GLUCOSE CLEARANCE IN THE ORAL CAVITY BEFORE AND DURING ORTHODONTIC TREATMENT

by

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Submitted as partial fulfillment of the requirement
for the degree of Master of Science

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INTRODUCTION

There are still differences of opinion as to the degree of usefulness of pH and lactobacillus measurements as indices of the success of dental caries control. It has been shown that the acidity of saliva can vary greatly from individual to individual and that many factors can affect the pH of saliva.[1]

Investigations show a significant relationship between dental caries and lactobacillus content of the saliva.[2-4] Numerous studies have been done concerning diet and caries control, and it has been established that refined carbohydrates play a major role in the etiology of dental caries.[5]

It is known that the nature of the bacterial flora and the acid production can vary greatly within each individual and that many factors can influence their concentration in saliva.[6]

Since the increased use of the multi-banded applicance in orthodontics, there has been some concern as to the environmental effect of these fixed objects on the teeth and their surrounding structures.

There is need for more research to determine the overall effect of orthodontic appliances on soft

tissue, root surfaces and growth and development.

It has sometimes been assumed that multi-banded orthodontic appliances automatically increase the caries susceptibility of the patient. There is some suggestive evidence to this effect. However, there are many who maintain that the protection afforded by a steel band on a tooth is enough to compensate for any increased caries susceptibility. An extensive longitudinal study must be done with a large sample size and strict controls to determine the quantitative effect of both factors.

Much of the tedium of the older laboratory methods dealing with salivary analysis and glucose content can now be eliminated by the Auto Analyzer developed by the Technicon Company of Chauncey, New York. This study will demonstrate the value of this instrument in measuring glucose clearance in saliva.

In this study, no attempt is made to record the number of carious lesions during or after orthodontic treatment. This study is designed as a pilot investigation of the relative effect of orthodontic appliances on carbohydrate clearance in the mouth. It will provide us with an enlightened measurement of glucose retention under orthodontic treatment and create a background for extensive study of caries susceptibility related to orthodontic treatment.

REVIEW OF LITERATURE

In 1897 one of the earliest investigations on caries susceptibility was done by Miller.[7] This was part of his great pioneering investigations and consisted of comparisons of the degree of acid production of different foods. Miller was the first to identify carbohydrates as the source of tooth decalcifying acid. Walkoff confirmed these results in 1917.[8]

Some significant studies have been done on the effect of carbohydrates in the diet and glucose clearance from the oral cavity. The most comprehensive of these was that of Lundquist,[9] who compiled the following table of caries potentiality indices:

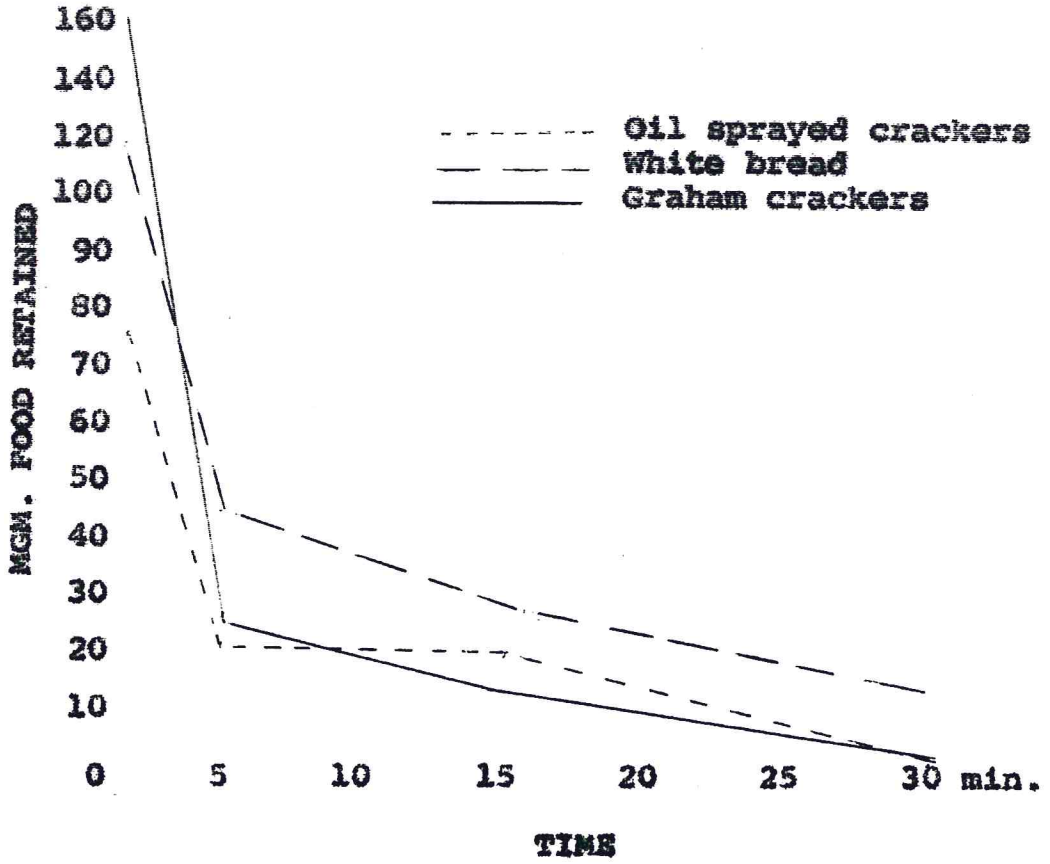
TABLE I

Lundquist's Table of Caries Potentiality Indices

Food	Total Sugar %	Sugar Concentration in Saliva Maximum %	Sugar Concentration in Saliva Av. Clearance time (min.) above 0.2%	Caries Potentiality Index
Caramel	64.0	18.8	5	27
Honey + bread + butter	19.0	4.6	7.5	24
Chocolate, light	47.5	10.1	6.25	21
Honey	72.8	5.6	5	18
Sweet cookies (biscuits)	9.0	1.9	5	18
Danish pastry	30.0	2.4	2.5	13
Wheaten bread	12.3	2.8	4	13
Ice cream	2.4	3.2	2.5	9
Marmalade	65.3	3.5	3.5	10
Marmalade + butter + bread	16.3	1.8	2.5	9
Potatoes (boiled)	0.8	1.6	2	7
Potatoes (fried)	3.9	0.4	2.5	7
White bread + butter	1.5	0.8	2	7
Coarse rye bread + butter	2.3	1.3	2	7
Milk	3.8	0.6	2	6
Apple	7.5	0.4	1	5
Orange	6.5	0.3	1	3
Fruit juice	11.5	1.2	1	3
Lemonade	9.3	0.5	0.75	2
Carrot (boiled)	2.4	0.1		1

Bibby later added to Lundquist's work, increasing the knowledge of the caries-producing potentialities of various foodstuffs. He studied the relative effect of different foods on the carbohydrate clearance of the oral cavity, this being measured by the glucose left in the salivary sediment of paraffin stimulated saliva. Glucose was used because of the ease with which it could be detected and its being a relatively good indicator of carbohydrate content.[10]

Bibby produced the following graph of three representative foodstuffs.[11]



Graph 1. Glucose Clearance of Three Representative Foodstuffs

Since the inception of Orthodontics, and particularly since the multi-banded appliance has been in wide use, there have been efforts to determine the effects of orthodontic appliances on caries susceptibility.

D. Y. Burrill, in 1940, made determinations of caries susceptibility by chemical and bacteriological tests before and during the course of orthodontic treatment.[12] Extreme caries susceptible patients became less susceptible during treatment. Patients of low susceptibility tended to become more susceptible. A third group of high average susceptibility demonstrated great variability, as changes occurred in both directions. An explanation suggested that the increased supervision afforded by the orthodontist kept the bad cases cleaner, while the appliances created increased food traps and regions of stagnation in naturally clean mouths.

In recent years, there has been further effort to determine the effect of orthodontic appliances on the oral environment. Arnold E. Bender, in May 1956, studied the effects of orthodontic appliances on the acid production potential and overall buffering capacity of saliva.[13]

A study by Bloom, Brown, and Lee of the change in the oral microbial flora produced by orthodontic appliances

METHOD OF PROCEDURE AND MATERIALS

Fifteen orthodontic patients were selected at random prior to the placement of multibanded orthodontic appliances.

Fasting saliva samples were taken from each patient prior to the start of each experiment to be used as controls.

Each patient was then given four ounces of Glucola, a beverage commonly used for glucose tolerance tests, which contains seventy-five mg. of glucose per bottle. One third were asked to drink from the bottle, one third from a glass, and one-third used a straw, the purpose being to determine if there was a difference in glucose retention in the oral cavity from these three methods.

After the beverage was consumed, paraffin stimulated saliva samples at one, three, five, and ten minutes were taken, with an attempt to obtain the same volume of saliva from each patient. The samples were filtered and analyzed for glucose by the glucose Auto Analyzer method often used in medical laboratories for analysis of blood sugar.

After a thorough brushing, without a dentifrice, and five rinses, each patient produced a second control

sample and then consumed a slice of ordinary white bread[1] spread with pure glucose jelly. The saliva samples were taken at the time intervals mentioned above. The same procedures were repeated as the patients consumed two commercially manufactured caramels.[2]

Two months after the initial experiments, the patients used for this study had a full complement of orthodontic bands placed on the teeth, and leveling arch wires in place. The same procedures from the early part of the experiment were repeated.

By comparing the data from the two analyses, we obtained a quantitative measure of increased food retention attributable to the orthodontic appliances. Furthermore, we had the opportunity to learn if there was any significant difference in glucose retention among the three methods used in consuming the liquid.

Care was taken so that the quantities of food consumed by the patients, the time intervals, and each laboratory analysis were kept identical in each set of experiments.

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1. Hough Bakery, Cleveland, Ohio.

2. Kraft Corporation.

Pure glucose in the beverage and jelly was used and the saliva samples frozen immediately following each experiment to minimize any variance due to enzymatic action of the carbohydrate. The experiment was done twice before appliances and three times following appliances as an increased check on our procedures.

In previous experiments involving salivary glucose content, a laborious and time consuming laboratory procedure was involved to obtain glucose measurement.

In Bibby, Goldberg, and Chen's paper on Evaluation of Caries Producing Potentialities of Various Food-stuffs,[17] the analytical procedure used was a typical example of those used in the past. The method in the above study used the Somogyi-Shaffer-Hartmann procedure.[18] The use of automatic analytical chemistry in this study can only be appreciated by comparison with previous techniques and is of great value in doing a large number of chemical analyses.

The Auto Analyzer continuously measures and compares on a moving graph the level of concentration of a given component in the test solution against a known concentration of that component in a standard solution. Test solutions used until this time for glucose determinations have been whole blood, serum, plasma, urine, and cerebrospinal fluid. Never before has saliva been

used as a test solution.

No volumetric or gravimetric measurement is involved using the autoanalyzer, rather it is a continuous plotting of ratios (the concentration of the sought material in the unknown against its known concentration ratio in the standard control).

Once the analyzer has been set up, it is completely automatic. The samples are picked up, pumped along, and mixed with a flowing stream of diluent. The diluted sample then moves through a dialyzer where its diffusible constituents are separated and fed into a flowing reagent stream. In glucose determinations the stream is passed through a colorimeter, and the result permanently recorded on a moving graph.

Figure 1 shows an autoanalyzer set up with all its component parts. From right to left are the reagent used in the test, sample plate, proportioning pump and mixing coil, dialyzer, heating bath, colorimeter, and recorder.

Sample plate - As the loaded sampling plate rotates (anywhere from 20, 40, or 60 analyses per hour) a hinged pick up crook dips into each sample cup in succession aspirating its contents for a given interval and feeding the sample into the system. At the proper instant, the pick up crook automatically lifts out while the sampling plate rotates, then dips into the next

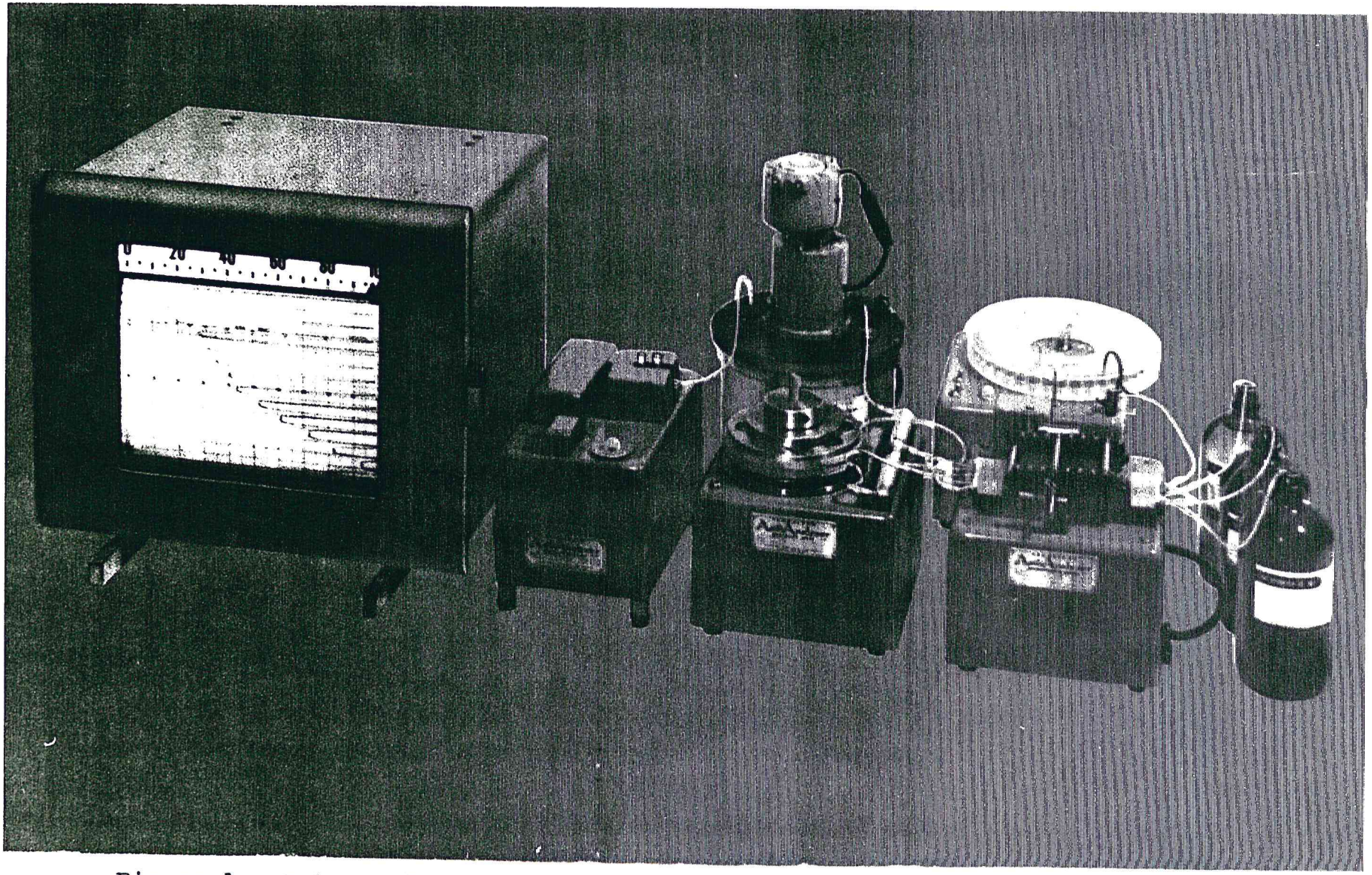


Figure 1. Auto Analyzer set up.

right to left: 1. reagents used in the test, 2. sample plate, 3. proportioning pump and mixing coil, 4. dialyzer, 5. heating bath, 6. colorimeter and recorder.

sample, and so on, repeating this in and out action until all the samples on the plate have entered the instrument following each other in a flowing stream.

During the periodic intervals, between its dips, the aspirating crook sucks in air which acts as an insulating barrier between samples as they flow along the analyzing route. The time ratio of drawing sample vs. drawing air is roughly 2-1. Thus each sample travels through the autoanalyzer as a separate entity kept from contact with its neighbors by air barriers. Known standards are interspersed with the unknown samples at periodic intervals. In their travel through the system, both knowns and unknowns will be subjected to exactly the same conditions from beginning to end, and the standards act as infallible control and afford a definitive check on the accuracy of the analytical reading.

Herein lies one of the salient advantages of the autoanalyzer concept. In conventional chemistry procedures, standards, are made up to correspond to the process after the separatory stages of filtration or precipitation or distillation. Thus losses or gains incurred in these preliminary stages before the standards are applied are reflected in inaccurate readings. In the autoanalyzer standards and unknowns are treated alike under identical conditions, so the

comparison is absolute.

The Proportioning Pump can continuously pump up to eight separate fluids and/or gases simultaneously, while varying their individual delivered output in any ratio up to 8 to 1. The pump consists of two parallel stainless steel roller chains with spaced roller thwarts that bear continuously against a spring loaded pump platen. Across this platen lies a set of flexible tubes whose different lumina determine the rate of flow through each. At each point in the system where two liquids come together there is a Mixing Coil. The purpose of this mixing is to provide a homogeneous diluted sample for dialysis.

Dialysis in the autoanalyzer is a continuous function of concentration. There are no variables other than the concentration of the sought material in the unknown samples.

The dialyzer is comprised of a matching pair of transparent plastic plates whose surfaces are mirror grooved to provide a continuous channel when the plates are brought into contact. With a cellophane membrane sandwiched between them, the plates are clamped together leaving the continuous pathway separated only by the semi-permeable membrane. The flowing stream of unknown enters the upper half of the spiral; while the stream of the color developing reagents enter the lower half.

In the course of their parallel travel, the diffusible constituents of the unknown pass through the semi-permeable membrane to enter into the flowing reagent stream. On completion of the circuit, the reagent stream emerges containing a proportion of the total volume of diffusible constituents in the unknown sample. The remainder of the sample passes off to waste. It is not necessary to bring dialysis to completion. If both time and area of dialysis exposure are the same for both standards and unknowns, the ratio of the total amount of material dialyzed will vary only as its concentration.

After leaving the dialyzer the flowing stream of reagent may join another reagent such as a catalyst or color developer. The mixed stream then flows to the Heating Bath. Here is where the color reaction is developed. The heating bath maintains a temperature of 95 degrees C plus or minus one-tenth of a degree. The important thing is to keep the temperature constant so both standards and unknown will react alike.

