival inflammation⁽²²⁾⁽¹¹²⁾, it is of interest that plaque has been reported to contain 47 to 128 ppm iron⁽¹⁷²⁾.

Although iron is a constituent of enamel and plaque, little is known about the relationship between iron and caries experience. The only two studies that have been conducted have been concerned with the possible relationship between the concentration of iron in water and caries experience in different geographic areas⁽⁸⁰⁾⁽¹³²⁾. Neither study provided evidence of a connection between the two.

Results of animal studies indicate that the effect of iron on caries incidence may be highly dependent on the chemical form in which it is administered. Navia⁽¹⁶³⁾, for example, classified iron as a "caries inert" element on the basis of studies with an unspecified iron compound⁽²⁴⁴⁾, an iron (II) - EDTA complex⁽⁹⁸⁾ and iron (III) citrate⁽¹⁴¹⁾. In each study, the test compound was added to the drinking water and/or the diet of rats during a caries test period. However, it is possible that the EDTA and citrate ligands, both of which form chelates with iron⁽⁷²⁾⁽²⁰⁹⁾, could have affected the cariostatic properties of the element, since both McClure⁽¹⁴¹⁾ and Emilson and Krasse⁽⁶⁰⁾ have obtained different results using simple iron salts. McClure⁽¹⁴¹⁾, whose study with iron (III) citrate was cited by Navia⁽¹⁶³⁾, also tested iron (III) chloride and found that this salt caused a marked decrease in experimental caries in rats. Subsequently, Emilson and Krasse⁽⁶⁰⁾ found that both iron (II) sulphate and iron (III) chloride significantly reduced the incidence of experimental caries in hamsters when applied topically or added to the drinking water or to the diet.

Both Fe^{2+} and Fe^{3+} cations have one particular property which could be significant in influencing the chain of events associated with the initiation and progression of carious lesions. Bachra and van Harskamp⁽⁷⁾ found that both of these cations had the ability to precipitate "a poorly crystallized, carbonate-containing, calcium hydroxyapatite" from solutions containing calcium and phosphate under conditions where precipitation would not otherwise occur. The authors reported that the addition of Fe^{2+} or Fe^{3+} cations at concentrations as low as $0.1\mu\text{M}$ and $0.001\mu\text{M}$ respectively, was sufficient to induce apatite formation in a metastable calcification buffer.

Therefore, in a situation where raised levels of ionised calcium and phosphate could be expected to be found, such as adjacent to a decalcification front in a carious lesion, the presence of Fe^{2+} or Fe^{3+} cations could prove to be beneficial by encouraging the precipitation of calcium and phosphate within the lesion.

Furthermore, it is possible that polyvalent cations such as Fe^{3+} may enhance the well established benefits of topical fluoride therapy by binding fluoride to enamel⁽¹⁴⁰⁾ and, in so doing, limit the fluoride loss that invariably occurs after such treatments⁽³⁰⁾⁽¹⁴⁵⁾⁽¹⁵⁰⁾⁽¹⁷⁸⁾.

Laboratory and clinical assessments of the cariostatic potential of solutions containing iron (III) chloride and sodium fluoride^a have been made⁽¹²⁾⁽⁶⁶⁾⁽²²²⁾⁽²²³⁾ and an undefined compound containing iron and fluoride has been tested for its ability to reduce the dissolution rate of enamel in acetate buffer⁽¹⁴³⁾. However, no published reports

^aOften referred to as "ferric fluoride" in the literature⁽²⁷⁾⁽²⁰³⁾.

of laboratory or clinical studies on iron (II) fluoride or iron (III) fluoride, as distinct from solutions containing iron (III) chloride and sodium fluoride, were found. This situation, together with the evidence suggesting that non-fluoride iron salts may have cariostatic properties, led to this study being undertaken.

Four laboratory screening tests were carried out to determine the cariostatic potential of iron (II) and iron (III) salts. The tests were designed to assess whether these salts:

1. Could be used to enhance fluoride uptake by enamel from fluoride solutions (Test 1).
2. Were effective in some of the areas in which fluoride is believed to exert its effect⁽¹¹⁶⁾, namely enamel solubility (Test 2), bacterial colonisation of enamel (Test 3) and acid production by cariogenic organisms (Test 4).

In this thesis, a separate section is devoted to each of the four tests. Each section contains a brief introduction, a description of the materials and methods used together with an analysis and a discussion of the results obtained. Finally, the significance of the findings in relation to the problem of caries prevention is discussed.

REVIEW OF LITERATURE

Over 30 years ago, Dean and co-workers⁽⁴⁹⁾⁽⁵⁰⁾ demonstrated an inverse association between the level of fluoride in the drinking water and the caries experience of human populations. Around the same time laboratory studies were undertaken in an attempt to define the mechanisms by which fluoride exerts its effect⁽¹⁵⁾⁽²²⁸⁾. These early developments appear to have set the general pattern for the field and laboratory investigations that have since been conducted in the search for other cariostatic agents. Much of the current knowledge about the cariostatic potential of iron has been derived from studies that have followed this pattern.

In one such study, Losee and Adkins⁽¹³²⁾ measured the concentration of iron and 20 other elements in the water supplies used by a group of caries-free subjects. The values obtained were compared with the published values for the same 21 elements in the water supplies of (i) neighbouring cities, (ii) cities where the caries prevalence was high and (iii) cities where the caries prevalence was low. It was found that the water supplies of the caries-free subjects and those of the cities did not differ significantly with respect to the concentrations of iron, aluminium, barium, beryllium, chromium, cobalt, copper, lead, manganese, nickel, rubidium, silver, tin, zinc or zirconium. However, the concentrations of boron, lithium, molybdenum, strontium, titanium and vanadium were significantly higher in the water supplies of the caries-free subjects than in the water supplies of the neighbouring cities. In addition, the concentrations of molybdenum and

strontium were significantly higher in the water supplies of the caries-free subjects than in the water supplies of the cities where the caries prevalence was low.

Rothman *et al.* ⁽¹⁸³⁾ found that the caries prevalence in children differed markedly in the neighbouring Colombian villages of Heliconia and Don Matias. Glass *et al.* ⁽⁸⁰⁾ reported subsequently that the village with the higher caries prevalence, Don Matias, had significantly higher concentrations of iron, copper, and manganese in its water supplies than Heliconia. Conversely, Heliconia had significantly higher concentrations of calcium, magnesium, molybdenum and vanadium in its water supplies than Don Matias. However, no direct causal relationship between any of the elements and caries prevalence was established.

No reports were found on the relationship between the concentration of iron in soil and caries prevalence. Most of the work concerning the cariostatic potential of iron has been related to its use as a topical agent and has included laboratory, animal and clinical studies. The remainder of this review is devoted to an examination of these studies.

ASSESSMENTS OF THE CARIOSTATIC POTENTIAL OF TOPICALLY APPLIED IRON SALT SOLUTIONS

LABORATORY STUDIES

Effect on Fluoride Uptake

In recent years, much attention has been given to ways of increasing fluoride uptake by enamel and ensuring that as much as possible of the

additional fluoride is retained⁽⁶⁾⁽⁵⁴⁾⁽¹¹⁹⁾⁽¹⁴⁰⁾⁽¹⁴⁴⁾⁽¹⁷¹⁾⁽¹⁷⁶⁾.

McCann⁽¹⁴⁰⁾ provided one approach to this problem by showing that fluoride uptake by enamel from acidulated phosphate-fluoride^a solutions could be markedly enhanced if the enamel was first treated with a solution containing a polyvalent metal, such as iron. Enamel treated for one minute with an iron (III) salt solution (0.05 M Fe^{3+}) and for three minutes with APF had a mean fluoride concentration of 12,300 ppm (S.D.^b = 2,310) in the "first layer" after 24 hours washing in pH 5.5 acetate buffer. McCann did not indicate the depth of the "first layer" nor did he use a control in this section of the experiment. However control enamel, subjected to the same washing procedure earlier in the experiment, had a mean fluoride concentration of 1,020 ppm (S.D. = 280) in the "first layer". This layer had a mean depth of 7.72 μm (S.D. = 1.98). The use of other polyvalent cations, Al^{3+} , Ti^{4+} or Sn^{4+} , prior to the use of APF was also found to markedly enhance fluoride uptake.

Clarkson *et al.*⁽⁴²⁾ subsequently confirmed that the treatment of enamel with solutions containing Fe^{3+} , Al^{3+} , Ti^{4+} or Sn^{4+} cations enhanced fluoride uptake from APF solutions.

Although McCann⁽¹⁴⁰⁾ suggested that some of the newly acquired fluoride could be released and react with hydroxyapatite to form fluorapatite, Grøn⁽⁸⁶⁾, in a recent paper, questioned whether fluorapatite was formed at all in this type of treatment. Instead, he suggested that the initial enamel treatment with polyvalent cations results in a hetero-ionic exchange with surface calcium in apatite

^a Referred to as APF throughout this thesis.

^b S.D. = Standard deviation.

crystals and that the fluoride then, initially at least, binds to the newly acquired cations.

Effect on Enamel Dissolution Rate

The work of Volker⁽²²⁸⁾ in 1939, showing that the treatment of enamel powder with sodium fluoride reduced its "solubility"^a in pH 4.0 acetate buffer, stimulated considerable interest in enamel dissolution rate studies as a means of screening compounds for cariostatic potential.

The basic method of Volker was subsequently used by Manly and Bibby⁽¹⁴³⁾ to screen 147 compounds for their ability to reduce the rate of enamel dissolution in an acid buffer. As far as can be ascertained from the literature, their report in 1949 contained the earliest findings on the effect of iron salts on enamel dissolution.

Manly and Bibby⁽¹⁴³⁾ treated enamel powder with the test compounds for either three or 20 minutes and then exposed the powder to pH 4.0 acetate buffer for 15 minutes. The weight loss of the enamel powder was determined and used to assess the effectiveness of the treatment. Although a complete ranking of the compounds in order of efficacy was not made, many of the compounds tested, including all the iron (III) salts, did reduce the dissolution rate of enamel. In contrast, the only iron (II) salt tested (ammonium iron (II) sulphate) was ineffective.

^aThe term 'solubility' has often been used incorrectly⁽²⁴⁾. Many 'enamel solubility studies' have been, in fact, kinetic studies in which enamel dissolution rates, not enamel solubility, have been measured. Solubility determinations can be made only when equilibrium conditions have been attained⁽²⁴⁾.

One of the earliest indications of a possible synergism between iron and fluoride in reducing the dissolution rate of enamel was obtained by Torell and co-workers⁽²²³⁾ in 1958. They found that enamel treated with solutions containing iron (III) chloride and sodium fluoride showed less enamel loss after 18 to 24 hour incubation in a saliva - sucrose mixture than did enamel surfaces treated with sodium fluoride alone. The degree of enamel loss was assessed from an examination of ground sections taken from the treated and control areas.

Recently Edgar and Jenkins⁽⁵⁹⁾ investigated the effect of three cations naturally occurring in 'raw' sugar, on the ability of this product to reduce the dissolution rate of enamel *in vitro*. The three cations, Fe^{3+} , Cu^{2+} and Ca^{2+} when added singly or in combination to sucrose in the concentration found in a 30 per cent solution of 'raw' sugar, reduced the dissolution rate of either enamel powder or calcium orthophosphate in pH 5.0 acetate buffer. The addition of all three cations reduced the enamel dissolution rate to a level approaching that obtained with 'raw' sugar alone. The reductions in dissolution rates were calculated from the phosphate levels and, occasionally, the calcium levels in the buffer solutions at the end of a 30 minute exposure period.

In addition to the studies just reviewed in which the authors set out deliberately to investigate the effect of iron salts on the rate of enamel dissolution, a number of other studies have provided indirect evidence that iron may affect enamel demineralisation.

In the period from 1954 to 1956, Torell published a number of papers relating to the effect of iron salts on "enamel destruction"⁽²¹⁰⁾⁽²¹¹⁾⁽²¹²⁾⁽²¹³⁾⁽²¹⁴⁾⁽²¹⁶⁾⁽²²⁰⁾. In general, the

treatments to which the enamel specimens were subjected tended to be quite harsh including, in many instances, prolonged exposures to low pH environments. The effects of the treatments were assessed solely on gross changes in the enamel surfaces.

It is noteworthy however, that in one of these studies Torell⁽²¹⁰⁾ found that the effect of iron (II) salts on enamel surfaces differed markedly from that of iron (III) salts. Enamel surfaces immersed in non-fluoride iron (II) salt solutions for 7 days showed no enamel loss at the end of the treatment, whereas those immersed in non-fluoride iron (III) salt solutions of the same concentration did show marked enamel loss. Subsequently Torell⁽²¹⁷⁾ presented a hypothesis relating to the reaction between iron salts and enamel. In his opinion, the topical application of such salts as iron (III) chloride and iron (III) nitrate resulted in the formation of a "ferric hydroxide - ferric phosphate gel" on the enamel surface and that, as long as the gel remained undersaturated with respect to phosphate ions, the underlying enamel dissolved. In contrast, the topical application of the equivalent iron (II) salts resulted in the formation of "insoluble defined ferric phosphates" which rendered the enamel surface resistant to acid attack. A number of the experiments carried out by Torell⁽²¹¹⁾⁽²¹³⁾⁽²¹⁸⁾⁽²¹⁹⁾⁽²²⁰⁾ were designed to confirm this hypothesis.

Two groups of investigators⁽¹³⁾⁽¹⁸⁸⁾ studying rat incisor enamel have made observations about its resistance to acid demineralisation which are of interest in view of the high concentrations of iron (1 to 10 per cent) found in the surface layers of these teeth⁽⁹¹⁾. Selvig and Halse⁽¹⁸⁸⁾ noted that the iron-rich areas were particularly resistant to the demineralisation procedures used to prepare specimens for

transmission electron microscopy. In a later study, Berkovitz and Heap⁽¹³⁾ found that the areas rich in iron, calcium and phosphorus were more resistant to etching with 50 per cent phosphoric acid than other areas.

Until 100 years ago, there existed a Japanese custom, Ohaguro, which involved staining teeth by the alternate application of iron salts and tannic acid⁽⁴⁾. As a result of reports suggesting that people who practised this custom had a low caries experience, investigations were undertaken to examine the effects of Ohaguro on enamel⁽⁹⁵⁾⁽¹⁰⁷⁾⁽¹⁰⁸⁾⁽¹⁸⁷⁾⁽²⁰⁶⁾. In one of the investigations, Seino *et al.*⁽¹⁸⁷⁾ treated extracted teeth, using the technique of Ohaguro, and then briefly exposed the teeth to dilute hydrochloric acid. An examination of the control and treated surfaces by electron microscopy showed that the control surfaces were etched whereas the treated surfaces were not.

Recently, Besic *et al.*⁽¹¹⁾ compared the trace element content of enamel specimens which differed in their resistance to acid demineralisation. The crowns of extracted teeth were cut in half and one half of each crown was immersed for eight hours in a decalcifying solution (pH 3.5) prepared from tricalcium phosphate and hydrochloric acid. At the end of this time, the enamel surfaces were examined for the presence of white spot lesions. Those that were free of such lesions were classified as being "acid resistant" while those that had lesions were classified as being "acid susceptible". The enamel surfaces of the other halves of the "acid resistant" and "acid susceptible" crowns were then analysed for trace elements using an ion microprobe. The concentrations of iron, vanadium, zinc, aluminium and

copper were all markedly higher (iron 2.2 times) in the surface enamel of the "acid resistant" specimens than in the surface enamel of the "acid susceptible" specimens.

Effect on Bacteria

There has been a marked paucity of work on the effect of iron salts on the metabolic activity of plaque organisms *in situ* or *in vitro*. Furthermore, no reports were found on the effect of iron salts on the bacterial colonisation of tooth surfaces *in vitro*.

Stephan⁽²⁰¹⁾, in a brief report in 1949, indicated that he had tested the effect of various elements, including iron, on the growth of oral lactobacilli, streptococci, staphylococci and some unspecified organisms. The only finding given in relation to iron was that it, as well as zinc, had "both stimulatory and inhibitory effects". However, some of the other elements tested, copper, cobalt, nickel, molybdenum and fluoride, showed "only inhibitory effects".

In 1952 Forbes and Smith⁽⁶⁷⁾ reported their findings on the effect of iron and other metal salts on acid production in saliva. Each salt was added to a mixture of whole saliva and sucrose and the mixture incubated at 37°C. The pH of the mixture was determined at unspecified intervals thereafter. Iron, aluminium, chromium, cobalt, magnesium and manganese salts had little or no effect on acid production, whilst palladium, platinum and zinc salts had a moderate effect. A marked inhibitory effect was noted when copper, gold, mercury and silver salts were tested. No details of the iron salts used were published.

Extracellular polysaccharides produced by *S. mutans* in the presence of sucrose, are believed to play a significant role in plaque

formation⁽⁷⁶⁾⁽⁷⁷⁾⁽⁸⁷⁾⁽⁸⁸⁾. In this context the studies of Carlsson⁽³⁷⁾ and Scales *et al.*⁽¹⁸⁴⁾ on the effect of metals, including iron, on enzymes responsible for the production of these polymers are of particular interest.

In the first of these studies, Carlsson⁽³⁷⁾ isolated a levansucrase from the culture broth of *S. mutans* strain JC2 and tested the effect of several cations, at concentrations of 1 mM and 10 mM, on the activity of the enzyme in the presence of a phosphate buffer. Under these conditions, Fe^{2+} and Fe^{3+} cations as well as Ba^{2+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Cr^{3+} , Li^{+} , Mg^{2+} , Sr^{2+} and Zn^{2+} cations when added singly to the buffer, had essentially no effect on enzyme activity at either concentration. However, Ag^{+} , Cu^{2+} , Hg^{2+} , Ni^{2+} and Pb^{2+} cations inhibited enzyme activity at both concentrations. Carlsson also tested sodium fluoride at a concentration of 50 mM and found that it had no inhibitory effect.

Subsequently, Scales *et al.*⁽¹⁸⁴⁾ isolated a glycosyltransferase complex containing a dextransucrase and a levansucrase from the culture broth of *S. mutans* strain FA1 and tested the effect of several cations on the activity of the complex in the presence of a cacodylate buffer. This buffer was deliberately chosen to avoid the complication, associated with a phosphate buffer, of metals forming precipitates in the presence of phosphate. Under these conditions, Fe^{2+} cations as well as Cd^{2+} , Co^{2+} , Mg^{2+} , Ni^{2+} , Pb^{2+} and Zn^{2+} cations partly inhibited the activity of the glycosyltransferase complex, when added singly to the buffer, at a concentration of 1.6 mM. However, Ag^{+} , Cu^{2+} and Hg^{2+} cations completely inhibited activity at this concentration. Fluoride

was tested at concentrations of 1.6 mM, 4.0 mM, 8.0 mM and 16 mM but had no demonstrable effect on the activity of the complex.

ANIMAL STUDIES

Effect of Iron Salts on Experimental Caries

The findings of McClure⁽¹⁴¹⁾ in 1948, obtained as a result of adding iron salts in two different forms to the drinking water of rats fed a cariogenic diet, suggest that the cariostatic potential of iron may depend on the chemical form, and the concentration, in which it is administered. Rats given drinking water supplemented with 250 ppm iron as iron (III) chloride had a 71 per cent decrease in experimental caries, whereas those given drinking water supplemented with 500 ppm iron as iron (III) citrate had a 21 per cent increase in caries. However, when both the drinking water and the diet were supplemented with 250 ppm iron as iron (III) citrate, a 19 per cent decrease in caries occurred.

In a well conducted study, Emilson and Krasse⁽⁶⁰⁾ assessed the cariostatic properties of iron (II) sulphate and iron (III) chloride when applied topically, or added to the drinking water or the diet of albino hamsters. The animals were infected with a streptomycin-resistant strain of *S. mutans* Ingbritt 1600 and were given drinking water supplemented with streptomycin and fed a cariogenic diet throughout the test period. At the end of 90 days the animals were sacrificed and the teeth scored for caries. All the treatments caused a reduction in caries.

Effect on Plaque

Emilson and Krasse⁽⁶⁰⁾ found that the formation of plaque deposits in hamsters infected with *S. mutans* strain Ingbritt 1600 was reduced when iron (II) sulphate or iron (III) chloride was applied topically, or added to the drinking water or to the diet. The authors noted that both iron salts cause a "brownish - black to reddish - brown stain which could be easily scraped off the tooth surface".

A recent brief report by Beighton⁽¹⁰⁾ suggests that iron may have a specific inhibitory effect on *S. mutans*. He found that the addition of iron to the drinking water of rats significantly reduced the proportion of *S. mutans* in established fissure plaque but did not affect the remaining plaque flora to any marked degree.

CLINICAL TRIALS

At present, there appears to be a complete absence of clinical data on the cariostatic properties of non-fluoride iron salts. However, during the period from 1958 to 1964 there were several reports from Sweden on the clinical efficacy of solutions containing iron (III) chloride and sodium fluoride⁽¹²⁾⁽⁶⁶⁾⁽⁹⁹⁾⁽²²²⁾.

Several small, short-term, clinical trials were conducted and, overall, the findings were equivocal⁽⁶⁶⁾⁽⁹⁹⁾⁽²²²⁾. Berggren and Welanders⁽¹²⁾, however, conducted a large clinical trial involving 1325 schoolchildren, 10 years of age, to assess the cariostatic properties of solutions containing either (i) iron (III) chloride and sodium fluoride,

(ii) zirconium oxychloride and sodium fluoride or (iii) sodium fluoride alone. The test solutions were applied with a toothbrush either twice or five times annually. The control group did not use a placebo solution or carry out the toothbrushing procedures. At the end of two years, there was only a slight reduction in caries in all groups that had brushed twice annually. In contrast, the group that had brushed five times annually with the solution containing iron (III) chloride and sodium fluoride had a 33 per cent reduction in caries which was statistically significant ($P < 0.01$)^a. The corresponding group that had brushed with the sodium fluoride solution had a 29 per cent reduction and the remaining group that had brushed with the solution containing zirconium oxychloride and sodium fluoride had a 17 per cent reduction in caries. The reduction obtained with sodium fluoride was statistically significant ($P < 0.01$)^a.

Although the effect of the treatments was particularly evident on the approximal surfaces of the maxillary teeth, Berggren and Welanders noted that the solution containing iron (III) chloride and sodium fluoride appeared to have some effect on the occlusal surfaces of maxillary teeth when it was used five times annually.

In 1976, a brief report was published by Gerdin and Torell⁽⁷⁴⁾ on a clinical trial in which an alkaline fluoride solution containing iron, aluminium and manganese was tested. Although the findings indicate that the solution may have cariostatic properties there was insufficient data presented to allow a full assessment to be made.

^aCalculated from the data presented by Berggren and Welanders⁽¹²⁾ in Table 3A.

SECTION 1

THE EFFECT OF IRON (II) AND IRON (III) SALTS ON THE UPTAKE OF FLUORIDE BY ENAMEL FROM FLUORIDE SOLUTIONS (TEST 1)

In 1969, McCann⁽¹⁴⁰⁾ showed that the pretreatment of enamel with Fe^{3+} cations increased fluoride uptake by enamel from APF solutions.

The work described in this section is an extension of the work of McCann. It was designed to assess the effect of Fe^{2+} cations, as well as Fe^{3+} cations, on fluoride uptake by enamel from two fluoride solutions, APF and neutral sodium fluoride..

For comparison purposes, fluoride uptake by enamel from single applications of either iron (II) fluoride, iron (III) fluoride, tin (II) fluoride or a solution containing iron (III) chloride and sodium fluoride was also determined.

In recent years scanning electron microscopy has been employed to help understand the nature of reactions between fluoride compounds and enamel⁽⁹⁰⁾⁽¹²⁰⁾⁽²³⁰⁾⁽²³¹⁾ and was used in this investigation for this purpose.

MATERIALS AND METHODS

MATERIALS

Teeth

Sound lower permanent central incisor teeth, extracted from freshly slaughtered 2.0 to 2.5 year old beef cattle, were obtained

from Homebush Abattoirs, Sydney, N.S.W., Australia^a.

Chemicals

All chemicals were the highest grade available commercially: Acidulated phosphate-fluoride (Orapharm Products Ltd., Melbourne, Vic., Australia); acetic acid, nitric acid and sodium fluoride (BDH Chemicals Ltd., Poole, England); iron (II) chloride, potassium acetate and potassium dihydrogen phosphate (E. Merck and Co., Darmstadt, West Germany); calcium nitrate and caesium chloride (Ajax Chemicals Ltd., Sydney, N.S.W., Australia); iron (III) chloride (Riedel-De Haën AG, Seelze-Hannover, West Germany); iron (II) fluoride and iron (III) fluoride (PCR Inc., Gainesville, Fla., U.S.A.); tin (II) fluoride (Ozark-Mahoning, Tulsa, Okla., U.S.A.).

All solutions were made up with distilled, deionised water.

Apparatus

The apparatus used for the treatment of enamel consisted of a vertically mounted electric motor with chuck, a glass beaker encased by a glass water jacket and a clamp for holding a plastic vial

^aBovine teeth were used because they possess large, relatively flat, sound facial surfaces and could be obtained in adequate numbers. They have been used by other workers in fluoride uptake⁽¹⁴⁸⁾⁽¹⁶⁰⁾ and enamel dissolution⁽⁴⁶⁾⁽⁶⁵⁾⁽¹¹⁰⁾⁽¹⁹²⁾⁽²³⁴⁾⁽²³⁵⁾ experiments.

containing the treatment solution in a fixed position in the glass beaker (Fig. 1). The plastic vials for the treatment solutions (15 mm diameter, 50 mm high) had plastic lids with a 10 mm hole in the centre to allow the introduction of acrylic rods which supported the enamel specimens.

METHODS

Preparation of Enamel

A hollow, 5 mm internal diameter, diamond drill (Horico, Hopf. Ringleb GmbH, Berlin, West Germany) was used to cut a cylindrical core from the centre of the facial surface of each tooth. All cutting was done under a water spray.

Before use the cores were stored at 4°C in quarter-strength Ringer solution^a (pH 7.1), with some thymol crystals added to suppress bacterial growth. None of the cores were stored for more than nine days.

Each core was mounted on an 8 mm diameter, 70 mm long acrylic rod with self-curing acrylic resin (DeTrey RR, A.D. International, London, England). The resin was extended up the sides of each core

^aPrepared from the formula in *The Oxoid Manual* (1973), p.205, Oxoid Ltd., London, England.

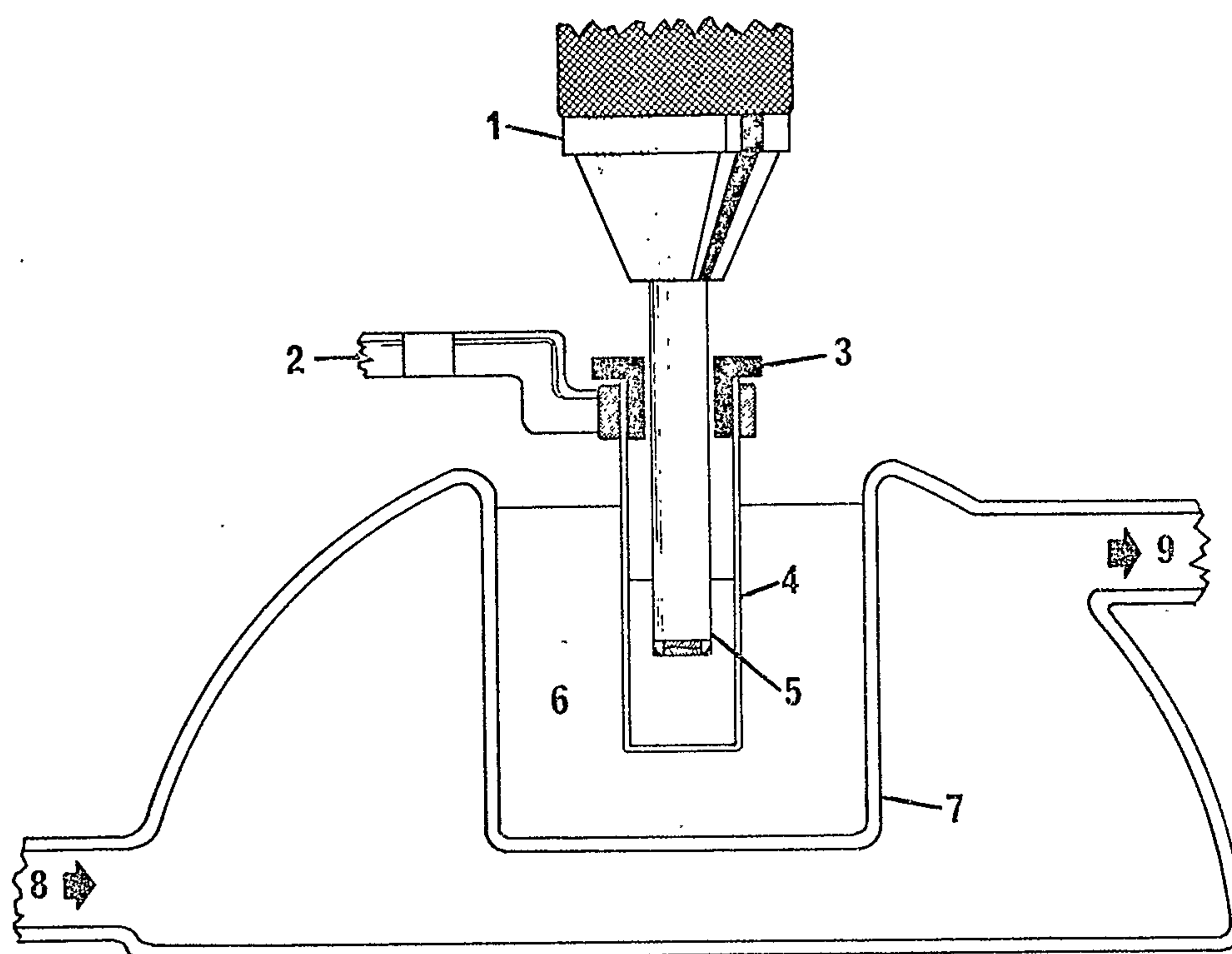
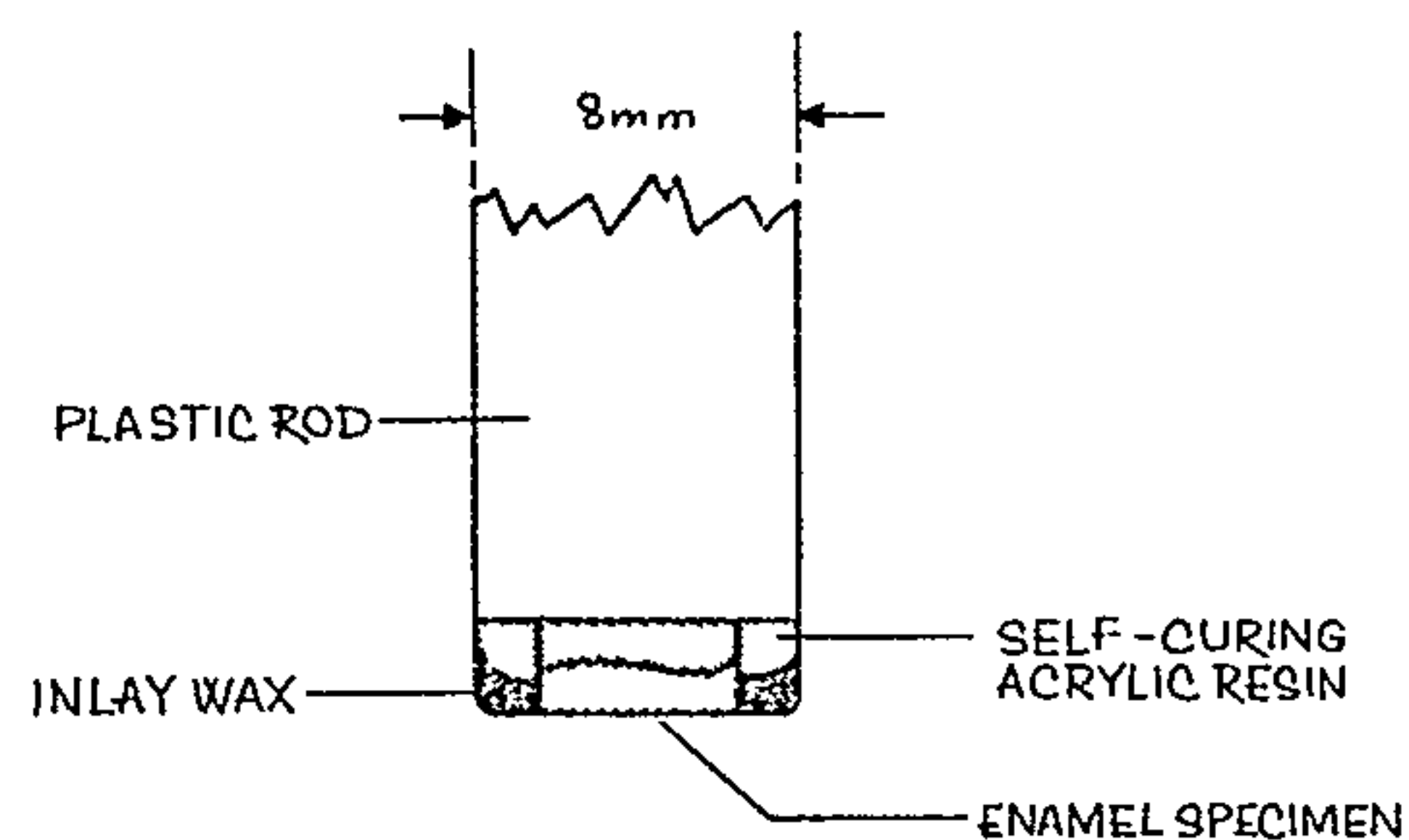


Fig. 1. Diagram of apparatus used for the treatment of enamel specimens: 1 = chuck attached to an electric motor (not shown); 2 = clamp for holding plastic vial; 3 = plastic lid with hole in centre; 4 = plastic vial containing treatment solution; 5 = acrylic rod supporting enamel specimen; 6 = water 37°C; 7 = glass beaker encased by glass water jacket; 8 = water inlet; 9 = water outlet.

Fig. 2. Diagram showing enamel specimen mounted on an acrylic rod.



as far as the dentino-enamel junction. Each rod was placed in the chuck of an electric drill mounted in a vertical drill press and the rotating specimen was then gently lowered onto a horizontal block covered with a graded series of wet and dry paper (Wetordry Tri-m-ite Paper, 3M Co., Sydney, N.S.W., Australia). Enamel was removed to a depth of approximately 0.5 mm under a continuous water spray. The finest grade paper (1200) was used to produce the final finish.

The edge of the enamel surface and any areas on the enamel wall not covered by resin were coated with blue inlay wax (Blue Inlay Casting Wax, Kerr Mfg. Co., Detroit, Mich., U.S.A.). The wax was smoothed and made level with the enamel surface (Fig. 2).

The specimens were stored in quarter-strength Ringer solution (pH 7.1) before use.

Treatment of Enamel

Details of the treatment solutions and treatment times used are given in Table 1. An outline of the treatment procedures is given in the flow diagram in Fig. 3.

Two millilitres of the appropriate solution at 37°C was pipetted into a plastic vial, the vial clamped in position in the treatment apparatus and the acrylic rod supporting an enamel specimen lowered until the enamel surface was 10 mm below the surface of the treatment solution. The specimen was then rotated at 2,000 rpm for the appropriate treatment period, the temperature of the solution being maintained at

TABLE 1

TREATMENT SOLUTIONS AND TREATMENT TIMES USED IN
FLUORIDE UPTAKE STUDY

A. Two Stage Treatments

Stage 1			Stage 2		
Solution	pH	Treatment Time (min)	Solution	pH	Treatment Time (min)
1 mM Fe Cl ₂ . 4 H ₂ O	5.1	1	APF (12,300 ppm F) used in all treatments	3.2	4
10 mM Fe Cl ₂ . 4 H ₂ O	4.3	1			
100mM Fe Cl ₂ . 4 H ₂ O	3.2	1			
1 mM Fe Cl ₃ . 6 H ₂ O	3.2	1			
10 mM Fe Cl ₃ . 6 H ₂ O	2.4	1			
100mM Fe Cl ₃ . 6 H ₂ O	1.6	1			
1 mM Fe Cl ₂ . 4 H ₂ O	5.1	1	476 mM NaF (9,050 ppm F) used in all treatments	7.0	4
10 mM Fe Cl ₂ . 4 H ₂ O	4.3	1			
100mM Fe Cl ₂ . 4 H ₂ O	3.2	1			
1 mM Fe Cl ₃ . 6 H ₂ O	3.2	1			
10 mM Fe Cl ₃ . 6 H ₂ O	2.4	1			
100mM Fe Cl ₃ . 6 H ₂ O	1.6	1			

TABLE 1 (cont.)

B. One Stage Treatments

Solution	pH	Treatment Time (min)
H ₂ O (Control)	5.3	5
15 mM Fe F ₂ (570 ppm F) ^a	4.4	5
10 mM Fe F ₃ (570 ppm F) ^b	3.5	5
50 mM Fe Cl ₃ · 6 H ₂ O + 190 mM NaF (3,620 ppm F) ^c	2.1	5
APF (12,300 ppm F) ^c	3.2	5
26 mM SnF ₂ (970 ppm F) ^c	3.4	5
511mM SnF ₂ (19,400 ppm F) ^c	2.8	5
48 mM NaF (905 ppm F) ^c	7.0	5
476mM NaF (9,050 ppm F) ^c	7.0	5

^a Prepared under nitrogen. Solution warmed to 60°C and continuously stirred to facilitate dissolution of salt. Solution cooled to 37°C before use.

^b Solution warmed to 60°C and continuously stirred to facilitate dissolution of salt. Solution cooled to 37°C before use.

^c Solutions have been tested clinically.

(A solution containing 50 mM FeCl₃ · 6 H₂O and 190 mM NaF has been clinically tested when self-applied with a toothbrush⁽¹²⁾.)

Solutions of APF, 511 mM SnF₂ and 476 mM NaF have each been clinically tested when professionally applied. See reviews by Horowitz and Heifetz⁽¹⁰⁴⁾ and Stookey⁽²⁰³⁾.

SnF₂ at a concentration of 26 mM has clinically tested as a component of dentifrices. For reviews see Duckworth⁽⁵⁸⁾ and Heifetz and Horowitz⁽⁹⁷⁾.

NaF at a concentration of 48 mM has been clinically tested as a mouthrinse. For review of trials see report of Council on Dental Therapeutics⁽⁴⁵⁾.)

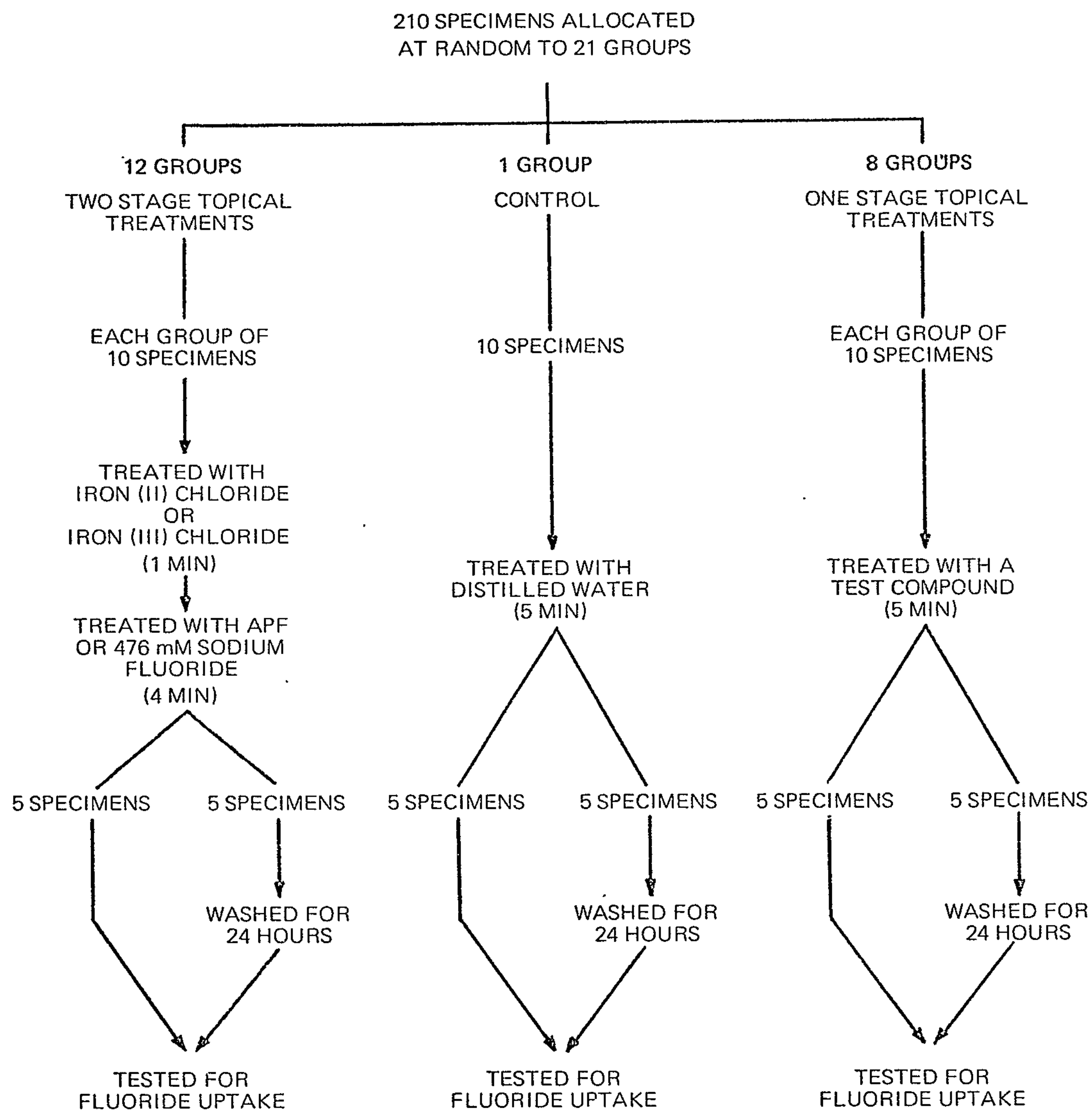


Fig. 3. Flow diagram outlining experimental procedures followed in fluoride uptake study.

37°C throughout.

No attempt was made to exclude oxygen during the treatments with iron (II) or tin (II) solutions.

At the end of each treatment period, the acrylic rod was removed from the apparatus and the enamel surface washed for 15 seconds under a fine stream of distilled, deionised water. One half the specimens in each group were tested immediately for fluoride uptake. The remaining half were then washed for 24 hours in a solution consisting of 1 mM calcium nitrate and 5 mM potassium dihydrogen phosphate buffered to pH 5.5 with 7 mM acetic acid and 50 mM potassium acetate. The composition of this solution was the same as that of the solution used by McCann⁽¹⁴⁰⁾ and subsequently by Caslavská *et al.*⁽³⁸⁾, for washing treated enamel specimens. The specimens were immersed, in groups of 10, in 500 ml of the solution which was constantly stirred.

The solution was changed after 30, 60 and 90 minutes and then left for the remainder of the 24 hour washing period.

Fluoride Determination

To determine the fluoride concentration in surface enamel an enamel biopsy was taken from each specimen.

Each acrylic rod was held in a vertical position, enamel surface upward, with soft dental wax (Investo Bite Wax, Investo Mfg. Co., Sydney, N.S.W., Australia). Twenty microlitres of 0.1 N nitric acid was pipetted onto the enamel surface and left for 30 seconds at which time the rod was tilted through 90 degrees and the acid washed into a plastic vial with 2 ml of TISAB (Orion Research Inc., Cambridge, Mass., U.S.A.) from a pipette. A plastic tip attached to a 20 µl repeating

pipette (Eppendorf, Gerätebau Netheler GmbH, Hamburg, West Germany) was used for aspirating any liquid remaining on the enamel surface. The aspirated liquid was added to the contents of the vial. All solutions were used at room temperature (23°C - 25°C).

The fluoride concentration in each vial was determined by the method of Frant and Ross⁽⁶⁹⁾ using a combination fluoride - specific ion electrode (Orion Model 96-09, Orion Research Inc., Cambridge, Mass., U.S.A.) connected to a mV/pH meter (Pye Unicam Model 290 Mk. 2, Pye Unicam Ltd., Cambridge, England).

To determine the precision of the method, six replicate estimations were made on the pooled contents of two vials. Each vial contained an enamel biopsy taken from a control specimen. The mean fluoride concentration was 0.049 ppm, S.D. = 0.011 (n = 5).

The accuracy of the method was checked by adding known amounts of fluoride to nine samples selected at random. The mean percentage recovery was 96.7 ± 2.1 .

Calculations

Fluoride Concentration

The following formula⁽⁶⁾ was used to calculate the fluoride content of each enamel biopsy:

$$\text{F content (ppm)} = \frac{\text{Wt. fluoride in biopsy}}{\text{Wt. enamel in biopsy}} \times 10^6$$

The weight of enamel in each biopsy specimen was based on the amount of calcium present assuming that the calcium content of enamel is 37 per cent⁽²⁸⁾.

The calcium content of each specimen was determined by atomic absorption spectroscopy (Model AA-5, Varian Techtron Pty. Ltd., Springvale, Vic., Australia) using a 50 μ l aliquot diluted 1 in 41 in a background solution containing 8 mM caesium chloride and 158 mM nitric acid. The details of the method used were identical to those described by Schamschula *et al.* (185).

To determine the precision of the method, five replicate estimations were made on the contents of one vial. The vial contained an enamel biopsy taken from a control specimen. The mean calcium concentration was 76.21 ppm, S.D. = 2.02 (n = 5).

The accuracy of the method was checked by adding known amounts of calcium to 9 samples selected at random. The mean percentage recovery was 97.3 per cent, S.D. = 2.4 (n = 9).

Depth of Etched Layer

The depth of the etched layer was calculated from the following formula:

$$\text{Depth } (\mu\text{m}) = \frac{\text{Wt. enamel in biopsy}}{\text{Area of enamel surface } (\text{mm}^2) \times 2.95}$$

It was assumed that the depth of etch was uniform over the entire enamel surface and that the density of enamel is 2.95⁽¹⁾.

The area of the enamel surface was determined using the method described below.

Enamel Surface Area

Although a standard instrument was used to cut each core, the cutting procedure did cause some minor chipping of the enamel margins. A method was developed which enabled the area of the exposed enamel surface to be determined.

Each acrylic rod was held in a vertical position with the enamel surface upwards and photographed under standardised lighting conditions. The final print was enlarged 3 X.

The area of the print covered by each enamel surface was cut out and weighed and a section of the same print of known areas was also cut out and weighed.

The area of the original enamel surface was calculated using the formula:

Original surface area =

$$\frac{\text{Wt. of enamel surface area cut from print}}{\text{Wt. of known surface area cut from print}} \times \frac{\text{Known surface area (mm}^2\text{)}}{9}$$

(N.B. A magnification of 3X gives a nine fold increase in surface area).

The assumption was made that the paper used for each individual print was of uniform density.

Scanning Electron Microscopy

An additional 54 specimens were prepared and assigned at random to 27 groups containing 2 specimens each. One treatment solution was used per group.

After being washed for 15 seconds in distilled, deionised water, the specimens were placed in a dessicator and allowed to air dry. Subsequently they were mounted on aluminium stubs with colloidal silver (M154, Polaron Equipment Ltd., Watford, England) and coated with carbon and then gold-palladium or gold (Mathey Garrett, Sydney, N.S.W.,

Australia) in a vacuum evaporator on a rotating-tilting stage (CE12/14 Coating Unit, Dynavac High Vacuum Pty. Ltd., Melbourne, Vic., Australia). The specimens were examined in a scanning electron microscope (Stereoscan 600, Cambridge Scientific Instruments, Cambridge, England) at an accelerating voltage of 25KV.

RESULTS

FLUORIDE UPTAKE

Data on fluoride uptake from APF by enamel pretreated with iron (II) chloride or iron (III) chloride solutions are presented in Table 2-A.

While the pretreatments with iron (II) chloride solutions appeared to have little effect on fluoride uptake by enamel from APF the pretreatments with 10 mM iron (III) chloride and 100 mM iron (III) chloride had a marked effect. Enamel pretreated with 10 mM iron (III) chloride had a mean fluoride concentration of 16,722 ppm (S.D. = 2,306) to a mean depth of 2.61 μ m (S.D. = 0.19) while enamel pretreated with 100 mM iron (III) chloride had a mean fluoride concentration of 23,032 ppm (S.D. = 5,886) to a mean depth of 2.40 μ m (S.D. = 0.70). In contrast, enamel treated with APF alone had a mean fluoride concentration of 4,500 ppm (S.D. = 926) to a mean depth of 3.78 μ m (S.D. = 0.13).

The 24 hour washing procedure appreciably reduced the levels of fluoride acquired by enamel from APF.

TABLE 2

FLUORIDE UPTAKE BY ENAMEL FROM
TOPICAL TREATMENTS

A. Fluoride Uptake from APF by Enamel Pretreated with Solutions Containing
Iron (II) Chloride or Iron (III) Chloride.

Pretreatment Solution	Fluoride Concentration in Enamel (ppm) ^a		Biopsy Depth (μm) ^a	
	Immediately Following Fluoride Treatment	After 24 Hours Washing	Immediately Following Fluoride Treatment	After 24 Hours Washing
None (APF alone)	4,500 (S.D. = 926)	1,204 (S.D. = 41)	3.78 (S.D. = 0.13)	3.88 (S.D. = 0.14)
1 mM FeCl ₂ · 4 H ₂ O	4,113 (S.D. = 337)	1,502 (S.D. = 228)	3.26 (S.D. = 0.54)	3.98 (S.D. = 0.21)
10 mM FeCl ₂ · 4 H ₂ O	4,076 (S.D. = 878)	1,993 (S.D. = 340)	3.03 (S.D. = 0.27)	4.56 (S.D. = 0.60)
100mM FeCl ₂ · 4 H ₂ O	6,167 (S.D. = 1,173)	1,974 (S.D. = 303)	3.04 (S.D. = 0.43)	4.41 (S.D. = 0.52)
1 mM FeCl ₃ · 6 H ₂ O	6,955 (S.D. = 3,433)	4,755 (S.D. = 1,968)	2.92 (S.D. = 0.33)	3.65 (S.D. = 0.39)
10 mM FeCl ₃ · 6 H ₂ O	16,722 (S.D. = 2,306)	6,114 (S.D. = 2,608)	2.61 (S.D. = 0.19)	4.06 (S.D. = 0.17)
100mM FeCl ₃ · 6 H ₂ O	23,032 (S.D. = 5,886)	14,633 (S.D. = 4,243)	2.40 (S.D. = 0.70)	3.56 (S.D. = 0.34)

^a Each value represents mean of five specimens (S.D. = Standard deviation).

B. Fluoride Uptake from 476 mM NaF by Enamel Pretreated with Solutions
Containing Iron (II) Chloride or Iron (III) Chloride.

Pretreatment Solution	Fluoride Concentration in Enamel (ppm) ^a		Biopsy Depth (μm) ^a	
	Immediately Following Fluoride Treatment	After 24 Hours Washing	Immediately Following Fluoride Treatment	After 24 Hours Washing
None (476mM NaF alone)	4,335 (S.D. = 1,076)	1,441 (S.D. = 190)	3.10 (S.D. = 0.56)	3.80 (S.D. = 0.56)
1 mM FeCl ₂ · 4 H ₂ O	1,622 (S.D. = 565)	1,412 (S.D. = 287)	3.58 (S.D. = 0.14)	5.48 (S.D. = 0.75)
10 mM FeCl ₂ · 4 H ₂ O	1,642 (S.D. = 555)	1,056 (S.D. = 224)	3.99 (S.D. = 0.30)	4.07 (S.D. = 0.13)
100mM FeCl ₂ · 4 H ₂ O	1,789 (S.D. = 626)	1,103 (S.D. = 116)	3.70 (S.D. = 0.29)	3.99 (S.D. = 0.37)
1 mM FeCl ₃ · 6 H ₂ O	1,572 (S.D. = 261)	1,399 (S.D. = 164)	3.64 (S.D. = 0.17)	3.57 (S.D. = 0.22)
10 mM FeCl ₃ · 6 H ₂ O	1,840 (S.D. = 419)	1,292 (S.D. = 109)	3.95 (S.D. = 0.41)	4.08 (S.D. = 0.23)
100mM FeCl ₃ · 6 H ₂ O	2,371 (S.D. = 510)	1,674 (S.D. = 191)	3.70 (S.D. = 0.53)	4.39 (S.D. = 0.41)

^a Each value represents mean of five specimens (S.D. = Standard deviation).

TABLE 2 (cont.)

C. Fluoride Uptake by Enamel from a Single Application of Test Compounds

Test Compound	Fluoride Concentration in Enamel (ppm) ^a		Biopsy Depth (μ m) ^a	
	Immediately Following Fluoride Treatment	After 24 Hours Washing	Immediately Following Fluoride Treatment	After 24 Hours Washing
H ₂ O (Control)	264 (S.D. = 104)	238 (S.D. = 70)	3.74 (S.D. = 0.33)	3.80 (S.D. = 0.29)
15 mM FeF ₂	1,085 (S.D. = 209)	726 (S.D. = 87)	3.92 (S.D. = 0.46)	4.02 (S.D. = 0.69)
10 mM FeF ₃	1,004 (S.D. = 309)	680 (S.D. = 63)	4.00 (S.D. = 0.30)	3.72 (S.D. = 0.26)
50 mM FeCl ₃ · 6 H ₂ O + 190mM NaF	5,206 (S.D. = 197)	1,893 (S.D. = 339)	3.56 (S.D. = 0.48)	3.87 (S.D. = 0.24)
26 mM SnF ₂	946 (S.D. = 233)	540 (S.D. = 87)	3.52 (S.D. = 0.24)	3.42 (S.D. = 0.46)
511mM SnF ₂	2,092 (S.D. = 348)	879 (S.D. = 123)	2.76 (S.D. = 0.35)	3.82 (S.D. = 0.24)
48 mM NaF	1,518 (S.D. = 330)	876 (S.D. = 277)	3.33 (S.D. = 0.25)	3.36 (S.D. = 0.15)

^a Each value represents mean of five specimens (S.D. = Standard deviation).

The data on fluoride uptake from 476 mM sodium fluoride by enamel pretreated with iron (II) chloride or iron (III) chloride solutions, shown in Table 2-B, indicates that the pretreatments tended to retard fluoride deposition in enamel. However, although the 24 hour washing procedure appreciably lowered the level of fluoride in enamel treated with 476 mM sodium fluoride alone, it did not have a marked effect on enamel pretreated with either iron (II) chloride or iron (III) chloride solutions.

Only one of the topical fluoride solutions shown in Table 2-C caused a fluoride deposition in enamel comparable to that obtained with APF or 476 mM sodium fluoride alone. Enamel treated with this solution, which contained 50 mM iron (III) chloride and 190 mM sodium fluoride, had a mean fluoride concentration of 5,206 ppm (S.D. = 197) to a mean depth of 3.56 μ m (S.D. = 0.48).

SCANNING ELECTRON MICROGRAPHS

The scanning electron micrographs of enamel treated with iron (II) chloride followed by APF (Figs. 5-A to 5-C) all show deposits on the enamel surface, similar to, but less dense than, those on the surface of enamel treated with APF alone (Fig. 4-B). Similar deposits are also present on the surface of enamel treated with 1 mM iron (III) chloride followed by APF (Fig. 6-A) however they appear to be virtually absent from the surface of enamel treated initially with 10 mM iron (III) chloride or 100 mM iron (III) chloride (Figs. 6-B and 6-C). The surface of enamel treated initially with 100 mM iron (III) chloride (Fig. 6-C) shows evidence of etching.

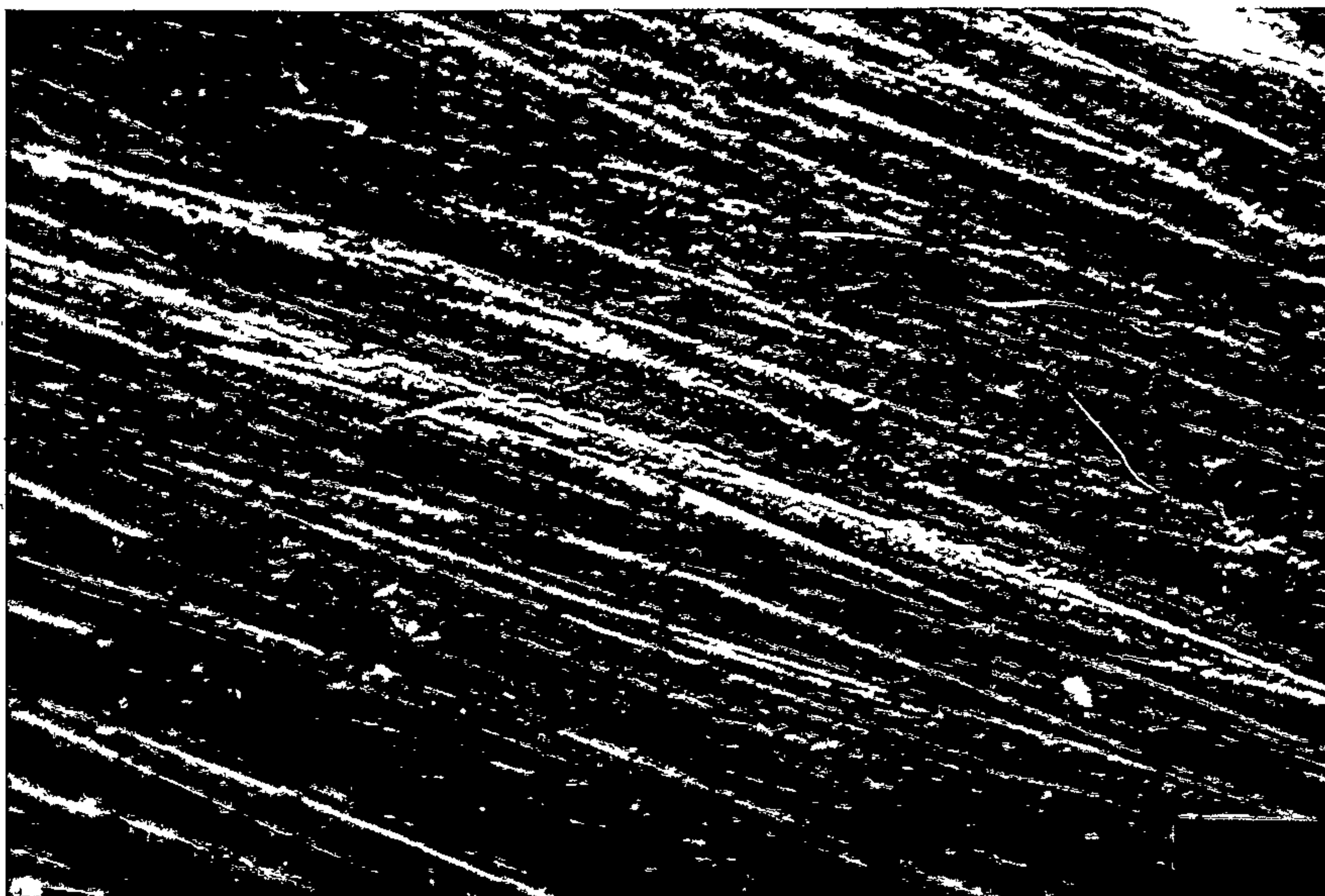
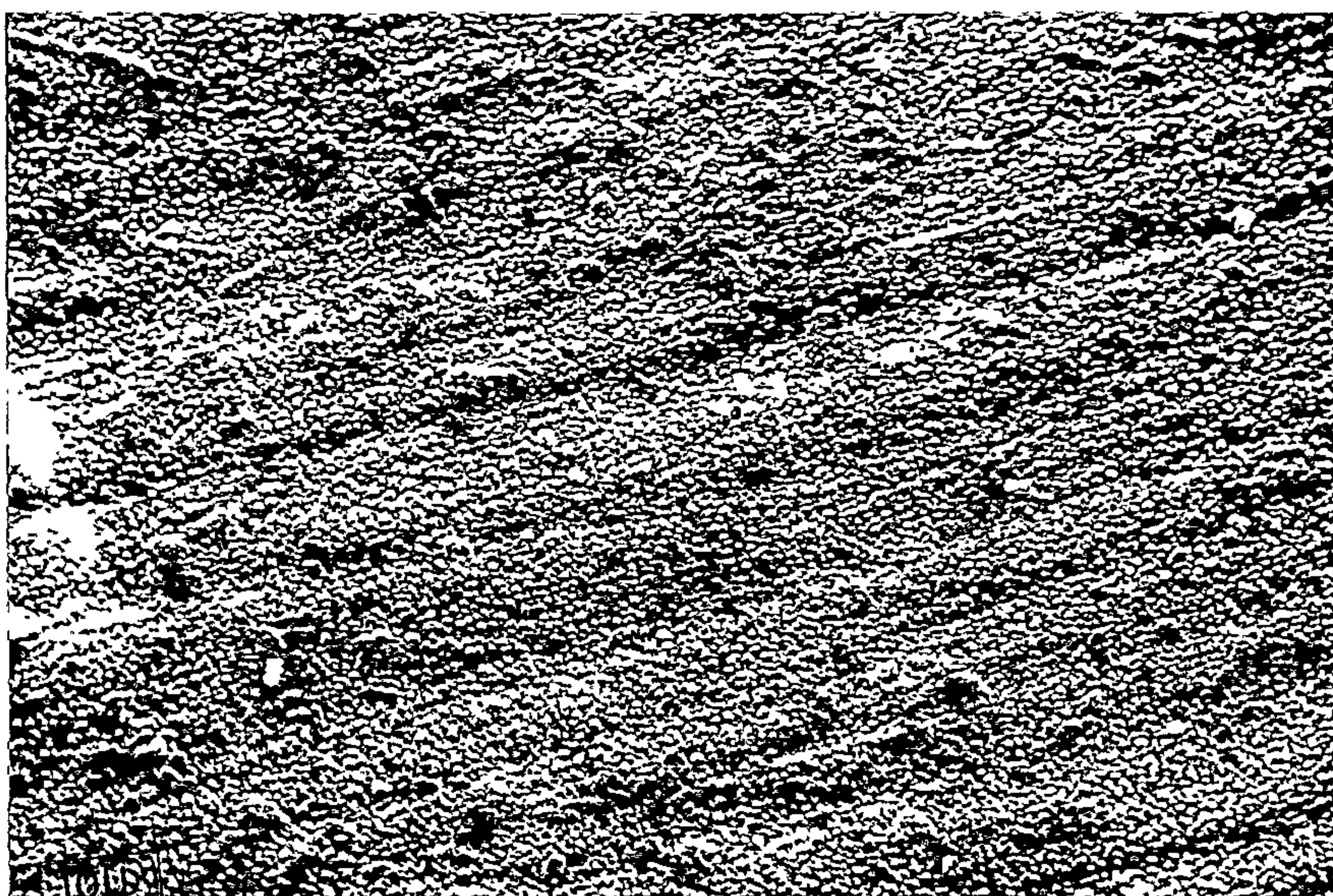


Fig. 4. Scanning electron micrographs of untreated and treated enamel.

A (*above*). Enamel immersed in distilled, deionised water for five minutes (control). The scratch marks left by the polishing procedure are evident. X 1,000.

B (*top right*). Enamel treated with APF for five minutes. The surface is covered with deposits of various shapes. X 2,000.

C (*right*). Enamel treated with 476 mM sodium fluoride for five minutes. The surface is covered with small globular deposits. X 2,000.



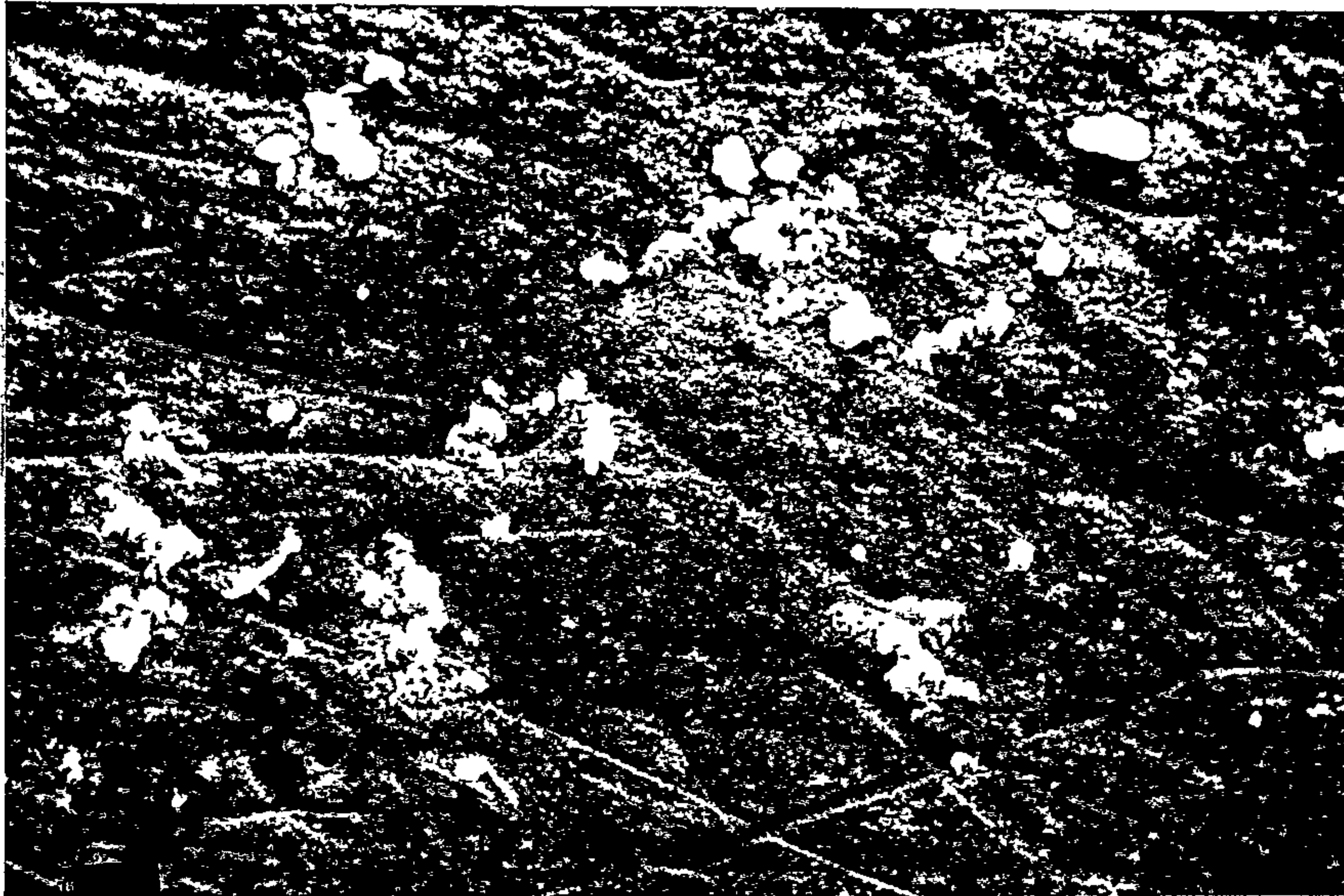
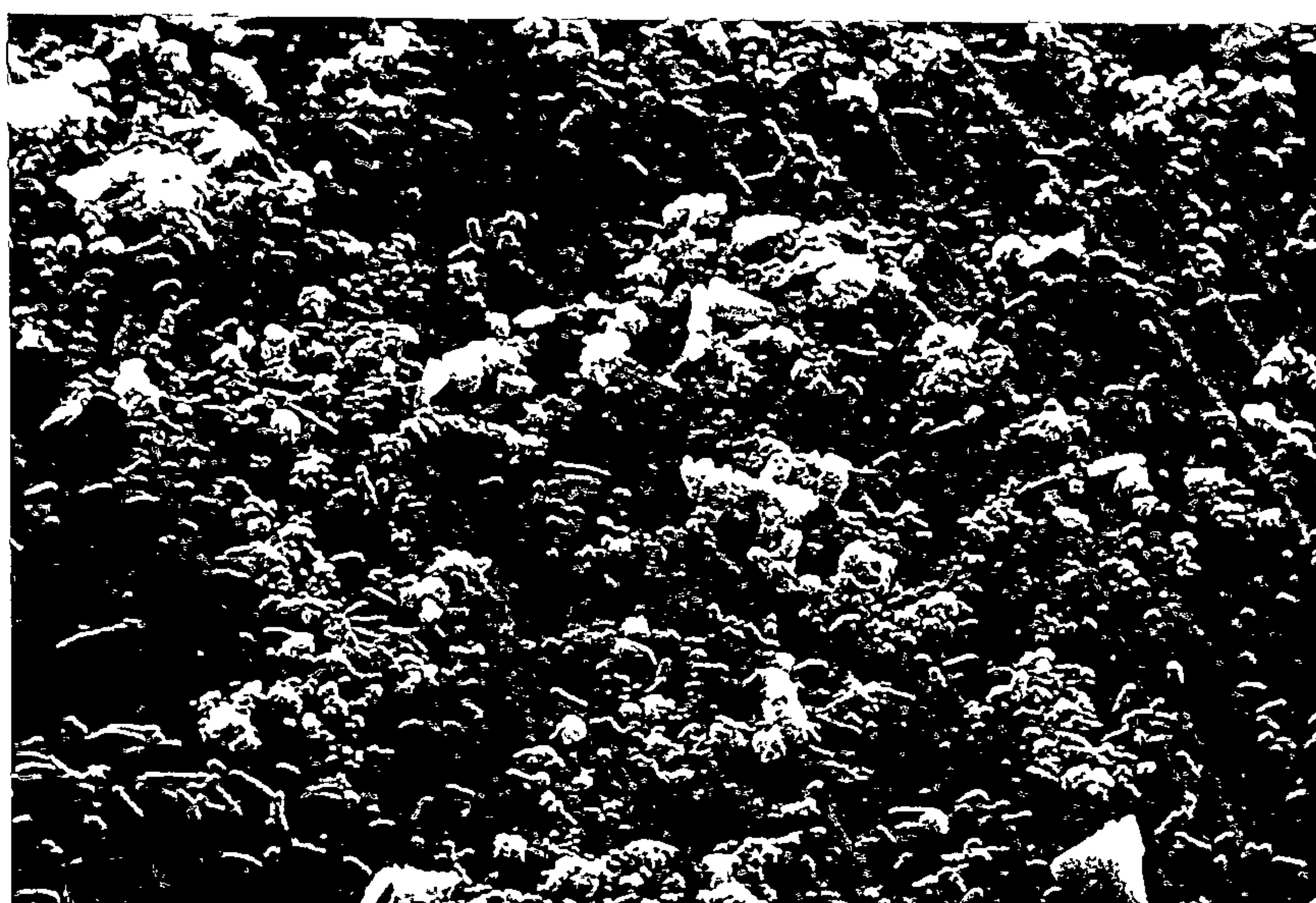
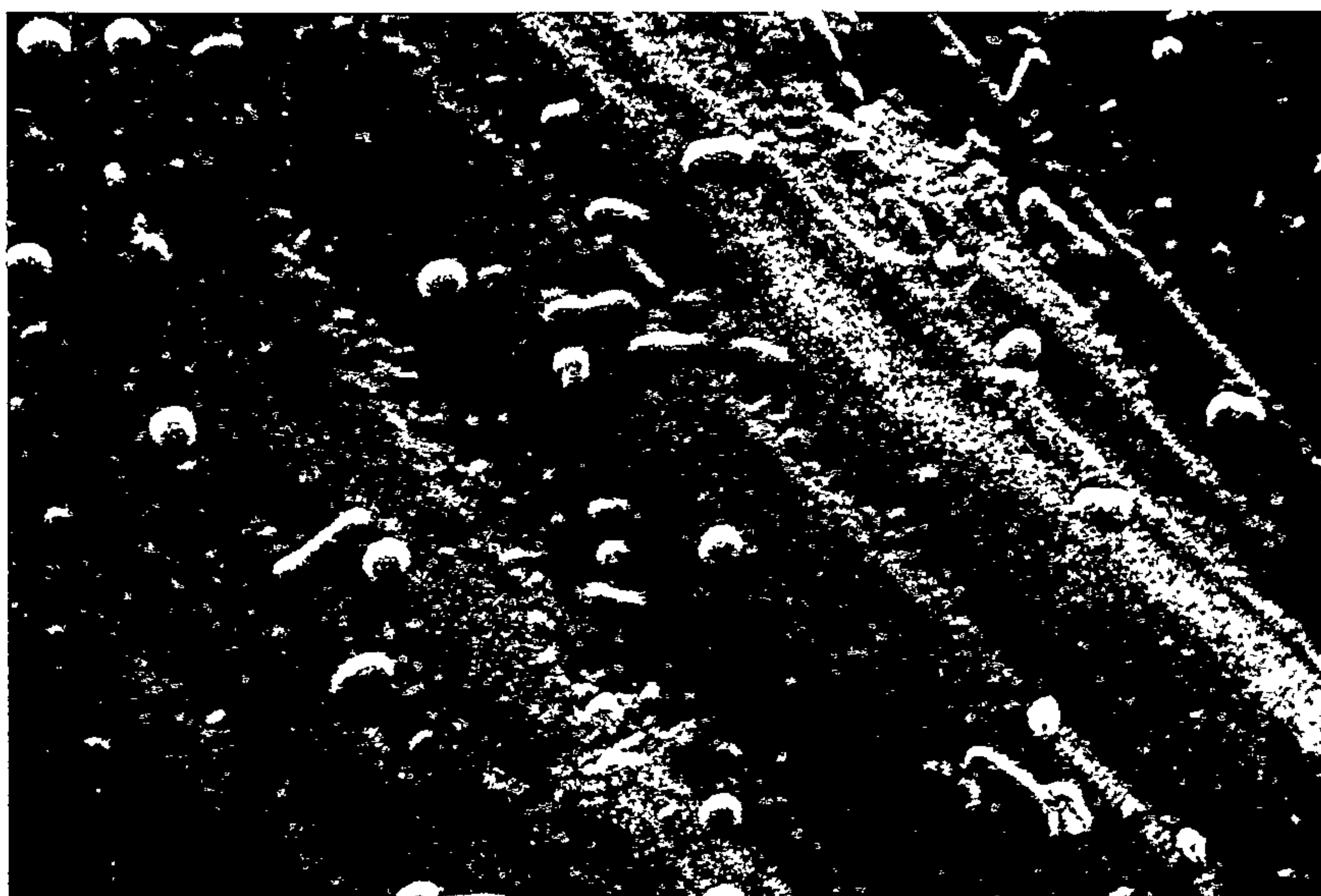


Fig. 5. Scanning electron micrographs of enamel treated with iron (II) chloride for one minute prior to a four minute topical treatment with APF (two stage treatments).

- A (*above*). First stage treatment with 1 mM iron (II) chloride. Some isolated surface deposits are evident. X 2,000.
- B (*top right*). First stage treatment with 10 mM iron (II) chloride. A few scattered rounded and rod-like deposits can be seen. X 5,000.
- C (*right*). First stage treatment with 100 mM iron (II) chloride. The surface is covered with deposits of various shapes. X 2,000.



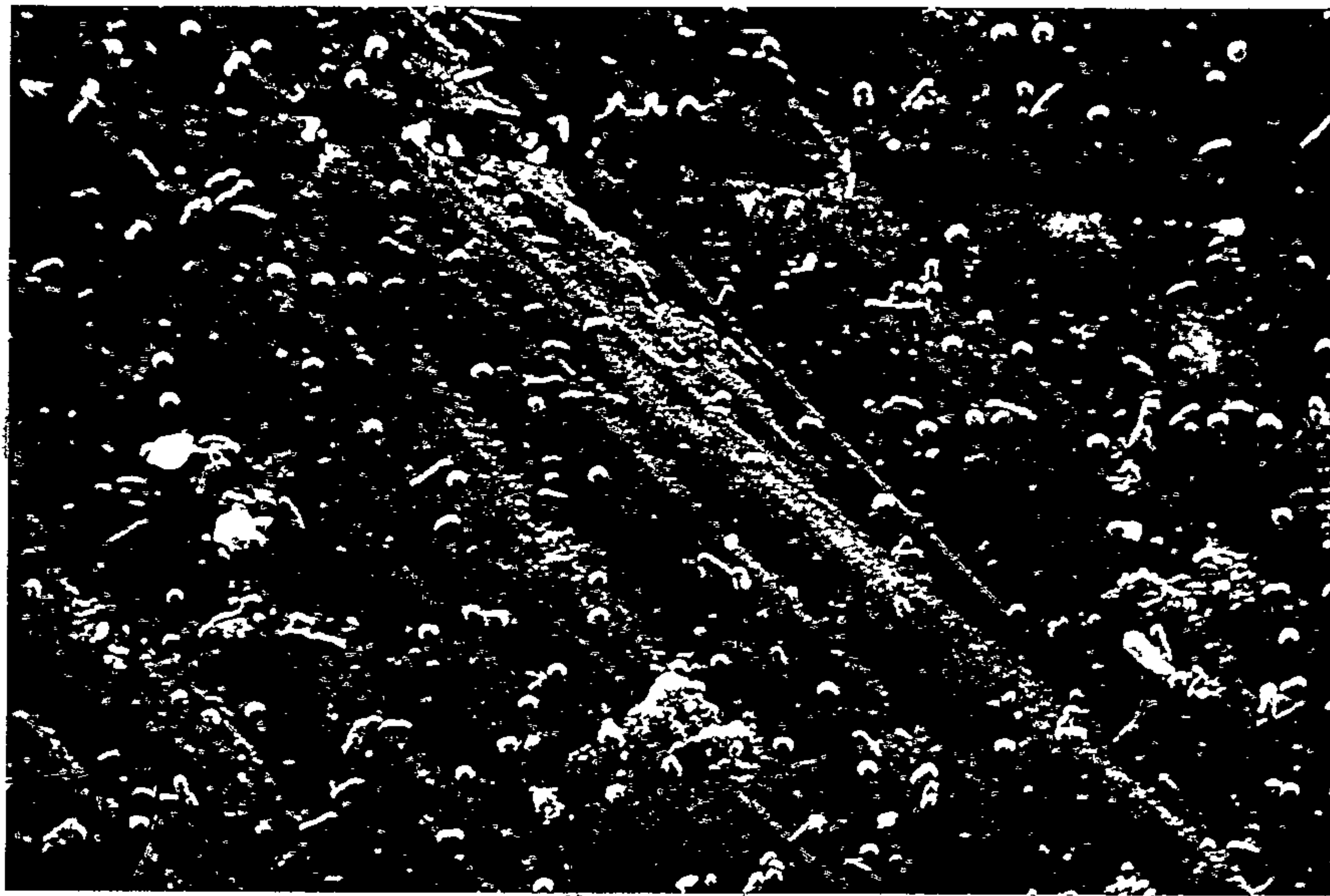


Fig. 6. Scanning electron micrographs of enamel treated with iron (III) chloride for one minute prior to a four minute topical treatment with APF (two stage treatments).

