The Bacterial Flora

OF THE

CHARLOTTESVILLE AND UNIVERSITY OF VIRGINIA

WATER SUPPLY.

A dissertation submitted to the Faculty of the University
of Virginia, April 28, 1901, for the degree
of Doctor of Philosophy.

BY
Dixon
LYMAN SKEEN, JR., M. D.

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Charlottesville and University of Virginia Water Supply.

Charlottesville and the University of Virginia have been supplied with water since 1885 from a reservoir located about five miles to the west of the city. A water shed of about 850 acres drains into a basin which is about 36 acres in area. Timber covers most of the region surrounding the reservoir, there being only a limited amount of cultivated soil, and one dwelling, which is some distance from the basin.

Finding the water supply inadequate for increasing demands, a pumping plant was established in 1897. The location of this plant is some distance below the reservoir and nearer to the city. The area of drainage for this source of supply is a region with two streams supplied by surface water. A large amount of cultivated soil and five or six dwellings are found along these streams. Two of the dwellings are located near the edges of streams.

This investigation was undertaken with the following objects:

I. A determination of the number of bacteria present, as indicated by samples taken at a number of points in the water system, using nutrient gelatin and agar under like conditions.

II. Observations as to the effects of ordinary seasonal changes and those of marked fluctuating weather upon the number of bacteria present.

III. A study of the most important characteristics of bacteria present in the water supply and the detection of those, if any, whose presence should cause doubts as to its safety.

IV. A consideration of the water supply in question from a sanitary standpoint in the light of the facts determined.

Samples of water were collected in small glass-stoppered bottles which had been washed in a mineral acid, rinsed in water, dried, and sterilized in the hot-air oven for two hours at 130°-150° C. Petrie dishes, after being properly cleaned, were dried and subjected to a dry heat of 130°-150° C. for two hours. After careful cleaning, pipettes were placed in the steam sterilizer for about twenty minutes.

At the reservoir and pumping station sampling bottles were opened and closed while entirely immersed in the water. Deep samples were obtained by lowering the bottle in a Lentz apparatus, which allows the partial removal of the stopper at any desired depth. Samples, when transported, were packed in ice soon after collection and kept at a low temperature until opened, which was in each case within two hours after being taken. After shaking well to stir up heavier solid matter, the necks of bottles were carefully cleaned before removal of stoppers. At the laboratory tap the water was allowed to run for ten minutes before collecting the sample to be examined.

To neutralize the constant acidity of the nutrient media used a four per cent. solution of sodium hydrate was employed. Litmus paper served as an indicator until a distinct alkaline reaction to the paper appeared. Phenolphthalein was then made use of because it is more delicate as an indicator than is ordinary litmus paper. In using phenolphthalein, about ten cubic centimeters of the medium was placed in a test tube and a few drops of the indicator added. Usually two tests, after the addition of the alkali to the whole mass and shaking each time, gave the slightly alkaline reaction desired.

Three tubes each of melted gelatin and agar were inoculated from each sample of water. To one tube of each medium .6 cc of water was added, and after shaking well, the contents poured into a sterilized Petrie dish; .3 cc of water was placed into two other tubes and treated the same as the above; .1 cc of water was added to each of the two remaining tubes; plating therefore gave a set of three plates for each of the two media. The use of the three quantities of water allows an average to be taken, and in case a very large number of colonies develop a more accurate count can be made where least water has been used. Previous bacteriological determinations of the water under examination showed the lack of necessity for dilution with sterilized water. In some cases tubes were kept after plating, and the number of colonies developing in the small amount of medium clinging to their sides counted. An average of two colonies to the cubic centimeter was obtained from the tubes so examined; this correction was therefore made in examinations. Average numbers from the three plates in a set are given. Fractions of one-half or less have been neglected; others are given as one.

In all cases sets of plates from each sample were placed in one vessel and given like conditions as to light and temperature. The chamber of the incubator was used to supply a dark place and to maintain a constant temperature of 18°-20° C. During warm weather, a small stream of tap water was run through the incubator, while in a very warm spell an ice coil was so arranged as to cool the tap water. The use of a gas flame was necessary to maintain the desired temperature when ice was thus employed.

Early liquefaction of gelatin after inoculation made it impossible in some cases to obtain an enumeration of all colonies that would have developed upon this medium.

The following table includes the number of colonies of bacteria to the cubic centimeter of water from the successive enumerations. The pumping station was not in use earlier than January, 1901, nor was it possible to obtain deep samples in all examinations at this place and the reservoir. In October enumerations were made during clear weather. The latter part of November was a time of light rain storms. December and January were unusually warm and dry for these months. In February there was ice present, and with it a large amount of suspended matter, especially in the basin at the pumping station and the stream supplying the reservoir. On March 23d samples were taken the next day after a heavy rain-storm, when the streams and the basin at the pumping station were very muddy. On April 6th the body of water in the settling basin at the pumping station contained little suspended matter, because no water had been run in for several days. A heavy rain-storm at this time caused the water of the stream to be very muddy. Clear weather prevailed in April from the 13th to the 20th. During the time that samples were taken neither extremely cold nor extremely warm weather occurred. For this reason variations that would be looked for from seasonal changes are slight.

No. 1.

	TAP.		PUMPING STATION.						RESERVOIR.									
	In Laboratory		Source.		Outlet.				Source.		Middle.				Outlet.			
					Surface.		Deep.				Surface.		Deep.		Surface.		Deep.	
	Gelatin	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin	Agar
1900. October 12 . . .	85	34																
16 . . .	79	34																
20 . . .	92	54																
27 . . .	38	29	303	221														
November 3 . . .	84	20																
10 . . .	17	20																
17 . . .	30	79																
24 . . .	104	48	876	807	. . .	. . .	. . .	. . .	137	284	. . .	. . .	. . .	. . .	29	36		
December 1 . . .	108	81																
8 . . .	281	110																
15 . . .	45	81																
22 . . .	162	153	425	687	. .	. . .	. . .	. . .	150	263	105	149	. . .	. .	145	173		
30 . . .	85	59																
1901. January 5 . . .	97	91																
12 . . .	46	39																
19 . . .	59	47																
26 . . .	30	31	460	422	670	. . .	1,347	. . .	64	68	42	59	95	60	40	55	37	90
February 2 . . .	35	29																
9 . . .	47	28																
16 . . .	31	42																
23 . . .	28	27	970	959	423	726	312	621	300	298	23	30	. . .	. . .	20	24	40	127
March 2 . . .	27	14																
9 . . .	22	19																
16 . . .	31	27																
23 . . .	473	312	4,110	3,733	3,240	2,288	4,440	5,376	295	303	77	77	138	99	73	76	89	55
30 . . .	72	53																
April 6 . . .	204	166	2,366	2,833	870	750	803	650										
13 . . .	59	69	377	336														
20 . . .	85	104	527	447	. . .	. .	. . .	. .	180	162	240	233	433	446	250	290	483	554

A comparison of results from surface samples with those taken from near the bottom of the basins is expressed in tabular form as follows: For outlet samples an effort was made to collect from as near the outlet pipe as possible. That differences are greater at the pumping station than at the reservoir is due to the fact that little sediment was stirred up in taking samples from near the bottom of the reservoir, while anything like complete sedimentation had not taken place at the pumping station. In cases where surface samples show more bacteria than do deep ones the cause may be due to the presence of material near the surface of the water, as was the condition at the outlet of the pumping basin on April 6th.

No. 2.

	Reservoir.								Pumping Station.			
	Middle.				Outlet.				Outlet.			
	Surface.		Deep.		Surface.		Deep.		Surface.		Deep.	
	Gelatin.	Agar.	Gelatin.	Agar.	Gelatin.	Agar.	Gelatin.	Agar.	Gelatin.	Agar.	Gelatin.	Agar.
1901.												
January 23	100	59	110	60	80	55	105	90				
February 27	42	30	. . .	. . .	28	24	. . .	. . .	400	812	700	620
March 23	77	77	138	99	73	76	89	55	1,386	2,288	4,440	5,376
April 6	. . .	. . .	. . .	. . .	. . .	.	. . .	. . .	870	750	808	650
13												
20	240	233	433	446	250	290	483	554				

To note the effect of reaction of media, a number of examinations, with double sets of plates, were made. One set contained the alkaline media used for the greater portion of this work, while the other set had media of an acid reaction, as indicated by phenolphthalein. All of the determinations were made from samples of tap water during the months of January, February, and March. The following table presents the results obtained:

No. 3.

GELATIN.		AGAR.	
Alkaline.	Acid.	Alkaline	Acid.
30	38	31	47
35	32	20	47
47	47	28	30
31	36	42	27
28	65	27	33
31	52	27	20

In order to compare graphically the number of colonies of bacteria upon gelatin and agar, a chart of curves giving results from a number of examinations is given. Dates being given will show at once comparative variations from seasonal and from somewhat violent weather changes. Some slight differences are possibly due to the variations between different samples of the same water. As the laboratory tap water comes almost entirely from the reservoir, no relation between the tap and the pumping basin can be obtained from these curves. Examinations of water as supplied from the stand pipe on Preston Heights gave about 650 colonies to the cubic centimeter of water, at which time samples from the pumping station gave about an equal number. **Chart No. 1.**

A chart giving only maximum numbers from samples taken at all points included in the determinations shows some interesting features. Here again a close parallel between nutrient gelatin and agar as media is expressed. Reference to the preceding tables and chart will show that the largest numbers appeared at nearly the same time at all places. **Chart No. 2.**

A careful selection of colonies presenting obvious macroscopic differences has given the list in the following tables. Colonies similar to those described were found upon plates from most samples. Most often, however, only three or four kinds would appear on each plate.

More careful study will doubtless show some in the list to be merely varities of others. It has been observed, in the study of characteristics of these colonies, that more chromogenic forms appear where suspended matter is most abundant. Perhaps there is some relation between the organic matter of vegetable origin and color production.

*The unsettled condition of our knowledge of the limits by which species of water bacteria are defined renders any attempt at their determination premature. Only those features, therefore, are here noted which will be of probable service in a future investigation having such determination in view. It seems probable that the large majority, if not all, the species here noted are simple aquatic saprophytes.

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*I have received, as this is in the press, Migula's "Compendium der bacteriologischen Wasseruntersuchung, but, unfortunately, too late for use in this connection.

Bacilli which Liquefy Gelatin.

No.	Place Found.	Size in Micra.	Motility.	Colonies on Agar.	Colonies on Gelatin.	Color Production.
1	Tap	3-4	Motile	White feathery mass spreading from one centre	Little growth on surface	
2	"	2-4	"	Irregular outline, club shaped uneven branches	Little growth on surface	
3	"	1-2	"	Round, dark red, well defined border	Like agar	Red.
4	Reservoir	2-3	"	Small, round translucent spot, white centre	Spreads much on surface, patchy	
5	"	1.5-2	"	Round, white patch, fusiform nucleus	Conical liquified area along puncture	Blue-green.
6	"	2-2.5	Non-motile	Small, brownish white mass, regular outline.	Dark, greyish patch on surface	Brown.
7	"	5-6	Motile	Irregularly spreading uneven mass	Thick, flaky patch on surface,	
8	"	2-3	"	Dark yellowish mass, circular nucleus	Feathery extension from stab	Orange-yellow.
9	"	2.5-3	"	Large, dark violet spot, colors medium	Medium becomes dark in color	Violet.
10	Pumping station	2-3	"	Radial spreading, darker nucleus	Inverted bell-glass area of liquifaction	
11	"	2.5-3	"	Concentric rings, triangular nucleus	Spreads rapidly, scum on gelatin	
12	"	2-2.5	"	Irregular branching, pointed processes from border	Yellow scum on surface, green tint	Green.
13	"	2-3	Non-motile	Large wooly branching mass, arms striated	White scum on surface	
14	"	2-3	" "	Radial spreading, dirty brown color	Brownish scum on surface	
15	"	4-5	Motile	Large, spreading, white mass	Saucer-shaped, suspended masses	
16	"	1.5-2	"	Elongated, light yellow band	Small depressed patch	Yellow.
17	"	1-2	"	Small, round, dark yellow spots	Brownish spots on surface	
18	"	1-2	"	Horn-like projections from border, yellow	Little depression of surface colonies	Yellow.
19	"	2-2.5	"	Small yellowish mass, depressed centre	Thin, white, depressed masses	Yellow.
20	"	2-3	"	Elongated translucent patch	Thin, white areas, much spreading	
21	"	3-4	"	Elevated, lobular, with patchy surface	Large, spreading patch	
22	"	2-2.5	"	Large, translucent patch, serrated edge	Very rapid spreading on surface	

Cocci which Liquefy Gelatin.

No.	Place Found.	Size in Micra.	Motility.	Colonies on Agar.	Colonies on Gelatin.	Color Production.
1	Reservoir	1-1.5	Motile . . .	Round, well defined, white colonies	Grows slowly on surface—well along stab	
2	"	1.5-2	" . . .	Glistening, brownish, well defined mass	Surface and puncture growths have pink tint	
3	"	1-1.5	" . . .	Large, spreading, white, branches end in bulbs	Abundant growth on surface and along stab	
4	"	.50-1	" . . .	Translucent, white, heavier border	Whitish masses in centre of depressed area	
5	Pumping Station	1-1.5	" . . .	Glistening, yellowish-white, irregular outline	Depressed with concentric rings	
6	"	2-2.5	" . . .	Yellowish-white, radial spreading	Radial spreading from stab . .	
7	"	1-1.5	"	Small, fusiform, orange color .	Indistinct translucent colonies in depressed area	Orange.
8	"	.75-1	"	Small, fusiform, white color . .	Granular patch in depressed area	

Cocci which do not Liquefy Gelatin.

No.	Place Found.	Size in Micra.	Motility.	Colonies on Agar.	Colonies on Gelatin.	Color Production.
1	Reservoir	1-1 5	Motile . . .	Round, well defined, lemon-yellow color	Grows slowly on surrace—well in stab	Lemon.
2	"	1-2	" . . .	Glistening patch, with yellow nucleus	Glistening yellow patch on surface	
3	"	.75 -1	" . . .	Brownish, well defined, with fusiform nucleus	White, with depressed centre .	
4	"	1-1.5	" . . .	Small, red, regular borders . . .	Fine granular, red, spreads much	Red.
5	Pumping station	.75-1	" .	Rounded, lobular, pink mass . .	Spreading, granular, pink . . .	Pink.
6	"	.50-1	" . . .	Round, glistening, lemon-yellow	Yellow, felted mass in depression	Yellow.
7	"	1-2	" . . .	Concentric rings, four-branched nucleus	Little growth on surface	Olive.

The preceding results show the reservoir water to be quite pure from a bacteriological standpoint under most circumstances. In numbers of bacteria it compares favorably with the best of water supplies. The purity of this water is due to the almost entire absence of inhabited dwellings from the water shed and the small area of cultivated soil thereon, as well as to quite effective sedimentation, which serves to remove much of the suspended material reaching it from supplying streams. An additional settling basin above the present one would cause even long continued storms to have little effect upon the water at the gate-house.

Judged by the number of bacteria present alone, the pumping station water is much less pure than is the reservoir water. The presence of inhabited dwellings and cultivated soil near supplying streams readily explains the larger number found. The occurrence of such a marked increase in numbers in March, with the subsequent decrease when there had been no storm for some time, is a fact of great significance in this connection. In this case, too, there is an absence of either effective natural or artificial means of purification of the water. The effect of surface flushing by storms emphasizes the need of some means of purification of water derived from this source. Additional basins for sedimentation would serve to clear and purify the water, and at the same time would add to the supply.