From the heating bath, the solution travels to the colorimeter which continuously monitors the concentration level of the samples flowing through it. The instrument is a dual beam type utilizing two separate photoelectric cells, one as a reference the other for measuring. The Recorder operates as an integral part of the colorimetric

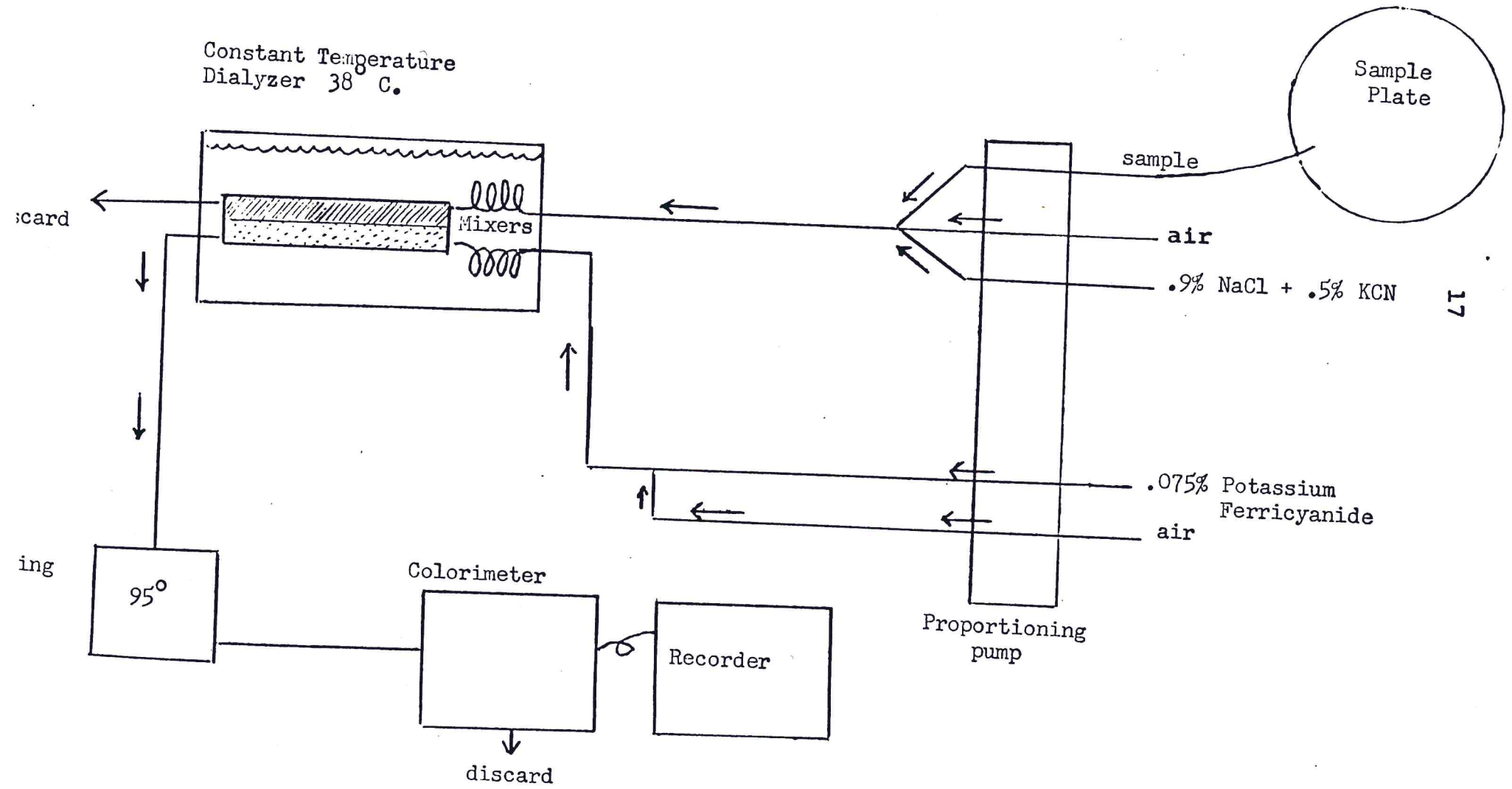
circuit. It comprises a balanced ratio system in which the unknown and standard samples are continuously recorded against a fixed reference.

The method employed here for glucose determination is a modification of one proposed by W. S. Hoffman.[18] The glucose is determined by a direct reading procedure utilizing the Potassium Ferricyanide-Potassium Ferrocyanide oxidation reduction reaction. This yellow solution of Potassium Ferricyanide is reduced to the colorless Ferrocyanide in the presence of Potassium Cyanide which acts as a sensitizing agent. In this procedure, one measures a loss of color of the Potassium Ferricyanide. (Inverse colorimetric technique) A calibration graph is obtained by plotting per cent transmissions of standards vs. concentration on semilog paper. The per cent transmission obtained for the sample is converted to mg. per 100 ml. by reading the corresponding value from the calibration graph. This gives mg. per 100 ml. of glucose present in the specimen.

Figure 2 shows a flow diagram of the analytical arrangement for glucose determination.

FIGURE 2. Auto Analyzer

flow diagram
for
glucose



STATISTICAL ANALYSIS

T tests for significance were conducted on the experimental data.

BEVERAGE

When the mean values before and after appliances for the time glucose concentration curve were tested, substantial differences were found. In every instance, the second mean was higher than the first, and the magnitude of the difference was highly significant, that is substantially below the .1% level.

BREAD AND JELLY

The after values were again higher than the before values. With the exception of the point at ten minutes on the curve, all the differences were highly significant at below .1% level. The point at ten minutes was significant at the .3% level.

CARAMEL

For the caramel curve, the three and five minute points were significant at the 10% level. The ten minute point was significant at the .1% level and the one minute and zero points were well below the .1% level.

$\bar{X}_1$ = average of sample before appliances

$\bar{X}_2$ = average of sample after appliances

^{2} = variance

N = number of samples

$\bar{X}$ = $\bar{X}_2$ minus $\bar{X}_1$

$\bar{X}$ = standard deviation between two means

Table 2

STATISTICAL ANALYSES BEFORE APPLIANCES

	Time	X	2	N
Beverage	0	1.11	11.18	27
	1	504.81	393820.59	27
	3	129.96	43841.91	27
	5	11.74	249.30	27
	10	1.69	14.39	26
Bread and jelly	0	0.74	5.20	27
	1	824.22	167625.22	27
	3	369.81	38212.63	27
	5	81.81	6487.67	27
	10	17.70	1133.81	27
Caramel	0	1.81	17.56	27
	1	1102.40	670100.13	27
	3	567.44	72439.57	27
	5	160.33	21562.19	27
	10	26.14	320.73	27

Table 3

STATISTICAL ANALYSES AFTER APPLIANCES

		X	2	N
Beverage	0	11.56	124.62	41
	1	1711.58	909,656.83	41
	3	326.82	67,811.50	40
	5	63.26	4,261.88	41
	10	18.36	450.10	41
Bread and jelly	0	10.58	64.87	41
	1	2540.31	1,263,468.16	41
	3	629.22	160,740.17	41
	5	238.43	37,825.91	41
	10	48.17	3,035.32	41
Caramel	0	21.48	830.30	41
	1	2540.70	1,174,005.72	41
	3	688.04	141,024.98	41
	5	211.54	38,160.26	41
	10	53.34	2,674.19	41

Table 4

PROBABILITY OF ERROR CHART - BEFORE AND AFTER SAMPLES

		$\bar{X}$	x^2	$\bar{X}$	s	
Beverage	0	9.75	3.54	1.88	5.18	.001
	1	1206.77	37,888.00	194.00	6.22	.001
	3	196.86	3,425.00	58.50	3.36	.001
	5	51.52	116.12	10.78	4.77	.001
	10	16.67	11.82	3.44	4.84	.001
Bread and jelly	0	9.84	1.82	1.35	7.28	.001
	1	1716.09	38,034.00	195.00	8.80	.001
	3	259.41	5,488.00	74.10	3.50	.001
	5	156.62	1,195.00	34.60	4.52	.001
	10	30.47	119.98	11.00	2.77	.003
Caramel	0	20.30	21.42	4.60	4.41	.001
	1	1973.26	55,123.00	234.70	8.40	.001
	3	120.60	6,312.00	79.40	1.51	.010
	5	51.20	1,783.00	42.20	1.21	.010
	10	27.20	79.18	8.90	3.05	.001

FINDINGS AND DISCUSSIONS

In doing a study employing a group of children between the ages of eleven and thirteen and having them perform certain functions relative to the development of the study, it became quite apparent that there would be a great deal of individual variation. Upon calculating the final results, our thoughts were substantiated. The reader will notice the individual patient graphs in the appendix and note these variations.

The reasons for such variations were many. Under normal circumstances it is very difficult to repeat an experiment in exactly the same way as the preceding one. The laboratory analysis presented little problem in duplication, but the actual collection of saliva involving the variable moods and attributes of the children presented a different problem. This is why we did the before-banding experimentation twice and the after-banding three times. It is evident from tables 5 through 9 that there was some variation in each experiment.

We attempted to obtain the same volume of saliva from each individual. For the most part, this was accomplished, but occasionally, one of the youngsters

Table 5

GLUCOSE CONCENTRATION IN SALIVA, 1st EXPERIMENT BEFORE APPLIANCES

	B4 Beverage	1 min	3 min	5 min	10 min	B4 Bread and jelly	1 min	3 min	5 min	10 min	B4 Caramel	1 min	3 min	5 min	10 min
A	0	55	0	0	0	0	428	392	71	0	0	625	480	207	22
B	0	120	37	0	0	0	630	522	210	150	0	740	420	139	10
C	0	29	0	0	0	0	520	239	120	54	0	836	627	421	55
D	0	250	94	20	14	0	681	364	14	0	0	720	432	36	8
E	0	960	195	70	0	0	584	147	10	0	0	820	640	400	36
F	0	228	25	0	0	0	408	250	18	0	0	980	832	164	20
G															
H	0	1000	17	5	0	0	1000	460	200	8	5	1500	550	30	8
I	0	420	75	24	63	10	1200	800	275	0	0	800	534	53	13
J	8	1300	640	15	5	5	1120	440	27	8	0	1000	592	100	8
K	0	250	80	0	0	0	1440	500	56	0	0	1200	252	25	8
L	0	1470	620	10	0	0	1660	400	36	0	0	5000	1680	250	31
M	0	162	99	34	0	0	665	440	36	0	0	1080	354	76	34
N	0	175	65	10	0	0	726	321	41	16	0	835	421	44	12
O	0	143	44	0	0	0	645	294	30	0	0	967	612	148	69

Table 6

GLUCOSE CONCENTRATION IN SALIVA, 2nd EXPERIMENT BEFORE APPLIANCES

	B4 Beverage	1 min	3 min	5 min	10 min	B4 Bread and jelly	1 min	3 min	5 min	10 min	B4 Caramel	1 min	3 min	5 min	10 min
A	0	78	20	0	0	0	550	420	210	50	6	640	450	350	48
B	0	136	24	0	0	0	439	254	178	84	0	900	721	625	28
C	0	150	21	10	0	0	720	96	12	0	0	1021	589	274	20
D															
E	0	349	59	14	0	0	629	125	26	0	0	821	436	150	49
F	0	244	39	0	0	0	360	22	20	8	8	950	780	162	29
G	0	109	21	0	0	0	328	75	41	6	0	821	638	180	34
H	14	1650	120	25	7	5	1800	500	250	18	10	2000	625	27	7
I	8	850	71	20	10	0	750	625	75	8	0	610	500	95	10
J	0	2650	820	30	8	0	1500	720	50	25	16	950	192	13	8
K	0	240	78	0	0	0	1280	629	62	38	10	1100	325	25	10
L															
M	0	275	88	10	0	0	589	235	45	0	0	950	425	65	20
N	0	187	95	20	0	0	824	430	68	5	0	1010	685	140	58
O	0	150	52	0	0	0	783	285	34	0	0	839	529	130	43

Table 7

GLUCOSE CONCENTRATION IN SALIVA, 1st EXPERIMENT AFTER APPLIANCES

	B4 Beverage	1 min	3 min	5 min	10 min	B4 Bread and jelly	1 min	3 min	5 min	10 min	B4 Caramel	1 min	3 min	5 min	10 min
A	26	1300	585	30	17	15	428	1260	450	30	10	4750	1560	320	70
B															
C	0	1600	150	50	10	10	3000	900	450	130	100	2300	400	200	95
D	25	784	420	24	15	6	3515	1420	75	15	10	3745	825	450	100
E	0	925	380	75	24	5	2980	875	358	43	15	4150	875	425	65
F	0	1700	150	30	16	15	3000	1250	455	50	14	4700	1175	500	110
G	18	3200	850	100	55	20	4100	1200	625	260	140	2050	1275	700	135
H	2	3800	300	40	22	7	3100	355	157	58	15	2450	600	112	27
I	0	3755	1355	210	42	40	3900	750	316	26	12	1150	950	600	265
J	0	1475	90	10	0	0	1480	67	35	0	0	1800	332	76	10
K	16	260	150	40	13	9	2200	350	150	14	10	2300	375	120	20
L															
M	0	1200	150	40	9	8	1400	125	40	8	7	1750	150	30	10
N	16	2150	185	35	6	0	2275	386	174	64	12	1981	732	148	96
O	0	2330	365	62	21	10	2400	850	70	25	10	3500	986	65	20

Table 8

GLUCOSE CONCENTRATION IN SALIVA, 2nd EXPERIMENT AFTER APPLIANCES

	B4 Beverage	1 min	3 min	5 min	10 min	B4 Bread and jelly	1 min	3 min	5 min	10 min	B4 Caramel	1 min	3 min	5 min	10 min
A															
B	0	5000	600	110	14	6	6250	700	500	70	30	1750	550	175	20
C	0	600	89	25	24	18	3100	725	345	75	25	1200	650	58	40
D	25	700	30	16	15	15	800	420	40	6	12	875	248	32	14
E															
F	18	1050	40	16	15	14	2600	185	100	68	22	3400	1125	300	100
G	25	2350		40	6	0	1600	455	250	25	22	1600	555	257	108
H	30	2350	120	23	20	16	1000	30	6	0	11	800	150	17	0
I	26	1000	422	25	20	15	1400	250	10	5	0	1900	250	30	5
J	0	1600	124	12	23	11	1400	50	50	0	0	800	400	56	19
K	25	1250	250	76	18	15	800	1500	58	23	10	1425	234	100	12
L	27	1280	463	270	40	25	1400	600	235	80	23	3000	1000	42	22
M	0	1325	276	54	8	8	2165	550	184	35	25	2630	334	17	20
N	12	1452	150	421	21	16	2500	389	150	7	7	2630	436	65	30
O	10	1900	285	74	10	0	3100	390	100	50	18	2435	620	155	40

Table 9

GLUCOSE DETERMINATION IN SALIVA, 3rd EXPERIMENT AFTER APPLIANCES

	B4 Beverage	1 min	3 min	5 min	10 min	B4 Bread and jelly	1 min	3 min	5 min	10 min	B4 Caramel	1 min	3 min	5 min	10 min
A	10	1420	567	19	10	5	3650	1425	555	28	10	4720	1500	350	85
B	0	1800	576	118	34	10	3550	737	516	64	27	2780	720	180	32
C	0	1200	130	42	16	5	2980	835	455	110	40	2500	534	140	52
D	14	737	257	25	9	6	3225	1280	62	12	8	3540	500	420	60
E	8	740	310	34	10	6	2560	930	584	47	12	3720	680	435	68
F	0	2250	94	38	19	12	2430	1090	475	59	23	4380	1200	580	112
G	12	2570	780	140	38	25	3640	1100	628	235	110	2300	1340	680	125
H	35	2750	130	37	16	5	2150	234	140	76	22	2000	724	115	42
I	10	1225	410	30	16	7	2400	310	25	6	5	2900	380	65	10
J	17	1525	110	20	6	0	1530	84	62	15	5	1930	540	131	20
K	26	1120	310	52	10	5	724	632	110	12	4	1228	334	94	16
L	0	1340	520	310	32	19	1239	580	210	62	19	3180	1220	50	25
M	4	1450	370	84	12	5	2400	620	289	15	8	3100	721	180	59
N	18	1500	180	37	25	15	2620	412	172	18	6	2750	480	69	28
O	29	2212	350	79	16	5	3250	368	110	49	22	2160	550	134	38

failed to produce the minimum volume of 2 cc between the one, three, and five minute intervals. Some patients would collect the minimum volume much sooner (C and F) while in others (G and K) they would just get finished with the three minute collection in time to begin the five minute collection.

This was noted in only a few instances, but nevertheless, it contributed to the variability of the sample.

The subjects were asked to present themselves fasting before each experiment so as to minimize the initial glucose concentration. In some cases a trace of glucose was found in the initial control sample. This was noted on table 5, page 24, sample J1, also table 6, page 25, samples H and I. This was increasingly apparent as seen in tables 7, 8, and 9. In these tables, many initial samples had traces of glucose. The fairly high trace of glucose in the initial sample (above 25 mg./100 cc.) probably betrayed poor brushing habits or the subject could have consumed food shortly before the experiment. It followed that in all three experiments after the appliances were placed, that in one or two individuals the initial samples showed that glucose concentration was always high.

The patients were required to brush their teeth thoroughly and rinse at least six times between each change of food, such as after the ten minute beverage sample and before the initial sample of the bread and jelly. The variability in doing this is evident when one studies the experimental results. Some patients exhibited poor brushing techniques consistently, as in all of the experiments their initial sample before each representative food, was high.

Methods of mastication and swallowing would also produce some variability in the experimental results. It is reasonable to assume that the longer a bolus of food remains in the oral cavity, the greater the chances of increased retention. Again variation was noted in the length of time one individual would masticate compared with another. One patient had an extremely difficult time swallowing caramels. She retained them in the mouth many times longer than the average. With some of the children, a couple of rapid mandibular movements were all that were necessary to prepare the food for esophageal entrance, while others would incessantly move the bolus from side to side, making maximum contact with all possible retentive surfaces. No attempt was made in this study to classify the subjects as to this category, but, needless to say, this contributed to the variability of our results.

With the use of the autoanalyzer, our laboratory procedures were quite consistent. The large differential of glucose concentrations involved made it necessary to dilute some of our extremely high samples, which of course increased the possibility of human error. The dilution involved measuring, then passing through the autoanalyzer, and calculating to compensate for the original dilution. If an error was made in dilution or in reading from the autoanalyzer graphs, the possibility of its being magnified many times via the calculation for the dilution was inherent.

As noted on tables 5 through 9 there are a number of specific glucose concentrations which appear irregular as compared to the sequence of the majority of the data. On table 8, page 27 B 2 is abnormally high; K 8 obviously is an error due to the human factors explained above. D 11 and H 11 on this same table are not consistent with the D 10 and H 10 of the ten minute samples previously mentioned, and the initial sample prior to ingestion of the caramels. The error is slight, to be sure, and of little significance in the over all picture.

The fact that most of these irregularities occurred in the same set of samples, namely the second time the experiment was performed after orthodontic appliances were present indicates perhaps a little less refined

procedure than the other groups, and demonstrates the value of repeated experimentation when there are a number of variables involved.

It is interesting to note, that in the repeated experiments, the actual concentration of glucose of the same individual differed each time, but for the most part the plateau or range of the initial spike on the graphs and the rates of descents from the one minute samples were fairly constant. On pages 56 through 100 are listed the average glucose concentration time graphs of each of the subjects studied.

On these graphs and all other graphs in the paper the numerals following the letters indicate which part of the experiment is shown. The numeral one indicates the beverage, two, the bread and jelly, three the caramels. The broken line represents the glucose concentration before orthodontic appliances, and the single line depicts the concentration after bands and archwires are in place. There is one exception. Patient J, whose graphs appear on pages 65, 80, and 95 was accepted for treatment and was used during the initial experiments for this study. It was later determined that his mal-occlusion and eruption sequence did not lend themselves to banding at that time, hence no appliances were placed. We decided to use him anyway during the second part of our experimentation as a check

on our procedures, as there was a two and a half month interval between them; although we did not use the results in averaging the graphs for the "after" portion of this study.