- A (above).** First stage treatment with 1 mM iron (III) chloride. Scattered surface deposits of various shapes are evident. X 2,000.
- B (top right).** First stage treatment with 10 mM iron (III) chloride. Some areas appear to be slightly etched. X 2,000.
- C (right).** First stage treatment with 100 mM iron (III) chloride. Surface etching is apparent. There is no evidence of the scratch marks left by the polishing procedure as seen in Figs. 6-A and 6-B. X 2,000.

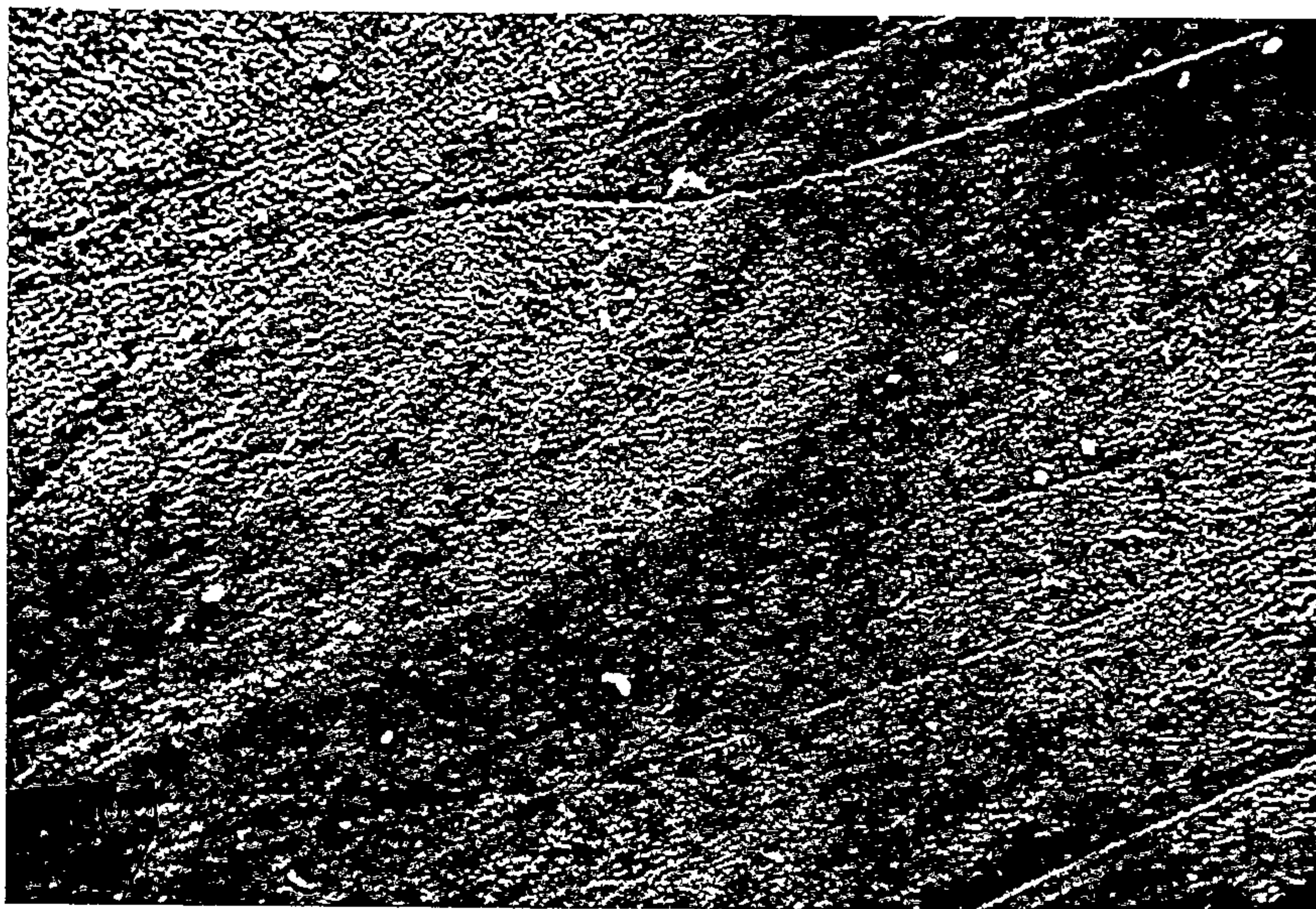




Fig. 7. Scanning electron micrographs of enamel treated with iron (II) chloride for one minute prior to a four minute topical treatment with 476 mM sodium fluoride (two stage treatments).

- A (*above*). First stage treatment with 1 mM iron (II) chloride. The surface is virtually free of deposits. X 2,000.
- B (*top right*). First stage treatment with 10 mM iron (II) chloride. Only isolated surface deposits are evident. X 2,000.
- C (*right*). First stage treatment with 100 mM iron (II) chloride. As in Figs. 7-A and 7-B, few surface deposits can be seen. X 2,000.



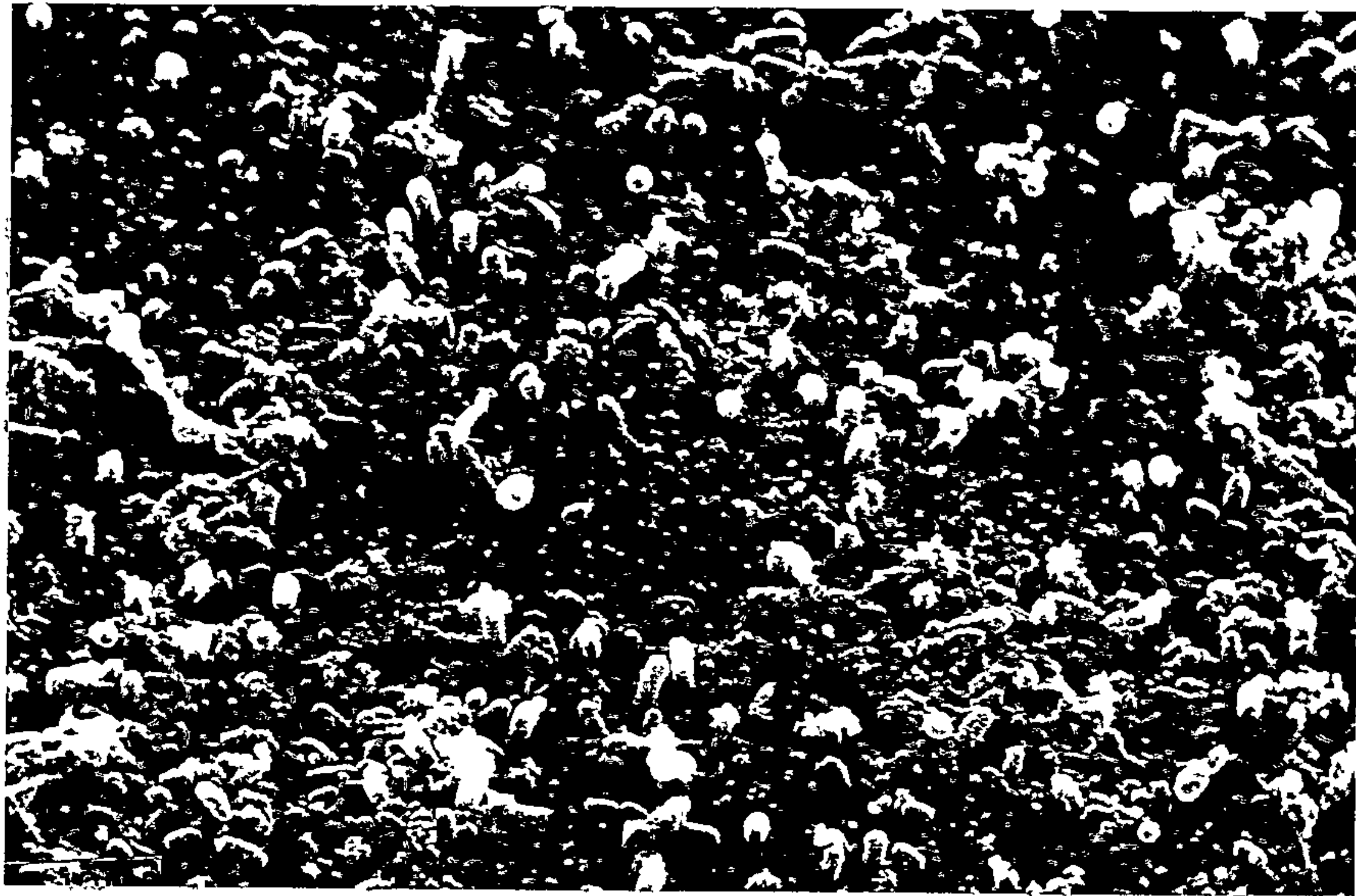


Fig. 8. Scanning electron micrographs of enamel treated with iron (III) chloride for one minute prior to a four minute topical treatment with 476 mM sodium fluoride (two stage treatments).

- A (*above*). First stage treatment with 1 mM iron (III) chloride. The surface is covered with deposits of various shapes. X 2,000.
- B (*top right*). First stage treatment with 10 mM iron (III) chloride. A relatively dense covering of deposits of various shapes is evident. X 2,000.
- C (*right*). First stage treatment with 100 mM iron (III) chloride. The surface has an etched appearance. X 2,000.

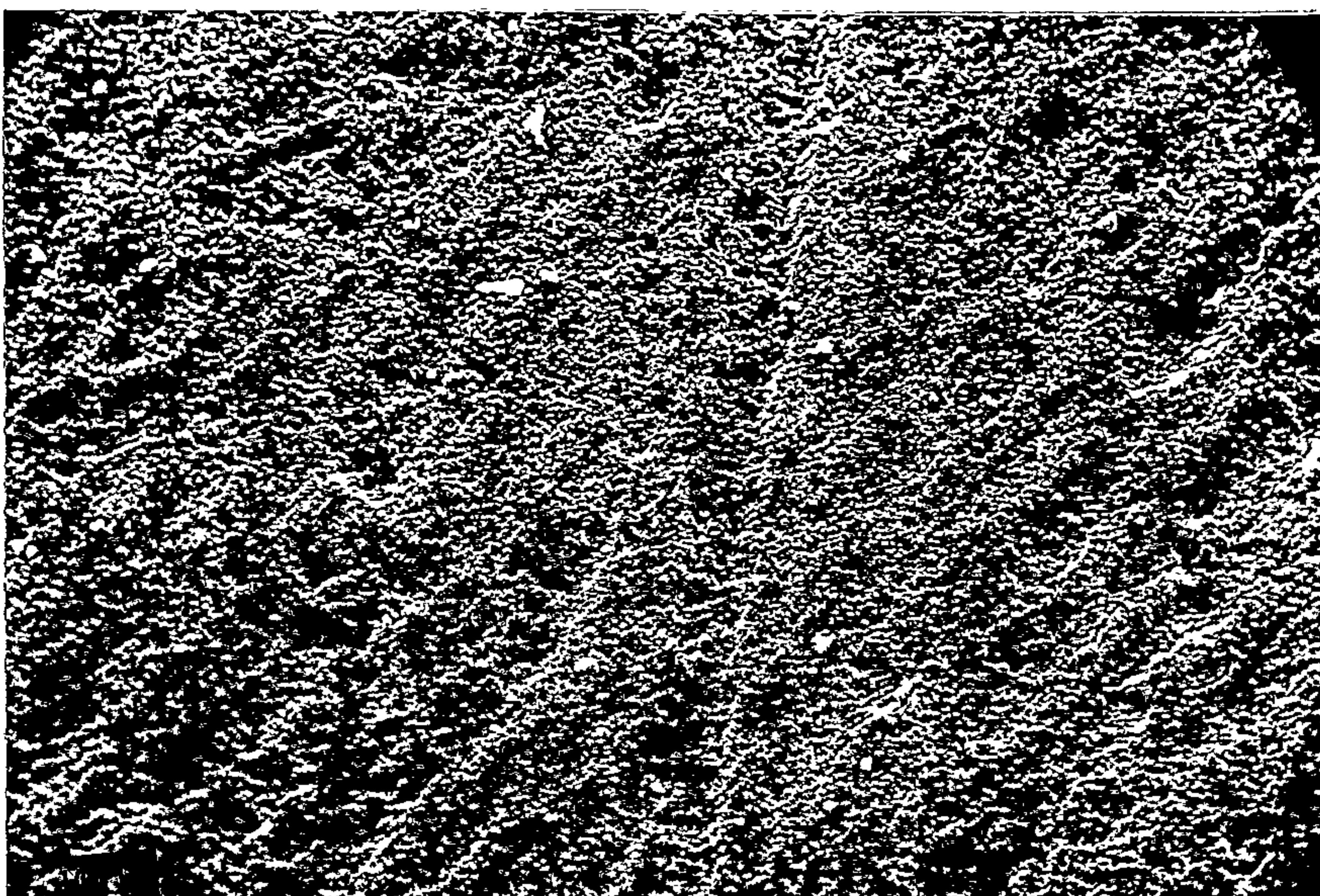
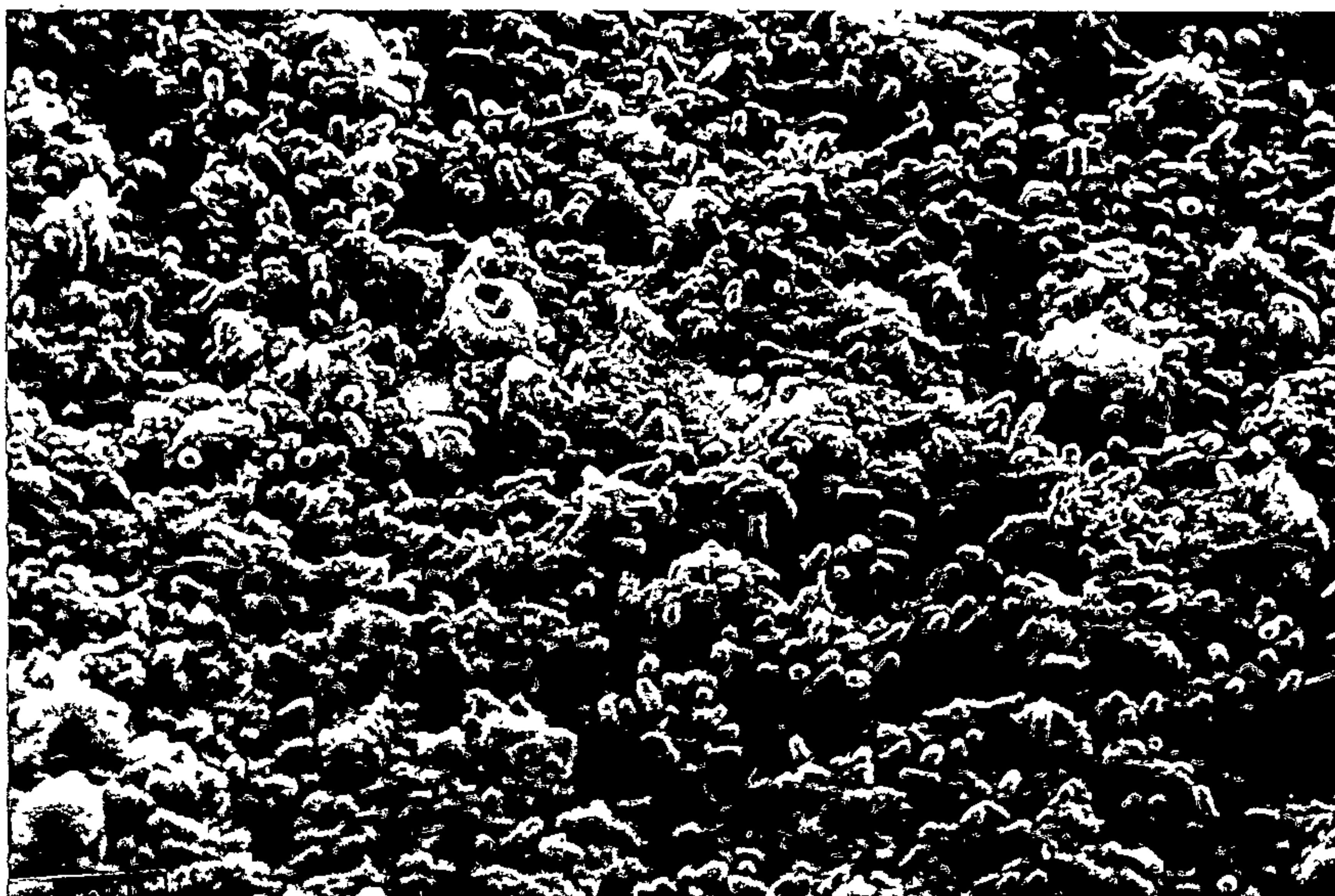
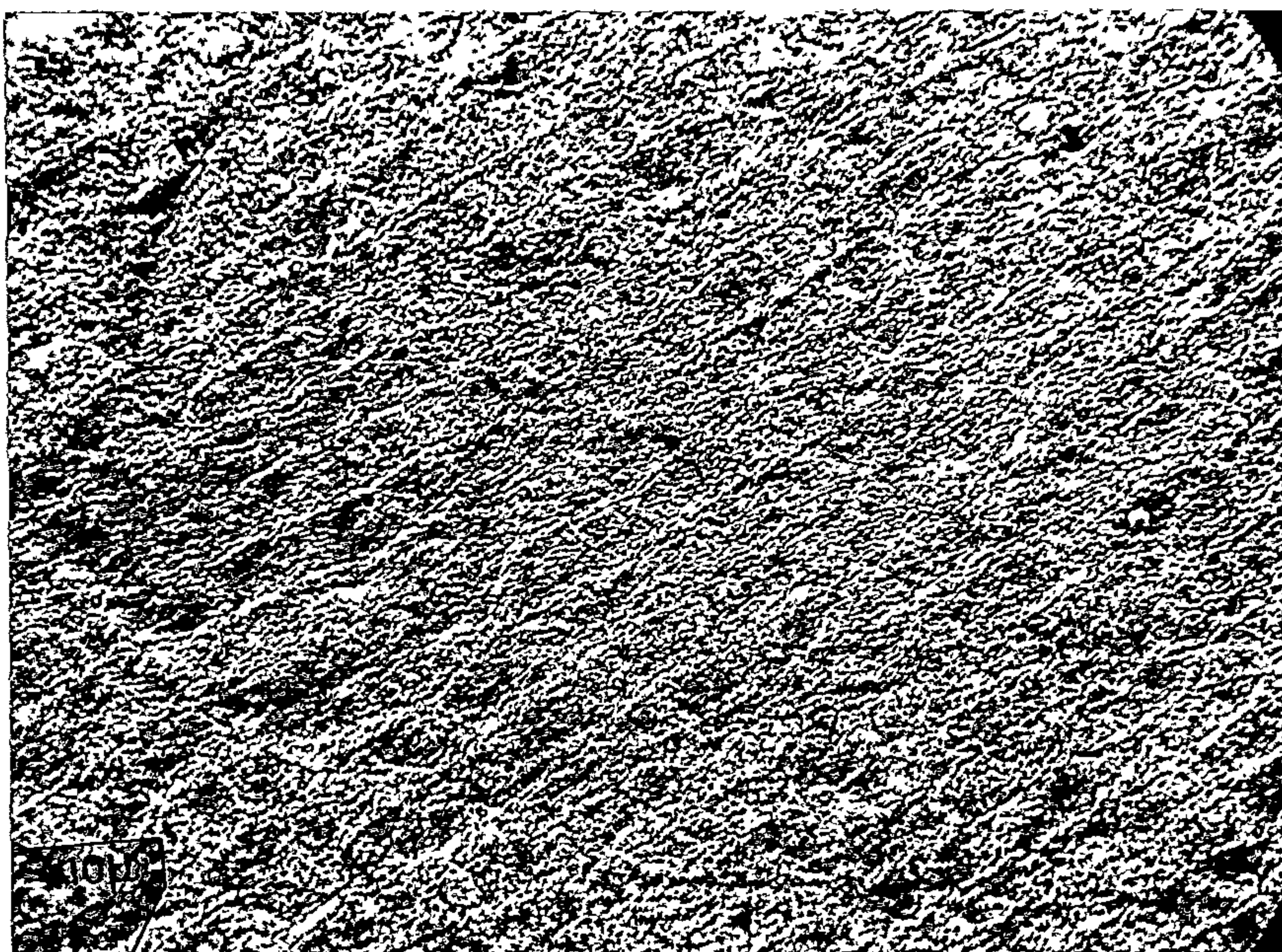




Fig. 9. Scanning electron micrographs of enamel treated with fluoride solutions containing iron.

- A (*above*). Enamel treated with 15 mM iron (II) fluoride for five minutes. Very few surface deposits can be seen. The scratch marks left by the polishing procedure are quite evident. X 2,000.
- B (*top right*). Enamel treated with 10 mM iron (III) fluoride for five minutes. The surface appears to be virtually free of deposits. The scratch marks from the polishing procedure are readily discernable. X 2,000.
- C (*right*). Enamel treated with a solution containing 50 mM iron (III) chloride and 190 mM sodium fluoride for five minutes. The surface has been etched by the treatment. X 2,000.



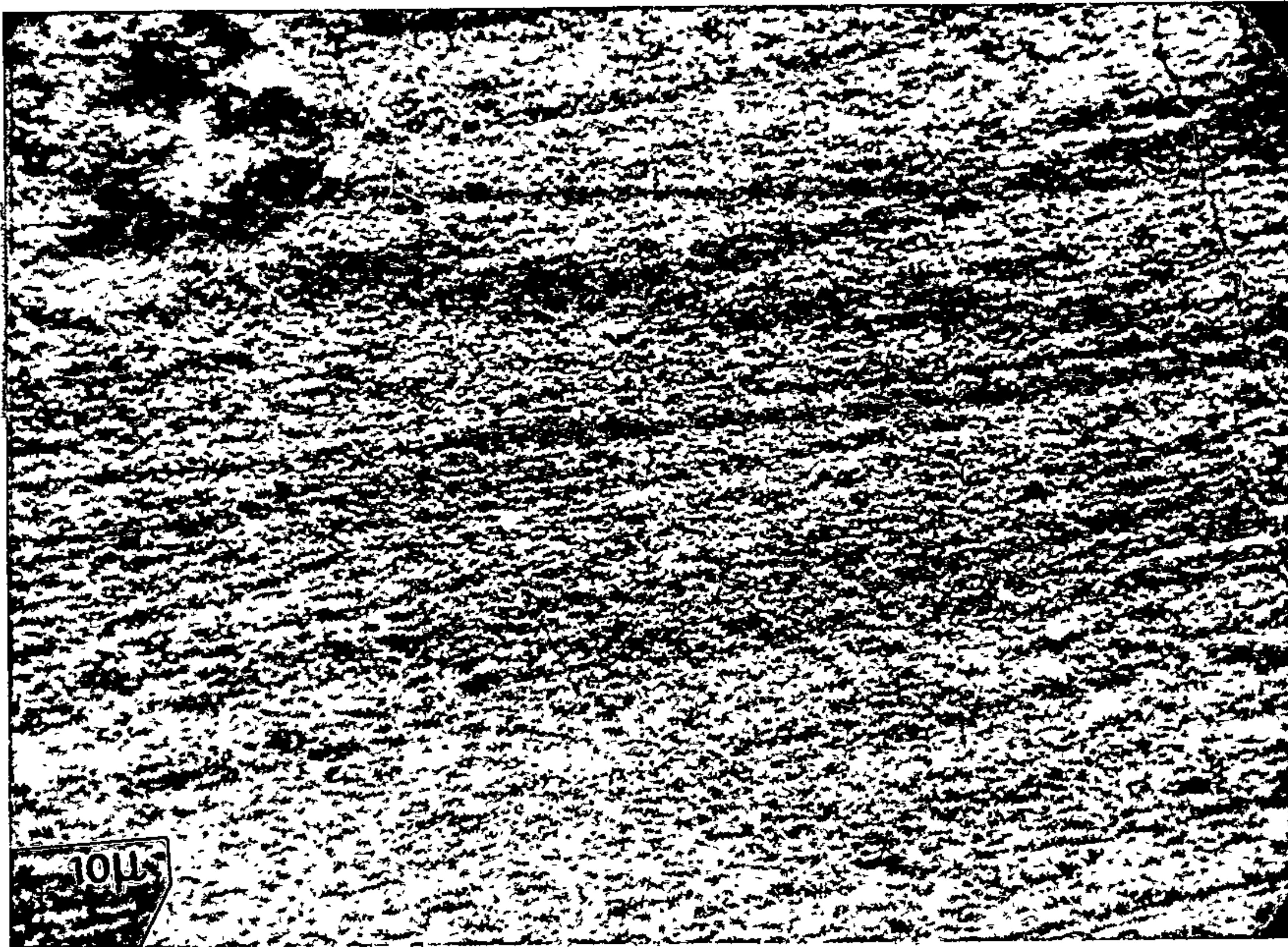


Fig. 10. Scanning electron micrographs of enamel treated with tin (II) fluoride or sodium fluoride.

- A** (*above*). Enamel treated with 26 mM tin (II) fluoride for five minutes. A slight etching of the surface is evident. X 2,000
- B** (*top right*). Enamel treated with 511 mM tin (II) fluoride for five minutes. The etching of the surface is more apparent than in Fig. 10-A. X 2,000.
- C** (*right*). Enamel treated with 48 mM sodium fluoride for five minutes. The surface is covered with small globules. The scratch marks left by the polishing procedure are quite evident. X 2,000.



Whereas the surface of enamel treated with 476 mM sodium fluoride alone is covered with globular deposits (Fig. 4-C), the surfaces of enamel treated with iron (II) chloride solutions prior to the topical treatment with 476 mM sodium fluoride (Figs. 7-A to 7-C), appear to be free of this type of deposit. In contrast, the surfaces of enamel treated with 1 mM iron (III) chloride or 10 mM iron (III) chloride followed by 476 mM sodium fluoride are covered with deposits (Figs. 8-A and 8-B). Although these deposits are not evident on the surface of enamel treated initially with 100 mM iron (III) chloride (Fig. 8-C) the surface does have a slightly etched appearance.

It is apparent that the enamel treated with 15 mM iron (II) fluoride or 10 mM iron (III) fluoride (Figs. 9-A and 9-B) differs little from the control enamel shown in Fig. 4-A. The scratch marks left by the polishing procedure are quite evident. A slight etching is apparent on the enamel treated with 26 mM tin (II) fluoride (Fig. 10-A) whilst the enamel treated with 48 mM sodium fluoride is covered with small globular deposits (Fig. 10-C). The surfaces of enamel treated with 511 mM tin (II) fluoride (Fig. 10-B) or the solution containing 50 mM iron (III) chloride and 190 mM sodium fluoride (Fig. 9-C) show clear evidence of etching.

DISCUSSION

To date, there appears to be little disagreement with the observation made by Brudevold *et al.*⁽³⁰⁾ in 1967, that the effectiveness of topical fluoride agents is directly related to their ability to

deposit fluoride in enamel as fluorapatite. These workers showed that a major portion of the fluoride deposited in enamel *in vivo* is lost within a period of 24 hours. They presented evidence to support their contention that only fluoride as fluorapatite is retained and that the portion lost consists essentially of unreacted fluoride and calcium fluoride.

Apart from the pretreatment of enamel with polyvalent cations as used in this study, a number of other methods have been developed in an attempt to increase the retention of topically applied fluoride, including the pretreatment of enamel with mild etching agents^{(2) (52)} and the application of sealants to retain the acquired fluoride *in situ*⁽⁵⁴⁾.

It has been a common finding that, despite the use of standardised etching procedures, the depth of enamel biopsies can vary from site to site^{(31) (171) (200)}. Under such circumstances, valid comparisons of treatment effects cannot be made without corrections for depth which are, in turn, complicated by the fact that fluoride concentrations in enamel decrease markedly from the surface towards the dentino-enamel junction⁽²⁹⁾. In recent years however, methods have been developed which take into account the presence of fluoride gradients and allow the calculation of fluoride concentrations at pre-determined depths^{(32) (118)}.

Although such procedures were not used in this study, the difference between some of the treatments were so marked that some general conclusions can be reached.

The effectiveness of iron (III) chloride, at concentrations of 10 mM and 100 mM, in increasing fluoride uptake by enamel from APF was most evident, whereas iron (II) chloride at equivalent concentrations

had little effect. These findings confirm those of McCann⁽¹⁴⁰⁾ regarding the use of Fe^{3+} cations prior to topical treatments with APF and appear to confirm his observations regarding the comparative ineffectiveness of divalent cations in such situations. McCann attributed his findings to the ability of polyvalent cations to form strong fluoride complexes and, at the same time, bind firmly to phosphate in enamel.

An examination of the scanning electron micrographs in Figs. 4 and 6 shows that enamel treated with 100 mM iron (III) chloride followed by APF (Fig. 6-C) and to a lesser extent enamel treated with 10 mM iron (III) chloride followed by APF (Fig. 6-B), are etched more than enamel treated with APF alone (Fig. 4-B). It appears therefore that the low pH of the iron (III) chloride solutions alone may have contributed to the enhanced fluoride uptake by causing an etching of enamel surfaces and, in so doing, increasing the number of reactive sites available.

In contrast to the findings with APF, the pretreatment of enamel with iron (II) chloride or iron (III) chloride solutions prior to the topical application of 476 mM sodium fluoride appeared to decrease fluoride uptake by enamel. Interestingly, Mühlemann and Regolati⁽¹⁵⁶⁾ have reported a similar situation occurring when they used aluminium chloride to pretreat molar teeth of rats prior to a topical application of neutral sodium fluoride. Apparently such pretreatments with iron chloride or aluminium chloride considerably modify the normal reactions between neutral sodium fluoride and enamel. However, in contrast to the situation with the other topical treatments, the 24 hour washing procedure did not appear to appreciably reduce the fluoride levels that were reached.

The finding that the fluoride uptake by enamel from 476 mM sodium fluoride and APF were comparable was unexpected. In a recent *in vitro* study, Kirkegaard⁽¹¹⁷⁾ determined the fluoride uptake by enamel from different topical fluoride compounds, including 476 mM sodium fluoride and APF. He found that a five minute application of 476 mM sodium fluoride and a five minute application of APF increased the fluoride levels in the outer 3 μ M of enamel by 2,073 ppm (S.E.^a = 229) and 3,541 ppm (S.E. = 235) respectively.

It is possible that the fluoride uptake by enamel from 476 mM sodium fluoride could have been enhanced by the stirring conditions used in this study together with the fact that all solutions were used at 37°C, not room temperature. Although Kirkegaard did not indicate the temperature of the solutions used, it has been shown by Mellberg and Loertscher⁽¹⁴⁷⁾ that fluoride uptake by enamel from topical fluoride solutions can be enhanced if they are warmed from room temperature to 35°C before use. Finally, the use of bovine teeth instead of human teeth, as used by Kirkegaard, may have affected the relative fluoride uptake from the two solutions.

Despite the widespread use of 476 mM sodium fluoride and APF in topical fluoride therapy⁽¹⁶¹⁾, it is surprising that until recently no direct comparison had been reported concerning the fluoride uptake by enamel from a single application of these two compounds⁽¹¹⁷⁾. Bruun⁽³¹⁾, in 1973, also commented on the lack of work in this area.

The appreciably lower fluoride uptake by enamel from 511 mM tin (II) fluoride than from either 476 mM sodium fluoride or APF seen in

^aS.E. = Standard error.

this study is in general agreement with the findings of Brudevold *et al.*⁽³⁰⁾, Cooley⁽⁴³⁾ and Kirkegaard⁽¹¹⁷⁾.

The scanning electron micrographs (Figs. 4 to 10) show that many of the treated surfaces had an appreciable accumulation of reaction products. Although no attempt was made to determine the nature of these deposits, on the basis of the findings of Gerould⁽⁷⁵⁾ it is possible that the globules on enamel treated with sodium fluoride at concentrations of 48 mM (Fig. 10-C) and 476 mM (Fig. 4-C) are essentially calcium fluoride. Gerould found that a globular layer formed on the surface of enamel after treatment with sodium fluoride and, using x-ray and electron diffraction, identified the layer as calcium fluoride.

Furthermore, on the basis of the finding of Baud and Bang⁽⁹⁾, calcium fluoride should predominate on enamel treated with APF (Fig. 4-B). These workers, using x-ray diffraction, found that calcium fluoride was the main reaction product when enamel was treated with APF and also found that this deposit could be completely removed by prolonged washing. However, another reaction product, fluorapatite, could not be removed by the washing procedure.

Wei and Forbes⁽²³²⁾ have established that tin-fluoride-phosphate ($\text{Sn}_3\text{F}_3\text{PO}_4$) can form on enamel during exposure to a tin (II) fluoride solution. Wei⁽²³⁰⁾, using scanning electron microscopy to follow the reaction, found that after four minutes of treatment the main change was a slight roughening of the enamel surface. In this study, enamel exposed to tin (II) fluoride at concentrations of 28 mM (Fig. 10-A) or 511 mM (Fig. 10-B) for five minutes showed evidence of some etching but little evidence of deposit formation.

An examination of the possible significance of these findings and of fluoride uptake by enamel in general, in relation to caries prevention, is presented in the general discussion section on page 142.

SECTION 2

THE EFFECT OF IRON (II) AND IRON (III) SALTS ON THE DISSOLUTION RATE OF ENAMEL (TEST 2)

In many laboratory studies, assessments of the 'solubility' reducing properties of test compounds have been based on the ability of the compounds to limit calcium and/or phosphorus loss, or occasionally weight loss, from enamel or hydroxyapatite during a fixed period in an organic acid buffer⁽³⁴⁾⁽³⁶⁾⁽¹⁴³⁾⁽¹⁵⁷⁾⁽¹⁵⁹⁾⁽¹⁹²⁾. However, a study by Gray⁽⁸²⁾ in 1965 clearly showed the limitations of using a single acid exposure time. He treated sound enamel with either 25 mM tin (II) fluoride, 25 mM tin (II) chloride or 50 mM sodium fluoride for five minutes and then exposed the treated specimens to pH 4.5 lactate buffer for periods up to four hours. The protective effect of the treatments changed as the acid exposure time was increased. The effectiveness of the treatments in descending order was:

At 16 minutes - $\text{SnF}_2 > \text{SnCl}_2 > \text{NaF}$

At 30 minutes - $\text{SnF}_2 > \text{NaF} > \text{SnCl}_2$

At 120 minutes - $\text{SnF}_2 = \text{NaF} > \text{SnCl}_2$ (no effect)

In the first experiment in this section, the basic method of Gray⁽⁸¹⁾⁽⁸²⁾ was used to determine the effect of topical treatments of iron (II) and iron (III) salts, as well as APF, sodium fluoride and tin (II) fluoride, on the dissolution rate of enamel in pH 4.5 lactate buffer.

The findings in Section 1 of this study showed that the pretreatment of enamel with iron (II) or iron (III) salts could

markedly affect the fluoride uptake by enamel from APF and sodium fluoride solutions. Therefore, an additional investigation was carried out to examine the effect of these treatments on the dissolution rate of enamel in pH 4.5 lactate buffer.

In the second experiment in this section, the effect on the enamel dissolution rate of adding iron (II) or iron (III) salts to pH 4.5 lactate buffer was examined. For comparison purposes, tin (II) chloride, tin (II) fluoride, sodium fluoride, as well as calcium and phosphate salts, were also tested.

MATERIALS AND METHODS

MATERIALS

Teeth

Sound lower permanent central incisor teeth, extracted from freshly slaughtered 2.0 to 2.5 year old beef cattle, were obtained from Homebush Abattoirs, Sydney, N.S.W., Australia.

Chemicals

All chemicals were the highest grade available commercially: Acidulated phosphate-fluoride (Orapfram Products Ltd., Melbourne, Vic., Australia); calcium chloride and sodium fluoride (BDH Chemicals Ltd., Poole, England); iron (II) chloride and potassium

dihydrogen phosphate (E. Merck and Co., Darmstadt, West Germany); iron (III) chloride (Riedel-De Haën AG, Seelze-Hannover, West Germany); iron (II) fluoride and iron (III) fluoride (PCR Inc., Gainesville, Fla., U.S.A.); tin (II) fluoride (Ozark-Mahoning, Tulsa, Okla., U.S.A.).

All solutions were made up with distilled, deionised water.

Apparatus

Experiment 1

The apparatus used for treating the enamel specimens with the test compounds and for exposing the specimens to the lactate buffer was identical to that shown in Fig. 1.

Under the stirring conditions used in this experiment (2,000 rpm) no special precautions were necessary to prevent vortex formation in the vicinity of the enamel surface.

Experiment 2

The apparatus employed in Experiment 1 was again used. However, the hollow lids of the plastic vials were replaced with solid PTFE^a lids, with a 9 mm diameter hole in the centre and a 1.25 mm diameter hole on either side. The centre hole, when coated with high-vacuum grease (Ajax Chemicals Ltd., Sydney, N.S.W., Australia), provided a snug fit for the plastic rods supporting the enamel specimens while

^aPolytetrafluorethylene

the smaller holes were used for nitrogen/oxygen intake and exhaust ports (Fig. 11).

METHODS

Preparation of Enamel

Experiment 1

A hollow, 5 mm internal diameter, diamond drill (Horico, Hopf. Ringleb GmbH, Berlin, West Germany) was used to cut a cylindrical core from the centre of the facial surface of each tooth. All cutting was done under a water spray.

The cores were stored together, for eight weeks at room temperature, in a sealed glass jar containing 500 ml of a 53 μ M sodium fluoride (1 ppm F^-) solution plus some thymol crystals to suppress bacterial growth. Preliminary studies showed that this ageing procedure markedly reduced the variation in dissolution rates within treatment groups.

At the end of eight weeks, the cores were mounted on acrylic rods and the specimens were prepared using the method described previously on page 21.

Experiment 2

Preliminary studies showed that the addition of certain of the test compounds to the lactate buffer reduced the enamel dissolution rate to such a degree that an approximately two fold increase in enamel surface area over that used in Experiment 1 was necessary to obtain calcium levels that could be readily measured.

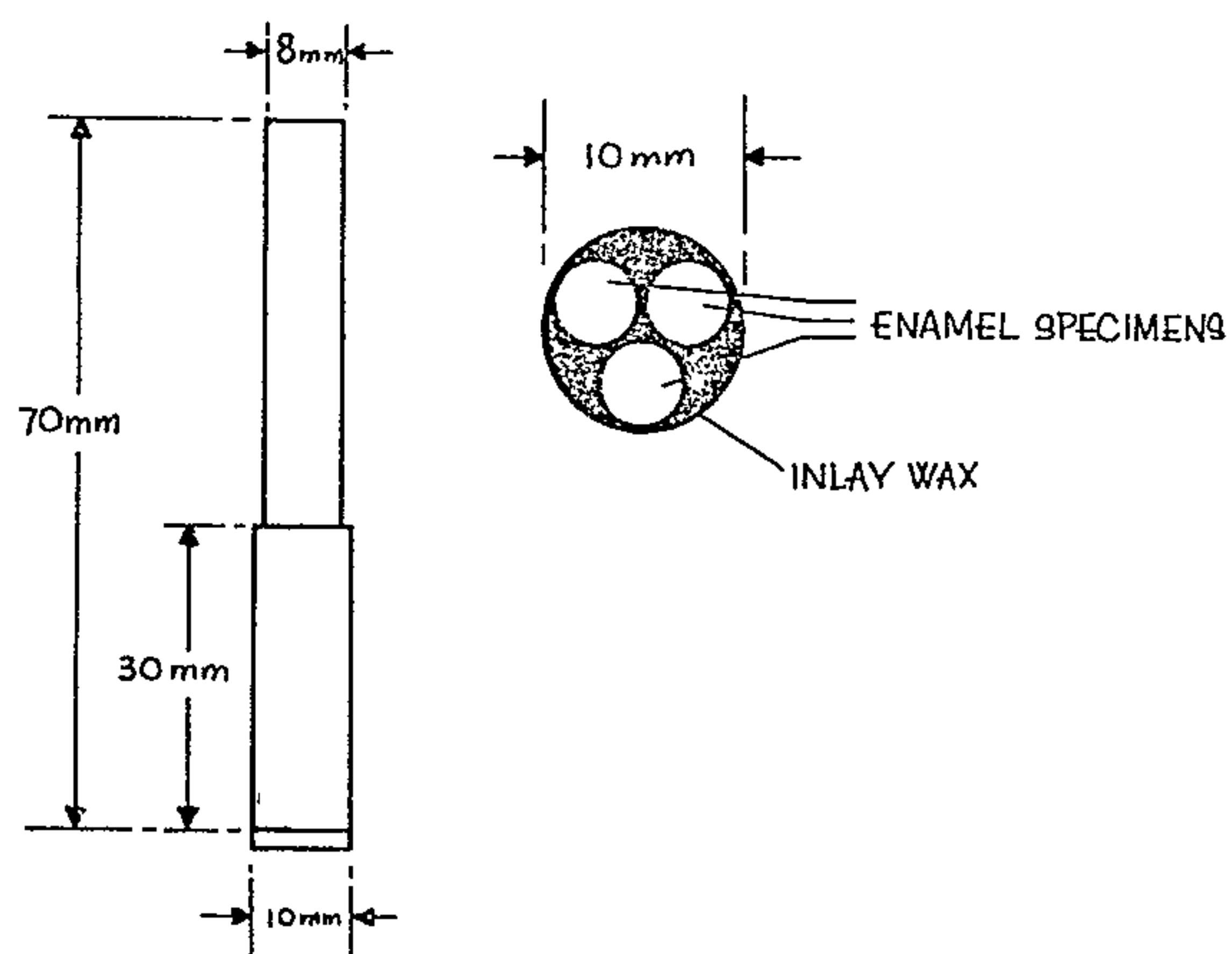
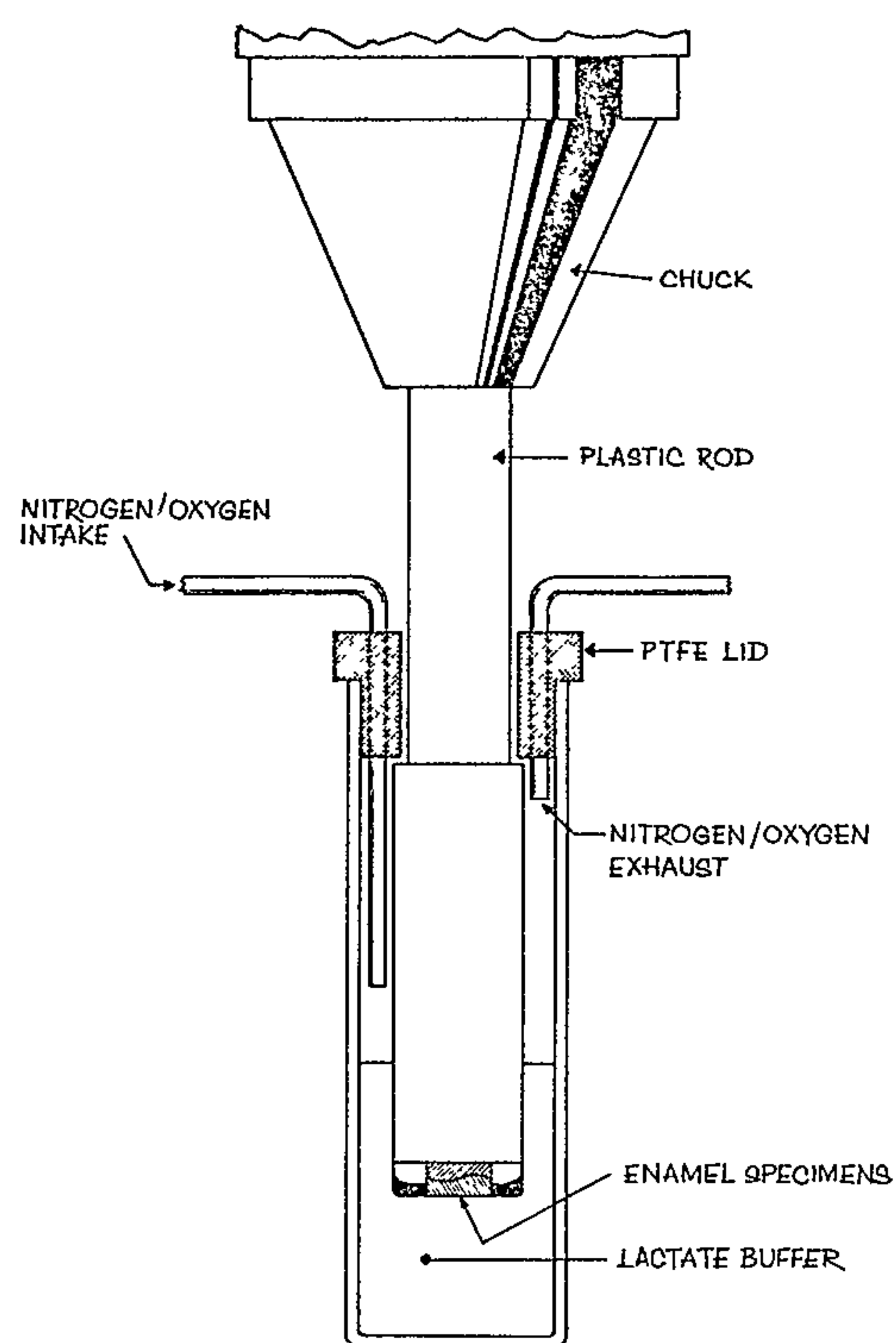


Fig. 11. (Top). Diagram showing an acrylic rod, supporting enamel specimens, in place in a plastic vial containing lactate buffer. The smaller diameter end of the rod is clamped in a chuck attached to an electric motor (not shown). The PTFE lid has gas inlet and exhaust ports. The remainder of the apparatus is not shown, but it was identical to that illustrated in Fig. 1. (Bottom). Diagram showing mounting of specimens on the 10 mm diameter end of the acrylic rod.

The preparation of enamel was carried out as in Experiment 1 with the exception that:

1. A hollow, 4 mm internal diameter, diamond drill was used to cut three cylindrical cores from the middle of the facial surface of each tooth.
2. Three cores were mounted on the 10 mm diameter end of 70 mm long acrylic rods. (The other end of each rod had previously been machined down to a diameter of 8 mm so that it would fit through the hole in the PTFE lid of each plastic vial and fit into the chuck of the dissolution apparatus [Fig. 11]).

Treatment of Enamel (Experiment 1)

Details of the treatment solutions and treatment times used are given in Table 3. The treatment procedures followed are shown in the flow diagram in Fig. 12.

Two millilitres of the appropriate solution at 37°C was pipetted into a plastic vial, the vial clamped in position in the treatment apparatus and an acrylic rod supporting an enamel specimen lowered until the enamel surface was 10 mm below the surface of the treatment solution. The specimen was then rotated at 2,000 rpm for the appropriate treatment period. The temperature of the solution was kept at a constant 37°C.

At the end of each treatment period, the acrylic rod was removed from the apparatus and the enamel surface washed for 15 seconds under a fine stream of distilled, deionised water. The volume of water used for each washing was 12 ml.

To determine the amount of calcium lost by the enamel during the

TABLE 3

TREATMENT SOLUTIONS AND TREATMENT TIMES USED IN
ENAMEL DISSOLUTION RATE STUDY (EXPERIMENT 1)

A. One Stage Treatments (Single Application of Test Compounds).

Solution	pH	Treatment Time (min)
H ₂ O (Control)	5.3	5
1 mM FeCl ₂ · 4 H ₂ O	5.1	5
10 mM FeCl ₂ · 4 H ₂ O	4.3	5
100mM FeCl ₂ · 4 H ₂ O	3.2	5
1 mM FeCl ₃ · 6 H ₂ O	3.2	5
10 mM FeCl ₃ · 6 H ₂ O	2.4	5
100mM FeCl ₃ · 6 H ₂ O	1.6	5
15 mM FeF ₂ (570 ppm F) ^a	4.4	5
10 mM FeF ₃ (570 ppm F) ^b	3.5	5
50 mM FeCl ₃ · 6 H ₂ O	2.1	5
+ 190mM NaF (3,620 ppm F) ^c		
APF (12,300 ppm F) ^c	3.2	5
26 mM SnF ₂ (970 ppm F) ^c	3.4	5
511mM SnF ₂ (19,400 ppm F) ^c	2.8	5
48 mM NaF (905 ppm F) ^c	7.0	5
476mM NaF (9,050 ppm F) ^c	7.0	5

^a Prepared under nitrogen. Solution warmed to 60°C and continuously stirred to facilitate dissolution of salt. Solution cooled to 37°C before use.

^b Solution warmed to 60°C and continuously stirred to facilitate dissolution of salt. Solution cooled to 37°C before use.

^c Solutions have been tested clinically (see footnote ^c Table 1).

TABLE 3 (cont.)

B. Two Stage Treatments

Stage 1			Stage 2		
Solution	pH	Treatment Time (min)	Solution	pH	Treatment Time (min)
1 mM FeCl ₂ · 4H ₂ O	5.1	1	APF (12,300 ppm F) used in all treatments	3.2	4
10 mM FeCl ₂ · 4H ₂ O	4.3	1			
100mM FeCl ₂ · 4H ₂ O	3.2	1			
1 mM FeCl ₃ · 6H ₂ O	3.2	1			
10 mM FeCl ₃ · 6H ₂ O	2.4	1			
100mM FeCl ₃ · 6H ₂ O	1.6	1			
1 mM FeCl ₂ · 4H ₂ O	5.1	1	476mM NaF (9,050 ppm F) used in all treatments	7.0	4
10 mM FeCl ₂ · 4H ₂ O	4.3	1			
100mM FeCl ₂ · 4H ₂ O	3.2	1			
1 mM FeCl ₃ · 6H ₂ O	3.2	1			
10 mM FeCl ₃ · 6H ₂ O	2.4	1			
100mM FeCl ₃ · 6H ₂ O	1.6	1			

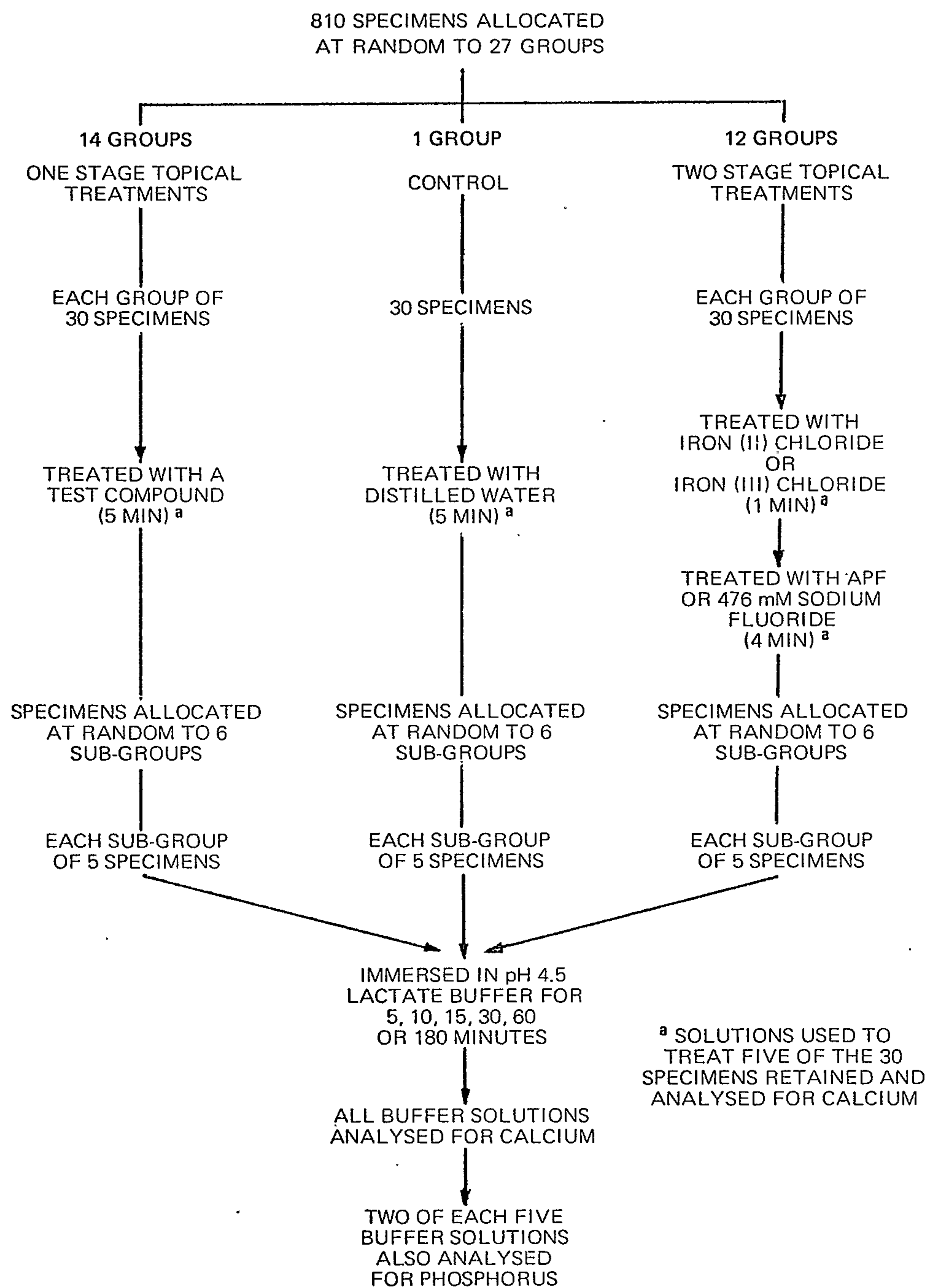


Fig. 12. Flow diagram outlining experimental procedures followed in enamel dissolution rate study.

treatment and subsequent washing procedure, the treatment solutions in the vials used for five of the 30 specimens in each group (Fig. 12), were mixed with the water used in the washing procedure, and retained for calcium analysis.

Dissolution of Enamel

Experiment 1

Two millilitres of 0.1 M sodium lactate - lactic acid buffer at 37°C was pipetted into a plastic vial, the vial clamped in position in the dissolution apparatus and an acrylic rod supporting a treated enamel specimen lowered until the enamel surface was 10 mm below the surface of the buffer. The specimen was then rotated at 2,000 rpm for either 5, 10, 15, 30, 60 or 180 minutes. The temperature of the buffer was kept at a constant 37°C. One sub-group (five specimens) of each group (Fig. 12) was used per time period.

The buffer solution in each of the five vials used for a sub-group was retained and analysed for calcium. In order to determine Ca/P ratios, the buffer solution in two of the five vials was analysed for phosphorus.

Experiment 2

Since the addition of ions to an acid buffer may markedly alter the ionic strength and therefore the activity coefficients of the ionic species dissolving from the enamel surface⁽²⁰⁾, it was necessary to add a relatively inert salt in sufficient concentration to provide a high and relatively constant ionic strength background. Sodium chloride was used to provide this background on the basis of the findings of Gray⁽⁸¹⁾ who showed that the addition of sodium chloride to a 0.1 M lactate

buffer, (pH 4.5) to a final concentration of 0.6 M, had little effect on the dissolution rate of enamel.

Sufficient sodium chloride was added to the 0.1 M sodium lactate - lactic acid buffer used in this experiment to give a final concentration of 0.5 M.

The test compounds added to the buffer are listed in Table 4.

Nitrogen was bubbled through the buffer solution for 30 minutes prior to the addition of iron (II) and tin (II) salts to minimise oxidation. Oxygen was bubbled through the buffer solution for 30 minutes prior to the addition of iron (III) salts to minimise reduction.

The dissolution procedure was identical to that used in Experiment 1, except that the lactate buffer exposure period for all specimens was 60 minutes. The experiments in which Fe^{2+} or Sn^{2+} cations were used were carried out in an atmosphere of moist nitrogen, whilst those in which Fe^{3+} cations were used were carried out in an atmosphere of moist oxygen.

Calcium Analysis

A 400 μl aliquot from each of the treatment solutions selected for calcium analysis and a 20 μl aliquot from each of the buffer solutions were diluted, 1 in 6 and 1 in 103 respectively, with a background solution containing 8 mM caesium chloride and 158 mM nitric acid. Calcium estimations were made using the method described previously on page 26.

To determine the precision of the method, six replicate estimations were made on one sample. The mean calcium concentration was 1.96 ppm, S.D. = 0.07 (n = 6).

TABLE 4

CATIONS AND ANIONS ADDED TO pH 4.5 LACTATE BUFFER IN
ENAMEL DISSOLUTION RATE STUDY (EXPERIMENT 2).

Added Cation or Anion ^a		
Na ⁺	as	NaCl ^b
Fe ²⁺	as	FeCl ₂ · 4H ₂ O
Fe ³⁺	as	FeCl ₃ · 6H ₂ O
Sn ²⁺	as	SnCl ₂
Ca ²⁺	as	CaCl ₂
PO ₄ ³⁻	as	KH ₂ PO ₄
F ⁻	as	NaF ^c
F ⁻	as	FeF ₂ ^c
F ⁻	as	FeF ₃ ^c
F ⁻	as	SnF ₂ ^c

a Each salt added singly to lactate buffer or give required cation or anion concentration (10μM, 100μM or 1mM).

b Added to control lactate buffer to give a final Na⁺ concentration 10μM, 100μM or 1mM above that already present in buffer.

c Sufficient salt added to give a final F⁻ concentration of 10μM, 100μM or 1mM.

The accuracy of the method was tested by adding known amounts of calcium to nine samples selected at random. The mean percentage recovery was 95.6 ± 3.6 .

Phosphorus Analysis

An aliquot of 1 ml from each of the buffer solutions selected for phosphorus analysis was analysed for this element following the colorimetric method of Lueck and Boltz⁽¹³⁵⁾ using a single beam spectrophotometer (Spectronic 20, Bausch and Lomb, Rochester, N.Y., U.S.A.). The instrument was used in accordance with the method described by Evenson⁽⁶³⁾ to increase the sensitivity of measurement.

To determine the precision of the method three samples were pooled and six replicate estimations were made on the pooled sample. The mean phosphorus concentration was 0.82 ppm, S.D. = 0.087 ($n = 6$).

The accuracy of the method was checked by adding known amounts of phosphorus to nine samples selected at random. The mean percentage recovery was 104.1 ± 4.8 .

Calculation of Enamel Surface Area

The area of each enamel surface was determined using the method described previously on pages 26 and 27.

RESULTS

EXPERIMENT 1 - EFFECT OF TOPICAL TREATMENTS ON ENAMEL DISSOLUTION RATE

One Stage Treatments (Single application of test compounds)

The dissolution rate curves for control and treated enamel shown in Figs. 13 and 14 were prepared from the data, summarised in the Appendix, on calcium loss from enamel during the three hour immersion period in pH 4.5 lactate buffer.

Overall, the dissolution rates of both control and treated enamel decreased with time, the main decrease tending to occur from 30 to 60 minutes onwards.

Whilst the dissolution rates for enamel treated with non-fluoride iron salts appeared to follow this general pattern, two of the treatments, 1 mM iron (III) chloride and 10 mM iron (III) chloride, tended to retard the initial dissolution rate appreciably (Fig. 13-B). Another two treatments, 100 mM iron (II) chloride (Fig. 13-A) and 100 mM iron (III) chloride (Fig. 13-B), appeared to exert their main effect from 60 minutes onwards and tended to noticeably retard the enamel dissolution rate during this period.

Except for 26 mM tin (II) fluoride (Fig. 14-A) and 511 mM tin (II) fluoride (Fig. 14-B), all the test compounds containing fluoride tended to noticeably retard the enamel dissolution rate from 30 to 60 minutes onwards. The two tin (II) fluoride solutions however, appeared to increase the dissolution rate slightly from 30 minutes onwards.

Presented in Table 5-A are data on calcium and phosphorus loss

Fig. 13. Dissolution rates of enamel (based on calcium loss) in pH 4.5 lactate buffer following one stage topical treatments with non-fluoride iron salts.

A (top). Effect of topical treatment with iron (II) chloride at a concentration of 1 mM, 10 mM, or 100 mM (treatment time — five minutes).

B (right). Effect of topical treatment with iron (III) chloride at a concentration of 1 mM, 10 mM or 100 mM (treatment time — five minutes).

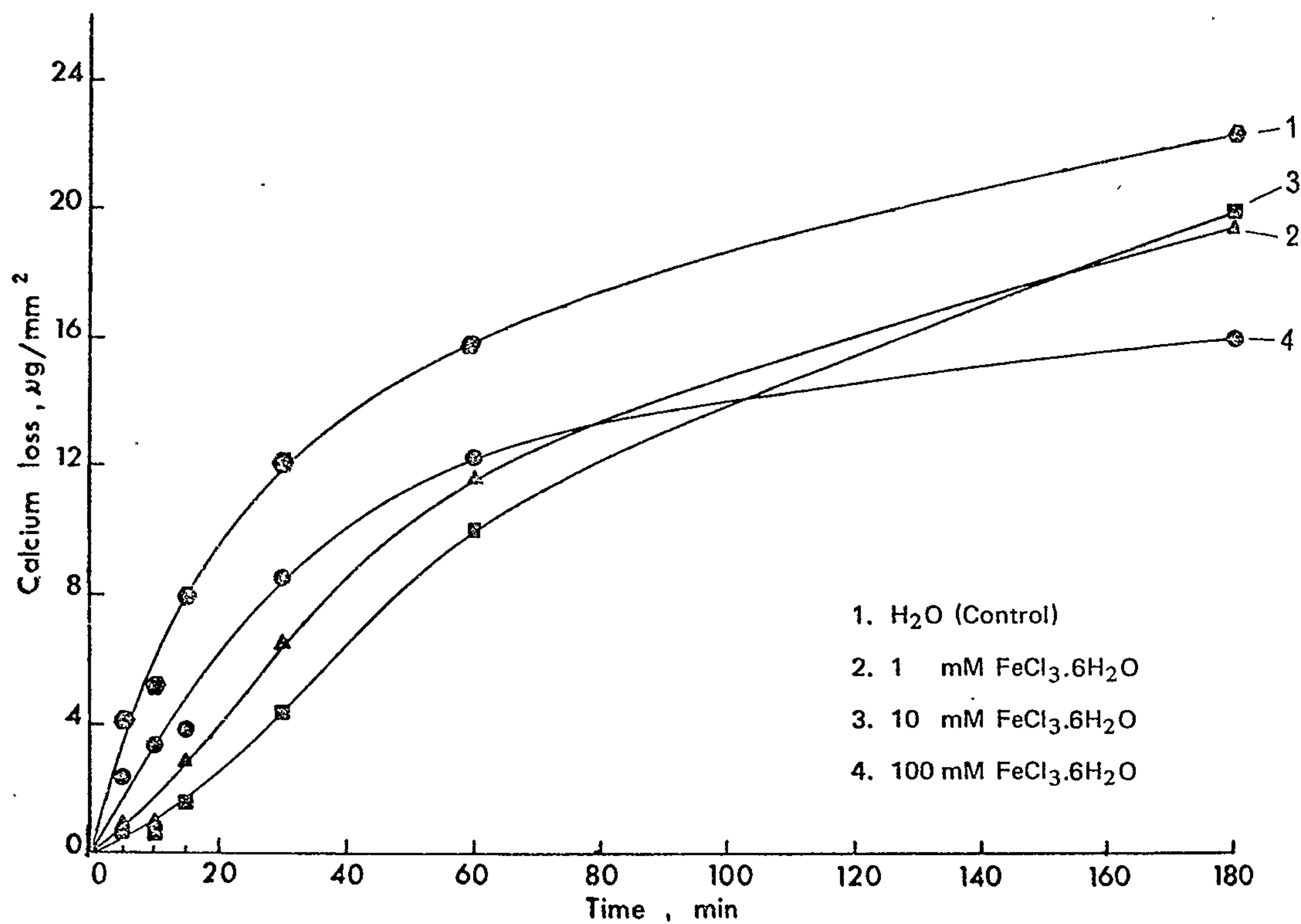
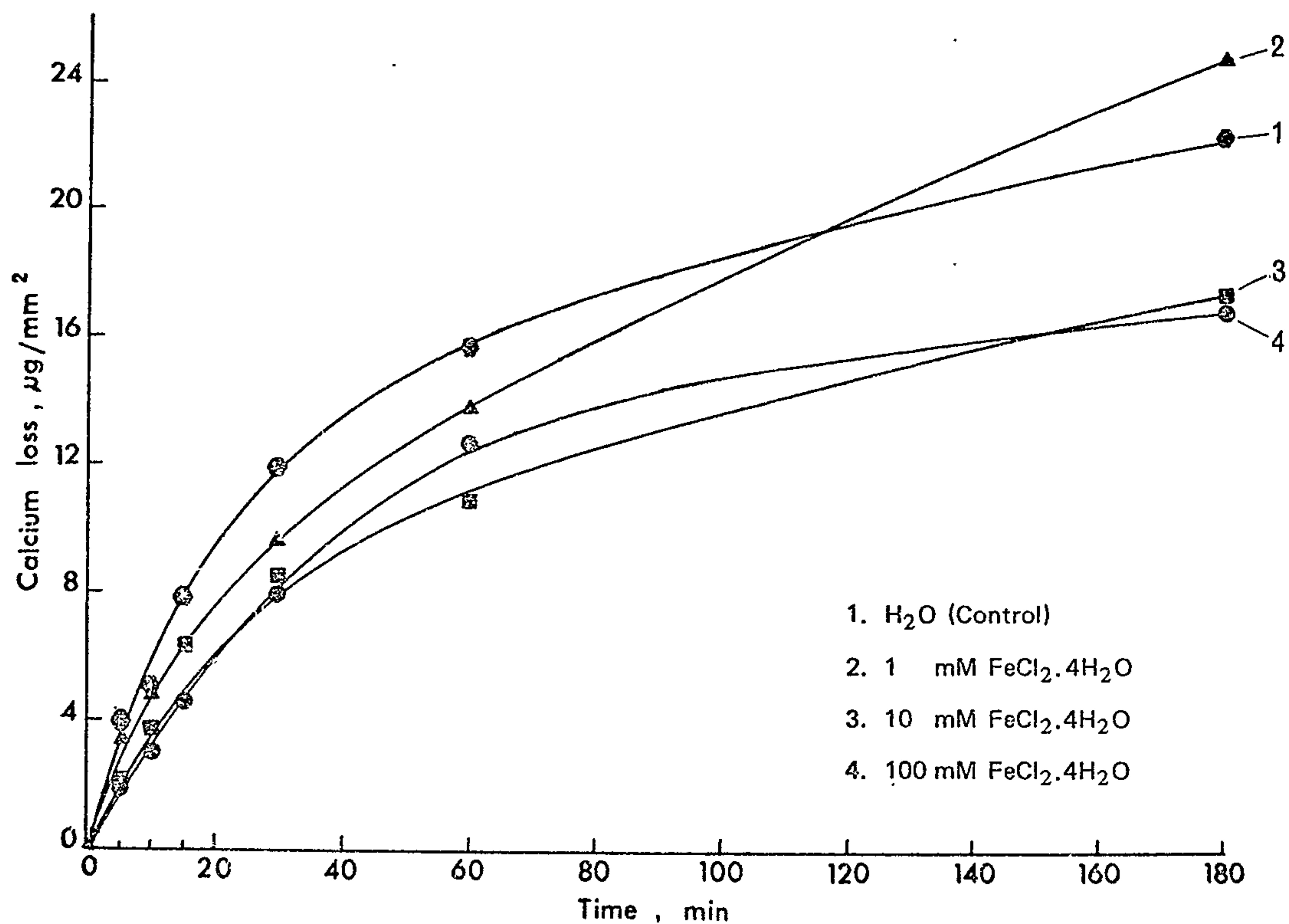
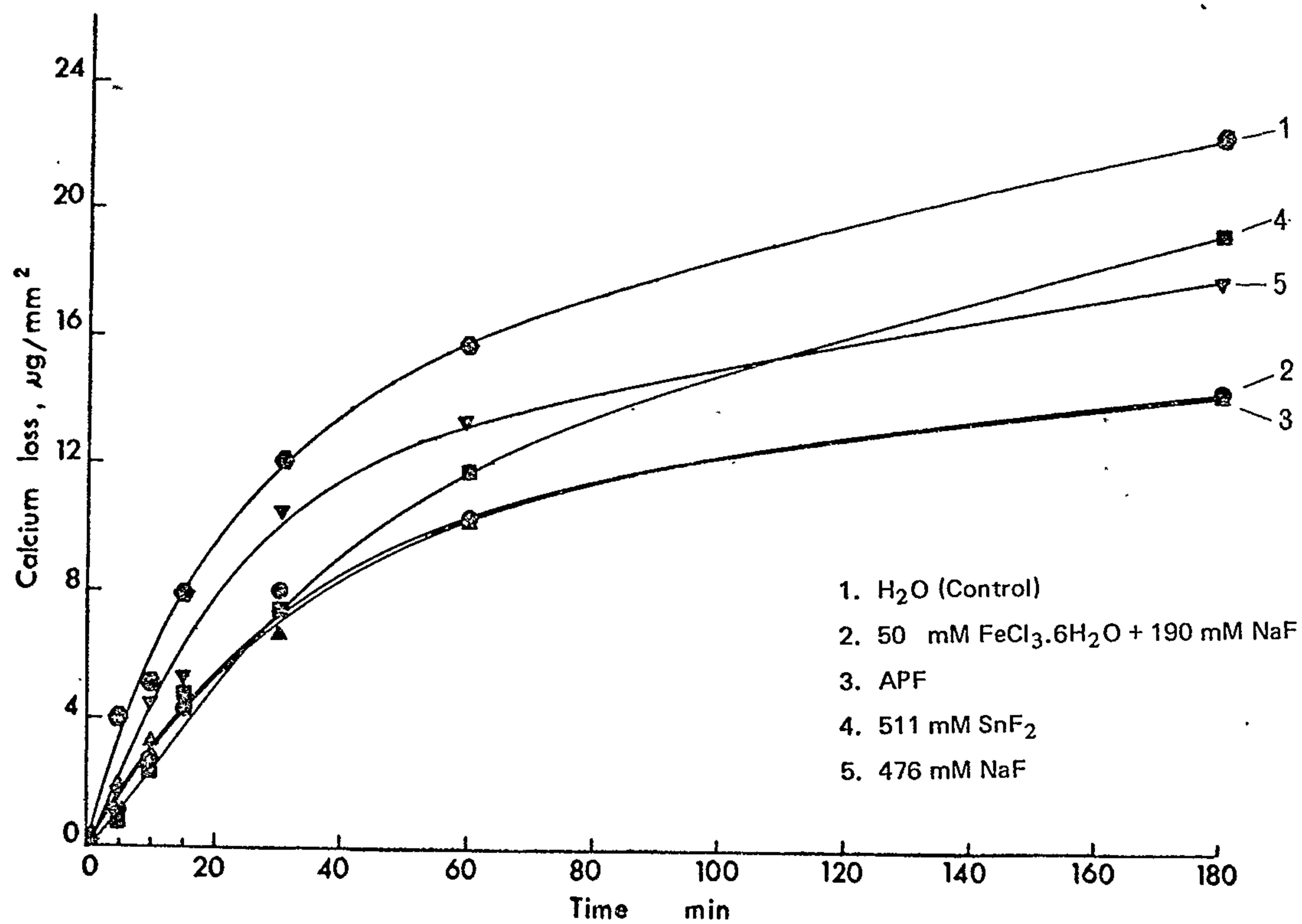
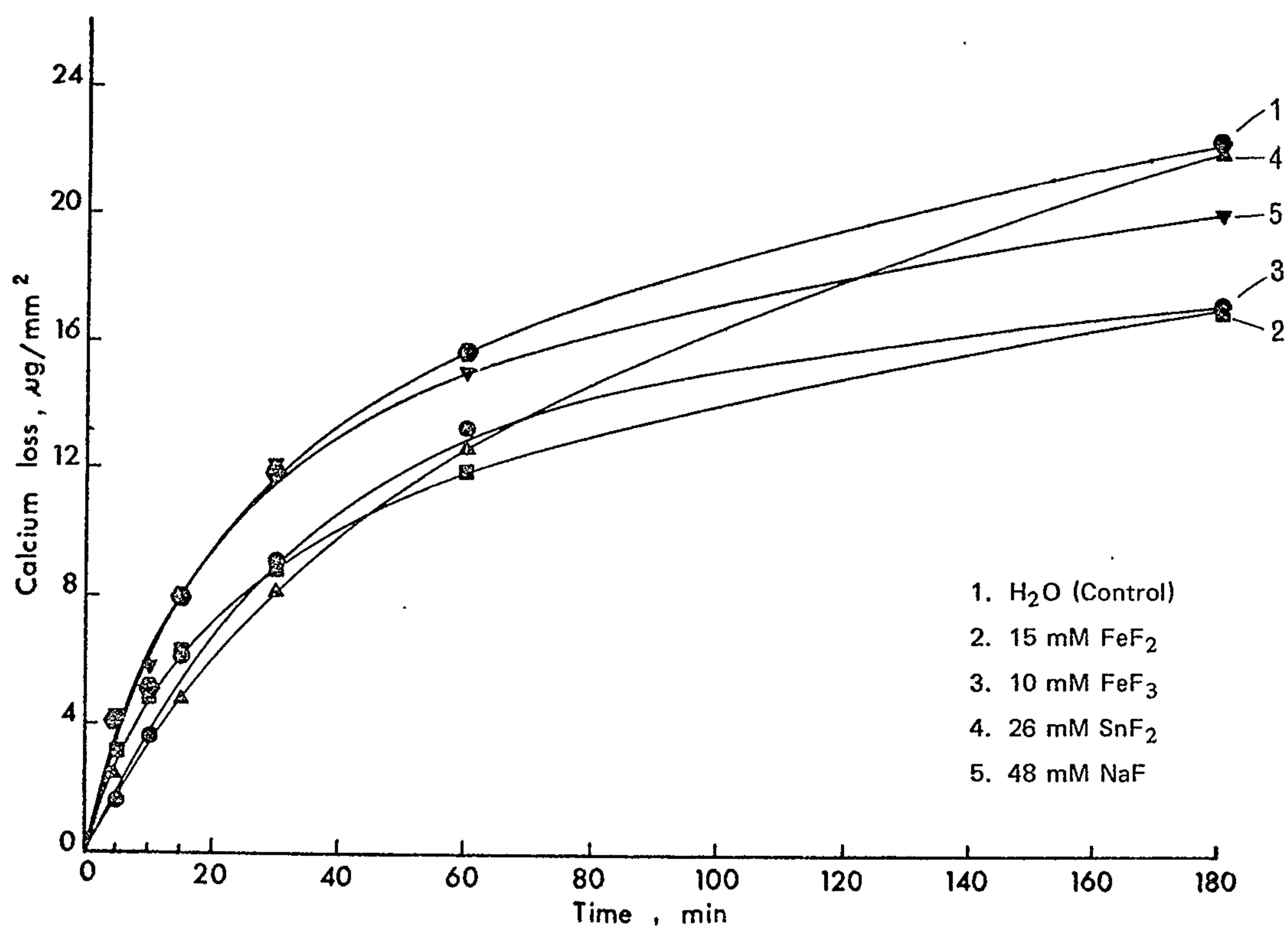


Fig. 14. Dissolution rates of enamel (based on calcium loss) in pH 4.5 lactate buffer following one stage topical treatments with test compounds containing fluoride.

A (top). Effect of topical treatment with 15 mM iron (II) fluoride, 10 mM iron (III) fluoride, 26 mM tin (II) fluoride or 48 mM sodium fluoride (treatment time — five minutes).

B (right). Effect of topical treatment with APF, 511 mM tin (II) fluoride, 476 mM sodium fluoride or a solution containing 50 mM iron (III) chloride and 190 mM sodium fluoride (treatment time — five minutes).



from enamel at the end of the three hour immersion period in lactate buffer. A comparison of the calcium loss values from treated enamel with that from control enamel was made using Dunnett's t test.

Table 5-A shows that three of the six test compounds without fluoride, and four of the eight test compounds with fluoride, significantly reduced calcium loss from enamel at three hours.