From a sanitary standpoint, it is evident that the reservoir is surrounded by conditions very favorable to a safe water supply. To make it absolutely safe, however, the last remaining habitation should be removed from the water shed. The larger number of bacteria from samples of pumping station water, when taken along with surrounding conditions, only justifies considering this source of supply a suspicious one. Efforts to demonstrate the presence of the bacillus coli communis have failed, but there is little doubt that animal excreta are included in the contaminating material.

While it is true that most bacteria from soil and decaying organic matter are harmless, yet obvious possible sources of animal and especially human contamination should be avoided.

CONCLUSIONS FROM THIS AND FORMER DETERMINATIONS.

I. The reservoir affords a comparatively safe water supply.

II. The pumping station water is suspicious, though not necessarily dangerous.

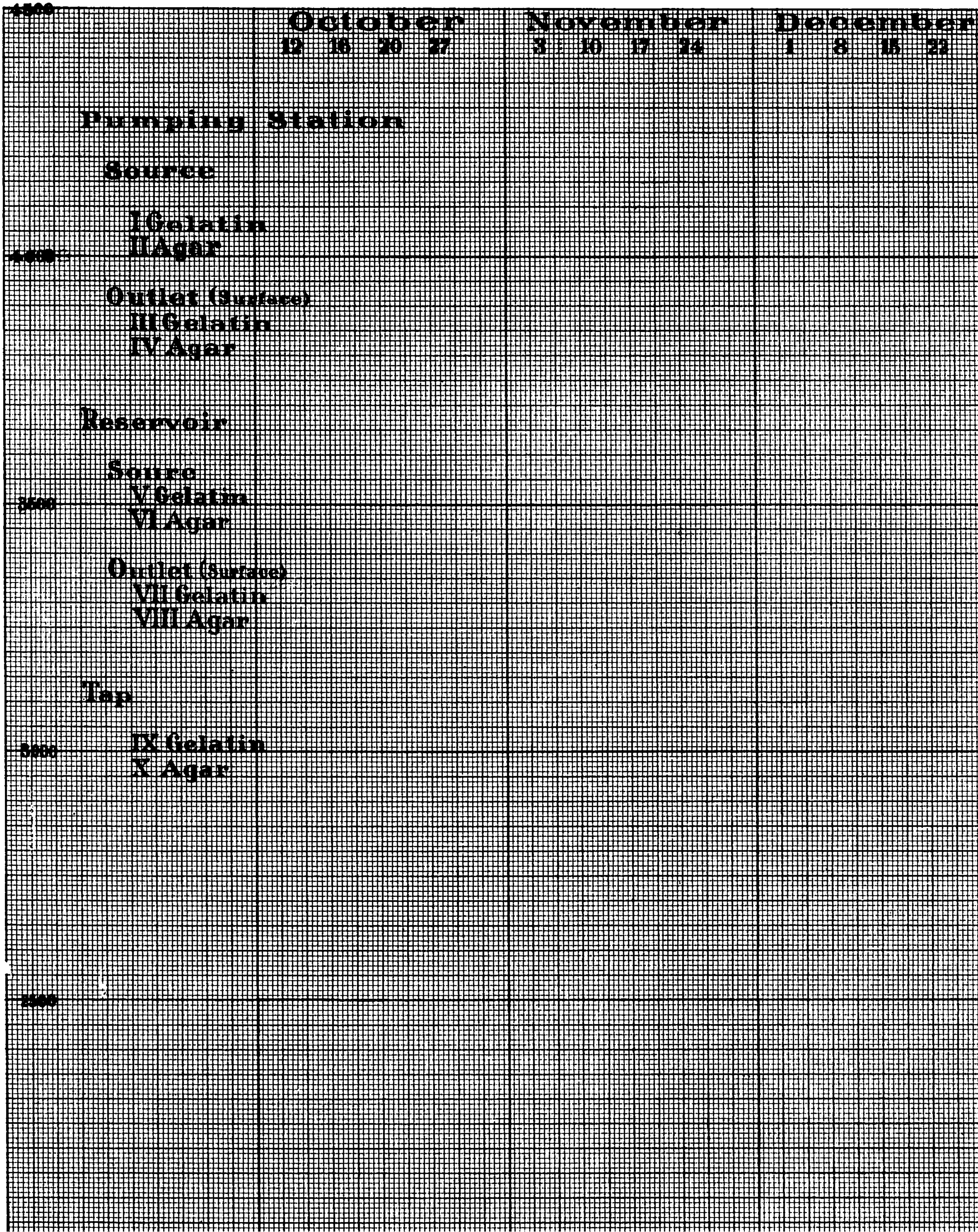
III. The effect of sedimentation upon the bacterial life in water is to give an increased number for deep samples.

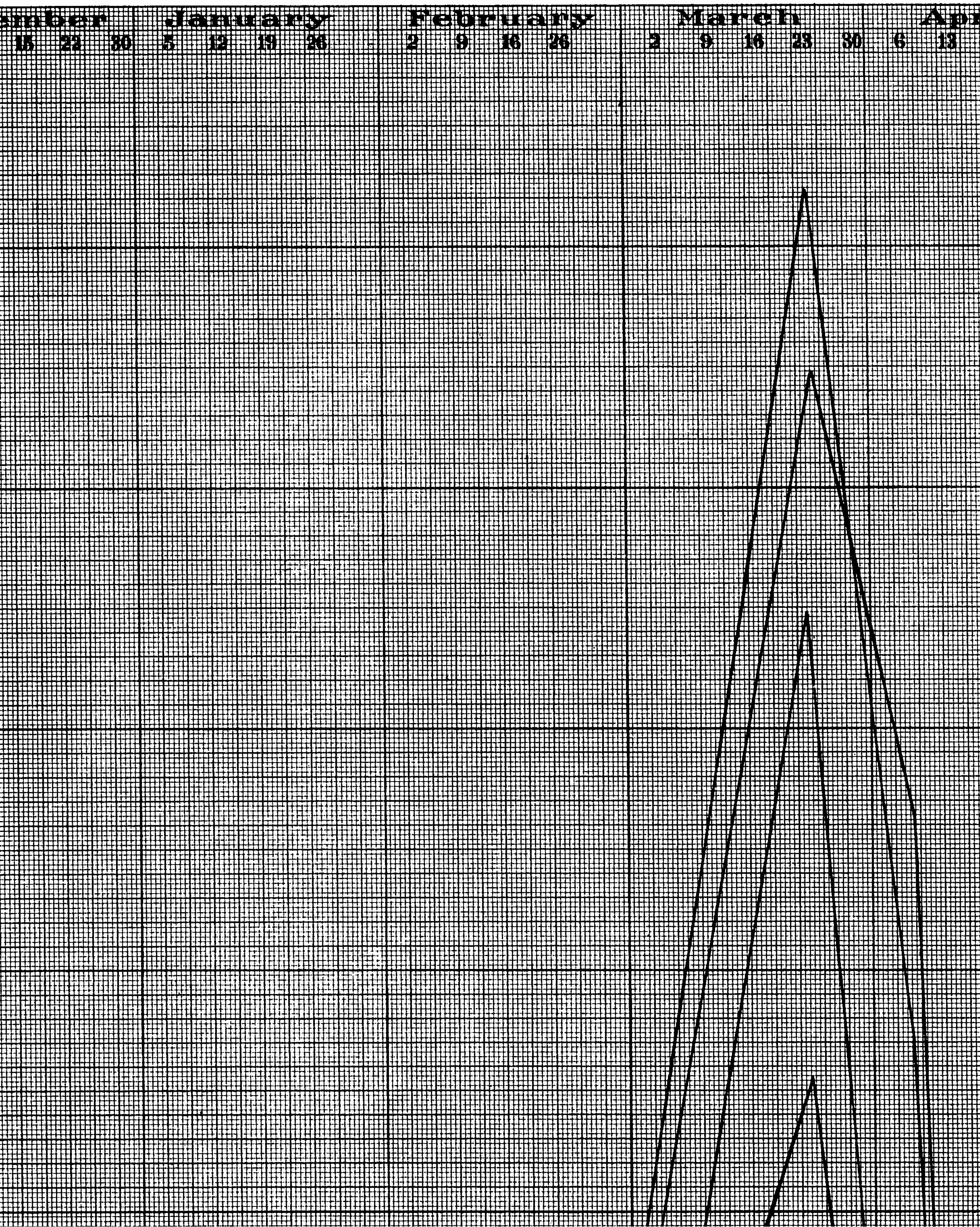
IV. Storms serve to add to the number of bacteria along with the increase in suspended solids.

V. When solids are present in large enough amount to give a muddy water, effective sedimentation will remove a very large percentage of the bacteria.

VI. Pumping water into the city main will not influence the purity of the resevoir water to any marked extent, little, if any, water being forced into the reservoir.

VII. The use of good domestic filters will remove a large part of the bacterial life from water. This conclusion is based upon examinations made before and after filtering tap water in the Biological Laboratory, a decrease from 500 to fewer than 80 colonies to the cubic centimeter of water being obtained.