J 1, page 65, indicates a higher concentration during the first experiment. J 2, page 80, is almost identical, and J 3, is nearly so except for a variation at the one minute level.

Table 10 represents the average glucose concentrations of the beverage part of the experiment. The patients are grouped into those who consumed the beverage by the methods listed. Graph 2, page 35 represents the three groups prior to banding. Graph 3, page 36 depicts the glucose concentration after appliances. The single line represents the bottle, a large broken line, the straw, and a small broken line represents the cup. In the first sequence before braces, it will be noted that those who drank directly from the bottle had the highest glucose retention. This was followed by those who used the straw and the lowest retention came from those who drank from a cup. In the sequence after braces, the results are inconclusive. This time the straw is slightly higher than the bottle, and again the cup is low. There is an indication, even from this small sample, that by drinking from a glass or cup, one will have less retention of caries-causing

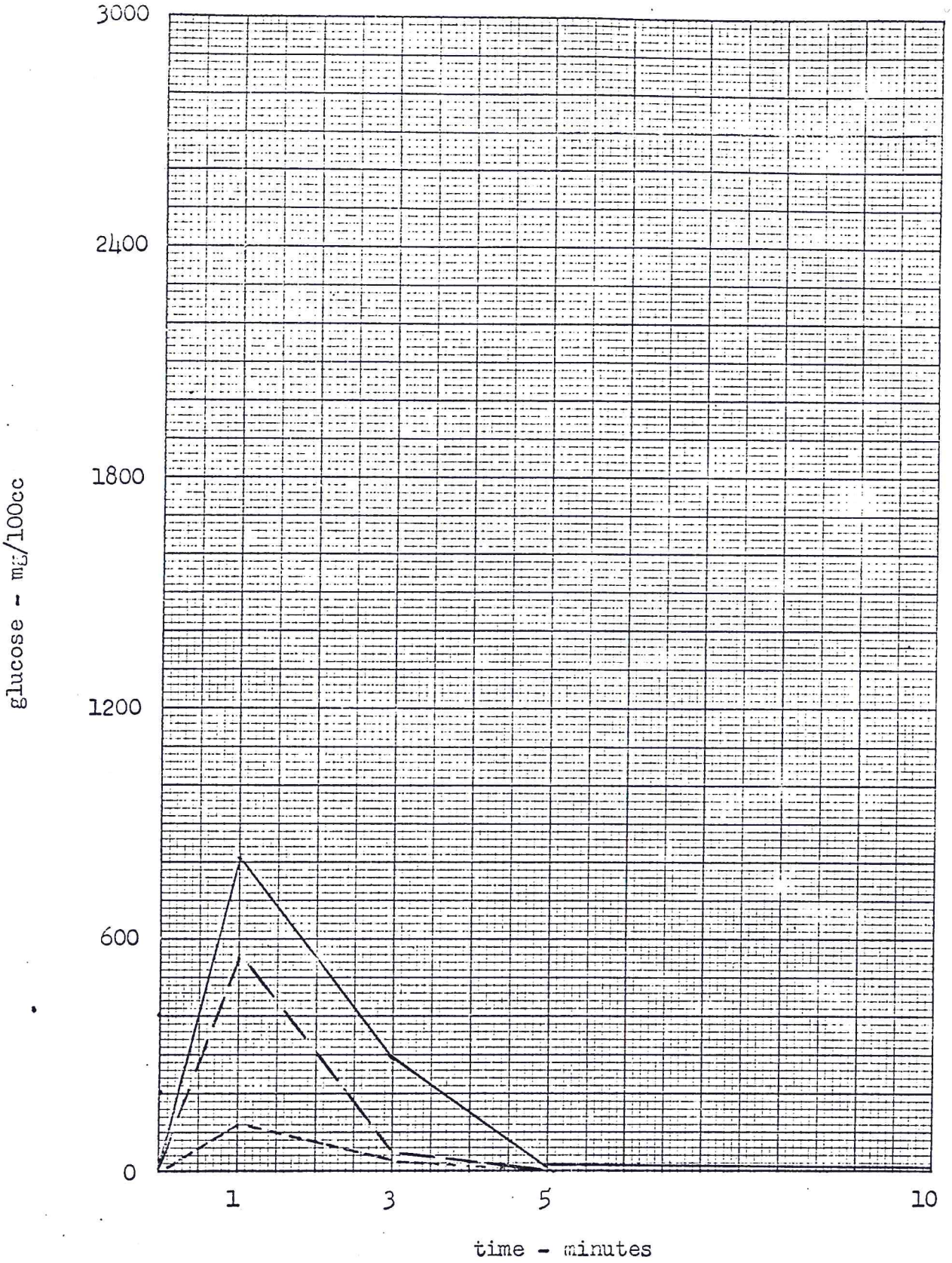
Table 10

AVERAGE GLUCOSE CONCENTRATIONS OF THE CUP, STRAW, AND BOTTLE METHODS OF CONSUMING THE BEVERAGE

	CUP					STRAW					BOTTLE				
Minutes	0	1	3	5	10	0	1	3	5	10	0	1	3	5	10
Glucose mg/100 cc.															
Before	0	131	40	7	0	2	559	71	5	1	2	329	284	13	3
After	12	1682	219	51	14	15	1916	249	49	21	6	1763	392	84	19

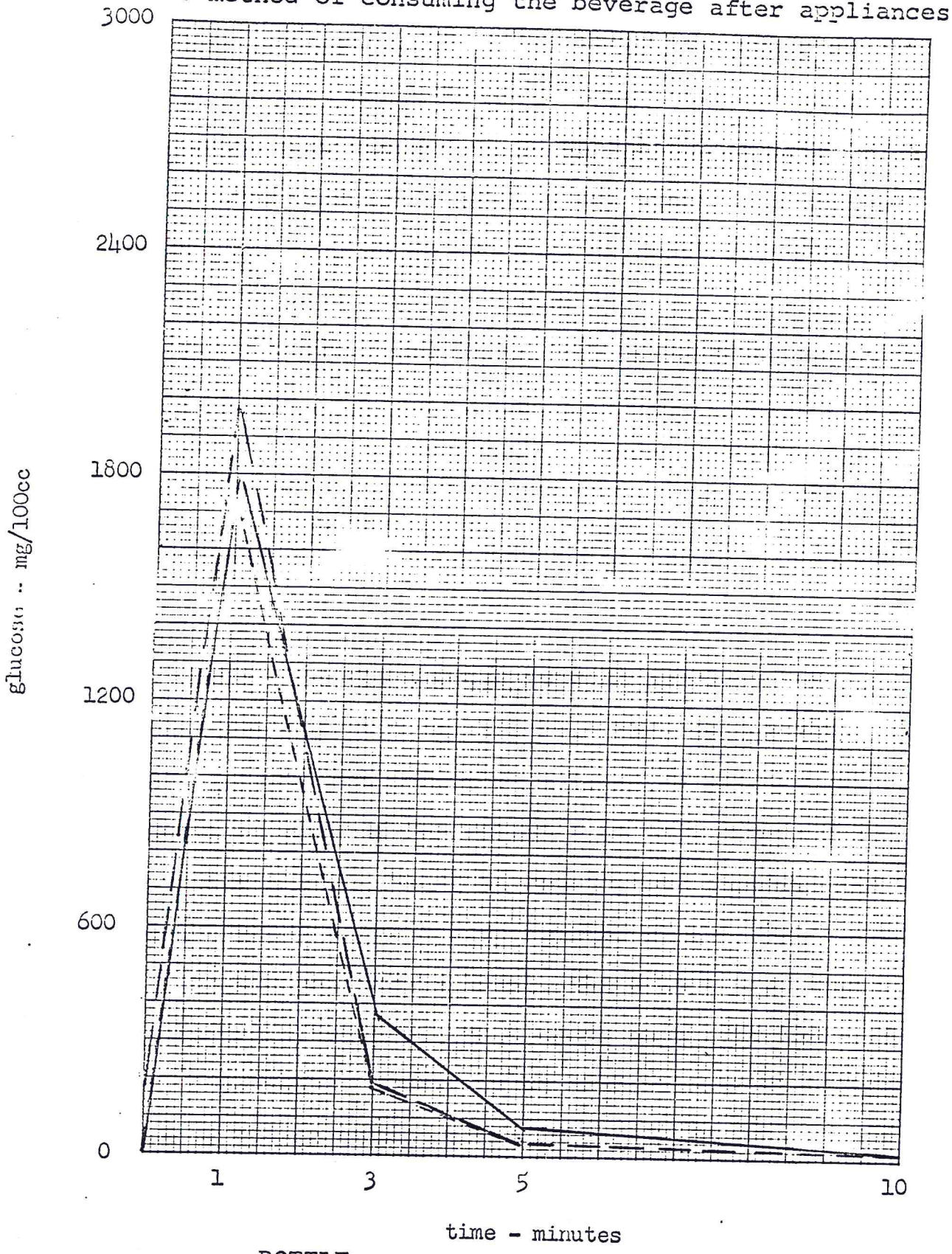
Graph 2

Time glucose concentration graph of cup, straw, and bottle method of consuming the beverage before appliances.



Graph 3

Time glucose concentration graph of cup, straw, and bottle method of consuming the beverage after appliances



substances than from the other methods.

Graph 4, page 38 shows the average before and after glucose concentrations of the patients as they consumed the beverage. Graph 5, page 39 is the same graph except that each line represents 10 mg/100 cc. instead of 20.

Below is a table showing the exact concentration in mg/100 cc. from which the average graph was made.

Table 11

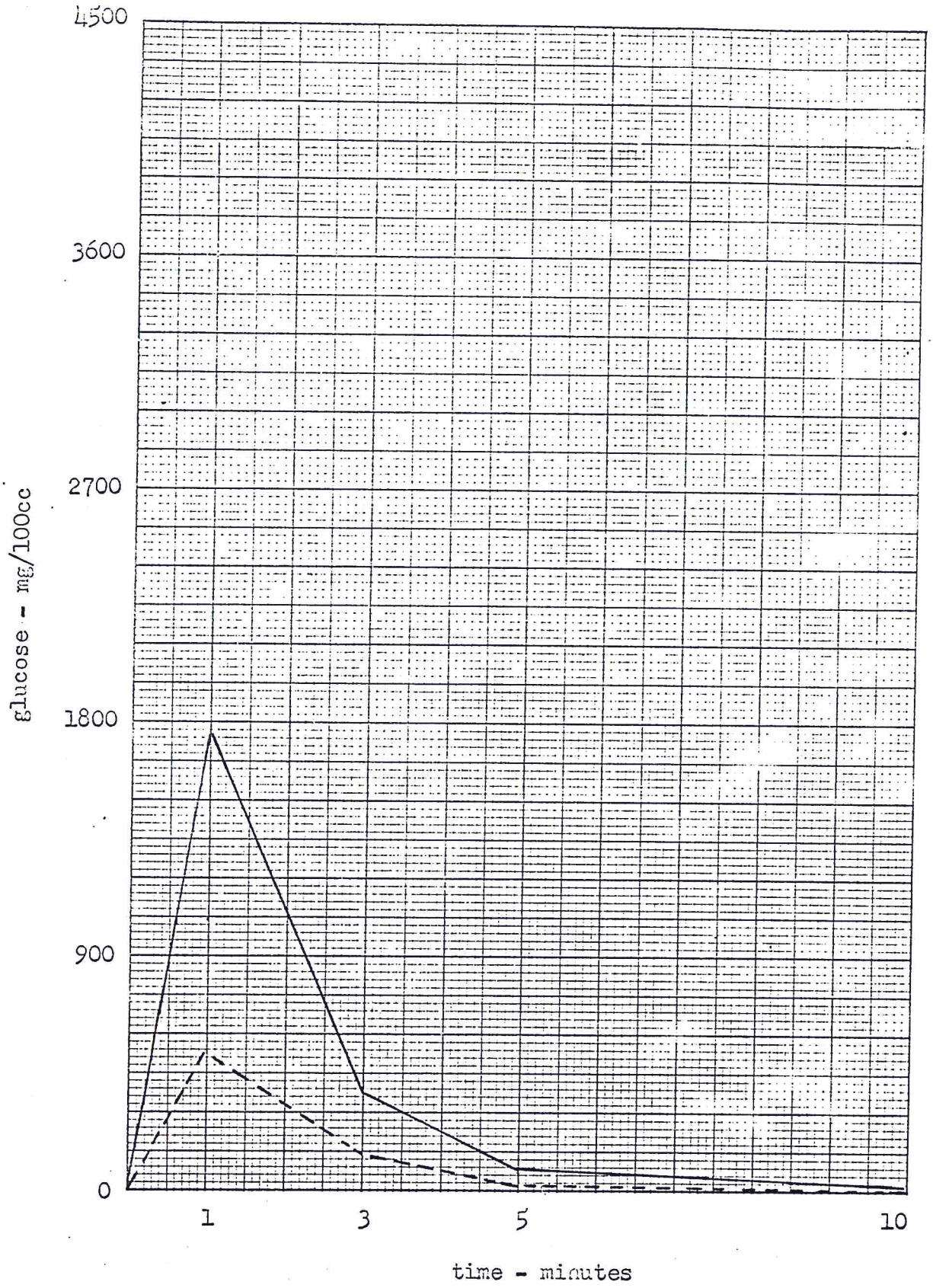
AVERAGE GLUCOSE CONCENTRATIONS OF BEVERAGE EXPERIMENT

	0	1 min.	3 min.	5 min.	10 min.
Before appliances	1	536	140	12	4
After appliances	11.5	1760	379	70	19

Note that the average initial sample after appliances was 11.5 mg./100 cc. This is a relatively small amount, but indicates a trace of glucose is present in some of the patients as they appeared for the experimentation. This, of course, was expected due to the increased surface area created by the appliances.

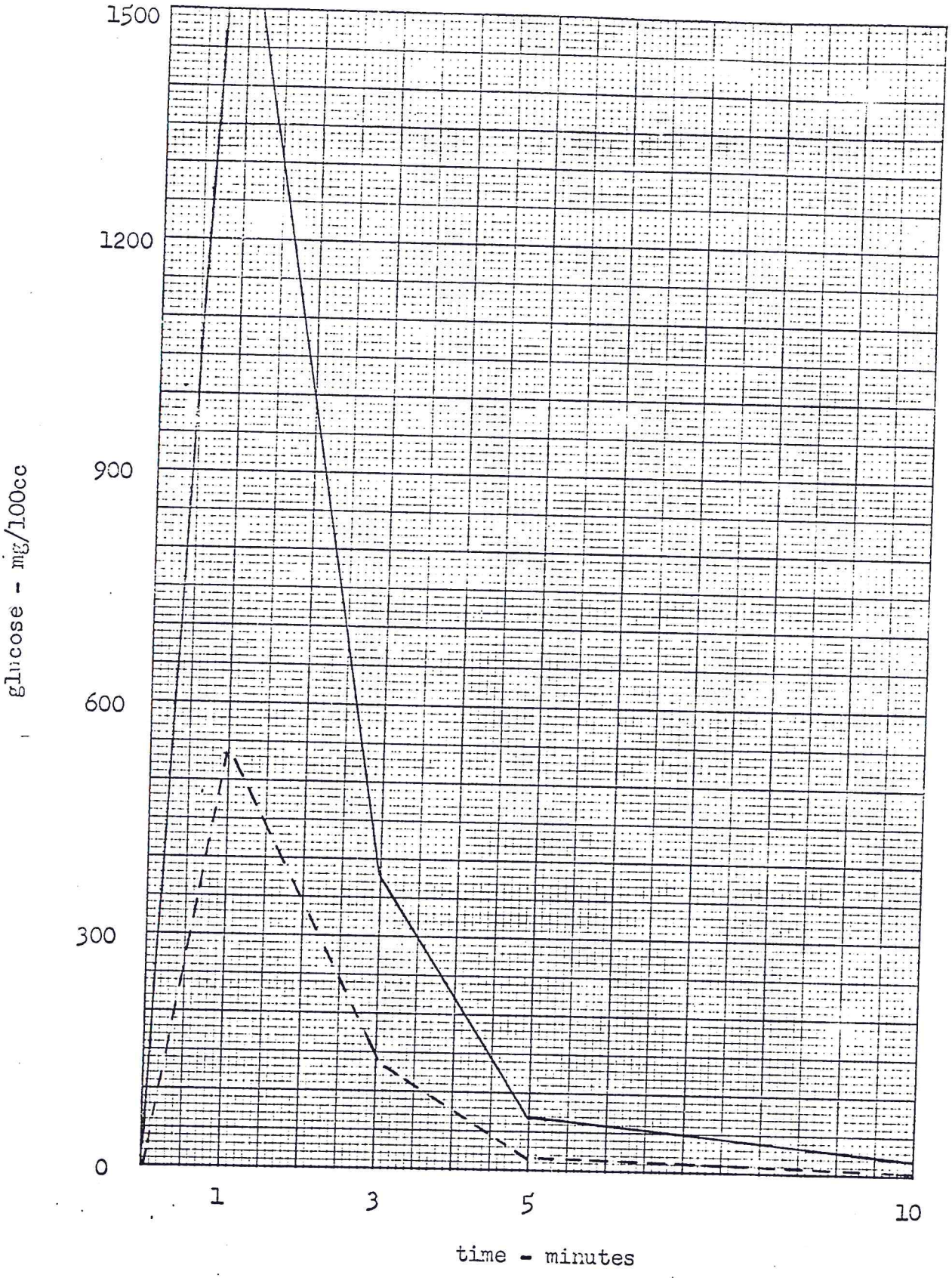
At the one minute level, there is a 228% increase in glucose retention. This drops to a 170% increase at the three minute level, up to 483% at five minutes and 475% at ten minutes. There is a rate of fall of 690 mg/100 cc. per minute between one and three minute levels. Between the three and five minute level, the

Average time glucose concentration graphs of beverage experiment



Graph 5

Average time glucose concentration graphs of beverage experiment, one line equals 10 mg./100 cc.



rate of fall slows to 150 mg./100 cc. per minute. Between the five and ten minute level, there is a drastic change in the rate of fall. It is 10 mg./100 cc. per minute. The rate of fall before appliances is 198 mg./100 cc. per minute between the one and three minute level, then to 65 mg./100 cc. per minute and finally 1.5 mg./100 cc. per minute between the five and ten minute intervals.

Pages 56 to 70 in the Appendix contain the individual graphs for the beverage experiment. They all show the same general pattern except for J, as already explained, and L. Patient L showed a slightly higher concentration before appliances with a steady drop. At the four minute level, the concentration of glucose without appliances drops below the concentration with appliances.

Below is a table showing the average concentrations following the ingestion of the bread and jelly.

Table 12

AVERAGE GLUCOSE CONCENTRATION OF BREAD AND JELLY EXPERIMENT

	0	1 min.	3 min.	5 min.	10 min.
Before appliances	0	815	362	73	16
After appliances	12	2745	724	264	49

The graph on pages 42 and 43 are the corresponding graphs.

Note that the zero concentration of glucose after appliances is nearly the same as with the beverage. At the one minute level the increase after appliances is 239%. This diminishes to 100% at the three minute level, up to 234% at the five minute interval, and 206% after ten minutes.