Although the topical treatment with 100 mM iron (III) chloride resulted in a 28.3 per cent reduction in calcium loss from enamel in lactate buffer (Table 5-A), which was significant at the 0.01 level, Table 6-A shows that the treatment itself caused a marked calcium loss from enamel. In contrast, the other two non-fluoride topical treatments that significantly reduced calcium loss from enamel in lactate buffer, 10 mM iron (II) chloride ($P < 0.05$) and 100 mM iron (II) chloride ($P < 0.01$), caused only a minor amount of calcium loss from enamel during the five minute topical treatment period (Table 6-A).

The APF solution and the solution containing 50 mM iron (III) chloride and 190 mM sodium fluoride were both quite effective in reducing calcium loss from enamel in lactate buffer (Table 5-A). The reductions obtained, 36.2 per cent and 35.7 per cent respectively, were both significant at the 0.01 level. However, both solutions, especially APF, caused an appreciable calcium loss from enamel at the topical treatment stage (Table 6-A).

The two test compounds with the lowest fluoride concentrations, 15 mM iron (II) fluoride and 10 mM iron (III) fluoride, reduced calcium loss by 22.8 per cent and 22.6 per cent respectively (Table 5-A). Both reductions were significant at the 0.05 level. Furthermore, neither solution caused appreciable calcium loss from enamel at the

TABLE 5

CALCIUM AND PHOSPHORUS LOSS FROM TREATED ENAMEL AFTER
THREE HOUR IMMERSION PERIOD IN pH 4.5 LACTATE BUFFER

A. Values ^a for Enamel Treated with a Single Application of
Test Compounds (One Stage Treatments)

Treatment	Calcium Loss ($\mu\text{g}/\text{mm}^2$) ^b	Difference ($\mu\text{g}/\text{mm}^2$)	Difference (per cent)	Ca/P (molar) Ratio ^c
H ₂ O (Control)	22.48 (S.D. = 2.07)	—	—	1.59
1 mM FeCl ₂ . 4H ₂ O	24.91 (S.D. = 3.41)	+2.43	- 10.8	1.48
10 mM FeCl ₂ . 4H ₂ O	17.61 (S.D. = 1.53)	4.87 *	21.7	1.48
100mM FeCl ₂ . 4H ₂ O	17.14 (S.D. = 1.24)	5.34 **	23.8	1.42
1 mM FeCl ₃ . 6H ₂ O	19.57 (S.D. = 3.38)	2.91	12.9	1.56
10 mM FeCl ₃ . 6H ₂ O	19.97 (S.D. = 3.78)	2.51	11.2	1.50
100mM FeCl ₃ . 6H ₂ O	16.11 (S.D. = 4.34)	6.37 **	28.3	1.44
15 mM FeF ₂	17.36 (S.D. = 1.62)	5.12 *	22.8	1.49
10 mM FeF ₃	17.40 (S.D. = 3.12)	5.08 *	22.6	1.50
50 mM FeCl ₃ . 6H ₂ O + 190mM NaF	14.45 (S.D. = 0.70)	8.03 **	35.7	1.58
APF	14.35 (S.D. = 2.10)	8.13 **	36.2	1.66
26 mM SnF ₂	22.18 (S.D. = 2.57)	0.30	1.3	1.45
511mM SnF ₂	19.53 (S.D. = 2.06)	2.95	13.1	1.53
48 mM NaF	20.34 (S.D. = 2.56)	2.14	9.5	1.50
476mM NaF	18.20 (S.D. = 1.46)	4.28	19.0	1.41

* Significant at 0.05 level (Dunnett's t test ^d).

** Significant at 0.01 level.

^a Phosphorus loss shown as Ca/P (molar) ratio.

^b Each value represents mean of five specimens (S.D. = Standard deviation).

^c Each value represents mean of two specimens.

^d Using tables in Biometrics 20, 482-491 (1964).

TABLE 5 (cont.)

B. Values ^a for Enamel Treated with Iron (II) Chloride or Iron (III) Chloride Prior to Topical Treatment with APF (Two Stage Treatments)

Pretreatment	Calcium Loss ($\mu\text{g}/\text{mm}^2$) ^b	Difference ($\mu\text{g}/\text{mm}^2$)	Difference (per cent)	Ca/P (molar) Ratio ^c
None (APF alone)	14.35 (S.D. = 2.10)	—	—	1.66
1 mM FeCl ₂ · 4H ₂ O	12.51 (S.D. = 3.06)	1.84	12.8	1.88
10 mM FeCl ₂ · 4H ₂ O	13.80 (S.D. = 1.48)	0.55	3.8	1.69
100mM FeCl ₂ · 4H ₂ O	13.54 (S.D. = 2.30)	0.81	5.6	1.89
1 mM FeCl ₃ · 6H ₂ O	13.48 (S.D. = 0.90)	0.87	6.1	1.52
10 mM FeCl ₃ · 6H ₂ O	12.70 (S.D. = 1.84)	1.65	11.5	1.76
100mM FeCl ₃ · 6H ₂ O	10.95 (S.D. = 2.04)	3.40 *	23.7	2.15

* Significant at 0.05 level (Dunnett's t test).

^a Phosphorus loss shown as Ca/P (molar ratio)

^b Each value represents mean of five specimens (S.D. = Standard deviation)

^c Each value represents mean of two specimens

C. Values ^a for Enamel Treated with Iron (II) Chloride or Iron (III) Chloride Prior to Topical Treatment with 476 mM NaF (Two Stage Treatments)

Pretreatment	Calcium Loss ($\mu\text{g}/\text{mm}^2$) ^b	Difference ($\mu\text{g}/\text{mm}^2$)	Difference (per cent)	Ca/P (molar) Ratio ^c
None (476 mM NaF alone)	18.20 (S.D. = 1.46)	—	—	1.41
1 mM FeCl ₂ · 4H ₂ O	16.65 (S.D. = 1.94)	1.55	8.5	1.69
10 mM FeCl ₂ · 4H ₂ O	19.82 (S.D. = 3.23)	+1.62	- 8.9	1.58
100mM FeCl ₂ · 4H ₂ O	17.28 (S.D. = 3.54)	0.92	5.1	1.46
1 mM FeCl ₃ · 6H ₂ O	16.48 (S.D. = 4.00)	1.72	9.5	1.46
10 mM FeCl ₃ · 6H ₂ O	15.17 (S.D. = 3.98)	3.03	16.6	1.50
100mM FeCl ₃ · 6H ₂ O	13.12 (S.D. = 1.48)	5.08 **	27.9	1.73

** Significant at the 0.01 level (Dunnett's t test).

^a Phosphorus loss shown as Ca/P (molar) ratio.

^b Each value represents mean of five specimens (S.D. = Standard deviation).

^c Each value represents mean of two specimens.

TABLE 6

CALCIUM LOSS FROM ENAMEL DURING TOPICAL TREATMENTS

A. One Stage Treatments (Single Application of Test Compounds)

Test Compounds	Calcium Loss ($\mu\text{g}/\text{mm}^2$) ^a
H ₂ O (Control)	0
1 mM FeCl ₂ · 4H ₂ O	0.08 (S.D. = 0.01)
10 mM FeCl ₂ · 4H ₂ O	0.03 (S.D. = 0.01)
100mM FeCl ₂ · 4H ₂ O	0.22 (S.D. = 0.02)
1 mM FeCl ₃ · 6H ₂ O	1.00 (S.D. = 0.68)
10 mM FeCl ₃ · 6H ₂ O	0.99 (S.D. = 0.34)
100mM FeCl ₃ · 6H ₂ O	5.50 (S.D. = 0.55)
15 mM FeF ₂	0.05 (S.D. = 0.01)
10 mM FeF ₃	0.02 (S.D. = 0.01)
50 mM FeCl ₃ · 6H ₂ O + 190mM NaF	1.16 (S.D. = 0.25)
APF	2.41 (S.D. = 0.30)
26 mM SnF ₂	0.37 (S.D. = 0.06)
511mM SnF ₂	0.57 (S.D. = 0.07)
48 mM NaF	0.12 (S.D. = 0.02)
476mM NaF	0.20 (S.D. = 0.06)

a Each value represents mean of five specimens
(S.D. = Standard deviation).

TABLE 6 (cont.)

**B. Enamel Treated with Iron (II) Chloride or Iron (III) Chloride
Prior to Topical Treatment with 476 mM NaF.**

Pretreatment	Total Calcium Loss ($\mu\text{g}/\text{mm}^2$) ^a
1 mM $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	2.00 (S.D. = 0.19)
10 mM $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	2.50 (S.D. = 0.53)
100mM $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	2.40 (S.D. = 0.51)
1 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	2.32 (S.D. = 0.32)
10 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	2.58 (S.D. = 0.11)
100mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	4.40 (S.D. = 0.25)

^a Calcium loss during pretreatment plus calcium loss during treatment with APF.
Each value represents mean of five specimens
(S.D. = Standard deviation).

**C. Enamel Treated with Iron (II) Chloride or Iron (III) Chloride
Prior to Topical Treatment with 476 mM NaF.**

Pretreatment	Total Calcium Loss ($\mu\text{g}/\text{mm}^2$) ^a
1 mM $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	0.29 (S.D. = 0.17)
10 mM $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	0.12 (S.D. = 0.08)
100mM $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	0.86 (S.D. = 0.48)
1 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.50 (S.D. = 0.09)
10 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.65 (S.D. = 0.18)
100mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	2.36 (S.D. = 0.40)

^a Calcium loss during pretreatment plus calcium loss during treatment with 476 mM NaF.
Each value represents mean of five specimens
(S.D. = Standard deviation).

topical treatment stage (Table 6-A).

Under the conditions of this experiment, neither sodium fluoride at concentrations of 48 mM or 476 mM, nor tin (II) fluoride at concentrations of 26 mM or 511 mM, significantly reduced calcium loss from enamel at three hours (Table 5-A).

Table 5 shows that at three hours the Ca/P ratios ranged from 1.41 to 1.66 and, as the data in the Appendix show, the Ca/P ratios remained reasonably constant throughout the three hour experimental period.

The effect of iron (II) chloride on enamel was so markedly different from that of iron (III) chloride during the five minute topical treatment period (Table 6-A) that an examination of enamel surfaces treated with these salts was made using scanning electron microscopy.

Additional enamel specimens were prepared and treated with either iron (II) chloride or iron (III) chloride at concentrations of 1 mM, 10 mM or 100 mM for five minutes. The specimens were air dried, coated with carbon then gold and examined in a scanning electron microscope.

The scanning electron micrographs presented in Figs. 15 to 18 show the different effects of the treatments on enamel. The surface of the enamel treated with 100 mM iron (II) chloride (Fig. 15-C) is covered with globular deposits whereas the surfaces treated with 1 mM iron (II) chloride and 10 mM iron (II) chloride (Figs. 15-A and 15-B respectively) have fewer deposits and are similar to the surface of the control enamel shown in Fig. 4-A in that the scratch marks left by the polishing procedure are still quite evident.

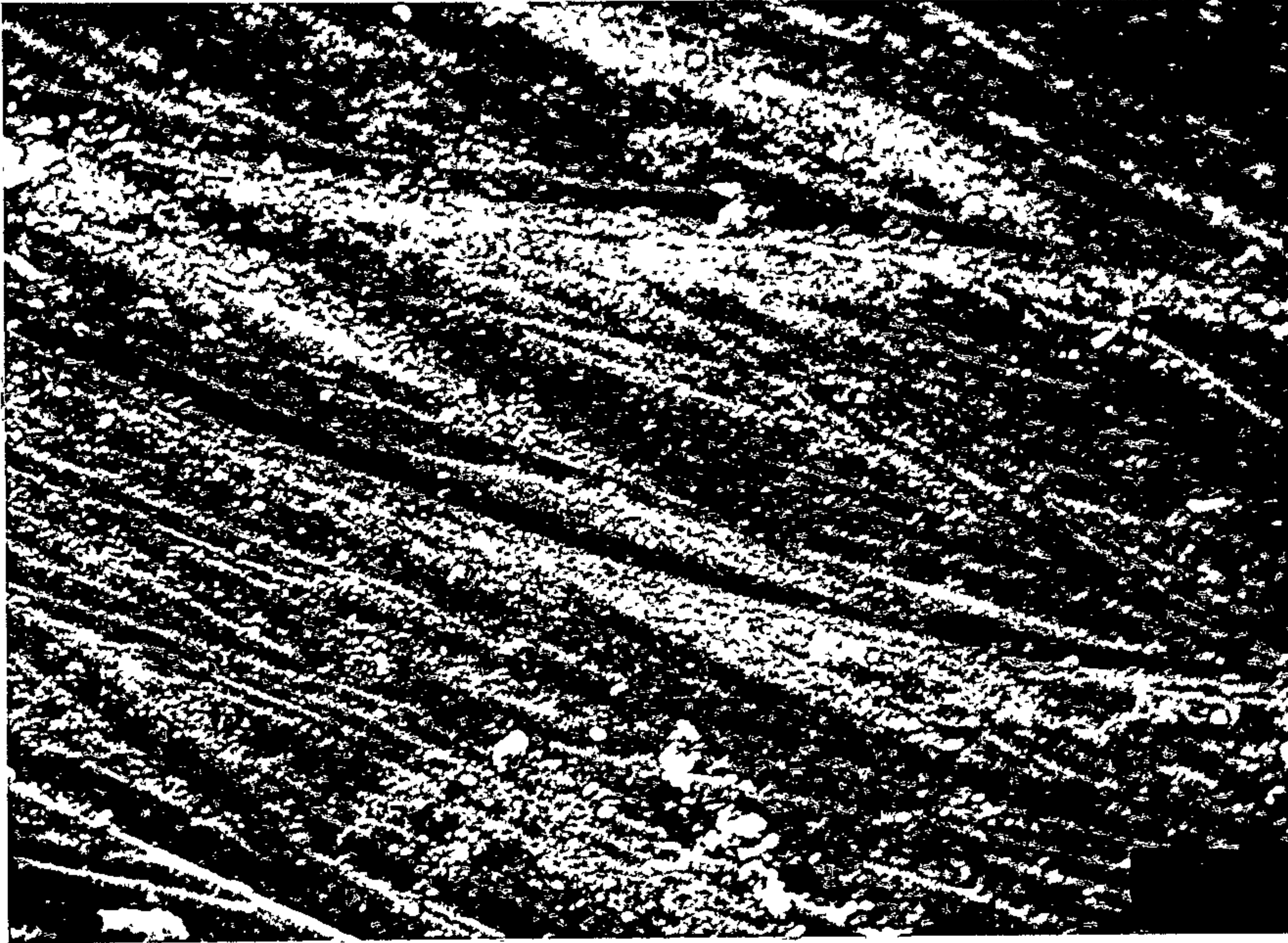


Fig. 15. Scanning electron micrographs of enamel treated with iron (II) chloride.

- A** (*above*). Enamel treated with 1 mM iron (II) chloride for five minutes. Some surface deposits are apparent. The scratch marks left by the polishing procedure are readily discernable. X 2,000.
- B** (*top right*). Enamel treated with 10 mM iron (II) chloride for five minutes. An appreciable clump as well as some isolated surface deposits can be seen. X 2,000.
- C** (*right*). Enamel treated with 100 mM iron (II) chloride for five minutes. The surface is covered with globular deposits. X 5,000.

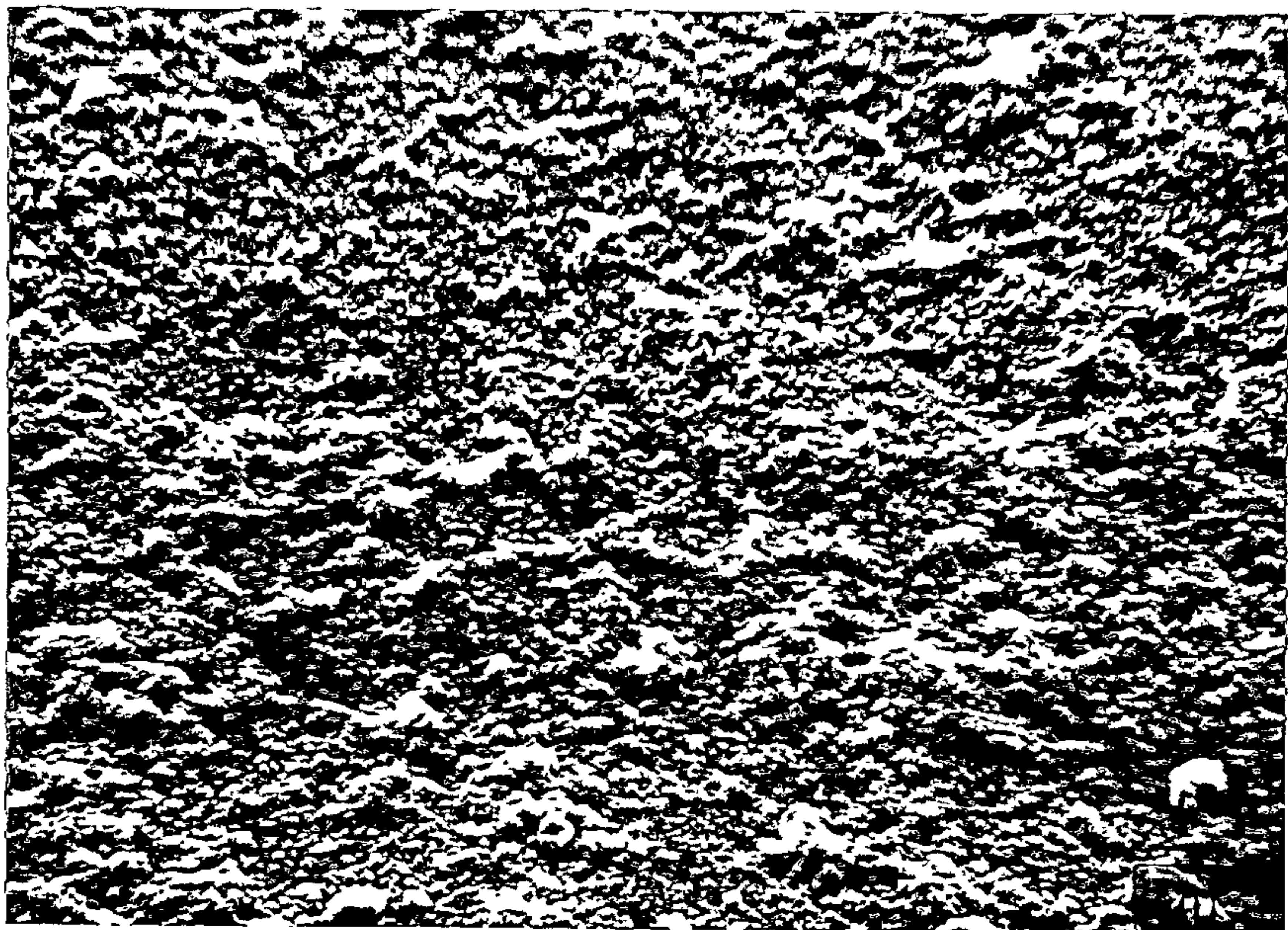


Fig. 16. Scanning electron micrographs of enamel treated with 1 mM iron (III) chloride for five minutes.

A (*top right*). Surface is covered with a deposit suggestive of a film that has cracked during drying. X 500.

B (*right*). A higher magnification view of Fig. 16-A. The portion of the surface not covered by the deposit appears to be etched. X 2,000.

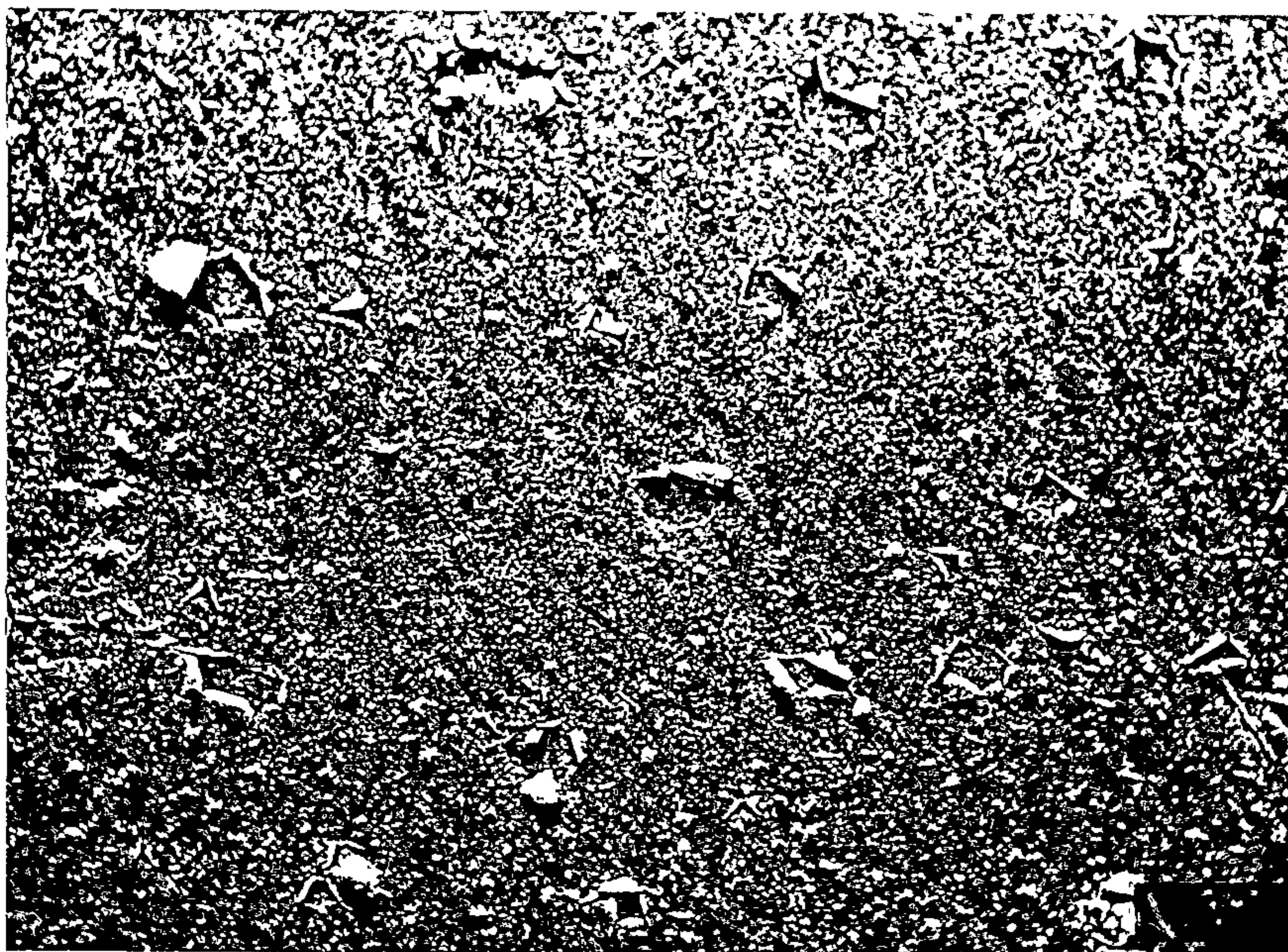


Fig. 17. Scanning electron micrographs of enamel treated with 10 mM iron (III) chloride for five minutes.

A (*top right*). As in Fig. 16 the surface is covered with a deposit suggestive of a film that has cracked during drying. X 200.

B (*right*). A higher magnification view of Fig. 17-A. Clear evidence of etching is seen on the surface not covered by deposits. X 2,000.

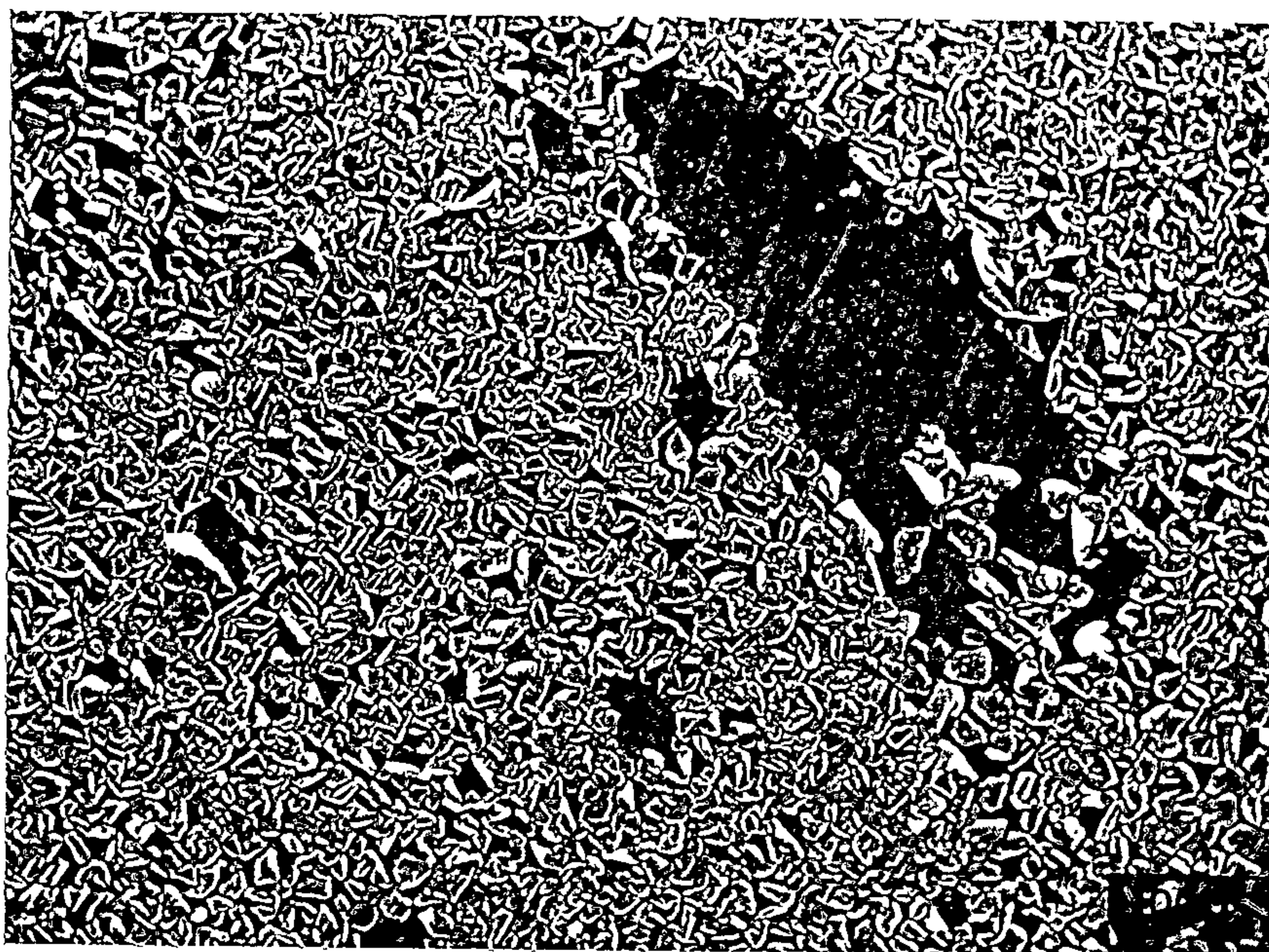
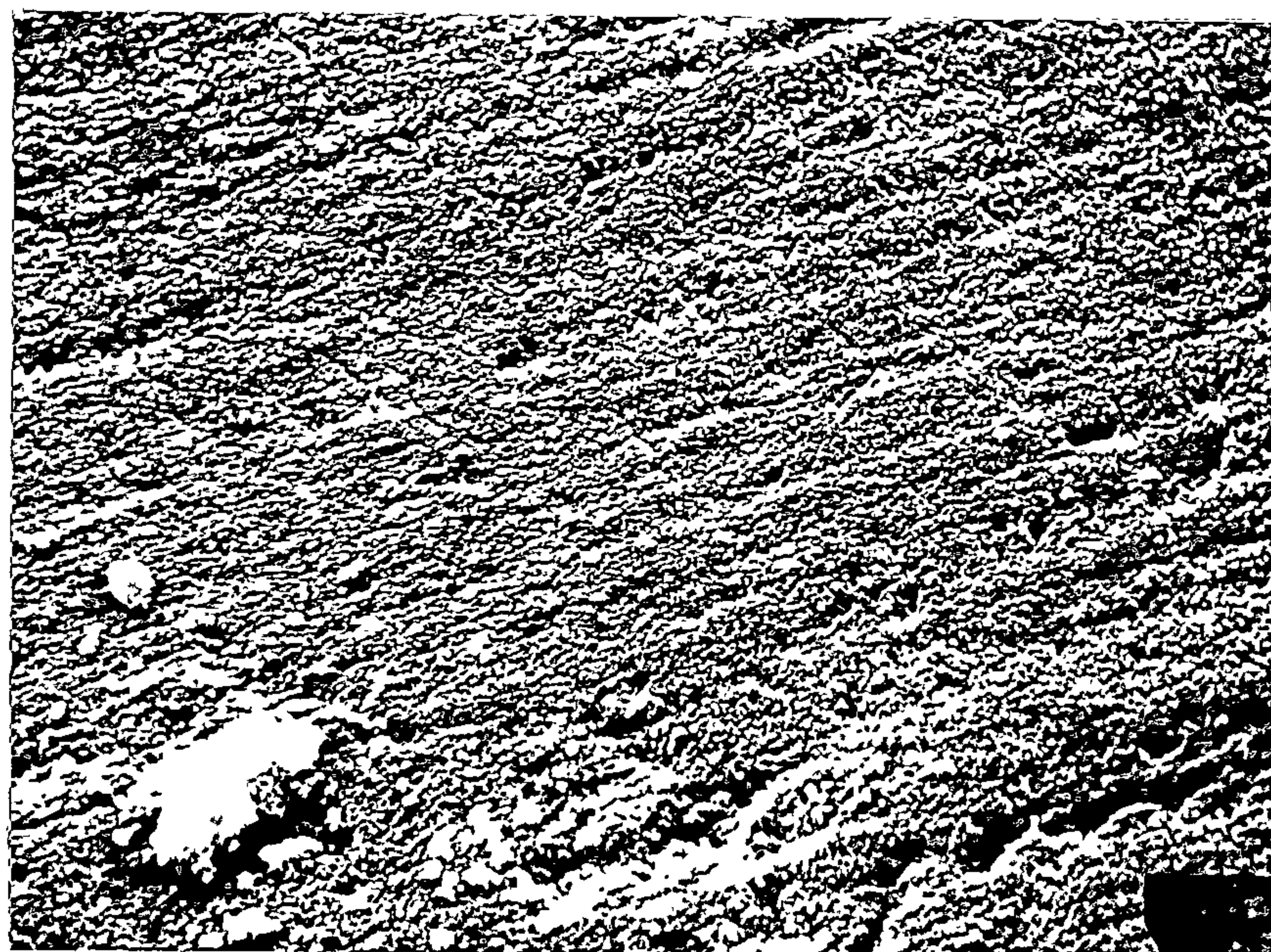


Fig. 18. Scanning electron micrograph of enamel treated with 100 mM iron (III) chloride for five minutes.

A (*top right*). A relatively thick clump of surface deposits and an area of roughened enamel can be seen. X 200.

B (*right*). A higher magnification view of Fig. 18-A showing an area not covered by deposits. The surface is appreciably etched. X 2,000.



In contrast, the surfaces of enamel treated with iron (III) chloride solutions (Figs. 16 to 18) all show deposits suggestive of a film that has cracked during drying. The deposit appears to be thickest on the enamel treated with 100 mM iron (III) chloride and thinnest on the enamel treated with 1 mM iron (III) chloride, whilst the areas not covered by deposits all appear to be etched.

Application of Iron Salts Followed by Topical Fluoride Treatment

Dissolution rate curves for enamel subjected to the various treatments are presented in Figs. 19 and 20. The curves were prepared from the data, summarised in the Appendix, on calcium loss from enamel during the three hour immersion period in pH 4.5 lactate buffer.

The curves show that after 60 minutes, the dissolution rates of enamel treated with iron salts prior to topical treatments with APF or 476 mM sodium fluoride were very similar and tended to parallel those of enamel treated with the respective fluoride solutions alone. In most instances, the main differences in dissolution rates occurred prior to this time.

Data on calcium and phosphorus loss from enamel after three hours immersion in pH 4.5 lactate buffer are presented in Tables 5-B and 5-C.

Comparisons between the calcium losses from pretreated enamel and from enamel treated with either APF or 476 mM sodium fluoride alone were made using Dunnett's t test.

Tables 5-B and 5-C show that of all the treatment combinations tested, only the treatments with 100 mM iron (III) chloride followed by APF or 476 mM sodium fluoride significantly reduced the calcium loss

Fig. 19. Dissolution rates of enamel (based on calcium loss) in pH 4.5 lactate buffer following two stage topical treatments with iron (II) chloride or iron (III) chloride and APF.

- A (top).** Effect of two stage treatments comprising a one minute topical treatment with iron (II) chloride at a concentration of 1 mM, 10 mM or 100 mM followed by a four minute topical treatment with APF.
- B (right).** Effect of two stage treatments comprising a one minute topical treatment with iron (III) chloride at a concentration of 1 mM, 10 mM or 100 mM followed by a four minute topical treatment with APF.

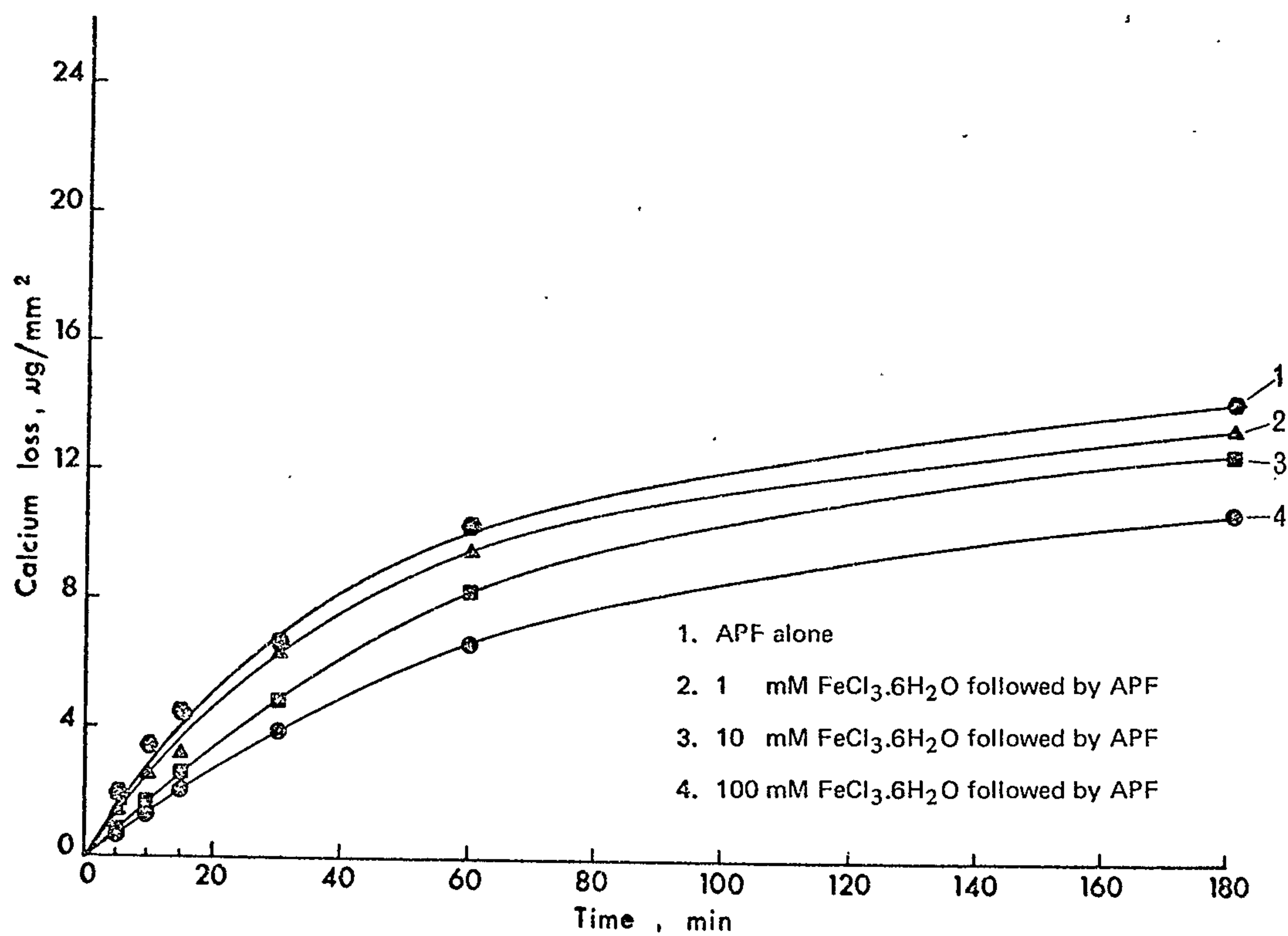
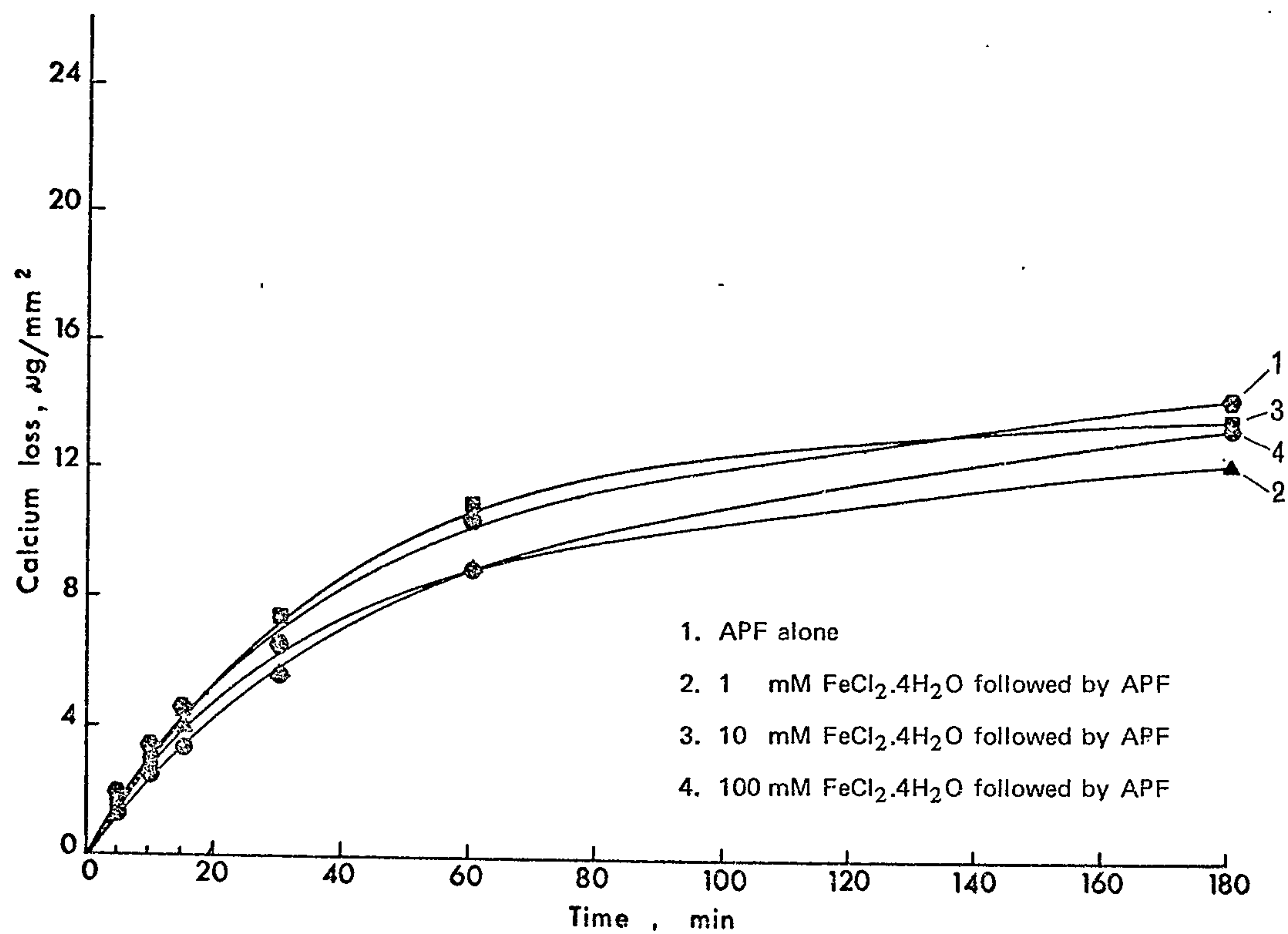
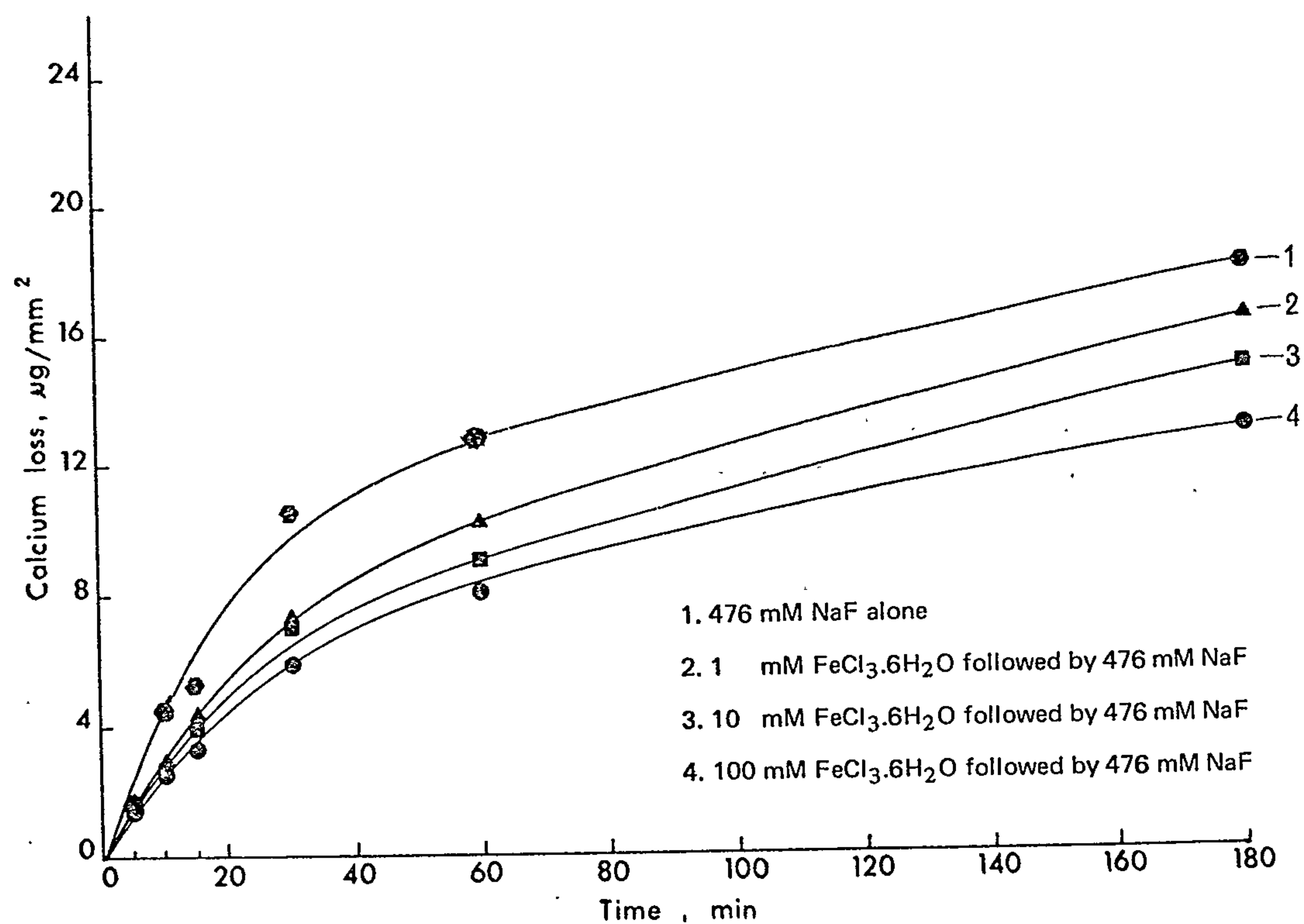
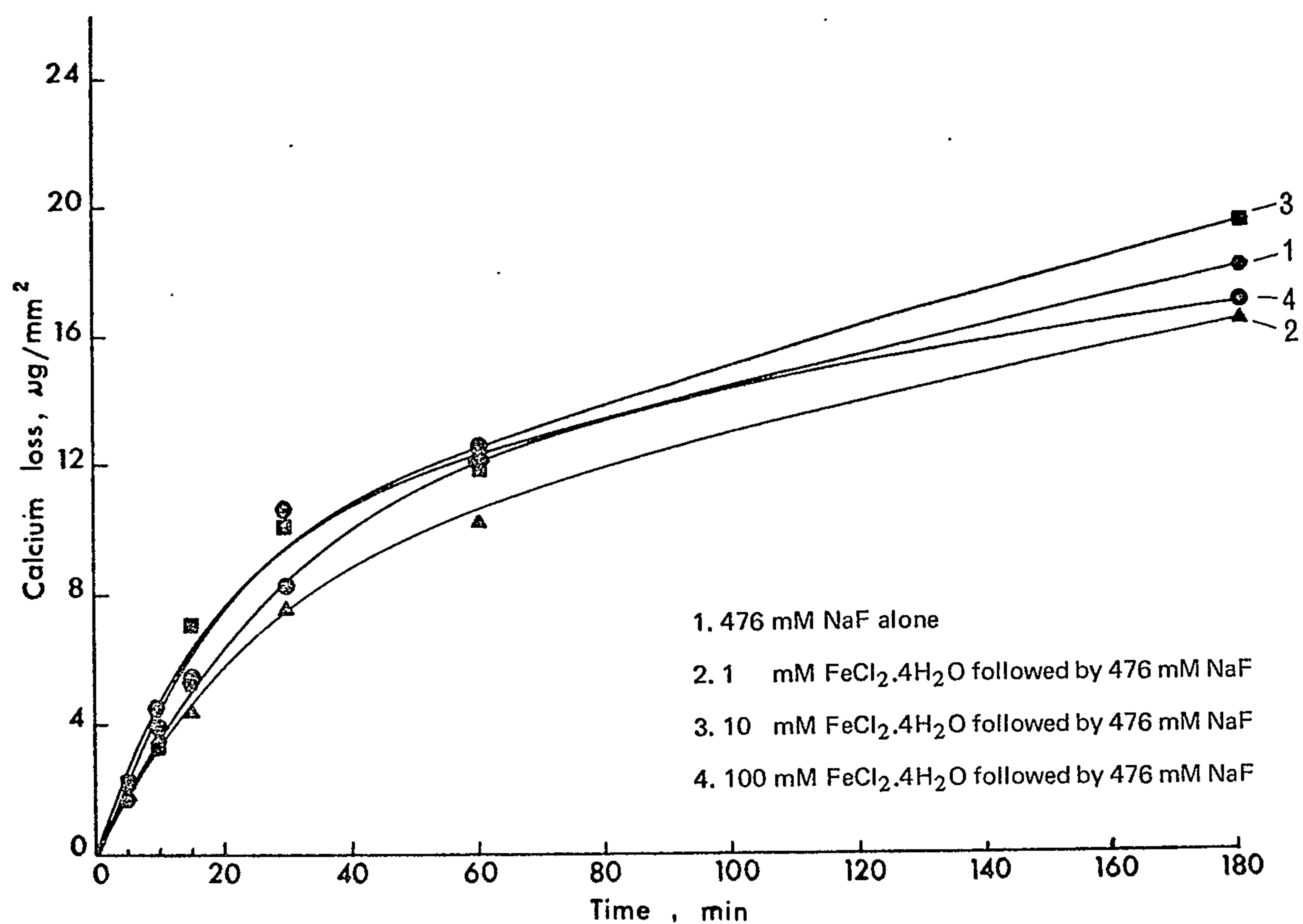


Fig. 20. Dissolution rates of enamel (based on calcium loss) in pH 4.5 lactate buffer following two stage topical treatments with iron (II) chloride or iron (III) chloride and 476 mM sodium fluoride.

- A (*top*). Effect of two stage treatments comprising a one minute topical treatment with iron (II) chloride at a concentration of 1 mM, 10 mM or 100 mM followed by a four minute topical treatment with 476 mM sodium fluoride.
- B (*right*). Effect of two stage treatments comprising a one minute topical treatment with iron (III) chloride at a concentration of 1 mM, 10 mM or 100 mM followed by a four minute topical treatment with 476 mM sodium fluoride.



from enamel more than the treatments with APF or 476 mM sodium fluoride alone ($P < 0.05$ and $P < 0.01$ respectively).

Tables 6-B and 6-C show the amount of calcium lost from enamel during the one minute application of iron (II) chloride or iron (III) chloride solutions and the subsequent four minute topical treatment with APF or 476 mM sodium fluoride.

It is noteworthy that the calcium loss from enamel during the treatment with 100 mM iron (III) chloride followed by APF was approximately twice that when APF was used alone. Furthermore, the calcium loss from enamel during the treatment with 100 mM iron (III) chloride followed by 476 mM sodium fluoride was approximately 12 times higher than that when 476 mM sodium fluoride was used alone.

The graphs in Fig. 21 are plotted from data on Ca/P ratios in the Appendix. Overall, there was a trend toward lower Ca/P ratios with time, especially from enamel treated with 10 mM iron (III) chloride or 100 mM iron (III) chloride followed by APF. However, the terminal Ca/P ratios still tended to be higher than those of enamel treated with APF or 476 mM sodium fluoride alone.

EXPERIMENT 2 - THE EFFECT OF ADDITION OF TEST COMPOUNDS TO LACTATE BUFFER ON ENAMEL DISSOLUTION RATE

Table 7 shows the dissolution rates of enamel in pH 4.5 lactate buffer containing Fe^{2+} , Fe^{3+} , Sn^{2+} , Ca^{2+} or PO_4^{3-} ions at a final concentration of 10 μM , 100 μM or 1 mM. Also presented in Table 7 are the corresponding data obtained when the buffer contained iron (II)

Fig. 21. Graphs showing Ca/P (molar) ratios in pH 4.5 lactate buffer following the immersion of enamel subjected to two stage topical treatments. Values at the end of six immersion periods (5, 10, 15, 30, 60 and 180 minutes) are presented.

- A. Enamel treated with iron (II) chloride at a concentration of 1 mM, 10 mM or 100 mM prior to a topical treatment with APF.
- B. Enamel treated with iron (III) chloride at a concentration of 1 mM, 10 mM or 100 mM prior to a topical treatment with APF.
- C. Enamel treated with iron (II) chloride at a concentration of 1 mM, 10 mM, or 100 mM prior to a topical treatment with 476 mM sodium fluoride.
- D. Enamel treated with iron (III) chloride at a concentration of 1 mM, 10 mM or 100 mM prior to a topical treatment with 476 mM sodium fluoride.

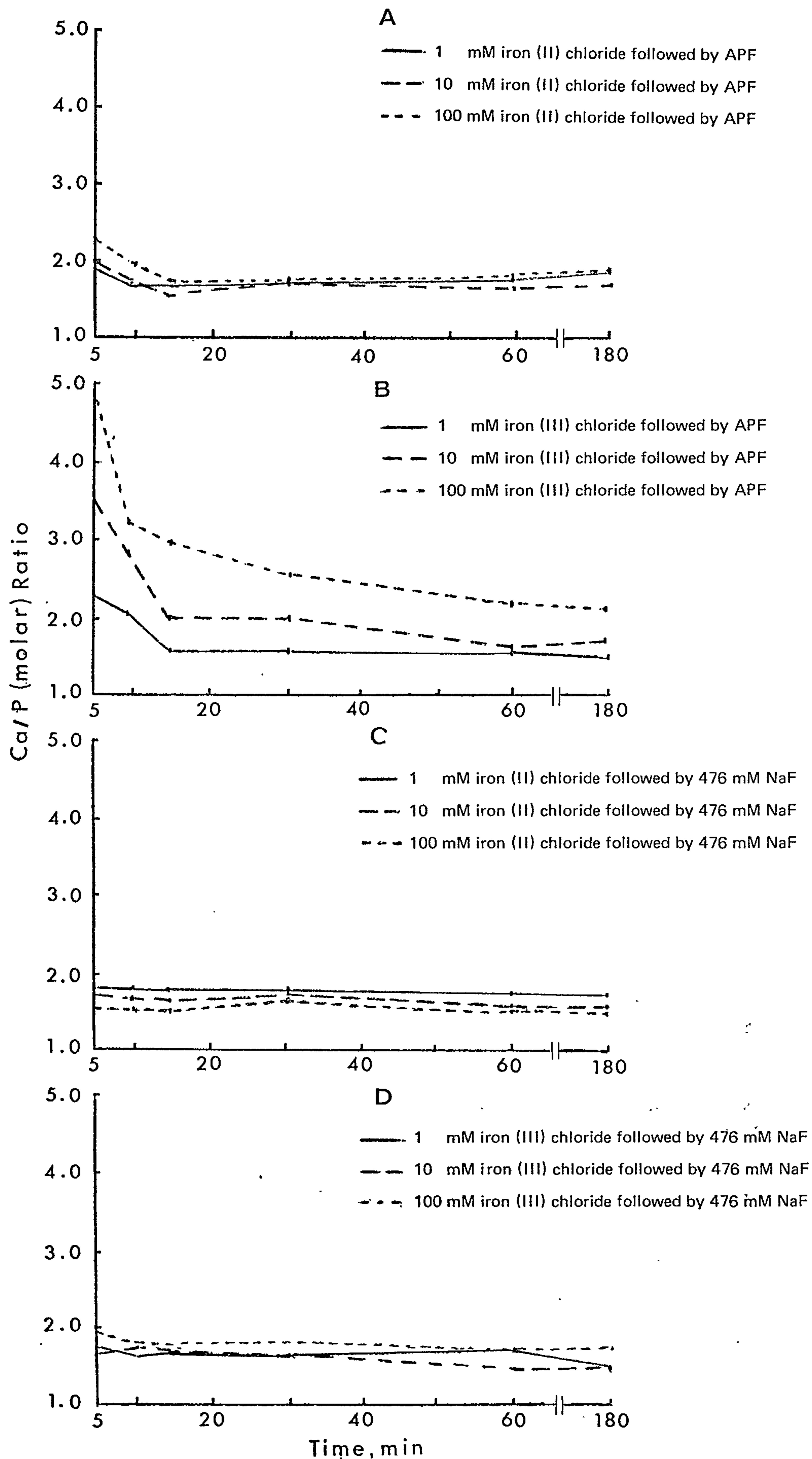


TABLE 7

DISSOLUTION RATE OF ENAMEL IMMERSSED^a IN pH 4.5 LACTATE BUFFER
CONTAINING ADDED CATIONS OR ANIONS

Added Cation or Anion	Concentration of Added Cation or Anion							
	10 μ M				100 μ M			
	Calcium Loss (μ g/mm ²) ^b	Difference (μ g/mm ²)	Difference (per cent)		Calcium Loss (μ g/mm ²) ^b	Difference (μ g/mm ²)	Difference (per cent)	
Na ⁺ (Control)	10.73 (S.D. = 0.44)	—	—		10.90 (S.D. = 0.46)	—	—	
Fe ²⁺	10.77 (S.D. = 1.19)	+ 0.04	- 0.4		8.76 (S.D. = 0.02)	2.14 *	19.6	43.4
Fe ³⁺	9.79 (S.D. = 1.32)	0.94	8.8		7.56 (S.D. = 0.51)	3.34 **	30.6	72.5
Sn ²⁺	10.58 (S.D. = 0.77)	0.15	1.4		7.11 (S.D. = 0.85)	3.79 **	34.8	88.9
Ca ²⁺	10.80 (S.D. = 1.01)	- 0.07	- 0.7		10.48 (S.D. = 1.37)	0.42	3.9	- 0.7
PO ₄ ³⁻	11.40 (S.D. = 1.16)	- 0.67	- 6.2		10.00 (S.D. = 0.96)	0.90	8.3	4.4
F ⁻ (as NaF)	9.37 (S.D. = 0.44)	1.36	12.7		8.43 (S.D. = 0.65)	2.47 *	22.7	37.0
F ⁻ (as FeF ₂)	9.11 (S.D. = 0.43)	1.62	15.1		6.73 (S.D. = 0.61)	4.17 **	38.3	71.6
F ⁻ (as FeF ₃)	9.00 (S.D. = 0.41)	1.73	16.1		6.78 (S.D. = 0.49)	4.12 **	37.8	81.4
F ⁻ (as SnF ₂)	8.25 (S.D. = 0.39)	2.48 *	23.1		3.61 (S.D. = 0.32)	7.29 **	66.9	90.2

* Significant at 0.05 level (Dunnett's t test^c).

** Significant at 0.01 level.

a Immersion time 60 minutes.

b Each value represents mean of three specimens (S.D. = Standard deviation).

c Using tables in Biometrics 20, 482 - 491 (1964).

fluoride, iron (III) fluoride, tin (II) fluoride or sodium fluoride at a final fluoride concentration of 10 μM , 100 μM or 1 mM.

Three of the cations, Fe^{2+} , Fe^{3+} and, especially, Sn^{2+} , depressed the enamel dissolution rate when added to lactate buffer at concentrations of 100 μM or 1 mM. The effect was particularly marked at the high concentration. All the reductions were significant at the 0.01 level except for the reduction obtained with Fe^{2+} cations at a concentration of 100 μM which was significant at the 0.05 level (Dunnett's t test). However, when these cations were added to the buffer at a concentration of 10 μM , they had little effect.

With one exception, tin (II) fluoride, none of the fluoride salts significantly reduced the enamel dissolution rate when added to lactate buffer at a fluoride concentration of 10 μM . The reduction obtained with tin (II) fluoride at this concentration was significant at the 0.05 level.

All the fluoride salts tested significantly reduced the enamel dissolution rate when added to lactate buffer at a fluoride concentration of 100 μM and 1 mM ($P < 0.01$).

It is noteworthy that, at a fluoride concentration of 1 mM, iron (II) fluoride, iron (III) fluoride and tin (II) fluoride depressed the enamel dissolution rate more markedly than sodium fluoride and that, at a fluoride concentration of 100 μM , tin (II) fluoride still exerted a very pronounced effect.

Most of the specimens exhibited a surface "glaze" after immersion in lactate buffer containing either Sn^{2+} cations at a concentration of 1 mM, or iron (II) fluoride, iron (III) fluoride or tin (II) fluoride at a fluoride concentration of 1 mM. In order to investigate this situation

Fig. 22. Scanning electron micrographs of enamel immersed for 60 minutes in pH 4.5 lactate buffer containing added Na^{2+} or Ca^{2+} cations at a concentration of 1 mM.

A (*top*). Enamel immersed in lactate buffer containing added Na^{+} cations (control). The surface is heavily etched. X 1,000.

B (*right*). Enamel immersed in lactate buffer containing added Ca^{2+} cations. Appreciable surface etching is apparent. X 1,000.

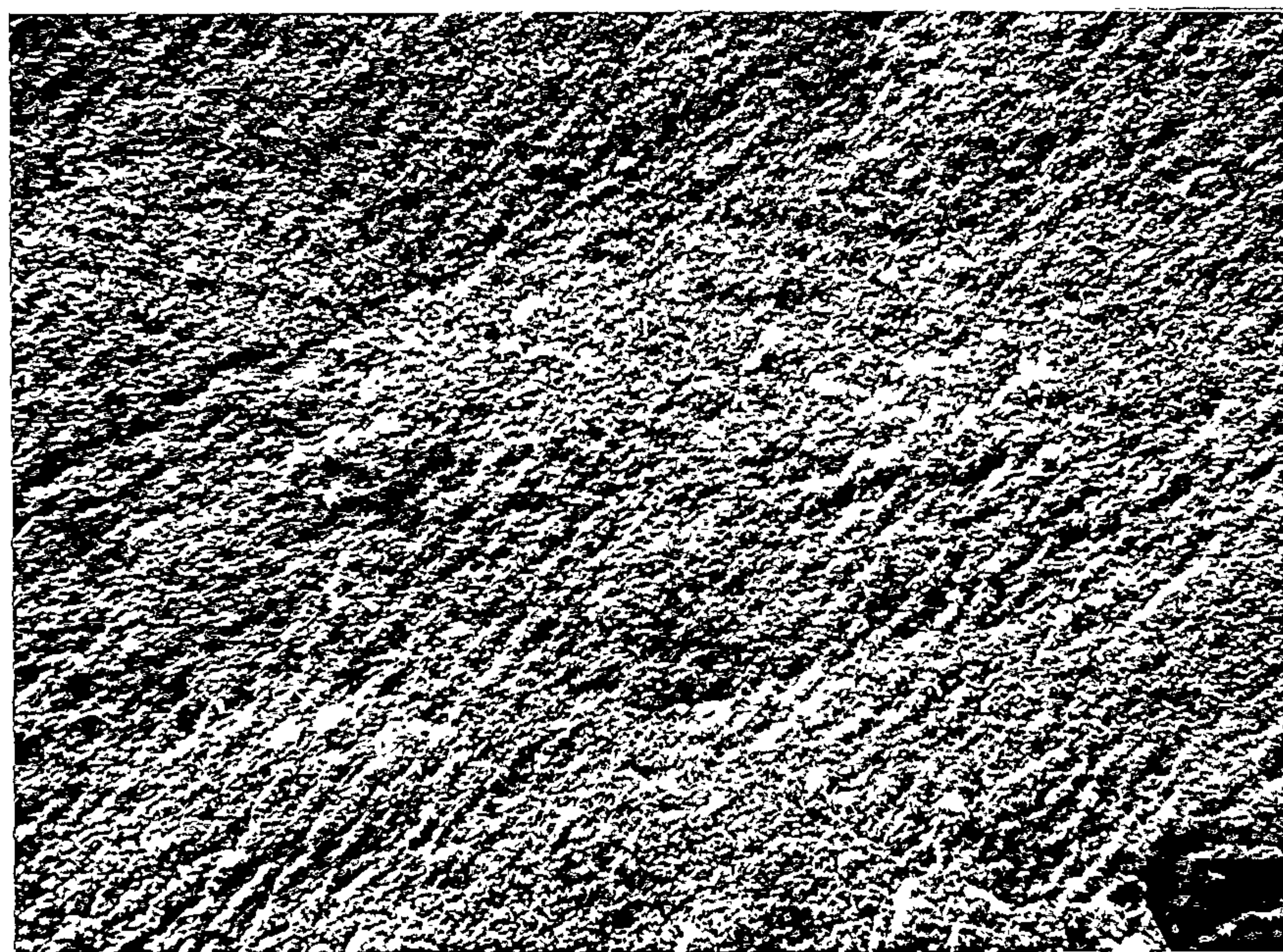
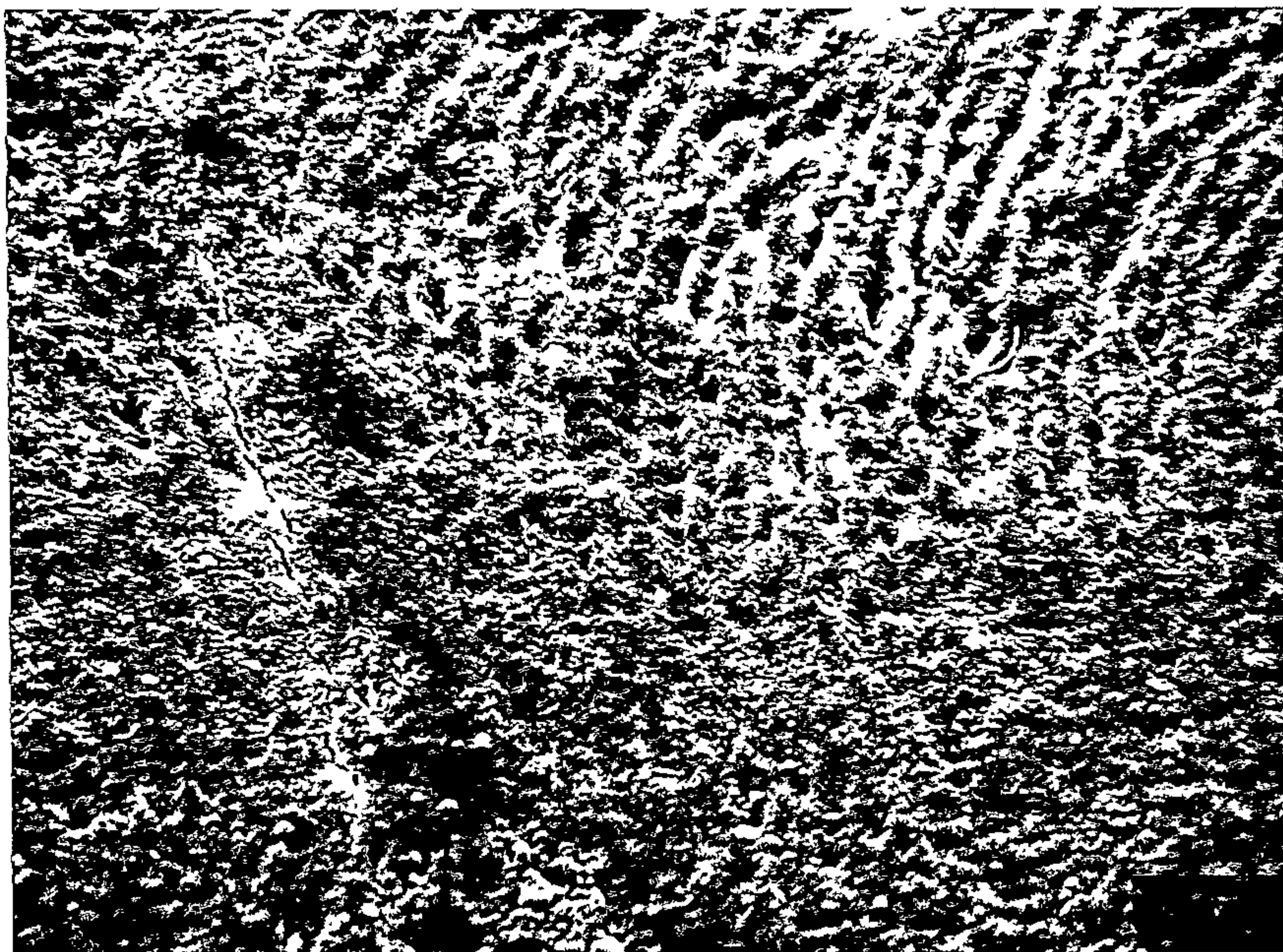


Fig. 23. Scanning electron micrographs of enamel immersed for 60 minutes in lactate buffer containing added PO_4^{3-} anions or Fe^{2+} cations at a concentration of 1 mM.

A (top). Enamel immersed in lactate buffer containing added PO_4^{3-} anions. The surface shows evidence of marked etching. X 1,000.

B (right). Enamel immersed in lactate buffer containing added Fe^{2+} cations. Some surface etching is evident. X 2,000.

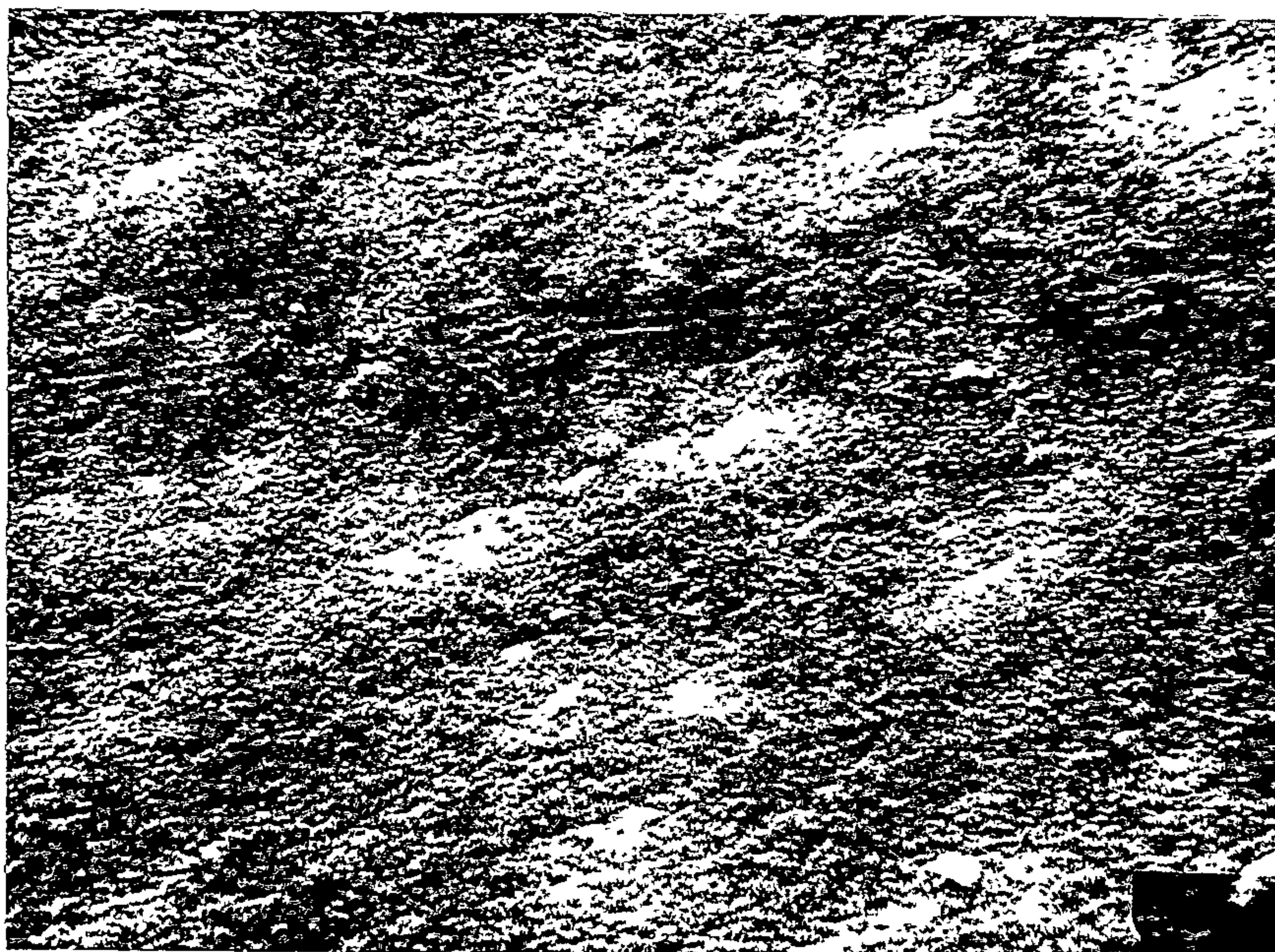
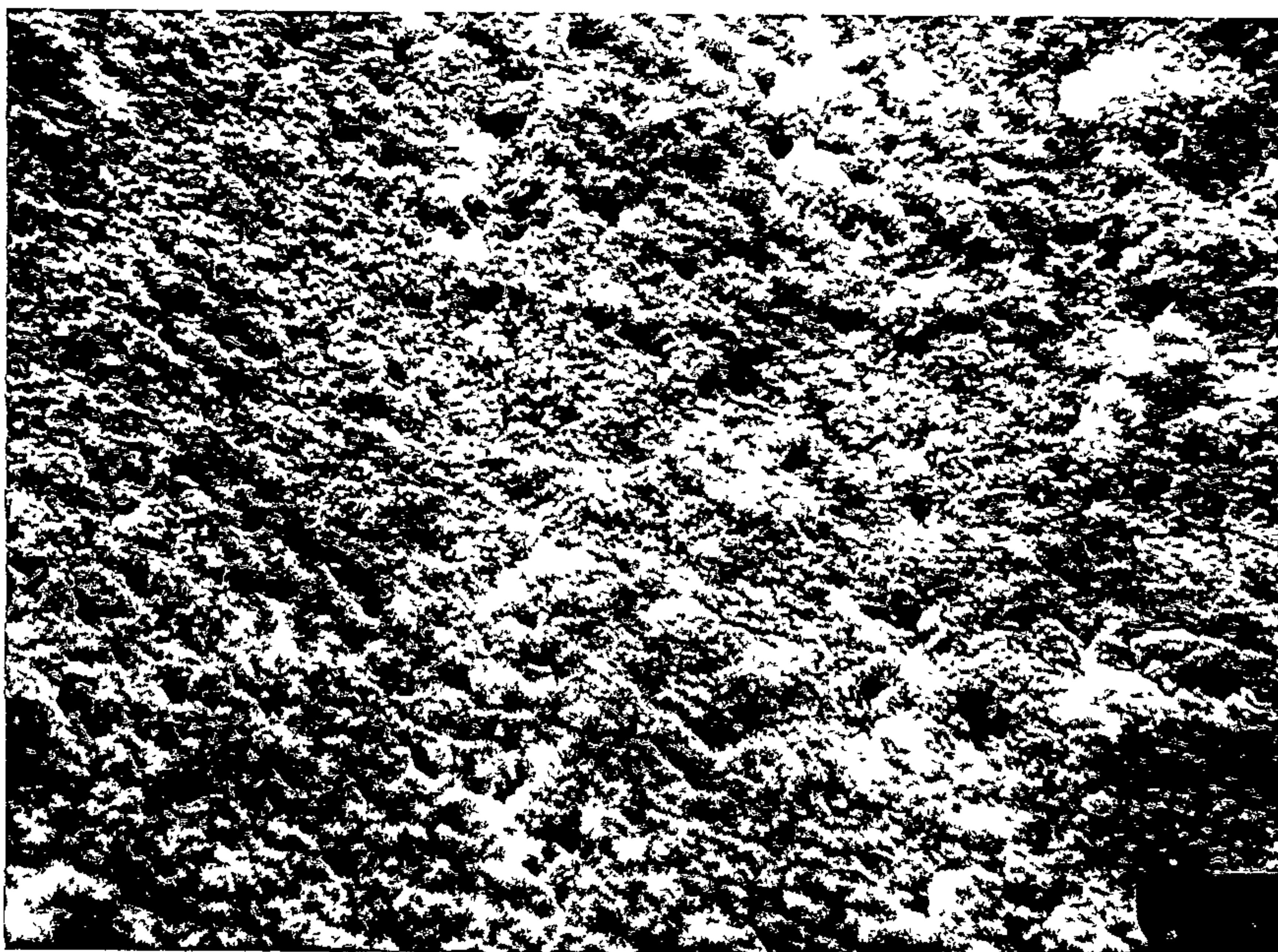


Fig. 24. Scanning electron micrographs of enamel immersed for 60 minutes in pH 4.5 lactate buffer containing added Fe^{3+} or Sn^{2+} cations at a concentration of 1 mM.

A (top). Enamel immersed in lactate buffer containing added Fe^{3+} cations. Some surface deposits, as well as some minor etching, can be seen. The scratch marks left by the polishing procedure are evident. X 2,000.

B (right). Enamel immersed in lactate buffer containing added Sn^{2+} cations. A few scattered deposits can be seen. The surface appears to be slightly etched. X 2,000.

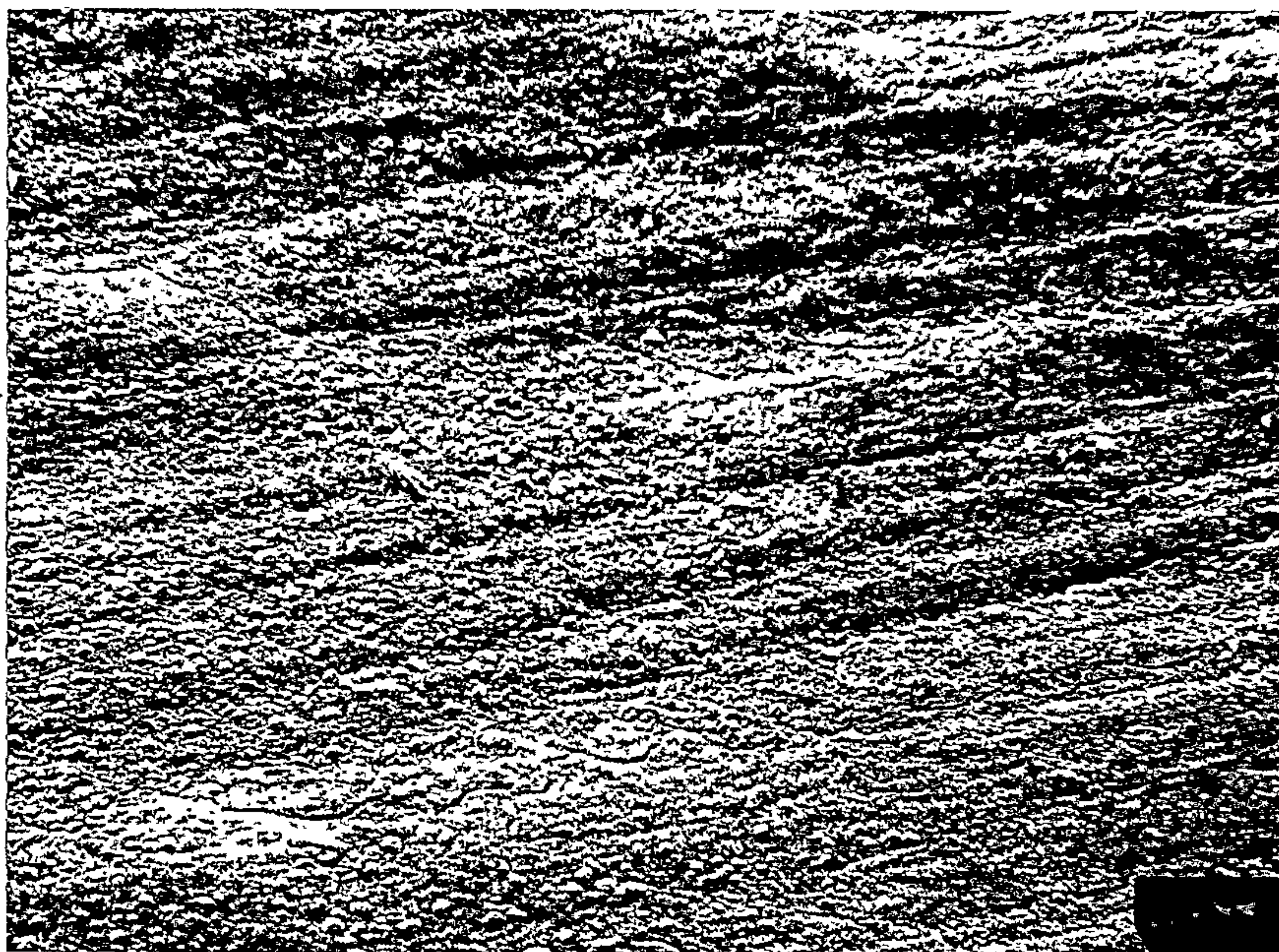


Fig. 25. Scanning electron micrographs of enamel immersed for 60 minutes in pH 4.5 lactate buffer containing added F^- anions at a concentration of 1 mM.