4500
4000
3500
3000
2500
October
12 16 20 27
November
3 10 17 24
December
1 8 15 22
Pumping Station
Source
I Gelatin
II Agar
Outlet (Surface)
III Gelatin
IV Agar
Reservoir
Soure
V Gelatin
VI Agar
Outlet (Surface)
VII Gelatin
VIII Agar
Tap
IX Gelatin
X Agar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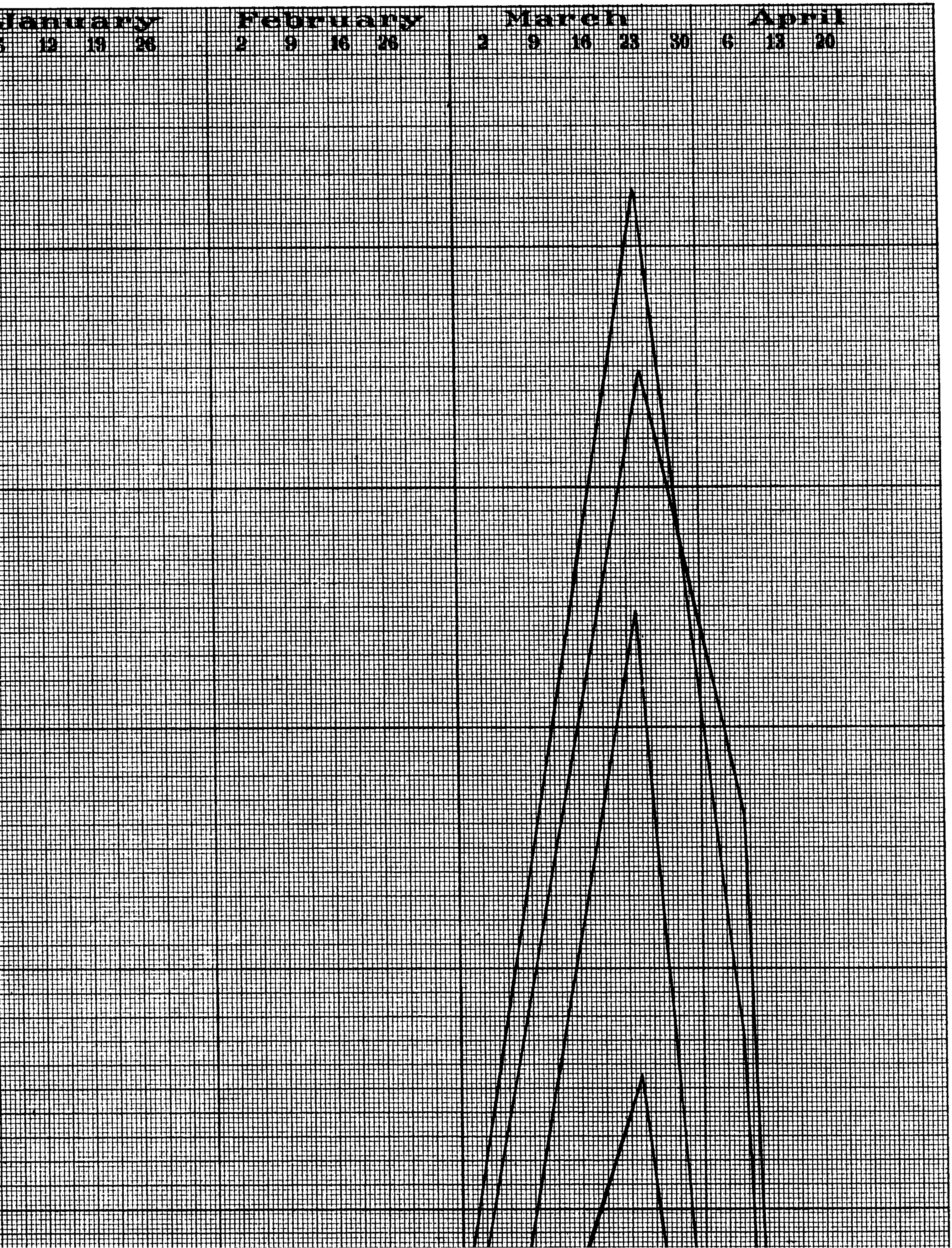
January
February
March
Apr
15 22 30
5 12 19 26
2 9 16 26
2 9 16 23 30
6 13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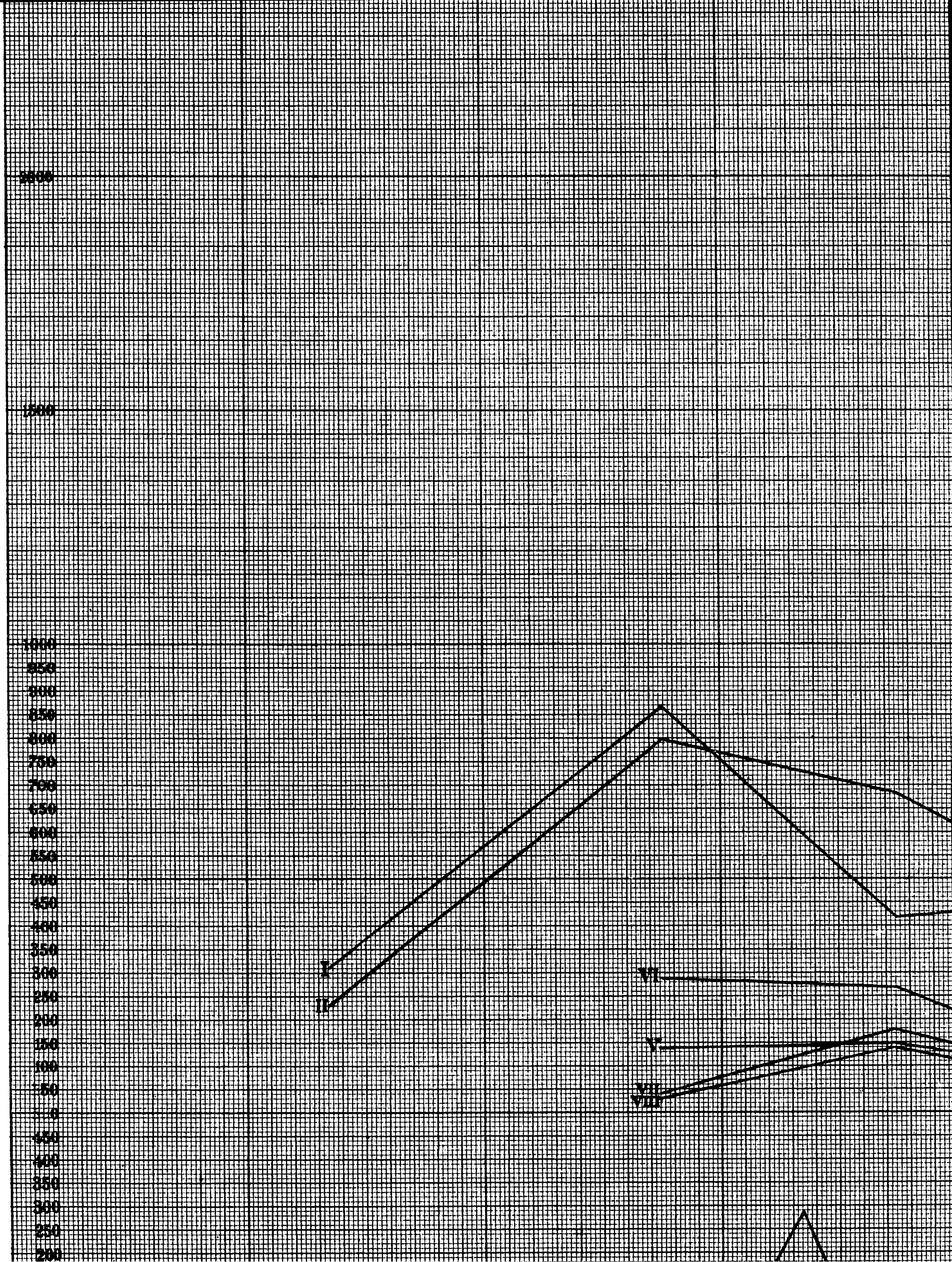
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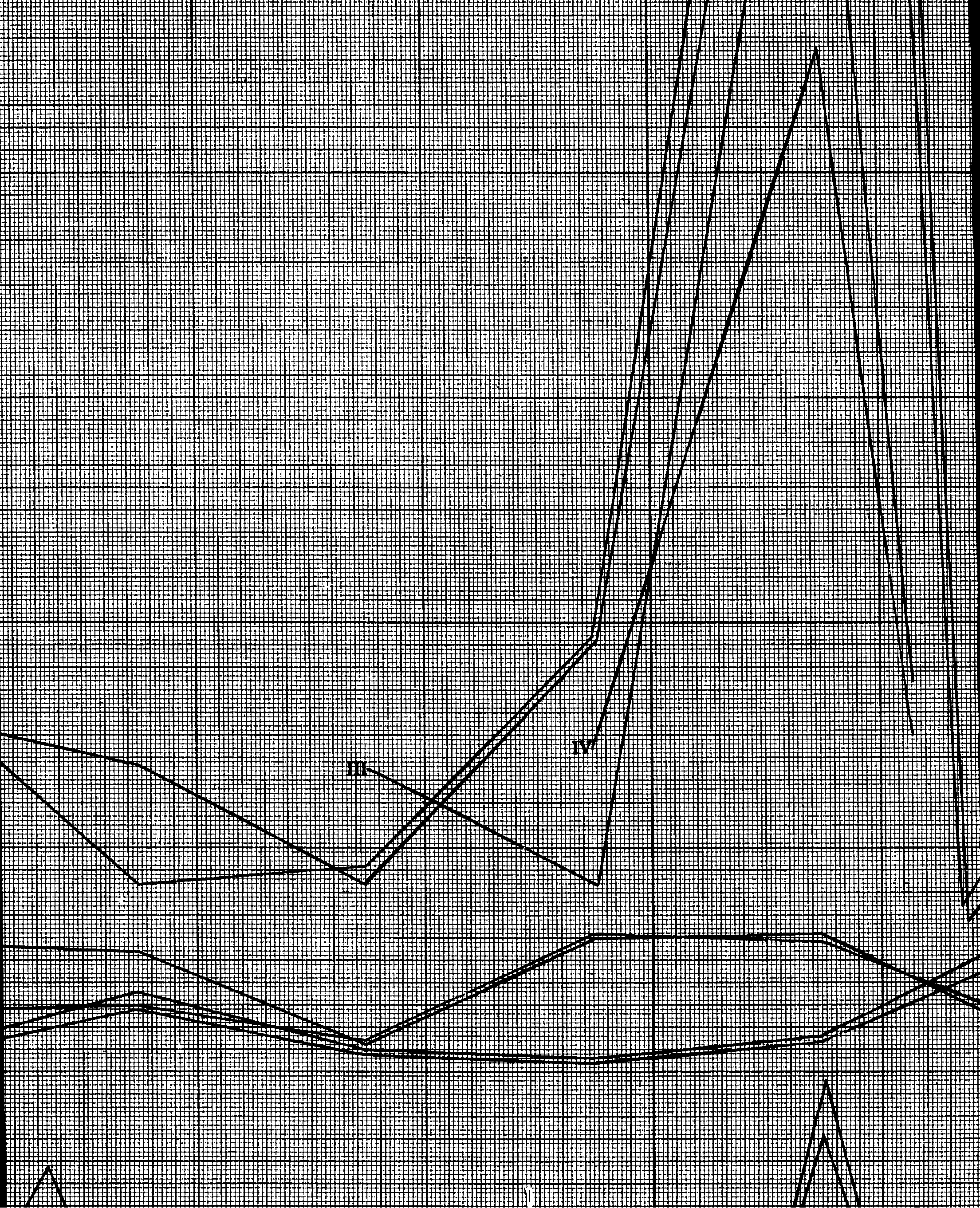
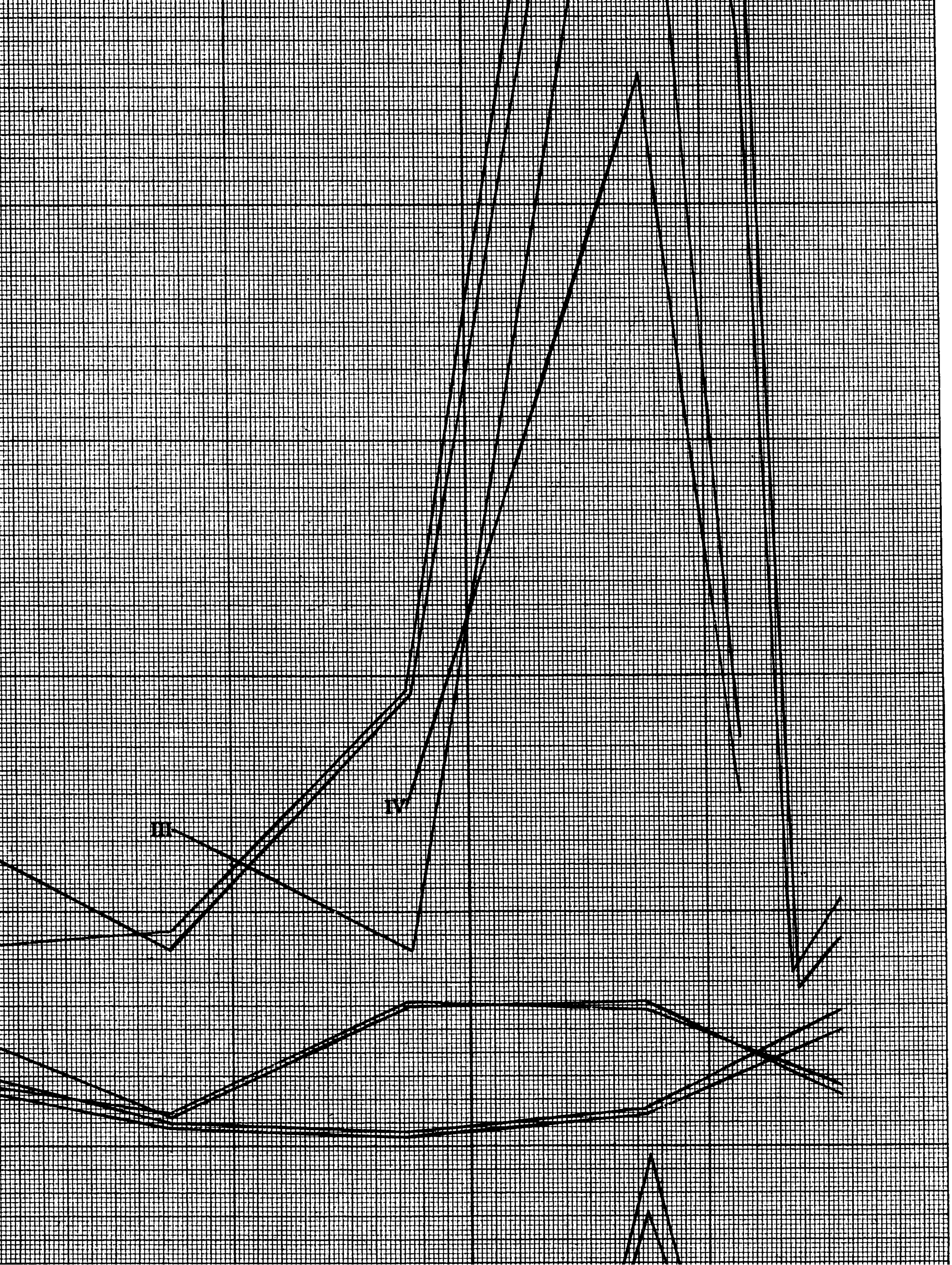
I
II
VI
V
VII
VIII