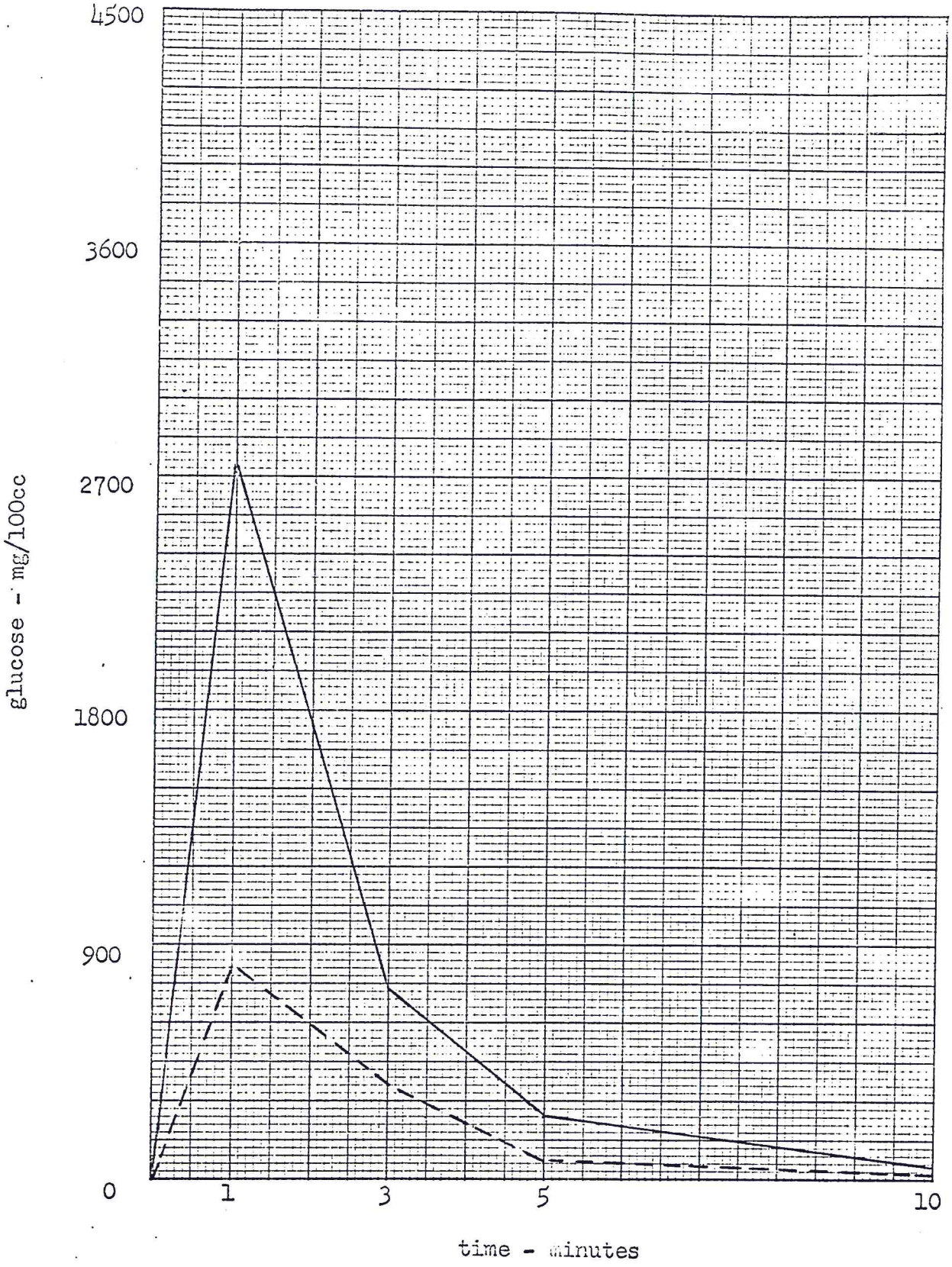
The rate of fall between one and three minutes before appliance is 226 mg./100 cc. per minute. From three to five minutes it slips to 145 mg./100 cc. per minute. Between five and ten minutes the rate of fall is 11 mg./100 cc. per minute. The rate of fall after appliances is 1,010 mg./100 cc. per minute at one and three minute intervals, 240 between three and five minutes and 37 between five and ten minutes.

The individual graphs for this part of the experiments can be found on pages 71 to 85 in the Appendix. Graphs H, I, K, and L deviate slightly from the majority.

Below is the table of averages after the mastication of the caramels.

Graph 6

Average time glucose concentration graphs of bread and jelly experiment



Graph 7

Average time glucose concentration graphs of bread and jelly experiment, one line equals 10 mg./100 cc.

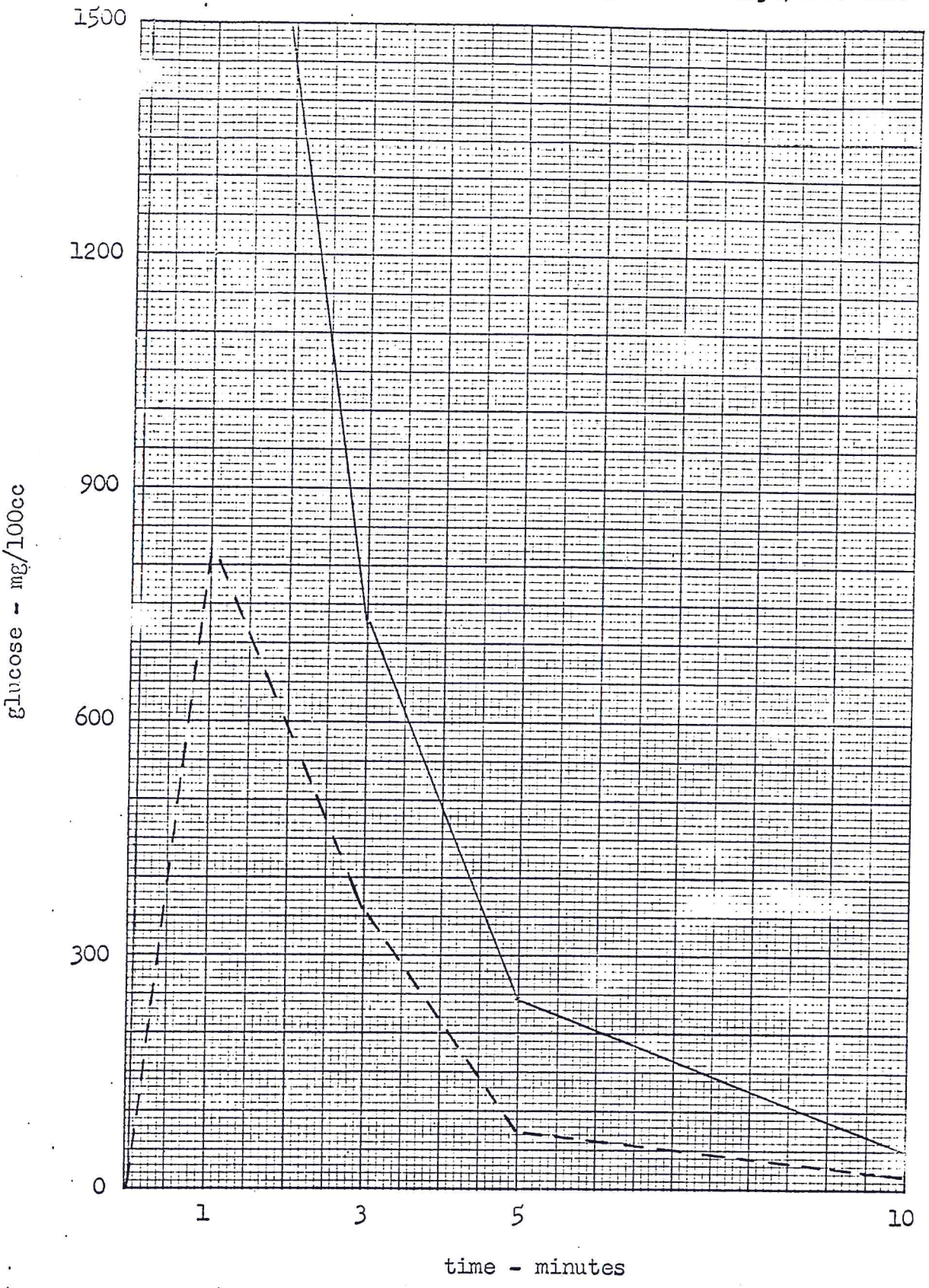


Table 13

AVERAGE GLUCOSE CONCENTRATION OF CARAMEL EXPERIMENT

	0	1 min.	3 min.	5 min.	10 min.
Before appliances	2	1208	587	155	36
After appliances	22	2558	714	209	57

On pages 45 and 46 are the corresponding graphs. This time the trace of glucose prior to beginning this experiment is double that of the beverage and bread and jelly.

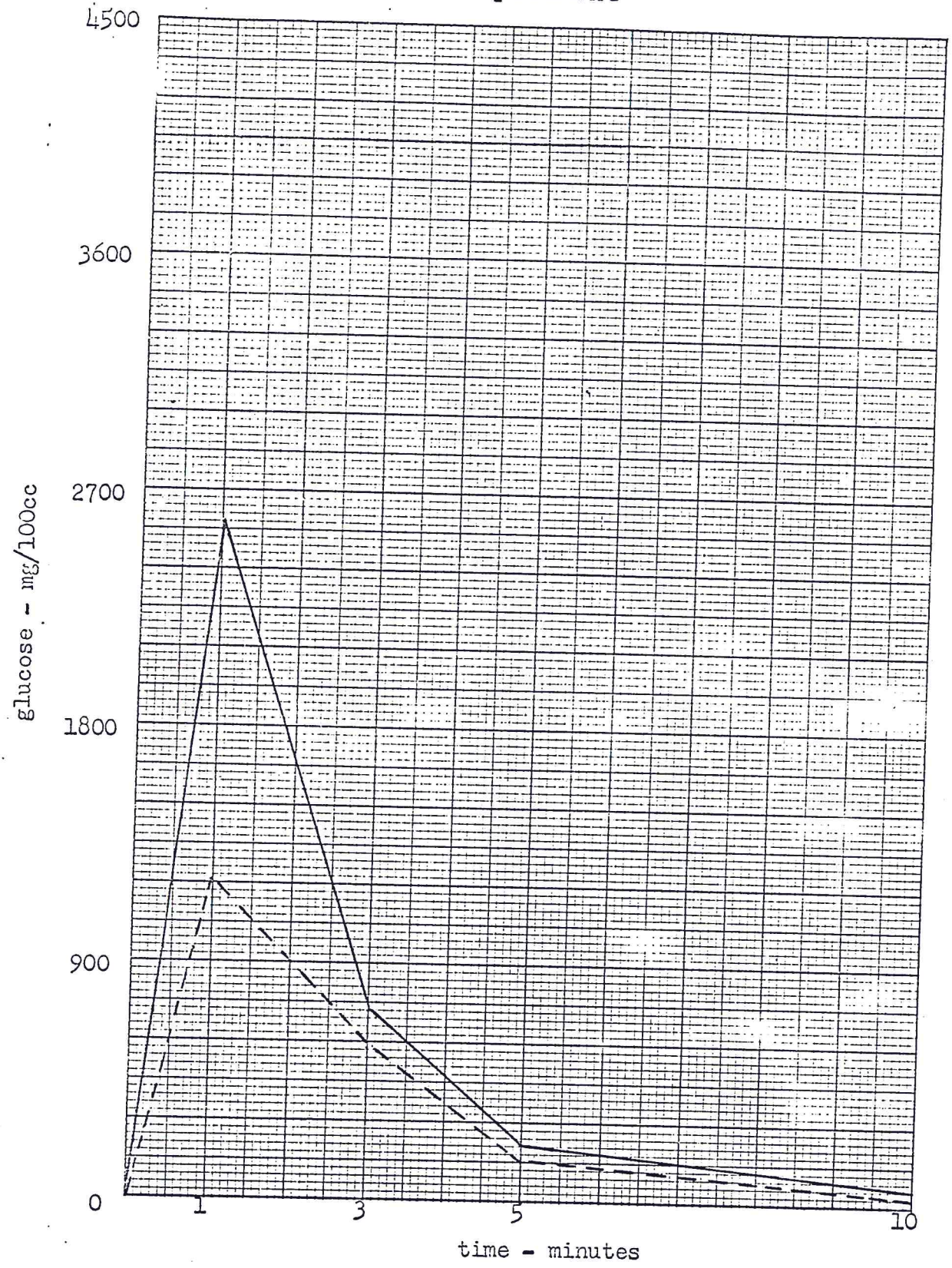
At one minute the increased concentration of glucose during orthodontic treatment was 112% that of before treatment. At three minutes 21%; five minutes 100%, and 119% at ten minutes.

Before appliances the rate of fall per minute was 335 mg./100 cc. between one and three minutes; 215 mg./100 cc. between three and five, and 26 mg./100 cc. between five and ten minutes. After appliances they were 911, 253, and 31 respectively.

By observing closely the individual graphs of the caramels portion of the experiment, on pages 86-100 of the Appendix, one can note there are more variations than in the previous two parts.

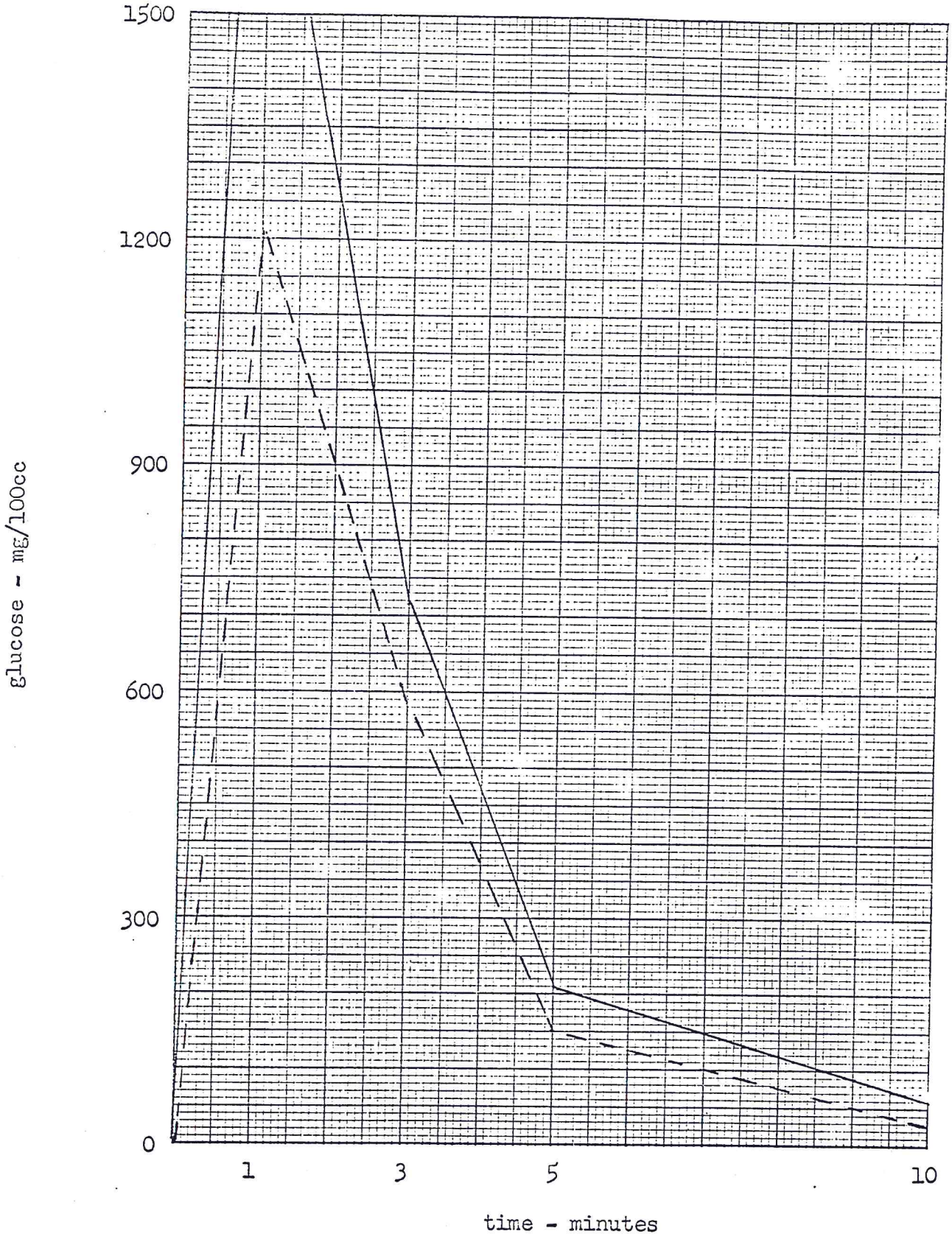
Graph 8

Average time glucose concentration graphs of caramel experiment



Graph 9

Average time glucose concentration graphs of caramel experiment, one line equals 10 mg./100 cc.



It is particularly interesting to note the similarities between B 3, C 3, J 3, M 3, O 3, as in each, the concentration during orthodontic treatment dips slightly below the glucose level after three to five minutes prior to treatment.

In graphs of D 3, F 3, G 3, I 3, the rate of fall prior to treatment is slower at the one to three minute interval than the three to five minute interval. This is the only time in the entire study where this type of reading occurred. The phenomenon was probably due to the tacky consistency of the caramels, causing the food to be retained in the mouth for a longer period of time before a more rapid dissemination.

SUMMARY AND CONCLUSIONS

Fifteen orthodontic patients were selected at random prior to treatment. They were asked to consume at stated intervals three representative foodstuffs, a beverage, glucola, containing 75 mg. of glucose per bottle, white bread spread with glucose jelly, and finally two commercially manufactured caramels.

For each type of food used, one initial and four subsequent saliva samples were taken at one, three, five, and ten minutes. These samples were analyzed for glucose concentration by the use of the Auto Analyzer. The same procedure was followed two months later, after each patient received a multi-banded orthodontic appliances cemented in place.

The glucose concentrations of the before and after samples were compared to determine the differences in the rate of clearance and the amount of retention created by the orthodontic appliances. A further study was done to analyze the various methods of consuming liquids, via bottle, straw, and cup, in relation to glucose clearance.

In the before portion of the study, the cup produced the least retention. The bottle produced the most. During orthodontic treatment, the results were incon-

clusive, as all were nearly the same, although the cup was still lower than the other two methods.

As would be expected, there was a large increase in glucose retention after the orthodontic appliance was placed. The table below summarizes the percent increase of each part of the experiment at the one, three, five, and ten minute intervals.

Table 14

PERCENT INCREASE OF GLUCOSE CONCENTRATION DURING ORTHODONTIC TREATMENT

		1 min.	3 min.	5 min.	10 min.
% increase of glucose during orthodontic treatment	beverage	228	170	483	475
	bread and jelly	273	100	244	49
	caramel	112	21	35	119

Note that at the one minute level the increased percentage of the bread and jelly was highest. The caramel was low due to the high retention rate of the before appliance samples. This follows also at the three and five minute levels. Since the sticky or tacky foods were well retained by unbanded teeth, the percentage increase in the one to five minute period due to banding, tends to be lower than that seen for the other two foods. However, they are retained as long and the actual glucose

concentrations are just as high. Again, the main difference is that these foods have a normally high retention without orthodontic appliances.