A (top). Enamel immersed in lactate buffer containing F^- anions added as sodium fluoride. Some surface etching is apparent. X 1,000.

B (right). Enamel immersed in lactate buffer containing F^- anions added as iron (II) fluoride. Some surface deposits are evident. The surface has a slightly etched appearance. X 1,000.

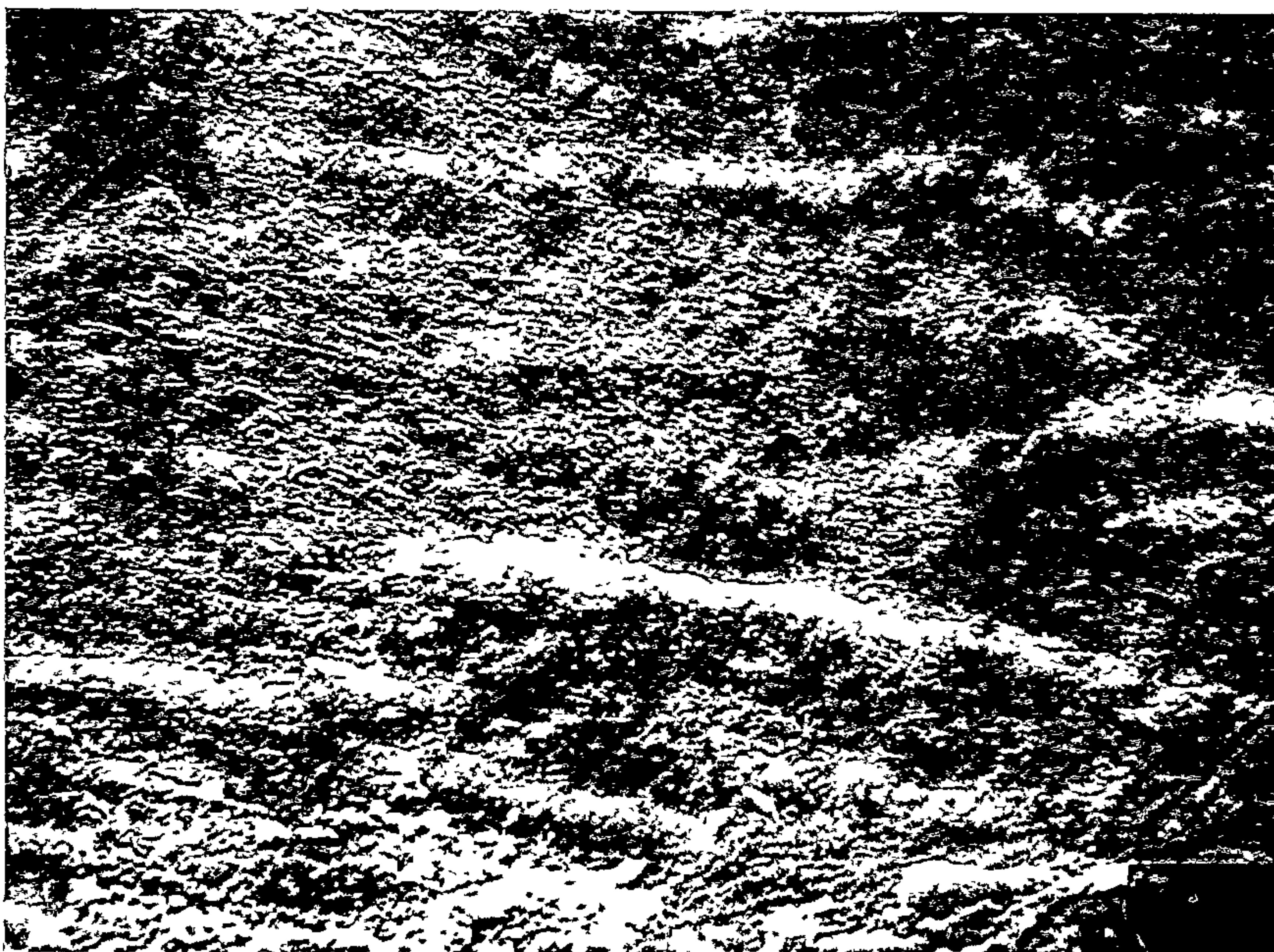
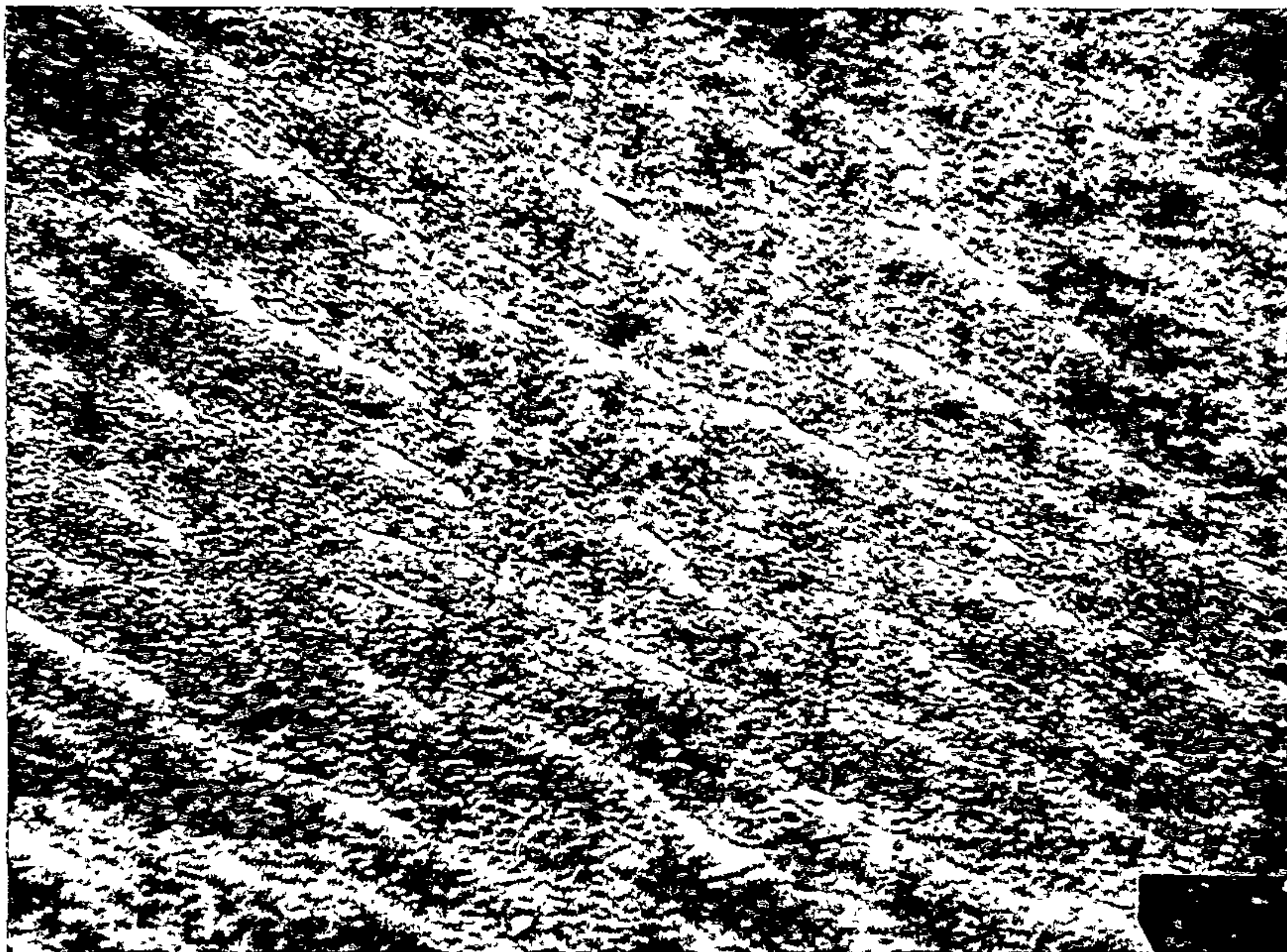
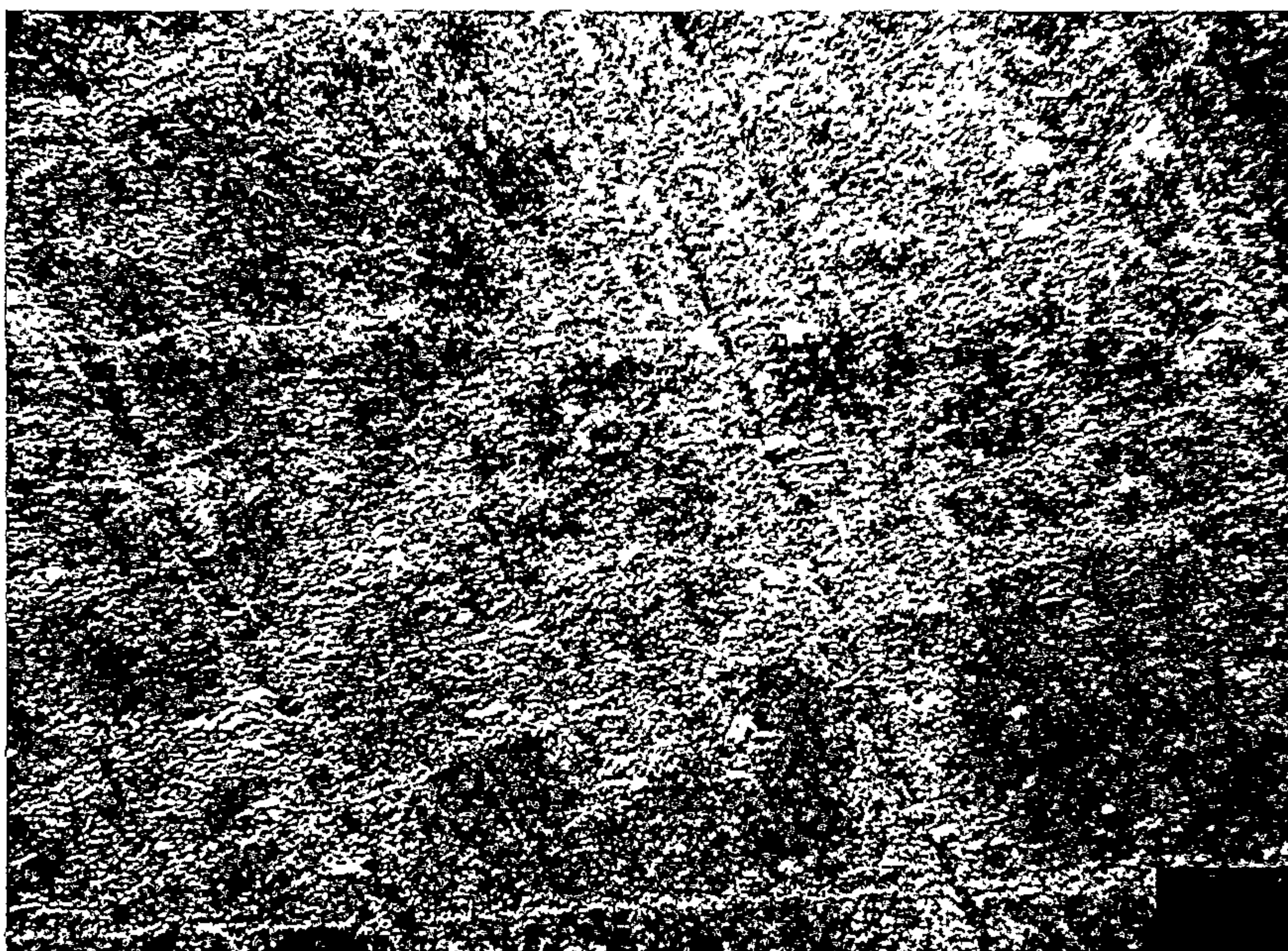
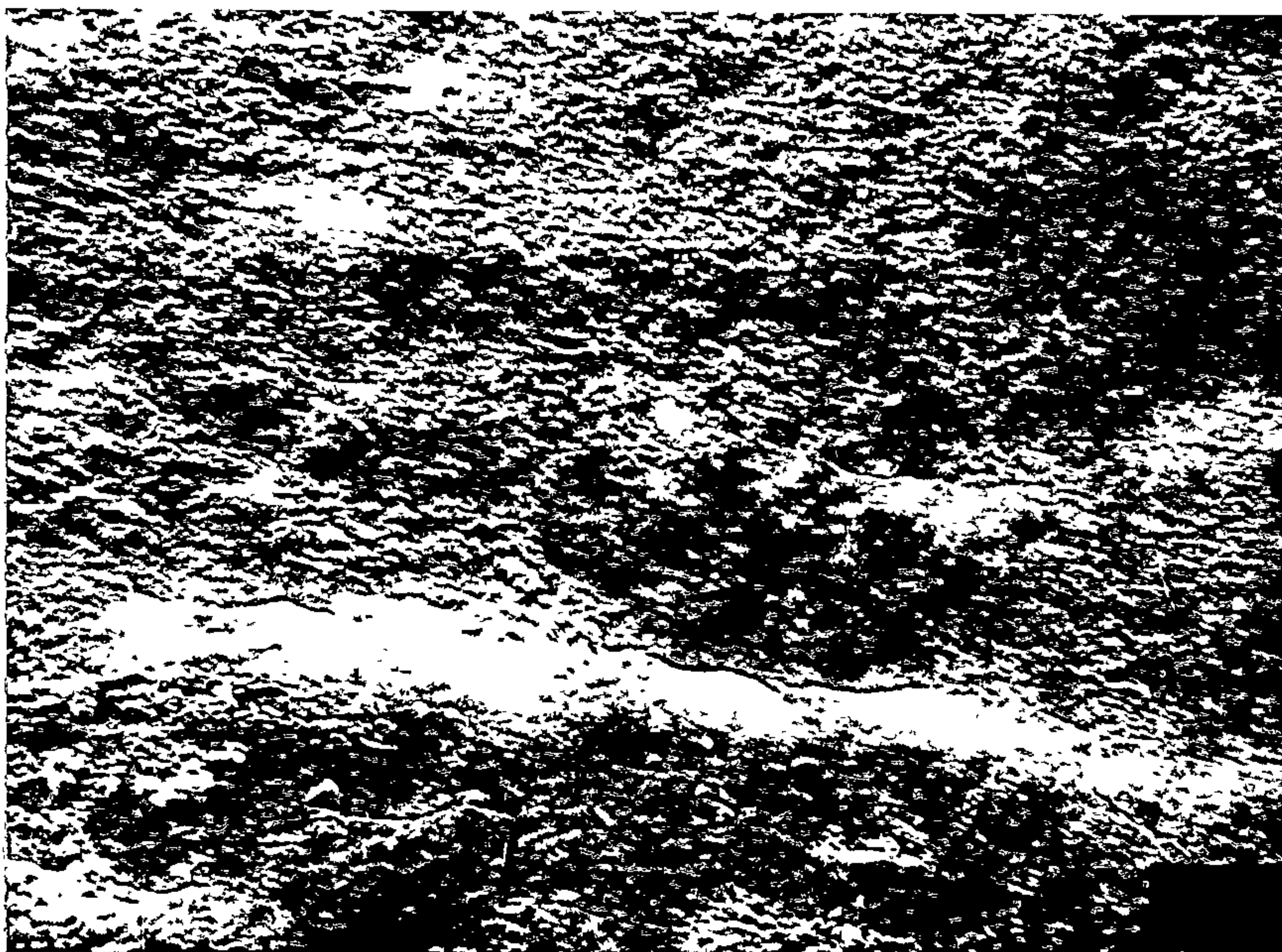


Fig. 26. Scanning electron micrographs of enamel immersed for 60 minutes in pH 4.5 lactate buffer containing added F^- anions at a concentration of 1 mM.

A (top). Enamel immersed in lactate buffer containing F^- anions added as iron (III) fluoride. There is an appreciable accumulation of surface deposits. There appears to be very little surface etching. X 2,000.

B (right). Enamel immersed in lactate buffer containing F^- anions added as tin (II) fluoride. Some surface deposits, as well as some very minor surface etching, are apparent. The scratch marks left by the polishing procedure are quite discernable. X 1,000.



further, all specimens immersed in buffer solutions containing test compounds at a concentration of 1 mM were air dried, coated with carbon then gold and examined in a scanning electron microscope. Examples of the findings are presented in Figs. 22 to 26.

Marked etching is evident on the control enamel (Fig. 22-A) and the enamel immersed in lactate buffer containing Ca^{2+} cations (Fig. 22-B) or PO_4^{3-} anions (Fig. 23-A) while some etching is evident on enamel immersed in buffer containing Fe^{2+} cations (Fig. 23-B) or sodium fluoride (Fig. 25-A).

Surface deposits, as well as some very minor etching, are evident on the surface of enamel immersed in buffer containing Fe^{3+} cations (Fig. 22-A), Sn^{2+} cations (Fig. 22-B), iron (II) fluoride (Fig. 25-B), iron (III) fluoride (Fig. 26-A) or tin (II) fluoride (Fig. 26-B).

DISCUSSION

Gray⁽⁸¹⁾, who developed the method used in this study, found that the dissolution rate of enamel in pH 4.5 lactate buffer decreases with time. No alternative explanation to the one presented by Gray⁽⁸¹⁾, Gray and Francis⁽⁸³⁾ and Francis *et al.*⁽⁶⁸⁾ was found in the literature to account for this situation. These workers suggested that as the reaction proceeds, the concentration of reaction products in the buffer increases to a level where some of these products are precipitated on the enamel as a new surface phase, possibly dicalcium phosphate dihydrate⁽⁸¹⁾⁽⁸³⁾ or calcium monohydrogen phosphate⁽⁶⁸⁾. As the new phase covers the more soluble underlying enamel, the reaction rate

decreases and the system then approaches equilibrium with the new phase, not with the underlying enamel.

In a later study, Gray⁽⁸²⁾, using a similar method, showed that enamel treated with sodium fluoride, tin (II) chloride or tin (II) fluoride tended to have an initial dissolution rate lower than that of untreated enamel. This was attributed to the presence of an apparently thin, protective layer of reaction products that had formed on enamel as a result of the topical treatments⁽⁶⁸⁾.

On the basis of these suggestions, it is possible to offer some tentative interpretations of the enamel dissolution rate curves obtained in this study.

Generally, the initial dissolution rates of enamel treated with test compounds tended to be less than that of control enamel. However, it was evident that the initial dissolution rate of enamel treated with 1 mM iron (III) chloride and that of enamel treated with 10 mM iron (III) chloride were particularly low. This suggests that the surface film seen in the scanning electron micrographs of enamel treated with these compounds (Figs. 16 and 17) may have been intact and have initially acted as a relatively effective protective layer.

Furthermore, it is noteworthy that the dissolution rates of enamel treated with fluoride compounds, except for tin (II) fluoride, tended to decrease noticeably from 30 to 60 minutes onwards. Therefore, it is possible that these treatments encouraged the precipitation of new surface phases which, in turn, reduced the enamel dissolution rate. This would be consistent with the observations of Kuyper and Kutnerian⁽¹²³⁾ that the precipitation of calcium and phosphate is enhanced in the presence of fluoride. In this respect the finding

that topical treatments with 100 mM iron (II) chloride as well as 100 mM iron (III) chloride tended to reduce the enamel dissolution rate appreciably after 30 to 60 minutes is of interest, in that it suggests that the iron moiety alone may have encouraged the precipitation process. This would be consistent with the findings of Bachra and van Harskamp⁽⁷⁾ who showed that the addition of Fe^{2+} or Fe^{3+} cations to a metastable calcification buffer caused the precipitation of calcium and phosphate under conditions where precipitation does not normally occur.

In contrast, it appears that the tin moiety in tin (II) fluoride may have tended to inhibit the precipitation of reaction products on enamel, since topical treatments with both 28 mM tin (II) fluoride and 511 mM tin (II) fluoride appeared to increase the enamel dissolution rate after 60 minutes. It has been found by Meyer and Nancollas⁽¹⁵²⁾ that both tin (II) fluoride and tin (II) chloride can inhibit crystal growth of hydroxyapatite, and it is possible that a similar inhibitory mechanism may have operated in this situation.

An examination of the findings of Gray⁽⁸²⁾ indicates that his dissolution rate curve for enamel treated with 50 mM sodium fluoride shows a similar trend to the one in this study for enamel treated with 48 mM sodium fluoride. However, in contrast to the results in this study with 26 mM tin (II) fluoride, Gray's dissolution rate curve for enamel treated with 25 mM tin (II) fluoride did not show an upward trend with time.

Gray⁽⁸²⁾ also tested a non-fluoride tin salt, 25 mM tin (II) chloride. Initially, the dissolution rate of enamel treated with 25 mM tin (II) chloride and that of enamel treated with 25 mM tin (II)

fluoride were comparable. However, as the reaction proceeded, the dissolution rate of enamel treated with 25 mM tin (II) chloride increased markedly.

In many of the studies designed to assess the effect of test compounds on enamel dissolution rates only a single acid exposure time has been used⁽³⁴⁾⁽³⁶⁾⁽¹⁴³⁾⁽¹⁵⁷⁾⁽¹⁵⁹⁾⁽¹⁹²⁾. The findings of this study support the observations of Gray⁽⁸²⁾ that widely differing results can be obtained by altering the acid exposure time. For example, the difference (per cent) in calcium loss between control enamel and enamel treated with 511 mM tin (II) fluoride (Appendix) varied with the acid exposure time as follows:

5 min	10 min	15 min	30 min	60 min	180 min
83.0	53.6	40.8	38.1	24.5	13.1

The use of short acid exposure times (15 minutes) by Muhler⁽¹⁵⁷⁾ and Shrestha *et al.*⁽¹⁹²⁾ may be one of the reasons these workers obtained reductions in enamel dissolution rates of 80 per cent and 54 per cent respectively after topical treatments with 511 mM (approx.) tin (II) fluoride.

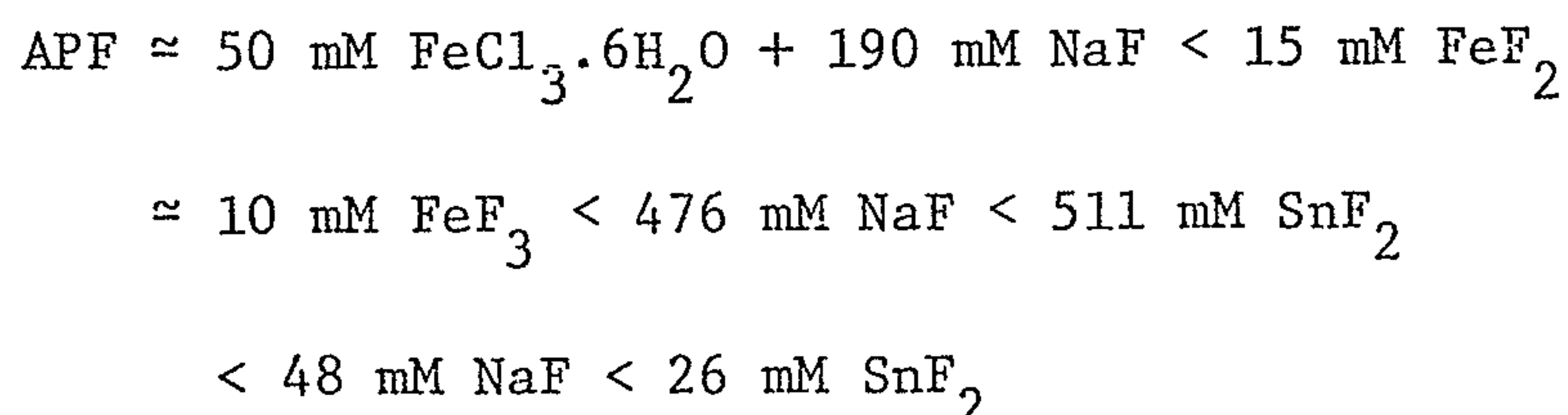
Of particular interest are the calcium loss values obtained at the end of the three hour experimental period. However, before discussing these findings, it must be emphasised that these values are not necessarily indicative of the effects of the treatments on enamel solubility *per se* because equilibrium conditions had not been reached at three hours.

The low calcium loss from enamel treated with 15 mM iron (II) fluoride and that from enamel treated with 10 mM iron (III) fluoride

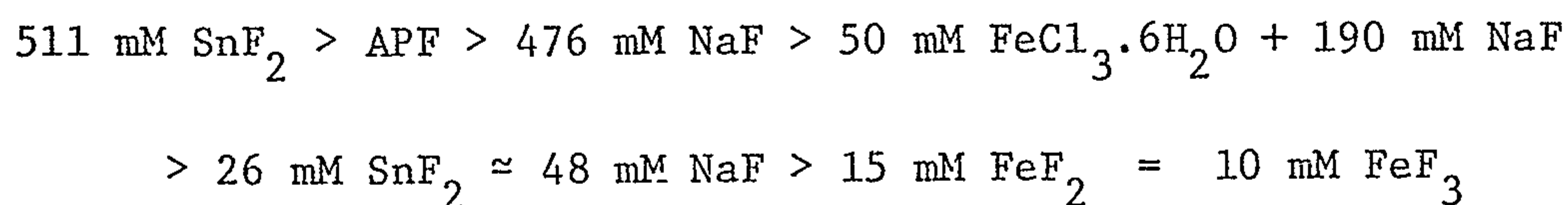
was unexpected in view of the comparatively low fluoride concentration of these solutions (570 ppm fluoride) and the relatively modest fluoride uptake by enamel from these solutions (Table 2).

Although no attempt was made to determine whether correlations existed between calcium loss from enamel and (i) fluoride concentrations in the treatment solutions or (ii) fluoride uptake by enamel, there did not appear to be any obvious relationship between these parameters. The possible exceptions were enamel specimens treated with APF or the solution containing 50 mM iron (III) chloride and 190 mM sodium fluoride.

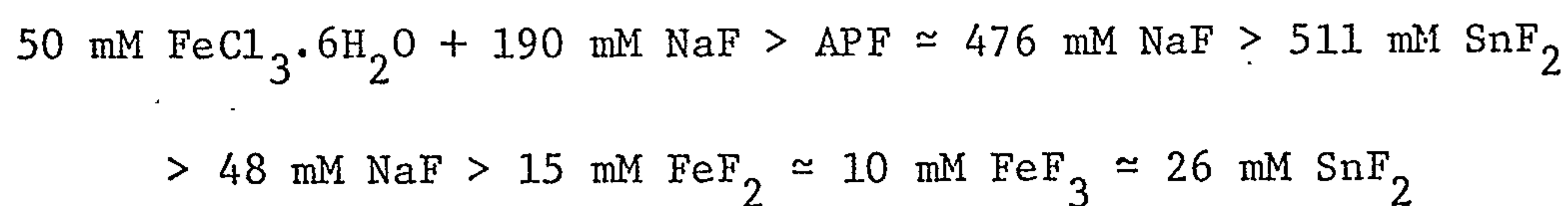
The calcium loss from treated enamel at three hours, in increasing order, was:



Fluoride concentration in the treatment solutions, in decreasing order, was:



Fluoride uptake by enamel (approximate only, because of the slight differences in enamel biopsy depths [Table 2]), in decreasing order, was:



The Ca/P (molar) ratios at three hours ranged from 1.41 to 1.66 which no doubt reflect, to some extent, the composition of the surface phases formed on the enamel surfaces. Comparisons between the Ca/P ratios obtained in this study and those of Gray⁽⁸²⁾ are not possible, because Gray did not publish this part of his findings. However Cutress⁽⁴⁷⁾, who used the method developed by Gray⁽⁸¹⁾ to assess the dissolution rates of human enamel from different geographic locations, obtained Ca/P (molar) ratios ranging from 1.1 to 1.3 at the end of three hours.

In general, enamel pretreatments with iron (II) chloride or iron (III) chloride followed by either APF or 476 mM sodium fluoride mainly affected the initial enamel dissolution rates. This suggests that they had little additional effect on the precipitation of new surface phases over that exerted by APF or 476 mM sodium fluoride alone. As in the case of 15 mM iron (II) fluoride and 10 mM iron (III) fluoride, it is difficult to explain the findings in terms of fluoride uptake, at least at the depths sampled. For example, the pretreatment of enamel with iron (II) chloride or iron (III) chloride tended to inhibit fluoride uptake by enamel from 476 mM sodium fluoride (Table 2). However, despite this, the calcium loss from enamel treated with 100 mM iron (III) chloride followed by 476 mM sodium fluoride, was significantly less than that from enamel treated with 476 mM sodium fluoride alone, at the end of the three hour immersion period in pH 4.5 lactate buffer.

Although 100 mM iron (III) chloride significantly reduced calcium loss from enamel during the three hour immersion period in pH 4.5 lactate buffer, the use of this solution in clinical situations may be limited, apart from any other factors, by the effect the solution itself

may have on enamel. The calcium loss from enamel during the five minute topical treatments with this solution was approximately 25 times higher than the calcium loss during the equivalent treatments with 100 mM iron (II) chloride. These findings are in accord with those of Torell⁽²¹⁰⁾ regarding the comparative 'erosive' properties of iron (II) and iron (III) salts.

In marked contrast to the findings of McCann⁽¹⁴⁰⁾, it was found that topical treatments with APF caused approximately four times more calcium loss from enamel than equivalent treatments with 511 mM tin (II) fluoride. These findings are, however, in general agreement with those of Stookey *et al.*⁽²⁰⁴⁾.

A number of workers have shown that the addition of certain ions, such as F^- ⁽¹⁶²⁾⁽¹⁹⁸⁾, Ca^{2+} ⁽⁸¹⁾ and Sn^{2+} ⁽¹⁴²⁾⁽¹⁹⁸⁾, to an acid buffer can appreciably reduce enamel dissolution rates. It is believed that the enamel dissolution rates are reduced because of the formation of a relatively adherent and coherent layer of sparingly soluble reaction products on the enamel surface⁽⁶⁸⁾.

Under the conditions of this study, Fe^{2+} , Fe^{3+} and Sn^{2+} cations significantly reduced the rate of enamel dissolution when added singly to pH 4.5 lactate buffer at concentrations of 100 μ M and 1 mM, whereas Ca^{2+} cations and PO_4^{3-} anions, at the same concentrations, had no appreciable effect. Gray⁽⁸¹⁾ however, found that the addition of both Ca^{2+} cations and PO_4^{3-} anions at concentrations of 3 mM and 18 mM, respectively, to pH 4.5 lactate buffer, markedly reduced the dissolution rate of enamel.

The addition of iron (II) fluoride, iron (III) fluoride or tin (II) fluoride to the buffer at a final fluoride concentration of 100 μ M

or 1 mM appeared to have an appreciably greater effect in reducing the enamel dissolution rate than the addition of sodium fluoride at the equivalent fluoride concentrations. The findings with tin (II) fluoride and sodium fluoride are in contrast to those of Speirs *et al.* ⁽¹⁹⁸⁾ who found that the effects of tin (II) fluoride and sodium fluoride when added singly to pH 4.2 acetate buffer were similar.

Presumably, the reaction products formed by iron (II) fluoride, iron (III) fluoride or tin (II) fluoride on enamel surfaces provided a better barrier against acid demineralisation than the reaction products formed by sodium fluoride.

These findings suggest that Fe^{2+} , Fe^{3+} or Sn^{2+} cations in the vicinity of an enamel surface can have a profound effect on enamel dissolution. However, Francis *et al.* ⁽⁶⁸⁾ have indicated that many protective layers so formed are only effective as long as the appropriate cation is present in the acid solution and that the protective effect can be lost once the layer is exposed to a fresh acid solution without the common cation.

An outline of the possible significance of the findings of this study are presented in the general discussion section on page 142.

SECTION 3

THE EFFECT OF IRON (II) AND IRON (III) SALTS ON *IN VITRO*

PLAQUE FORMATION BY *S. MUTANS*, *S. SANGUIS* AND

A. VISCOSUS (TEST 3)

Since it is now well established that plaque is a prerequisite for the initiation of caries⁽²²⁾⁽¹¹²⁾, any agent capable of inhibiting bacterial colonisation of tooth surfaces could be regarded as having definite cariostatic potential.

The formation of plaque is a complex process⁽⁷⁷⁾ and a number of model systems have been developed to help understand some of the mechanisms involved⁽⁶²⁾⁽¹¹³⁾⁽¹³⁹⁾⁽¹⁶⁴⁾⁽¹⁶⁸⁾⁽²⁰⁵⁾. One of the earliest systems, that developed by McCabe *et al.*⁽¹³⁹⁾ has been widely used to screen compounds for their ability to inhibit bacterial colonisation on solid surfaces⁽⁵⁵⁾⁽⁸⁵⁾⁽¹¹⁵⁾⁽¹⁸⁹⁾⁽²²⁹⁾. It was used in this study to assess the effect of iron (II) and iron (III) salts on *in vitro* plaque formation by *S. mutans*, *S. sanguis* and *A. viscosus* on smooth enamel, ground (roughened) enamel and stainless steel wire.

The three bacterial species used are amongst the predominant organisms isolated from human plaque⁽⁹⁴⁾ and are of particular interest in view of their ability to form adherent deposits on solid surfaces⁽¹⁵³⁾⁽²²⁷⁾.

For comparison purposes, the effect of APF, tin (II) fluoride and sodium fluoride on *in vitro* plaque formation was also assessed.

MATERIALS AND METHODS

MATERIALS

Teeth

Sound lower permanent central incisor teeth, extracted from freshly slaughtered 2.0 to 2.5 year old beef cattle, were obtained from Homebush Abattoirs, Sydney, N.S.W., Australia.

Organisms

The three organisms used, *S. mutans* strain GS5, *S. sanguis* strain 804 and *A. viscosus* strain NY1, were obtained from the Institute of Dental Research, Sydney, N.S.W., Australia. The names of the original donors are shown in parentheses below.

S. mutans strain GS5 (A. Bleiweis, Gainesville, Fla., U.S.A.), *S. sanguis* strain 804 (J. Carlsson, Umeå, Sweden) and *A. viscosus* strain NY1 (J. van der Hoeven, Nijmegen, Holland) were maintained during the period of the experiment on blood agar slopes and transferred every two weeks.

Media

Trypticase soy broth was obtained from Baltimore Biological Labs., Cockeysville, Md., U.S.A. and brain heart infusion broth from Oxoid Ltd., London, England.

Chemicals

All chemicals were the highest grade available commercially:

Acidulated phosphate-fluoride (Orapharm Products Ltd., Melbourne, Vic., Australia); iron (II) chloride (E. Merck and Co., Darmstadt, West Germany); iron (III) chloride (Riedel-De Haën AG, Seelze-Hannover, West Germany); iron (II) fluoride and iron (III) fluoride (PCR Inc., Gainesville, Fa., U.S.A.); sodium fluoride (BDH Chemicals Ltd., Poole, England); sucrose (Ajax Chemicals Ltd., Sydney, N.S.W., Australia); tin (II) fluoride (Ozark-Mahoning, Tulsa, Okla., U.S.A.).

All solutions were made up with distilled, deionised water.

METHODS

Preparation of Specimens

The facial surface of each tooth was cleaned with pumice on a rotating brush. A triangular section was cut from the middle of the facial surface with a diamond disc (Horico, Hopf. Ringleb GmbH, Berlin, West Germany) in a straight handpiece and a 1 mm diameter hole was drilled through the dentine parallel to the dentino-enamel junction (Fig. 27). The sides of each section were smoothed with fine sandpaper discs (A.D. International, London, England) in a straight handpiece.

One end of a 16 cm long, 0.9 mm diameter, piece of stainless steel orthodontic wire (Dentaurum, H.P. Windelstroeter KG, Pforzheim, West Germany) was passed through the hole and the end looped over as shown in Fig. 27. The other end of the wire was inserted into a 14 mm

diameter rubber stopper for a distance of 2 cm. A 0.8 mm. bore hypodermic needle was pushed through the stopper and a pellet of cotton wool placed in the hub.

The rubber stopper was fitted into a 17 mm internal diameter, 15 cm long test tube containing 12 ml of distilled, deionised water and the assembly autoclaved at 121°C for 15 minutes.

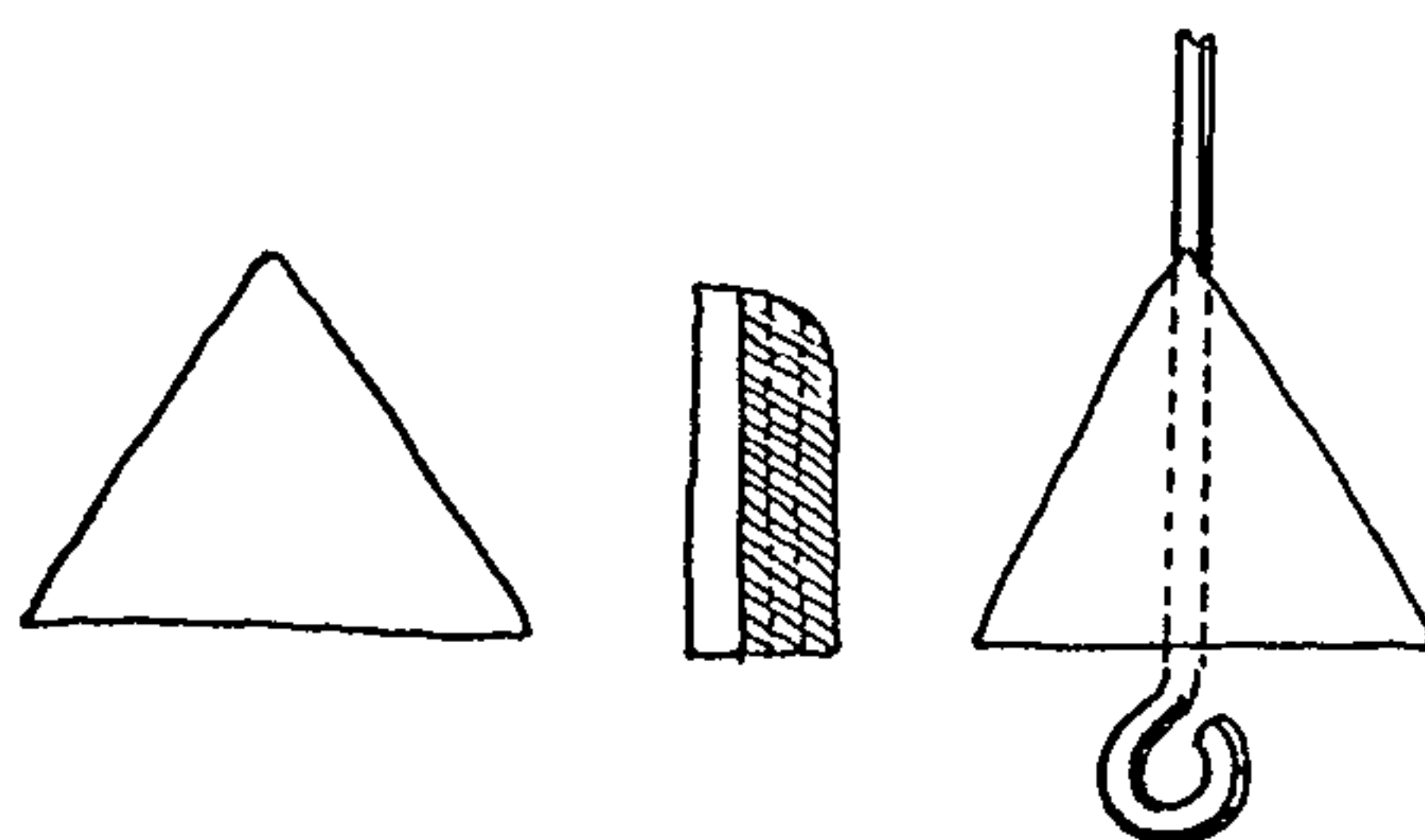


Fig. 27. Diagrams illustrating three stages in the preparation of specimens.
(Left). Triangular section of enamel cut from facial surface of bovine tooth.
(Middle). Hole drilled through dentine parallel to the dentino-enamel junction.
(Right). Stainless steel wire inserted through hole and end looped over.

Treatment of Specimens

The treatment solutions used are listed in Table 8. Immediately before use each solution was sterilised by vacuum filtration through a 0.22 μ m cellulose membrane filter (GS WP 047 00, Millipore Corp., Bedford, Mass., U.S.A.).

Two hundred and twenty five specimens were assigned at random to 15 groups each containing 15 specimens. One treatment solution was used per group of 15 specimens.

Each specimen was taken out of the test tube in which it was autoclaved and placed in a sterile test tube containing 12 ml of a

TABLE 8
TREATMENT SOLUTIONS AND TREATMENT TIMES USED IN
IN VITRO PLAQUE FORMATION STUDY

Solution	pH	Treatment Time (min)
H ₂ O (Control)	5.3	5
1 mM FeCl ₂ · 4H ₂ O	5.1	5
10 mM FeCl ₂ · 4H ₂ O	4.3	5
100mM FeCl ₂ · 4H ₂ O	3.2	5
1 mM FeCl ₃ · 6H ₂ O	3.2	5
10 mM FeCl ₃ · 6H ₂ O	2.4	5
100mM FeCl ₃ · 6H ₂ O	1.6	5
15 mM FeF ₂ (570 ppm F) ^a	4.4	5
10 mM FeF ₃ (570 ppm F) ^b	3.5	5
50 mM FeCl ₃ · 6H ₂ O + 190mM NaF (3,620 ppm F) ^c	2.1	5
APF (12,300 ppm F) ^c	3.2	5
26 mM SnF ₂ (970 ppm F) ^c	3.4	5
511mM SnF ₂ (19,400 ppm F) ^c	2.8	5
48 mM NaF (905 ppm F) ^c	7.0	5
476mM NaF (9,050 ppm F) ^c	7.0	5

^a Prepared under nitrogen. Solution warmed to 60°C and continuously stirred to facilitate dissolution of salt. Solution cooled to 37°C before use.

^b Solution warmed to 60°C and continuously stirred to facilitate dissolution of salt. Solution cooled to 37°C before use.

^c Solutions have been tested clinically (see footnote c Table 1).

treatment solution at room temperature. After five minutes it was removed, washed with sterile distilled, deionised water and placed in a sterile test tube containing 10 ml of culture broth inoculated with 2 ml of an overnight culture of one of the test organisms grown anaerobically at 37°C.

Five of the 15 specimens in each group were placed in test tubes containing trypticase soy broth and five per cent sucrose and inoculated with *S. mutans* strain GS5; another five were placed in test tubes containing trypticase soy broth and five per cent sucrose and inoculated with *S. sanguis* strain 804 and the remaining five were placed in test tubes containing brain heart infusion broth and five per cent sucrose and inoculated with *A. viscosus* strain NY1.

The test tubes were placed in anaerobic jars (A. Gallenkamp and Co. Ltd., London, England) which were then flushed with a gas mixture comprising 95 per cent nitrogen and five per cent carbon dioxide and placed in an incubator at 37°C.

The specimens in broth inoculated with *S. mutans* strain GS5 were transferred every 24 hours for five days into freshly inoculated broth and for another three days into uninoculated broth. The specimens in broth inoculated with either *S. sanguis* strain 804 or *A. viscosus* strain NY1 were transferred every 24 hours for nine days into freshly inoculated broth and for another four days into uninoculated broth.

Assessment of Plaque Formation

Each specimen was examined macroscopically for plaque formation under standardised lighting conditions at the end of the nine day (*S. mutans* strain GS5) or 14 day (*S. sanguis* strain 804 and *A. viscosus*

strain NY1) experimental period.

The amount of plaque formed on smooth enamel (facial surface of each triangular section), ground enamel (sides of each triangular section) and the wire supporting each triangular section was graded as being either none, very light, light, moderate or heavy. Specimens from a preliminary study, with light, moderate and heavy accumulations of *in vitro* plaque were used as standards. The specimens had been preserved in five per cent formalin.

RESULTS

IN VITRO PLAQUE FORMATION ON SURFACES EXPOSED TO *S. MUTANS* STRAIN GS5

Fig. 28 shows that none of the treatments completely inhibited plaque formation on any of the three test surfaces, smooth enamel, ground enamel or wire. It appeared that the type of surface had more effect on plaque accumulation than the treatments, smooth enamel having the lightest deposits and wire the heaviest.

The smooth enamel in all specimens had either a light or moderate accumulation of plaque. A light accumulation similar to that shown in Fig. 29-B occurred in all specimens treated with 10 mM iron (III) fluoride and predominated in the control specimens and the specimens treated with either 100 mM iron (II) chloride or 100 mM iron (III) chloride. Two or more of the specimens in each of the remaining treatment groups had a moderate accumulation of plaque on smooth enamel similar to that shown in Fig. 29-C, the maximum incidence occurring in

Treatment	Smooth Enamel					Ground Enamel					Wire				
	None	V.Light	Light	Moderate	Heavy	None	V.Light	Light	Moderate	Heavy	None	V.Light	Light	Moderate	Heavy
H ₂ O (Control)			4 a												
1 mM FeCl ₂ ·4H ₂ O			3	2											
10 mM FeCl ₂ ·4H ₂ O			3	2											
100 mM FeCl ₂ ·4H ₂ O			4	1											
1 mM FeCl ₃ ·6H ₂ O			2	3											
10 mM FeCl ₃ ·6H ₂ O			3	2											
100 mM FeCl ₃ ·6H ₂ O			4	1											
15 mM FeF ₂															
10 mM FeF ₃			5												
50 mM FeCl ₃ ·6H ₂ O + 190 mM NaF															
APF			2	3											
26 mM SnF ₂			3	2											
511 mM SnF ₂			3	2											
48 mM NaF			2	3											
476 mM NaF			3	2											

a Number of Specimens

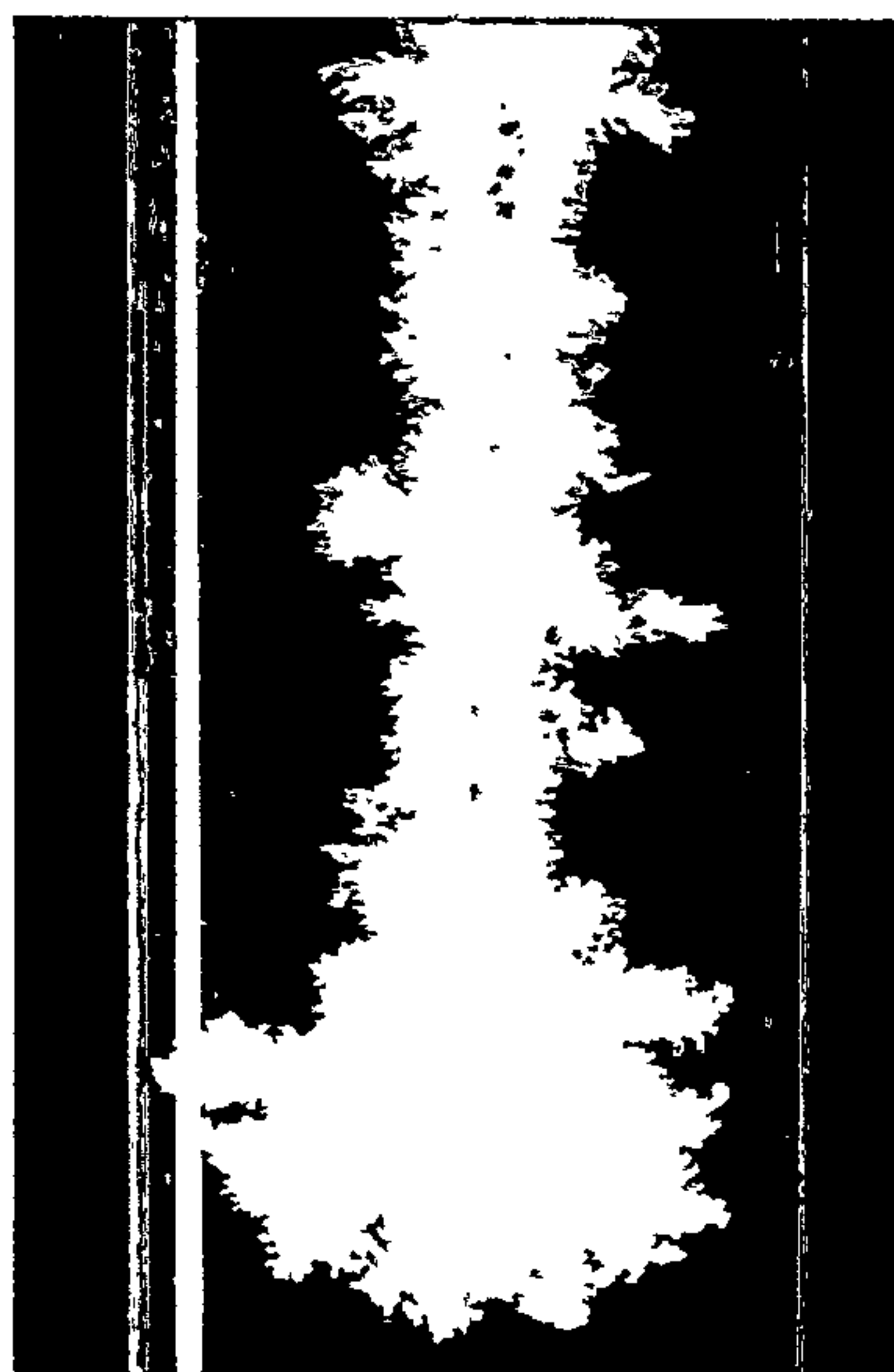
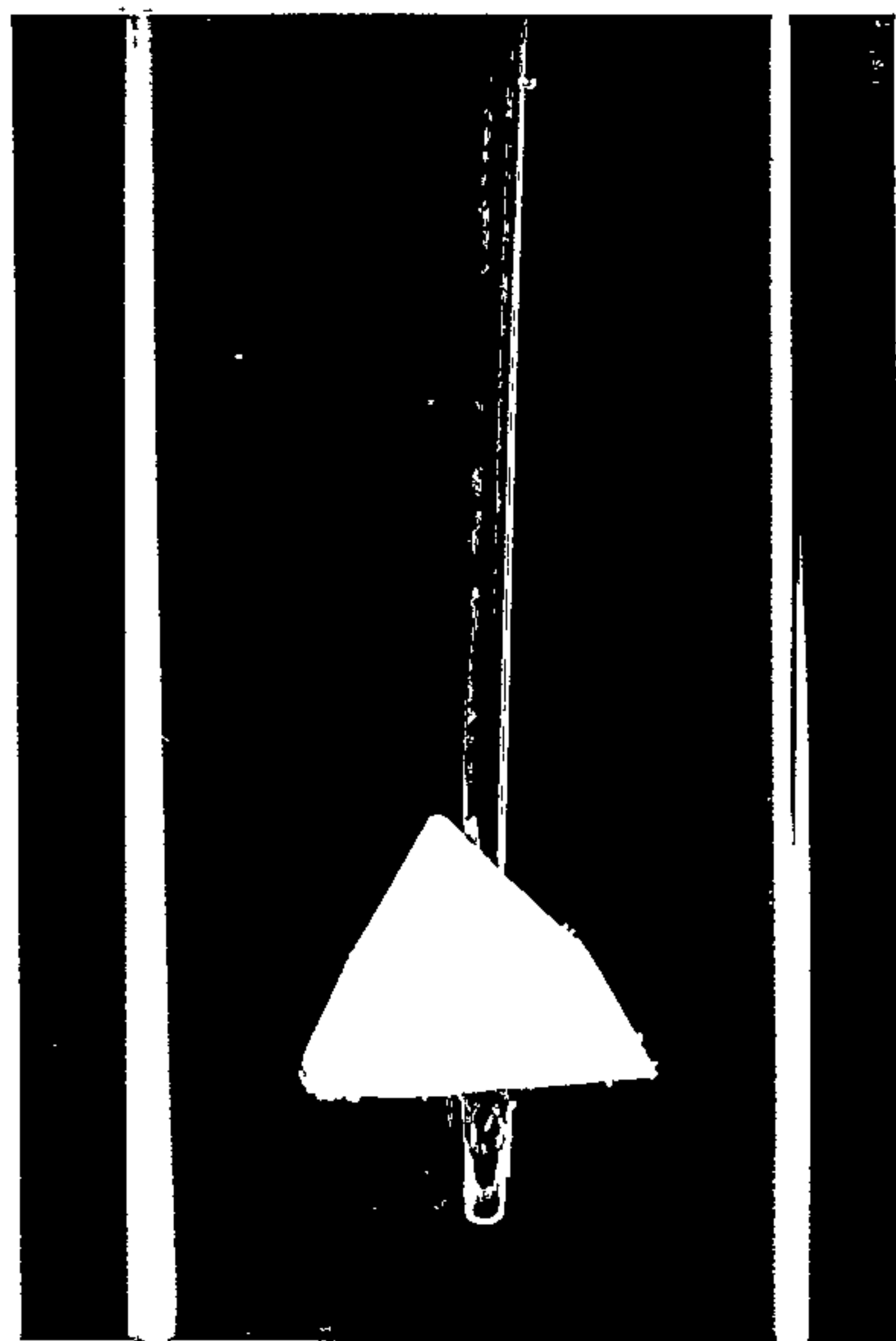
Fig. 28. *In vitro* plaque formation on three test surfaces (smooth enamel, ground enamel and stainless steel wire) exposed to *S. mutans* strain GS5 following treatment with test compounds (treatment time — five minutes). Diagram shows number of test surfaces with none, very light, light, moderate or heavy deposits of *in vitro* plaque at the end of the nine day experimental period.

Fig. 29. Photographs showing an untreated specimen and *in vitro* plaque formation on two specimens exposed to *S. mutans* strain GS5.

A (top left). Appearance of a specimen before treatment with a test compound. Two of the three test surfaces, smooth enamel (facial surface of triangular section) and stainless steel wire, can be seen. The sides of the triangular section comprise the third test surface, ground enamel. X2.

B (top right). An example of *in vitro* plaque formation on a specimen exposed to *S. mutans* strain GS5 at the end of the nine day experimental period. Plaque formation was graded, as 'light' on smooth enamel, 'moderate' on ground enamel and 'heavy' on stainless steel wire. X2.

C (right). A nine-day-old accumulation of plaque on a specimen exposed to *S. mutans* strain GS5. In this instance, two of the test surfaces, smooth enamel and ground enamel, have deposits which were graded as 'moderate'. The deposit on the stainless steel wire was graded as 'heavy'. X2.



the specimens treated with either 15 mM iron (II) fluoride or the solution containing 50 mM iron (III) chloride and 190 mM sodium fluoride.

Plaque formation on ground enamel was similar to those shown in Figs. 29-B and 29-C, although slightly heavier deposits occurred on all specimens treated with 476 mM sodium fluoride. The heavier deposits predominated in the specimens treated with either the solution containing 50 mM iron (III) chloride and 190 mM sodium fluoride or APF.

The majority of the wires supporting the enamel specimens had heavy accumulations of plaque, comparable to that depicted in Figs. 29-B and 29-C.

Although no one treatment either markedly inhibited or stimulated plaque formation on all three surfaces, it is noteworthy that the solution containing 50 mM iron (III) chloride and 190 mM sodium fluoride tended to stimulate plaque formation on both smooth enamel and ground enamel.

IN VITRO PLAQUE FORMATION ON SURFACES EXPOSED TO *S. SANGUIS* STRAIN 804

The majority of specimens exposed to *S. sanguis* strain 804 had the three test surfaces, smooth enamel, ground enamel and wire, covered with a light deposit of relatively smooth plaque similar to that shown in Fig. 31-A.

Although none of the treatments completely inhibited plaque formation (Fig. 30), the majority of specimens treated with 1 mM iron

Treatment	Smooth Enamel					Ground Enamel					Wire				
	None	V. Light	Light	Moderate	Heavy	None	V. Light	Light	Moderate	Heavy	None	V. Light	Light	Moderate	Heavy
H ₂ O (Control)			5	2				5	2				5	2	
1 mM FeCl ₂ 4H ₂ O			3					5					5		
10 mM FeCl ₂ 4H ₂ O			3					5					5		
100 mM FeCl ₂ 4H ₂ O			3					5					5		
1 mM FeCl ₃ 6H ₂ O		3	2					5				3	2		
10 mM FeCl ₃ 6H ₂ O			5					5					5		
100 mM FeCl ₃ 6H ₂ O			5					5					5		
15 mM FeF ₂		2	3					5					5		
10 mM FeF ₃				2				1	4				2	3	
50 mM FeCl ₃ 6H ₂ O + 190 mM NaF			3	2				2	3				2	3	
APF			4	1				4	1				4	1	
26 mM SnF ₂			4	1				3					5		
511 mM SnF ₂			3					3					3		
48 mM NaF			3					3					3		
476 mM NaF			5					5					5		

a Number of Specimens

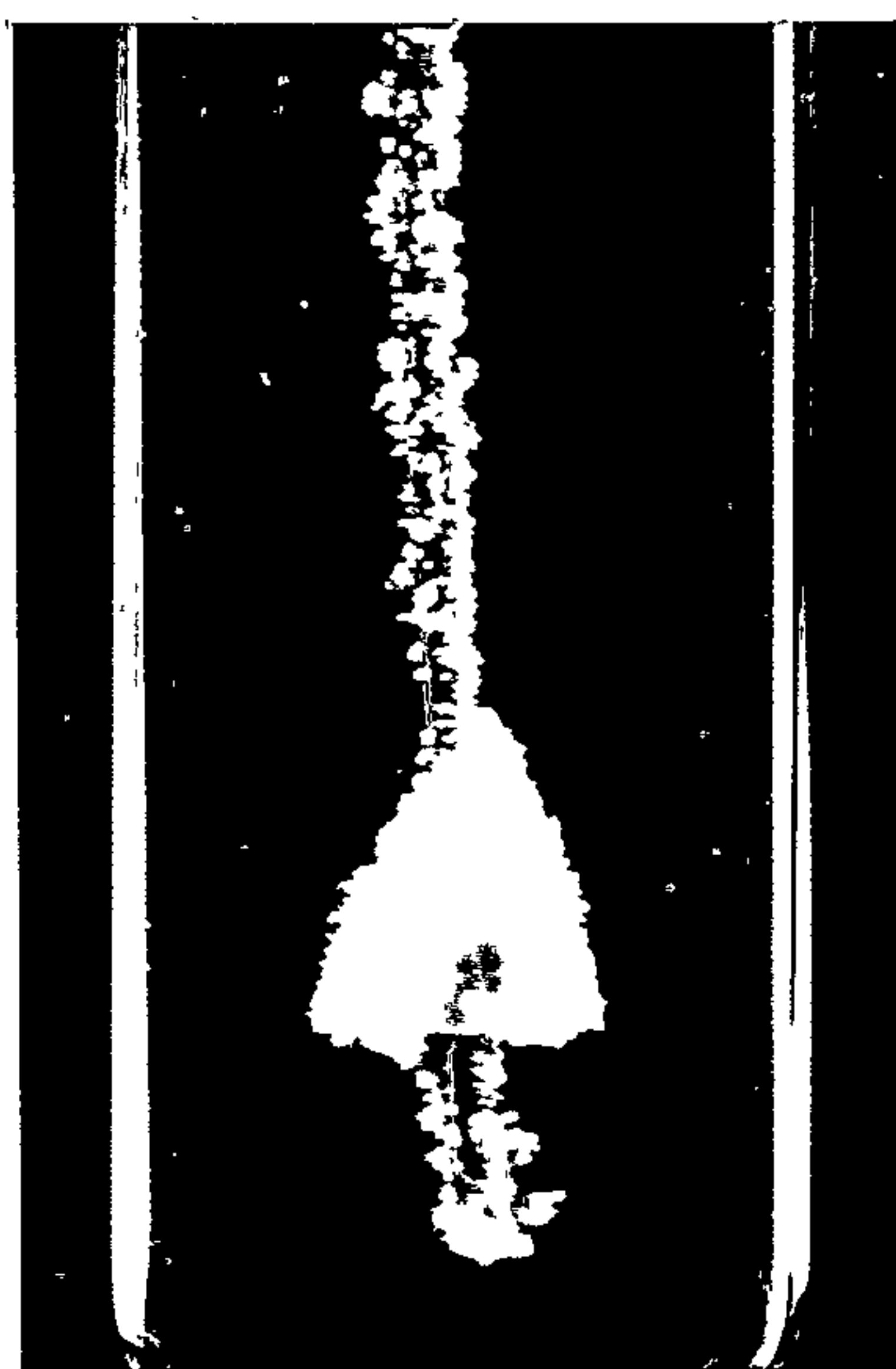
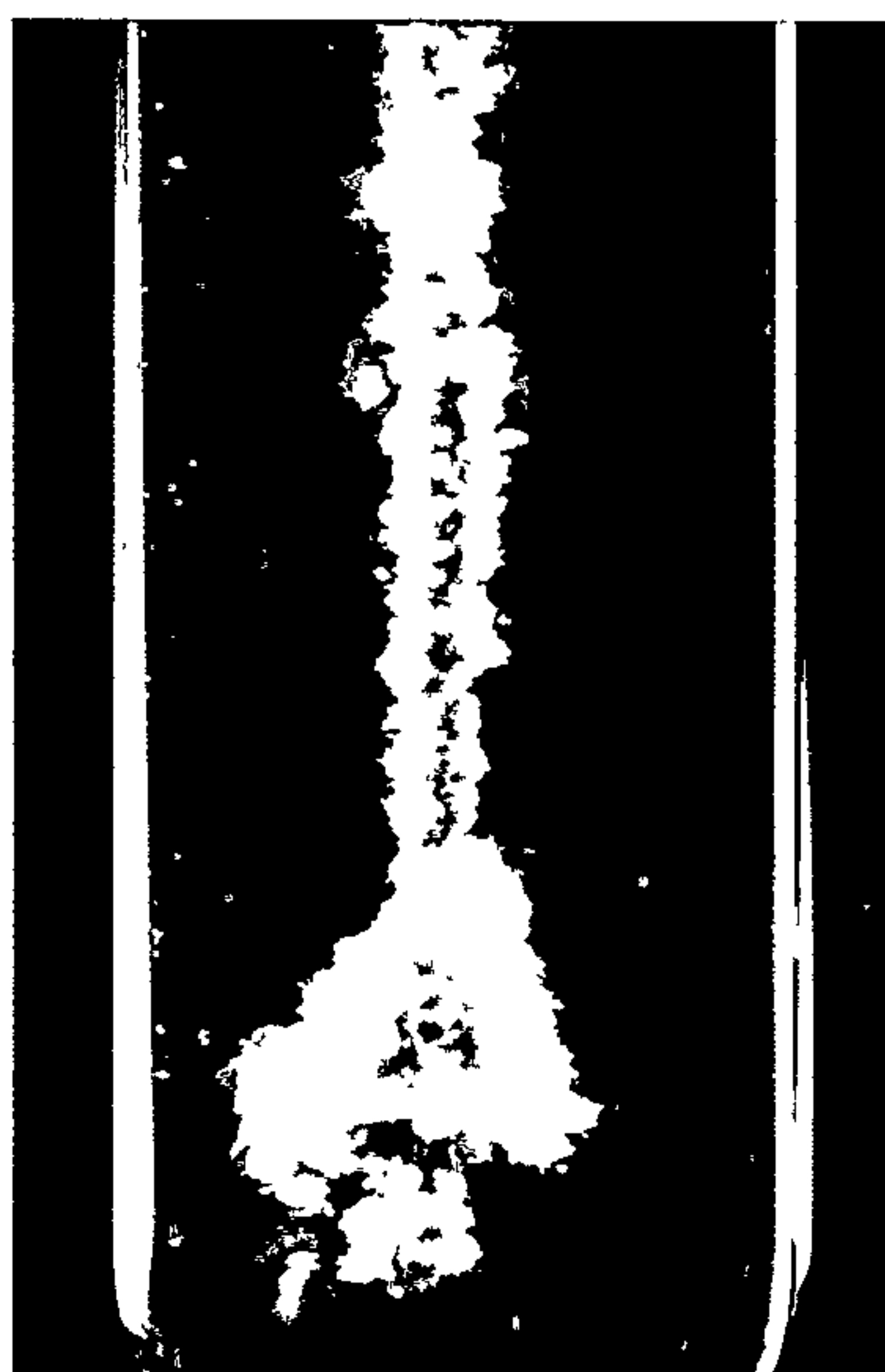
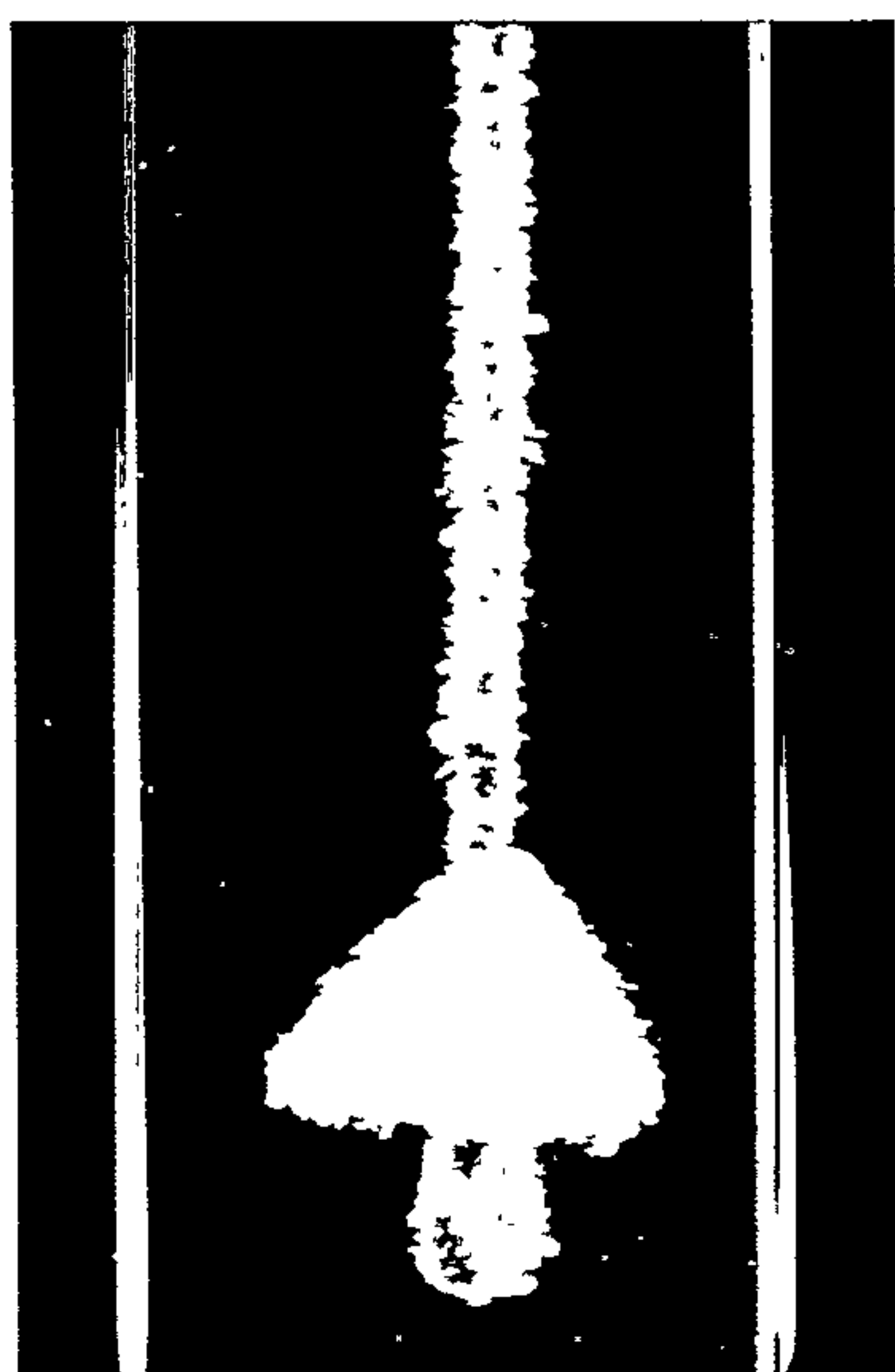
Fig. 30. *In vitro* plaque formation on three test surfaces (smooth enamel, ground enamel and stainless steel wire) exposed to *S. sanguis* strain 804 following treatment with test compounds (treatment time — five minutes). Diagram shows number of test surfaces with none, very light, light, moderate or heavy deposits of *in vitro* plaque at the end of the 14 day experimental period.

Fig. 31. Examples of *in vitro* plaque formation on specimens exposed to *S. sanguis* strain 804 or *A. viscosus* strain NY1.

A (top left). A 14-day-old accumulation of plaque on a specimen exposed to *S. sanguis* strain 804. All three test surfaces, smooth enamel (facial surface of triangular section), ground enamel (sides of triangular section) and stainless steel wire, have a light deposit of bacteria. X2.

B (top right). An example of a control specimen exposed to *A. viscosus* strain NY1 at the end of the 14 day experimental period. Clumps of bacteria can be seen on all three test surfaces. X2.

C (right). Typical appearance of treated specimens exposed to *A. viscosus* strain NY1 at the end of the 14 day experimental period. Only isolated clumps of bacteria can be seen on smooth enamel. Slightly heavier deposits are apparent on ground enamel and stainless steel wire. X2.



(III) chloride only had very light deposits of plaque on smooth enamel and wire. In contrast, 10 mM iron (III) fluoride appeared to stimulate plaque formation on smooth enamel, ground enamel and wire, whereas the solution containing 50 mM iron (III) chloride and 190 mM sodium fluoride tended to stimulate plaque formation on ground enamel and wire.

IN VITRO PLAQUE FORMATION ON SURFACES EXPOSED TO A. VISCOSUS STRAIN NY1

While no one treatment completely inhibited plaque formation on all three test surfaces, smooth enamel, ground enamel and wire, 476 mM sodium fluoride completely inhibited plaque formation on smooth enamel (Fig. 32). In addition, 10 mM iron (III) fluoride and APF completely inhibited plaque formation on smooth enamel in four of the five specimens in the respective treatment groups.

The majority of the specimens in the treatment groups had appreciably less plaque on smooth enamel and wire than the control specimens. This was not the case with ground enamel. However, 100 mM iron (III) chloride and the solution containing 50 mM iron (III) chloride and 190 mM sodium fluoride markedly inhibited plaque formation on this surface.

Three treatments had an appreciable inhibitory effect on plaque formation on two of the test surfaces. Apart from their pronounced effect on plaque formation on smooth enamel, 10 mM iron (III) fluoride and 476 mM sodium fluoride also inhibited plaque formation on wire, while 100 mM iron (III) chloride inhibited plaque formation on both ground enamel and wire.

Treatment	Smooth Enamel					Ground Enamel					Wire				
	None	V. Light	Light	Moderate	Heavy	None	V. Light	Light	Moderate	Heavy	None	V. Light	Light	Moderate	Heavy
H ₂ O (Control)			5 a												
1 mM FeCl ₂ .4H ₂ O	3	2										4			
10 mM FeCl ₂ .4H ₂ O	1	3	1				4					4			
100 mM FeCl ₂ .4H ₂ O	2	2	1				1					3			
1 mM FeCl ₃ .6H ₂ O	2	3					1					1			
10 mM FeCl ₃ .6H ₂ O	1	4					2					2			
100 mM FeCl ₃ .6H ₂ O		5					5					5			
15 mM FeF ₂		5													
10 mM FeF ₃	4	1													
50 mM FeCl ₃ .6H ₂ O + 190 mM NaF	1	4					4								
APF	4	1										3			
26 mM SnF ₂	1	4										1			
511 mM SnF ₂	2	2	1				1					1			
48 mM NaF		5										4	1		
476 mM NaF	5											5			

^a Number of Specimens

Fig. 32. *In vitro* plaque formation on three test surfaces (smooth enamel, ground enamel and stainless steel wire) exposed to *A. viscosus* strain NY1 following treatment with test compounds (treatment time – five minutes). Diagram shows number of test surfaces with none, very light, light, moderate or heavy deposits of *in vitro* plaque at the end of the 14 day experimental period.

An example of the difference between plaque formation on control and treated specimens is shown in Figs. 31-B and 31-C. The isolated clumps of bacteria seen on the smooth enamel and wire in the treated specimen (Fig. 31-C) are typical of the type of deposit seen on most of the treated smooth enamel and wire surfaces.

DISCUSSION

In this study, none of the test compounds completely inhibited *in vitro* plaque formation by *S. mutans* strain GS5, *S. sanguis* strain 804 or *A. viscosus* strain NY1 on all three test surfaces, smooth enamel, ground enamel and stainless steel wire. Although some of the compounds tended to have some inhibitory effects, these were not constant overall and appeared to differ with the nature of the test surface and the type of organism used. For example, 10 mM iron (III) fluoride tended to inhibit *in vitro* plaque formation on smooth enamel and wire exposed to *A. viscosus* strain NY1 but appeared to stimulate plaque formation on smooth enamel, ground enamel and wire exposed to *S. sanguis* strain 804. Furthermore, 476 mM sodium fluoride completely inhibited *in vitro* plaque formation on smooth enamel exposed to *A. viscosus* strain NY1, but tended to stimulate plaque formation on ground enamel exposed to *S. mutans* strain GS5.

Two of the test compounds, tin (II) fluoride and sodium fluoride, have been evaluated by other workers using essentially the same system employed in this study⁽⁵⁵⁾⁽¹¹⁵⁾⁽¹⁸⁹⁾. Neither compound inhibited *in vitro* plaque formation by *Streptococcus* species on enamel⁽¹¹⁵⁾, root surfaces⁽¹¹⁵⁾ or stainless steel wire⁽⁵⁵⁾⁽¹¹⁵⁾⁽¹⁸⁹⁾. To date, only one fluoride compound, amine fluoride, has been found to inhibit *in vitro* plaque formation by *Streptococcus* species on enamel⁽¹¹⁵⁾ and stainless steel wire⁽⁵⁵⁾⁽¹⁸⁹⁾. However, it is believed that these inhibitory properties are mainly due to the long chain aliphatic amine moiety in amine fluoride⁽⁸⁾⁽²²⁹⁾.

The rate of *in vitro* plaque formation on specimens exposed to *S. mutans* strain GS5 appeared to be influenced by the nature of the test surface. At the end of the experimental period, the amount of *in vitro* plaque accumulated on the test surfaces appeared to be, in ascending order:

smooth enamel < ground enamel < stainless steel wire

Glantz⁽⁷⁹⁾ has shown that a relationship exists between the wettability of test surfaces and plaque accumulation *in vivo* and it is possible that variations in wettability may account for the differences seen.

In contrast to the situation with *S. mutans* strain GS5, *in vitro* plaque formation on specimens exposed to *S. sanguis* strain 804, although light, appeared to develop on all three test surfaces with equal facility. These findings are consistent with those of Olsson and Krasse⁽¹⁶⁸⁾ who found that *S. sanguis* adsorbed equally well to enamel and glass *in vivo*. These workers also found that the treatment of whale dentine with tin (II) fluoride or amine fluoride markedly reduced the number of *S. sanguis* and *S. salivarius* that adsorbed to this surface during a 40 minute incubation in a saliva-buffer mixture containing these organisms.

Ørstavik *et al.*⁽¹⁷⁰⁾ reported that the adsorption of *S. sanguis* to enamel *in vitro* was markedly enhanced if the enamel was first coated with a salivary pellicle. However, the adsorption of *S. mutans* was only slightly enhanced under the same conditions. Similar findings have been reported by other workers⁽¹⁰²⁾⁽¹²⁷⁾.

Although, in general, there was not a pronounced formation of *in vitro* plaque on test surfaces exposed to *A. viscosus* strain NY1,

strains of this organism could be important in plaque formation *in vivo* through their ability to form large aggregates with such dextran-synthesising organisms as *S. mutans* and *S. sanguis*⁽¹⁸⁾. Furthermore, as has been pointed out by Gibbons and van Houte⁽⁷⁷⁾, filamentous organisms present in plaque provide a large surface area to which other bacteria may become attached.

A number of reports have supported the proposition that the adsorption of bacteria to surfaces is biphasic, consisting initially of a reversible phase and subsequently an irreversible phase⁽⁴¹⁾⁽¹⁴⁶⁾⁽²⁴⁶⁾. In the reversible phase bacteria are believed to be held weakly, at a short distance from the surface, by a balance between the repulsive energies due to similar surface charges and the attractive energies due to van der Waal's forces⁽¹⁴⁶⁾. A firm attachment (irreversible phase) may then be achieved by the bacteria elaborating a substance, such as an extracellular polysaccharide, which serves to bridge the bacteria-surface interspace⁽¹⁴⁶⁾.