III
IV



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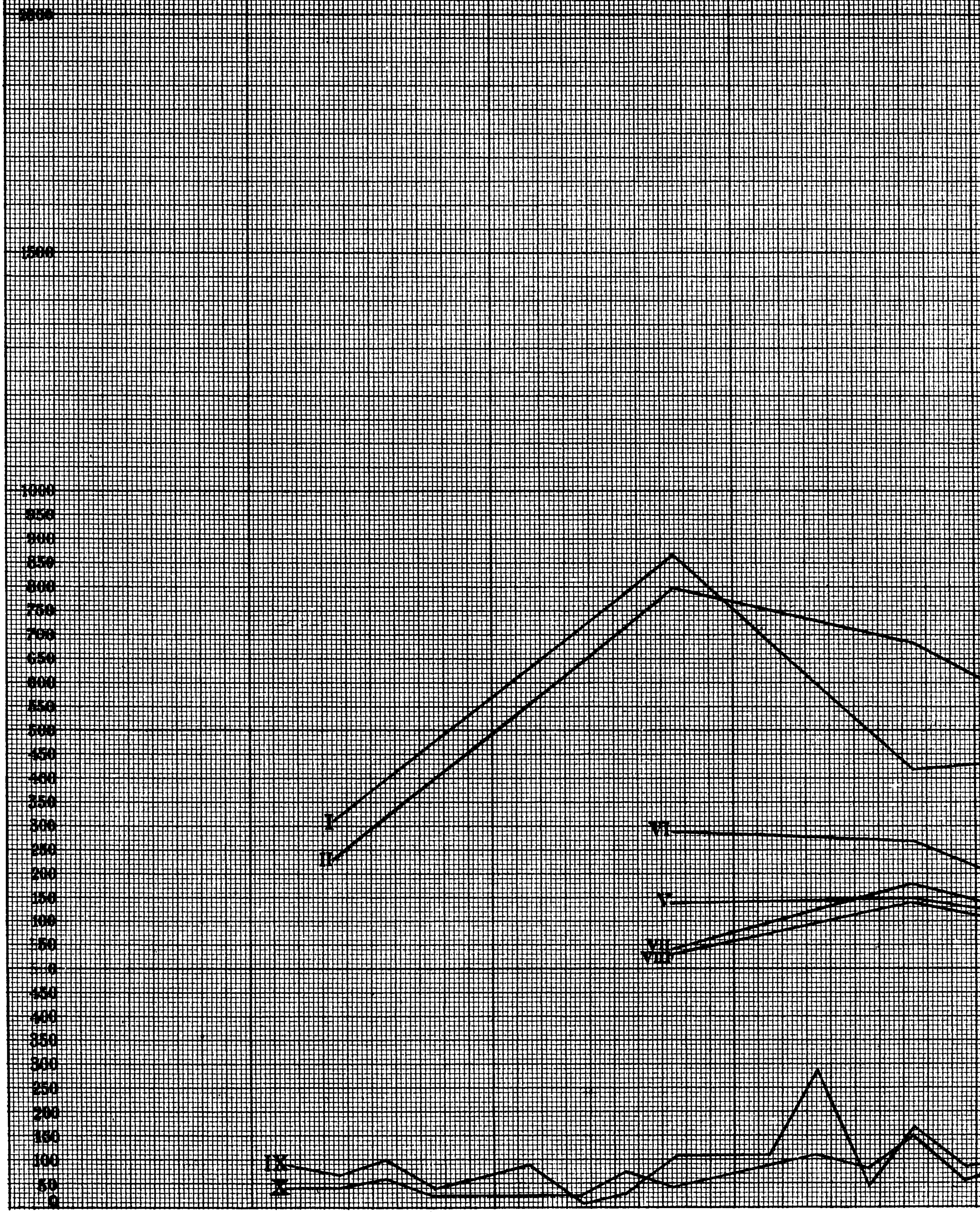
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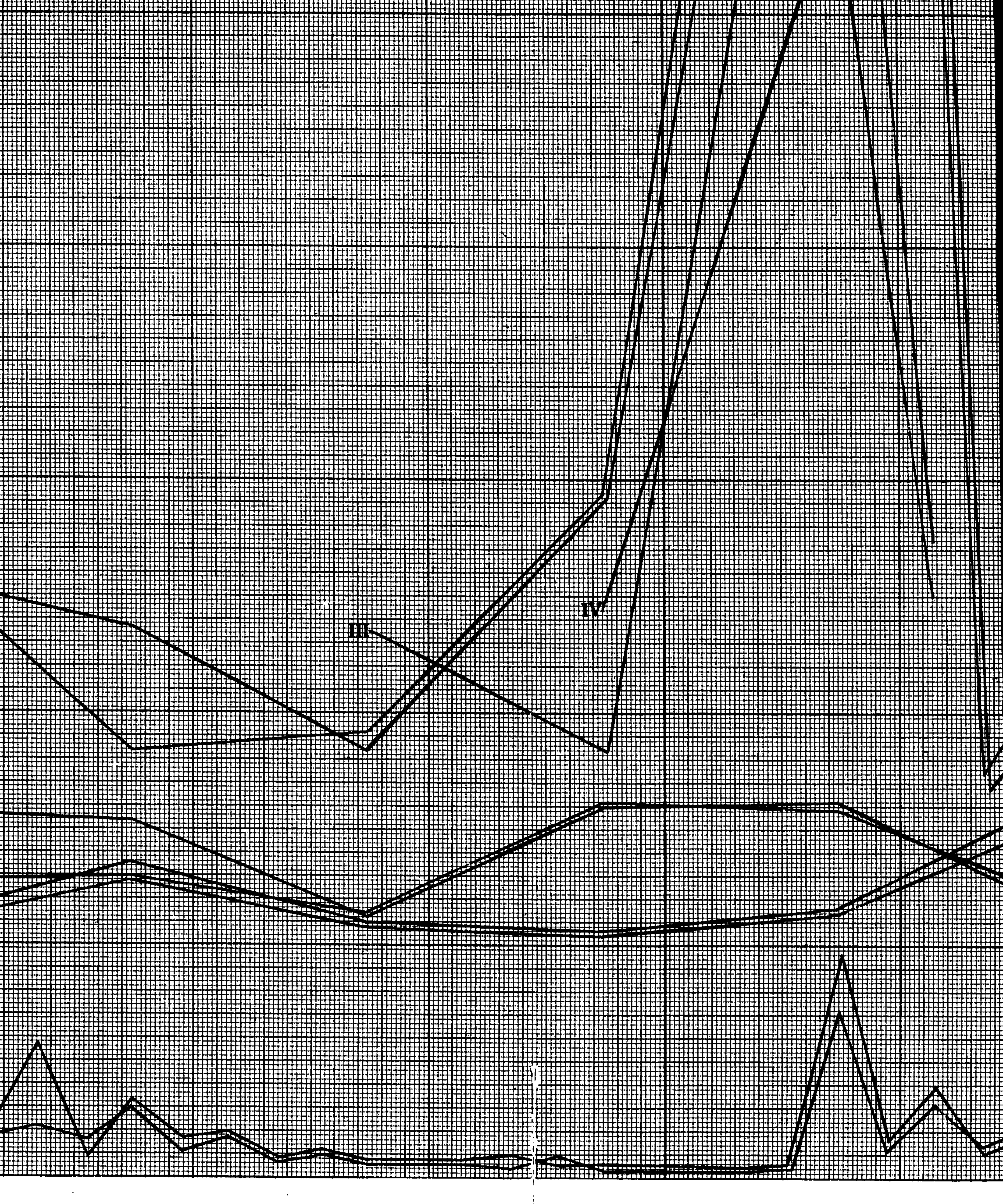
Chart No. I.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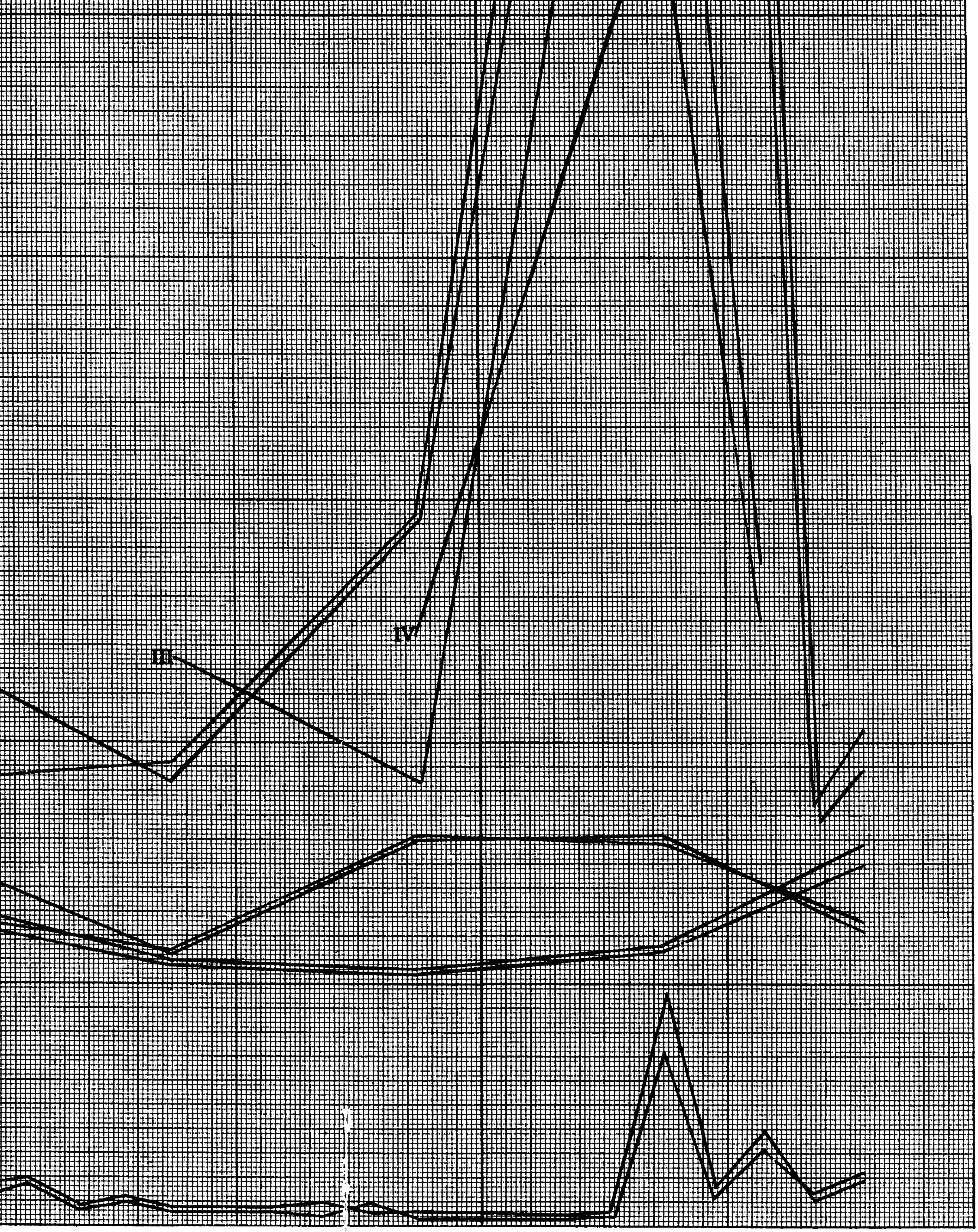
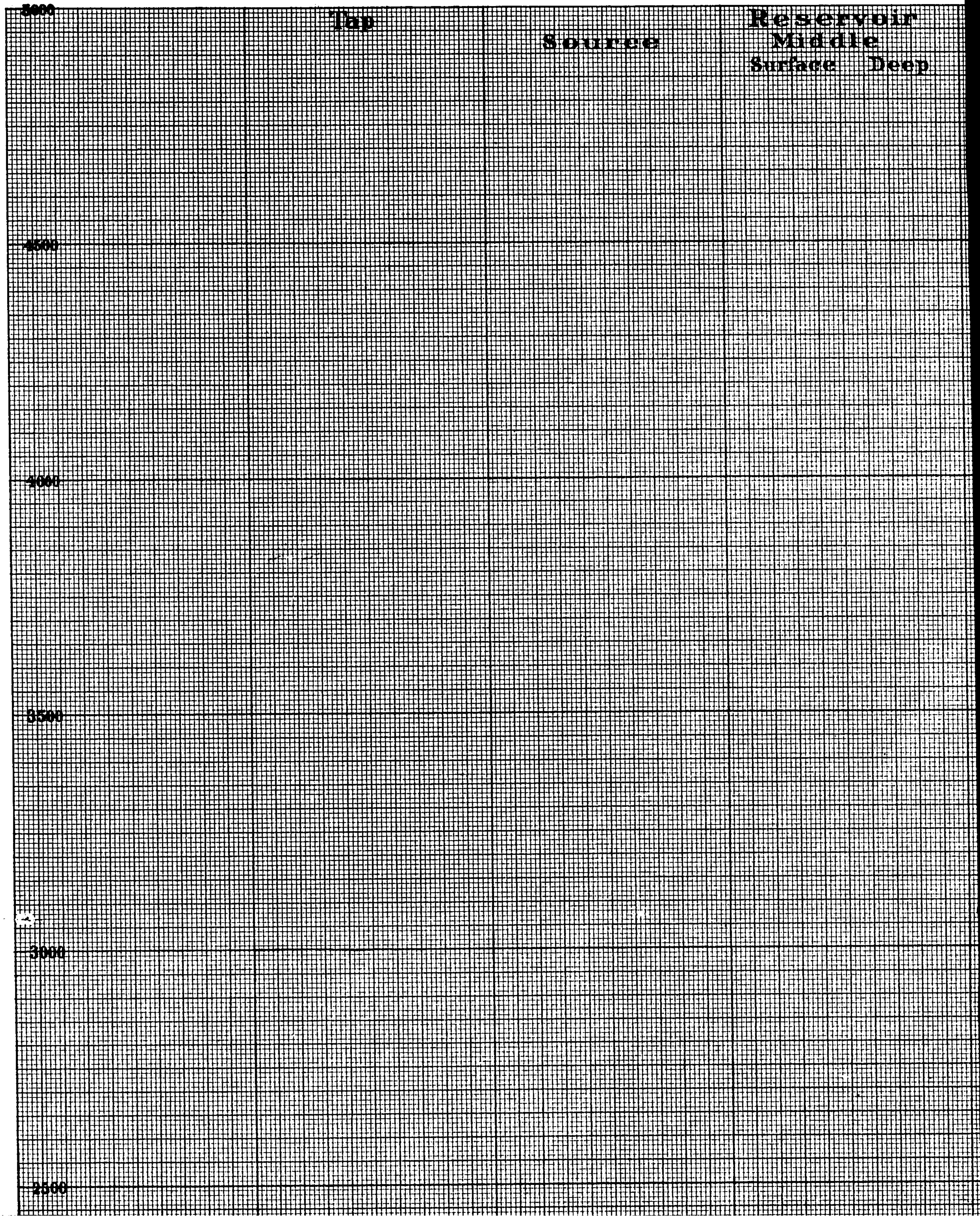
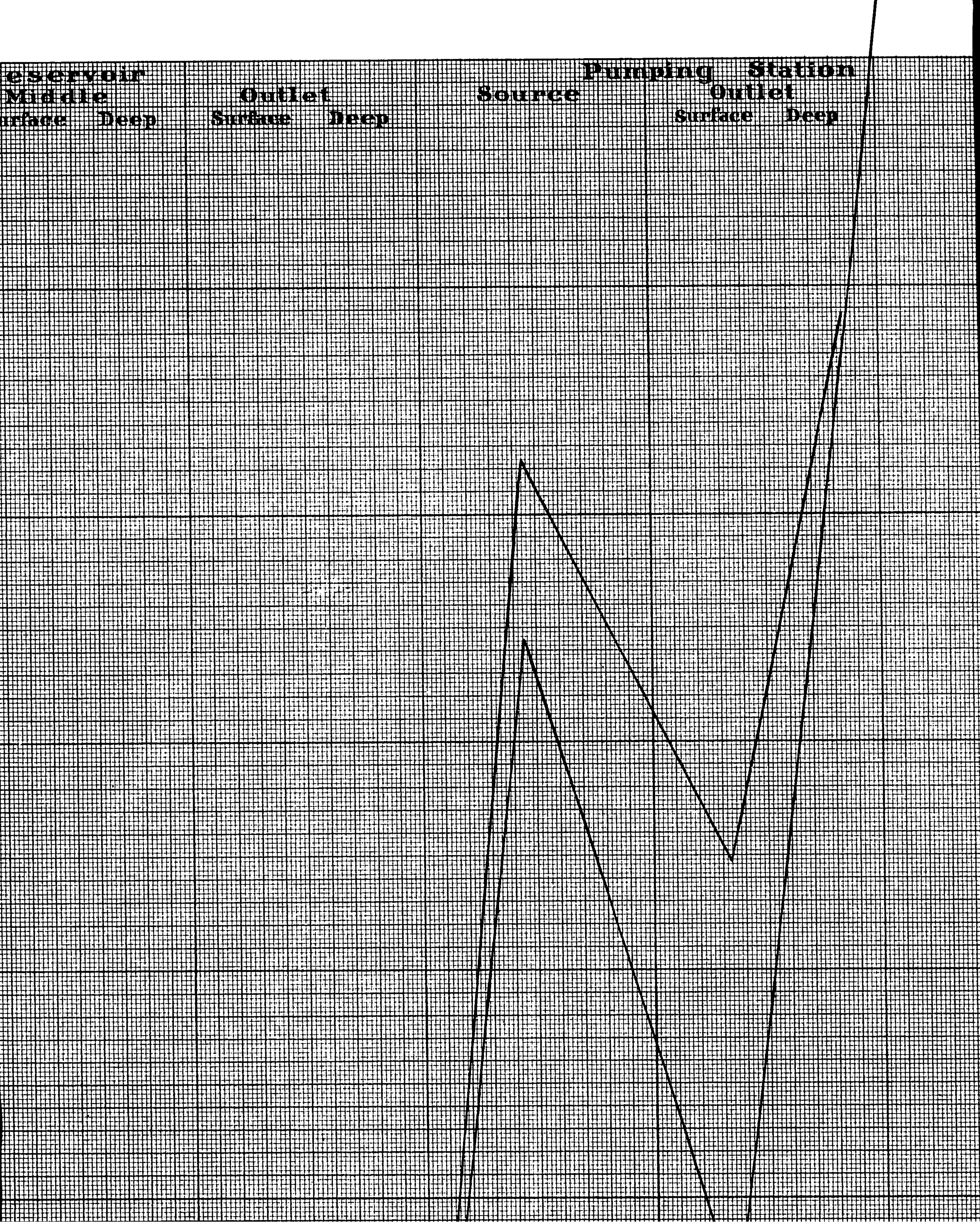
IV


III
IV


Tap
Source
Reservoir
Middle
Surface Deep
5000
4500
4000
3500
3000
2500



eservoir
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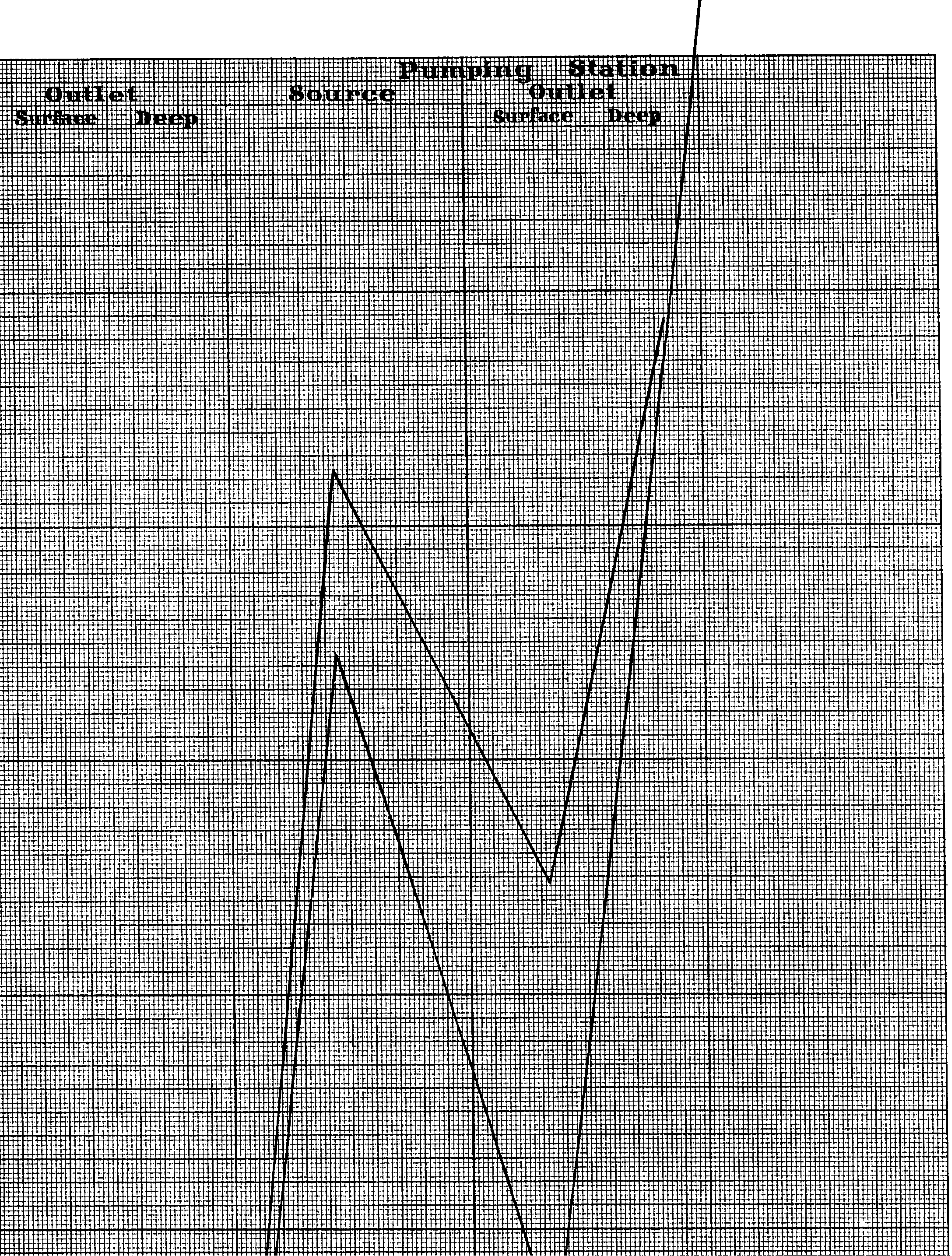
Surface Deep
Source
Pumping Station
Outlet
Surface Deep