In all of the samples there was a high increased percentage at the one minute level, a quick drop off at the three minute level, an increase at the five minute level. This increase after 5 minutes in all three foods was due to a faster rate of clearance in the before samples and a slower rate of the "after" samples, causing a higher percent increase at the five minute interval. At the ten minute level, the beverage showed a very high retention again, the cause being due to the quick clearance of the beverage without any appliances. The glucose concentrations of the beverage sample was still lower, but the percent increase from the original was higher.

Table 15 below shows the rates of fall per minute of the samples. The numerals are in mg./100 cc.

Table 15

AVERAGE RATE OF FALL PER MINUTE AS GLUCOSE CONCENTRATION BEFORE AND DURING ORTHODONTIC TREATMENT

		Intervals of Time		
		1-3 min.	3-5 min.	5-10 min.
Beverage	Before	198	65	1
	After	690	150	10
Bread and jelly	Before	226	145	11
	After	1010	240	39
Caramels	Before	335	216	26
	After	911	253	31

In all cases the rate of fall at the one to three minute level was high. It decreased considerably at the three to five minute interval and tapered off gradually after five minutes.

In summary, we can say that:

1. In the early stages of mastication the effect of orthodontic appliances on glucose retention is rather marked. Within one minute glucose concentration increases roughly 200%.
2. The rate of clearance is also very great, so at three minutes it decreased by half to about a 100% increase.
3. The rate of fall after three minutes is quite constant for both the before and after samples.
4. At five and ten minutes, the saliva, after appliances, contains about twice as much glucose as the before appliances.
5. Tacky foods such as caramels, are not retained at the same high increase as the other representative foods due to their large retentive qualities without orthodontic appliances.
6. It is significant to note that there are some influences counteracting the effects of increased carbohydrate retention during orthodontic treatment. It is evident from clinical experience that ortho-

REFERENCES

1. Haggard, H. A., and Greenberg, L. A.: Acidity of Saliva after Ingestion Carbohydrate and Acid Containing Substances, J. D. Res. 30: 126, Feb. 1951.

2. Orland, F. J.: An evaluation of Bacteria and Antibiotics in the Dental Caries Process, Mil. Surgeon 106: 345, May 1950.

3. Rogosa, M.: A Review of Current Studies in the U. S. Concerning the Relation of Micro-organisms to Certain Dental Diseases, J. D. Res. 39: 1105, 1960 (Abst.).

4. Bibby, B. G.: Lactobacillus and Dental Caries, J.A.M.A. 175: 739, 1961.

5. Jay, P.: The Role of Sugar in the Etiology of Dental Caries, J.A.D.A. 27: 393, March, 1940.

6. Bloom, Rodney H., Brown, Lee R., Jr.: A Study of the Effects of Orthodontic Appliances on the Oral Microbial Flora, Oral Sur., Oral Med., Oral Path., May 1964.

7. Miller, W. D.: Die Mikro-organisms der mundhohleed, Leipzig George Themes 1962, page 199.

8. Walkoff, Otto: Unser Brot als wichtigste Ursache der Zahnkaries. Muenchner med. Wachschrft. 64: 1007, 1917.

9. Lundquist, Claes: Oral Sugar Clearance, its Influence on Dental Caries Activity, Odont Revy 3 Supplement 1, 1952.

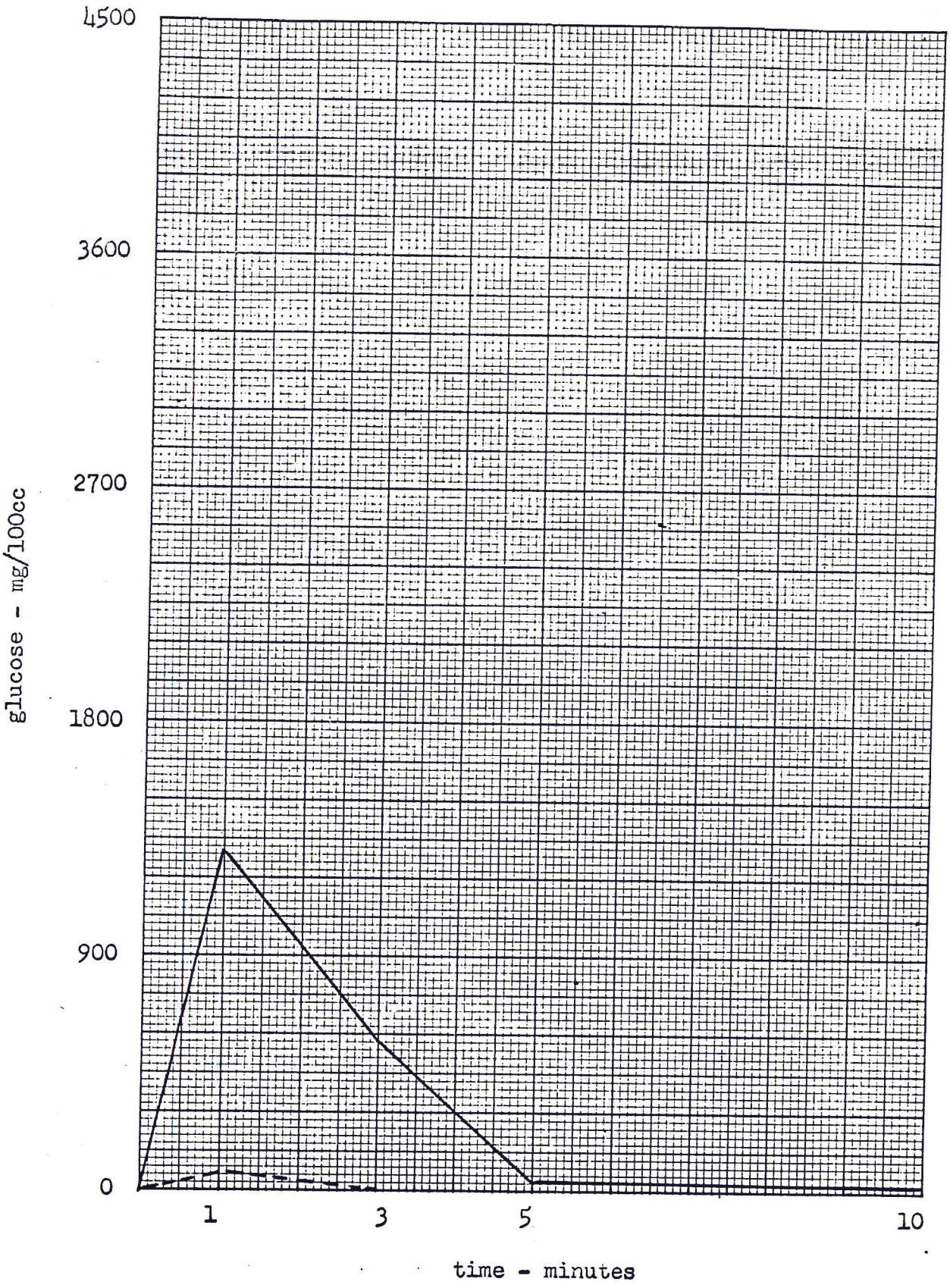
10. Bibby, B. C.: Effect of Sugar Content of Foodstuffs on their Caries Producing Potentialities, J.A.D.A. Vol. 51, page 293, 1955.

11. Bibby, Hyman, Goldberg, and Chen: Evaluation of Caries Producing Potentialities of Various Foodstuffs, J.A.D.A. Vol. 42, May 1951.