Olsson *et al.*⁽¹⁶⁹⁾ have reported that the presence of Ga^{3+} or Al^{3+} cations in the suspending medium increased the number of streptococci (*S. mutans*, *S. sanguis* and *S. salivarius*) adsorbed to dentine surfaces *in vitro*. Therefore, it is possible that Fe^{2+} cations and, especially, Fe^{3+} cations if present in the vicinity of a tooth surface, may act as counter ions and thus increase the number of organisms adsorbed in the reversible phase.

S. mutans, *S. sanguis* and *A. viscosus* can all produce extracellular polysaccharides which may play some role in firmly attaching these organisms to a surface. Although *S. mutans* can produce levans

from the fructose moiety of sucrose⁽⁷⁶⁾⁽¹⁸⁰⁾, it is the dextrans produced from the glucose moiety and, in particular, those with predominantly α -1,3 linkages, that are believed to be the most important in respect to plaque formation⁽⁷⁷⁾⁽⁸⁷⁾⁽⁸⁸⁾⁽²³⁸⁾. *S. sanguis* can also synthesise dextrans from sucrose⁽⁹⁶⁾⁽¹⁶⁷⁾ while *A. viscosus* can synthesise levans from sucrose⁽¹⁰⁵⁾⁽¹²²⁾ and can form a capsular polysaccharide from glucose⁽¹⁸¹⁾⁽²²⁵⁾.

Although it is known that Fe^{2+} cations can inhibit the activity of glycosyltransferase from *S. mutans* strain FA1 in a cacodylate buffer, little is known about the effect of iron salts on bacterial synthesis of extracellular polymers. However, Emilson and Krasse⁽⁶⁰⁾ have shown that iron (II) sulphate and iron (III) chloride can both inhibit the formation of *S. mutans* induced plaque deposits in hamsters when applied topically or added to the drinking water or to the diet.

Recent work by Beighton⁽¹⁰⁾ suggests that iron may have a specific inhibitory effect on *S. mutans*. He found that the addition of iron to the drinking water of rats significantly reduced the proportion of *S. mutans* in established fissure plaque but did not affect the rest of the plaque flora to any marked degree. Beighton⁽¹⁰⁾ reported a similar finding when fluoride was added to the drinking water. This is consistent with the findings from clinical studies showing that the topical application of high concentration fluoride preparations such as APF⁽¹²⁹⁾⁽¹³⁰⁾ or 511 mM tin (II) fluoride⁽²³⁹⁾ reduces the proportion of *S. mutans* in plaque.

Clearly more work is needed to determine the effect of iron salts on plaque formation *in vitro* and *in vivo*. The findings of

Emilson and Krasse⁽⁶⁰⁾ and Beighton⁽¹⁰⁾ indicate that a follow up study to the one in this section is warranted to determine whether repeated applications of low concentration iron salt solutions affect the growth of *S. mutans* induced plaque deposits *in vitro*.

SECTION 4THE EFFECT OF IRON (II) AND IRON (III) SALTS ON ACID
PRODUCTION BY *S. MUTANS*, *A. VISCOSUS* AND *L. CASEI* (TEST 4)

To date, one of the most frequently used methods for determining the effect of a compound on acid production by a specific organism has entailed the addition of the test compound to a broth culture of the organism and the measuring the subsequent change in pH or titratable acidity⁽⁴⁴⁾⁽⁹³⁾⁽¹³⁶⁾⁽¹³⁷⁾. However a preliminary study showed that the addition of Fe^{2+} cations or Fe^{3+} cations at a concentration of 1 mM to liquid media commonly used for the growth of *S. mutans*, such as Todd-Hewitt broth (Oxoid Ltd., London, England), trypticase soy broth (Baltimore Biological Labs., Cockeysville, Md., U.S.A.), brain heart infusion broth (Oxoid) and defined medium FMC⁽²⁰⁸⁾, resulted in precipitates being formed, examples of which are shown in Fig. 33. The addition of tin (II) salts also caused the formation of precipitates.

Although the nature of these precipitates was not determined, it is well known that heavy metals can cause precipitation of proteins on the alkaline side of their iso-electric points⁽²³⁷⁾.

Since the results of acid production experiments conducted in the presence of such precipitates could be suspect, the method used in this study was developed to allow washed cells to be treated with the test compounds in the absence of liquid media.

The three bacterial species used, *S. mutans*, *A. viscosus* and *L. casei*, have been found in human plaque⁽⁹⁴⁾ and have been shown

Fig. 33. Examples of precipitate formation in liquid culture media following addition of iron salts.

- A (top).** Trypticase soy broth following addition of Fe^{2+} cations, Fe^{3+} cations or F^- anions: 1 = Fe^{2+} cations added at a concentration of 1 mM; 2 = Fe^{3+} cations added at a concentration of 1 mM; 3 = F^- anions added at a concentration of 1 mM; 4 = no additive (control). (Similar results to those shown in Fig. 33-A were obtained with Todd-Hewitt broth and brain heart infusion broth)
- B (right).** Defined medium FMC following addition of Fe^{2+} cations, Fe^{3+} cations or F^- anions: 1 = Fe^{2+} cations added at a concentration of 1 mM; 2 = Fe^{3+} cations added at a concentration of 1 mM; 3 = F^- anions added at a concentration of 1 mM; 4 = no additive (control).



1

2

3

4



1

2

4

5

to produce caries in monoinfected gnotobiotic rats⁽⁵⁷⁾⁽¹⁸²⁾⁽²²⁷⁾. It is considered that *S. mutans* may be involved in the initiation of smooth surface and pit and fissure caries in humans⁽⁷⁸⁾⁽¹⁰⁶⁾⁽¹³¹⁾⁽¹⁹¹⁾, *A. viscosus* in the initiation of root surface caries⁽¹¹⁴⁾⁽²⁰⁷⁾ and *L. casei* in the progression of carious lesions⁽¹⁰⁶⁾.

For comparison purposes, the effect of APF, sodium fluoride and tin (II) fluoride on acid production was also determined.

MATERIALS AND METHODS

MATERIALS

Organisms

The three organisms used, *S. mutans* strain GS5, *A. viscosus* strain NY1 and *L. casei* var. *casei*, were obtained from the Institute of Dental Research, Sydney, N.S.W, Australia. The names of the original donors are shown in parentheses below.

S. mutans strain GS5 (A. Bleiweis, Gainesville, Fla., U.S.A.) and *A. viscosus* strain NY1 (J. van der Hoeven, Nijmegen, Holland) were maintained during the experiment on blood agar slopes and transferred every two weeks. *L. casei* var. *casei* was originally isolated from human saliva at the Institute of Dental Research, Sydney and, during the experiment, was maintained on Rogosa agar (Oxoid) slopes and transferred every two weeks.

Media

Trypticase soy broth was obtained from Baltimore Biological Labs., Cockeysville, Md., U.S.A. and brain heart infusion broth from Oxoid Ltd., London, England. M.R.S. broth was prepared using the formula of de Man, Rogosa and Sharpe⁽⁵¹⁾.

Chemicals

All chemicals were the highest grade available commercially: Acidulated phosphate-fluoride (Orapharm Products Ltd., Melbourne, Vic., Australia); glucose and sucrose (Ajax Chemicals Ltd., Sydney, N.S.W., Australia); iron (II) chloride (E. Merck and Co., Darmstadt, West Germany); iron (III) chloride (Riedel-De Haën AG, Seelze-Hannover, West Germany); iron (II) fluoride and iron (III) fluoride (PCR Inc., Gainesville, Fla., U.S.A.); sodium fluoride (BDH Chemicals Ltd., Poole, England); tin (II) fluoride (Ozark-Mahoning, Tulsa, Okla., U.S.A.).

All solutions were made up with distilled, deionised water.

Apparatus

Fermenter

A fermenter of 160 ml capacity was constructed for this experiment (Fig. 34). The main body consisted of a 250 ml culture vessel (Quickfit EV 250, Quickfit and Quartz Ltd., Stone, Staffs., England) fitted with a side port for inoculation and a five socket

flat flange lid (Quickfit MAF 0/50) secured to the culture vessel with a clip (Quickfit JC 50 F). Two of the sockets were used for the intake and exhaust of gases, a third for the delivery of alkali, a fourth for a temperature sensor (P7, Electro-Chemical Engineering Pty. Ltd., Sydney, N.S.W., Australia) and the remaining socket was enlarged to take a combination pH electrode (CA14/02 Philips, Eindhoven, Holland). The pH electrode was connected to a pH controller (Simac Instrumentation Ltd., Hersham, Walton-on-Thames, England) which activated a peristaltic pump connected to the alkali reservoir. The temperature sensor was attached to a temperature controller (Ether Mini Type 1990/1, Pye Ether Ltd., Stevenage, England) which activated the heat source, a 150 watt infra-red lamp (Infraphil, Philips, Eindhoven, Holland).

The fermenter was mounted on a magnetic stirrer which controlled the stirring rate of a PTFE coated spin bar inside the culture vessel.

The main body of the fermenter with the glass and rubber fittings *in situ*, except for the pH electrode, was autoclaved at 121°C for 20 minutes before use. The stem of the electrode was sterilised, using a modification of the method of Jacques⁽¹⁰⁹⁾, by immersion in a 5 per cent formaldehyde solution for 5 minutes. The electrode was washed thoroughly with sterile, distilled, deionised water before being fitted into the fermenter.

Holder for Organisms

A holder was constructed to allow the organisms to be

- (i) treated with the test compounds, (ii) washed and

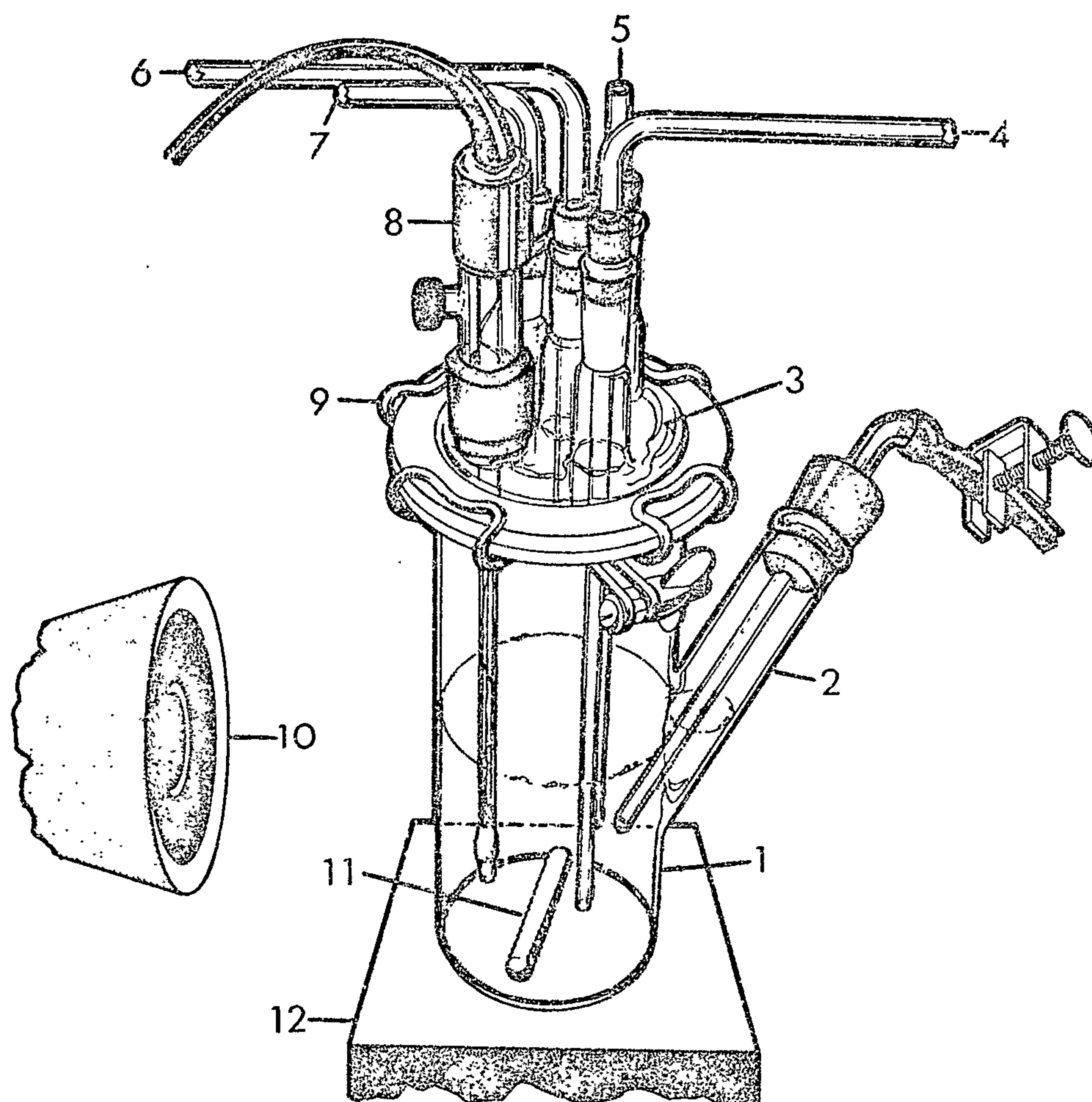
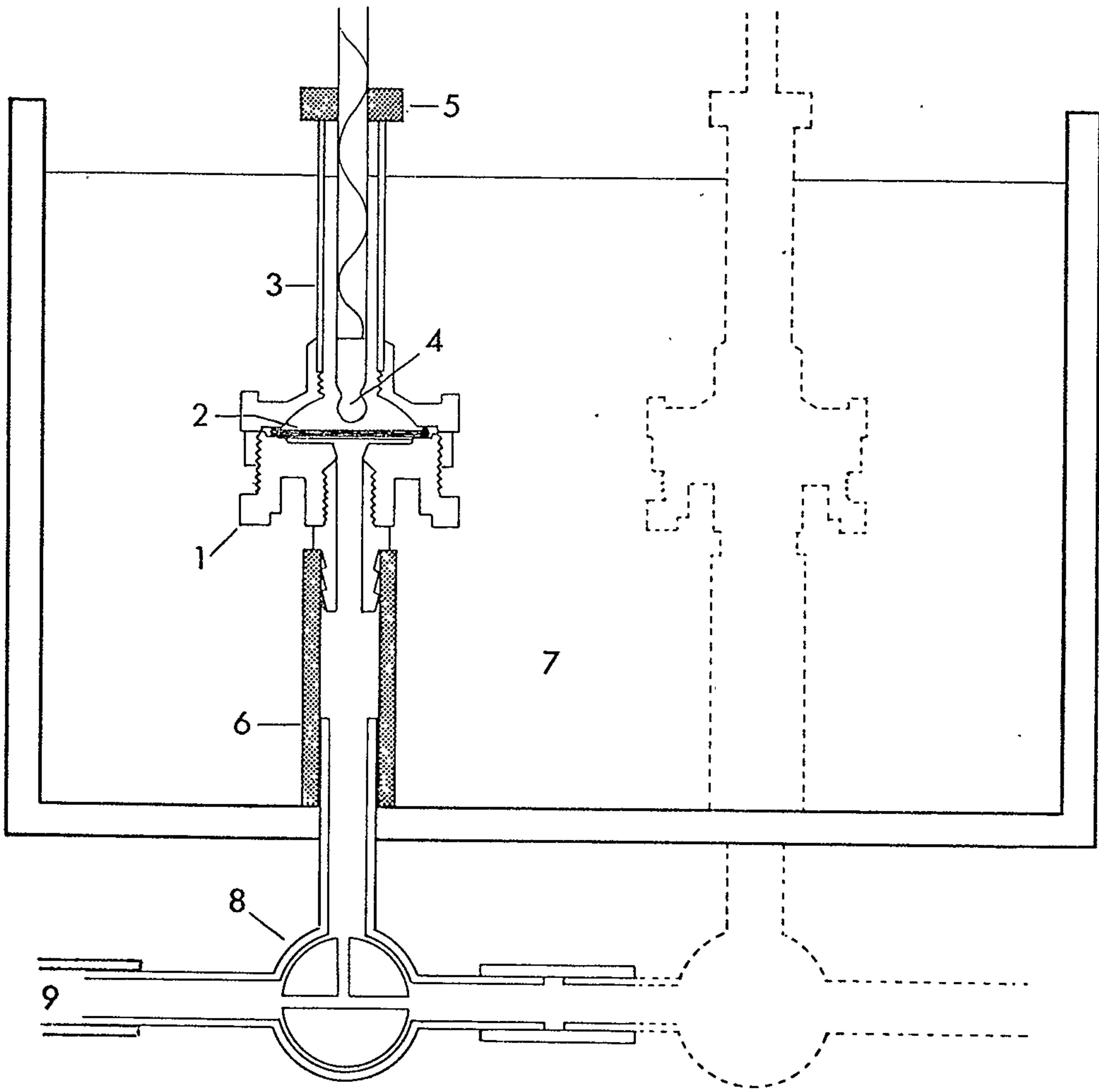
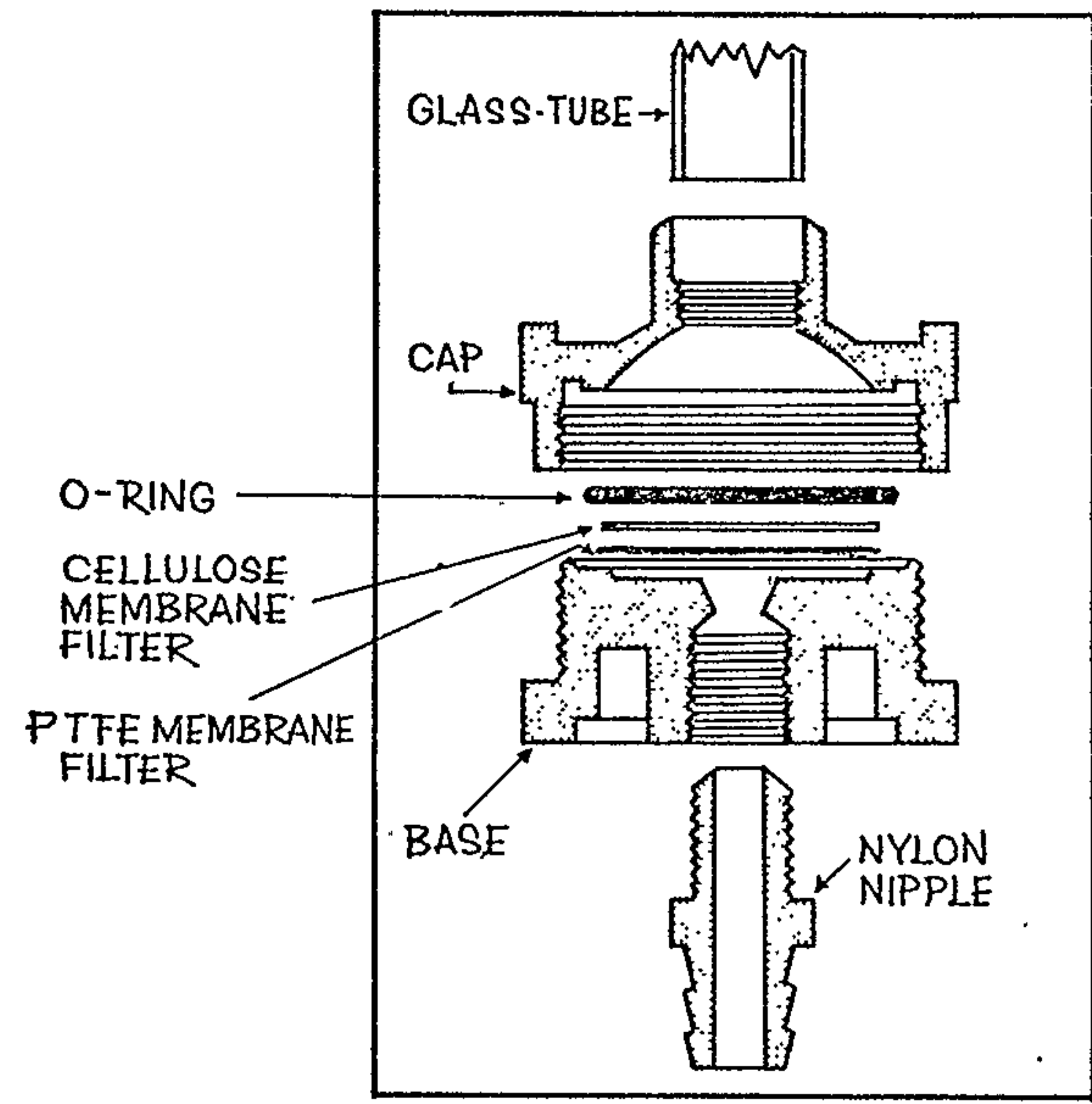


Fig. 34. Design of fermenter used in culture of test organisms: 1 = culture vessel; 2 = inoculation port; 3 = five socket flat flange lid; 4 = gas intake; 5 = housing for temperature sensor; 6 = alkali intake; 7 = gas exhaust; 8 = pH electrode; 9 = clip; 10 = infra-red lamp; 11 = spin bar; 12 = magnetic stirrer.

Fig. 35. Diagram of apparatus which allowed organisms to be (i) treated with test compounds, (ii) washed and (iii) monitored for acid production, whilst they remained *in situ*: 1 = autoclavable filter holder; 2 = 'mat' of organisms on top of membrane filters; 3 = glass tube containing culture broth; 4 = pH electrode; 5 = rubber stopper; 6 = rubber tubing; 7 = water at 37°C; 8 = 3-way stopcock; 9 = rubber tube connected to vacuum pump. (*Inset*). Exploded diagram of filter holder.



(iii) monitored for acid production, whilst they remained *in situ*.

It consisted of two autoclavable filter holders (Filter Holder-in-Line 25 mm, Gelman Instrument Co., Ann Arbor, Mich., U.S.A.)

connected to two 5 cm lengths of thick walled, 6 mm internal diameter, rubber tubing inside a water bath. Two 3-way stopcocks were each connected by one arm to a length of the rubber tubing through a hole in the floor of the water bath while another arm was connected to a vacuum pump (Fig. 35).

A 5 cm length of glass tubing (7 mm internal diameter) was inserted into the top of each filter holder.

A 0.45 μ m pore size cellulose membrane filter with grid (HAWG 025 00, Millipore Corp., Bedford, Mass., U.S.A.) and a 5 μ m pore size PTFE membrane filter (LSWP 025 00, Millipore) were used in the filter holder. The PTFE filter, which was placed underneath the cellulose filter, was only permeable to liquid when the vacuum pump was operating; when the pump was not operating liquid remained in the top part of the filter holder.

METHODS

Growth of Organisms

S. mutans strain GS5 was grown anaerobically at 37°C in trypticase soy broth. Ten millilitres of a 15 hour growth was added to 150 ml of trypticase soy broth, supplemented with 146 mM glucose^a,

^aAutoclaved separately from trypticase soy broth.

in the fermenter. The culture was grown at 37°C in a nitrogen - carbon dioxide atmosphere (95 per cent nitrogen - five per cent carbon dioxide) at pH 6.2 and was continuously stirred. The pH of the culture was kept constant by the addition of 2N sodium hydroxide. The cells were harvested at the exponential growth phase by centrifugation at 7,000 g for 10 minutes at 4°C.

A. viscosus strain NY1 was grown anaerobically at 37°C in brain heart infusion broth. Twenty millilitres of a 24 hour growth was added to 140 ml of brain heart infusion broth, supplemented with 146 mM glucose^a, in the fermenter. The culture was grown under the same stirring and atmospheric conditions as used for *S. mutans* strain GS5. However, the pH level was maintained at 7.0 throughout. The cells were also harvested at the exponential growth phase employing the method used for *S. mutans* strain GS5.

L. casei var. *casei* was grown anaerobically at 37°C in M.R.S. broth. Ten millilitres of a 24 hour growth was added to 150 ml of M.R.S. broth in the fermenter. The stirring, atmospheric and pH conditions were the same as those used for *S. mutans* strain GS5. The cells were harvested at the exponential growth phase employing the method used for *S. mutans* strain GS5.

Treatment of Bacterial Pellets

After centrifuging each pellet was suspended in quarter-strength Ringer solution^b at 4°C and centrifuged again at 7,000 g for 10

^a Autoclaved separately from brain heart infusion broth.

^b See footnote page 19.

minutes at 4°C. This procedure was then repeated and approximately 0.35 cm³ of the final pellet was placed in a beaker containing 2 ml of quarter-strength Ringer solution.

A measuring device consisting of a 7 mm diameter flat-ended PTFE plunger fitted in a hole in the centre of a flat PTFE disc (Kerr Mfg. Co., Detroit, Mich., U.S.A.) was used to obtain the packed cell volume of 0.35 cm³. Increments of the pellet remaining after the final centrifugation were added to the well formed in the centre of the disc when the plunger was withdrawn to a depth of 3 mm. Once the well had been filled the plunger was returned to its original position level with the disc surface. This procedure was repeated three times.

The beaker containing the organisms was placed in a shaker bath filled with water at 37°C and the beaker was gently agitated for 15 minutes.

Exposure of Organisms to Treatment Solutions and Measurement of Acid Production

The treatment solutions used are given in Table 9. All solutions were warmed to 37°C before use.

The filter holders and filters were autoclaved and placed *in situ* in the water bath which was then filled with water at 37°C. One millilitre of the quarter-strength Ringer solution, containing the suspended organisms, was pipetted into the glass tube on top of each filter holder and the vacuum applied^a. Once the liquid had been removed from the top part of the holder the vacuum was stopped and 1 ml

^aThe resulting 'mat' of organisms was approximately 0.7 mm thick.

TABLE 9
TREATMENT SOLUTIONS AND TREATMENT TIMES USED IN
ACID PRODUCTION STUDY

Solution	pH	Treatment Time (min)
Ringer Solution ^a (Control)	5.3	5
1 mM FeCl ₂ · 4H ₂ O	5.1	5
10 mM FeCl ₂ · 4H ₂ O	4.3	5
100mM FeCl ₂ · 4H ₂ O	3.2	5
1 mM FeCl ₃ · 6H ₂ O	3.2	5
10 mM FeCl ₃ · 6H ₂ O	2.4	5
100mM FeCl ₃ · 6H ₂ O	1.6	5
15 mM FeF ₂ (570 ppm F) ^b	4.4	5
10 mM FeF ₃ (570 ppm F) ^c	3.5	5
50 mM FeCl ₃ · 6H ₂ O		
+ 190mM NaF (3,620 ppm F) ^d	2.1	5
APF (12,300 ppm F) ^d	3.2	5
26 mM SnF ₂ (970 ppm F) ^d	3.4	5
511mM SnF ₂ (19,400 ppm F) ^d	2.8	5
48 mM NaF (905 ppm F) ^d	7.0	5
476mM NaF (9,050 ppm F) ^d	7.0	5

a Quarter - strength .

b Prepared under nitrogen. Solution warmed to 60°C and continuously stirred to facilitate dissolution of salt. Solution cooled to 37°C before use.

c Solution warmed to 60°C and continuously stirred to facilitate dissolution of salt. Solution cooled to 37°C before use.

d Solutions have been tested clinically (see footnote **c** Table 1).

of treatment solution at 37°C was pipetted into the glass tube and left for 5 minutes. At the end of this period the vacuum was re-applied and kept on until the treatment solution and the subsequent washing solution (2 ml of quarter-strength Ringer solution at 37°C) had passed through the filter.

After the vacuum had been stopped 0.5 ml of culture broth^a was pipetted into the glass tube and the vacuum re-applied for approximately 15 seconds. A further 1 ml of broth was then added to the glass tube and a combination electrode (CA14/02, Philips, Eindhoven, Holland), connected to a pH meter (Model 290 Mk.2, Pye Unicam Ltd., Cambridge, England) was inserted in the tube, with the tip of the electrode approximately 4 mm above the surface of the top membrane filter. The electrode system was standardised before use with a pH 6.88 phosphate buffer (E. Merck, Darmstadt, Germany) and a pH 4.62 acetate buffer (Merck).

No attempt was made to remove iron from the media.

No buffers, apart from those normally present in liquid media⁽²¹⁾, were necessary to obtain a reproducible initial pH with this system. However, it was particularly important to add then filter 0.5 ml of broth before adding the final 1 ml. Without this step, it was found difficult to obtain a reproducible initial pH after the test organisms had been treated with low pH solutions such as 100 mM iron (III) chloride.

The pH values were recorded at 10 minute intervals for 90

^aThe same type of broth used to grow the organisms supplemented with either 146 mM glucose or 146 mM sucrose.

minutes when *S. mutans* strain GS5 or *L. casei* var. *casei* were the test organisms and at 10 minute intervals for 150 minutes when *A. viscosus* strain NY1 was used.

Each experiment was performed in duplicate.

RESULTS

The initial and terminal pH values, and the mean hydrogen ion concentrations calculated from these values, are presented in Tables 10, 11 and 12.

Also shown in Tables 10, 11 and 12 are data on changes in hydrogen ion concentrations in culture broths during the 90 and 150 minute experimental periods. The data for the controls were used as base line values from which the relative effectiveness of the other treatments were assessed. These findings are presented in Figs. 36, 40 and 44.

The mean hydrogen ion concentration at each 10 minute interval in the 90 and 150 minute experimental periods was calculated from the original pH data and the values, thus obtained, were re-converted into pH and used to prepare the acid production rate graphs shown in Figs. 37 to 39, 41 to 43 and 45 to 47.

ACID PRODUCTION BY S. MUTANS STRAIN GS5

In general, iron (II) chloride appeared to be less effective than iron (III) chloride, at equivalent concentrations, in inhibiting acid production by *S. mutans* strain GS5 (Fig. 36). The difference was particularly evident at concentrations of 10 mM and 100 mM.

With the exception of 10 mM iron (III) fluoride and 48 mM sodium fluoride, all the fluoride solutions reduced acid production by more than 98 per cent. Treatments with 10 mM iron (III) fluoride and those with 48 mM sodium fluoride reduced acid production by 35.2 per cent and 89.5 per cent respectively when the culture broth was supplemented with glucose, and by 33.1 per cent and 74.3 per cent respectively when the culture broth was supplemented with sucrose (Fig. 36).

Whilst only the most general of comparisons can be made as to the relative effect of glucose and sucrose on acid production by *S. mutans* strain GS5 it does appear from Table 10 that, in the majority of tests, the terminal hydrogen ion concentrations in culture broth supplemented with glucose were higher than those in culture broth supplemented with sucrose.

The graphs in Fig. 37 show that iron (II) chloride at concentrations of 1 mM and 10 mM tended to increase the initial rates of acid production slightly. The graphs in Fig. 38 show that 10 mM iron (III) fluoride also tended to increase the initial rates of acid production slightly.

TABLE 10

ACID PRODUCTION BY *S. MUTANS* STRAIN GS5 AFTER
TREATMENT WITH TEST COMPOUNDS

A. Initial and Terminal pH Values

Treatment ^a	Medium							
	TSB ^b + 146 mM Glucose				TSB ^b + 146 mM Sucrose			
	Initial pH Replicate		Terminal pH ^c Replicate		Initial pH Replicate		Terminal pH ^c Replicate	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Ringer Solution ^d (Control)	7.35	7.35	4.40	4.38	7.35	7.35	4.58	4.63
1 mM FeCl ₂ · 4H ₂ O	7.34	7.35	4.65	4.71	7.35	7.32	4.54	4.76
10 mM FeCl ₂ · 4H ₂ O	7.35	7.35	4.58	4.73	7.35	7.32	5.04	4.73
100 mM FeCl ₂ · 4H ₂ O	7.35	7.34	4.95	4.86	7.34	7.35	5.01	5.05
1 mM FeCl ₃ · 6H ₂ O	7.35	7.35	4.68	4.80	7.35	7.35	4.68	4.84
10 mM FeCl ₃ · 6H ₂ O	7.35	7.35	5.64	5.51	7.34	7.35	5.52	5.60
100 mM FeCl ₃ · 6H ₂ O	7.33	7.34	7.21	7.25	7.35	7.33	7.29	7.14
15 mM FeF ₂	7.35	7.35	6.23	6.12	7.35	7.35	6.57	6.26
10 mM FeF ₃	7.35	7.35	4.54	4.62	7.35	7.35	4.69	4.89
50 mM FeCl ₃ · 6H ₂ O + 190 mM NaF	7.32	7.35	7.19	7.23	7.35	7.34	7.26	7.22
APF	7.35	7.35	7.20	7.30	7.33	7.34	7.27	7.25
26 mM SnF ₂	7.33	7.35	6.40	6.36	7.35	7.35	6.75	6.78
511 mM SnF ₂	7.32	7.35	7.09	7.25	7.34	7.35	7.20	7.31
48 mM NaF	7.35	7.34	5.77	5.16	7.35	7.35	5.73	4.96
476 mM NaF	7.35	7.37	7.20	7.12	7.36	7.35	7.29	7.25

^a Treatment time — five minutes.^b TSB — trypticase soy broth.^c At 90 minutes.^d Quarter-strength.

TABLE 10 (cont.)

B. Initial and Terminal Mean Hydrogen Ion Concentrations (g ions/l)^a

Treatment	Medium					
	TSB + 146 mM Glucose			TSB + 146 mM Sucrose		
	Initial Mean H ⁺ Concentration	Terminal Mean H ⁺ Concentration	Increase in H ⁺ Concentration	Initial Mean H ⁺ Concentration	Terminal Mean H ⁺ Concentration	Increase in H ⁺ Concentration
Ringer Solution (Control)	4.467×10^{-8} (7.35) ^b	4.075×10^{-5} (4.39)	4.070×10^{-5c}	4.467×10^{-8} (7.35)	2.487×10^{-5} (4.60)	2.483×10^{-5c}
1 mM FeCl ₂ · 4H ₂ O	4.519×10^{-8} (7.34)	2.094×10^{-5} (4.68)	2.089×10^{-5}	4.627×10^{-8} (7.33)	2.311×10^{-5} (4.64)	2.306×10^{-5}
10 mM FeCl ₂ · 4H ₂ O	4.467×10^{-8} (7.35)	2.246×10^{-5} (4.65)	2.241×10^{-5}	4.626×10^{-8} (7.33)	1.387×10^{-5} (4.86)	1.382×10^{-5}
100mM FeCl ₂ · 4H ₂ O	4.519×10^{-8} (7.34)	1.300×10^{-5} (4.89)	1.295×10^{-5}	4.519×10^{-8} (7.34)	9.342×10^{-6} (5.03)	9.297×10^{-6}
1 mM FeCl ₃ · 6H ₂ O	4.467×10^{-8} (7.35)	1.837×10^{-5} (4.74)	1.832×10^{-5}	4.467×10^{-8} (7.35)	1.689×10^{-5} (4.77)	1.685×10^{-5}
10 mM FeCl ₃ · 6H ₂ O	4.467×10^{-8} (7.35)	2.690×10^{-6} (5.57)	2.645×10^{-6}	4.510×10^{-8} (7.34)	2.766×10^{-6} (5.56)	2.721×10^{-6}
100mM FeCl ₃ · 6H ₂ O	4.624×10^{-8} (7.33)	5.895×10^{-8} (7.23)	1.271×10^{-8}	4.572×10^{-8} (7.34)	6.186×10^{-8} (7.21)	1.614×10^{-8}
15 mM FeF ₂	4.467×10^{-8} (7.35)	6.737×10^{-7} (6.17)	6.290×10^{-7}	4.467×10^{-8} (7.35)	4.093×10^{-7} (6.39)	3.646×10^{-7}
10 mM FeF ₃	4.467×10^{-8} (7.35)	2.641×10^{-5} (4.58)	2.637×10^{-5}	4.467×10^{-8} (7.35)	1.665×10^{-5} (4.78)	1.661×10^{-5}
50 mM FeCl ₃ · 6H ₂ O +190mM NaF	4.627×10^{-8} (7.33)	6.172×10^{-8} (7.21)	1.545×10^{-8}	4.519×10^{-8} (7.34)	5.760×10^{-8} (7.24)	1.240×10^{-8}
APF	4.467×10^{-8} (7.35)	5.661×10^{-8} (7.25)	1.194×10^{-8}	4.624×10^{-8} (7.33)	5.497×10^{-8} (7.26)	0.870×10^{-8}
26 mM SnF ₂	4.627×10^{-8} (7.33)	4.173×10^{-7} (6.38)	3.710×10^{-7}	4.467×10^{-8} (7.35)	1.719×10^{-7} (6.76)	1.272×10^{-7}
511mM SnF ₂	4.627×10^{-8} (7.33)	6.876×10^{-8} (7.16)	2.249×10^{-8}	4.519×10^{-8} (7.34)	5.604×10^{-8} (7.25)	1.085×10^{-8}
48 mM NaF	4.519×10^{-8} (7.34)	4.308×10^{-6} (5.37)	4.263×10^{-6}	4.467×10^{-8} (7.35)	6.413×10^{-6} (5.19)	6.368×10^{-6}
476mM NaF	4.366×10^{-8} (7.36)	6.948×10^{-8} (7.16)	2.582×10^{-8}	4.467×10^{-8} (7.35)	5.376×10^{-8} (7.27)	0.910×10^{-8}

^a Calculated from data in Table 10-A.^b Values in parentheses obtained by converting mean hydrogen ion concentration into pH.^c Used as base line values for findings shown in Fig. 36.

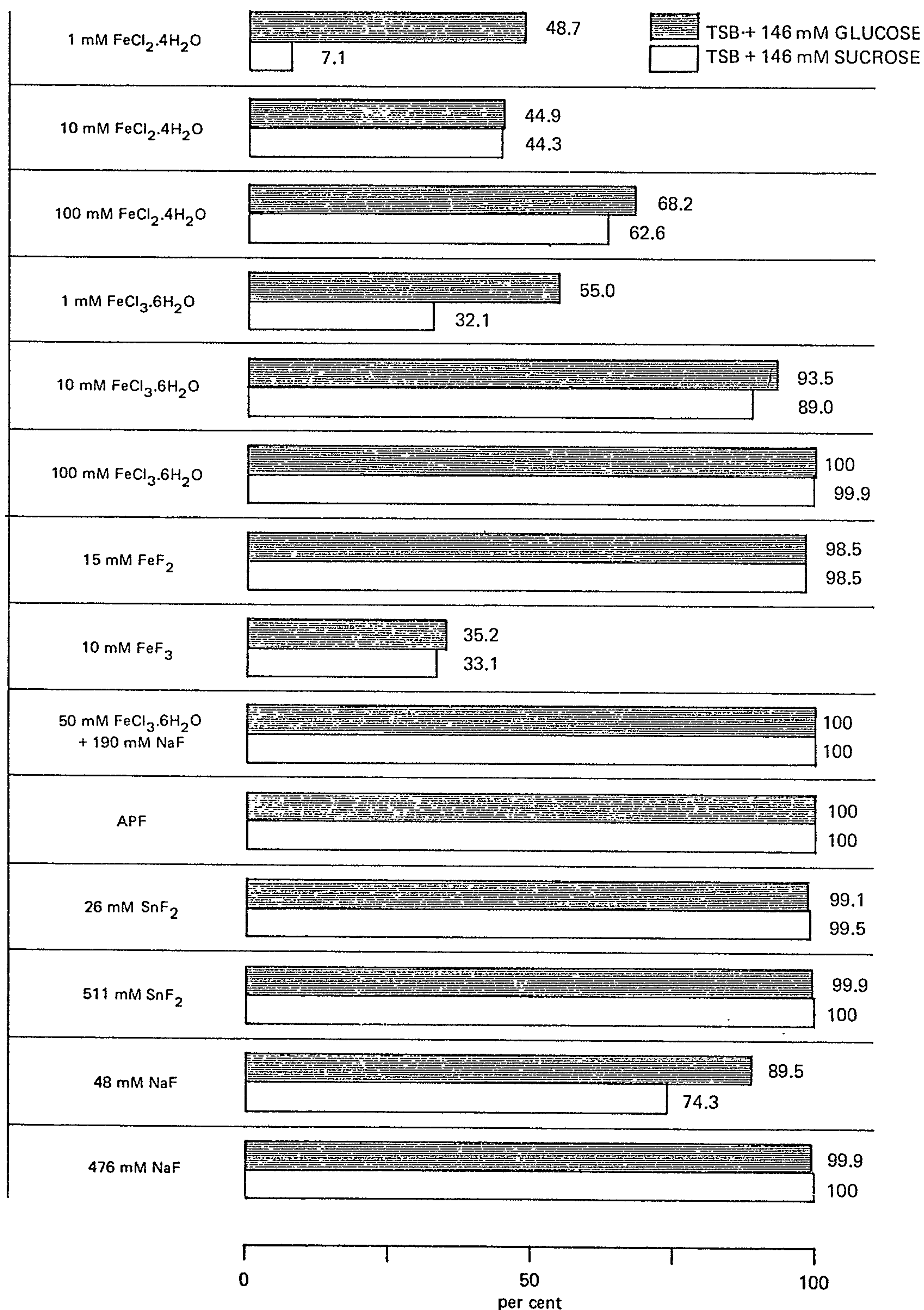


Fig. 36. Reductions in acid production by *S. mutans* strain GS5 in trypticase soy broth (TSB) supplemented with 146 mM glucose or 146 mM sucrose following treatment with test compounds (treatment time – five minutes).

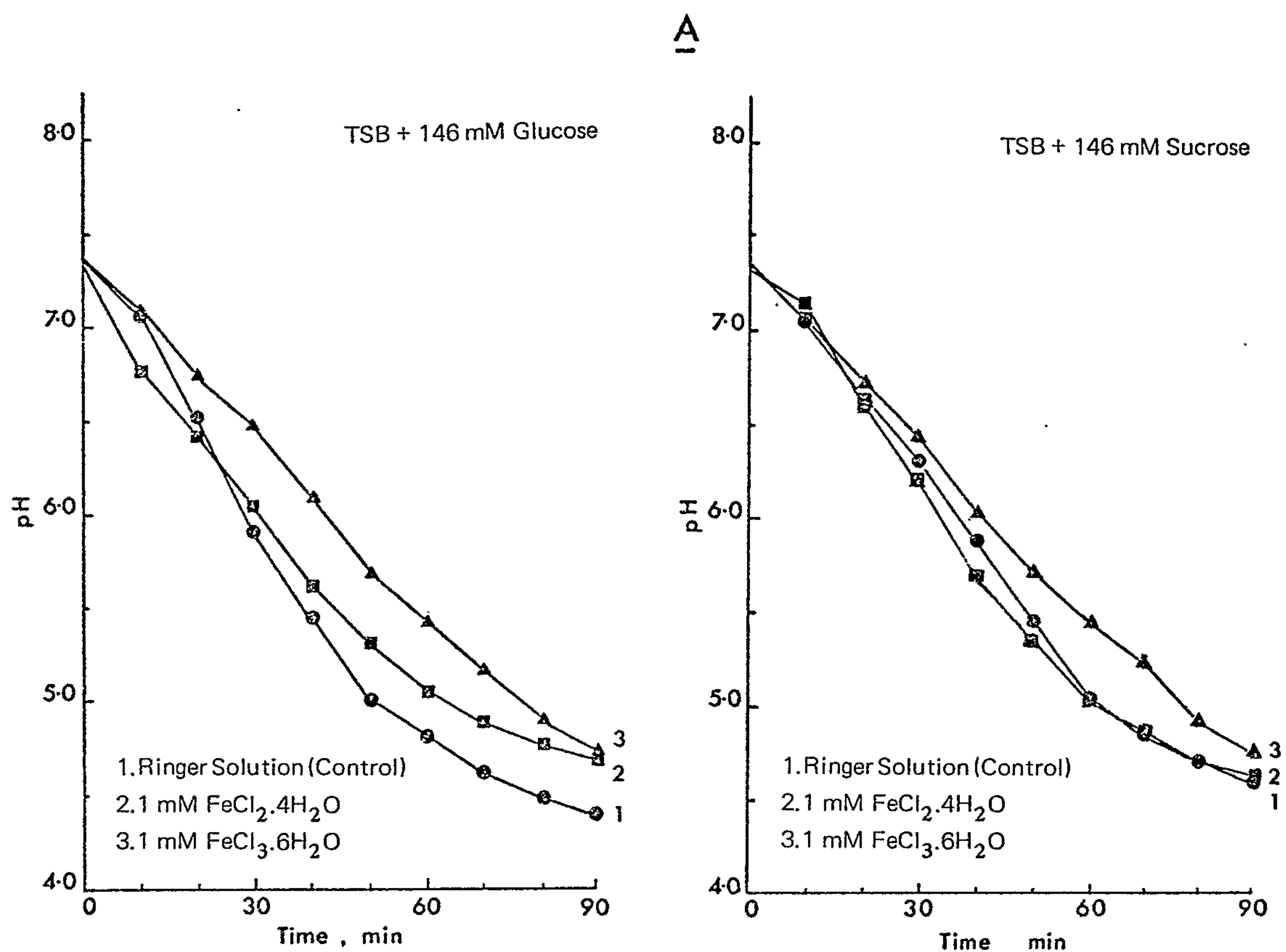
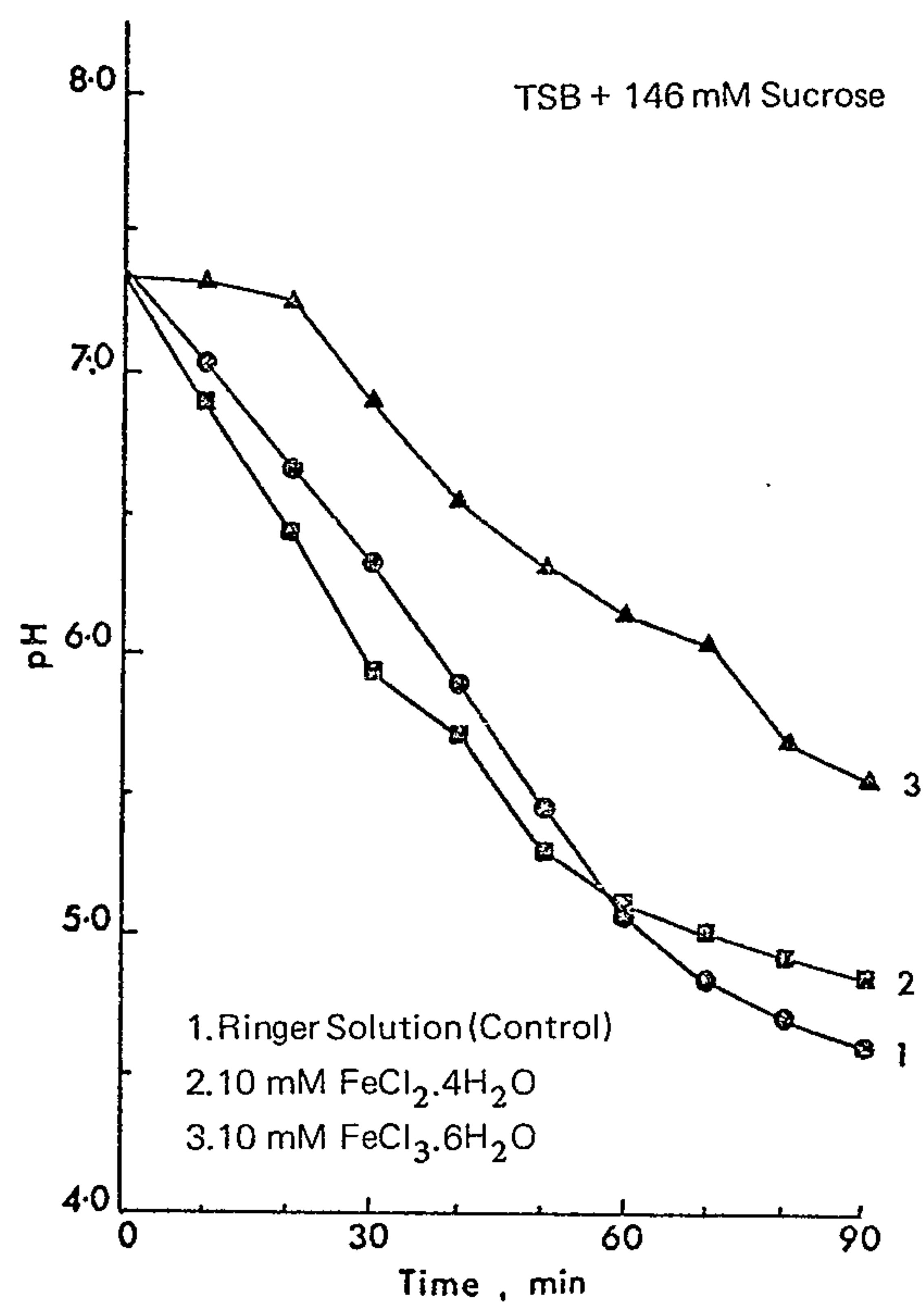
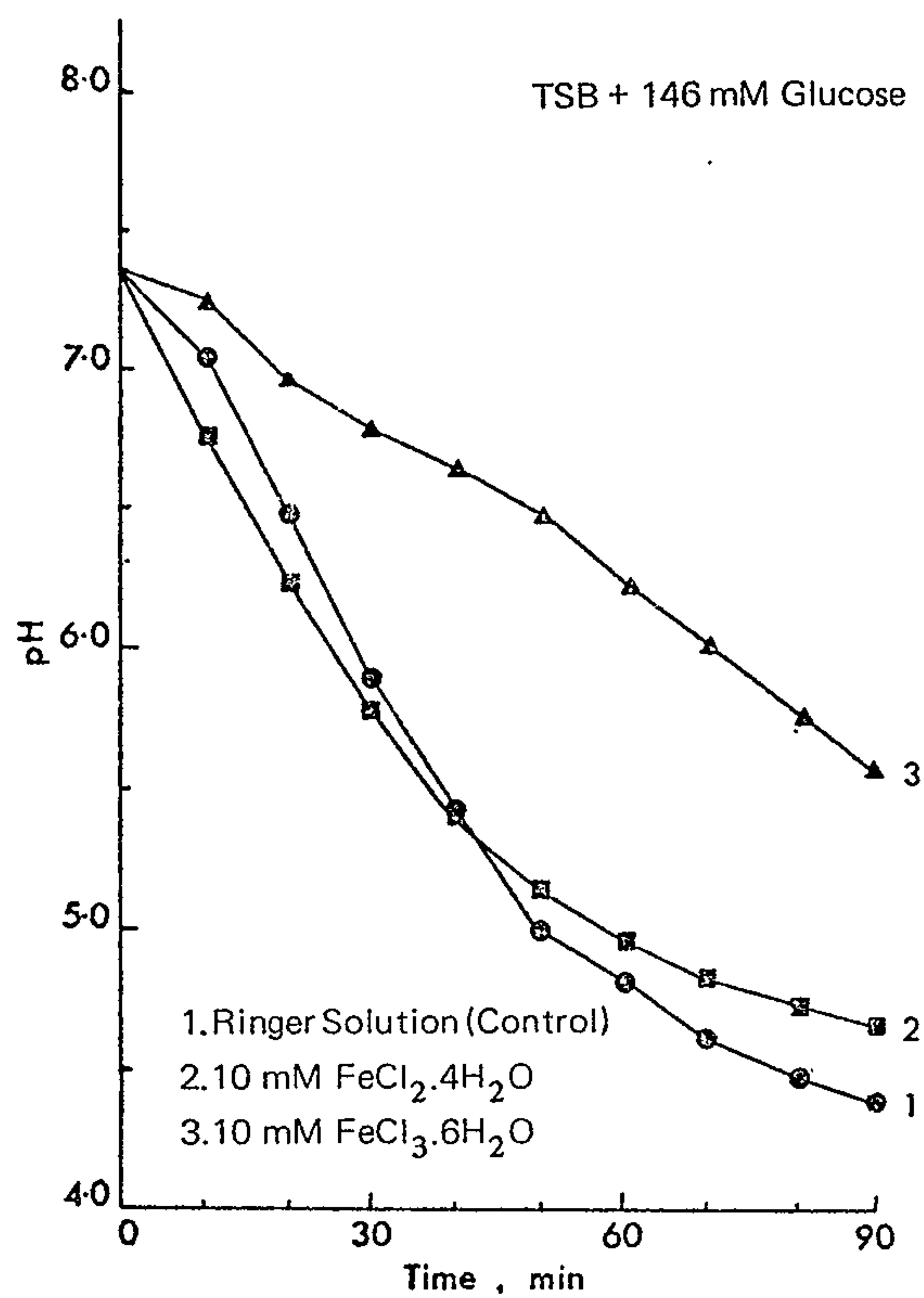


Fig. 37. Rates of acid production by *S. mutans* strain GS5 in TSB supplemented with glucose or sucrose following treatment with iron (II) chloride or iron (III) chloride.

- A. Effect of five minute treatments with iron (II) chloride or iron (III) chloride at a concentration of 1 mM. (Left). TSB supplemented with 146 mM glucose. (Right). TSB supplemented with 146 mM sucrose.
- B. Effect of five minute treatments with iron (II) chloride or iron (III) chloride at a concentration of 10 mM. (Left). TSB supplemented with 146 mM glucose. (Right). TSB supplemented with 146 mM sucrose.
- C. Effect of five minute treatments with iron (II) chloride or iron (III) chloride at a concentration of 100 mM. (Left). TSB supplemented with 146 mM glucose. (Right). TSB supplemented with 146 mM sucrose.

B



C

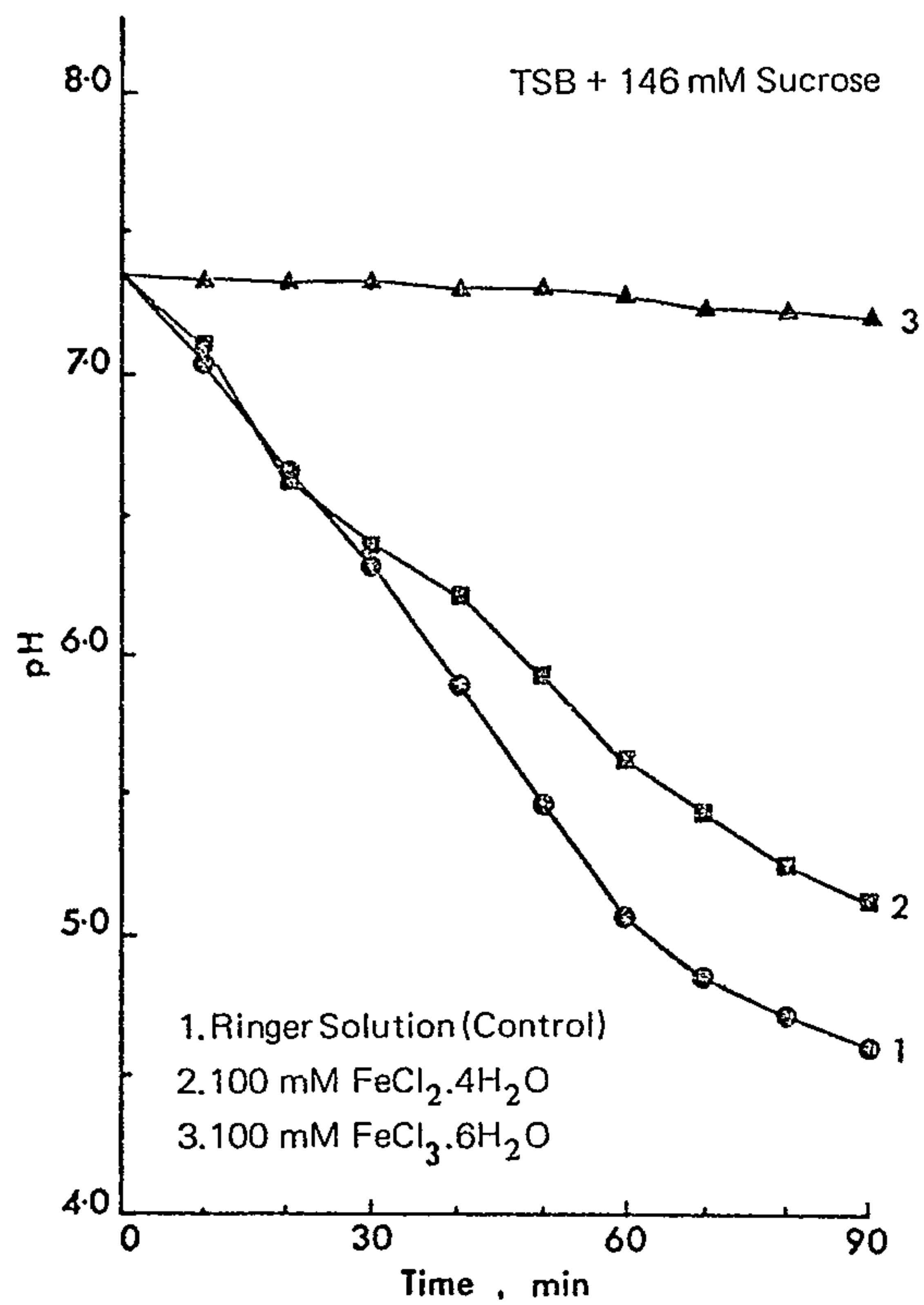
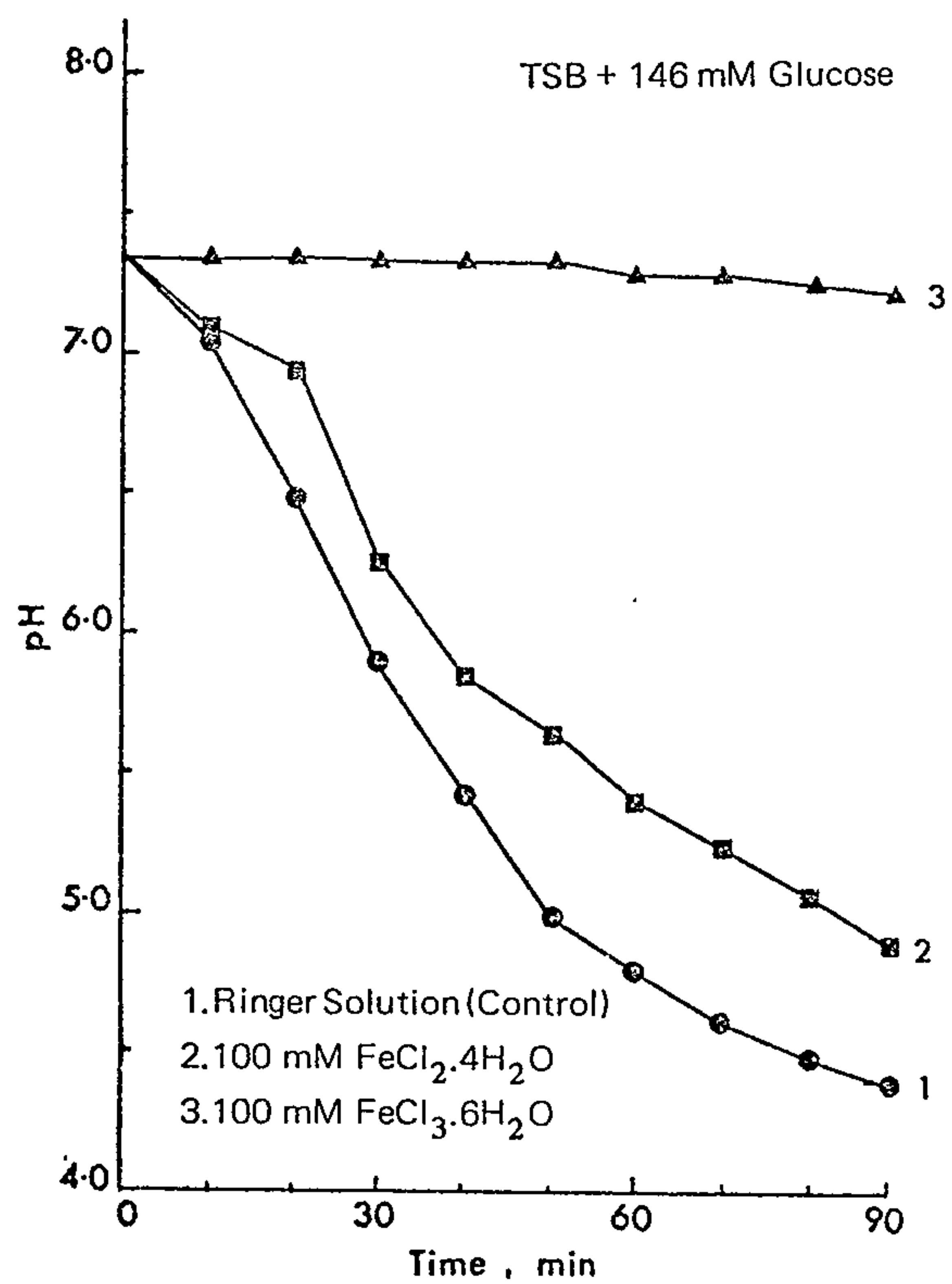
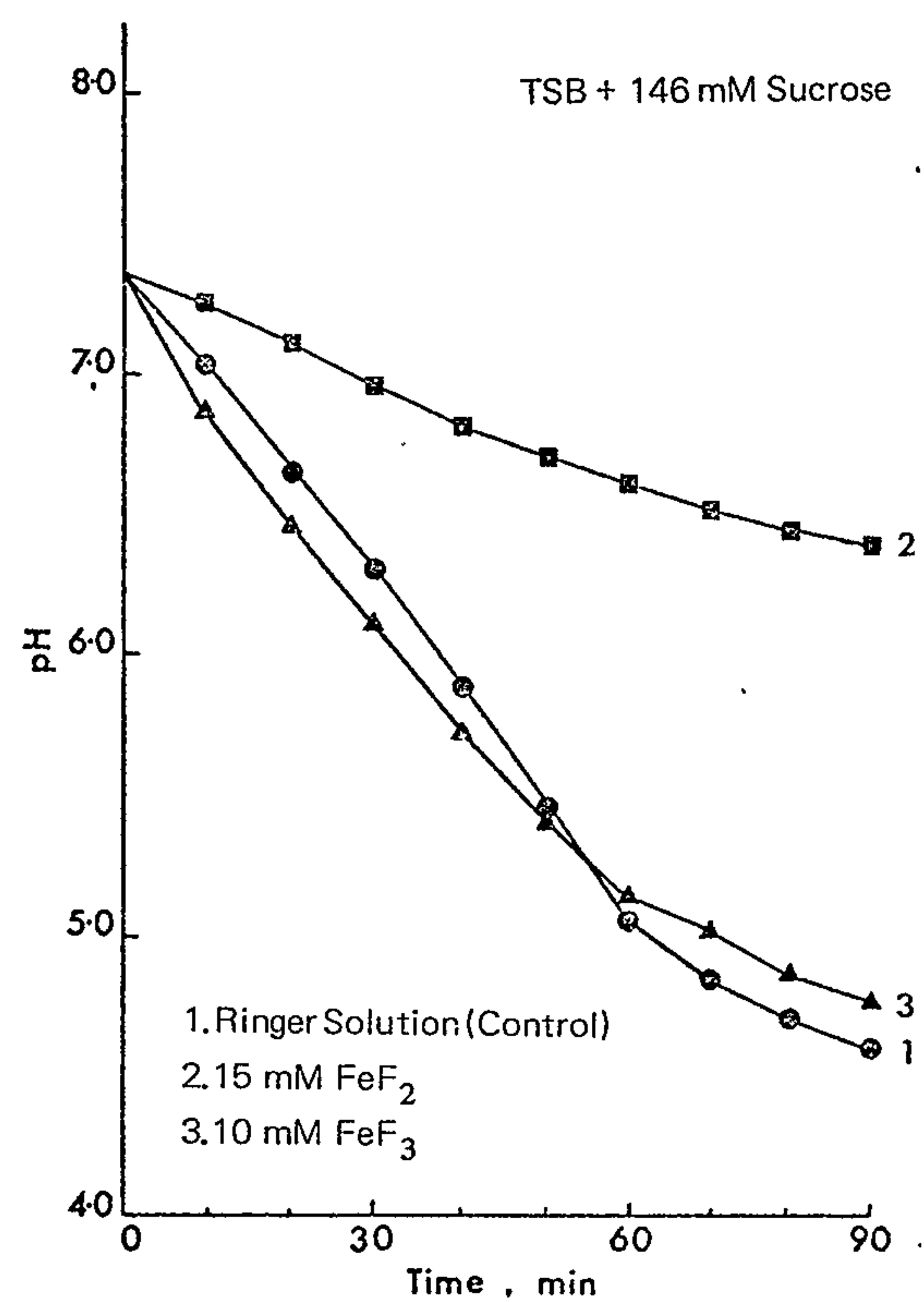
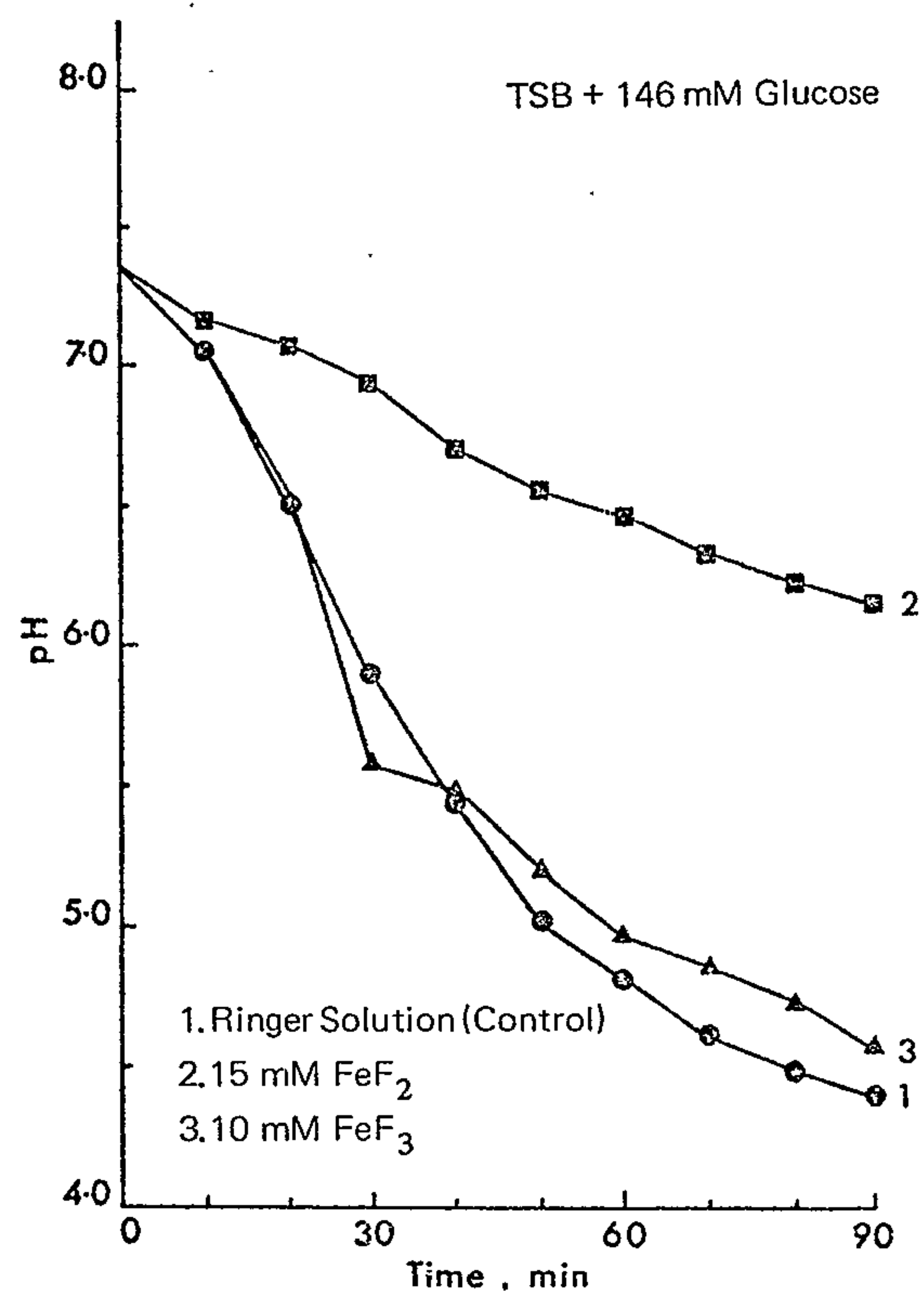


Fig. 38. Rates of acid production by *S. mutans* strain GS5 in TSB supplemented with glucose or sucrose following treatment with iron (II) fluoride, iron (III) fluoride, APF or a solution containing iron (III) chloride and sodium fluoride.

- A. Effect of five minute treatments with 15 mM iron (II) fluoride or 10 mM iron (III) fluoride. (*Left*). TSB supplemented with 146 mM glucose. (*Right*). TSB supplemented with 146 mM sucrose.
- B. Effect of five minute treatments with APF or a solution containing 50 mM-iron (III) chloride and 190 mM sodium fluoride. (*Left*). TSB supplemented with 146 mM glucose. (*Right*). TSB supplemented with 146 mM sucrose.