Pumping Station
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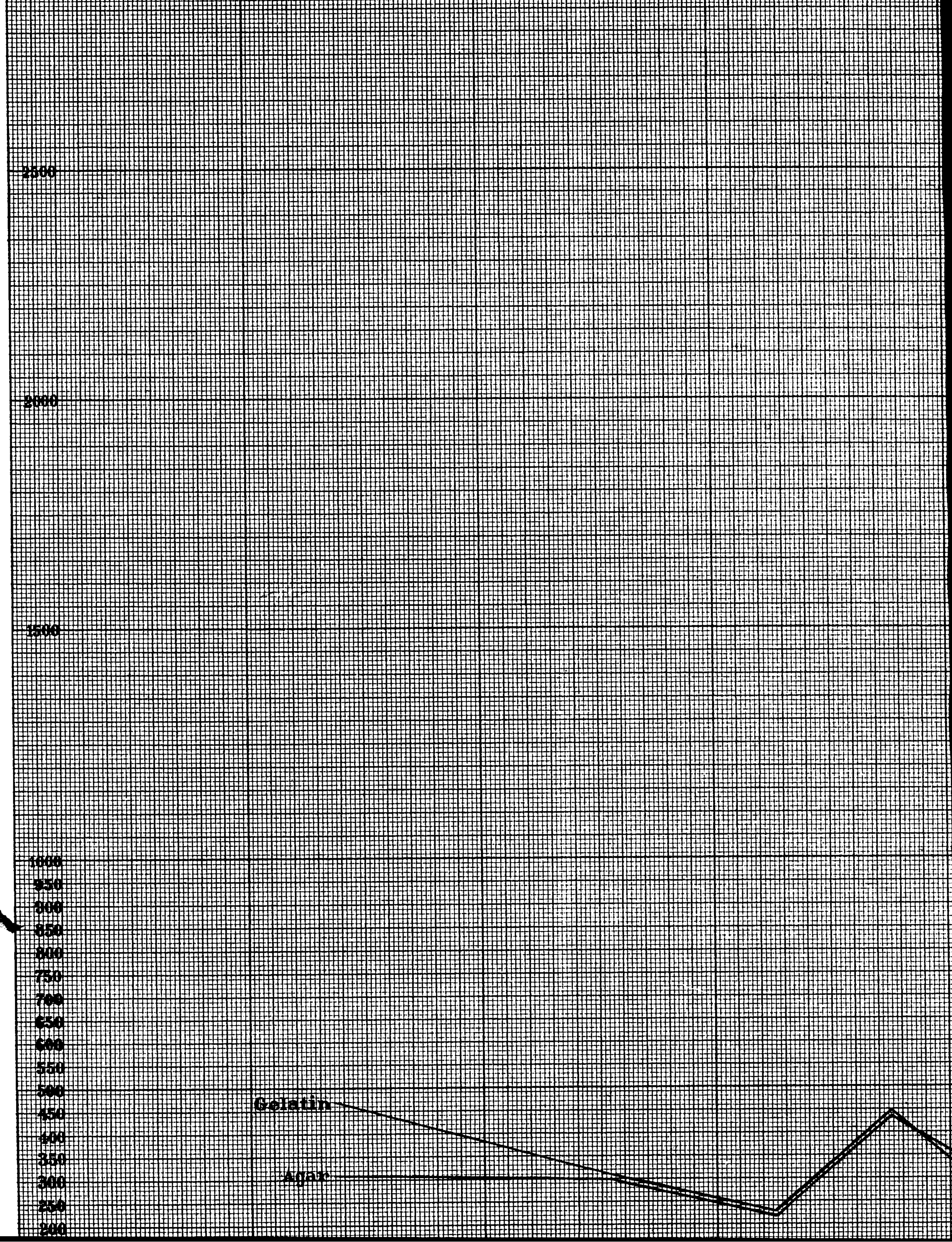
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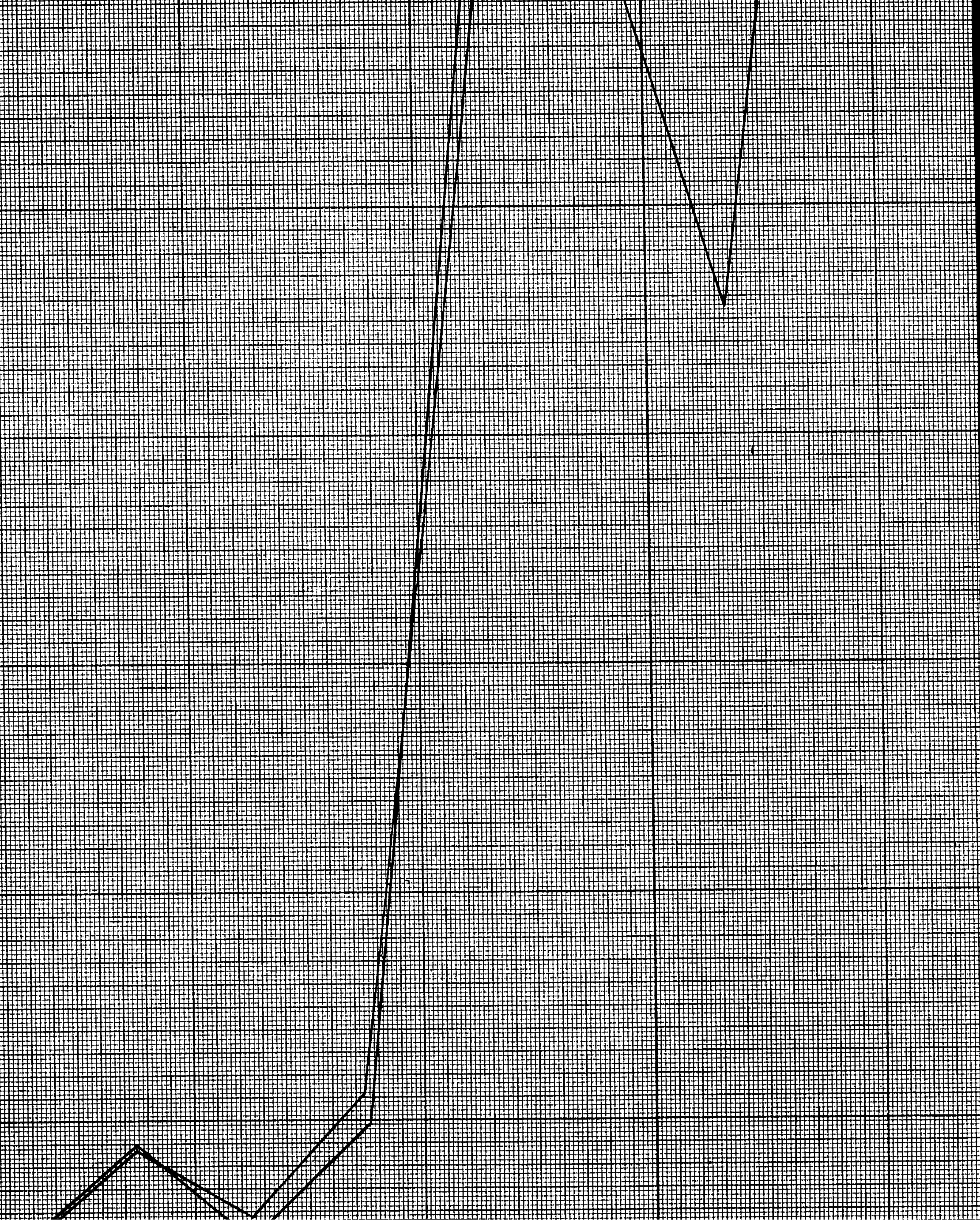
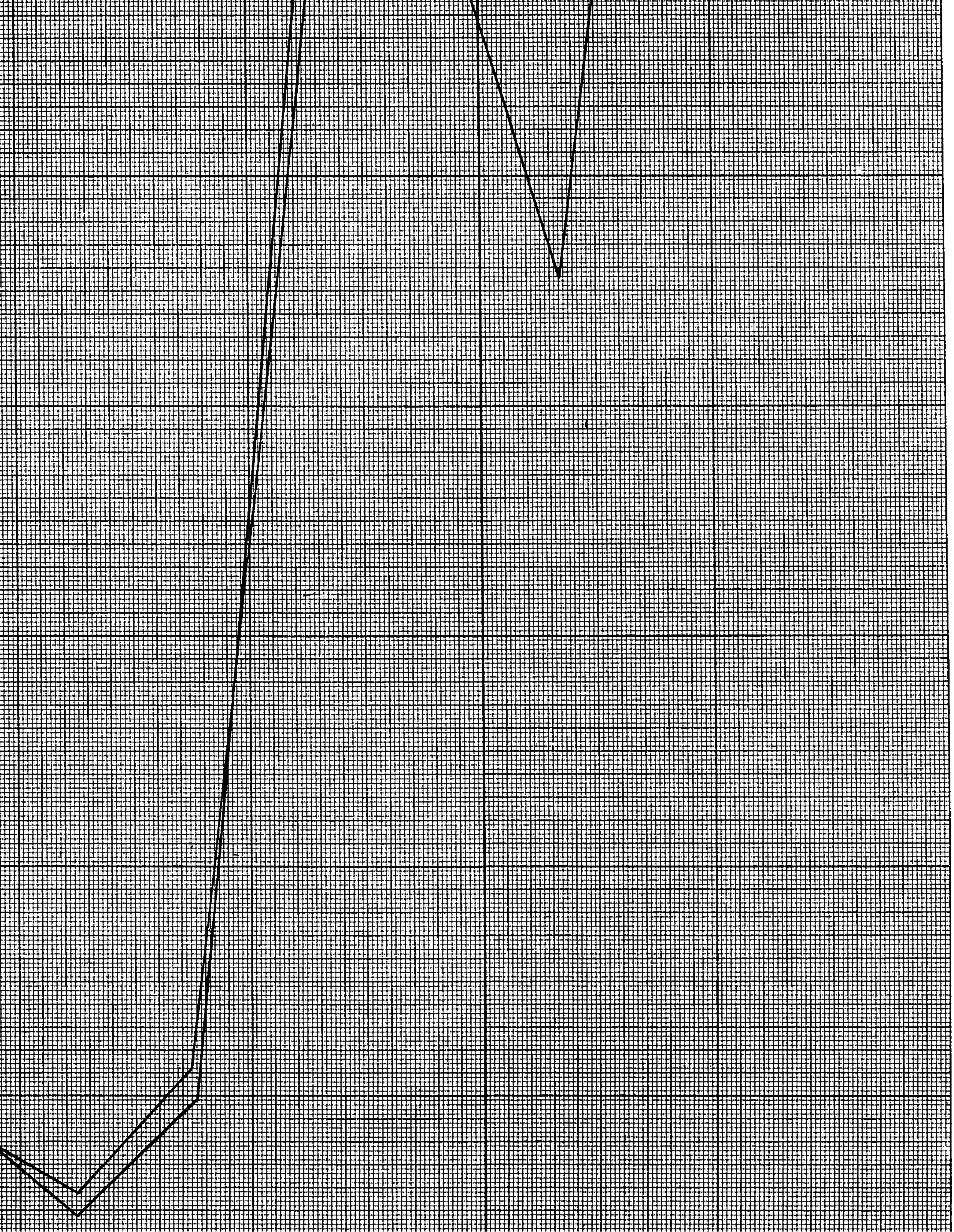
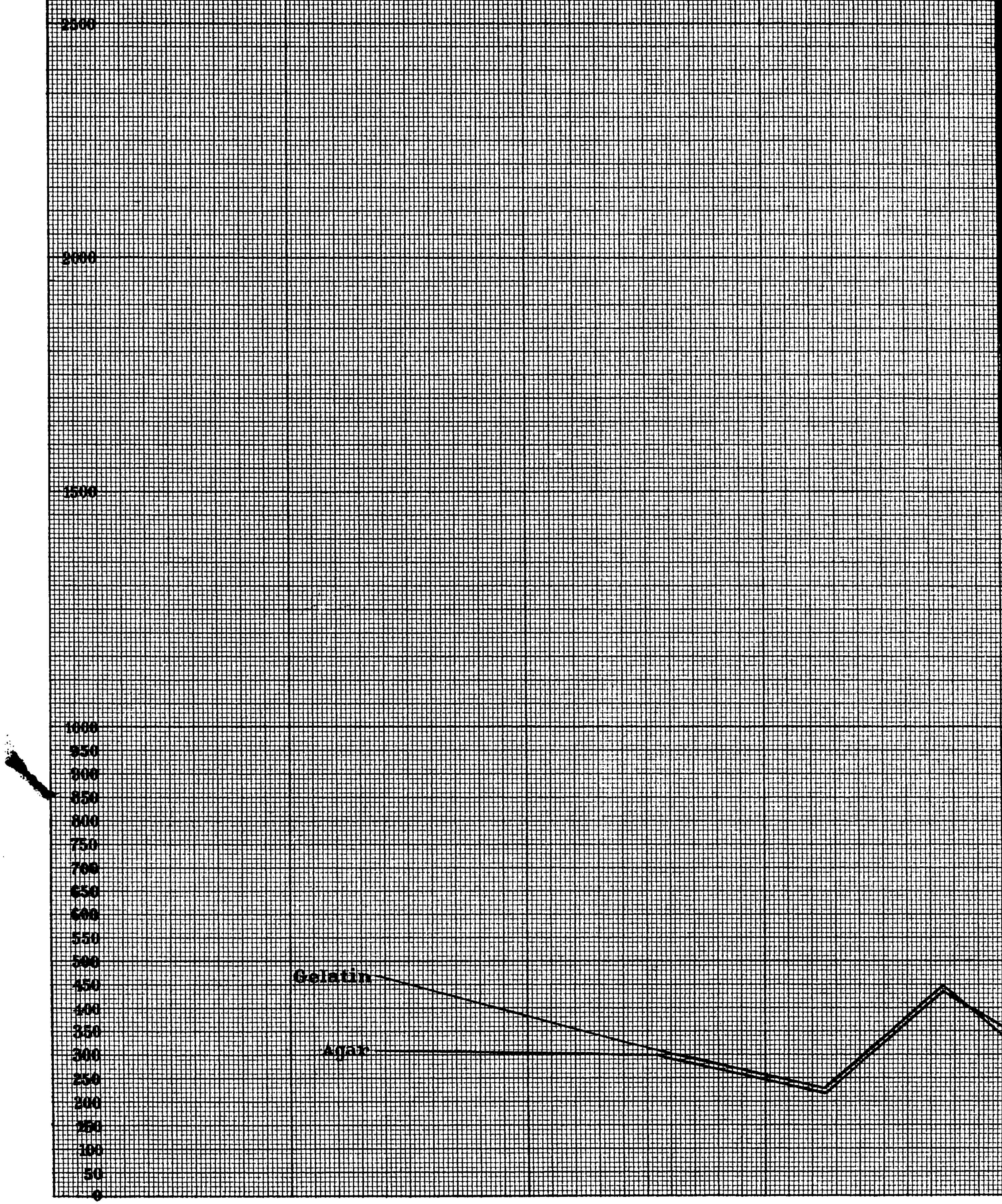
Agar