APPENDIX

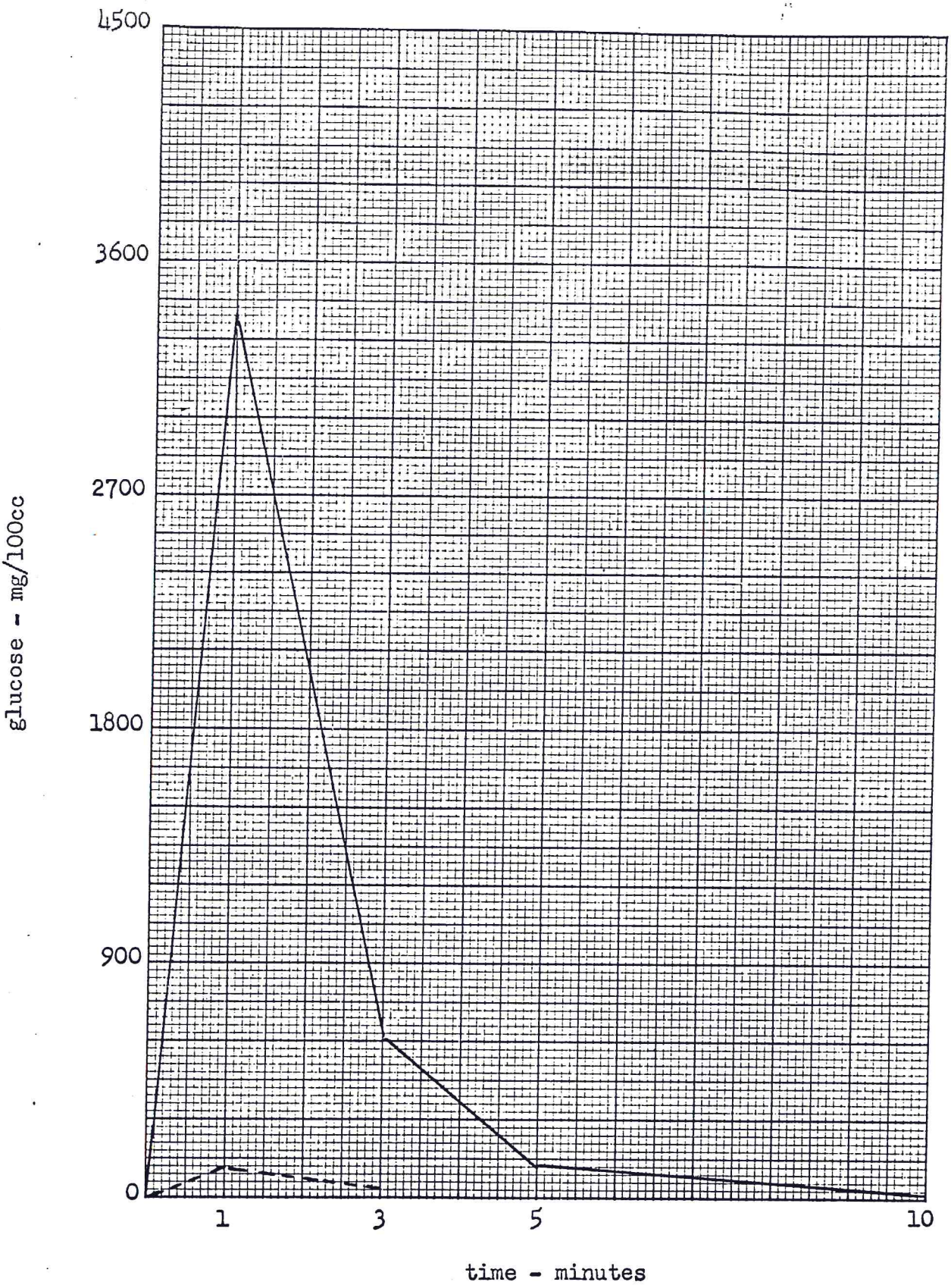
Subject A1

Time glucose concentration graph of the beverage experiment



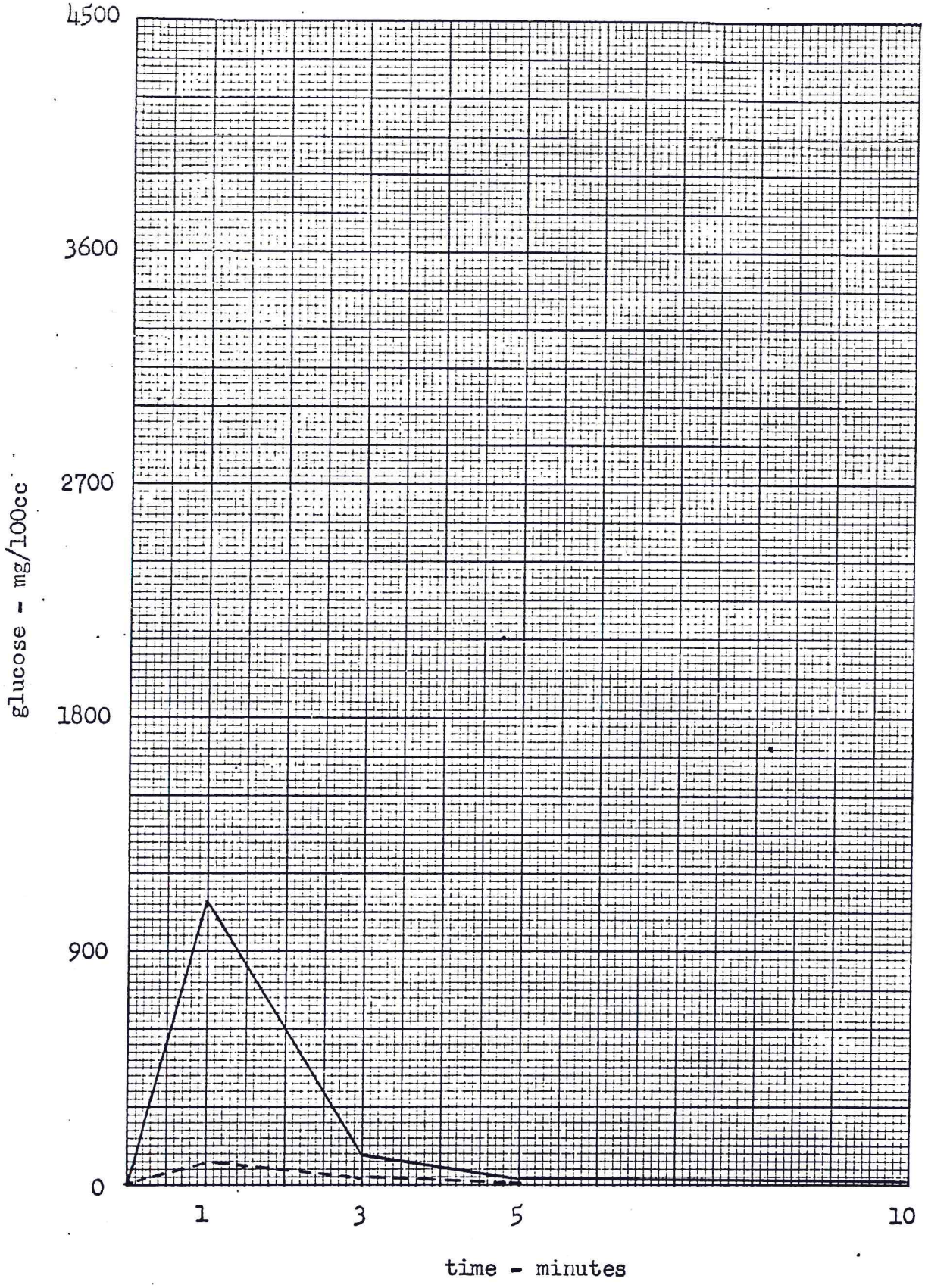
Subject B1

Time glucose concentration graph of the beverage experiment



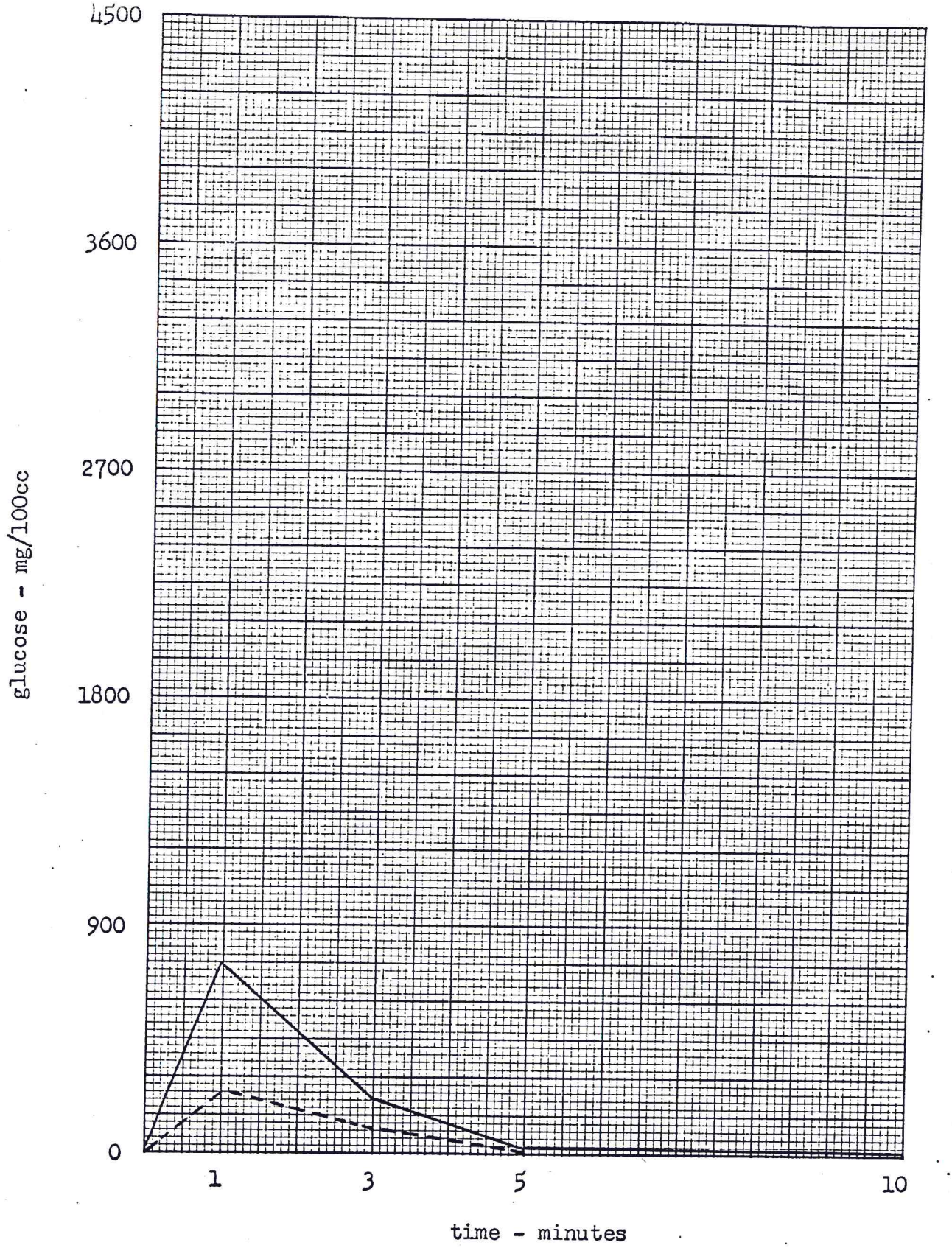
Subject C1

Time glucose concentration graph of the beverage experiment



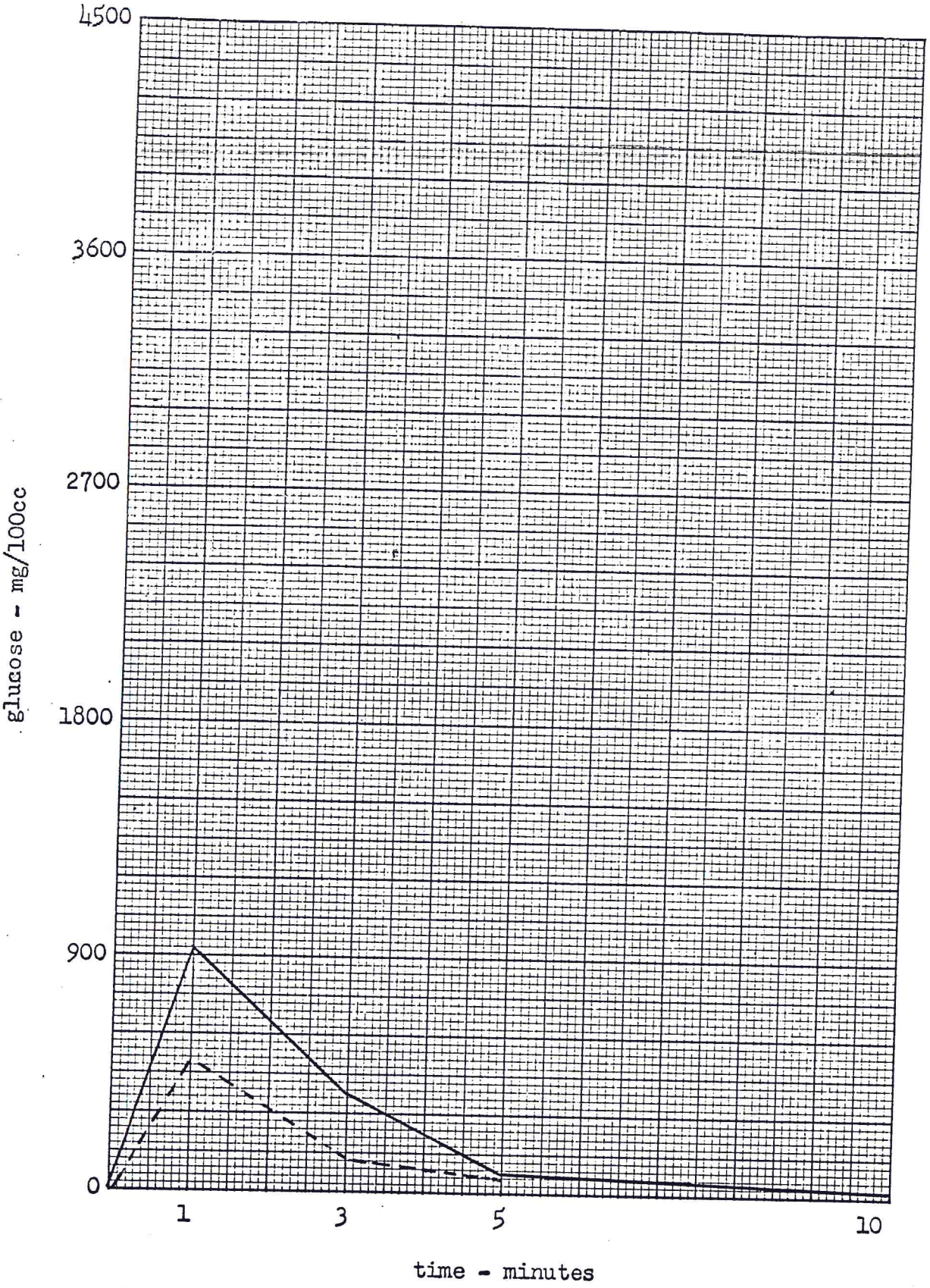
Subject D1

Time glucose concentration graph of the beverage experiment



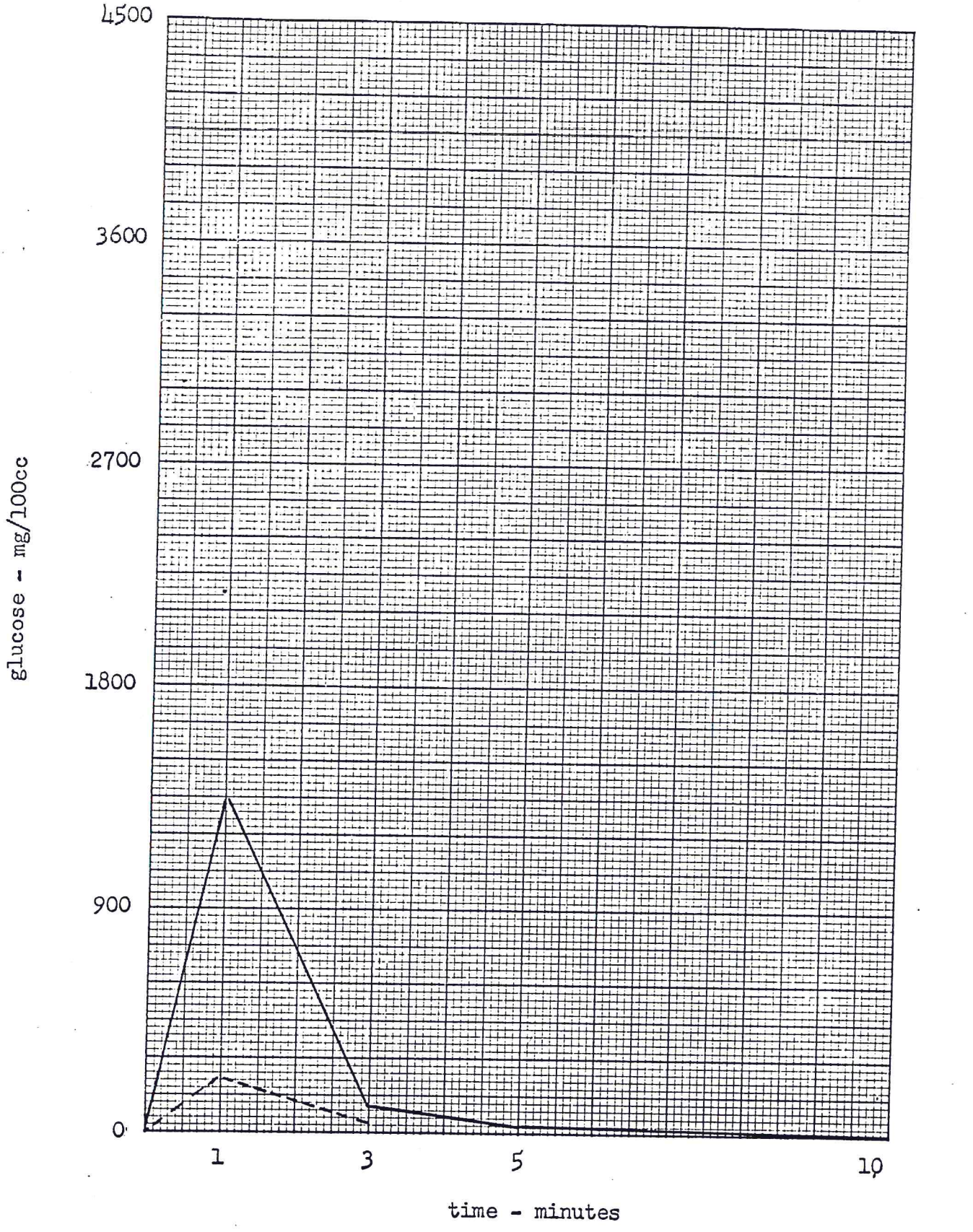
Subject E1

Time glucose concentration graph of the beverage experiment



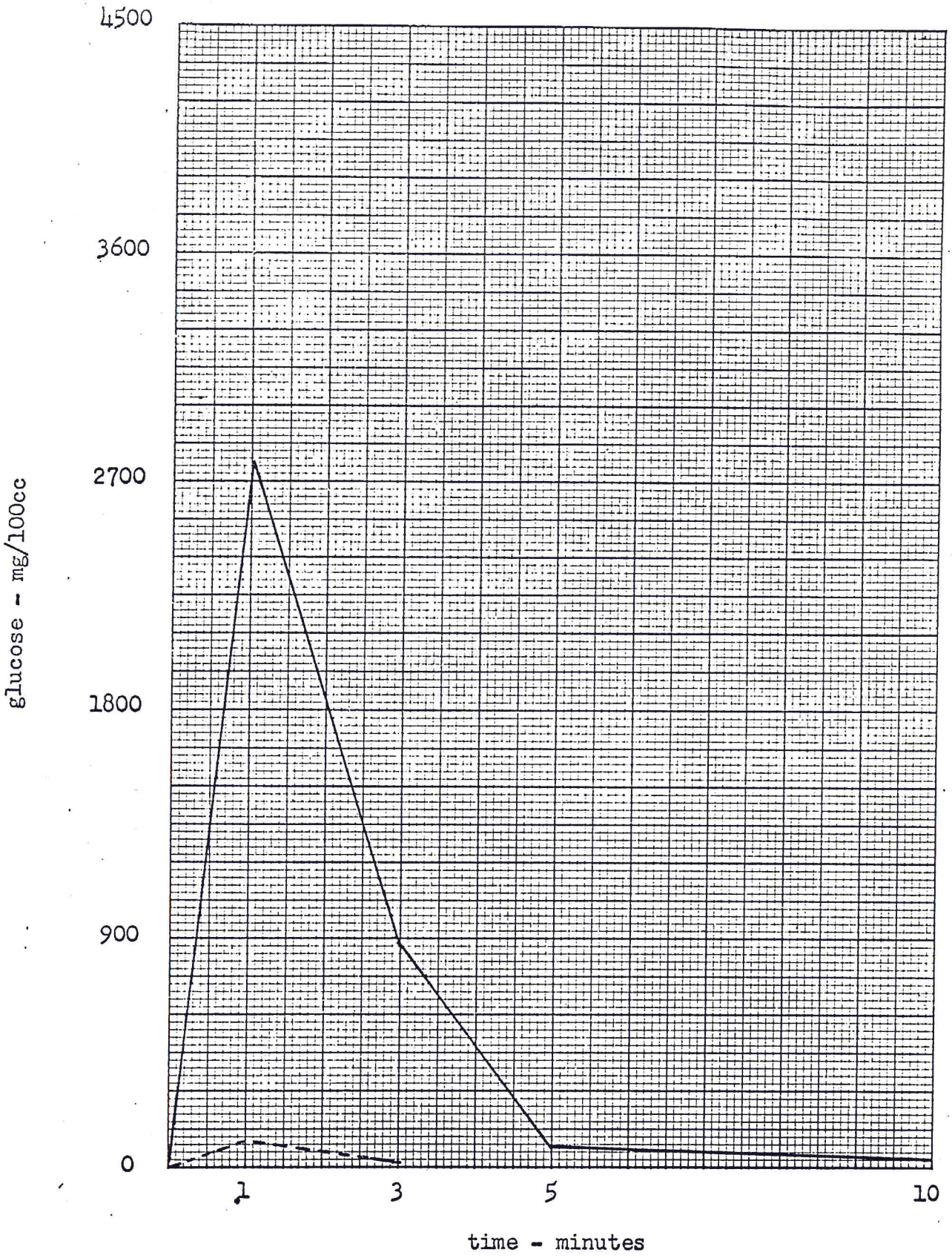
Subject F1

Time glucose concentration graph of the beverage experiment