A



B

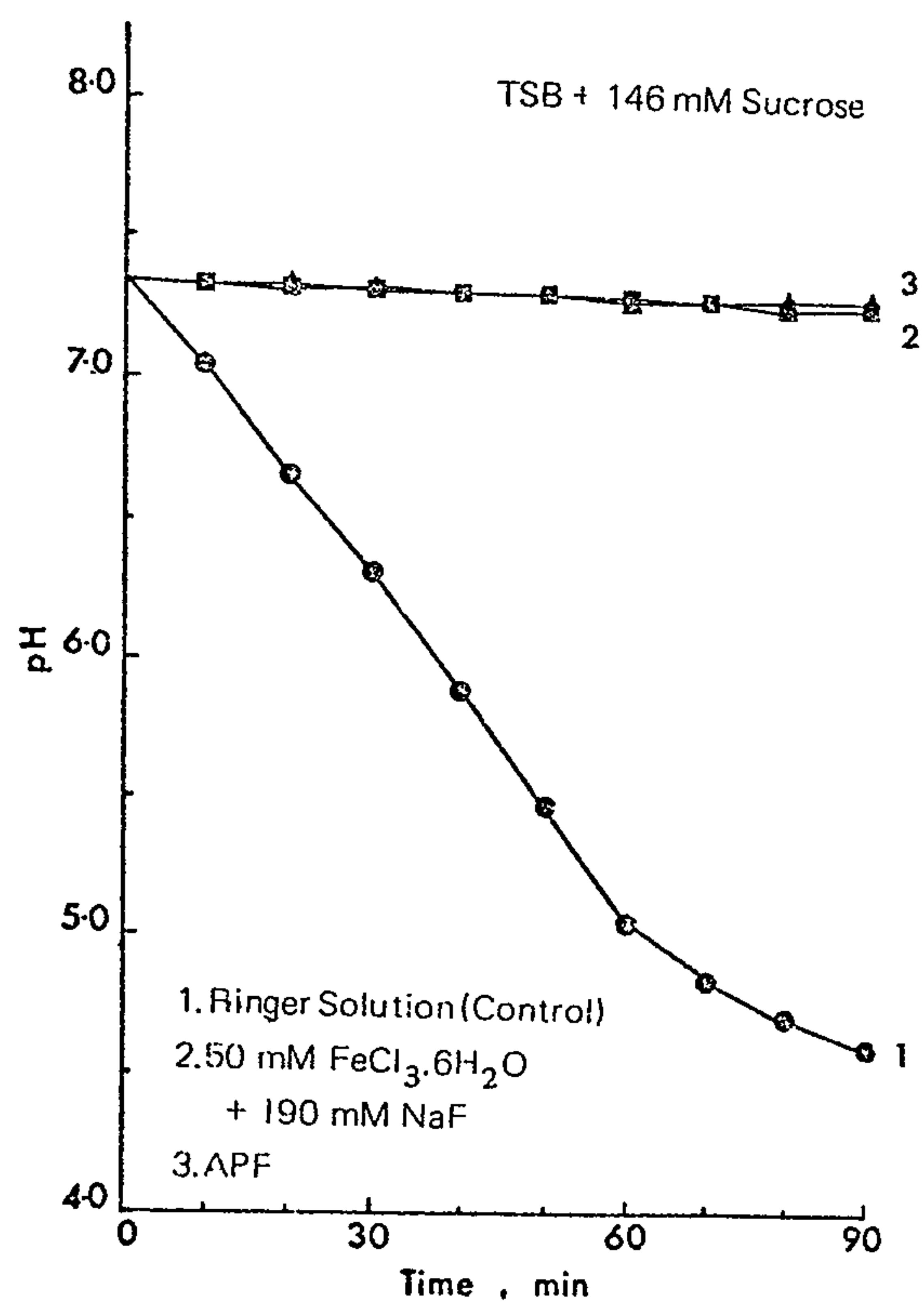
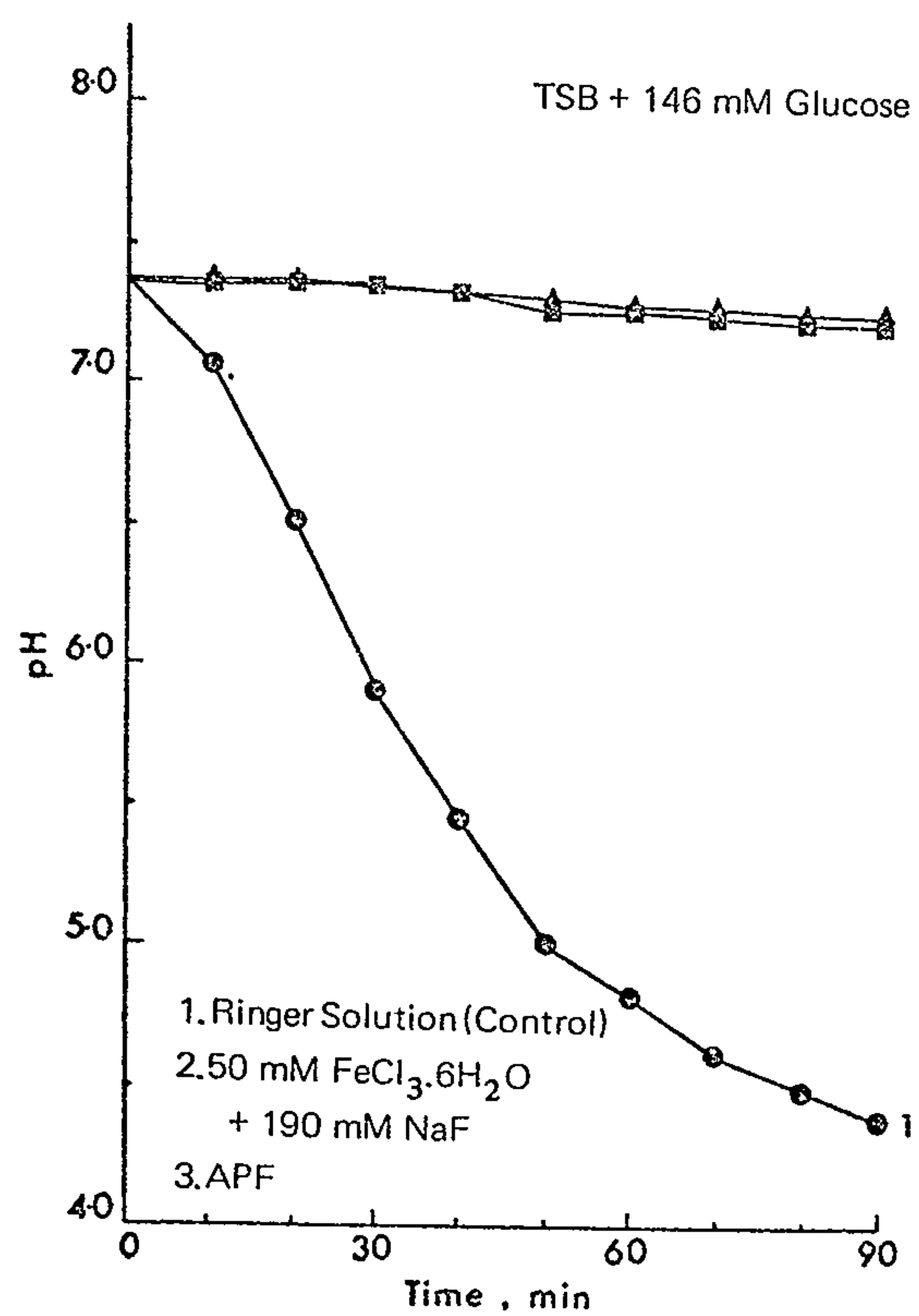
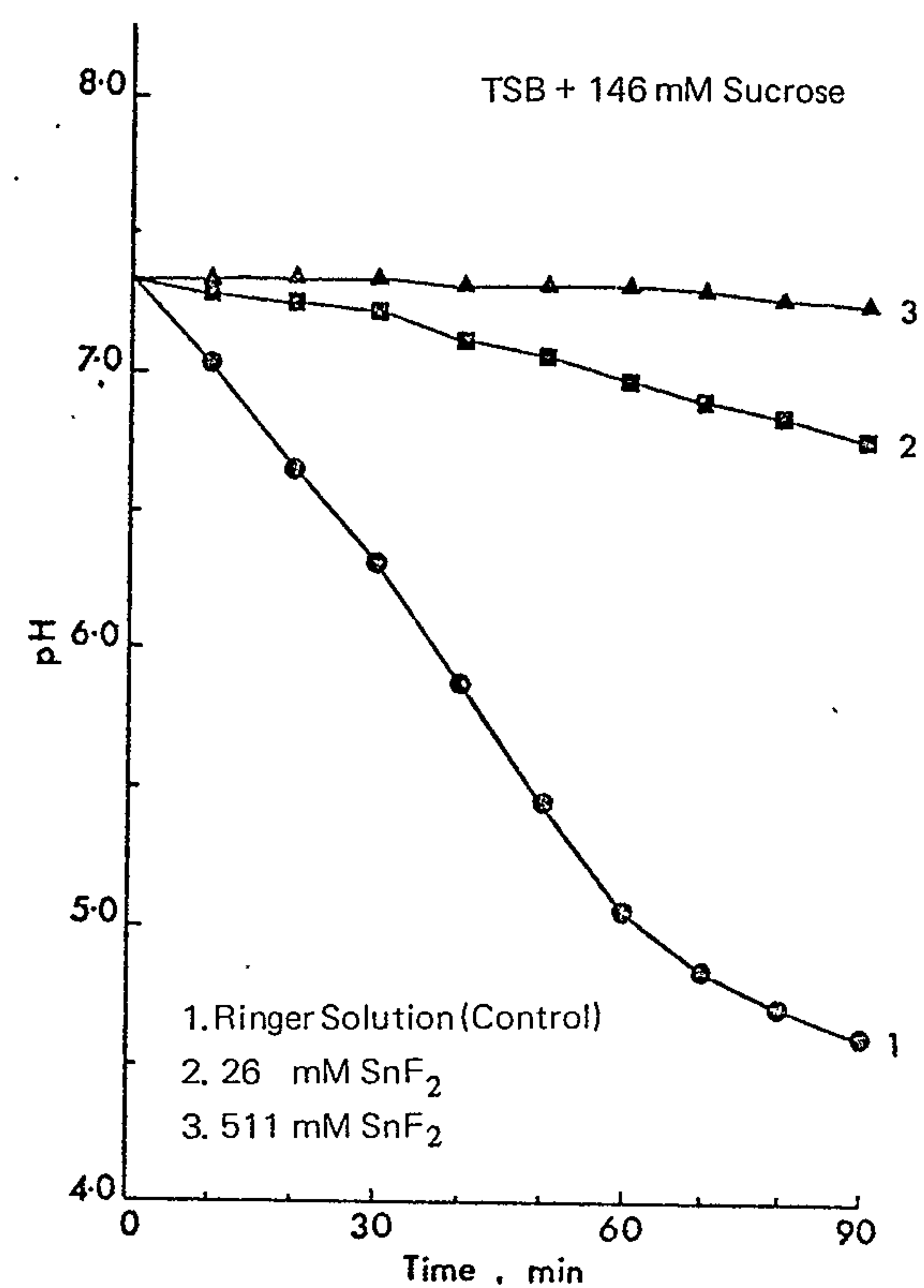
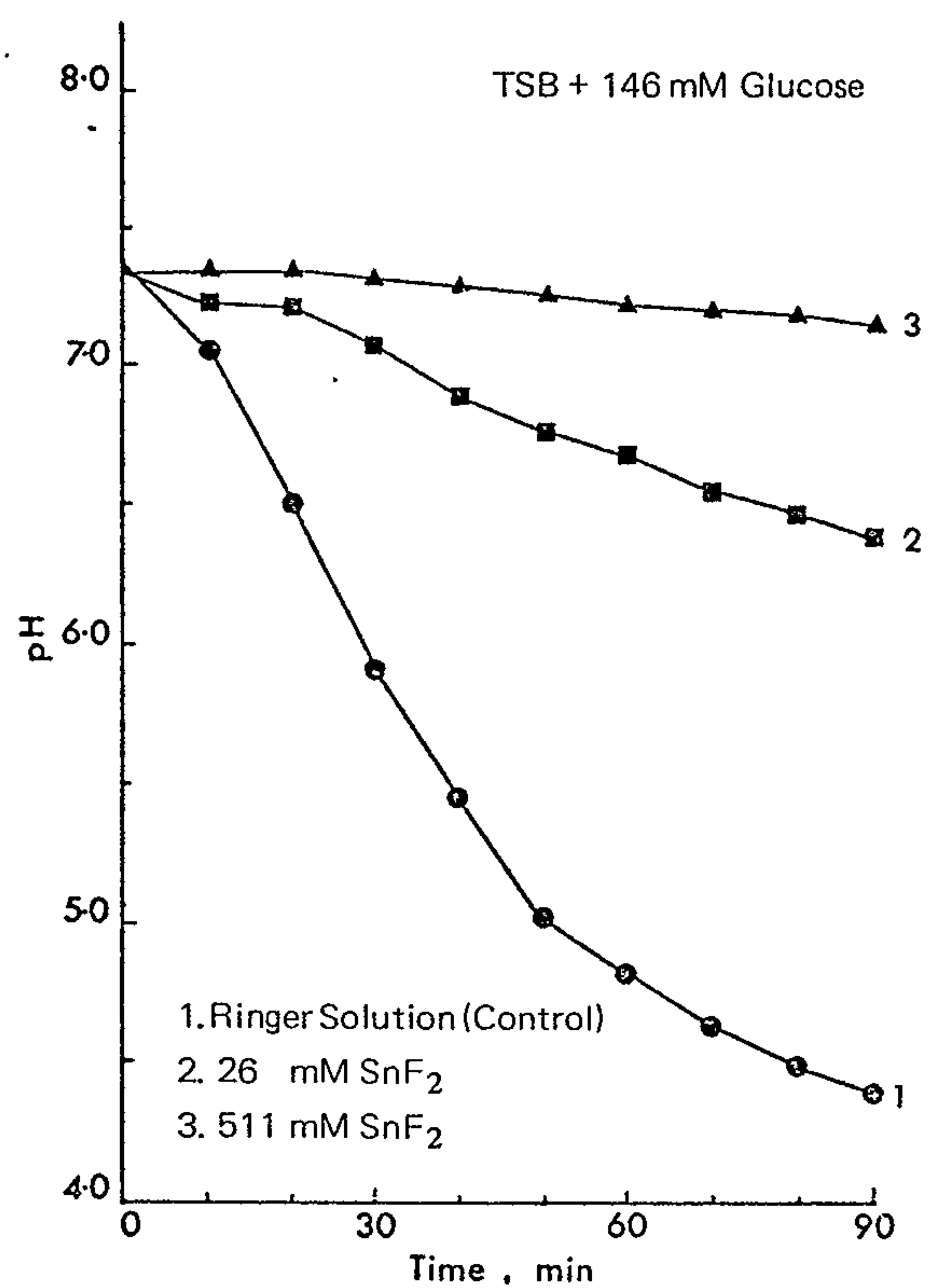


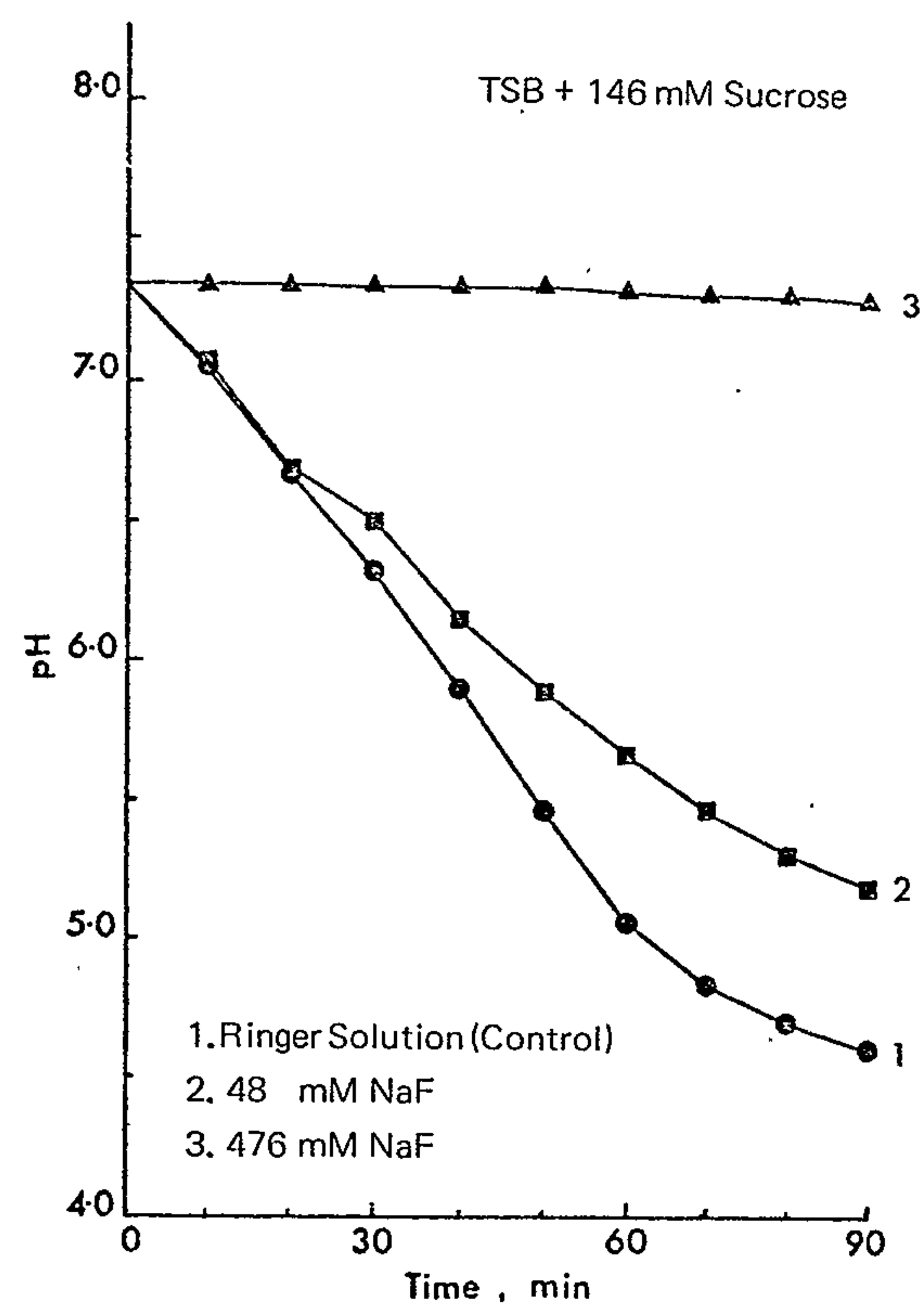
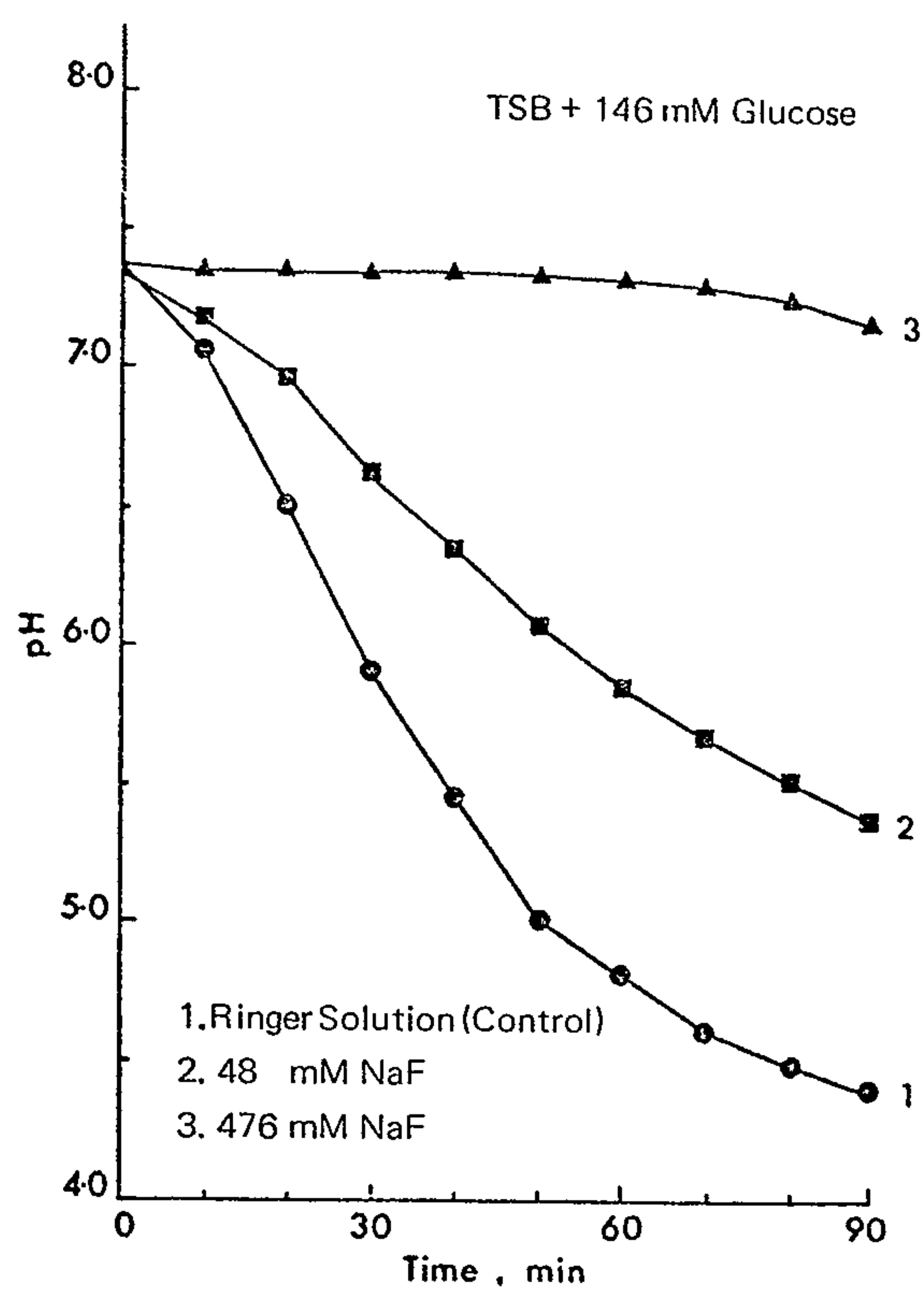
Fig. 39. Rates of acid production by *S. mutans* strain GS5 in TSB supplemented with glucose or sucrose following treatment with tin (II) fluoride or sodium fluoride.

- A. Effect of five minute treatments with tin (II) fluoride at a concentration of 26 mM or 511 mM. (*Left*). TSB supplemented with 146 mM glucose. (*Right*). TSB supplemented with 146 mM sucrose.
- B. Effect of five minute treatments with sodium fluoride at a concentration of 48 mM or 476 mM. (*Left*). TSB supplemented with 146 mM glucose. (*Right*). TSB supplemented with 146 mM sucrose.

A



B



ACID PRODUCTION BY A. VISCOSUS STRAIN NY1

With one exception, 1 mM iron (II) chloride, all the test compounds inhibited acid production by *A. viscosus* strain NY1 (Fig. 40).

Although, 1 mM iron (II) chloride increased acid production by 9.9 per cent when the culture broth was supplemented with glucose it did reduce acid production by 41.4 per cent when the culture broth was supplemented with sucrose.

It is evident from Fig. 40 that lower reductions in acid production occurred with treatments with iron (II) chloride than with treatments with iron (III) chloride at equivalent concentrations. Iron (III) chloride at a concentration of 10 mM reduced acid production by 82.1 per cent when the culture broth was supplemented with glucose and by 84.6 per cent when the culture broth was supplemented with sucrose. At a concentration of 100 mM, iron (III) chloride virtually inhibited acid production completely.

All the fluoride solutions reduced acid production markedly (Fig. 40). The reductions in acid production ranged from 80.9 per cent (10 mM iron (III) fluoride) to 99.9 per cent (511 mM tin (II) fluoride and 476 mM sodium fluoride).

The graphs in Figs. 41 to 43 show that the rates of acid production by organisms treated with the test compounds remained reasonably constant over the 150 minute experimental period.

TABLE 11

ACID PRODUCTION BY *A. VISCOSUS* STRAIN NY1 AFTER
TREATMENT WITH TEST COMPOUNDS

A. Initial and Terminal pH Values

Treatment ^a	Medium							
	BHI ^b + 146 mM Glucose				BHI ^b + 146 mM Sucrose			
	Initial pH Replicate		Terminal pH ^c Replicate		Initial pH Replicate		Terminal pH ^c Replicate	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Ringer Solution ^d (Control)	7.45	7.45	5.01	4.90	7.44	7.45	4.71	5.01
1 mM FeCl ₂ · 4H ₂ O	7.45	7.45	4.80	5.06	7.45	7.45	5.19	4.97
10 mM FeCl ₂ · 4H ₂ O	7.45	7.44	5.08	5.15	7.45	7.45	5.10	5.18
100mM FeCl ₂ · 4H ₂ O	7.44	7.44	5.27	5.11	7.45	7.44	5.45	5.06
1 mM FeCl ₃ · 6H ₂ O	7.45	7.44	5.25	5.20	7.45	7.44	5.14	5.10
10 mM FeCl ₃ · 6H ₂ O	7.45	7.45	5.73	5.66	7.45	7.45	5.50	5.85
100mM FeCl ₃ · 6H ₂ O	7.43	7.44	7.12	7.18	7.44	7.42	7.15	7.19
15 mM FeF ₂	7.45	7.45	6.23	6.16	7.43	7.45	5.90	6.01
10 mM FeF ₃	7.43	7.45	5.74	5.60	7.45	7.45	5.82	5.75
50 mM FeCl ₃ · 6H ₂ O + 190mM NaF	7.43	7.45	7.20	7.35	7.44	7.44	7.15	7.29
APF	7.45	7.45	7.16	7.32	7.43	7.43	7.24	7.17
26 mM SnF ₂	7.45	7.45	7.01	6.36	7.45	7.45	6.33	6.94
511mM SnF ₂	7.42	7.44	7.30	7.24	7.45	7.45	7.28	7.19
48 mM NaF	7.44	7.45	6.21	5.99	7.44	7.45	6.27	6.24
476mM NaF	7.45	7.45	7.25	7.11	7.45	7.45	7.22	7.21

^a Treatment time — five minutes.^b BHI — brain heart infusion broth.^c At 150 minutes.^d Quarter-strength.

TABLE 11 (cont.)

B. Initial and Terminal Mean Hydrogen Ion Concentrations (g ions/l) ^a

Treatment	Medium					
	BHI + 146 mM Glucose			BHI + 146 mM Sucrose		
	Initial Mean H ⁺ Concentration	Terminal Mean H ⁺ Concentration	Increase in H ⁺ Concentration	Initial Mean H ⁺ Concentration	Terminal Mean H ⁺ Concentration	Increase in H ⁺ Concentration
Ringer Solution (Control)	3.548×10^{-8} (7.45)	1.118×10^{-5} (4.95)	1.114×10^{-5c}	3.589×10^{-8} (7.44)	1.463×10^{-5} (4.83)	1.459×10^{-5c}
1 mM FeCl ₂ · 4H ₂ O	3.548×10^{-8} (7.45)	1.228×10^{-5} (4.91)	1.224×10^{-5}	3.548×10^{-8} (7.45)	8.586×10^{-5} (5.07)	8.550×10^{-6}
10 mM FeCl ₂ · 4H ₂ O	3.589×10^{-8} (7.44)	7.698×10^{-6} (5.11)	7.662×10^{-6}	3.548×10^{-8} (7.45)	7.275×10^{-6} (5.14)	6.920×10^{-6}
100mM FeCl ₂ · 4H ₂ O	3.631×10^{-8} (7.44)	6.566×10^{-6} (5.18)	6.530×10^{-6}	3.589×10^{-8} (7.44)	6.129×10^{-6} (5.21)	6.093×10^{-6}
1 mM FeCl ₃ · 6H ₂ O	3.589×10^{-8} (7.44)	5.966×10^{-6} (5.22)	5.930×10^{-6}	3.589×10^{-8} (7.44)	7.594×10^{-6} (5.12)	7.558×10^{-6}
10 mM FeCl ₃ · 6H ₂ O	3.548×10^{-8} (7.45)	2.025×10^{-6} (5.69)	1.990×10^{-6}	3.548×10^{-8} (7.45)	2.287×10^{-6} (5.64)	2.252×10^{-6}
100mM FeCl ₃ · 6H ₂ O	3.673×10^{-8} (7.43)	7.149×10^{-8} (7.15)	3.476×10^{-8}	3.716×10^{-8} (7.43)	6.768×10^{-8} (7.17)	3.052×10^{-8}
15 mM FeF ₂	3.548×10^{-8} (7.45)	6.403×10^{-7} (6.19)	6.048×10^{-7}	3.632×10^{-8} (7.44)	1.118×10^{-6} (5.95)	1.082×10^{-6}
10 mM FeF ₃	3.632×10^{-8} (7.44)	2.166×10^{-6} (5.66)	2.130×10^{-6}	3.548×10^{-8} (7.45)	1.646×10^{-6} (5.78)	1.611×10^{-6}
50 mM FeCl ₃ · 6H ₂ O + 190mM NaF	3.632×10^{-8} (7.44)	5.388×10^{-8} (7.27)	1.756×10^{-8}	3.890×10^{-8} (7.44)	6.104×10^{-8} (7.21)	2.214×10^{-8}
APF	3.548×10^{-8} (7.45)	5.852×10^{-8} (7.23)	2.300×10^{-8}	3.715×10^{-8} (7.43)	6.258×10^{-8} (7.20)	2.543×10^{-8}
26 mM SnF ₂	3.548×10^{-8} (7.45)	2.671×10^{-7} (6.57)	2.316×10^{-7}	3.548×10^{-8} (7.45)	2.913×10^{-7} (6.54)	2.558×10^{-7}
511mM SnF ₂	3.716×10^{-8} (7.43)	5.383×10^{-8} (7.27)	1.667×10^{-8}	3.548×10^{-8} (7.45)	5.852×10^{-8} (7.23)	2.304×10^{-8}
48 mM NaF	3.589×10^{-8} (7.44)	8.199×10^{-7} (6.09)	7.840×10^{-7}	3.589×10^{-8} (7.44)	5.562×10^{-7} (6.25)	5.203×10^{-7}
476mM NaF	3.548×10^{-8} (7.45)	6.693×10^{-8} (7.18)	3.145×10^{-8}	3.548×10^{-8} (7.45)	5.532×10^{-8} (7.26)	1.984×10^{-8}

^a Calculated from data in Table 11-A.^b Values in parenthesis obtained by converting mean hydrogen ion concentration into pH.^c Used as base line values for findings shown in Fig. 40.

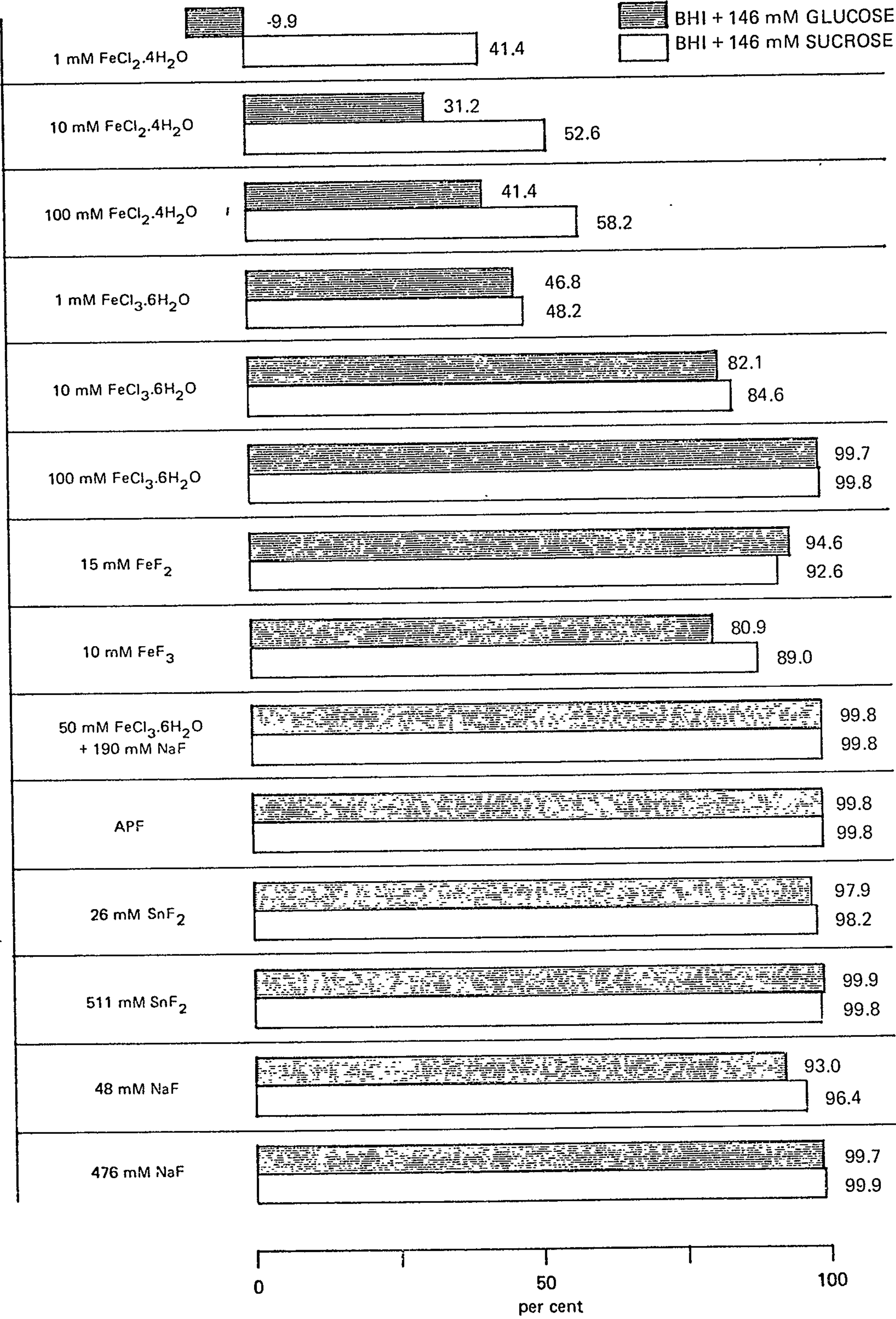


Fig. 40. Reductions in acid production by *A. viscosus* strain NY1 in brain heart infusion broth (BHI) supplemented with 146 mM glucose or 146 mM sucrose following treatment with test compounds (treatment time — five minutes).

A

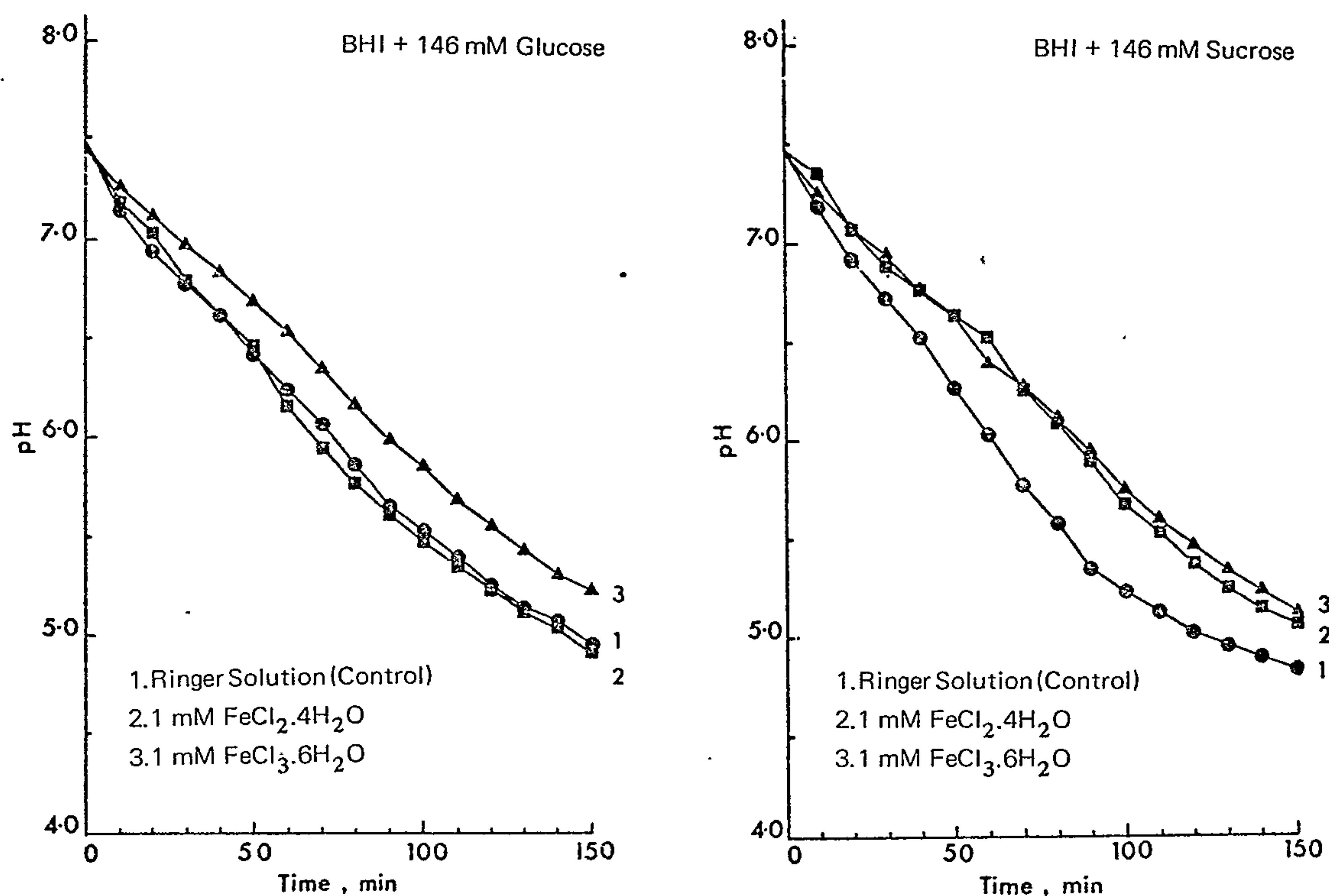


Fig. 41. Rates of acid production by *A. viscosus* strain NY1 in BHI broth supplemented with glucose or sucrose following treatment with iron (II) chloride or iron (III) chloride.

- A. Effect of five minute treatments with iron (II) chloride or iron (III) chloride at a concentration of 1 mM. (Left). BHI broth supplemented with 146 mM glucose. (Right). BHI broth supplemented with 146 mM sucrose.
- B. Effect of five minute treatments with iron (II) chloride or iron (III) chloride at a concentration of 10 mM. (Left). BHI broth supplemented with 146 mM glucose. (Right). BHI broth supplemented with 146 mM sucrose.
- C. Effect of five minute treatments with iron (II) chloride or iron (III) chloride at a concentration of 100 mM. (Left). BHI broth supplemented with 146 mM glucose. (Right). BHI broth supplemented with 146 mM sucrose.

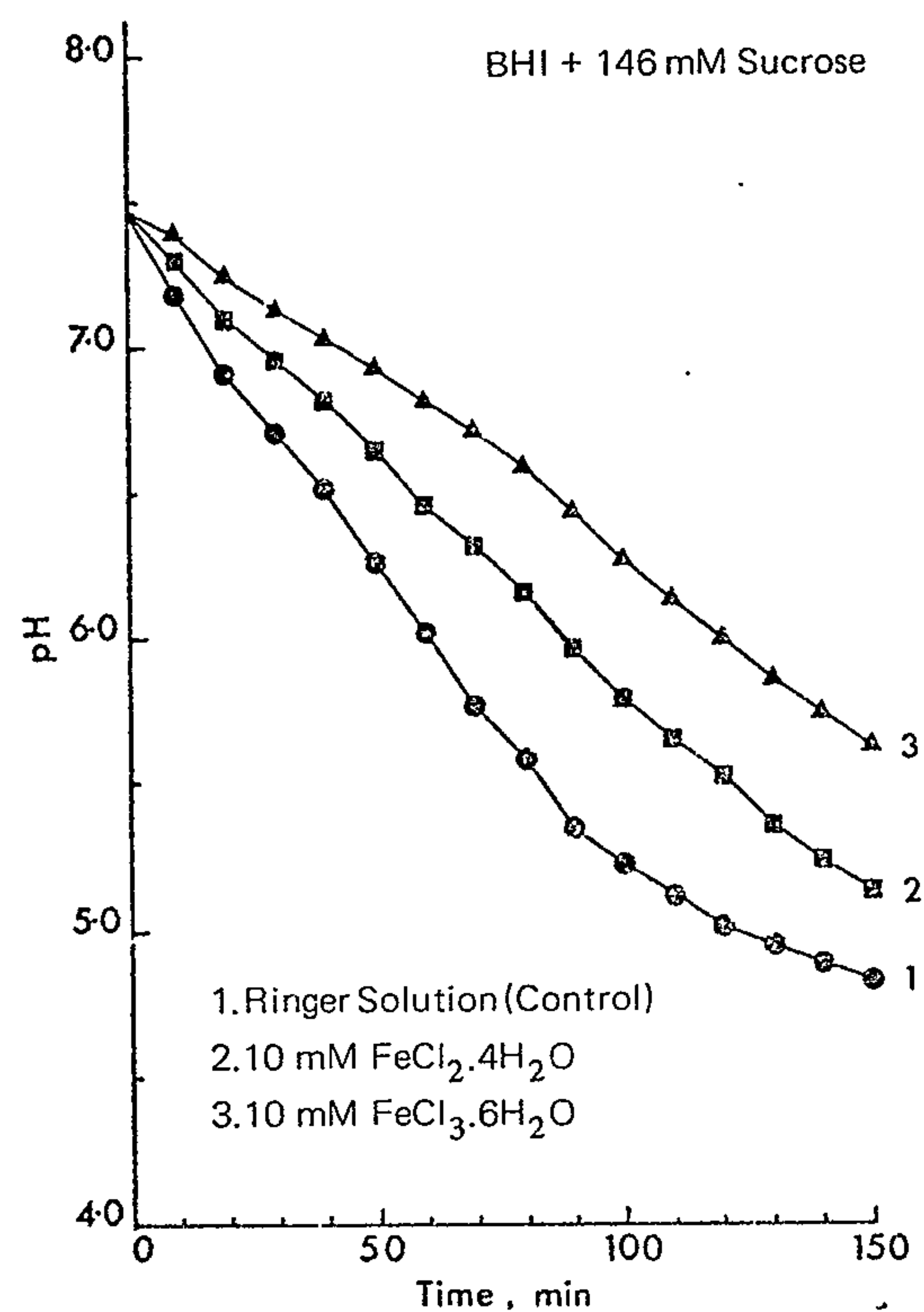
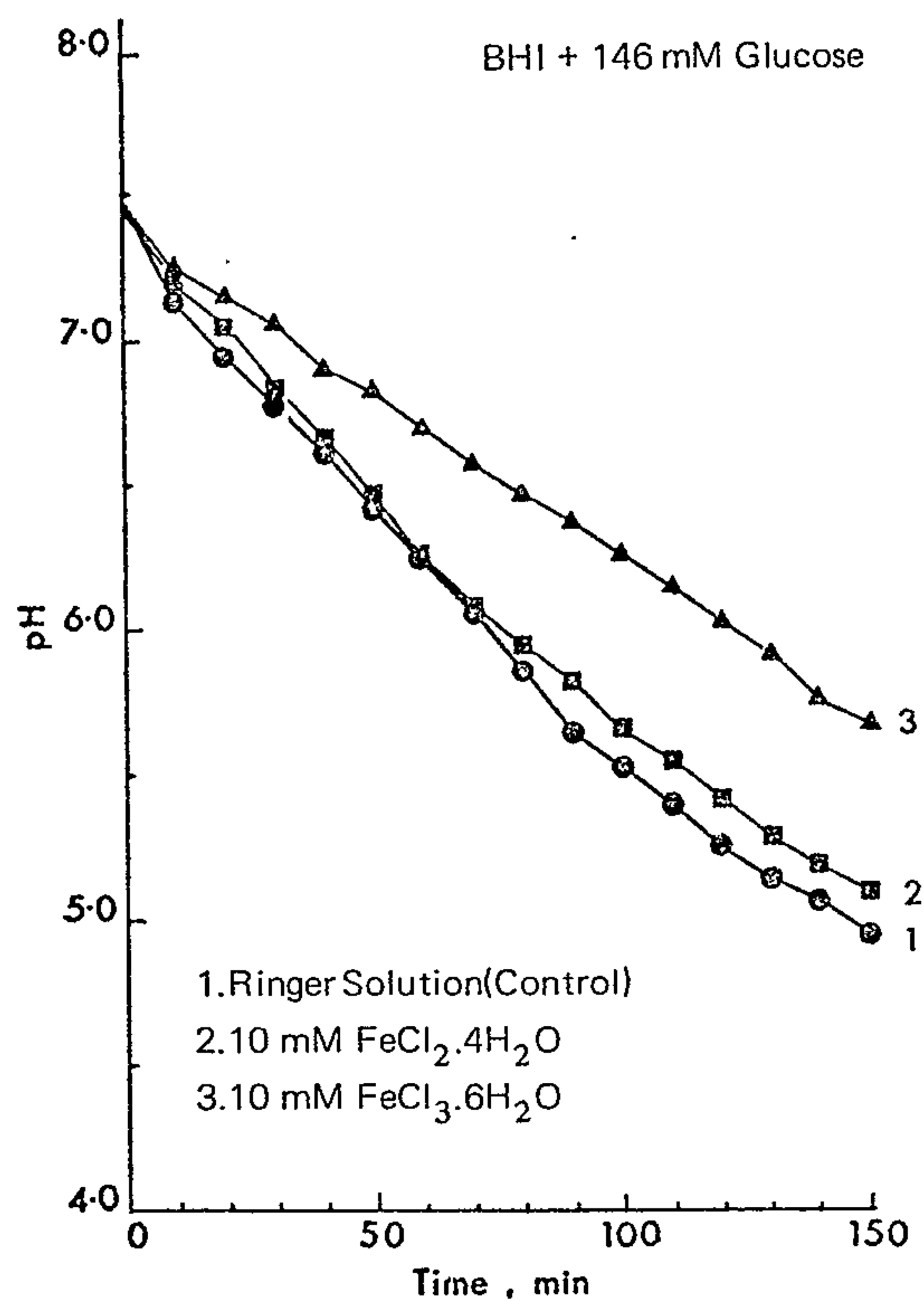
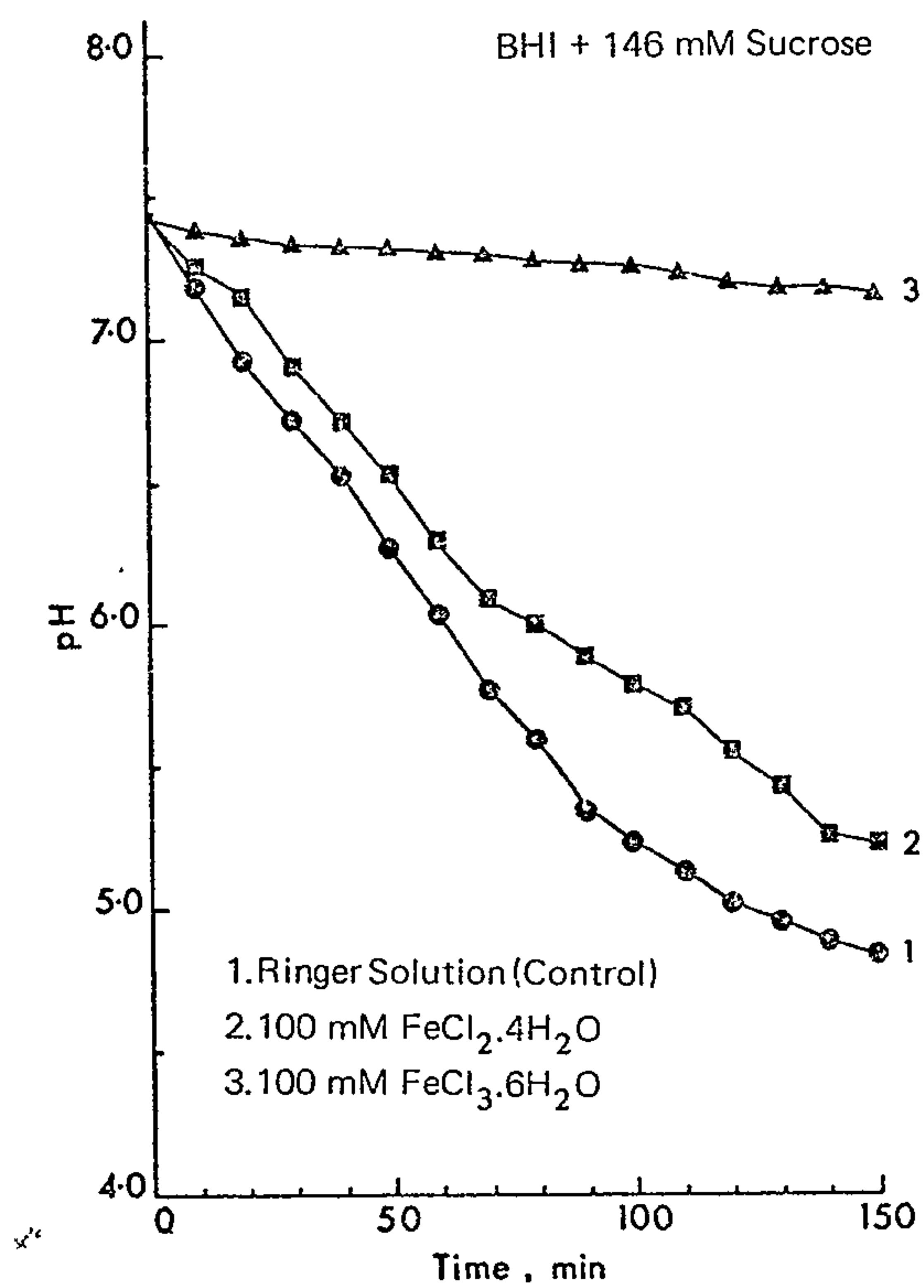
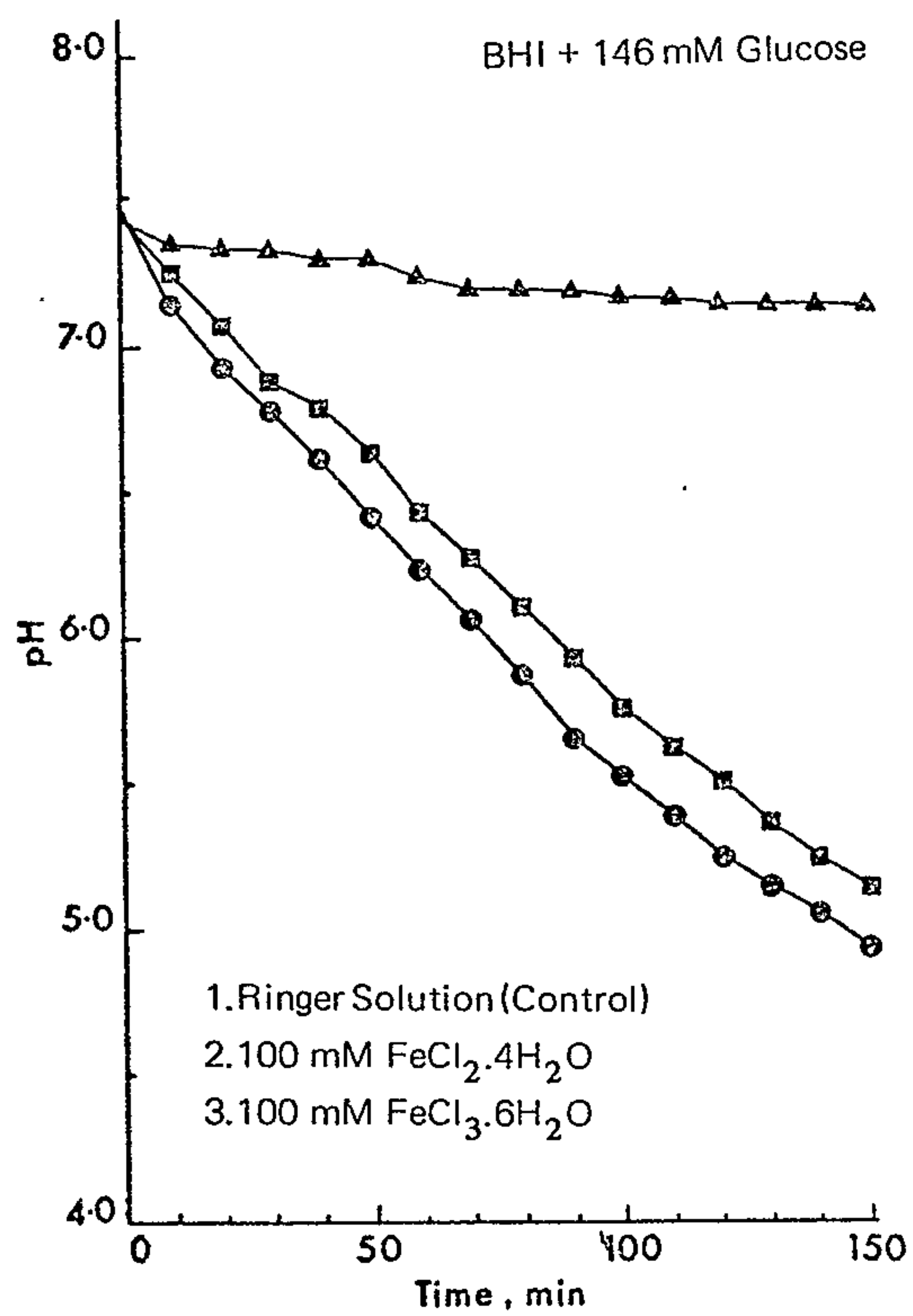
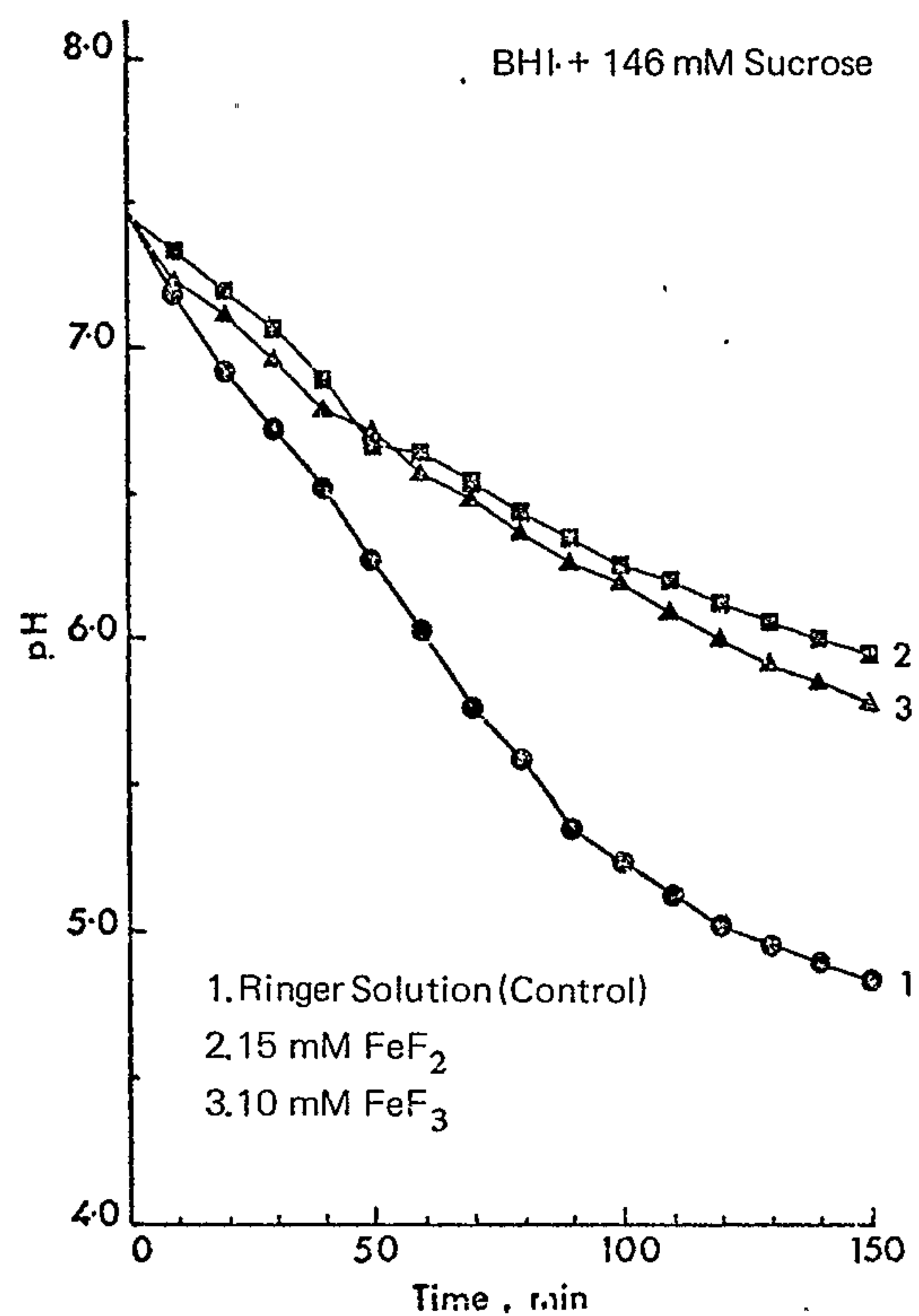
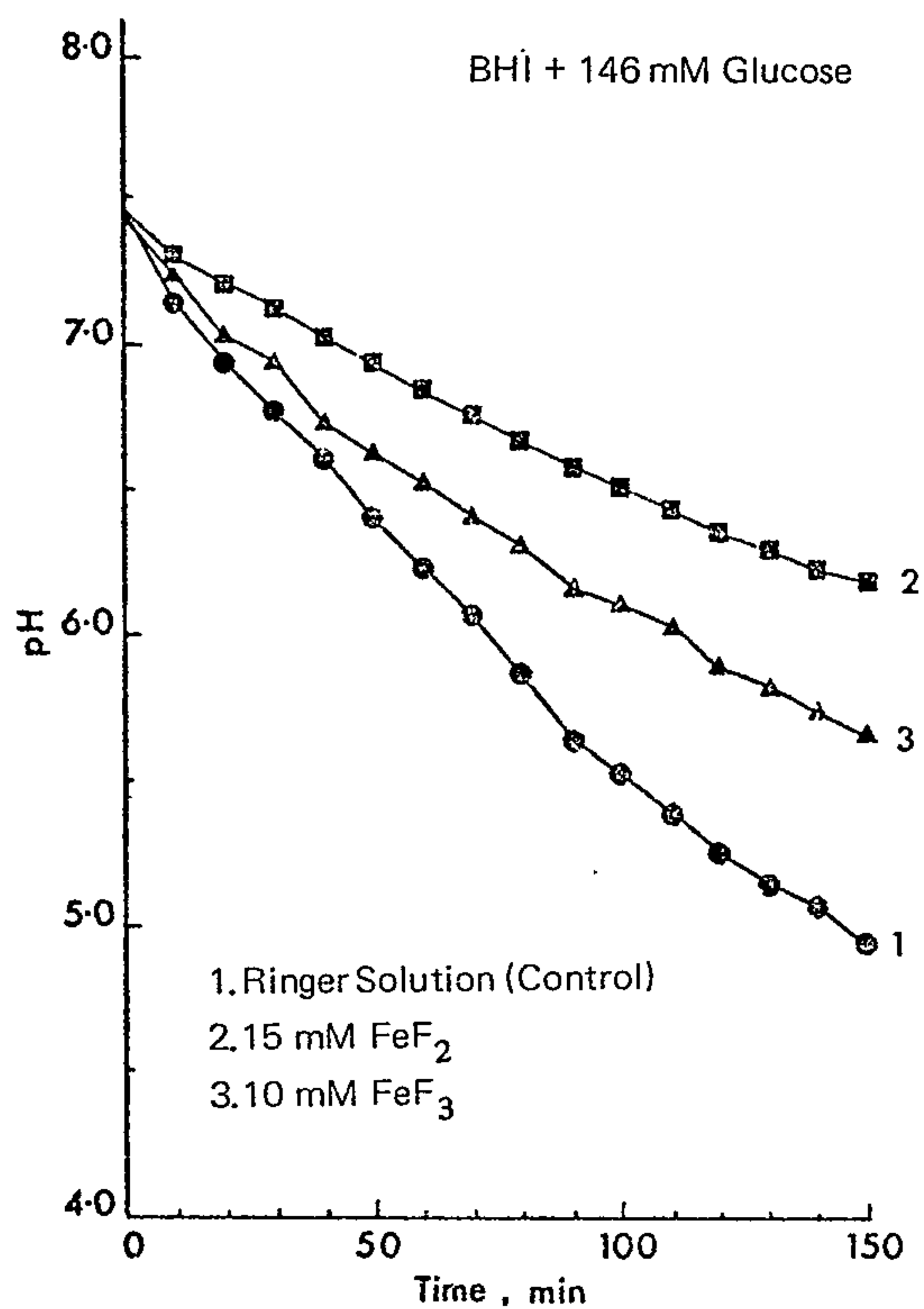
BC

Fig. 42. Rates of acid production by *A. viscosus* strain NY1 in BHI broth supplemented with glucose or sucrose following treatment with iron (II) fluoride, iron (III) fluoride, APF or a solution containing iron (III) chloride and sodium fluoride.

- A. Effect of five minute treatments with 15 mM iron (II) fluoride or 10 mM iron (III) fluoride. (*Left*). BHI broth supplemented with 146 mM glucose. (*Right*). BHI broth supplemented with 146 mM sucrose.
- B. Effect of five minute treatments with APF or a solution containing 50 mM iron (III) chloride and 190 mM sodium fluoride. (*Left*). BHI broth supplemented with 146 mM glucose. (*Right*). BHI broth supplemented with 146 mM sucrose.

A



B

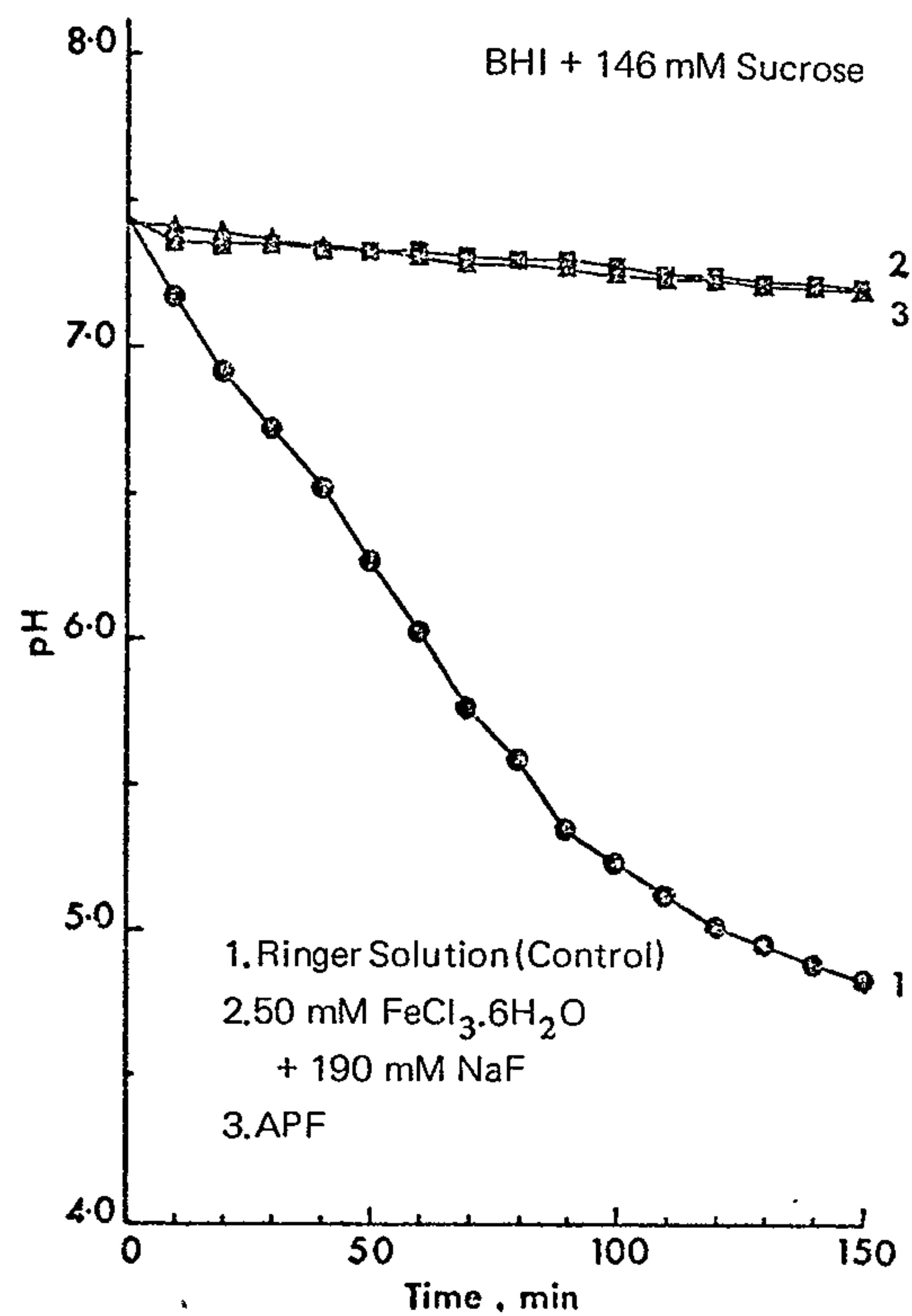
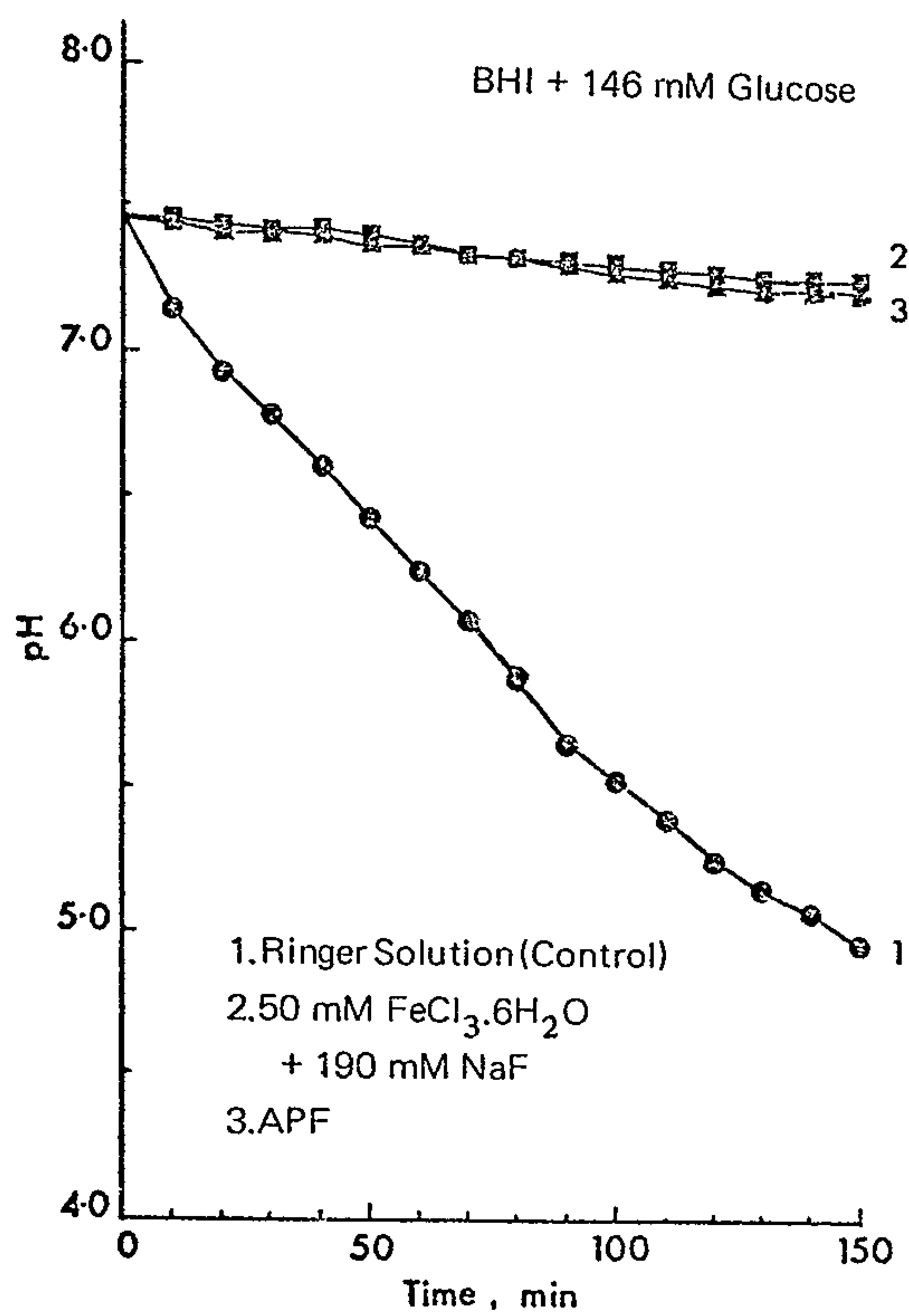
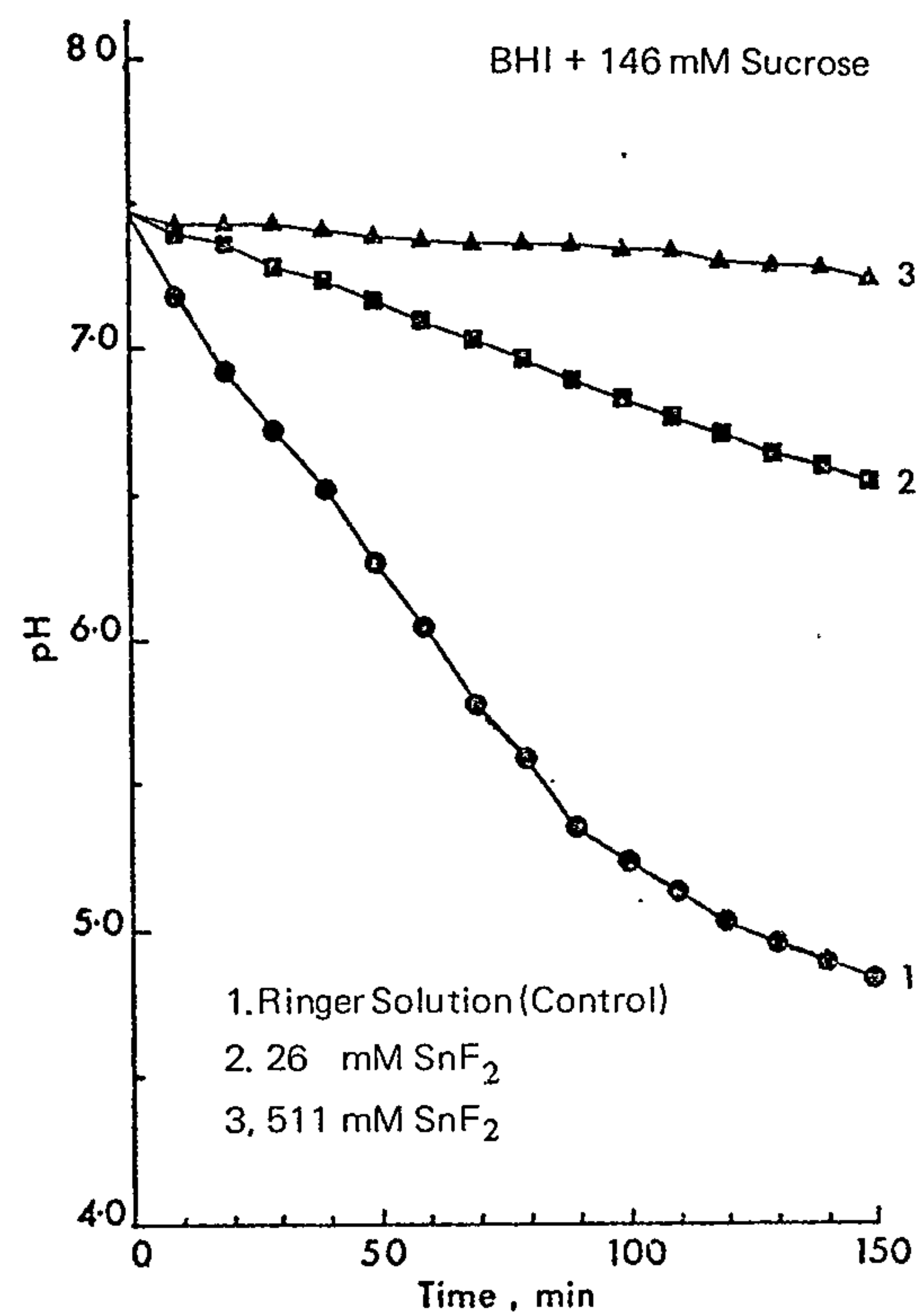
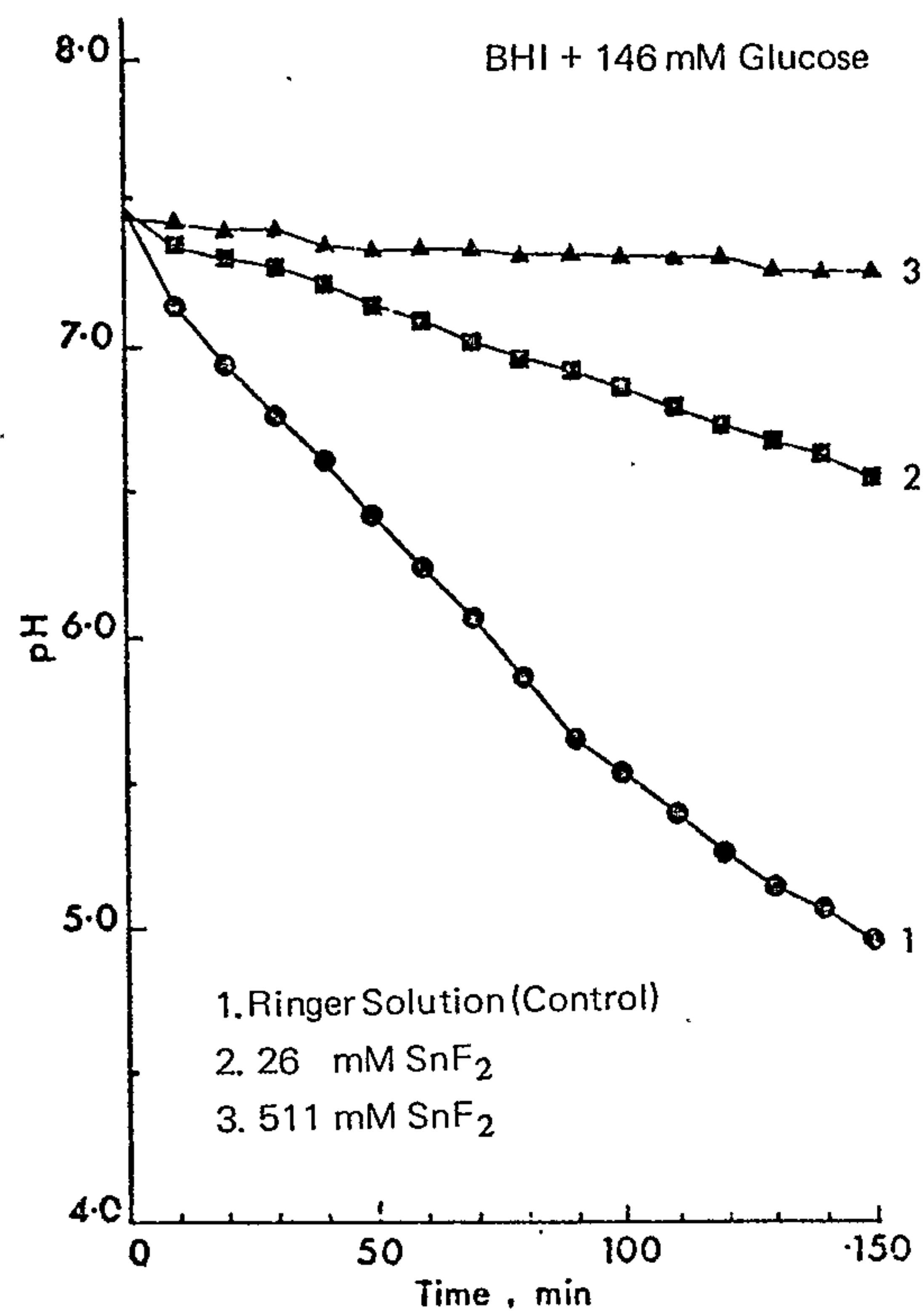


Fig. 43. Rates of acid production by *A. viscosus* strain NY1 in BHI broth supplemented with glucose or sucrose following treatment with tin (II) fluoride or sodium fluoride.

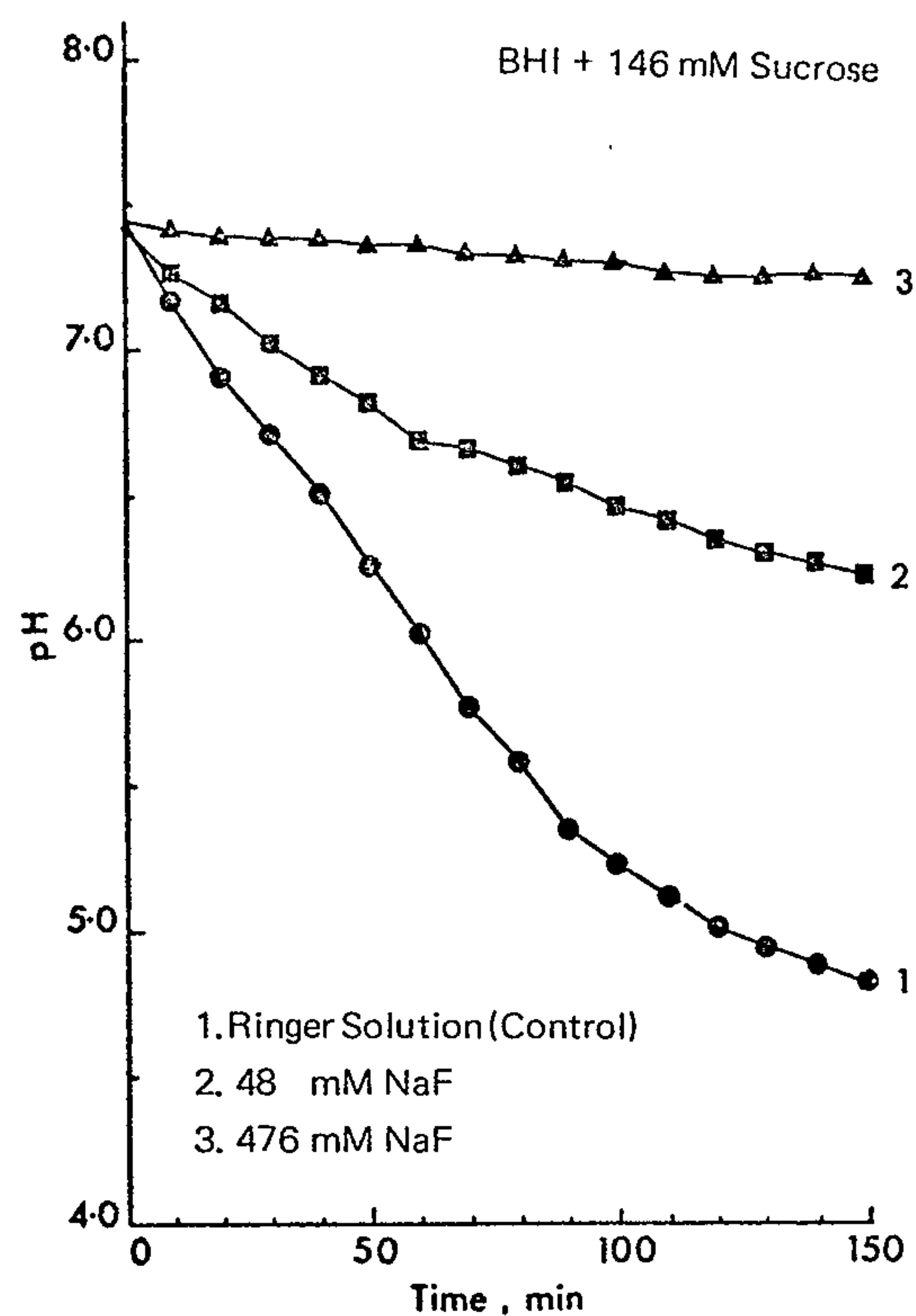
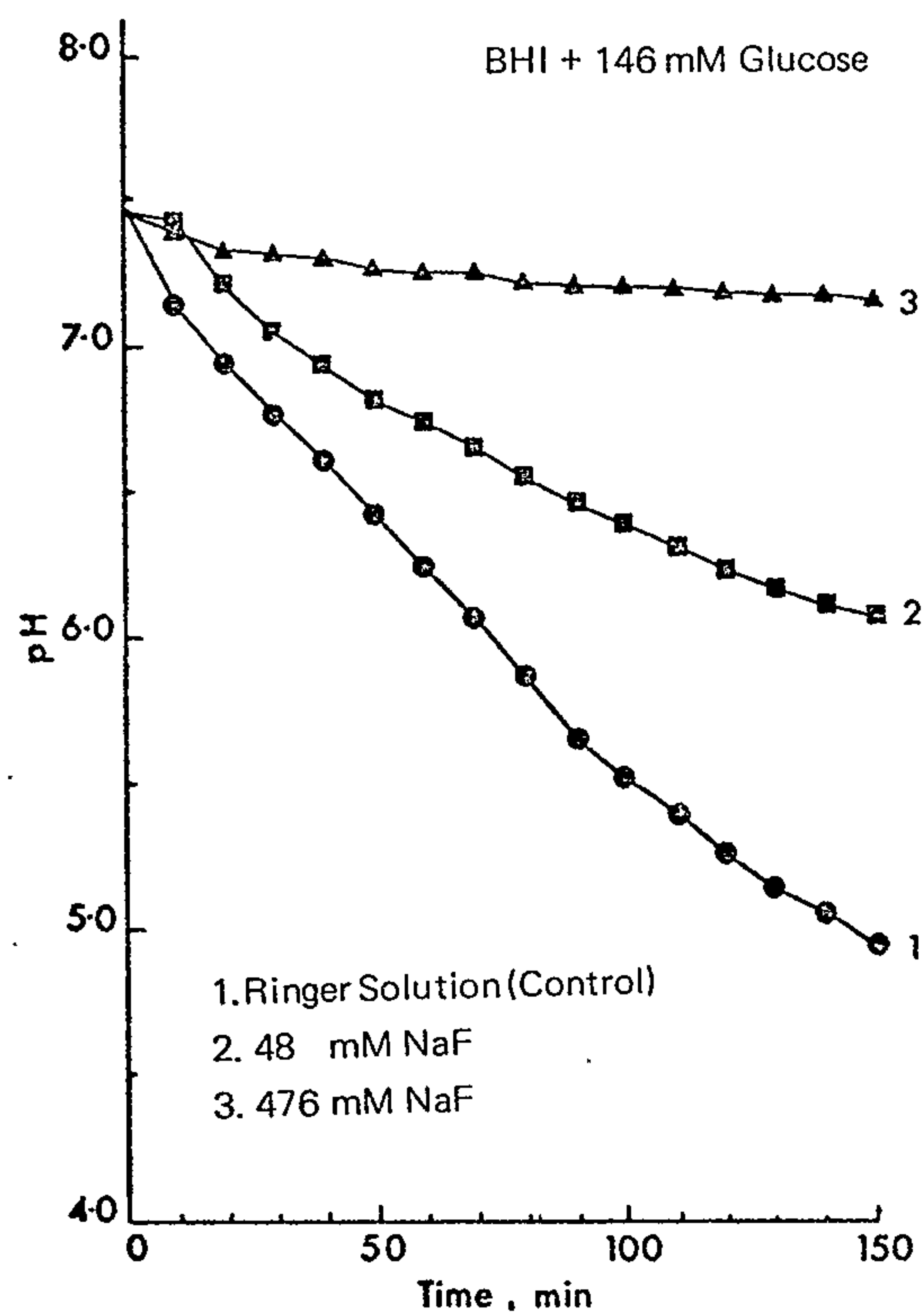
A. Effect of five minute treatments with tin (II) fluoride at a concentration of 26 mM or 511 mM. (*Left*). BHI broth supplemented with 146 mM glucose. (*Right*). BHI broth supplemented with 146 mM sucrose.

B. Effect of five minute treatments with sodium fluoride at a concentration of 48 mM or 476 mM. (*Left*). BHI supplemented with 146 mM glucose. (*Right*). BHI supplemented with 146 mM sucrose.

A



B



ACID PRODUCTION BY L. CASEI VAR. CASEI

Three of the six non-fluoride test compounds (1 mM iron (II) chloride, 10 mM iron (II) chloride and 1 mM iron (III) chloride) had little or no inhibitory effect on acid production by *L. casei* var *casei* (Fig. 44). Although 10 mM iron (III) chloride only reduced acid production by 1.9 per cent when the culture broth was supplemented with glucose it did reduce acid production by 48.0 per cent when the culture broth was supplemented with sucrose. Iron (III) chloride at a concentration of 100 mM almost completely inhibited acid production.