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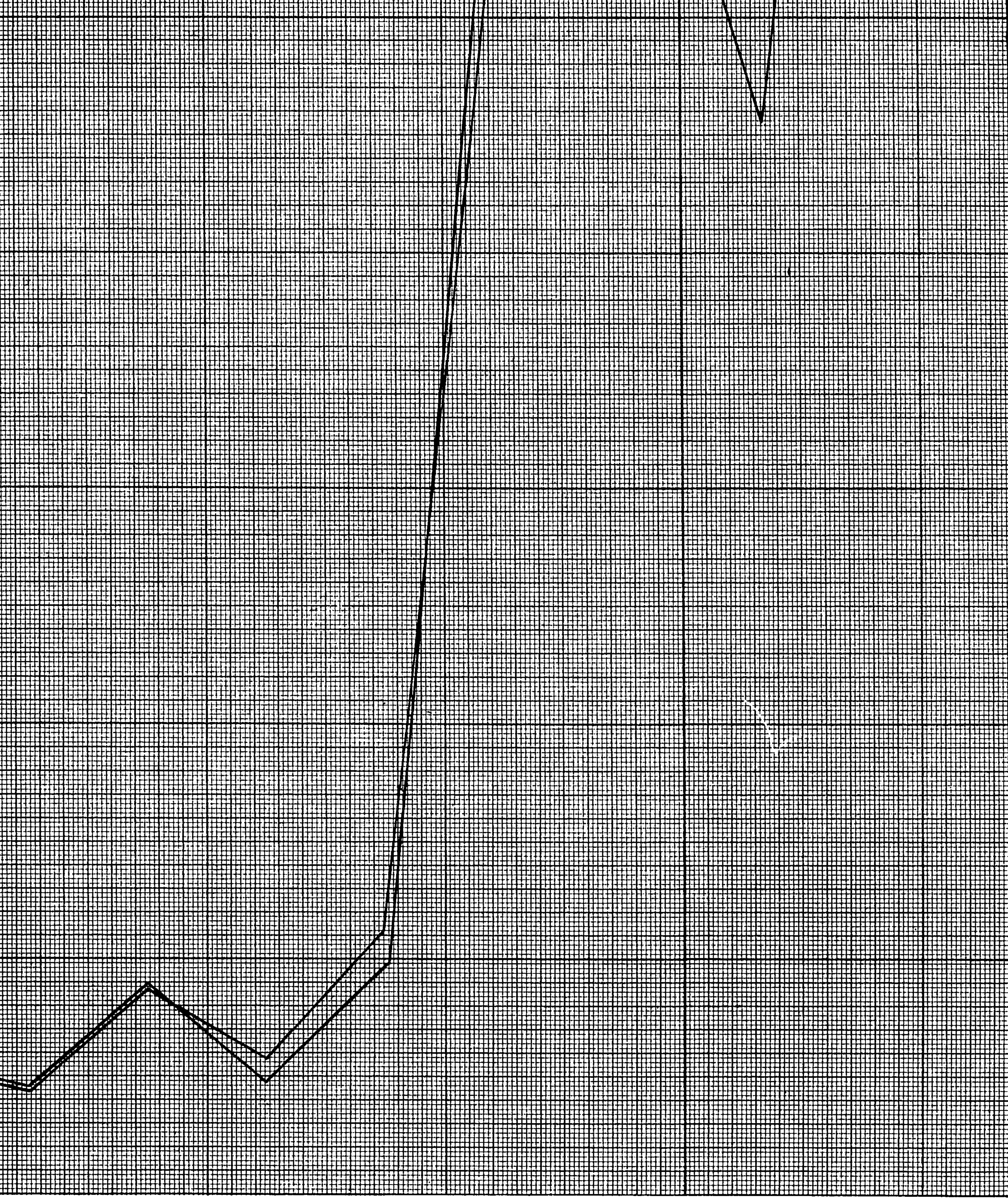
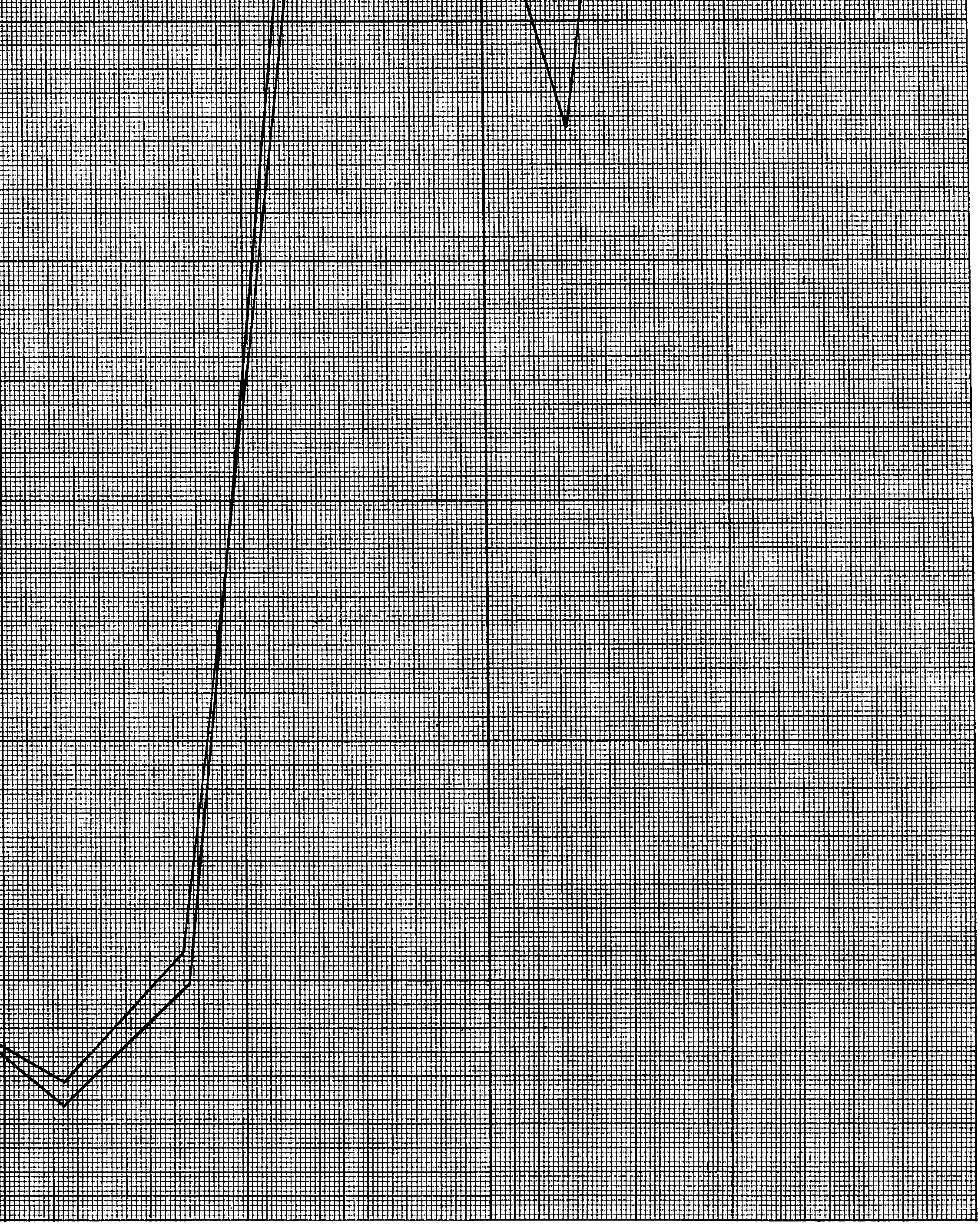
Gelatin
Agar

Chart No. 2.





VIRGINIA GEOLOGICAL SURVEY
THOMAS LEONARD WATSON, DIRECTOR

LIST OF MINES AND PROSPECTS

1. Benton
2. Fleming
3. Waller
4. Payne
5. Busby
6. Moss
7. Bowles (Tellurium veins)
8. Bowles (Back Field vein)
9. Shaw
10. McGloam
11. Tellurium
12. Scotia (Tellurium veins)
13. Scotia (Hodges vein)
14. Young American
15. Morgan
16. Belzoro
17. Collins
18. Grannison
19. Atmore
20. Kent
21. Bertha and Edith
22. Stockton Tunnel
23. Page
24. Snead
25. Hughes
26. Margaret
27. McKenna
28. Johnson
29. Hudgins
30. Ore Bank (Iron)
31. Lightfoot
32. Anaconda
33. Morton
34. Burnett
35. London and Virginia
36. Buckingham
37. Williams
38. Morrow
39. Bondurant
40. Greeley
41. Ayre (Iron)

Detailed maps are given of the areas inclosed by the heavy black lines. See Plate II and Fig. 1.

LEGEND

TRIASSIC (Newark)

Sediments

PRE-CAMBRIAN and CAMBRIAN

Granite Areas
(chiefly granodiorite, with hornblende schists, pegmatite, and some granite)

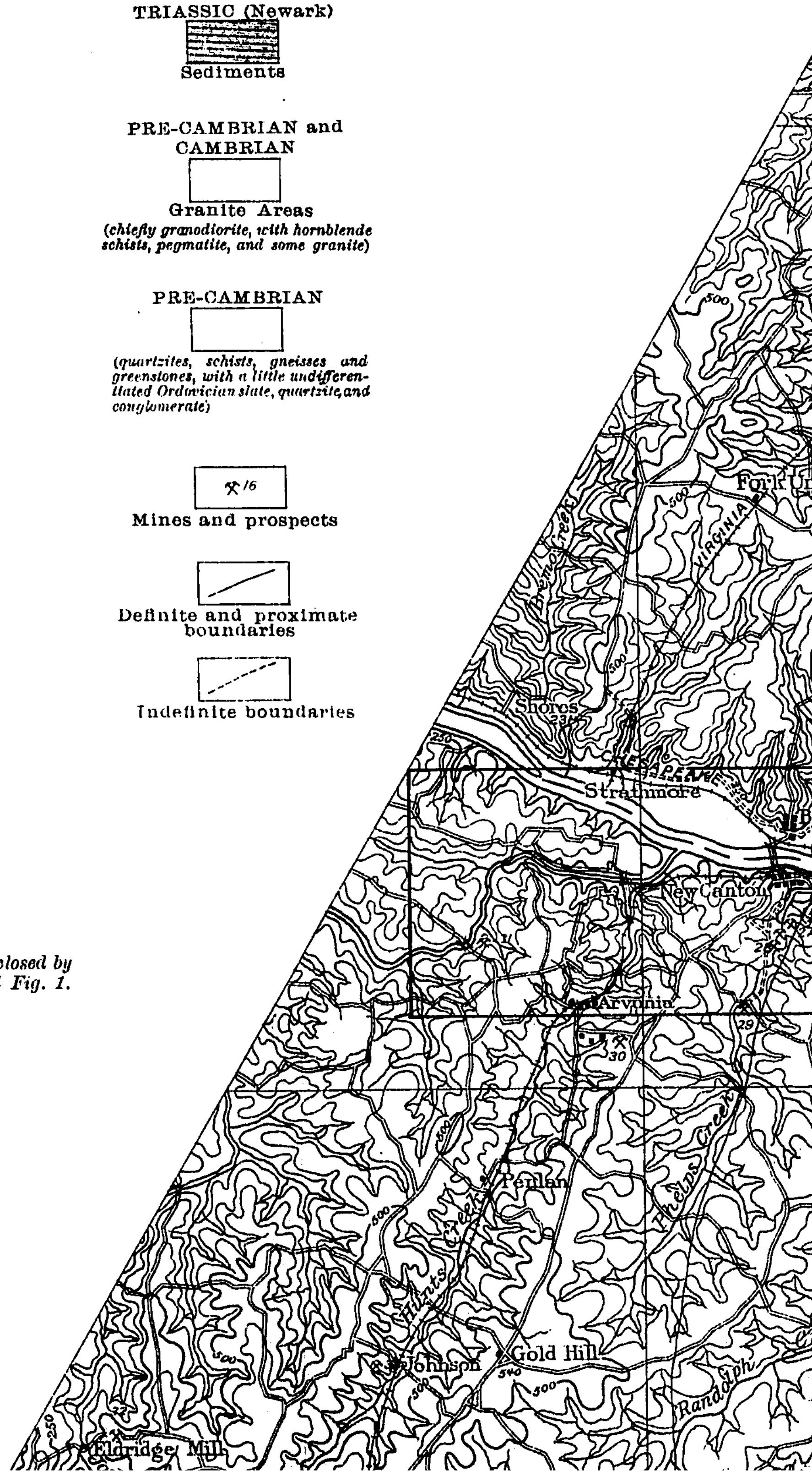
PRE-CAMBRIAN

(quartzites, schists, gneisses and greenstones, with a little undifferentiated Ordovician slate, quartzite, and conglomerate)

⚒ 16
Mines and prospects

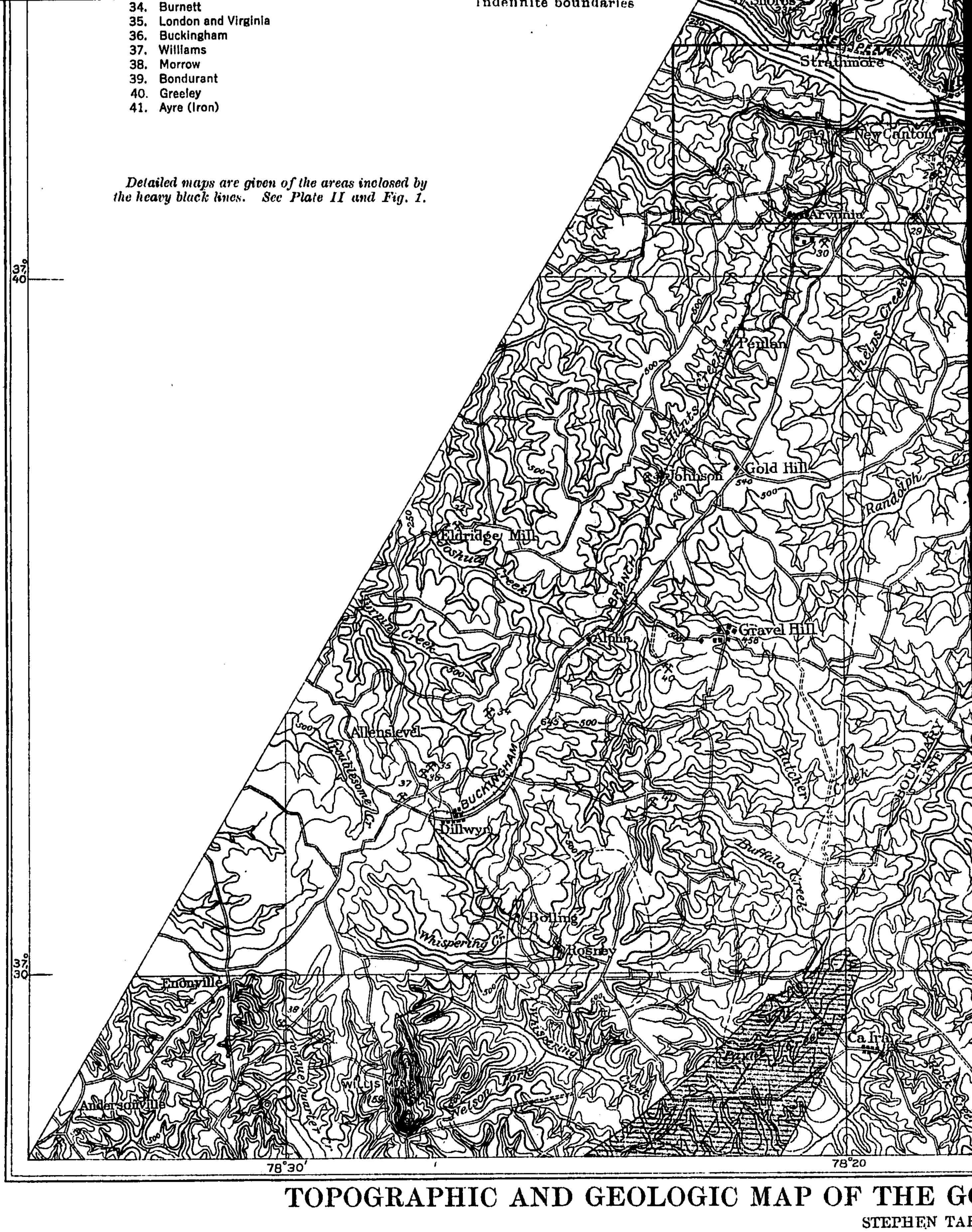
Definite and proximate boundaries

Indefinite boundaries




78°20'
78°10'
Kents Store
Yancys Store
PALMYRA
Wilmington
Tabscott
Caledonia
Carys Brook
Lantana
Stage Junction
Dixie
Fork Union Station
Fork Union
Columbia
FLUVANNA
GOOCHLAND
BOUNDARY LINE
CUMBERLAND CO
Fife
Island
Elk Island
Elk Hill
Cremona
Strathmore
Bremo Bluff
New Canton
Stearnes
Boswell
Arvonia
Gilliamsville
Flanagan Mills
Cartersville
Pemberton
BUCKINGHAM
CUMBERLAND
Bush Park
Trenton Mills
Gold Hill
Lawford
Randolph Creek
Willis River
Bolling
POWHATAN CO
GOOCHLAND CO
VIRGINIA
Shores
Bula