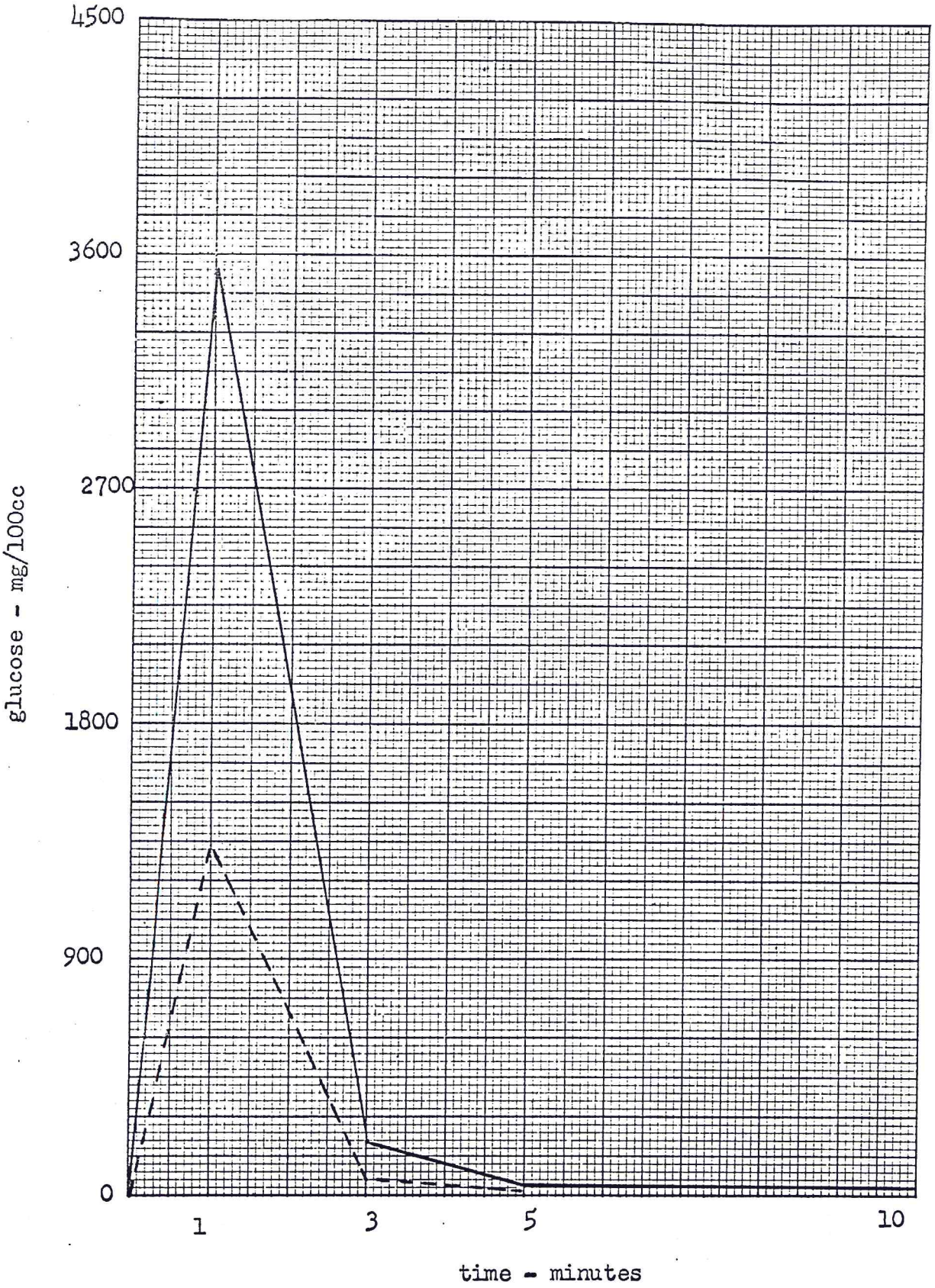
Subject G1

Time glucose concentration graph of the beverage experiment



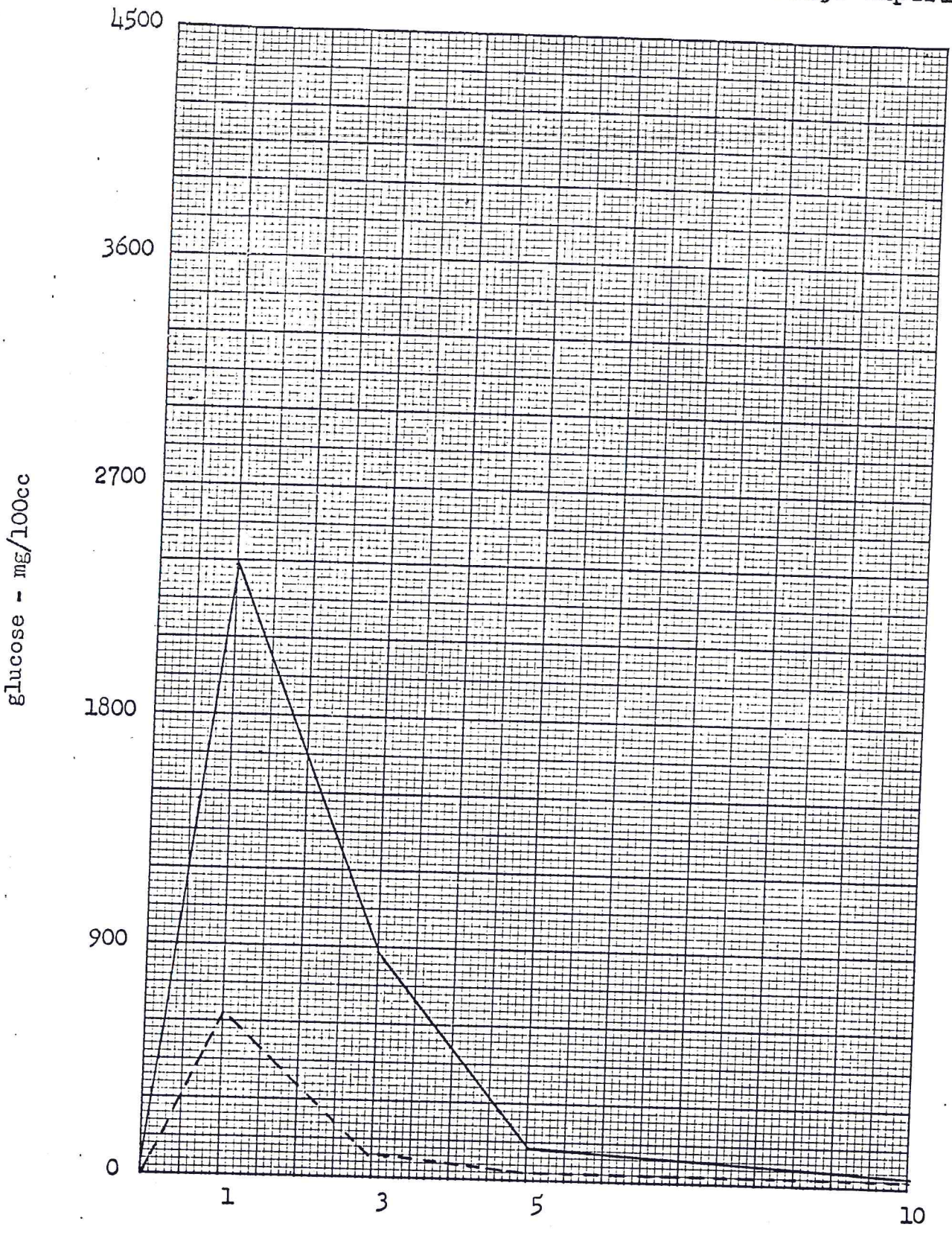
Subject H1

Time glucose concentration graph of the beverage experiment



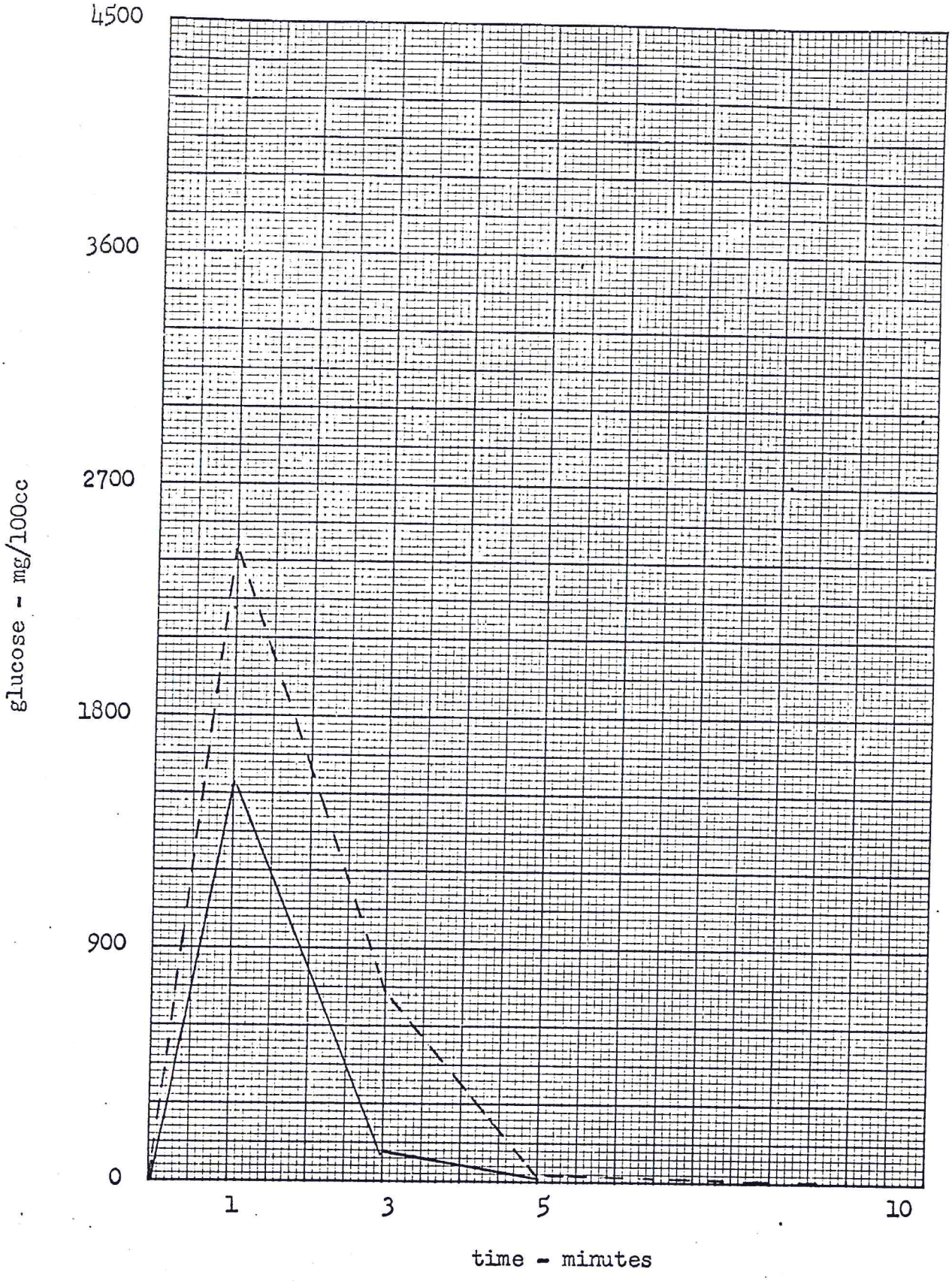
Subject II

Time glucose concentration graph of the beverage experiment



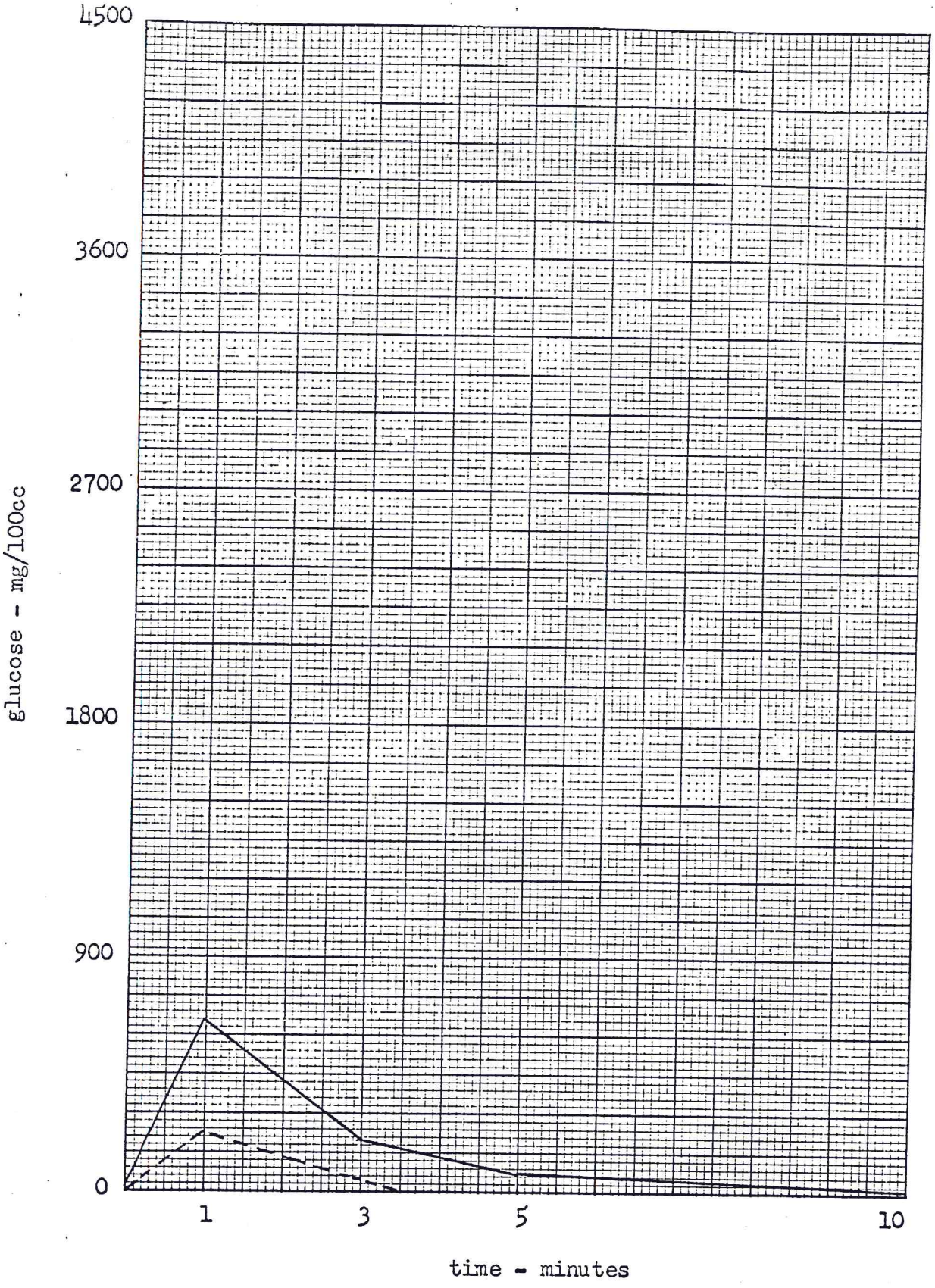
Subject J1

Time glucose concentration graph of the beverage experiment



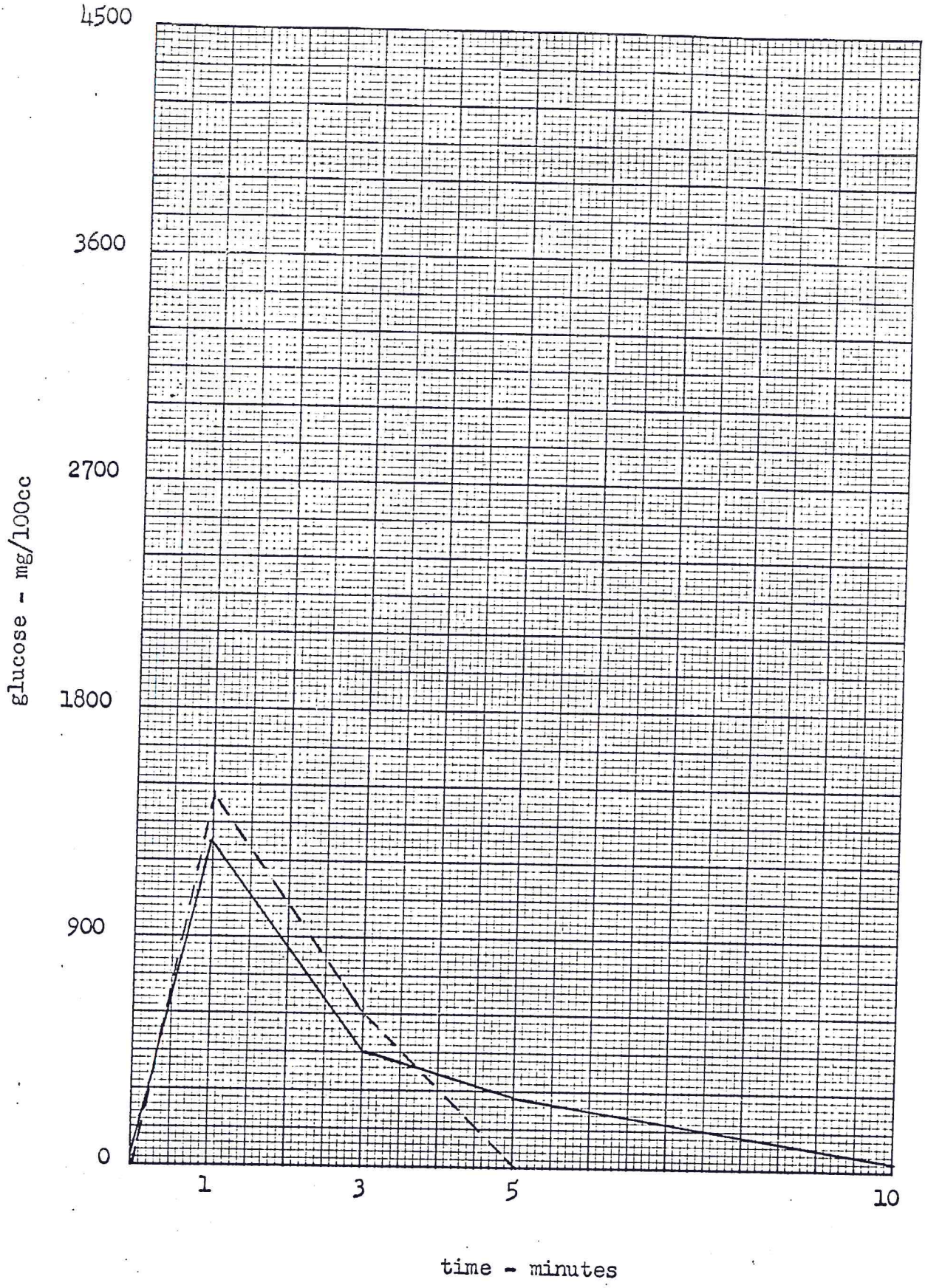
Subject K1

Time glucose concentration graph of the beverage experiment



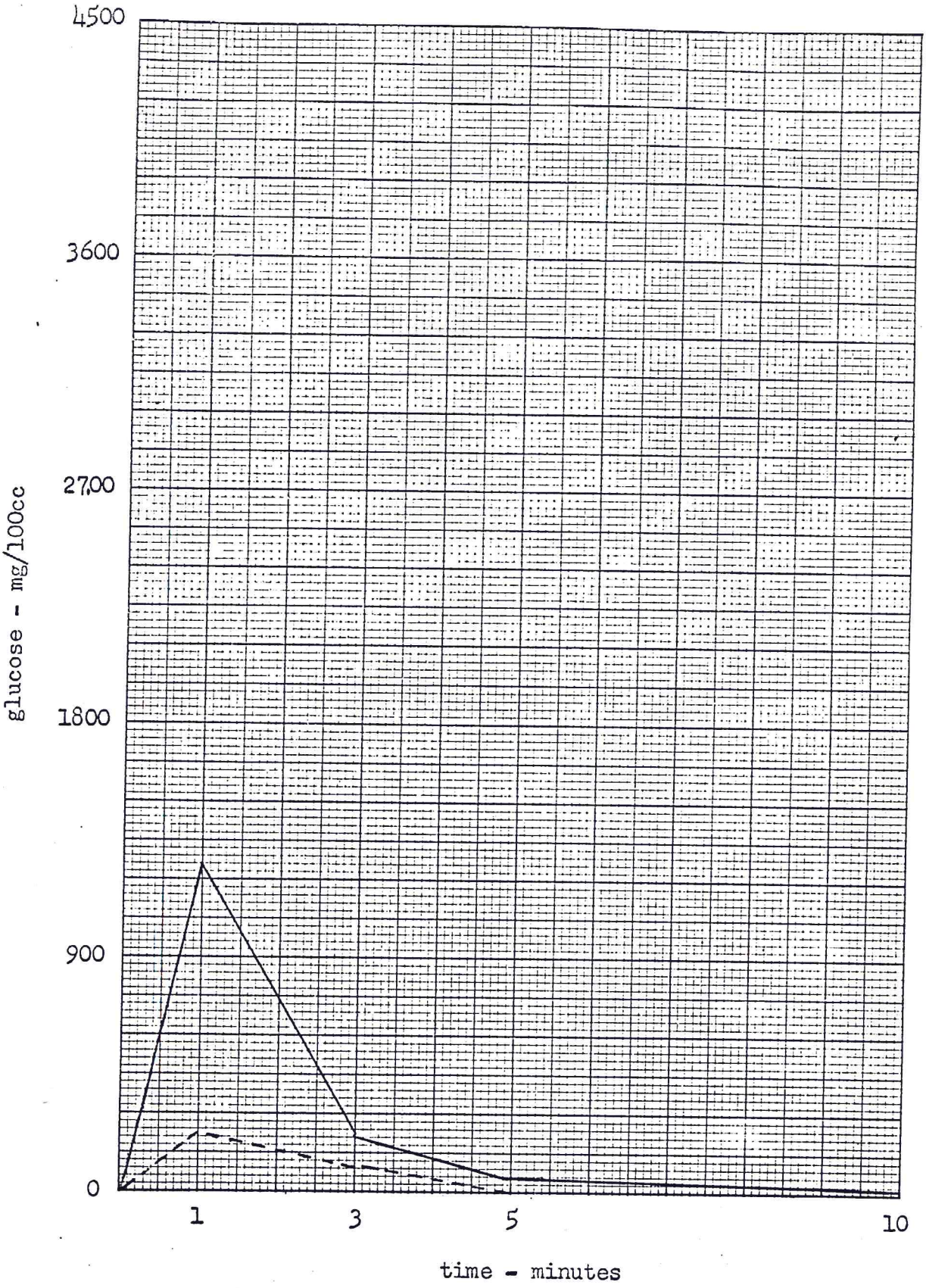
Subject L1

Time glucose concentration graph of the beverage experiment