Two of the four fluoride solutions with a fluoride concentration below 1,000 ppm (10 mM iron (III) fluoride and 48 mM sodium fluoride) had little or no inhibitory effect on acid production (Fig. 44). The remaining two fluoride solutions in this category (15 mM iron (III) fluoride and 26 mM tin (II) fluoride) had a moderate inhibitory effect. The reductions in acid production obtained with these solutions ranged from 38.1 per cent (15 mM iron (II) fluoride) to 67.9 per cent (26 mM tin (II) fluoride).

Three of the four fluoride solutions with a fluoride concentration above 3,000 ppm (APF, 511 tin (II) fluoride and the solution containing 50 mM iron (III) chloride and 190 mM sodium fluoride) inhibited acid production by more than 92 per cent (Fig. 44). However, the remaining solution in this category (476 mM sodium fluoride) had a less pronounced inhibitory effect on acid production. This solution reduced acid production by 58.7 per cent when the culture broth was supplemented with glucose and by 74.5 per cent when the culture broth was supplemented with sucrose.

TABLE 12

ACID PRODUCTION BY *L. CASEI* VAR. *CASEI* AFTER
TREATMENT WITH TEST COMPOUNDS

A. Initial and Terminal pH values

Treatment ^a	Medium							
	M.R.S. ^b + 146 mM Glucose				M.R.S. ^b + 146 mM Sucrose			
	Initial pH Replicate		Terminal pH ^c Replicate		Initial pH Replicate		Terminal pH ^c Replicate	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Ringer Solution ^d (Control)	6.70	6.69	4.34	4.51	6.70	6.67	4.38	4.32
1 mM FeCl ₂ · 4H ₂ O	6.70	6.70	4.39	4.43	6.70	6.70	4.35	4.38
10 mM FeCl ₂ · 4H ₂ O	6.70	6.70	4.48	4.40	6.70	6.69	4.37	4.40
100 mM FeCl ₂ · 4H ₂ O	6.70	6.70	4.58	4.54	6.70	6.70	4.41	4.44
1 mM FeCl ₃ · 6H ₂ O	6.70	6.70	4.35	4.47	6.70	6.70	4.37	4.31
10 mM FeCl ₃ · 6H ₂ O	6.68	6.70	4.42	4.43	6.70	6.70	4.69	4.58
100 mM FeCl ₃ · 6H ₂ O	6.70	6.70	6.45	6.51	6.70	6.70	6.20	5.86
15 mM FeF ₂	6.71	6.70	4.66	4.59	6.70	6.70	4.64	4.70
10 mM FeF ₃	6.70	6.70	4.50	4.35	6.70	6.69	4.37	4.39
50 mM FeCl ₃ · 6H ₂ O + 190mM NaF	6.70	6.68	6.65	6.60	6.70	6.70	6.62	6.63
APF	6.70	6.69	5.46	5.53	6.70	6.67	5.73	5.85
26 mM SnF ₂	6.70	6.70	4.84	4.70	6.71	6.70	4.77	4.92
511 mM SnF ₂	6.70	6.70	6.60	6.53	6.70	6.70	6.50	6.59
48 mM NaF	6.70	6.70	4.40	4.35	6.70	6.70	4.40	4.40
476 mM NaF	6.70	6.70	4.67	4.98	6.72	6.70	5.31	4.74

^a Treatment time — five minutes.^b Prepared from the formula of de Man, Rogosa and Sharp⁽⁵¹⁾.^c At 90 minutes.^d Quarter-strength.

TABLE 12 (cont.)

B. Initial and Terminal Mean Hydrogen Ion Concentration (g ions/l)^a

Treatment	Medium					
	M.R.S. + 146 mM Glucose			M.R.S. + 146 mM Sucrose		
	Initial Mean H ⁺ Concentration	Terminal Mean H ⁺ Concentration	Increase in H ⁺ Concentration	Initial Mean H ⁺ Concentration	Terminal Mean H ⁺ Concentration	Increase in H ⁺ Concentration
Ringer Solution (Control)	2.018 × 10 ⁻⁷ (6.69) ^b	3.831 × 10 ⁻⁵ (4.42)	3.811 × 10 ^{-5c}	2.067 × 10 ⁻⁷ (6.68)	4.477 × 10 ⁻⁵ (4.35)	4.456 × 10 ^{-5c}
1 mM FeCl ₂ · 4H ₂ O	1.995 × 10 ⁻⁷ (6.70)	3.895 × 10 ⁻⁵ (4.41)	3.875 × 10 ⁻⁵	1.995 × 10 ⁻⁷ (6.70)	4.582 × 10 ⁻⁵ (4.36)	4.562 × 10 ⁻⁵
10 mM FeCl ₂ · 4H ₂ O	1.995 × 10 ⁻⁷ (6.70)	3.646 × 10 ⁻⁵ (4.44)	3.626 × 10 ⁻⁵	1.818 × 10 ⁻⁷ (6.69)	4.123 × 10 ⁻⁵ (4.38)	4.105 × 10 ⁻⁵
100 mM FeCl ₂ · 4H ₂ O	1.995 × 10 ⁻⁷ (6.70)	2.757 × 10 ⁻⁵ (4.56)	2.737 × 10 ⁻⁵	1.995 × 10 ⁻⁷ (6.70)	3.936 × 10 ⁻⁵ (4.40)	3.916 × 10 ⁻⁵
1 mM FeCl ₃ · 6H ₂ O	1.995 × 10 ⁻⁷ (6.70)	3.928 × 10 ⁻⁵ (4.41)	3.908 × 10 ⁻⁵	1.995 × 10 ⁻⁷ (6.70)	4.318 × 10 ⁻⁵ (4.34)	4.298 × 10 ⁻⁵
10 mM FeCl ₃ · 6H ₂ O	1.995 × 10 ⁻⁷ (6.70)	3.759 × 10 ⁻⁵ (4.42)	3.739 × 10 ⁻⁵	2.067 × 10 ⁻⁷ (6.68)	2.336 × 10 ⁻⁵ (4.63)	2.315 × 10 ⁻⁵
100 mM FeCl ₃ · 6H ₂ O	1.995 × 10 ⁻⁷ (6.70)	3.319 × 10 ⁻⁵ (6.48)	1.324 × 10 ⁻⁷	1.995 × 10 ⁻⁷ (6.70)	1.006 × 10 ⁻⁶ (6.00)	0.806 × 10 ⁻⁶
15 mM FeF ₂	1.995 × 10 ⁻⁷ (6.70)	2.379 × 10 ⁻⁵ (4.62)	2.359 × 10 ⁻⁵	1.995 × 10 ⁻⁷ (6.70)	2.143 × 10 ⁻⁵ (4.67)	2.123 × 10 ⁻⁵
10 mM FeF ₃	1.995 × 10 ⁻⁷ (6.70)	3.815 × 10 ⁻⁵ (4.42)	3.795 × 10 ⁻⁵	2.018 × 10 ⁻⁷ (6.69)	4.170 × 10 ⁻⁵ (4.38)	4.150 × 10 ⁻⁵
50 mM FeCl ₃ · 6H ₂ O +190 mM NaF	2.042 × 10 ⁻⁷ (6.69)	2.375 × 10 ⁻⁷ (6.62)	0.330 × 10 ⁻⁷	1.995 × 10 ⁻⁷ (6.70)	2.371 × 10 ⁻⁷ (6.62)	0.376 × 10 ⁻⁷
APF	2.018 × 10 ⁻⁷ (6.69)	3.029 × 10 ⁻⁶ (5.49)	2.827 × 10 ⁻⁶	2.067 × 10 ⁻⁷ (6.68)	1.637 × 10 ⁻⁶ (5.79)	1.430 × 10 ⁻⁶
26 mM SnF ₂	1.995 × 10 ⁻⁷ (6.70)	1.720 × 10 ⁻⁵ (4.76)	1.700 × 10 ⁻⁵	1.975 × 10 ⁻⁷ (6.70)	1.450 × 10 ⁻⁵ (4.84)	1.430 × 10 ⁻⁵
511 mM SnF ₂	1.995 × 10 ⁻⁷ (6.70)	2.732 × 10 ⁻⁷ (6.56)	0.737 × 10 ⁻⁷	1.995 × 10 ⁻⁷ (6.70)	2.866 × 10 ⁻⁷ (6.54)	0.871 × 10 ⁻⁷
48 mM NaF	1.995 × 10 ⁻⁷ (6.70)	4.224 × 10 ⁻⁵ (4.37)	4.204 × 10 ⁻⁵	1.995 × 10 ⁻⁷ (6.70)	3.981 × 10 ⁻⁵ (4.40)	3.961 × 10 ⁻⁵
476 mM NaF	1.995 × 10 ⁻⁷ (6.70)	1.592 × 10 ⁻⁵ (4.80)	1.572 × 10 ⁻⁵	1.950 × 10 ⁻⁷ (6.71)	1.155 × 10 ⁻⁵ (4.94)	1.135 × 10 ⁻⁵

^a Calculated from data in Table 12-A^b Values in parentheses obtained by converting mean hydrogen ion concentration into pH^c Used as base line values for findings shown in Fig. 44.

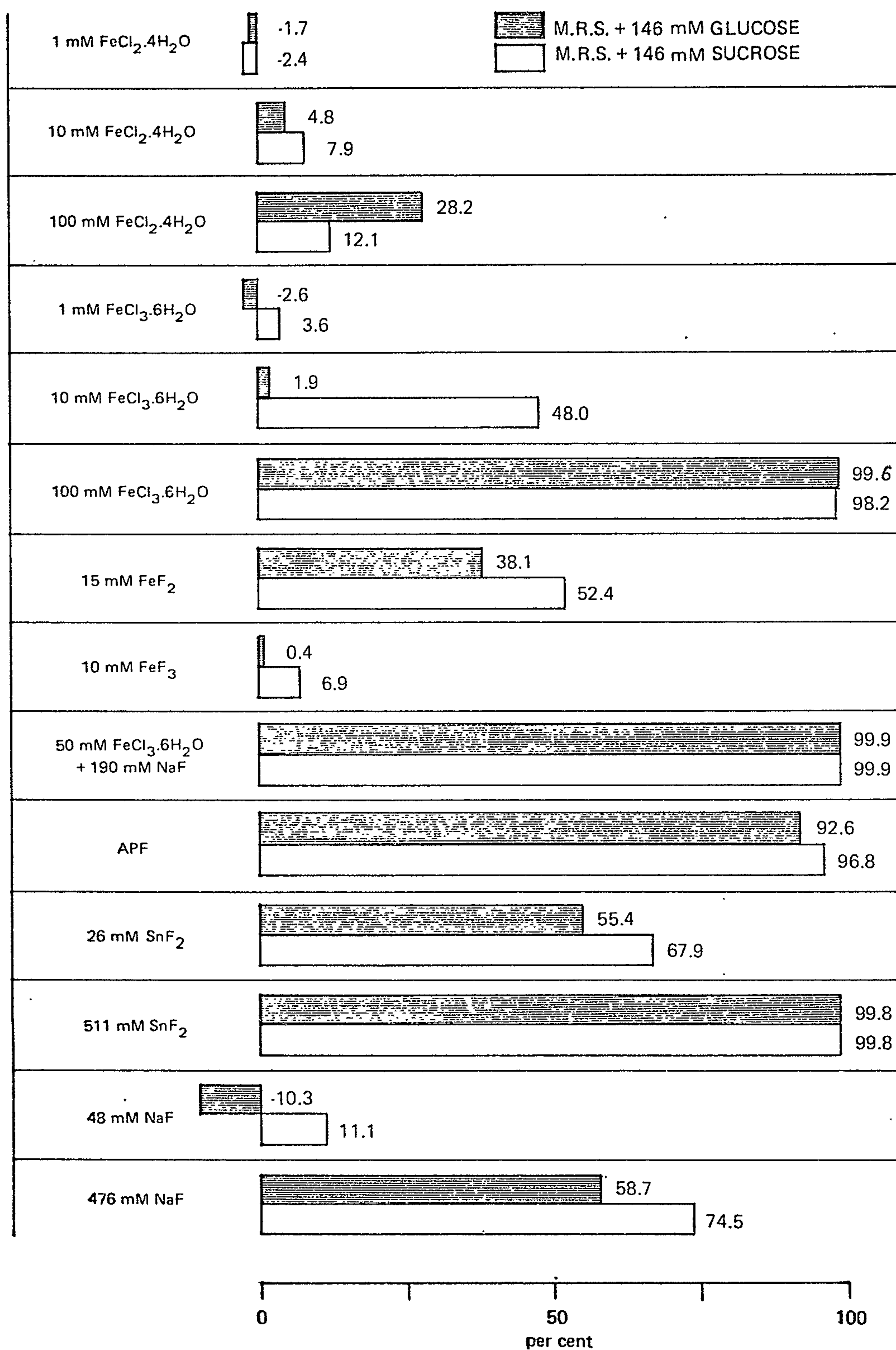


Fig. 44 . Reductions in acid production by *L. casei* var *casei* in M.R.S. broth supplemented with 146 mM glucose or 146 mM sucrose following treatment with test compounds (treatment time – five minutes).

A

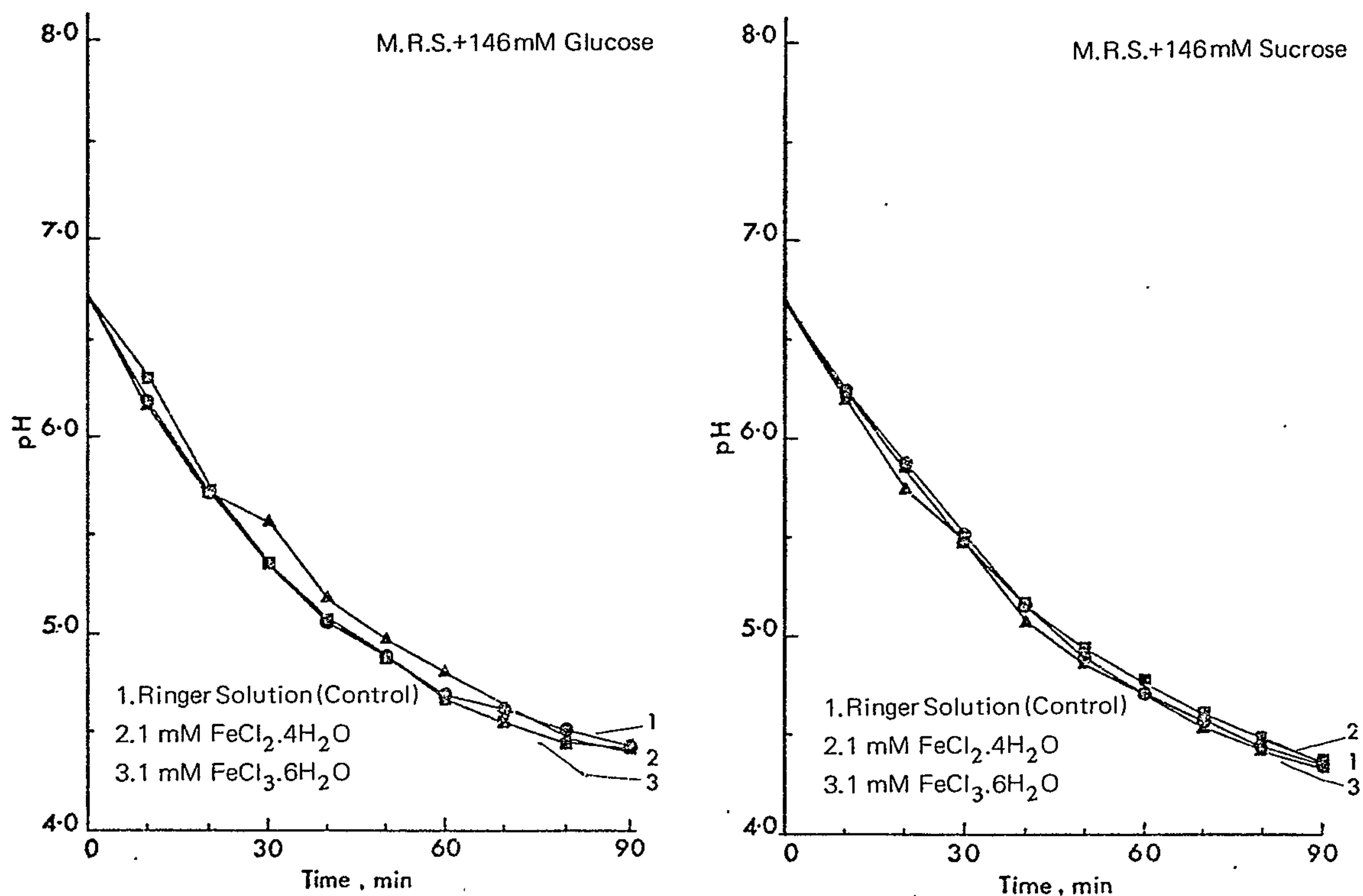
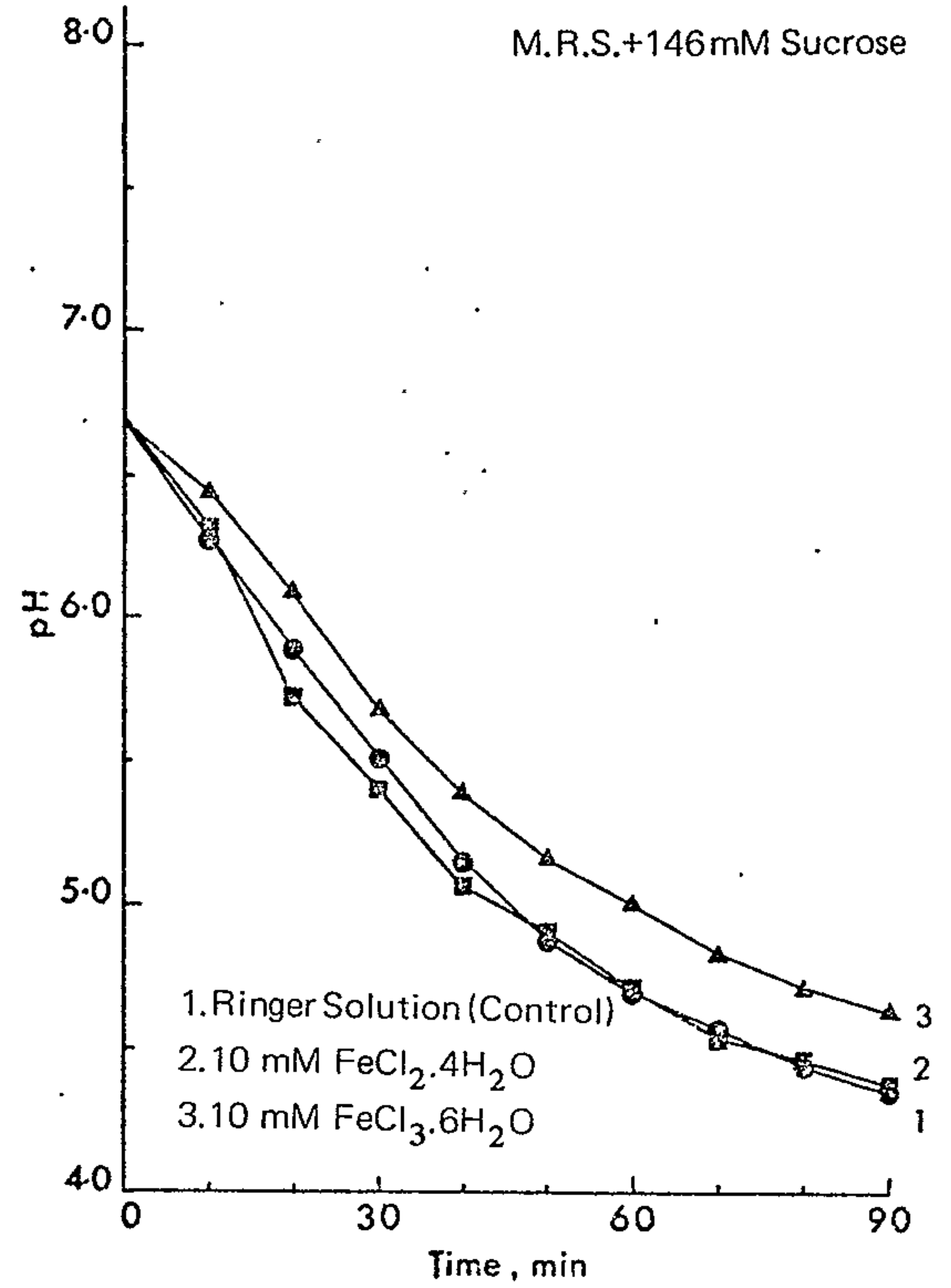
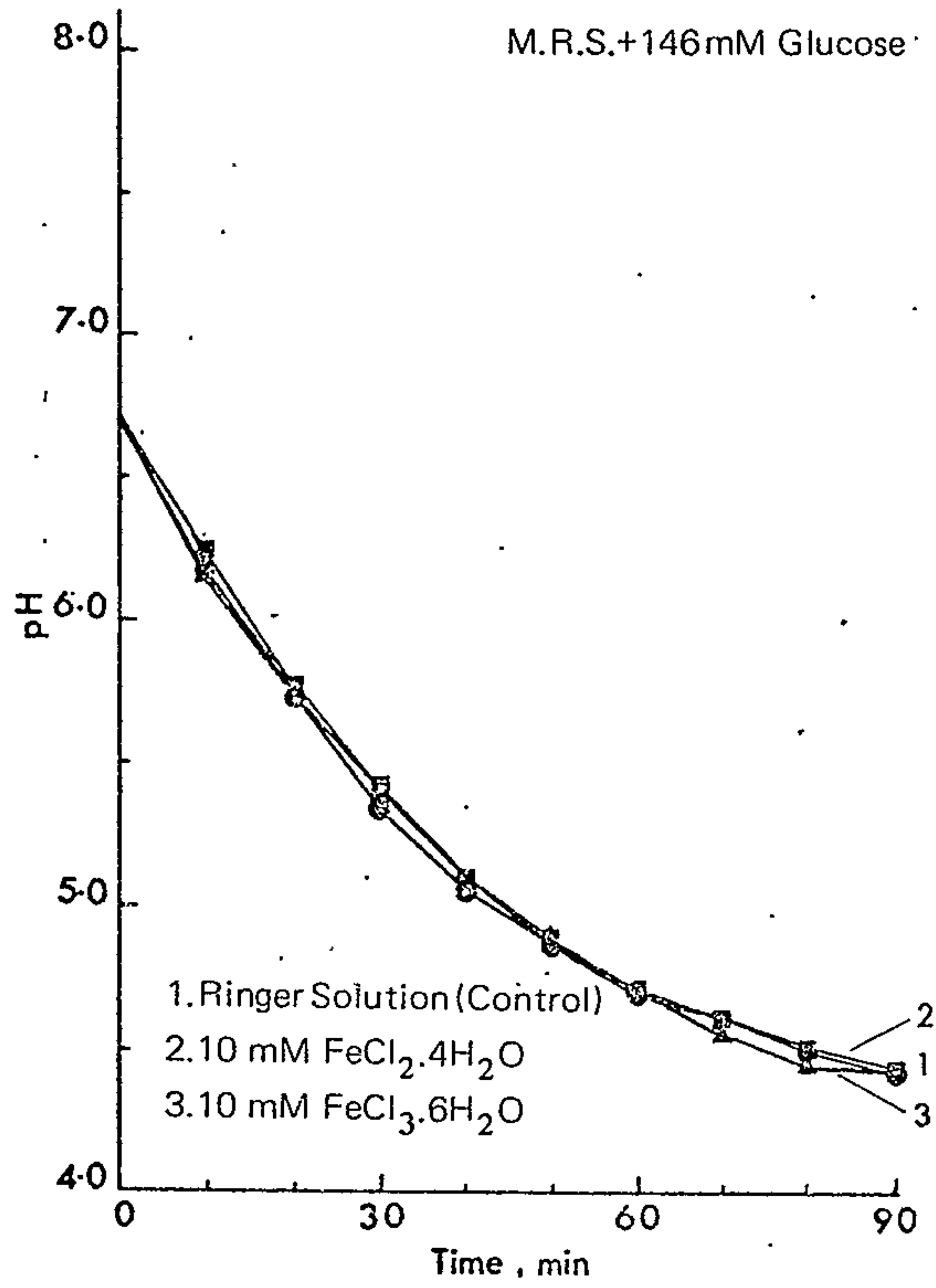


Fig. 45. Rates of acid production by *L. casei* var. *casei* in M.R.S. broth supplemented with glucose or sucrose following treatment with iron (II) chloride or iron (III) chloride.

- A. Effect of five minute treatments with iron (II) chloride or iron (III) chloride at a concentration of 1 mM. (Left). M.R.S. broth supplemented with 146 mM glucose. (Right). M.R.S. broth supplemented with 146 mM sucrose.
- B. Effect of five minute treatments with iron (II) chloride or iron (III) chloride at a concentration of 10 mM. (Left). M.R.S. broth supplemented with 146 mM glucose. (Right). M.R.S. broth supplemented with 146 mM sucrose.
- C. Effect of five minute treatments with iron (II) chloride or iron (III) chloride at a concentration of 100 mM. (Left). M.R.S. broth supplemented with 146 mM glucose. (Right). M.R.S. broth supplemented with 146 mM sucrose.

B



C

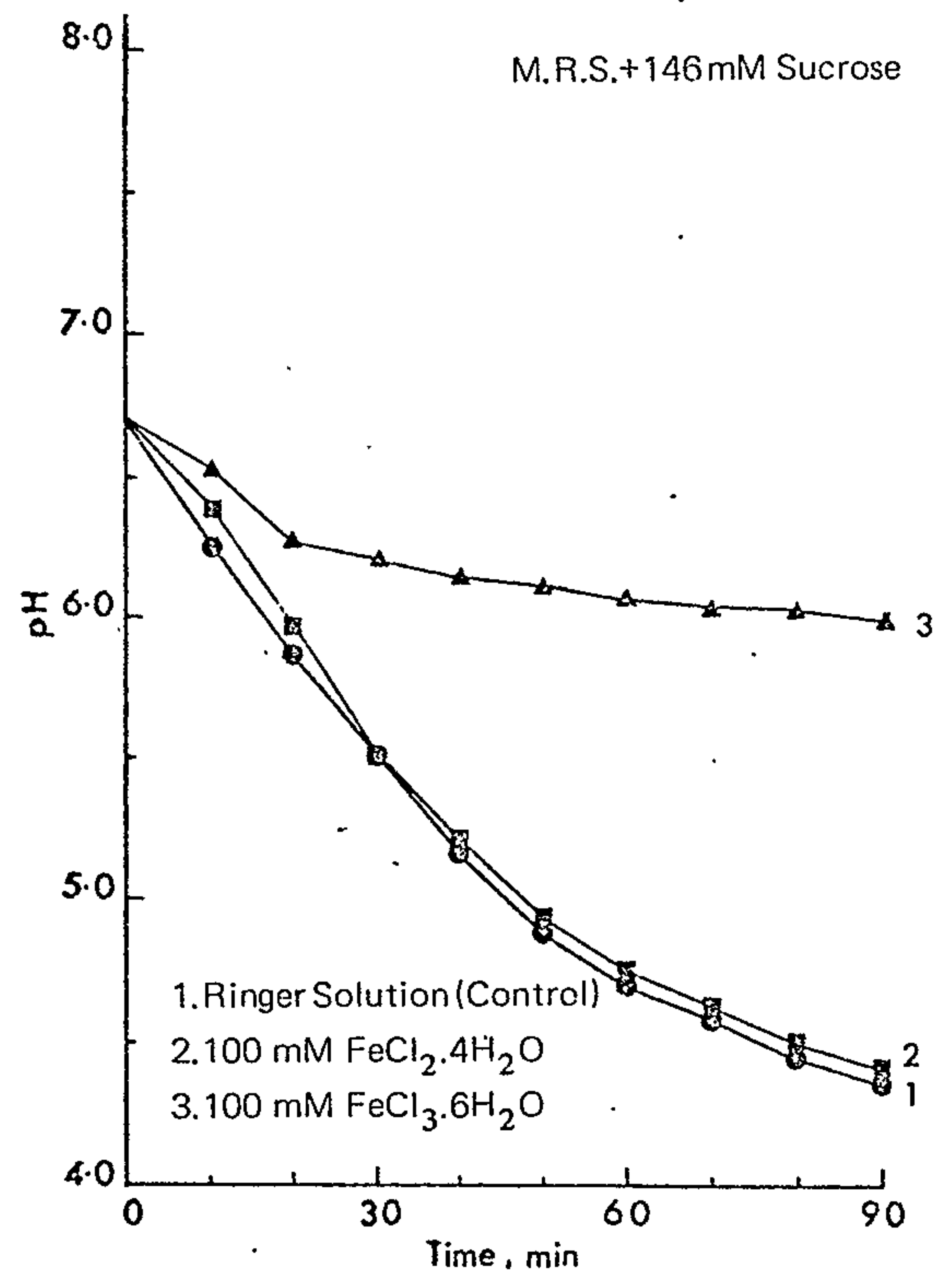
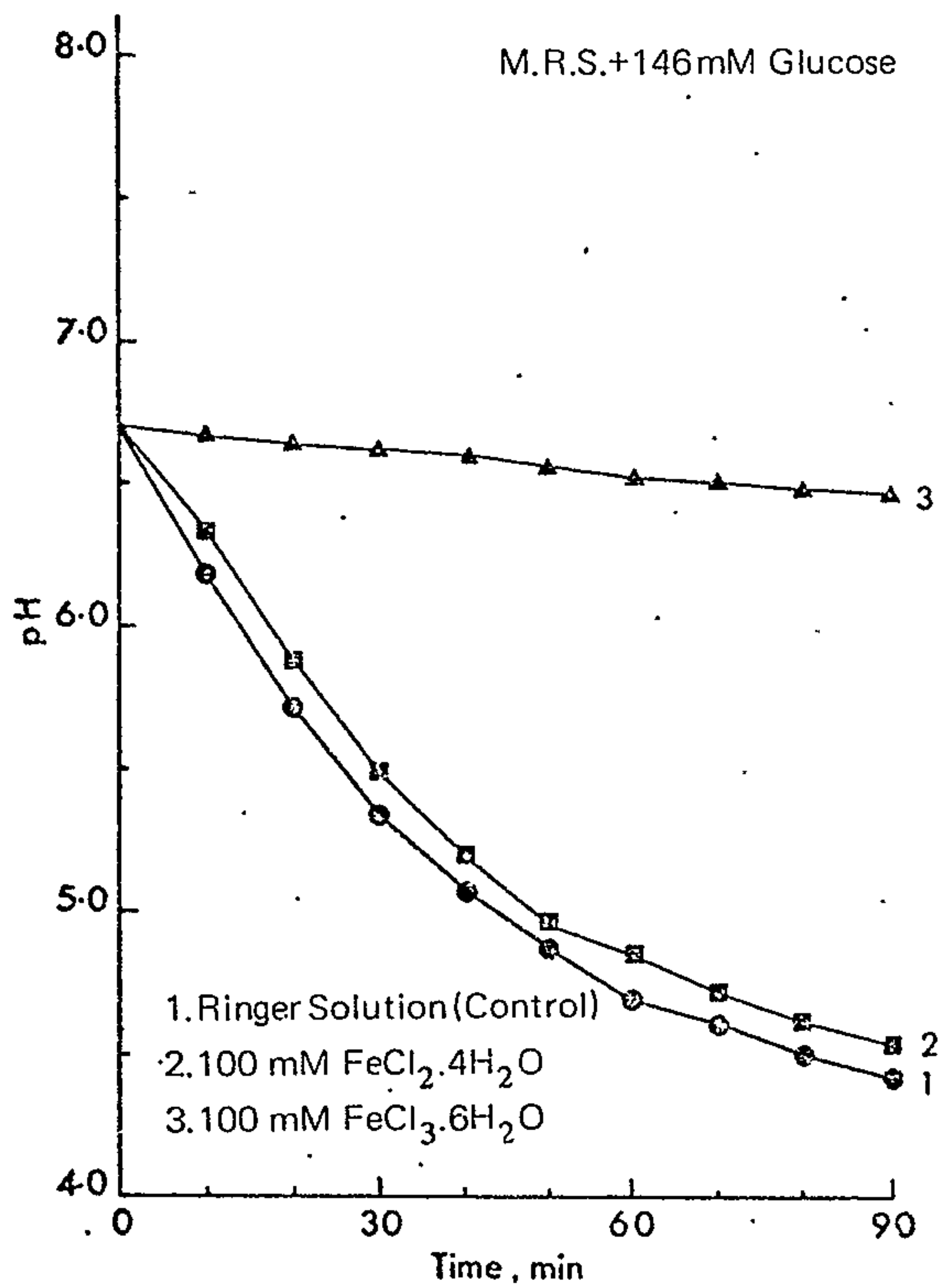
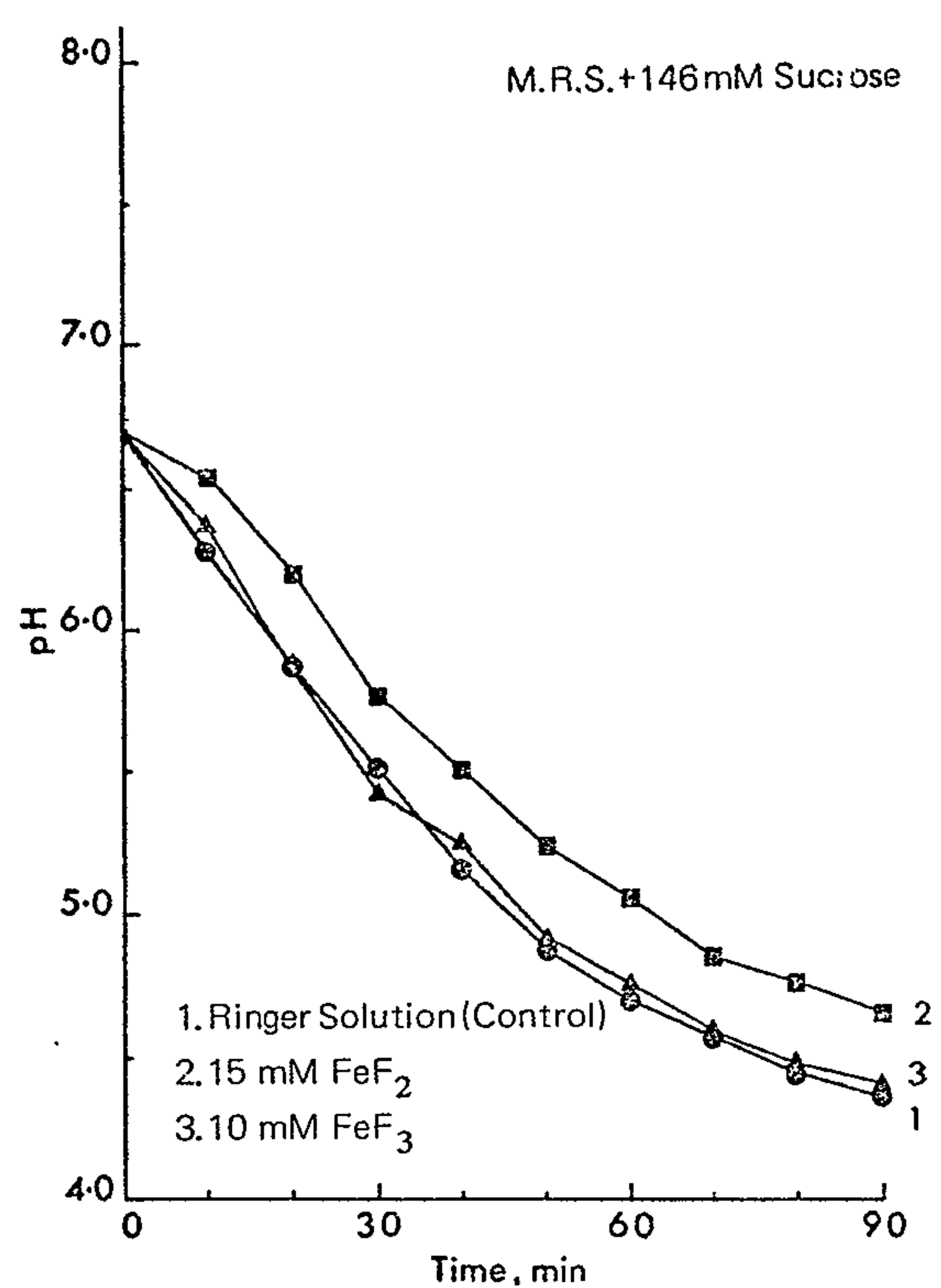
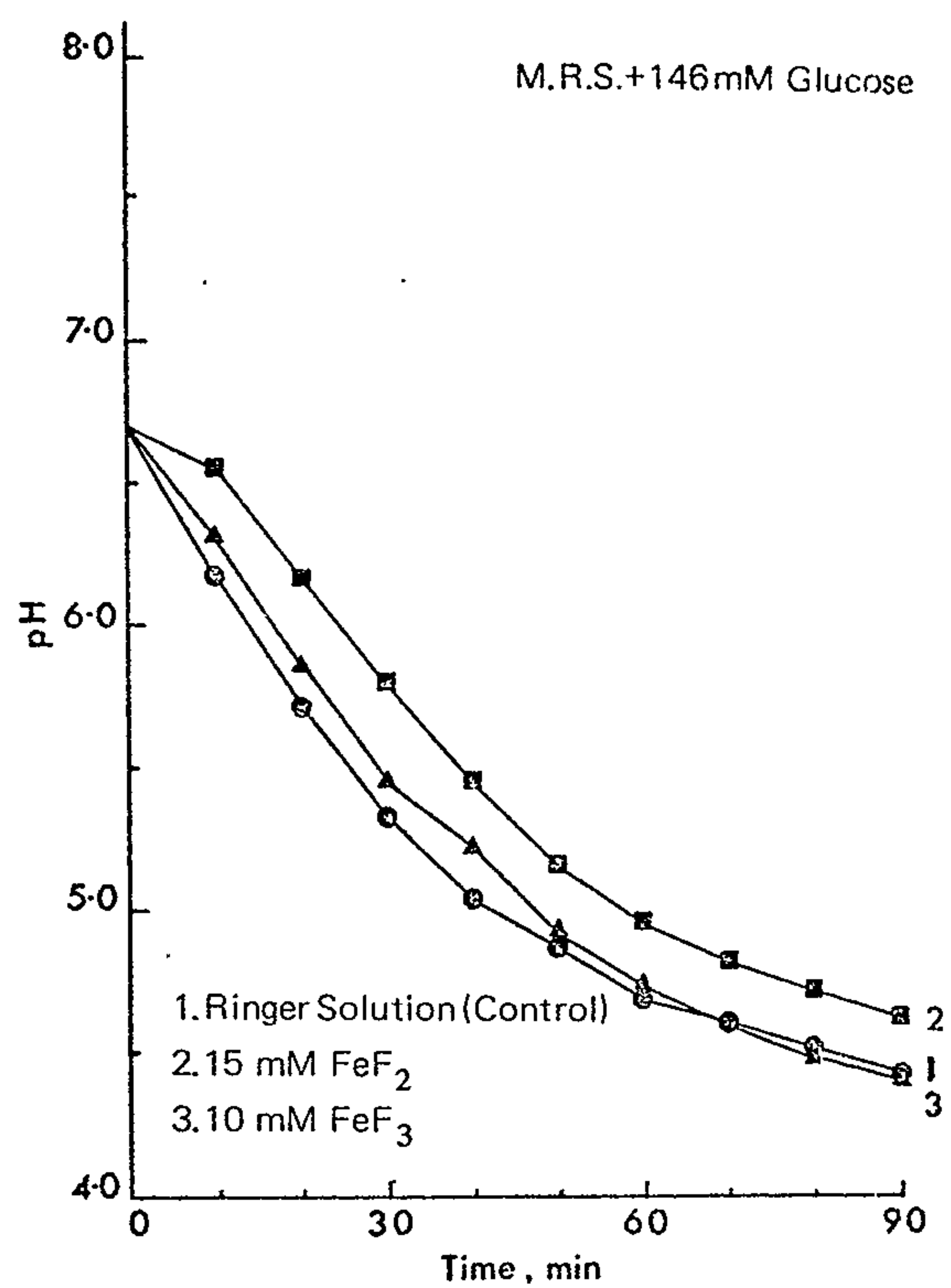


Fig. 46. Rates of acid production by *L. casei* var. *casei* in M.R.S. broth supplemented with glucose or sucrose following treatment with iron (II) fluoride, iron (III) fluoride, APF or a solution containing iron (III) chloride and sodium fluoride.

- A.** Effect of five minute treatments with 15 mM iron (II) fluoride or 10 mM iron (III) fluoride. (*Left*). M.R.S. broth supplemented with 146 mM glucose. (*Right*). M.R.S. broth supplemented with 146 mM sucrose.
- B.** Effect of five minute treatments with APF or a solution containing 50 mM iron (III) chloride and 190 mM sodium fluoride. (*Left*). M.R.S. broth supplemented with 146 mM glucose. (*Right*). M.R.S. broth supplemented with 146 mM sucrose.

A



B

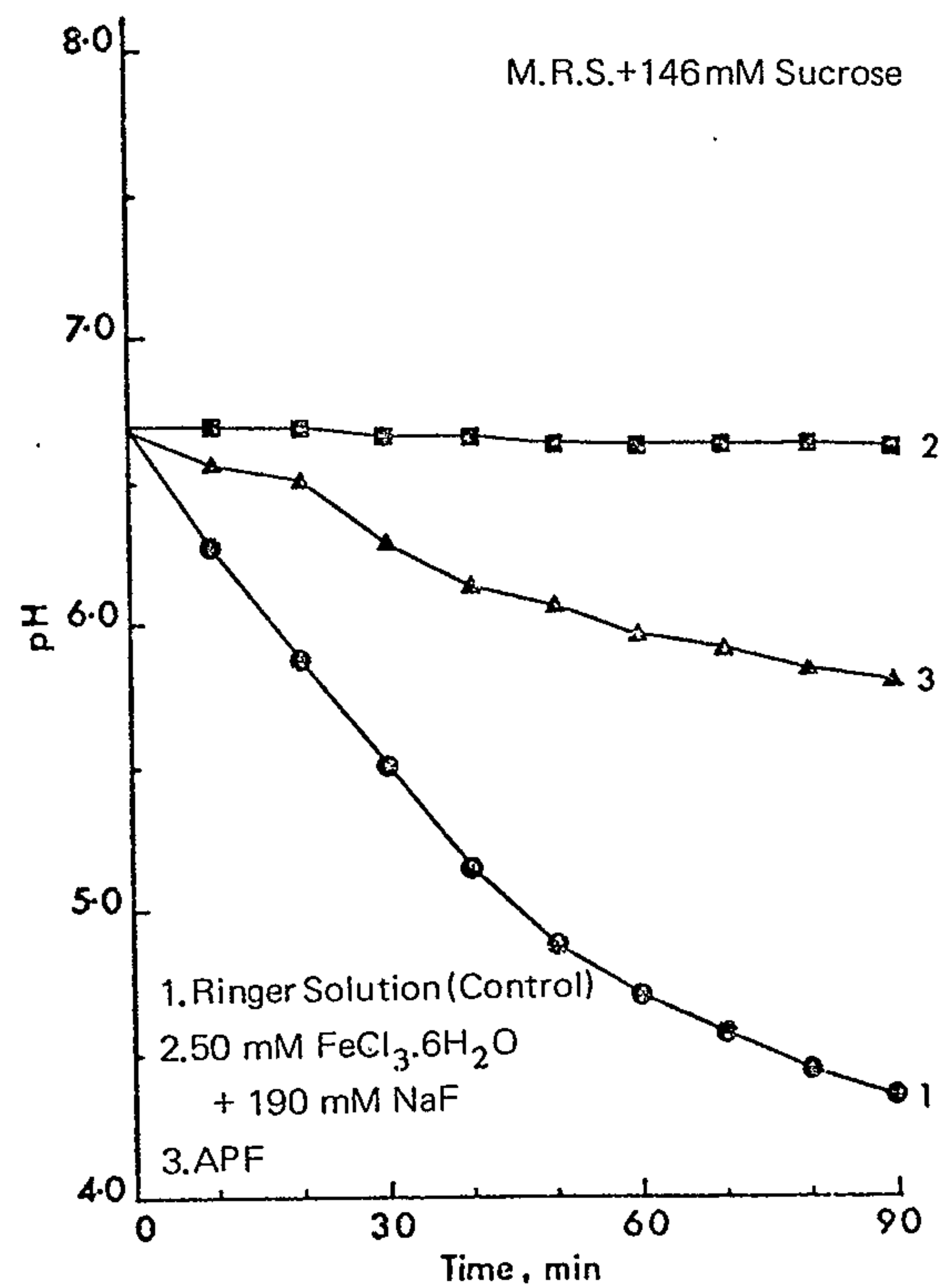
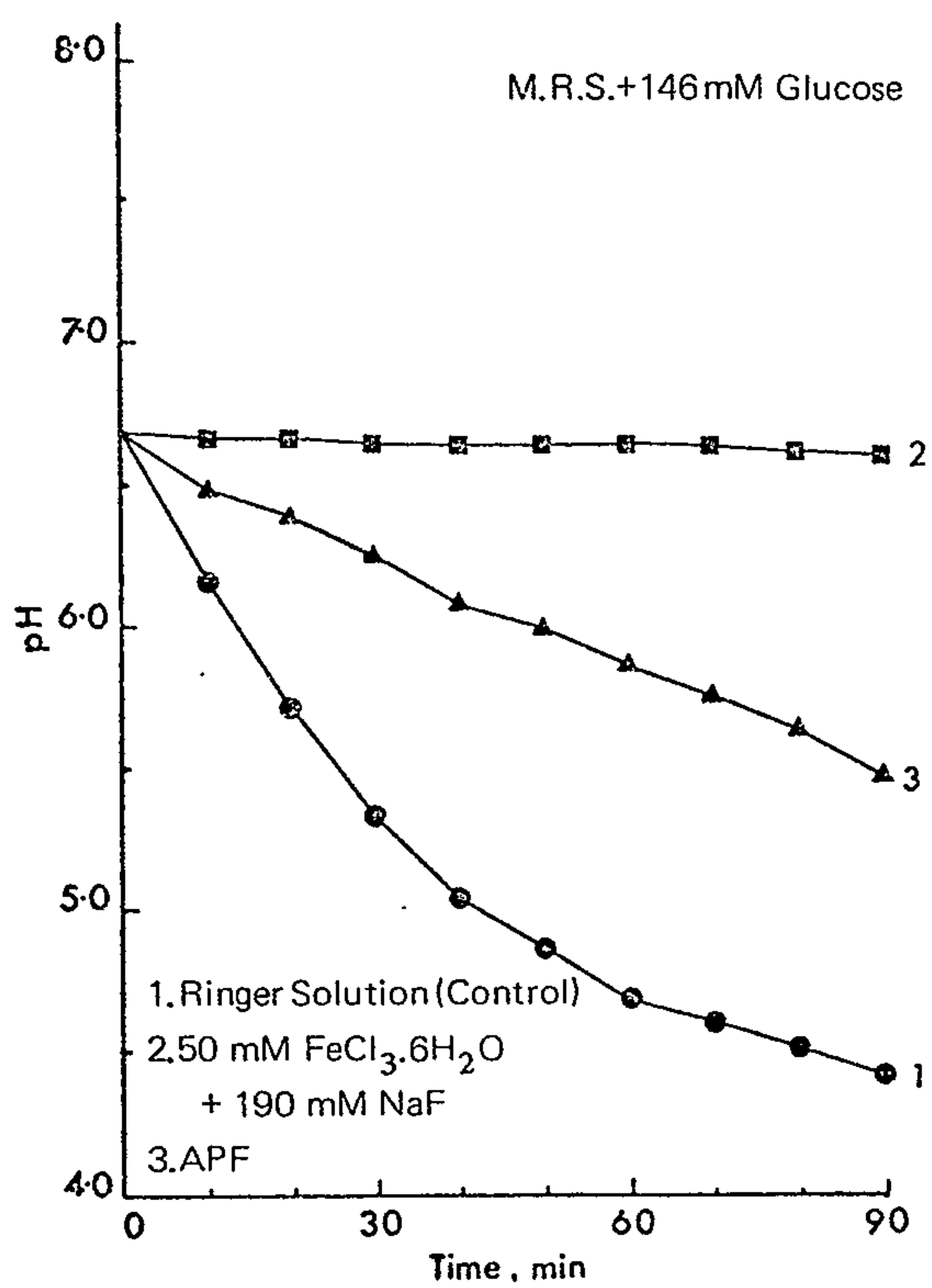
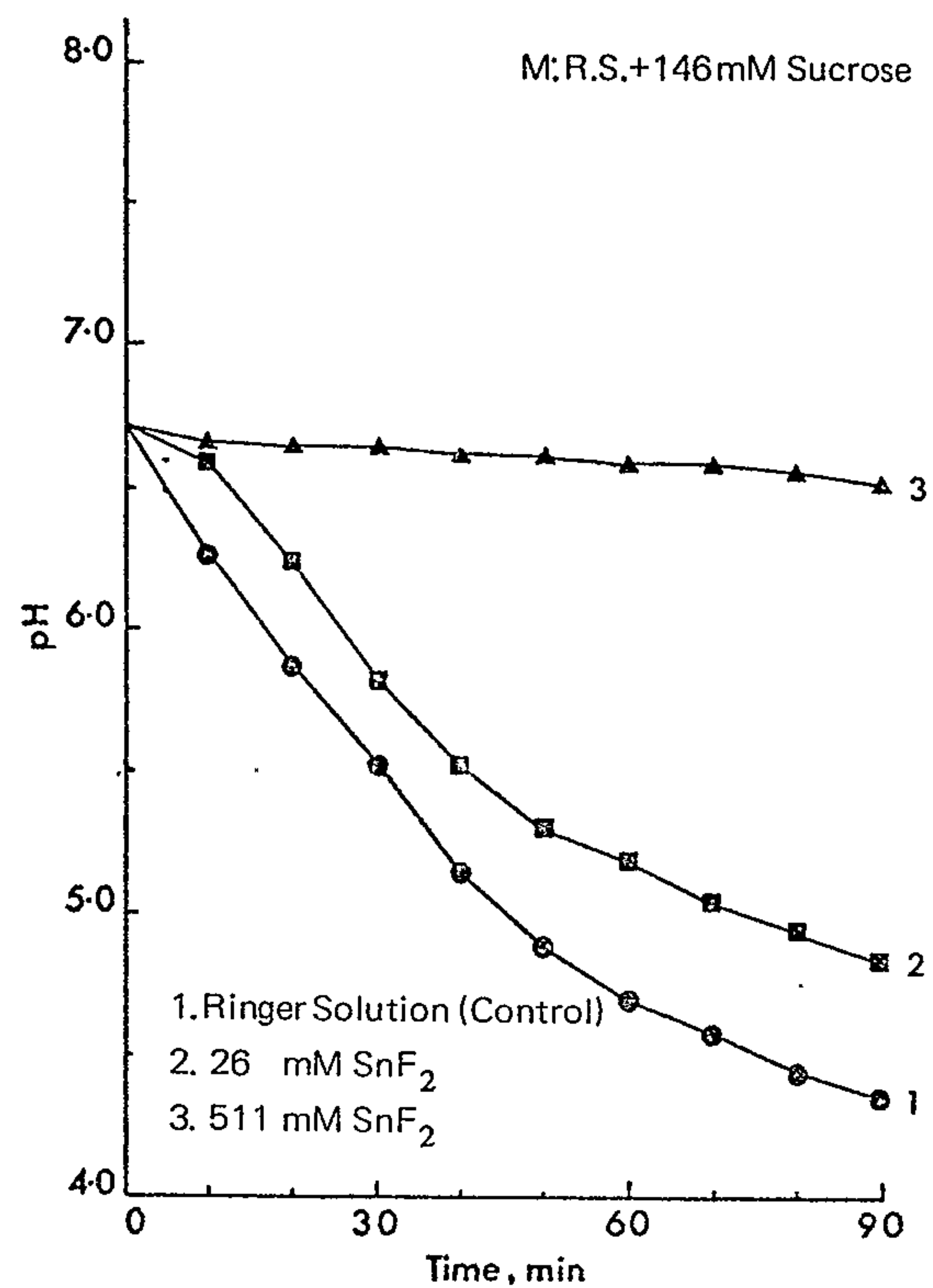
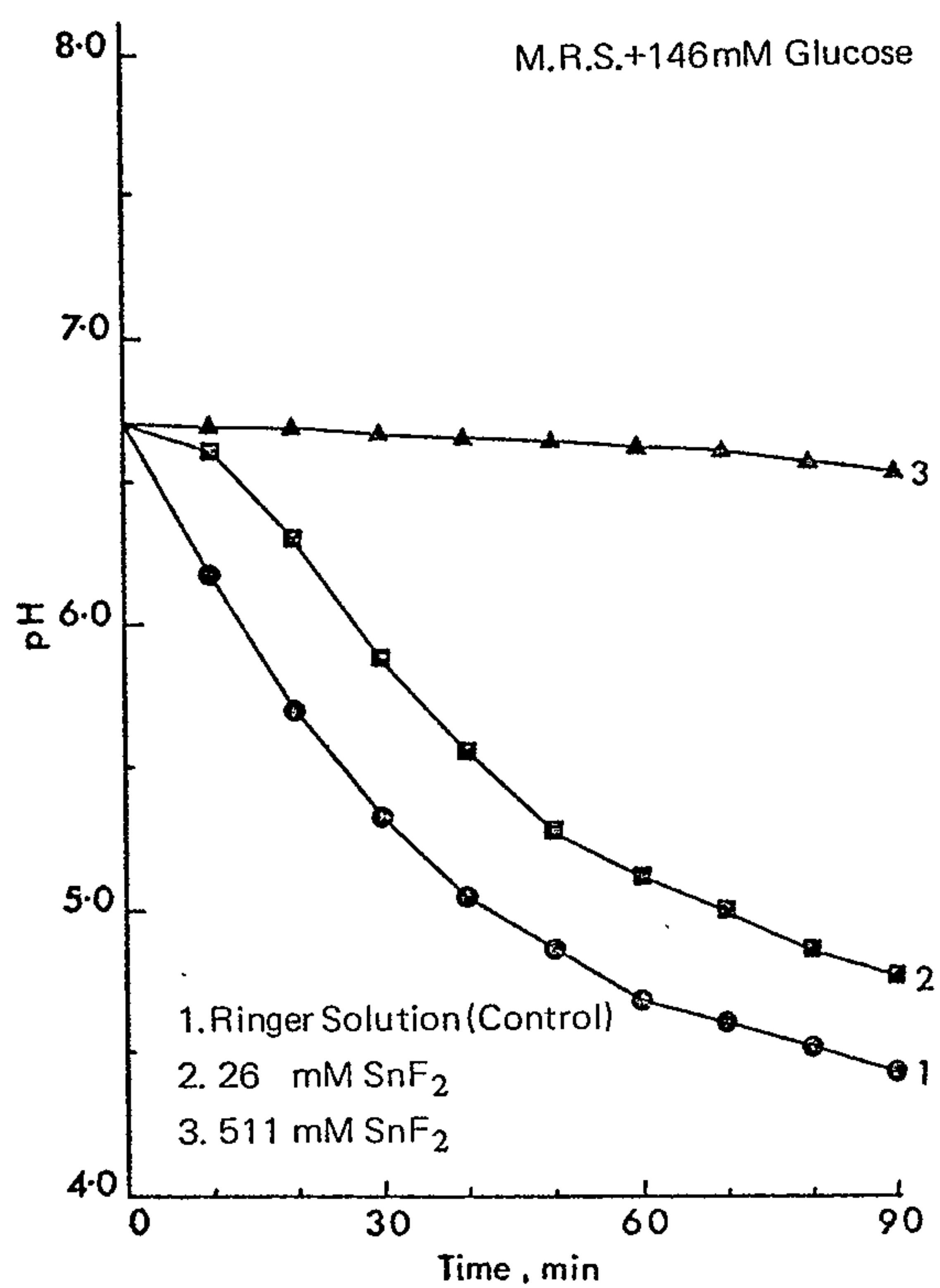


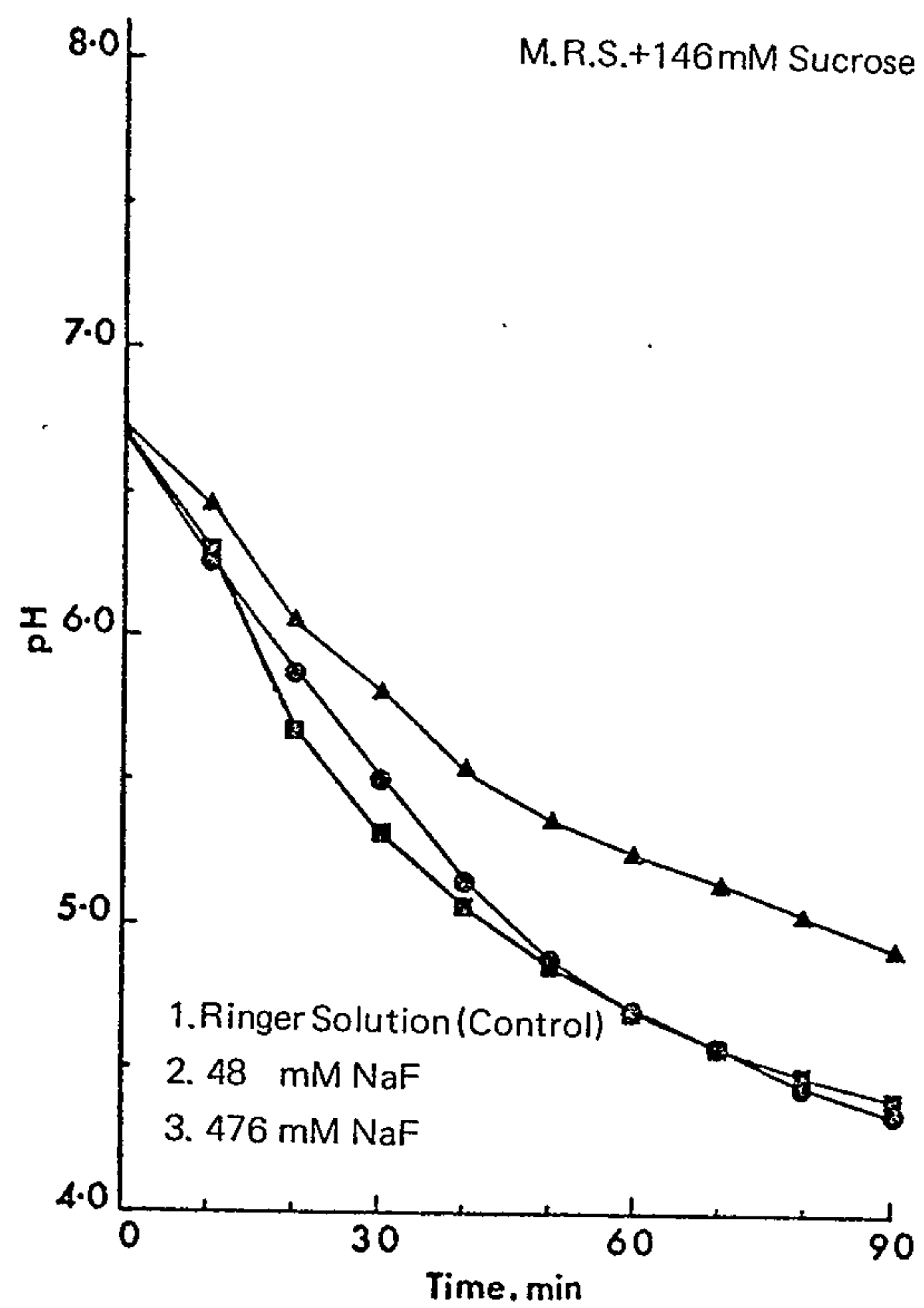
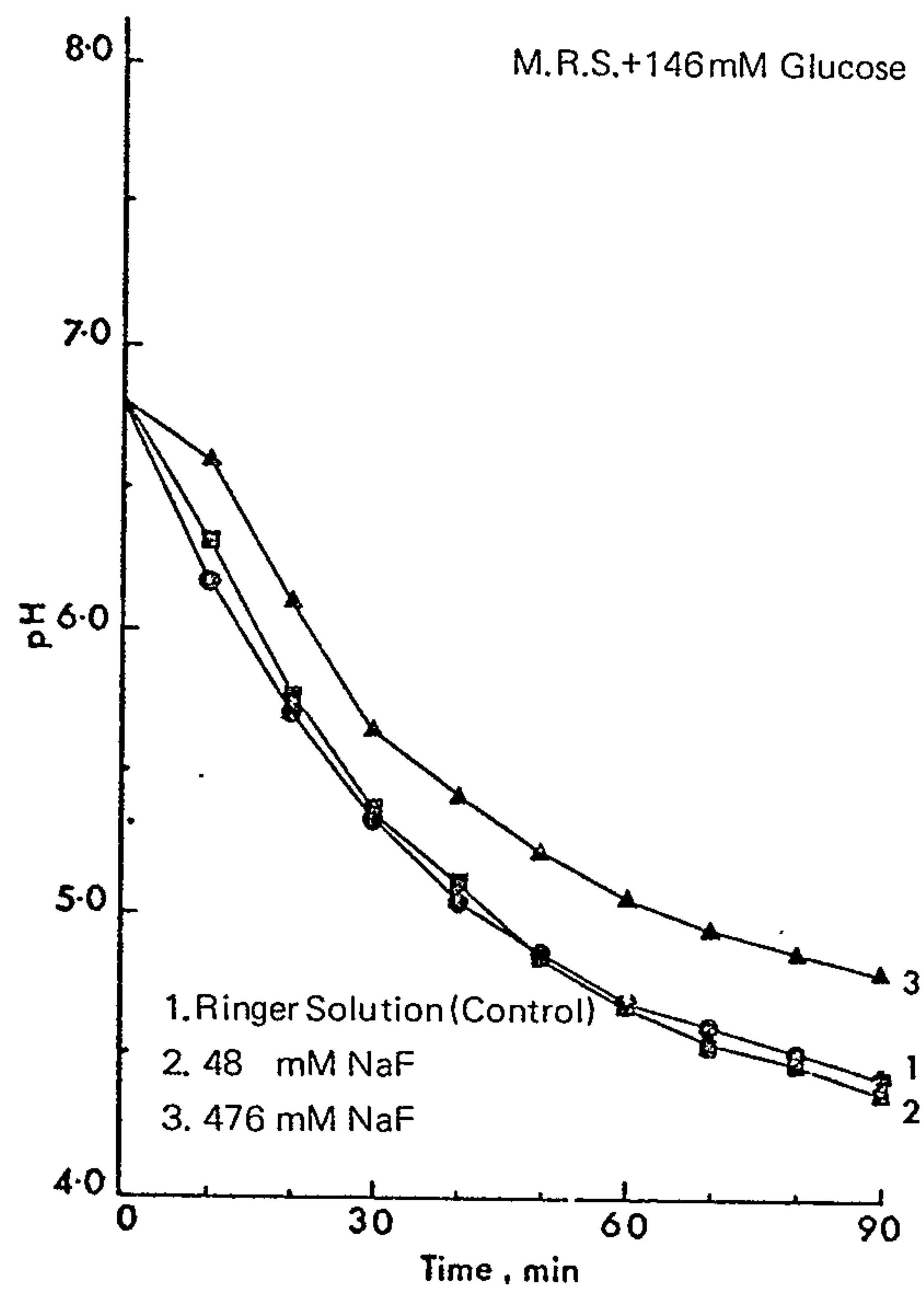
Fig. 47. Rates of acid production by *L. casei* var. *casei* in M.R.S. broth supplemented with glucose or sucrose following treatment with tin (II) fluoride or sodium fluoride.

- A. Effect of five minute treatments with tin (II) fluoride at a concentration of 26 mM or 511 mM. (*Left*). M.R.S. broth supplemented with 146 mM glucose. (*Right*). M.R.S. broth supplemented with 146 mM sucrose.
- B. Effect of five minute treatments with sodium fluoride at a concentration of 48 mM or 476 mM. (*Left*). M.R.S. broth supplemented with 146 mM glucose. (*Right*). M.R.S. broth supplemented with 146 mM sucrose.

A



B



The similarity is evident in Figs. 45 to 47 between the rates of acid production by *L. casei* var. *casei* treated with the control solution and those of *L. casei* var. *casei* treated with 1 mM iron (II) chloride, 10 mM iron (II) chloride, 1 mM iron (III) chloride, 10 mM iron (III) chloride (culture broth supplemented with glucose) 10 mM iron (III) chloride or 48 mM sodium fluoride.

DISCUSSION

In general, the five minute treatments of the test organisms with iron (II) chloride appeared to be less effective in inhibiting acid production than the corresponding treatments with iron (III) chloride at equivalent concentrations. These findings appear to be consistent with the observations of Guest and Salle⁽⁸⁹⁾ that the antimicrobial properties of iron (II) chloride are appreciably less than those of iron (III) chloride. However, it is noteworthy that these workers found that the use of iron (II) chloride and iron (III) chloride in combination was far more effective than the use of either compound alone.

With two exceptions, 10 mM iron (III) fluoride and 48 mM sodium fluoride, all the fluoride treatments inhibited acid production by the three test organisms. While 10 mM iron (III) fluoride and 48 mM sodium fluoride had little or no inhibitory effect on acid production by *L. casei* var. *casei*, both treatments did inhibit acid production by the other two organisms, *S. mutans* strain GS5 and *A. viscosus* strain NY1.

element for bacteria⁽²³³⁾, essential elements may inhibit metabolic activity if present in the wrong concentration or in the wrong proportion relative to other nutrients⁽¹⁵¹⁾.

Bowen⁽¹⁹⁾ has proposed a number of ways by which metals may exert a toxic effect on bacteria. Some of these are of interest in that they could provide some indication of the mechanisms by which iron and tin may affect bacterial metabolism:

1. Metal cations, especially those of the more electro-negative metals such as tin, could have a direct anti-enzyme effect due to their affinity for vital amino, imino and sulphhydryl groups.
2. Stable precipitates or chelates could be formed through the reaction of metal cations with essential metabolites such as phosphate.
3. Essential transport processes could be affected as a result of changes in cell permeability induced by metal cations.

In 1940, Bibby and Van Kesteren⁽¹⁵⁾ reported that 1 ppm fluoride inhibited glycolysis by pure cultures of streptococci and lactobacilli. Subsequent studies with mixed oral bacteria⁽¹⁰⁰⁾⁽¹¹¹⁾⁽²⁴²⁾, dental plaque⁽¹⁶⁵⁾⁽¹⁸⁶⁾⁽²⁴⁰⁾ and pure cultures of organisms including *L. casei*⁽¹⁵⁾⁽⁴⁰⁾⁽¹⁹⁰⁾⁽¹⁹³⁾, and *S. mutans*⁽¹³⁶⁾⁽¹³⁷⁾ have confirmed the antiglycolytic action of fluoride. This effect is attributed to the ability of fluoride to inhibit glucose transport into the cell through its inhibitory effect on the activity of the enzyme enolase⁽⁹²⁾. Enolase is involved in one of the reactions of the Embden - Meyerhof pathway, namely the conversion of 2-phosphoglycerate

to phosphoenolpyruvate⁽¹⁹⁶⁾. Since phosphoenolpyruvate is the phosphate donor in a reaction responsible for the transport of glucose into the cell⁽⁹²⁾, any reduction in the amount of phosphoenolpyruvate available for phosphorylation will, in turn, affect the amount of glucose available for glycolysis⁽⁹²⁾.

It is possible that some of the differences noted earlier between the antiglycolytic action of 48 mM sodium fluoride and that of 15 mM iron (II) fluoride or 26 mM tin (II) fluoride may have been due to the pH of the solutions. Whilst 48 mM sodium fluoride had a neutral pH, 15 mM iron (II) fluoride and 26 mM tin (II) fluoride had pH values of 4.4 and 3.4 respectively. Roberts and Rahn⁽¹⁷⁹⁾ in 1946 and subsequently Shiota⁽¹⁹⁰⁾ showed that the inhibitory effects of fluoride were enhanced at pH values below neutrality which they attributed to the formation of undissociated hydrogen fluoride. Interestingly, Sims⁽¹⁹³⁾, in a later experiment, obtained data which suggested that acid production by surface aggregates of streptococci or lactobacilli in the presence of glucose ceased once a critical concentration of undissociated hydrogen fluoride had been reached.

Although only the most general of comparisons can be made between the acid production rates of the three test organisms, the rate of acid production by *A. viscosus* strain NY1 appeared to be much less than that of either *S. mutans* strain GS5 or *L. casei* var. *casei*. These findings are consistent with those of Charlton *et al.*⁽³⁹⁾ who reported that the plaque of hamsters monoinfected with *A. viscosus* produced acid from sucrose, glucose or fructose, at lower rates than plaque from hamsters monoinfected with *S. mutans*.

Studies on the pH of plaque deposits *in vivo* have shown that the decrease in pH in the presence of sucrose is similar to the decrease in pH in the presence of glucose⁽¹⁵⁴⁾⁽¹⁶⁵⁾. However, a different situation occurs when sucrose or glucose is added to pure cultures of streptococci grown in essentially sucrose-free media⁽¹⁷⁴⁾⁽¹⁹⁵⁾⁽²⁰²⁾⁽²⁴⁵⁾. Under these circumstances, the acid production rate from sucrose is lower than that from glucose, which may mean that a period of time is required for the induction of enzymes involved in sucrose utilization⁽⁷³⁾. Similarly, in this study, the acid production rate of *S. mutans* strain GS5, which was also grown in an essentially sucrose-free medium, tended to be lower in the presence of sucrose than in the presence of glucose. In contrast, there was no appreciable difference, overall, in the rates of acid production of *A. viscosus* strain NY1 or *L. casei* var. *casei* in the presence of sucrose or glucose.

The finding that *L. casei* var. *casei* was more resistant to many of the treatments than *S. mutans* strain GS5, and especially *A. viscosus* strain NY1, is of interest, in that it may have implications in the selection of compounds for use in areas, such as carious lesions⁽¹⁰⁶⁾, where appreciable concentrations of lactobacilli are likely to be found.

A further discussion of these findings in relation to the problem of caries prevention is presented in the next section.

GENERAL DISCUSSION

It has been shown (Section 1) that fluoride deposition in enamel from APF can be enhanced by pretreating enamel with iron (III) chloride. However, the clinical significance of this finding remains to be determined.

It appears from laboratory and clinical studies that the relationship between fluoride uptake by enamel and caries resistance is somewhat tenuous⁽³⁾⁽⁶¹⁾⁽¹²⁴⁾⁽¹⁷⁵⁾. Regolati *et al.*⁽¹⁷⁵⁾ showed that the treatment of rat molar teeth with aluminium or titanium salts prior to a topical application of neutral sodium fluoride resulted in markedly higher fluoride levels in surface enamel over those obtained by the use of neutral sodium fluoride alone. However, despite this, the two stage treatments did not provide any additional protection against experimental caries. In 1967 Englander *et al.*⁽⁶¹⁾ found that children who used an APF gel daily had an appreciably higher fluoride level in surface enamel than children who followed the same programme using a neutral sodium fluoride gel with the same fluoride concentration. However, a comparison of the DMFS increments at the end of the two year study showed that the efficacy of the two gels was comparable. A similar situation was noted by Aasenden *et al.*⁽³⁾ in a report on a three year programme that had involved children rinsing with, and then swallowing, either a low fluoride concentration APF solution or a neutral sodium fluoride solution with an equivalent fluoride concentration. It was found that the protection against caries afforded by the solutions was comparable even though the APF solution deposited more fluoride in surface enamel.

Recent work by Larsen *et al.*⁽¹²⁴⁾ also shows that high fluoride concentrations in surface enamel are not necessarily equated with lower caries susceptibility. These workers found that rats which had acquired high concentrations of fluoride in surface enamel prior to a caries test period, had a higher incidence of experimental caries than rats which had received a continuous supply of fluoride, at a relatively low concentration, throughout the caries test period.

Mellberg and Singer⁽¹⁴⁹⁾ suggested that the poor correlation between fluoride uptake and caries susceptibility may be due to the additional fluoride being deposited mainly in the outer enamel. Under these circumstances, little protection is given to the area where the main portion of mineral is lost in the early lesion, namely the subsurface zone.

However to date, there does not appear to be any record of laboratory studies having been carried out to determine the effect of two stage treatments with iron salts and topical fluoride preparations on experimental caries in animals. Therefore, in light of the results obtained in this study with iron (III) chloride and APF, a pilot study with animals is warranted to determine whether there is any potential in this form of topical fluoride therapy.

Despite the wide use of enamel dissolution tests to screen compounds for cariostatic potential, to date no direct relationship between enamel dissolution rates and caries experience has been established.

Brudevold and McCann⁽²⁷⁾ have cited several examples where compounds have been effective in reducing enamel dissolution rates

in laboratory tests but ineffective when used topically in clinical trials. Amongst the compounds in this category are indium nitrate⁽¹⁴⁾⁽¹⁴³⁾, lead fluoborate⁽²⁶⁾⁽¹⁴³⁾ and tin (II) sulphate⁽⁸⁴⁾⁽¹⁵⁸⁾. An examination of the laboratory procedures used in the assessment of these three compounds shows that, in each instance, treated enamel was exposed to an acid buffer for only 5⁽⁸⁴⁾ or 15 minutes⁽¹⁴³⁾. The findings of this study (Section 2) suggest that an entirely different interpretation of their potential effectiveness may have been obtained if multiple and longer acid exposure times had been used. With the exception of tin (II) fluoride, all the test compounds in this study with proven cariostatic efficacy tended to exert a noticeable depressive effect on the enamel dissolution rate as the concentration of reaction products in the lactate buffer increased. If the interpretation of this situation presented earlier (page 80) is correct, it indicates that these compounds may have encouraged the precipitation of reaction products on the enamel surface. One of the test compounds with proven cariostatic properties, sodium fluoride, had little effect on the initial dissolution rate of enamel at both concentrations, 48 mM and 476 mM. Such a situation suggests that laboratory assessments of the cariostatic potential of compounds, based solely on their effect on the initial dissolution rate of enamel, may be misleading.

The method used in Section 2 can be refined to provide detailed information on the kinetics of crystal dissolution⁽⁷¹⁾. Such a 'rotating disc system' has been used to study the dissolution of enamel⁽¹²⁸⁾⁽²⁴³⁾. However, because of mathematical constraints

The solubility product of iron (III) hydroxide ($10^{-38.7}$ M at 25°C ⁽¹⁶⁾) only allows a maximum concentration of Fe^{3+} cations at pH 7 of approximately 10^{-18} M, whereas the solubility product of iron (II) hydroxide ($10^{-15.1}$ M at 25°C ⁽¹²⁵⁾) allows a maximum concentration of Fe^{2+} cations at pH 7 of approximately 10^{-1} M.

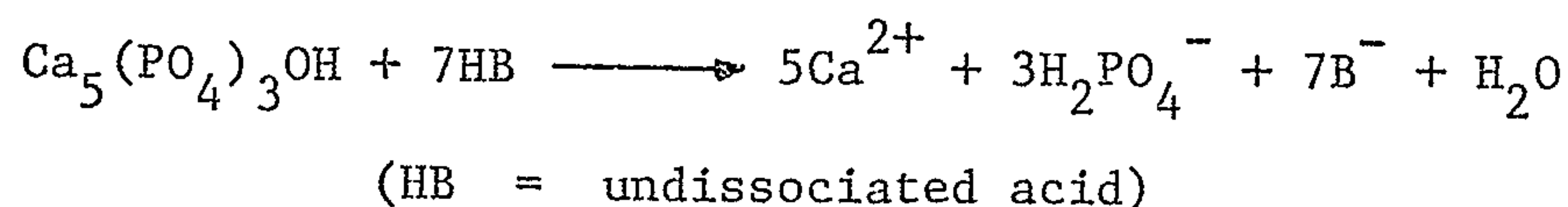
Since very low concentrations of Fe^{2+} or Fe^{3+} cations can cause the precipitation of calcium and phosphate under conditions where precipitation would not otherwise occur⁽⁷⁾, the presence of free iron in plaque, particularly at pH levels around neutrality, could have a stimulatory effect on remineralisation processes. This aspect is worthy of investigation. In addition, much information could be obtained by the use of well defined *in vitro* systems.

Several *in vitro* techniques have been developed to assess the remineralising properties of fluoride which have involved the use of artificially softened enamel or white-spot enamel (see reviews by Koulourides⁽¹²¹⁾ and Silverstone⁽¹⁹⁴⁾). However, it may be possible to obtain a better understanding of the effect of various ions on the remineralisation process by studying their effect on the growth of hydroxyapatite crystals since, in essence, the remineralisation process involves the 're-growth' of crystals destroyed by acid. By using pure hydroxapatite crystals, some of the uncertainties associated with the use of undefined surface or subsurface enamel can be avoided. In 1974, Meyer and Nancollas⁽¹⁵²⁾ reported on the use of such a method to study the effect of Sn^{2+} cations and F^{-} anions on the kinetics of crystal growth of hydroxyapatite.

Whilst the screening tests in Section 4 showed that a number of

the test compounds were effective in inhibiting acid production by *S. mutans* strain GS5, *A. viscosus* strain NY1 and *L. casei* var. *casei*, the question remains as to their effectiveness *in vivo* in inhibiting glycolytic activity in plaque. Ideally, *in vivo* studies should be aimed at obtaining data relating to the pathological consequence of acid production, namely the initiation of carious lesions.