78°10'
78°00
37° 50'
37° 40'
Kents Store
Shannon Hill
Yancys Store
PALMYRA
Tabscott
Wilmington
Caledonia
Hadensville
Cary's Brook
Lantana
Stage Junction
Dixie
Bula
Fork Union
Columbia
Fife
CUMBERLAND CO
Island
Elk Island
Elk Hill
Cremona
Stearnes
Boswell
LUVANNA
Pemberton
Cartersville
Flanagan Mills
Stokes
BUCKINGHAM
CUMBERLAND
Bush Park
Bolling I
CUMBERLAND CO
POWHATAN CO
GOOCHLAND CO
Trenton Mills
WILLIS
Creek
Little Byrd Creek
Little Lickinghole Creek
BOUNDARY LINE
FLUVANNA
GOOCHLAND



TOPOGRAPHIC AND GEOLOGIC MAP OF THE G

STEPHEN TA

Sca

1 ½ 0 1

Contou

Datum

MAP OF THE GOLD BELT IN THE JAMES RIVER BASIN, VIRGINIA

STEPHEN TABER, Assistant Geologist

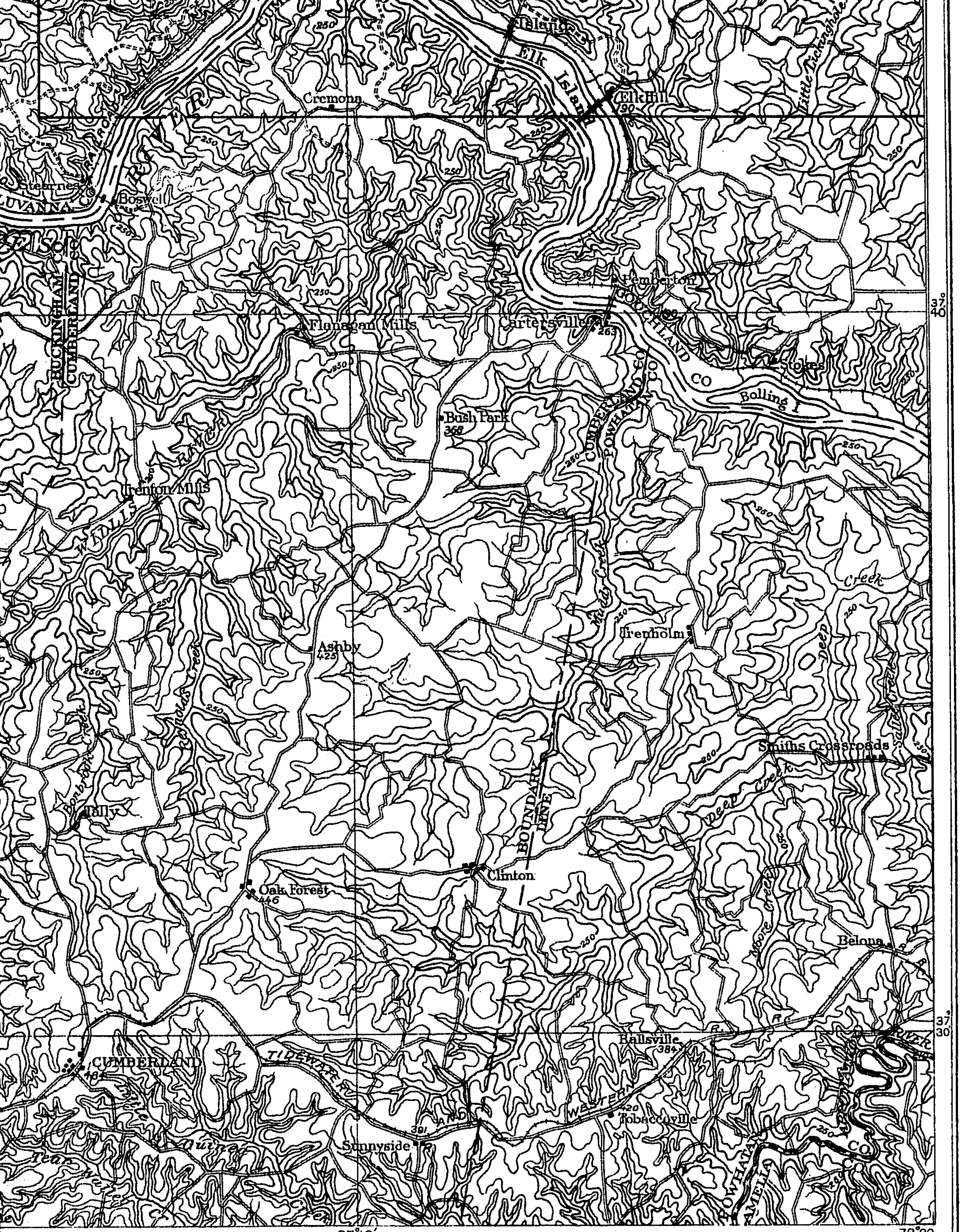
Scale: 1:137,115

1 ½ 0 1 2 3 4 5 Miles

Contour interval, 20 feet

Datum is mean sea level

1912

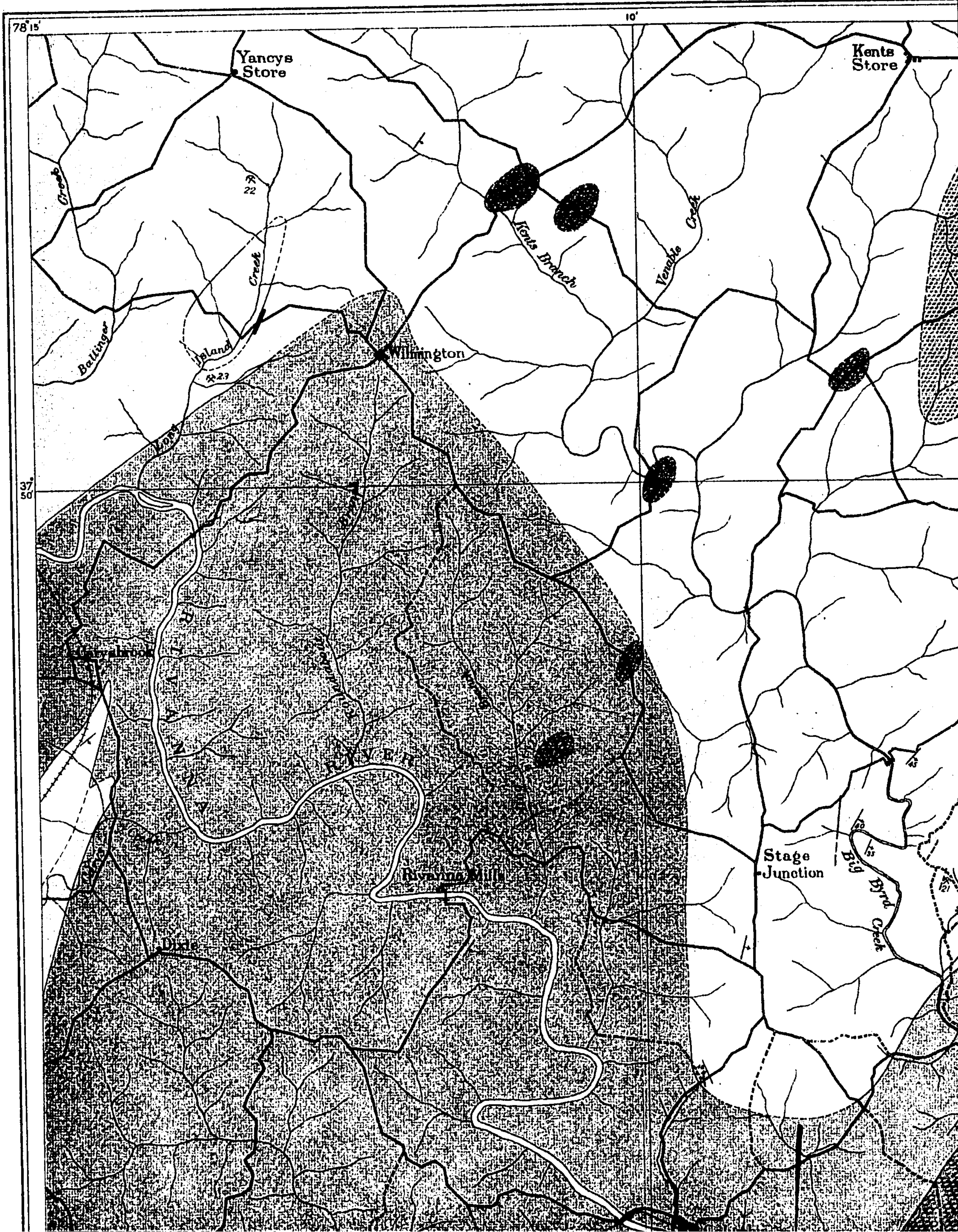


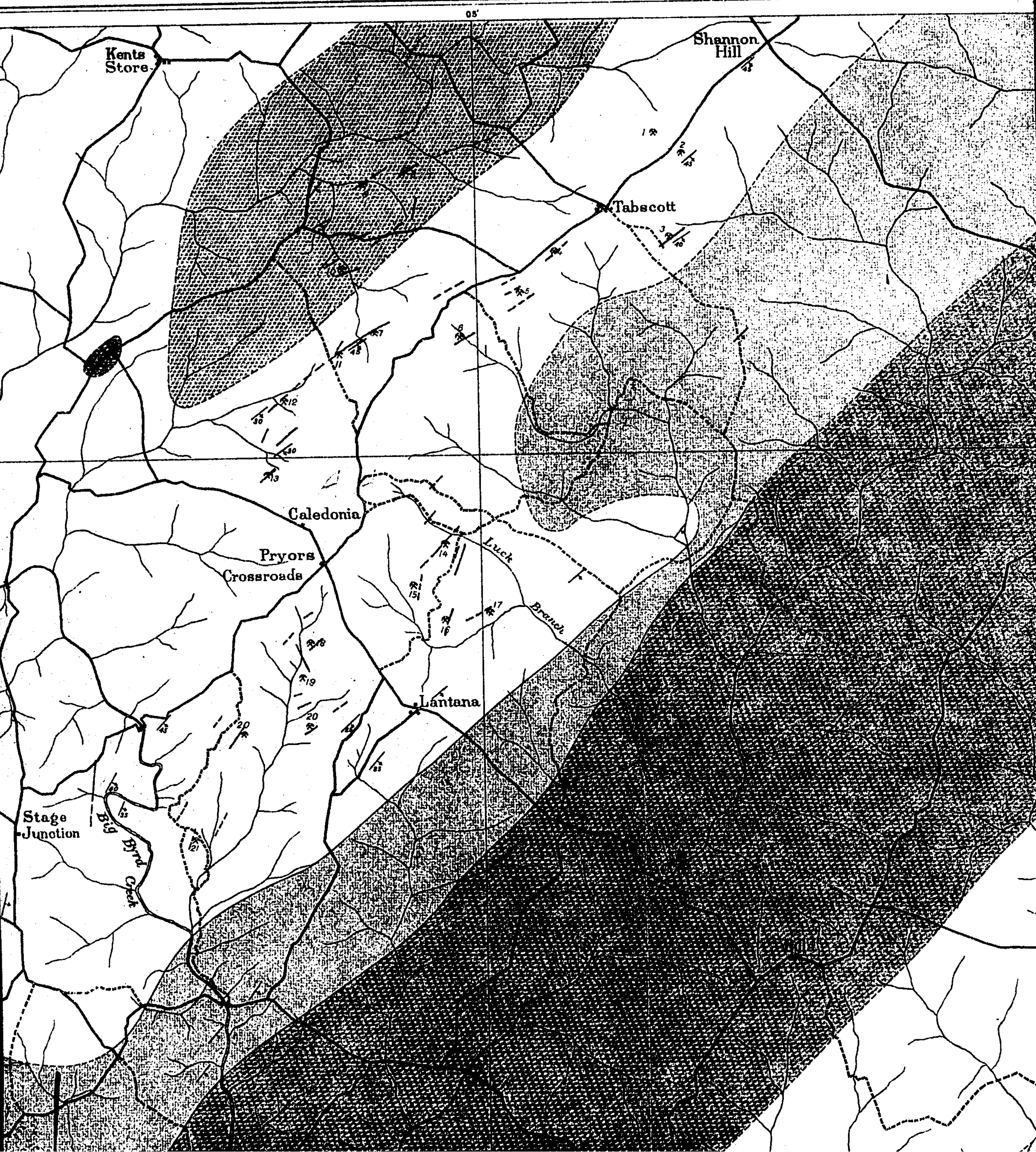
ELT IN THE JAMES RIVER BASIN, VIRGINIA

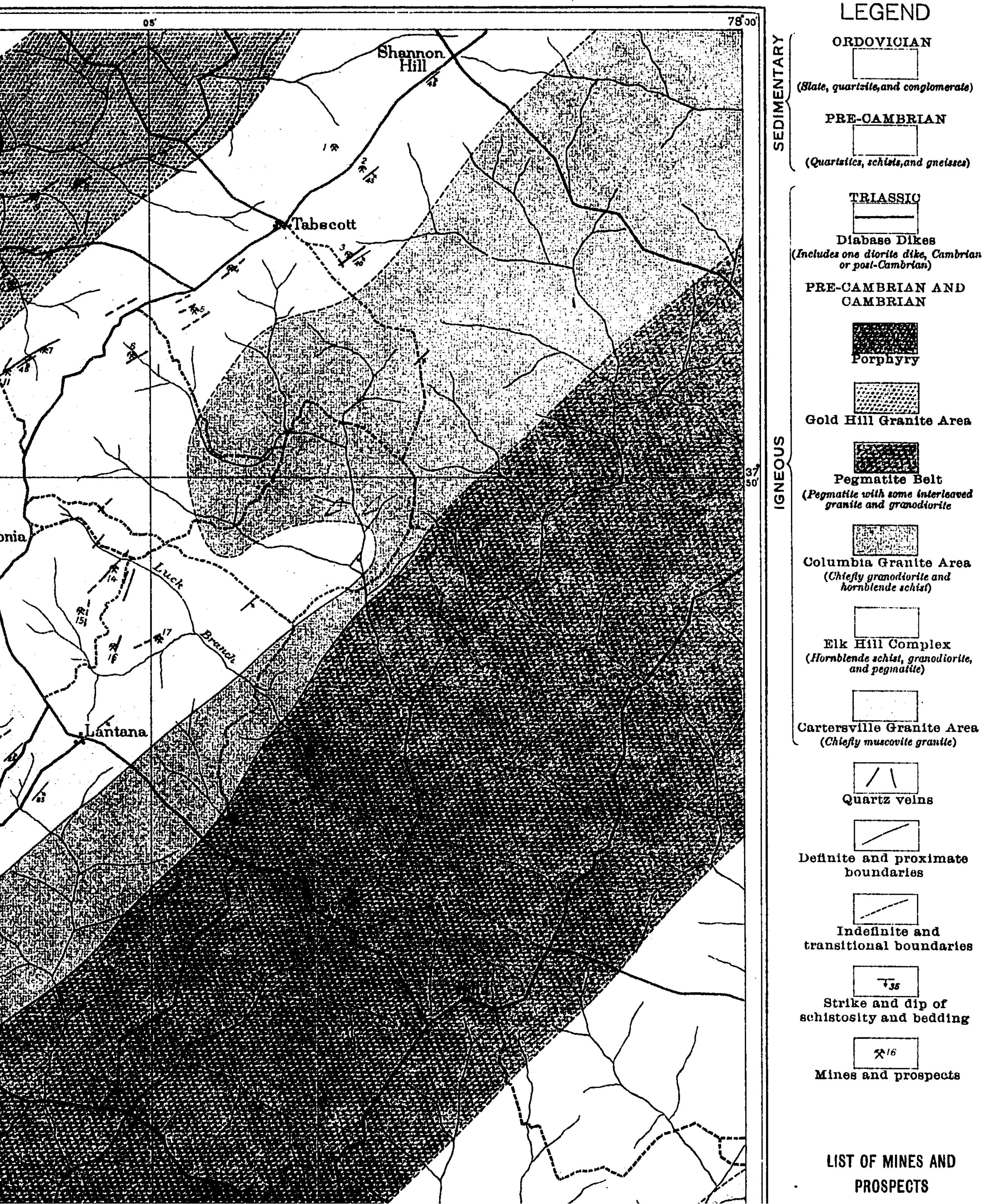
nt Geologist

4 5 Miles

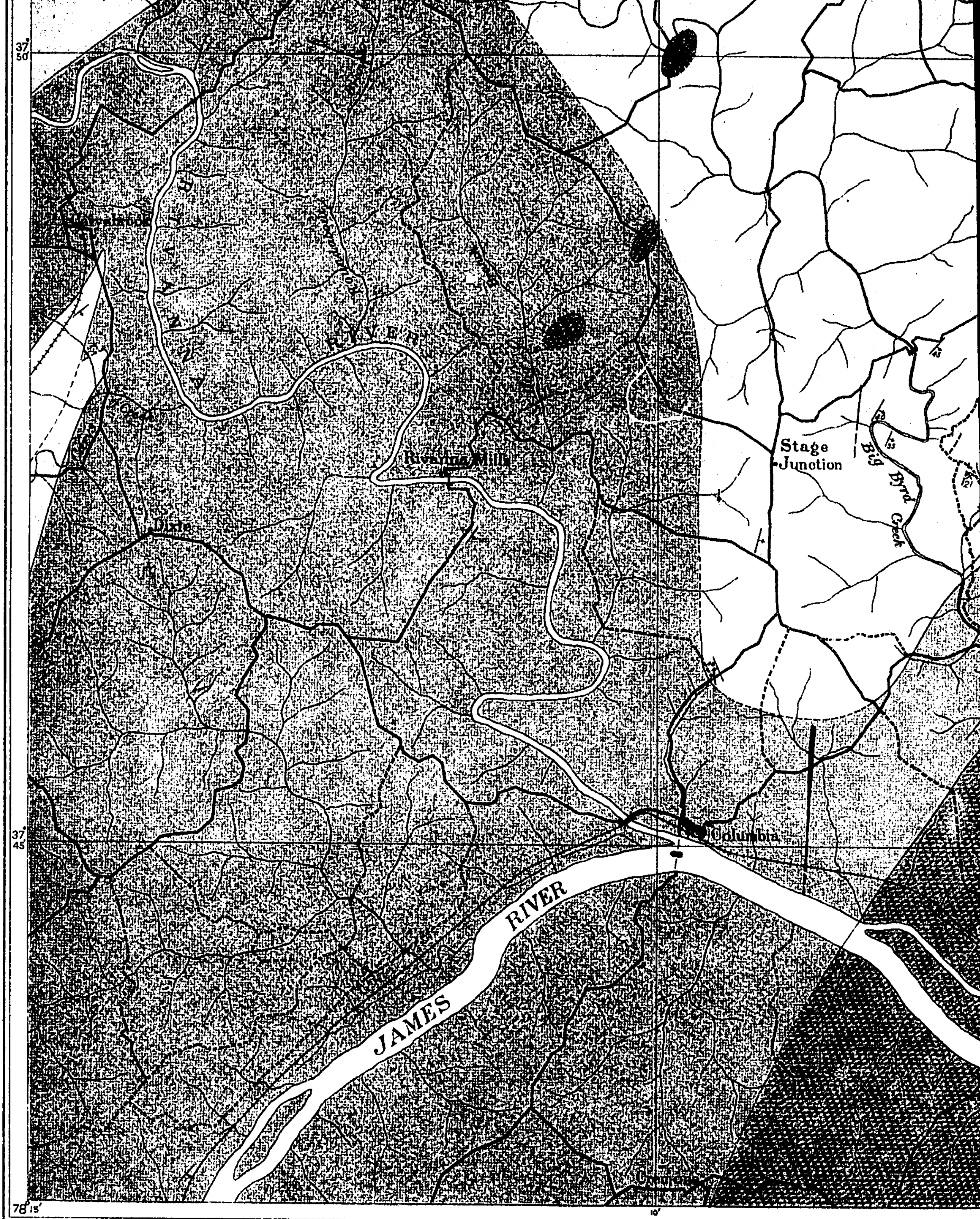
VIRGINIA GEOLOGICAL SURVEY
THOMAS LEONARD WATSON, DIRECTOR




05'
Kents Store
Shannon Hill
Tabscott
Caledonia
Pryors Crossroads
Luck Branch
Lantana
Stage Junction
Big Byrd Creek



LIST OF MINES AND PROSPECTS