In this respect, it is noteworthy that several workers have emphasised the importance of undissociated acid as well as hydrogen ions in controlling the rate of enamel dissolution during incipient lesion formation *in vitro*⁽⁶⁴⁾⁽¹⁰¹⁾⁽¹⁰³⁾⁽¹⁵⁵⁾. Holly and Gray⁽¹⁰³⁾ have indicated that a reservoir of undissociated acid is needed near a decalcification front to replace hydrogen ions lost through reactions with phosphate and hydroxyl ions, as shown in the following equation:



One of the factors affecting the availability of hydrogen ions is the pK values of the acids produced by plaque bacteria. Geddes⁽⁷³⁾ has reported that acetic, butyric and propionic acids, which have pK values of 4.73, 4.83 and 4.87 respectively⁽²³⁶⁾, tend to predominate in plaque around neutral pH. However, lactic acid, which she found to be the main acid responsible for decreasing plaque pH in the presence of glucose or sucrose, is a stronger acid having a pK of 3.8⁽²³⁶⁾.

It appears therefore that in order to obtain a full understanding of the effect of test compounds on the glycolytic activity of plaque, such parameters as pH, undissociated acid and acid type should be monitored. The desirability of such an approach is emphasised by the

recent findings of van der Hoeven⁽²²⁶⁾. He reported that the relative proportions of lactic acid, acetic acid and ethanol produced by *S. mutans* strain C67-1 in gnotobiotic rats varied appreciably with time following exposure of the organisms to a one minute 'pulse' of a sucrose-food extract mixture.

The antiglycolytic properties of one of the test compounds used in this study, 476 mM sodium fluoride, has been tested by Woolley and Rickles⁽²⁴⁰⁾. A single application of the solution was found to significantly inhibit acid production in the plaque of schoolchildren for 3 to 4 days. However these workers only measured one parameter, the decrease in plaque pH following a 'sugar' rinse.

One of the most promising compounds tested in this study was iron (II) fluoride. Although this compound is only sparingly soluble in water⁽¹⁶⁶⁾ it was, none the less, quite effective in reducing acid production by *S. mutans* strain GS5, *A. viscosus* strain NY1 and *L. casei* var. *casei* (Section 4). In addition, it was markedly effective in reducing the dissolution rate of enamel in pH 4.5 lactate buffer when either applied topically or added to the buffer (Section 2).

This latter property of iron (II) fluoride is of particular interest considering the relatively low fluoride concentration (570 ppm) in the solutions used for topical treatments. Despite the limited fluoride uptake by enamel from these solutions (Section 1), five minute topical treatments with 15 mM iron (II) fluoride significantly reduced calcium loss from enamel during a three hour immersion in pH 4.5 lactate buffer ($P < 0.05$). In contrast, under these experimental conditions, neither 511 mM tin (II) fluoride (19,400 ppm fluoride) nor 476 mM sodium fluoride (9,050 ppm fluoride) significantly reduced calcium loss from enamel. Furthermore, it is interesting that, in

common with most of the test compounds with proven cariostatic properties, 15 mM iron (II) fluoride tended to exert a noticeable depressive effect on the dissolution rate of enamel in pH 4.5 lactate buffer as the concentration of reaction products in the buffer increased. On the basis of the explanation presented earlier (page 80), this finding suggests that 15 mM iron (II) fluoride may have encouraged the precipitation of reaction products on the enamel surface.

Another test compound that has not been assessed clinically, iron (III) fluoride, was also markedly effective in reducing the dissolution rate of enamel in pH 4.5 lactate buffer when applied topically or added to the buffer. However, it did not appear to be as effective as iron (II) fluoride in inhibiting acid production by *S. mutans* strain GS5, *A. viscosus* strain NY1 and, particularly, *L. casei* var. *casei*.

These findings indicate a future possibility of replacing some of the present high concentration fluoride preparations with a compound of equivalent effectiveness containing a relatively low concentration of fluoride.

Clearly further testing of iron (II) fluoride is warranted. A follow up study to determine the effect of this compound on experimental caries in animals would be of considerable value.

SUMMARY AND CONCLUSIONS

A review of the literature indicated that non-fluoride iron salts may have cariostatic properties and that these salts may enhance the well established benefits of topical fluoride therapy. However despite this, it was found that no clinical assessments of these compounds as topical agents had been made and that only a limited number of pertinent laboratory studies had been carried out. Furthermore, no reports were found concerning laboratory or clinical studies on iron (II) fluoride or iron (III) fluoride (as distinct from solutions containing iron (III) chloride and sodium fluoride).

In view of this situation, four laboratory screening tests were carried out to determine the cariostatic potential of iron (II) and iron (III) salts. The tests were designed to assess whether these salts:

1. Could be used to enhance fluoride uptake by enamel from fluoride solutions (Test 1).
2. Could reduce the dissolution rate of enamel (Test 2), the formation of *in vitro* plaque (Test 3) and acid production by cariogenic organisms (Test 4).

Test 1 The effect of enamel pretreatments with iron (II) chloride or iron (III) chloride on the subsequent fluoride uptake by enamel from APF^a or 476 mM sodium fluoride was determined. Cores of bovine enamel were treated with an iron salt solution for one minute and then with APF or 476 mM sodium fluoride for four minutes. One half of the specimens were tested immediately for fluoride uptake. The other half were washed

^aAcidulated phosphate-fluoride.

for 24 hours in pH 5.5 acetate buffer containing calcium and phosphate, before being tested for fluoride uptake.

For comparison purposes, fluoride uptake by enamel from single five minute applications of either APF, sodium fluoride, iron (II) fluoride, iron (III) fluoride, tin (II) fluoride, or a solution containing iron (III) chloride and sodium fluoride was also determined.

A further group of specimens were prepared, treated with the same test compounds and the treated surfaces examined using scanning electron microscopy.

Pretreatment of enamel with iron (III) chloride, particularly at concentrations of 10 mM and 100 mM, enhanced fluoride uptake from APF by enamel. The mean fluoride concentration in enamel pretreated with 100 mM iron (III) chloride at a mean depth of 2.40 μm (S.D. = 0.70) was 5.1 times higher than that in enamel treated with APF alone at a mean depth of 3.78 μm (S.D. = 0.13). In contrast to the situation with iron (III) chloride, the mean fluoride levels in enamel pretreated with iron (II) chloride did not differ markedly from the mean fluoride level in enamel treated with APF alone. The 24 hour washing procedure reduced the levels of acquired fluoride.

The treatment of enamel with iron (II) chloride or iron (III) chloride prior to topical treatment with 476 mM sodium fluoride appeared to reduce the fluoride uptake by enamel from this solution. However, the fluoride levels that were attained were not appreciably reduced by the 24 hour washing procedure.

Under the conditions of this test, the fluoride uptake by enamel from a five minute treatment with APF was comparable to that from a five minute treatment with 476 mM sodium fluoride. The solution containing

iron (III) chloride and sodium fluoride deposited approximately five times more fluoride in enamel than either iron (II) fluoride or iron (III) fluoride.

An examination of treated surfaces using scanning electron microscopy showed that the treatment with 100 mM iron (III) chloride followed by APF caused etching of enamel. Etching was also seen on surfaces treated with the solution containing iron (III) chloride and sodium fluoride and on surfaces treated with tin (II) fluoride at a concentration of 511 mM. Surface deposits were evident on enamel treated with 100 mM iron (II) chloride followed by APF and on enamel treated with iron (III) chloride at a concentration of 1 mM or 10 mM and then treated with 476 mM sodium fluoride. Enamel treated with APF alone also had an accumulation of surface deposits.

The following conclusions can be drawn from the findings in this test:

1. The treatment of enamel with iron (II) chloride or iron (III) chloride prior to the topical application of APF or 476 mM sodium fluoride can affect the fluoride uptake by enamel from these solutions. The degree of fluoride uptake is influenced by the oxidation state and the concentration of the iron salt used and the type of fluoride solution employed.
2. With few exceptions, an appreciable portion of the fluoride deposited in enamel by fluoride solutions can be removed by washing the treated surfaces for 24 hours with pH 5.5 acetate buffer containing calcium and phosphate.

Test 2 - Experiment 1 An assessment was made of the effect of topical treatments with iron salts on the dissolution rate of enamel in pH 4.5

1. Iron (II) chloride at concentrations of 10 mM and 100 mM reduced calcium loss from enamel by 21.7 per cent ($P < 0.05$) and 23.8 per cent ($P < 0.01$) respectively.
2. Iron (III) chloride at a concentration of 100 mM reduced calcium loss from enamel by 28.3 per cent ($P < 0.01$).
3. Iron (II) fluoride and iron (III) fluoride reduced calcium loss from enamel by 22.8 per cent ($P < 0.05$) and 22.6 per cent ($P < 0.05$) respectively.
4. The solution containing iron (III) chloride and sodium fluoride reduced calcium loss from enamel by 35.7 per cent ($P < 0.01$) whilst APF reduced calcium loss by 36.2 per cent ($P < 0.01$).

Although tin (II) fluoride at concentrations of 26 mM and 511 mM, and sodium fluoride at concentrations of 48 mM and 476 mM, did reduce the calcium loss from enamel immersed in lactate buffer for three hours, the reductions were not statistically significant.

An assessment was made of the calcium loss from enamel during the actual five minute topical treatment with the test compounds. The highest calcium loss occurred during topical treatments with iron (III) chloride at a concentration of 100 mM whilst the second highest calcium loss occurred during topical treatments with APF.

The dissolution rates of enamel treated with iron (II) chloride or iron (III) chloride prior to topical treatments with APF or 476 mM sodium fluoride were very similar from 60 minutes onwards and tended to parallel those of enamel treated with the respective fluoride solutions alone. In most instances, the differences in dissolution rates occurred prior to this time. Of all the treatment combinations

tested, only the treatments with 100 mM iron (III) chloride followed by APF or 476 mM sodium fluoride afforded a greater protection against calcium loss at three hours than the respective fluoride solutions alone.

Test 2 - Experiment 2 The effect on the enamel dissolution rate of adding iron (II) or iron (III) salts to pH 4.5 lactate buffer was determined.

For comparison purposes, tin (II) chloride, tin (II) fluoride, sodium fluoride, as well as calcium and phosphate salts, were also tested.

Cores of bovine enamel mounted on plastic rods were immersed for 60 minutes in pH 4.5 lactate buffer containing the added salts. At the end of the immersion period the buffer was analysed for calcium and the data, thus obtained, were used to determine the effect of each salt on the enamel dissolution rate.

The addition of salts that gave Fe^{2+} , Fe^{3+} or Sn^{2+} cation concentrations of 100 μM or 1 mM caused a significant decrease in the dissolution rate of enamel. However, the addition of salts that gave similar concentrations of Ca^{2+} cations or PO_4^{3-} anions had no appreciable effect on the enamel dissolution rate.

All the fluoride salts significantly reduced the enamel dissolution rate when added to lactate buffer at a fluoride concentration of 100 μM or 1 mM. At a fluoride concentration of 1 mM, iron (II) fluoride, iron (III) fluoride and tin (II) fluoride depressed the enamel dissolution rate more markedly than sodium fluoride.

Most of the specimens exhibited a surface "glaze" after immersion in lactate buffer containing either Sn^{2+} cations at a concentration of

1 mM, or iron (II) fluoride, iron (III) fluoride or tin (II) fluoride at a fluoride concentration of 1 mM.

On the basis of the findings in this test, it is concluded that:

1. Iron (II) chloride, iron (III) chloride, iron (II) fluoride and iron (III) fluoride can each reduce the calcium loss from enamel in pH 4.5 lactate buffer when either applied topically or added to the buffer.
2. Iron (III) chloride solutions can cause an appreciable calcium loss from enamel when applied topically and this may limit their use clinically.
3. The relative effects of test compounds on the dissolution rate of enamel in pH 4.5 lactate buffer can vary with the length of the immersion period in the buffer.
4. The addition of fluoride to pH 4.5 lactate buffer can reduce the dissolution rate of enamel immersed in the buffer. Higher reductions can be obtained if Fe^{2+} , Fe^{3+} or Sn^{2+} cations are also present in the buffer.

Test 3 The effect of topical treatments with iron (II) or iron (III) salts on *in vitro* plaque formation by *S. mutans* strain GS5, *S. sanguis* strain 804 or *A. viscosus* strain NY1 on smooth enamel, ground enamel and stainless steel wire was studied. Triangular pieces of enamel were prepared from bovine teeth and suspended on stainless steel wires. Each piece of enamel and its supporting wire was immersed for five minutes in a solution containing either iron (II) chloride, iron (III) chloride, iron (II) fluoride or iron (III) fluoride or a solution containing iron (III) chloride and sodium fluoride. For comparison purposes, APF, tin (II) fluoride, and sodium fluoride solutions were

also used. Each specimen was then placed in culture broth inoculated with one of the test organisms and transferred daily into freshly inoculated broth. In the later stage of the experiment the daily transfers were made into uninoculated broth. At the end of the nine day (*S. mutans* strain GS5) or 14 days (*S. sanguis* strain 804 and *A. viscosus* strain NY1) experimental period, the amount of plaque formed on smooth enamel, ground enamel and the wire supporting each section was assessed.

None of the test compounds completely inhibited *in vitro* plaque formation by *S. mutans* strain GS5, *S. sanguis* strain 804 or *A. viscosus* strain NY1 on all three test surfaces. Although some of the test compounds appeared to have some inhibitory effects, these were not constant overall and appeared to differ with the nature of the test surface and the type of organism used.

The rate of *in vitro* plaque formation on specimens exposed to *S. mutans* strain GS5 appeared to be influenced by the nature of the test surface. In contrast, *in vitro* plaque formation by *S. sanguis* strain 804, although light, appeared to develop on all three test surfaces with equal facility. Overall, there was not a pronounced formation of *in vitro* plaque on test surfaces exposed to *A. viscosus* strain NY1, especially on smooth enamel.

In view of the equivocal findings in this test, and the results of recent work suggesting that iron salts may have a specific inhibitory effect on *S. mutans*, it is concluded that further studies are required to determine the effect of iron salts on plaque formation *in vitro* and *in vivo*. In addition, it is suggested that a follow-up study be undertaken to determine whether repeated applications of low concentrations

iron salt solutions affect the growth of *S. mutans* induced plaque deposits *in vitro*.

Test 4 The effect of iron (II) and iron (III) salts on acid production by *S. mutans* strain GS5, *A. viscosus* strain NY1 and *L. casei* var. *casei* was investigated. Iron (II) chloride, iron (III) chloride, iron (II) fluoride, iron (III) fluoride and solution containing iron (III) chloride and sodium fluoride were tested. For comparison purposes, the effect of APF, tin (II) fluoride, and sodium fluoride on acid production by these organisms was also studied. The organisms were grown anaerobically in a fermenter at constant pH and were harvested by centrifugation at the exponential growth phase. A suspension of washed cells was pipetted into a holder, specifically designed for this experiment, which allowed the cells to be (i) treated with the test compounds, (ii) washed and (iii) monitored for acid production, whilst they remained *in situ*. The cells were treated with a test compound for five minutes, washed and then covered with culture broth supplemented with glucose or sucrose. The pH value of the broth was recorded at regular intervals for 90 minutes when *S. mutans* strain GS5 or *L. casei* var. *casei* were the test organisms and at regular intervals for 150 minutes when *A. viscosus* strain NY1 was used.

In general, the five minute treatments of the test organisms with iron (II) chloride appeared to be less effective in reducing acid production than the corresponding treatments with iron (III) chloride at equivalent concentrations. Iron (III) chloride at a concentration of 100 mM reduced acid production by all three organisms by more than 98 per cent whilst the solution containing iron (III) chloride and sodium fluoride reduced acid production by all three organisms by

more than 99 per cent. APF, tin (II) fluoride at a concentration of 511 mM and, to a lesser extent, sodium fluoride at a concentration of 476 mM, were all markedly effective in reducing acid production by the test organisms. With all organisms, higher reductions in acid production occurred after treatments with iron (II) fluoride than after treatments with iron (III) fluoride.

Two of the findings from these laboratory screening tests could be of particular importance clinically. Firstly, the results obtained by treating enamel with iron (III) chloride prior to a topical treatment with APF show that this form of treatment can deposit large amounts of fluoride in surface enamel and that the treated enamel is relatively resistant to acid demineralisation. Secondly, the results with iron (II) fluoride indicate a possibility of being able to replace some of the currently used high fluoride preparations with a low fluoride compound of equal effectiveness. Therefore, it is recommended that further testing of (i) two stage topical treatments with iron (III) chloride and APF, and (ii) topical treatment solutions containing iron (II) fluoride, be carried out.

BIBLIOGRAPHY

1. AASENDEN R. AND MORENO, E.C. (1971). Evaluation of biopsy data in human enamel fluoride studies. Arch. Oral Biol. 16, 1413 - 1426.
2. AASENDEN, R., BRUDEVOLD, F. AND McCANN, H. (1968). The response of intact and experimentally altered enamel to topical fluoride. Arch. Oral Biol. 13, 543 - 552.
3. AASENDEN, R., DE PAOLA, P.F. and BRUDEVOLD, F. (1972). Effects of daily rinsing and ingestion of fluoride solutions upon dental caries and enamel fluoride. Arch. Oral Biol. 17, 1705 - 1714.
4. AI, S., ISHIKAWA, T. and SEINO, A. (1965). 'Ohaguro' traditional tooth staining custom in Japan. Int. Dent. J. 15, 426 - 441.
5. ÅKESON, Å., BIÖRCK, G. and SIMON, R. (1968). On the content of myoglobin in human muscles. Acta Med. Scand. 183, 307 - 316.
6. ARENDS, J. and SCHUTHOF, J. (1975). Fluoride content in human enamel after fluoride application and washing - an *in vitro* study. Caries Res. 9, 363 - 372.
7. BACHRA, B.N. and VAN HARSKAMP, G.A. (1970). The effect of polyvalent metal ions on the stability of a buffer system for calcification *in vitro*. Calcif. Tiss. Res. 4, 359 - 365.
8. BALMELLI, O.P., REGOLATI, B. and MÜHLEMANN, H.R. (1974). Inhibition of streptococcal deposits on rat molars by amine fluoride. Helv. Odontol. Acta 18, Supplement 8, 45 - 53.
9. BAUD, C.A. and BANG, S. (1970). Electron probe and x-ray diffraction microanalyses of human enamel treated *in vitro* by fluoride solution. Caries Res. 4, 1 - 13.
10. BEIGHTON, D. (1976). The effect of various trace elements on percentage bacterial composition of rat plaque. Preprinted abstracts of papers presented at Internat. Ass. Dent. Res. (Aust. and N.Z. Division) 16th Annual Meeting. Abstract 29.

11. BESIC, F.C., BAYARD, M., WIEMANN, M.R. and BURRELL, K.H. (1975). Composition and structure of dental enamel: elemental composition and crystalline structure of dental enamel as they relate to its solubility. J. Am. Dent. Assoc. 91, 594 - 601.
12. BERGGREN, H. and WELANDER, E. (1964). The caries - inhibiting effect of sodium, ferric and zirconium fluorides. Acta Odontol. Scand. 22, 401 - 413.
13. BERKOVITZ, B.K.B. and HEAP, P.F. (1976). The effect of fluoride on iron, calcium and phosphorus distribution in the rat incisor. Caries Res. 10, 337 - 351.
14. BIBBY, B.G. (1947). Effect of topical application of indium nitrate solution on caries. Unpublished data. Cited in Brudevold, F. and McCann, H.G. (1968). Enamel solubility tests and their significance in regard to dental caries. Ann. N.Y. Acad. Sci. 153, 20 - 51.
15. BIBBY, B.G. and VAN KESTEREN, M. (1940). The effect of fluorine on mouth bacteria. J. Dent. Res. 19, 391 - 402.
16. BIEDERMANN, G. and SCHINDLER, P. (1957). On the solubility product of iron (III) hydroxide. Acta Chem. Scand. 11, 731 - 740.
17. BOTHWELL, T.H. and FINCH, C.A. (1962). Iron Metabolism. pp. 268 - 298. Boston: Little, Brown.
18. BOURGEAU, G. and McBRIDE, B.C. (1976). Dextran - mediated interbacterial aggregation between dextran-synthesizing streptococci and *Actinomyces viscosus*. Infect. Immun. 13, 1228 - 1234.
19. BOWEN, H.J.M. (1966). Trace Elements in Biochemistry. pp. 111 - 114. London: Academic Press.
20. BRADY, B.H.G., NAPPER, D.H. and SMYTHE, B.M. (1968). Effect of additives on the rate of dissolution of hydroxyapatite in unstirred acid buffers. J. Dent. Res. 47, 603 - 612.
21. BRIDSON, E.Y. and BRECKER, A. (1970). Design and formulation of microbial culture media. In Methods in Microbiology. Vol. 3A. pp. 229 - 295. Edited by J.N. Norris and D.W. Ribbons. London: Academic Press.
22. BRINER, W.W. (1971). Plaque in relation to dental caries and periodontal disease. Int. Dent. J. 21, 293 - 301.

23. BRINER, W.W. and FRANCIS, M.D. (1962). The effect of enamel fluoride on acid production by *Lactobacillus casei*. Arch. Oral Biol. 7, 541 - 550.
24. BROWN, W.E., GREGORY, T.M. and CHOW, L.C. (1977). Effects of fluoride on enamel solubility and cariostasis. Caries Res. 11, Supplement 1, 118 - 141.
25. BROWN, A.T. and WITTENBERGER, C.L. (1972). Fructose - 1:6 - diphosphate-dependent lactate dehydrogenase from a cariogenic streptococcus: purification and regulatory properties. J. Bacteriol. 110, 604 - 615.
26. BRUDEVOLD, F. (1948). Topical applications with lead fluoborate. Unpublished data. Cited in Brudevold, F. and McCann, H.G. (1968). Enamel solubility tests and their significance in regard to dental caries. Ann. N.Y. Acad. Sci. 153, 20 - 51.
27. BRUDEVOLD, F. and McCANN, H.G. (1968). Enamel solubility tests and their significance in regard to dental caries. Ann. N.Y. Acad. Sci. 153, 20 - 51.
28. BRUDEVOLD, F. and SÖREMARK, R. (1967). Chemistry of the mineral phase of enamel. In Structural and Chemical Organisation of Teeth. Vol. 2. pp. 247 - 277. Edited by A.E.W. Miles. New York: Academic Press.
29. BRUDEVOLD, F., McCANN, H.G. and GRØN, P. (1968). An enamel biopsy method for the determination of fluoride in human teeth. Arch. Oral Biol. 13, 877 - 885.
30. BRUDEVOLD, F., McCANN, H.G., NILSSON, R., RICHARDSON, B. and COKLICA, V. (1967). The chemistry of caries inhibition problems and challenges in topical treatments. J. Dent. Res. 46, Supplement to No. 1, 37 - 45.
31. BRUUN, C. (1973). Uptake and retention of fluoride by intact enamel *in vivo* after application of neutral sodium fluoride. Scand. J. Dent. Res. 81, 92 - 100.
32. BRUUN, C., MUNKSGAARD, E.C. and STOLTZE, K. (1975). A field biopsy method for fluoride determinations in human surface enamel. Community Dent. Oral Epidemiol. 3, 217 - 222.
33. BUCHANAN, B.B. and PINE, L. (1967). Path of glucose breakdown and cell yields of a facultative anaerobe, *Actinomyces naeslundii*. J. Gen. Microbiol. 46, 225 - 236.
34. BUONOCORE, M.G. and BIBBY, B.G. (1945). The effects of various ions on enamel solubility. J. Dent. Res. 24, 103 - 108.

35. BURMAN, D. (1974). Iron metabolism in infancy and childhood.
In Iron in Biochemistry and Medicine. pp. 543 - 562.
Edited by A. Jacobs and M. Worwood. London:
Academic Press.
36. BUTTNER, G. and MUHLER, J.C. (1958). The solubility of intact
dental enamel surfaces treated with potential
anticariogenic agents. J. Dent. Res. 37, 412 - 414.
37. CARLSSON, J. (1970). A levansucrase from *Streptococcus mutans*.
Caries Res. 4, 97 - 113.
38. CASLAVSKA, V., BRUDEVOLD, F., VRBIC V. and MORENO, E.C. (1971).
Response of human enamel to topical application of
ammonium fluoride. Arch. Oral Biol. 16, 1173 - 1180.
39. CHARLTON, G., FITZGERALD, D.B. and KEYES, P.H. (1971). Hydrogen
ion activity in dental plaques of hamsters during
metabolism of sucrose, glucose and fructose. Arch.
Oral Biol. 16, 655 - 661.
40. CLAPPER, W.E. (1947). The effect of sodium fluoride on the
metabolism of oral lactobacilli. Proc. Soc. Exp.
Biol. Med. 65, 333 - 336.
41. CLARK, W., GIBBONS, R.J. and SKOBE, Z. (1974). Studies of the
attachment of *Streptococcus mutans* to apatite surfaces.
J. Dent. Res. 53, Special Issue, Abstract 749.
42. CLARKSON, B.H., SPIERS R.L. and SILVERSTONE, L.M. (1975).
Uptake of a number of metal ions after topical fluoride
application and their subsequent effects on fluoride
uptake from APF and on caries lesion formation.
Abstracts of papers presented at 21st ORCA Congress.
Caries Res. 9, 293 - 294.
43. COOLEY, W.E. (1961). Reactions of tin (II) and fluoride ions
with etched enamel. J. Dent. Res. 40, 1199 - 1210.
44. COULTER, W.A. and RUSSELL, C. (1974). Effect of molybdenum on
growth and metabolism of *Veillonella parvula* and
Streptococcus mutans. J. Dent. Res. 53, 1445 - 1449.
45. COUNCIL ON DENTAL THERAPEUTICS. (1975). Council classifies
fluoride mouthrinses. J. Am. Dent. Assoc. 91,
1250 - 1251.
46. CUTRESS, T.W. (1966). Effect of sodium fluoride solutions of
varying pH on solubility of bovine enamel. Arch.
Oral Biol. 11, 121 - 130.
47. CUTRESS, T.W. (1972). The inorganic composition and solubility
of dental enamel from several specified population
groups. Arch. Oral Biol. 17, 93 - 109.

48. CURZON, M.E.J., LOSEE, F.L. and MACALISTER, A.D. (1975). Trace elements in the enamel of teeth from New Zealand and U.S.A. N.Z. Dent. J. 71, 80 - 83.
49. DEAN, H.T., ARNOLD, F.A. Jr. and ELVOVE, E. (1942). Domestic water and dental caries. V. Additional studies of the relation of fluoride domestic waters to dental caries experience in 4,425 white children aged 12 to 14 years, of 13 cities in 4 states. Public Health Rep. 57, 1155 - 1179.
50. DEAN, H.T., JAY, P., ARNOLD, F.A. Jr. and ELVOVE, E. (1939). Domestic water and dental caries including certain epidemiological aspects of oral *L. acidophilus*. Public Health Rep. 54, 862 - 888.
51. DE MAN J.C., ROGOSA, M. and SHARPE, M.E. (1960). A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23, 130 - 133.
52. DE PAOLA, P.F., AASENDEN, R. and BRUDEVOLD, F. (1971). The use of topically applied acidulated phosphate-fluoride preceded by mild etching of the enamel. A one-year clinical trial. Arch. Oral Biol. 16, 1155 - 1163.
53. DE VRIES, W., KAPTEIJN, W.M.C., VAN DER BEEK, E.G. and STOUTHAMER, A.H. (1970). Molar growth yields and fermentation balances of *Lactobacillus casei* L3 in batch cultures and in continuous cultures. J. Gen. Microbiol. 63, 333 - 345.
54. DOGON, I.L., VAN LEEUWEN, M. and KIRKLIN, M. (1973). *In vivo* studies on the "sealing" of fluoride in teeth: Two year clinical results. Preprinted abstracts of papers presented at Internat. Ass. Dent. Res. 51st General Meeting. Abstract 240.
55. DOLAN, M.M., KAVANAGH, B.J. and YANKELL, S.L. (1972). Artificial plaque prevention with organic fluorides. J. Periodontol. 43, 561 - 563.
56. DREIZEN, S., LEVY, B.M., NIEDERMEIER, W. and GRIGGS, J.H. (1970). Comparative concentrations of selected trace metals in human and marmoset saliva. Arch. Oral Biol. 15, 179 - 188.
57. DUANY, L.F., ZINNER, D.D. and LANDY, J.J. (1971). Bone loss, and caries in rats infected with human streptococci. J. Dent. Res. 50, 460 - 465.
58. DUCKWORTH, R. (1968). Fluoride dentrifices. A review of clinical trials in the United Kingdom. Br. Dent. J. 124, 505 - 509.

59. EDGAR, W.M. and JENKINS, G.N. (1974). Solubility - reducing agents in honey and partly-refined crystalline sugar. Br. Dent. J. 136, 7 - 14.
60. EMILSON, C.G. and KRASSE, B. (1972). The effect of iron salts on experimental dental caries in the hamster. Arch. Oral Biol. 17, 1439 - 1443.
61. ENGLANDER, H.R. KEYES, P.H., GESTWICKI, M. and SULTZ, H.A. (1967). Clinical anticaries effect of repeated sodium fluoride applications by mouthpieces. J. Am. Dent. Assoc. 75, 638 - 644.
62. ERICSON, Th., SANDHAM, J. and MAGNUSSON, I. (1975). Sedimentation method for studies of adsorption of micro-organisms onto apatite surfaces *in vitro*. Caries. Res. 9, 325 - 332.
63. EVENSON, M.A. (1970). Analytical procedures and instrumentation. In Fundamentals of Clinical Chemistry. pp. 77 - 126. Edited by N.W. Tietz. Philadelphia: W.B. Saunders.
64. FEATHERSTONE, J.D.B. (1976). Surface layer phenomena during artificial carious lesion formation in human enamel. Preprinted abstracts of papers presented at Internat. Ass. Dent. Res. (Aust. and N.Z. Division) 16th Annual Meeting. Abstract 26.
65. FEAGIN, F.F., JEANSONNE, B.G. and McCAGHREN, A. (1972). Kinetic calcium, phosphate, and fluoride dissolution after topical fluoride application to intact enamel surfaces. J. Dent. Res. 51, 1457 - 1463.
66. FJAESTAD-SEGER, M., NORSTEDT-LARSSON, K. and MORELL, P. (1961). Försök med enkla metoder för klinisk fluoapplikation. Sv. Tandl. förb. Tidning. 53, 169. Cited from Hals, E. (1961). Review of clinical and *in vitro* research with zirconium and ferrum fluorides. In Caries Symposium Zürich. pp. 165 - 171. Edited by H.R. Mühlemann and K.G. König. Berne: Hans Huber.
67. FORBES, J.C. and SMITH, J.D. (1952). Studies on the effect of metallic salts on acid production in saliva. J. Dent. Res. 31, 129 - 131.
68. FRANCIS, M.D., GRAY, J.A. and GRIEBSTEIN, W.J. (1968). The formation and influence of surface phases on calcium phosphate solids. In Advances in Oral Biology. Vol. 3. pp. 83 - 120. Edited by P.H. Staple. New York: Academic Press.

69. FRANT, M.S. and ROSS, J.W. Jr. (1966). Electrode for sensing fluoride ion activity in solution. Science 154, 1553 - 1555.
70. FRIEDEN, E. (1972). The chemical elements of life. Sci. Am. 227, 52 - 60.
71. GALLILY, I. and FRIEDLANDER, S.K. (1965). Kink poisons and the reduction of dissolution rate at crystal-liquid interfaces. J. Chem. Phys. 42, 1503 - 1511.
72. GARVAN, R.L. (1964). Metal chelates of ethylenediaminetetraacetic acid and related substances. In Chelating Agents and Metal Chelates. pp. 283 - 333. Edited by F.P. Dwyer and D.P. Mellor. New York: Academic Press.
73. GEDDES, D.A.M. (1975). Acids produced by human dental plaque metabolism *in situ*. Caries Res. 9, 98 - 109.
74. GERDIN, P.O. and TORELL, P. (1976). Al, Fe and Mn ions in fluoride solutions for topical applications (Abstract) Caries Res. 10, 143.
75. GEROULD, C.H. (1945). Electron microscope study of the mechanism of fluorine deposition in teeth. J. Dent. Res. 24, 223 - 233.
76. GIBBONS, R.J. and BANGHART, S.B. (1967). Synthesis of extra-cellular dextran by cariogenic bacteria and its presence in human dental plaque. Arch. Oral Biol. 12, 11 - 24.
77. GIBBONS, R.J. and VAN HOUTE, J. (1973). On the formation of dental plaque. J. Periodontol. 44, 347 - 360.
78. GIBBONS, R.J. DE PAOLA, P.F., SPINELL, D.M. and SKOBE, Z. (1974). Interdental localization of *Streptococcus mutans* as related to dental caries experience. Infect. Immun. 9, 481 - 488.
79. GLANTZ, P-O. (1969). On wettability and adhesiveness. Odontol. Revy 20, Supplement 17.
80. GLASS, R.L., ROTHMAN, K.J., ESPINAL, F., VÉLEZ, H. and SMITH, N.J. (1973). The prevalence of human dental caries and water-borne trace metals. Arch. Oral Biol. 18, 1099 - 1104.
81. GRAY, J.A. (1962). Kinetics of the dissolution of human dental enamel in acid. J. Dent. Res. 41, 633 - 645.
82. GRAY, J.A. (1965). Acid dissolution rate of sound and white-spot enamel treated with tin (II) and fluoride compounds. J. Dent. Res. 44, 493 - 501.

83. GRAY, J.A. and FRANCIS, M.D. (1963). Physical chemistry of enamel dissolution. In Mechanisms of Hard Tissue Destruction. pp. 213 - 260. Edited by R.F. Sognnaes. Washington, D.C.: American Association for the Advancement of Science.
84. GRAY, J.A., SCHWEIZER, H.C., ROSEVEAR, F.B. and BROGE, R.W. (1958). Electron microscopic observations of the differences in the effects of stannous fluoride and sodium fluoride on dental enamel. J. Dent. Res. 37, 638 - 648.
85. GREENBERG, J., TURESKY, S.S. and WARNER, V.D. (1976). Effect of metal salts in prolonging antibacterial activity of teeth treated with 8-hydroxyquinoline. J. Periodontol. 47, 664 - 666.
86. GRØN, P. (1977). Chemistry of topical fluorides. Caries Res. 11, Supplement 1, 172 - 204.
87. GUGGENHEIM, B. (1970). Enzymatic hydrolysis and structure of water-insoluble glucan produced by glucosyltransferases from a strain of *Streptococcus mutans*. Helv. Odontol. Acta 14, Supplement V, 89 - 108.
88. GUGGENHEIM, B. and SCHROEDER, H.E. (1967). Biochemical and morphological aspects of extracellular polysaccharides produced by cariogenic streptococci. Helv. Odontol. Acta 11, 131 - 152.
89. GUEST, H.L. and SALLE, A.J. (1942). Effect of oxidation-reduction on germicidal efficiency of some metallic salts. Proc. Soc. Exp. Biol. Med. 51, 272 - 273.
90. GWINNETT, A.J., BUONOCORE, M.G. and SHEYKHOLESLAM, Z. (1972). Effect of fluoride on etched human and bovine tooth enamel surfaces as demonstrated by scanning electron microscopy. Arch. Oral Biol. 17, 271 - 278.
91. HALSE, A. (1974). Elemental composition of the superficial layer of rat incisor enamel. Calcif. Tiss. Res. 16, 139 - 144.
92. HAMILTON, I.R. (1977). Effects of fluoride on enzymatic regulation of bacterial carbohydrate metabolism. Caries Res. 11, Supplement I, 262 - 291.
93. HANDELMAN, S.L. and KREINCES, G.H. (1973). Effect of phosphate and pH on *Streptococcus mutans* acid production and growth. J. Dent. Res. 52, 651 - 657.

94. HARDIE, J.M. and BOWDEN, G.H. (1975). Bacterial flora of dental plaque. Br. Med. Bull. 31, 131 - 135.
95. HAYAKAWA, M., AI, S. and ISHIKAWA, T. (1964). A study of the 'Ohaguro' stain (part 6). Presented at Toshi - Gakkai. Cited from Ai, S., Ishikawa, T. and Seino, A. (1965). 'Ohaguro' traditional tooth staining custom in Japan. Int. Dent. J. 15, 426 - 441.
96. HEHRE, E.J. and NEILL, J.M. (1946). Formation of serologically reactive dextrans by streptococci from subacute bacterial endocarditis. J. Exp. Med. 83, 147 - 162.
97. HEIFETZ, S.B. and HOROWITZ, H.S. (1972). Fluoride dentifrices. In Fluorides and Dental Caries. pp 22 - 33. Edited by E. Newbrun. Springfield: Charles C. Thomas.
98. HENDERSHOT, L.C. and FORSAITH, J. (1958). Effect of various metal salts of ethylenediamine tetraacetic acid on dental caries in the rat (Abstract). J. Dent. Res. 37, 32 - 33.
99. HENRIKSON, M., OLDÉN, I. and TORELL, P. (1958). Ett års erfarenhet av järnfluoridpensling. Göteborgs Tandl. Sällskaps Årsbok. Cited from Hals, E. (1961). Review of clinical and *in vitro* research with zirconium and ferrum fluorides. In Caries Symposium Zürich. pp. 165 - 171. Edited by H.R. Mühlemann and K.G. König. Berne: Hans Huber.
100. HERMANN, U. and MÜHLEMANN, H.R. (1958). Inhibition of salivary respiration and glycolysis by an organic fluoride. Helv. Odontol. Acta 2, 28 - 33.
101. HIGUCHI, W.I. (1974). Kinetic factors in the tooth - plaque - saliva model systems. J. Dent. Res. 53, Supplement to No. 2, 236 - 238.
102. HILLMAN, J.D., VAN HOUTE, J. and GIBBONS, R.J. (1970). Sorption of bacteria to human enamel powder. Arch. Oral Biol. 15, 899 - 903.
103. HOLLY, F.J. and GRAY, J.A. (1968). Mechanism for incipient carious lesion growth utilizing a physical model based on diffusion concepts. Arch. Oral Biol. 13, 319 - 334.
104. HOROWITZ, H.S. and HEIFETZ, S.B. (1972). The current status of topical fluorides in preventive dentistry. In Fluorides and Dental Caries. pp. 34 - 59. Edited by E. Newbrun. Springfield: Charles C. Thomas.

105. HOWELL, A. Jr. and JORDAN, H.Y. (1967). Production of an extracellular levan by *Odontomyces viscosus*. Arch. Oral Biol. 12, 571 - 573.
106. IKEDA, T., SANDHAM, H.J. and BRADLEY, E.L. Jr. (1973). Changes in *Streptococcus mutans* and lactobacilli in plaque in relation to the initiation of dental caries in negro children. Arch. Oral Biol. 18, 555 - 566.
107. ISHIKAWA, T., SEINO, A., SEKIMACHI, H., TAMURA, H. and AI, S. (1962). A study of the 'Ohaguro' stain (part 3) (part 4). Presented at Toshi-Gakkai. Cited from Ai, S., Ishikawa, T. and Seino, A. (1965). 'Ohaguro' traditional tooth staining custom in Japan. Int. Dent. J. 15, 426 - 441.
108. ISHIKAWA, T., SEINO, A., TAMURA, H. and AI, S. (1963). A study of the 'Ohaguro' stain. Presented at Japan Res. Soc. Dent. Materials and Appliances. Cited from Ai, S., Ishikawa, T. and Seino, A. (1965). 'Ohaguro' traditional tooth staining custom in Japan. Int. Dent. J. 15, 426 - 441.
109. JACQUES, N.A. (1976). Respiratory regulation of cyclopropane fatty acid synthesis in *Pseudomonas denitrificans*. Ph.D. thesis, University of Sydney.
110. JEANSONNE, B.G. and FEAGIN, F.F. (1972). Effects of various topical fluorides on subsequent mineralization and dissolution of enamel. J. Dent. Res. 51, 767 - 772.
111. JENKINS, G.N. (1959). The effect of pH on the fluoride inhibition of salivary acid production. Arch. Oral Biol. 1, 33 - 41.
112. JENKINS, G.N. (1972). Current concepts concerning the development of dental caries. Int. Dent. J. 22, 350 - 362.
113. JORDAN, H.V. and KEYES, P.H. (1966). *In vitro* methods for the study of plaque formation and carious lesions. Arch. Oral Biol. 11, 793 - 801.
114. JORDAN, H.V., KEYES, P.H. and BELLACK, S. (1972). Periodontal lesions in hamsters and gnotobiotic rats infected with actinomyces of human origin. J. Periodontol. Res. 7, 21 - 28.
115. KAVANAGH, B.J., MURPHY, C.V., DOLAN, M.M. and YANKELL, S.L. (1974). *In vitro* plaque prevention with fluorides: comparison of teeth with nichrome wires. J. Periodontol. 45, 314 - 315.

116. KEYES, P.H. and ENGLANDER, H.R. (1975). Fluoride therapy in the treatment of dentomicrobial plaque diseases. J. Am. Soc. Prev. Dent. 5, 17 - 26.
117. KIRKEGAARD, E. (1977). *In vitro* fluoride uptake in human dental enamel from various fluoride solutions. Caries Res. 11, 16 - 23.
118. KIRKEGAARD, E., MØLLER, I.J. and SKOV JENSEN, E. (1976). A method for *in vitro* studies on fluoride uptake in enamel using single tooth surfaces. Caries Res. 10, 370 - 378.
119. KOCH, G. and PETERSSON, L.G. (1972). Fluoride content of enamel surface treated with a varnish containing sodium fluoride. Odontol. Revy 23, 437 - 446.
120. KOCHAVI, D., GEDALIA, I. and ANAISE, J. (1975). Effect of conditioning with fluoride and phosphoric acid on enamel surfaces as evaluated by scanning electron microscopy and fluoride incorporation. J. Dent. Res. 54, 304 - 309.
121. KOULOURIDES, T. (1968). Experimental changes of enamel mineral density. In Art and Science of Dental Caries Research. pp. 355 - 378. Edited by R.S. Harris. New York: Academic Press.
122. KRICHEVSKY, M.I., HOWELL, A. Jr. and LIM, S. (1969). Levan formation by *Odontomyces viscosus*. J. Dent. Res. 48, 938 - 942.
123. KUYPER, A.C. and KUTNERIAN, K. (1962). Mechanism of incorporation of fluoride into bone salt. J. Dent. Res. 41, 345 - 350.
124. LARSON, R.H., MELLBERG, J.R., ENGLANDER, H.R. and SENNING, R. (1976). Caries inhibition in the rat by water-borne and enamel-bound fluoride. Caries Res. 10, 321 - 331.
125. LEUSSING, D.L. and KOLTHOFF, I.M. (1953). The solubility product of ferrous hydroxide and the ionization of the aquo-ferrous ion. J. Am. Chem. Soc. 75, 2476 - 2479.
126. LEVICH, V.G. (1942). Acta Physicochim. U.R.S.S. 17, 257. Cited from Riddiford, A.C. (1966). The rotating disk system. In Advances in Electrochemistry and Electrochemical Engineering. Vol. 4. pp. 47 - 116. Edited by P. Delahay. New York: Wiley - Interscience.

127. LILJEMARK, W.F. and SCHAUER, S.V. (1975). Studies on the bacterial components which bind *Streptococcus sanguis* and *Streptococcus mutans* to hydroxyapatite. Arch. Oral Biol. 20, 609 - 615.
128. LINGE, H.G. and NANCOLLAS, G.H. (1973). A rotating disc study of the dissolution of dental enamel. Calcif. Tiss. Res. 12, 193 - 208.
129. LOESCHE, W.J., MURRAY, R.J. and MELLBERG, J.R. (1973). The effect of topical acidulated fluoride on percentages of *Streptococcus mutans* and *Streptococcus sanguis* in interproximal plaque samples. Caries Res. 7, 283 - 296.
130. LOESCHE, W.J., SYED, S.A., MURRAY, R.J. and MELLBERG, J.R. (1975). Effect of topical acidulated phosphate fluoride on percentage of *Streptococcus mutans* and *Streptococcus sanguis* in plaque. Caries Res. 9, 139 - 155.
131. LOESCHE, W.J., ROWAN, J., STRAFFON, L.H. and LOOS, P.J. (1975). Association of *Streptococcus mutans* with human dental decay. Infect. Immun. 11, 1252 - 1260.
132. LOSEE, F.L. and ADKINS, B.L. (1969). A study of the mineral environment of caries-resistant navy recruits. Caries Res. 3, 23 - 31.
133. LOSEE, F.L., CURZON, M.E.J. and LITTLE, M.F. (1974). Trace element concentrations in human enamel. Arch. Oral Biol. 19, 467 - 470.
134. LOSEE, F.L., CUTRESS, T.W. and BROWN, R. (1974). Natural elements of the periodic table in human dental enamel. Caries Res. 8, 123 - 134.
135. LUECK, C.H. and BOLTZ, D.F. (1956). Spectrophotometric study of modified heteropoly blue method for phosphorus. Analyt. Chem. 28, 1168 - 1171.
136. LUOMA, H. (1972). The effects of chlorhexidine and fluoride combinations on the potassium, sodium and phosphorus content and acid production of cariogenic streptococci. Arch. Oral Biol. 17, 1431 - 1437.
137. LUOMA, H. and TUOMPO, H. (1975). The relationship between sugar metabolism and the potassium translocation by caries-inducing streptococci and the inhibitory role of fluoride. Arch. Oral Biol. 20, 749 - 755.

138. LYNCH, S.R., LIPSCHITZ, D.A., BOTHWELL, T.H. and CHARLTON, R.W. (1974). Iron and the reticulo-endothelial system. In Iron in Biochemistry and Medicine, pp. 563 - 587. Edited by A. Jacobs and M. Worwood. London: Academic Press.
139. McCABE, R.M., KEYES, P.H. and HOWELL, A. Jr. (1967). An *in vitro* method for assessing the plaque forming ability of oral bacteria. Arch. Oral Biol. 12, 1653 - 1656.
140. McCANN, H.G. (1969). The effect of fluoride complex formation on fluoride uptake and retention in human enamel. Arch. Oral Biol. 14, 521 - 531.
141. McClure, F.J. (1948). Observations on induced caries in rats. VI. Summary results of various modifications of food and drinking water. J. Dent. Res. 27, 34 - 40.
142. McLUNDIE, A.C. (1971). Studies on the influence of certain ions on enamel solubility *in vitro*. In Tooth Enamel II. pp. 149 - 153. Edited by R.W. Fearnhead and M.V. Stack. Bristol: John Wright.
143. MANLY, R.S. and BIBBY, B.G. (1949). Substances capable of decreasing the acid solubility of tooth enamel. J. Dent. Res. 28, 160 - 171.
144. MANLY, R.S. and HARRINGTON, D.P. (1968). Release of fluoride to enamel by an adhesive bandage. J. Am. Coll. Dent. 35, 34 - 44.
145. MARGALIT, D. and GEDALIA, I. (1969). Release of fluoride into saliva after topical fluoride application. J. Dent. Res. 48, 92 - 96.
146. MARSHALL, K.C., STOUT, R. and MITCHELL, R. (1971). Mechanism of the initial events in the sorption of marine bacteria to surfaces. J. Gen. Microbiol. 68, 337 - 348.
147. MELLBERG, J.R. and LOERTSCHER, K.L. (1972). Fluoride acquisition *in vitro* by sound tooth enamel from sodium fluoride - and ammonium silicofluoride - phosphate solutions. Arch. Oral Biol. 17, 1107 - 1116.
148. MELLBERG, J.R. and LOERTSCHER, K.L. (1974). Comparison of *in vitro* fluoride uptake by human and bovine enamel from acidulated phosphate - fluoride solutions. J. Dent. Res. 53, 64 - 67.

149. MELLBERG, J.R. and SINGER, L. (1977). Discussion paper in Cariostatic Mechanisms of Fluorides. pp. 101 - 115. Edited by W.E. Brown and K.G. König. Caries Res. 11, Supplement 1.
150. MELLBERG, J.R., LAAKSO, P.V. and NICHOLSON, C.R. (1966). The acquisition and loss of fluoride by topically fluoridated human tooth enamel. Arch. Oral Biol. 11, 1213 - 1220.
151. MELVILLE, T.H. and RUSSELL, C. (1975). Microbiology for Dental Students. p. 87. London: William Heinemann Medical Books.
152. MEYER, J.L. and NANCOLLAS, G.H. (1972). Effect of stannous and fluoride ions on the rate of crystal growth of hydroxyapatite. J. Dent. Res. 51, 1443 - 1450.
153. MILLER, C.H. and KLEINMAN, J.L. (1974). Effect of microbial interactions on *in vitro* plaque formation by *Streptococcus mutans*. J. Dent. Res. 53, 427 - 434.
154. MÖRCH, T. (1961). The acid potentiality of carbohydrates. An investigation of some common dietary components in Norway. Acta Odontol. Scand. 19, 355 - 385.
155. MORENO, E.C. and ZAHRADNIK, R.T. (1974). Chemistry of enamel subsurface demineralization *in vitro*. J. Dent. Res. 53, Supplement to No. 2, 226 - 235.
156. MÜHLEMANN, H.R. and REGOLATI, B. (1974). Aluminium - a cariostatic agent? In Forum Medici. pp. 51 - 58. Edited by T.M. Marthaler. Nyon: Zyma.
157. MUHLER, J.C. (1957). The effect of different fluorides on the solubility of intact dental enamel surfaces. J. Dent. Res. 36, 889 - 894.
158. MUHLER, J.C. (1958). Effects of fluoride and nonfluoride containing tin salts on the dental caries experience in children. J. Dent. Res. 37, 422 - 426.
159. MUHLER, J.C., BOYD, T.M. and VAN HUYSSEN, G. (1950). Effect of fluorides and other compounds on the solubility of enamel, dentine and tricalcium phosphate in dilute acids. J. Dent. Res. 29, 182 - 193.
160. MUNDORFF, S.A., LITTLE, M.F. and BIBBY, B.G. (1972). Enamel dissolution: II. Action of titanium tetrafluoride. J. Dent. Res. 51, 1567 - 1571.

161. MURRAY, J.J. (1976). Fluorides in Caries Prevention. pp. 99 - 113. Bristol: John Wright.
162. NAPPER, D.H. and SMYTHE, B.M. (1966). The dissolution kinetics of hydroxyapatite in the presence of kink poisons. J. Dent. Res. 45, 1775 - 1783.
163. NAVIA, J.M. (1970). Effect of minerals on dental caries. In Dietary Chemicals vs. Dental Caries. Advances in Chemistry Series 94, pp. 123 - 160. Edited by R.F. Gould. Washington: American Chemical Society.
164. NAYLOR, M.N., WILSON, R.F. and MELVILLE, M.R.B. (1970). Mono- and di-saccharide solutions and the formation of plaque *in vitro*. In Dental Plaque. pp. 41 - 47. Edited by W.D. McHugh. Edinburgh: E. and S. Livingstone.
165. NEFF, D. (1967). Acid production from different carbohydrate sources in human plaque *in situ*. Caries Res. 1, 78 - 87.
166. NICHOLLS, D. (1973). The Chemistry of Iron, Cobalt and Nickel. p. 1007. Oxford: Pergamon.
167. NIVEN, C.F. Jr., KIZIUTA, Z. and WHITE, J.C. (1946). Synthesis of a polysaccharide from sucrose by *Streptococcus S.B.E.* J. Bacteriol. 51, 711 - 716.
168. OLSSON, J. and KRASSE, B. (1976). A method for studying adherence of oral streptococci to solid surfaces. Scand. J. Dent. Res. 84, 20 - 28.
169. OLSSON, J., GLANTZ, P-O. and KRASSE, B. (1976). Surface potential and adherence of oral streptococci to solid surfaces. Scand. J. Dent. Res. 84, 240 - 242.
170. ØRSTAVIK, D., KRAUS, F.W. and HENSHAW, L.C. (1974). *In vitro* attachment of streptococci to the tooth surface. Infect. Immun. 9, 794 - 800.
171. PETERSSON, L.G. (1975). *In vivo* fluorine uptake in human enamel following treatment with a varnish containing sodium fluoride. Odontol. Revy 26, 253 - 266.
172. PUTTNAM, N.A., BRADSHAW, F. and PLATT, P. (1966). X-ray spectroscopic determination of the constituent elements of dental plaque. In Advances in Fluorine Research and Dental Caries Prevention. Vol. 4. pp. 157 - 162. Edited by P.M. James, K.G. König and H.R. Held. Oxford: Pergamon.

173. RAE, J.J. and CLEGG, C.T. (1948). The effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel and whole teeth in lactic acid. J. Dent. Res. 27, 52 - 53.
174. RANKE, A and RANKE, B. (1968). The fermentation of glucose and sucrose by strains of α -haemolytic streptococci from human dental plaque. Caries Res. 2, 193 - 200.
175. REGOLATI, B., SCHAIT, A., SCHMID, R. and MÜHLEMANN, H.R. (1974). The effect of titanium, aluminium and fluoride on rat caries. Helv. Odontol. Acta, 18, 92 - 96.
176. RICHARDSON, B. (1967). Fixation of topically applied fluoride in enamel. J. Dent. Res. 46, Supplement to No. 1, 87 - 91.
177. RIDDIFORD, A.C. (1966). The rotating disk system. In Advances in Electrochemistry and Electrochemical Engineering. Vol. 4. pp. 47 - 116. Edited by P. Delahay. New York: Wiley-Interscience.
178. RINDERER, L., SCHAIT, A. and MÜHLEMANN, H.R. (1965). Loss of fluoride from dental enamel after topical fluoridation. Helv. Odontol. Acta 9, 148 - 150.
179. ROBERTS, M.H. and RAHN, O. (1946). Antisepsis and ionization of sodium fluoride. J. Bacteriol. 52, 612 - 613.
180. ROBRISH, S.A., REID, W. and KRICHEVSKY, M.I. (1972). Distribution of enzymes forming polysaccharide from sucrose and the composition of polysaccharide synthesised by *Streptococcus mutans*. Appl. Microbiol. 24, 184 - 190.
181. ROSAN, B. and HAMMOND, B.F. (1974). Extracellular polysaccharides of *Actinomyces viscosus*. Infect. Immun. 10, 304 - 308.
182. ROSEN, S., LENNY, W.S. and O'MALLEY, J.E. (1968). Dental Caries in rats inoculated with *Lactobacillus casei*. J. Dent. Res. 47, 358 - 363.
183. ROTHMAN, K.J., GLASS, R.L., ESPINAL, F. and VÉLEZ, H. (1972). Caries free teeth in the absence of fluoride ion. J. Public Health Dent. 32, 225 - 228.
184. SCALES, W.R., LONG, L.W. and EDWARDS, J.R. (1975). Purification and characterization of a glycosyltransferase complex from the culture broth of *Streptococcus mutans* FA1. Carbohydrate Res. 42, 325 - 338.

185. SCHAMSCHULA, R.G., AGUS, H., BLAINEY, B., CHARLTON, G. and DAVEY, B. (1975). Laboratory methods. Document 7. WHO Study of Dental Caries Etiology, Papua New Guinea. DNH/ETIOL/75.7. Geneva: WHO.
186. SCHNEIDER, Ph. and MÜHLEMANN, H.R. (1974). The antiglycolytic action of amine fluorides on dental plaque. Helv. Odontol. Acta 18, Supplement VIII, 63 - 70.
187. SEINO, A., SEKIMACHI, H., ISHIKAWA, T., TAMURA, H. and AI, S. (1961). A study of the 'Ohaguro' stain. Japan Res. Soc. Dental Materials and Appliances, 6, 119. Cited from Ai, S., Ishikawa, T. and Seino, A. (1965). 'Ohaguro' traditional tooth staining custom in Japan. Int. Dent. J. 15, 426 - 441.
188. SELVIG, K.A. and HALSE, A. (1975). The ultrastructural localization of iron in rat incisor enamel. Scand. J. Dent. Res. 83, 88 - 95.
189. SHERN, R., SWING, K.W. and CRAWFORD, J.J. (1970). Prevention of plaque formation by organic fluorides. J. Oral Med. 25, 93 - 97.
190. SHIOTA, T. (1956). Effect of sodium fluoride on oral lactobacilli isolated from the rat. J. Dent. Res. 35, 939 - 946.
191. SHKLAIR, I.L., KEENE, H.J. and CULLEN, P. (1974). The distribution of *Streptococcus mutans* on the teeth of two groups of naval recruits. Arch. Oral Biol. 19, 199 - 202.
192. SHRESTHA, B.M., MUNDORFF, S.A. and BIBBY, B.G. (1972). Enamel dissolution: I. Effect of various agents and titanium tetrafluoride. J. Dent. Res. 51, 1561 - 1566.
193. SIMS, W. (1966). The effect of sodium fluoride on the rate of acid production of surface aggregates of streptococci and lactobacilli. J. Dent. Res. 45, 915 - 920.
194. SILVERSTONE, L.M. (1977). Remineralization phenomena. Caries Res. 11, Supplement 1, 59 - 84.
195. SKINNER, A. and NAYLOR, M.N. (1972). Influence of sugar type on the pattern of acid production by *Streptococcus mutans*. J. Dent. Res. 51, 1022 - 1024.
196. SOKATCH, J.R. (1969). Bacterial Physiology and Metabolism. pp. 72 - 111. London: Academic Press.

197. SÖREMARK, R. and LUNDBERG, M. (1964). Gamma-ray spectrometric analysis of the concentrations of Cr, Ag, Fe, Co, Rb and Pt in normal human enamel. Acta Odontol. Scand. 22, 255 - 259.
198. SPIERS, R.L., SPINELLI, M. and BRUDEVOLD, F. (1963). Solution rate of hydroxyapatite in acetate buffer containing low concentrations of foreign ions. J. Dent. Res. 42, 811 - 820.
199. SPIRO, T.G. and SALTMAN, P. (1974). Inorganic chemistry. In Iron in Biochemistry and Medicine. pp. 1 - 28. Edited by A. Jacobs and M. Worwood. London: Academic Press.
200. SPÖRRI, St., BELSER, U. and MÜHLEMANN, H.R. (1975). The precision of three enamel biopsy methods for fluoride determination. Helv. Odontol. Acta 19, 65 - 68.
201. STEPHAN, R.M. (1949). *In vitro* studies of the effects of some chemical substances on the growth of oral microorganisms and on their ability to dissolve tooth salts (Abstract). J. Dent. Res. 28, 652.
202. STEPHAN, R.M. and HEMMENS, E.S. (1947). Studies in changes in pH produced by pure cultures of oral microorganisms. II. Comparison of different microorganisms and different substrates. J. Dent. Res. 26, 25 - 33.
203. STOOKEY, G.K. (1970). Fluoride therapy. In Improving Dental Practice Through Preventive Measures. pp. 92 - 156. Edited by J.L. Bernier and J.C. Muhler. St. Louis: C.V. Mosby.
204. STOOKEY, G.K., HUDSON, J.T. and MUHLER, J.C. (1967). Laboratory studies concerning the effectiveness and safety of various fluoride and fluoride-phosphate systems. J. Dent. Res. 46, 503 - 513.
205. SUDO, S.Z., GUTFLEISCH, J.R., SCHOTZKO, N.K. and FOLKE, L.E.A. (1975). Model system for studying colonization and growth of bacteria on a hydroxyapatite surface. Infect. Immun. 12, 576 - 585.
206. SUZUKI, M., KOBAYASHI, T., ISHIKAWA, T., TAMURA, H. and AI, S. (1964). A study of the 'Ohaguro' stain (part 5). Presented at Toshi-Gakkai. Cited from Ai, S., Ishikawa, T. and Seino, A. (1965) 'Ohaguro' traditional tooth staining custom in Japan. Int. Dent. J. 15, 426 - 441.

207. SYED, S.A., LOESCHE, W.J., PAPE, H.L. Jr. and GRENIER, E. (1975). Predominant cultivable flora isolated from human root surface caries plaque. Infect. Immun. 11, 727 - 731.
208. TERLECKYJ, B., WILLETT, N.P. and SHOCKMAN, G.D. (1975). Growth of several cariogenic strains of oral streptococci in a chemically defined medium. Infect. Immun. 11, 649 - 655.
209. TIMBERLAKE, C.F. (1964). Iron-maleate and iron-citrate complexes. J. Chem. Soc. 5078 - 5085.
210. TORELL, P. (1954). Effect on enamel of iron salts of strong mineral acids. Odontol. Tidskr. 62, 247 - 261.
211. TORELL, P. (1954). Influence of saliva on reactions between iron and dental enamel. Odontol. Tidskr. 62, 335 - 347.
212. TORELL, P. (1954). Influence of sugars on reactions between divalent iron and dental enamel. Odontol. Tidskr. 62, 403 - 407.
213. TORELL, P. (1954). Influence of ferric thiocyanate complexes in reactions between iron and dental enamel. Odontol. Tidskr. 62, 409 - 418.
214. TORELL, P. (1954). Action of ferric-fluoride complex on dental enamel. Odontol. Tidskr. 62, 419 - 427.
215. TORELL, P. (1955). Presence of iron in saliva from homo. Odontol. Tidskr. 63, 7 - 10.
216. TORELL, P. (1955). Action of ferric-citrate complex on dental enamel. Odontol. Tidskr. 63, 11 - 17.
217. TORELL, P. (1955). Iron and dental hard tissues. Odontol. Tidskr. 63, 131 - 172.
218. TORELL, P. (1956). The possible caries activating effect of chemical reactions between sucrose and ferric iron. Odontol. Tidskr. 64, 165 - 183.
219. TORELL, P. (1956). The influence of fluoride ions on the fixation of phosphate ions to ferric-salivary precipitates. Odontol. Tidskr. 64, 184 - 188.
220. TORELL, P. (1956). The possibility of using iron fluorides in topical applications. Odontol. Tidskr. 64, 189 - 194.
221. TORELL, P. (1957). Determination of iron in dental enamel. Odontol. Tidskr. 65, 20 - 23.

222. TORELL, P. (1960). Munnskjölning med natriumfluorid - och järnfluoridlösningar. Ett års erfarenhet. Göteborgs Tandl.-Sällskaps Årskbok. Cited from Hals, E. (1961). Review of clinical and *in vitro* research with zirconium and ferrum fluorides. In Caries Symposium Zürich. pp. 165 - 171. Edited by H.R. Mühlemann and K.G. König. Berne: Hans Huber.
223. TORELL, P., MÖRCH, T. and HALS, E. (1959). Effect of topically applied agents on enamel. IV. Experiments *in vitro* with iron fluoride solutions. Acta Odontol. Scand. 17, 267 - 276.
224. TURESKY, S., GLICKMAN, I. and SANDBERG, R. (1972). *In vitro* chemical inhibition of plaque formation. J. Periodontol. 43, 263 - 269.
225. VAN DER HOEVEN, J.S. (1974). A slime-producing microorganism in dental plaque of rats, selected by glucose feeding. Caries Res. 8, 193 - 210.
226. VAN DER HOEVEN, J.S. (1976). Carbohydrate metabolism of *Streptococcus mutans* in dental plaque in gnotobiotic rats. Arch. Oral Biol. 21, 431 - 433.
227. VAN DER HOEVEN, J.S., MIKX, F.H.M., KÖNIG, K.G. and PLASSCHAERT, A.J.M. (1974). Plaque formation and dental caries in gnotobiotic and SPF Osborne-Mendel rats associated with *Actinomyces viscosus*. Caries Res. 8, 211 - 223.
228. VOLKER, J.F. (1939). Effect of fluorine on solubility of enamel and dentin. Proc. Soc. Exp. Biol. Med. 42, 725 - 727.
229. WARNER, V.D., WARNER, A.M., MIRTH, D.B., SANE, J.N., TURESKY, S.S. and SOLOWAY, B. (1976). A physicochemical approach to the study of amines as antiplaque agents. J. Dent. Res. 55, 130 - 134.
230. WEI, S.H.Y. (1974). Scanning electron microscope study of stannous fluoride-treated enamel surfaces. J. Dent. Res. 53, 57 - 63.
231. WEI, S.H.Y. (1975). Effect of topical fluoride solutions on the enamel surface as studied by scanning electron microscopy. Caries Res. 9, 445 - 458.
232. WEI, S.H.Y. and FORBES, W.C. (1974). Electron microprobe investigations of stannous fluoride reactions with enamel surfaces. J. Dent. Res. 52, 51 - 56.

233. WEINBERG, E.D. (1974). Iron and susceptibility to infectious disease. Science 184, 952 - 956.
234. WEISS, M.E. and BIBBY, B.G. (1966). Effects of milk on enamel solubility. Arch. Oral Biol. 11, 49 - 57.
235. WEISS, M.E. and BIBBY, B.G. (1966). Some protein effects on enamel solubility. Arch. Oral Biol. 11, 59 - 63.
236. WEST, E.S. (1963). Textbook of Biophysical Chemistry. p. 111. New York: Macmillan.
237. WHITE, A., HANDLER, P. and SMITH, E.L. (1968). Principles of Biochemistry p. 121. New York: McGraw-Hill.
238. WOOD, J.M. and CRITCHLEY, P. (1966). The extracellular polysaccharide produced from sucrose by a cariogenic streptococcus. Arch. Oral Biol. 11, 1039 - 1042.
239. WOODS, R.A. (1971). The short-term effect of topical fluoride applications on the concentration of *Streptococcus mutans* in dental plaque. Aust. Dent. J. 16, 152 - 155.
240. WOOLLEY, L.H. and RICKLES, N.H. (1971). Inhibition of acidogenesis in human dental plaque *in situ* following the use of topical sodium fluoride. Arch. Oral Biol. 16, 1187 - 1194.
241. WORWOOD, M. (1974). Iron and trace metals. In Iron in Biochemistry and Medicine pp. 335 - 367. Edited by A. Jacobs and M. Worwood. London: Academic Press.
242. WRIGHT, D.E. and JENKINS, G.N. (1954). The effect of fluoride on the acid production of saliva-glucose mixtures. Br. Dent. J. 96, 30 - 33.
243. WU, M-S., HIGUCHI, W.I., FOX, J.L. and FRIEDMAN, M. (1976). Kinetics and mechanism of hydroxyapatite crystal dissolution in weak acid buffers using the rotating disk method. J. Dent. Res. 55, 496 - 505.
244. WYNN, W., HALDI, J., LAW, M.L. and BENTLEY, K.D. (1958). Studies on the cariogenicity of the Emory and Harvard diets. (Abstract). J. Dent. Res. 37, 33.
245. YAMADA, T., HOJO, S., KOBAYASHI, Y., ASANO, Y., and ARAYA, S. (1970). Studies of the carbohydrate metabolism of cariogenic *Streptococcus mutans* strain PK-1. Arch. Oral Biol. 15, 1205 - 1217.
246. ZOBELL, C.E. (1943). The effect of solid surfaces upon bacterial activity. J. Bacteriol. 46, 39 - 56.

APPENDIX

CALCIUM AND PHOSPHORUS^a LOSS FROM TREATED ENAMEL DURING 5, 10, 15, 30, 60 AND 180 MINUTE IMMERSION PERIODS IN pH 4.5 LACTATE BUFFER (SECTION 2, EXPERIMENT 1)

A. Values for Enamel Treated with a Single Application of Test Compounds (One Stage Treatments)

Treatment	Time (min)					
	5	10	15	30	60	180
H ₂ O (Control)	4.02 (S.D. = 0.99) ^b (1.52) ^c	5.09 (S.D. = 1.10) (1.54)	7.89 (S.D. = 0.92) (1.55)	11.93 (S.D. = 1.22) (1.48)	15.69 (S.D. = 1.56) (1.52)	22.48 (S.D. = 2.07) (1.59)
1 mM FeCl ₂ · 4H ₂ O	3.41 (S.D. = 1.13) (1.39)	4.65 (S.D. = 1.20) (1.46)	6.43 (S.D. = 2.33) (1.56)	9.74 (S.D. = 2.17) (1.49)	13.83 (S.D. = 2.62) (1.41)	24.91 (S.D. = 3.41) (1.48)
10 mM FeCl ₂ · 4H ₂ O	1.77 (S.D. = 1.01) (1.62)	3.70 (S.D. = 0.84) (1.48)	6.25 (S.D. = 0.82) (1.45)	8.30 (S.D. = 1.54) (1.42)	10.77 (S.D. = 1.65) (1.44)	17.61 (S.D. = 1.53) (1.48)
100 mM FeCl ₂ · 4H ₂ O	1.79 (S.D. = 0.46) (1.44)	3.04 (S.D. = 0.79) (1.60)	4.52 (S.D. = 0.85) (1.56)	7.91 (S.D. = 0.76) (1.39)	12.58 (S.D. = 0.96) (1.46)	17.14 (S.D. = 1.24) (1.42)
1 mM FeCl ₃ · 6H ₂ O	0.71 (S.D. = 0.97) (1.56)	0.76 (S.D. = 0.62) (1.49)	2.92 (S.D. = 1.30) (1.40)	6.51 (S.D. = 1.00) (1.37)	11.63 (S.D. = 2.42) (1.46)	19.57 (S.D. = 3.38) (1.56)
10 mM FeCl ₃ · 6H ₂ O	0.66 (S.D. = 0.61) (1.47)	0.74 (S.D. = 0.82) (1.59)	1.48 (S.D. = 1.42) (1.36)	4.28 (S.D. = 2.49) (1.50)	10.07 (S.D. = 1.25) (1.47)	19.97 (S.D. = 3.78) (1.50)
100 mM FeCl ₃ · 6H ₂ O	1.18 (S.D. = 0.85) (1.38)	3.15 (S.D. = 0.99) (1.49)	3.50 (S.D. = 1.00) (1.42)	8.59 (S.D. = 1.76) (1.56)	12.31 (S.D. = 1.39) (1.39)	16.11 (S.D. = 4.34) (1.44)
15 mM FeF ₂	3.61 (S.D. = 1.29) (1.58)	4.75 (S.D. = 1.41) (1.40)	6.27 (S.D. = 1.27) (1.53)	8.82 (S.D. = 1.54) (1.36)	11.90 (S.D. = 1.26) (1.41)	17.36 (S.D. = 1.62) (1.49)

10 mM FeF_3	1.64 (S.D. = 0.79) (1.43)	3.55 (S.D. = 1.21) (1.47)	5.97 (S.D. = 1.56) (1.56)	9.11 (S.D. = 1.25) (1.49)	13.26 (S.D. = 1.10) (1.69)	17.40 (S.D. = 3.12) (1.50)
50 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ +190 mM NaF	1.06 (S.D. = 0.10) (1.39)	2.60 (S.D. = 0.60) (1.50)	4.39 (S.D. = 1.08) (1.68)	8.01 (S.D. = 0.70) (1.58)	10.29 (S.D. = 0.92) (1.46)	14.45 (S.D. = 0.70) (1.58)
APF	1.83 (S.D. = 0.62) (1.58)	3.29 (S.D. = 0.89) (1.50)	4.40 (S.D. = 0.69) (1.68)	6.56 (S.D. = 1.14) (1.58)	10.31 (S.D. = 1.78) (1.72)	14.35 (S.D. = 2.10) (1.66)
26 mM SnF_2	2.50 (S.D. = 0.94) (1.38)	3.63 (S.D. = 0.49) (1.53)	4.78 (S.D. = 1.45) (1.58)	8.23 (S.D. = 0.84) (1.55)	12.67 (S.D. = 0.42) (1.54)	22.18 (S.D. = 2.57) (1.45)
511 mM SnF_2	0.68 (S.D. = 0.48) (1.59)	2.36 (S.D. = 1.37) (1.45)	4.67 (S.D. = 0.42) (1.59)	7.39 (S.D. = 1.85) (1.42)	11.84 (S.D. = 1.52) (1.36)	19.53 (S.D. = 2.06) (1.53)
48 mM NaF	4.32 (S.D. = 1.35) (1.51)	5.79 (S.D. = 1.43) (1.67)	7.91 (S.D. = 1.33) (1.54)	12.14 (S.D. = 1.71) (1.45)	15.02 (S.D. = 2.84) (1.69)	20.34 (S.D. = 2.56) (1.50)
476 mM NaF	2.68 (S.D. = 0.21) (1.47)	4.52 (S.D. = 0.50) (1.53)	6.27 (S.D. = 0.84) (1.68)	10.58 (S.D. = 0.71) (1.51)	12.10 (S.D. = 1.49) (1.42)	18.20 (S.D. = 1.46) (1.41)

a Shown as Ca/P.(molar) ratios

b Each value represents mean of five specimens (S.D. = Standard deviation)

c Each value represents mean of two specimens

APPENDIX (cont.)

B. Values for Enamel Treated with Iron (II) Chloride or Iron (III) Chloride Prior to the Topical Application of APF (Two Stage Treatments)

Pretreatment Solution	Time (min)					
	5	10	15	30	60	180
Nil (APF alone)	1.83 (S.D. = 0.62) ^a (1.58) ^b	3.29 (S.D. = 0.89) (1.50)	4.40 (S.D. = 0.69) (1.68)	6.56 (S.D. = 1.14) (1.58)	10.31 (S.D. = 1.78) (1.72)	14.35 (S.D. = 2.10) (1.66)
1 mM FeCl ₂ · 4H ₂ O	1.79 (S.D. = 0.61) (1.90)	2.70 (S.D. = 0.67) (1.67)	4.14 (S.D. = 0.98) (1.68)	5.58 (S.D. = 1.00) (1.70)	8.76 (S.D. = 1.34) (1.77)	12.51 (S.D. = 3.06) (1.88)
10 mM FeCl ₂ · 4H ₂ O	1.35 (S.D. = 0.32) (1.97)	2.53 (S.D. = 0.33) (1.74)	4.03 (S.D. = 1.15) (1.60)	7.51 (S.D. = 1.90) (1.75)	11.04 (S.D. = 1.74) (1.67)	13.80 (S.D. = 1.48) (1.69)
100 mM FeCl ₂ · 4H ₂ O	1.42 (S.D. = 0.60) (2.27)	2.69 (S.D. = 0.52) (1.98)	3.35 (S.D. = 0.43) (1.72)	5.63 (S.D. = 0.64) (1.78)	9.06 (S.D. = 1.98) (1.77)	13.54 (S.D. = 2.30) (1.89)
1 mM FeCl ₃ · 6H ₂ O	1.44 (S.D. = 0.76) (2.22)	2.45 (S.D. = 0.60) (2.04)	3.19 (S.D. = 0.58) (1.56)	6.35 (S.D. = 1.01) (1.59)	9.54 (S.D. = 1.23) (1.59)	13.48 (S.D. = 0.90) (1.52)
10 mM FeCl ₃ · 6H ₂ O	0.78 (S.D. = 0.18) (3.52)	1.51 (S.D. = 0.22) (2.81)	2.48 (S.D. = 0.33) (2.05)	4.98 (S.D. = 0.71) (2.03)	8.25 (S.D. = 1.13) (1.66)	12.70 (S.D. = 1.84) (1.76)
100 mM FeCl ₃ · 6H ₂ O	0.74 (S.D. = 0.04) (4.79)	1.35 (S.D. = 0.14) (3.21)	2.13 (S.D. = 0.38) (2.99)	3.88 (S.D. = 0.94) (2.60)	6.59 (S.D. = 0.98) (2.21)	10.95 (S.D. = 2.04) (2.15)

a Each value represents mean of five specimens (S.D. = Standard deviation)

b Each value represents mean of two specimens

C. Values for Enamel Treated with Iron (II) Chloride or Iron (III) Chloride Prior to the Topical Application of 476 mM Sodium Fluoride (Two Stage Treatments)

Pretreatment Solution	Time (min)					
	5	10	15	30	60	180
Nil (476 mM NaF alone)	2.68 (S.D. = 0.21) ^a (1.47) ^b	4.52 (S.D. = 0.50) (1.53)	6.27 (S.D. = 0.84) (1.68)	10.58 (S.D. = 0.71) (1.51)	12.10 (S.D. = 1.49) (1.42)	18.20 (S.D. = 1.46) (1.41)
1 mM FeCl ₂ · 4H ₂ O	1.78 (S.D. = 0.72) (1.80)	3.55 (S.D. = 1.05) (1.76)	4.38 (S.D. = 1.36) (1.72)	7.56 (S.D. = 2.36) (1.71)	10.30 (S.D. = 2.29) (1.72)	16.65 (S.D. = 1.94) (1.69)
10 mM FeCl ₂ · 4H ₂ O	2.08 (S.D. = 0.46) (1.72)	3.33 (S.D. = 1.09) (1.62)	7.11 (S.D. = 1.44) (1.70)	10.11 (S.D. = 1.50) (1.67)	11.87 (S.D. = 3.07) (1.56)	19.82 (S.D. = 3.23) (1.58)
100 mM FeCl ₂ · 4H ₂ O	1.86 (S.D. = 0.48) (1.50)	3.90 (S.D. = 1.59) (1.47)	5.51 (S.D. = 1.17) (1.53)	8.32 (S.D. = 1.55) (1.65)	12.18 (S.D. = 1.84) (1.56)	17.28 (S.D. = 3.54) (1.46)
1 mM FeCl ₃ · 6H ₂ O	1.79 (S.D. = 0.67) (1.76)	2.97 (S.D. = 1.00) (1.61)	4.41 (S.D. = 0.83) (1.68)	7.43 (S.D. = 1.58) (1.72)	10.19 (S.D. = 1.92) (1.65)	16.48 (S.D. = 4.00) (1.46)
10 mM FeCl ₃ · 6H ₂ O	1.62 (S.D. = 0.72) (1.63)	2.72 (S.D. = 0.74) (1.71)	3.93 (S.D. = 1.19) (1.73)	6.99 (S.D. = 1.50) (1.64)	9.00 (S.D. = 0.88) (1.50)	15.17 (S.D. = 3.98) (1.50)
100 mM FeCl ₃ · 6H ₂ O	1.47 (S.D. = 0.95) (1.96)	2.63 (S.D. = 1.37) (1.80)	3.22 (S.D. = 1.27) (1.77)	5.93 (S.D. = 0.30) (1.82)	8.12 (S.D. = 1.06) (1.73)	13.12 (S.D. = 1.48) (1.73)

a Each value represents mean of five specimens (S.D. = Standard deviation)

b Each value represents mean of two specimens