DETAILED GEOLOGIC MAP OF GOLD BELT ON NORTH SIDE OF

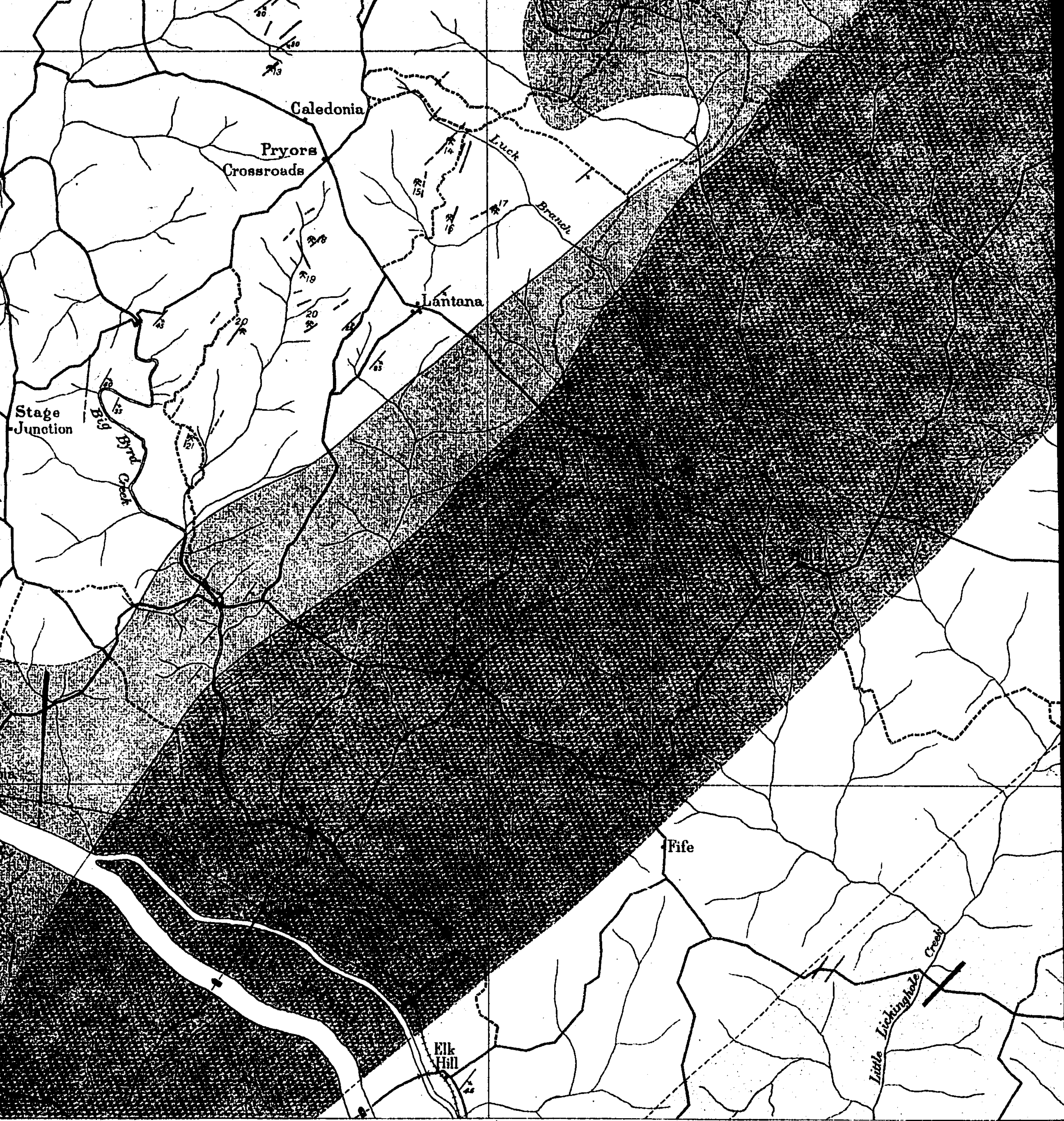
STEPHEN TABER, A

Scale: 1:62

1 ½ 0 1

1 ½ 0 1 2

191



ON NORTH SIDE OF JAMES RIVER, IN GOOCHLAND AND FLUVANNA COUNTIES

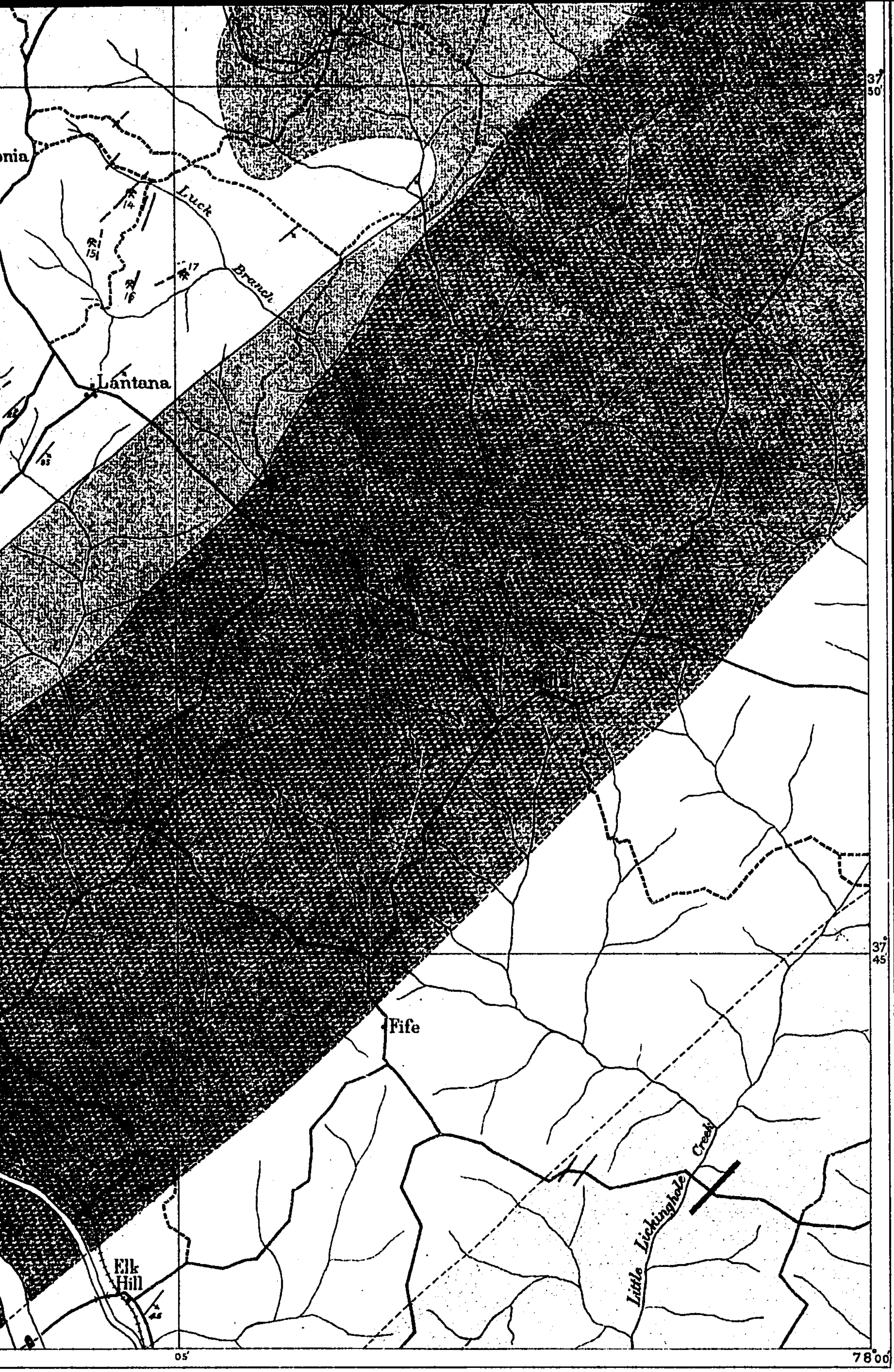
STEPHEN TABER, Assistant Geologist

Scale: 1:62,500

1 2 3 4 Miles

0 1 2 3 4 5 Kilometers

1912



Gold Hill Granite Area

IGNEOUS

Pegmatite Belt
(Pegmatite with some interleaved granite and granodiorite)

Columbia Granite Area
(Chiefly granodiorite and hornblende schist)

Elk Hill Complex
(Hornblende schist, granodiorite, and pegmatite)

Cartersville Granite Area
(Chiefly muscovite granite)

Quartz veins

Definite and proximate boundaries

Indefinite and transitional boundaries

Strike and dip of schistosity and bedding

Mines and prospects

LIST OF MINES AND PROSPECTS

1. Benton
2. Fleming
3. Waller
4. Payne
5. Busby
6. Moss
7. Bowles (Tellurium veins)
8. Bowles (Back Field vein)
9. Shaw
10. McGloam
11. Tellurium
12. Scotia (Tellurium veins)
13. Scotia (Hodges vein)
14. Young American
15. Morgan
16. Belzoro
17. Collins
18. Grannison
19. Atmore
20. Kent
21. Bertha and Edith
22. Stockton Tunnel
23. Page

VER, IN GOOCHLAND AND FLUVANNA COUNTIES

3 4 Miles

4 5 Kilometers