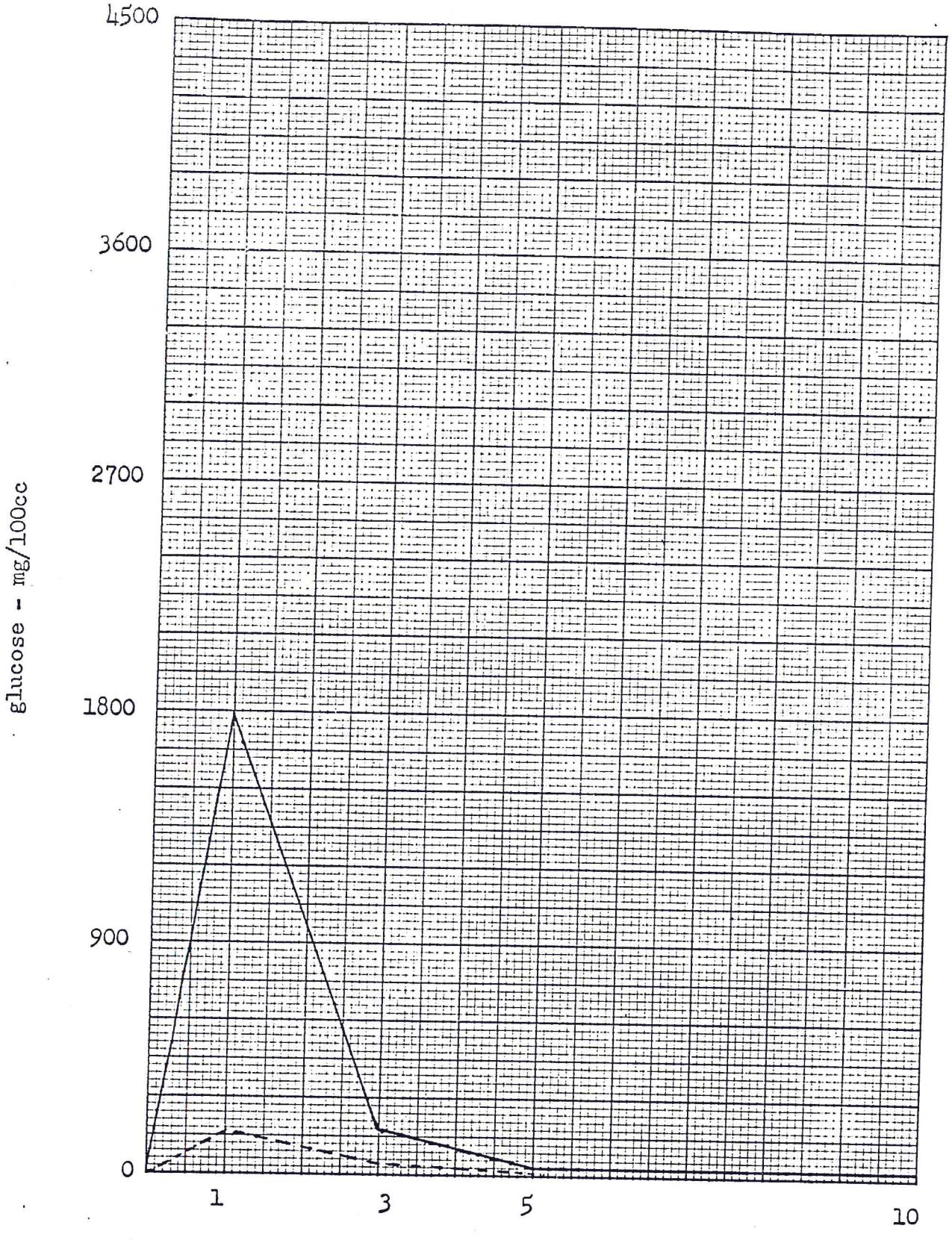
Subject ML

Time glucose concentration graph of the beverage experiment



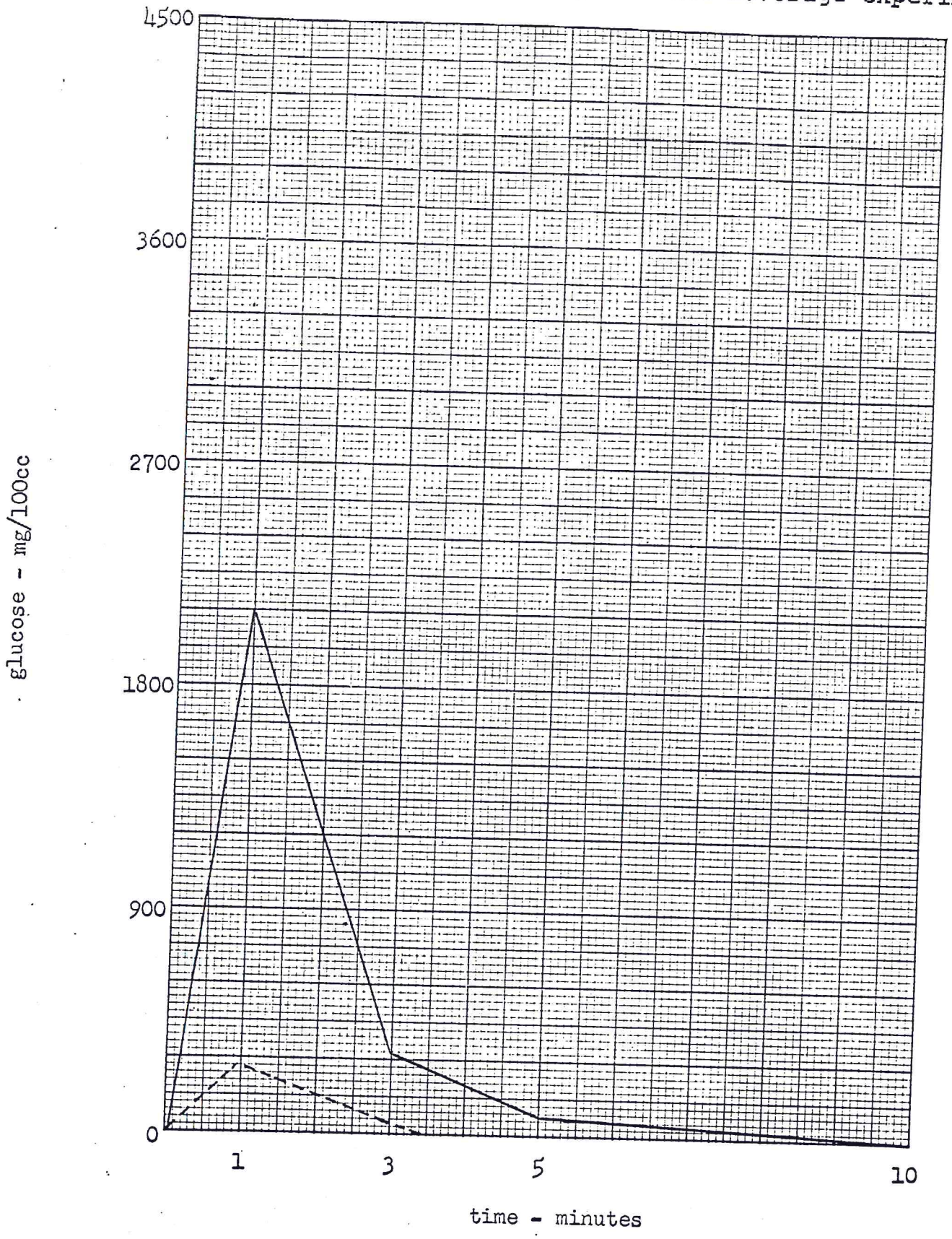
Subject N1

Time glucose concentration graph of the beverage experiment



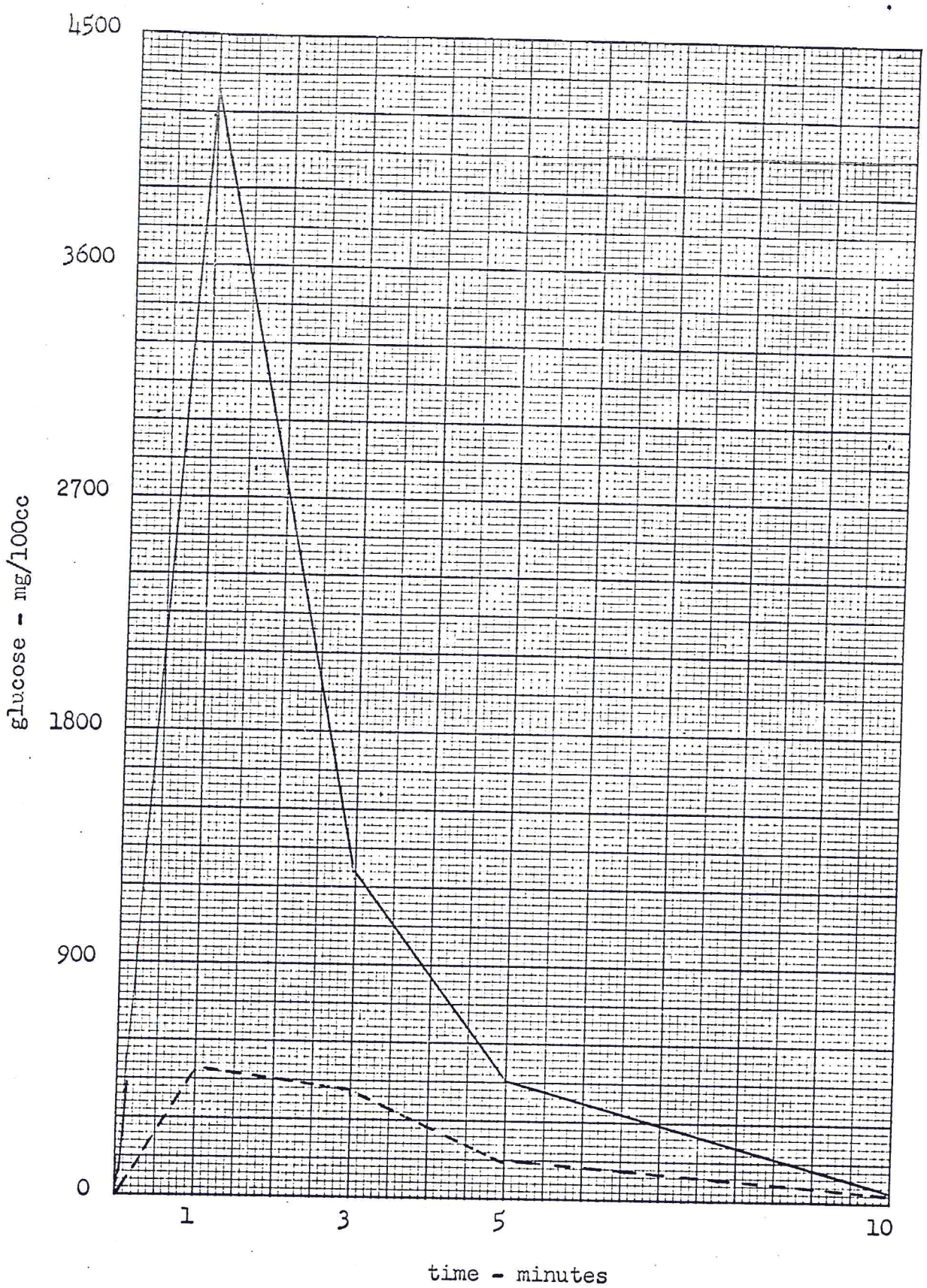
Subject 01

Time glucose concentration graph of the beverage experiment



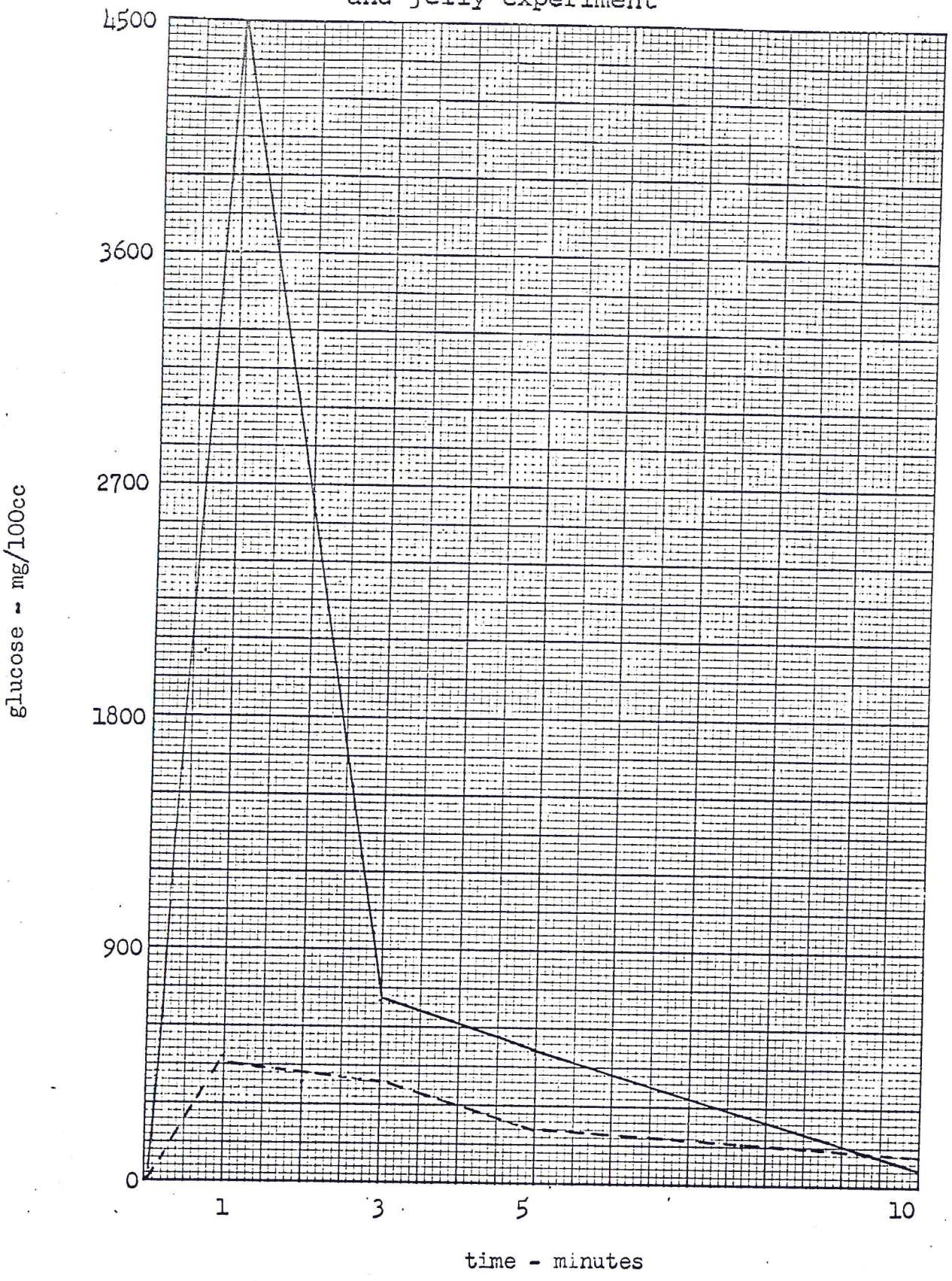
Subject A2

Time glucose concentration graph of the bread and jelly experiment



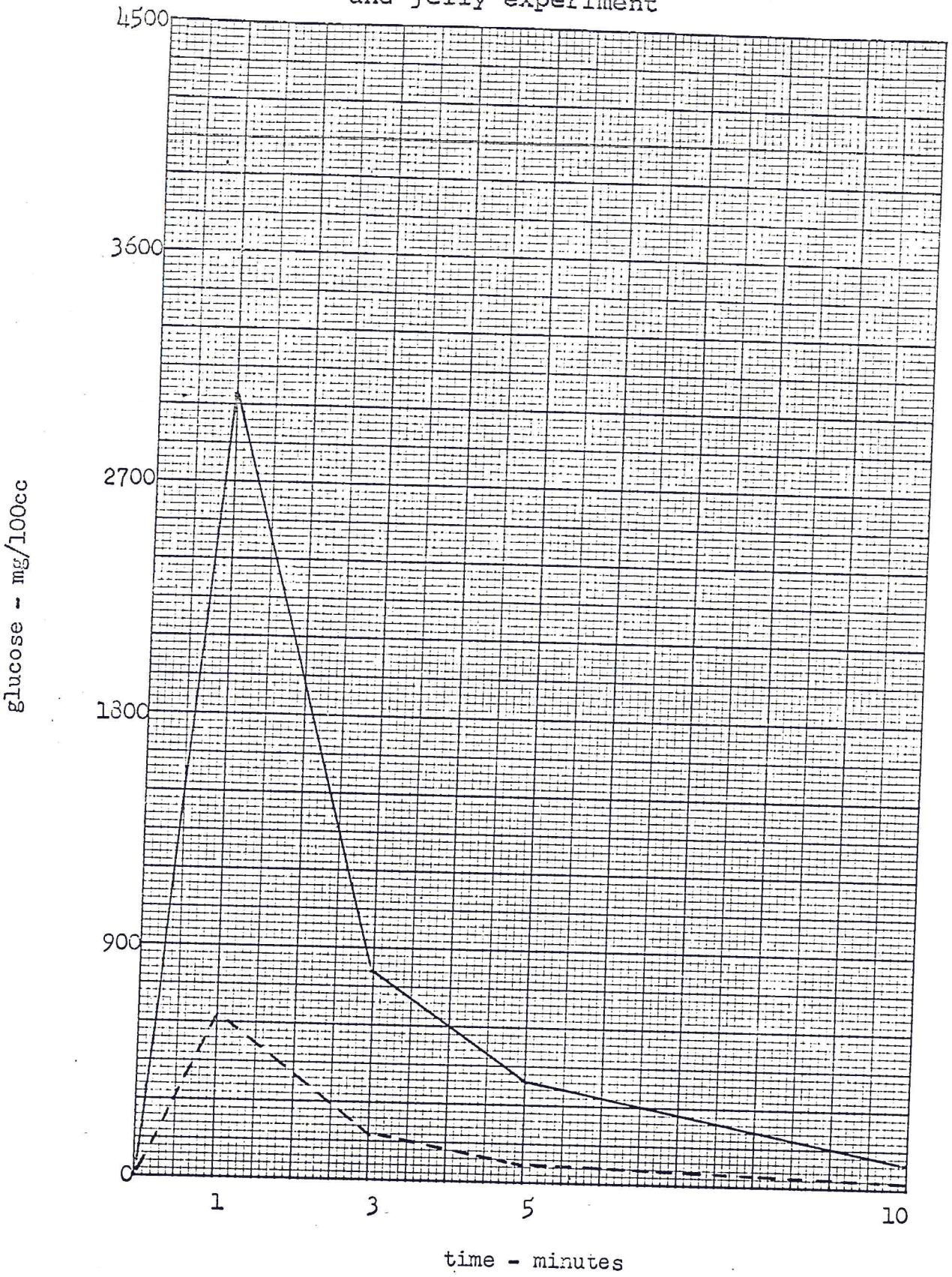
Subject B2

Time glucose concentration graph of the bread and jelly experiment



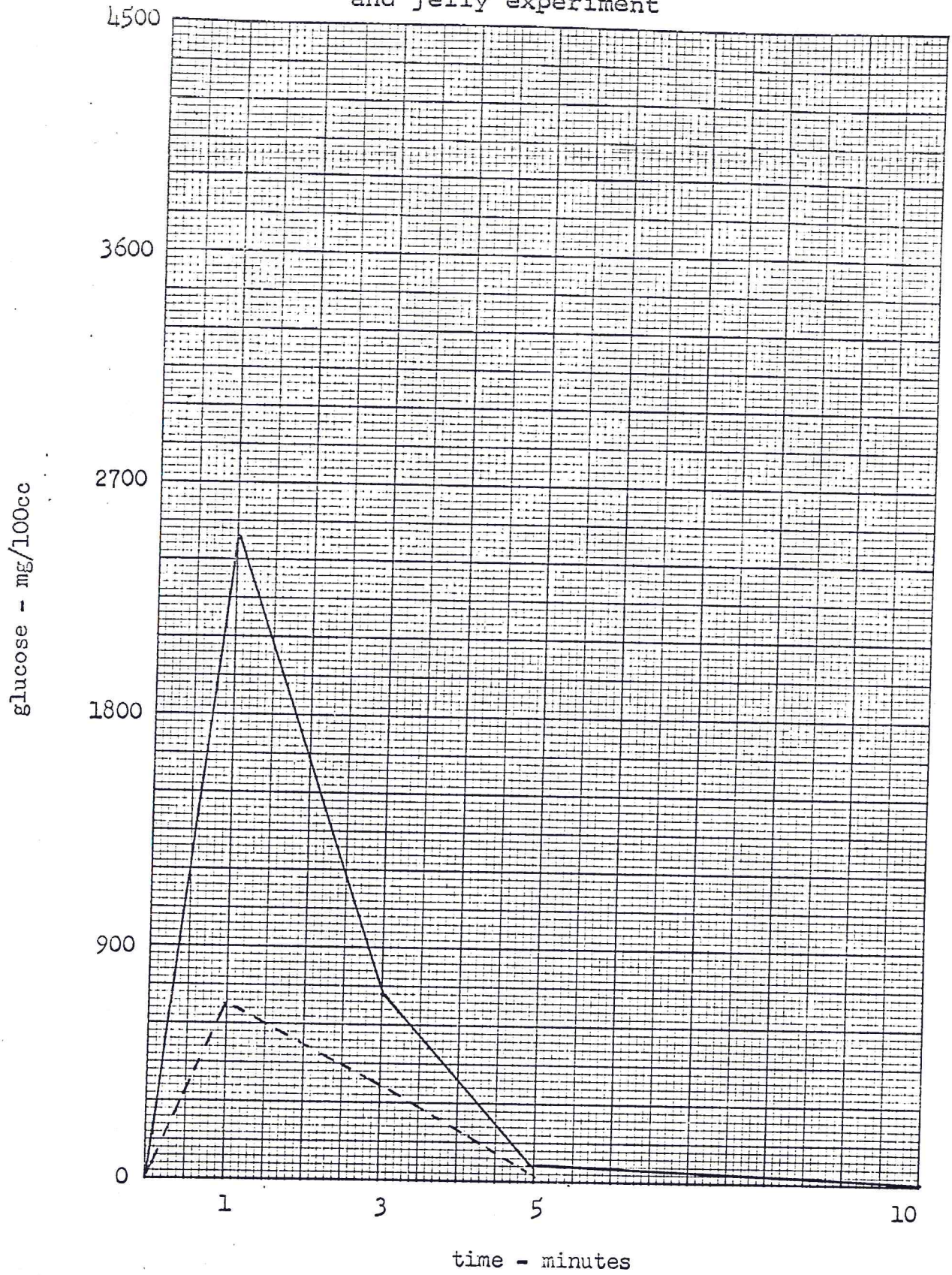
Subject C2

Time glucose concentration graph of the bread and jelly experiment



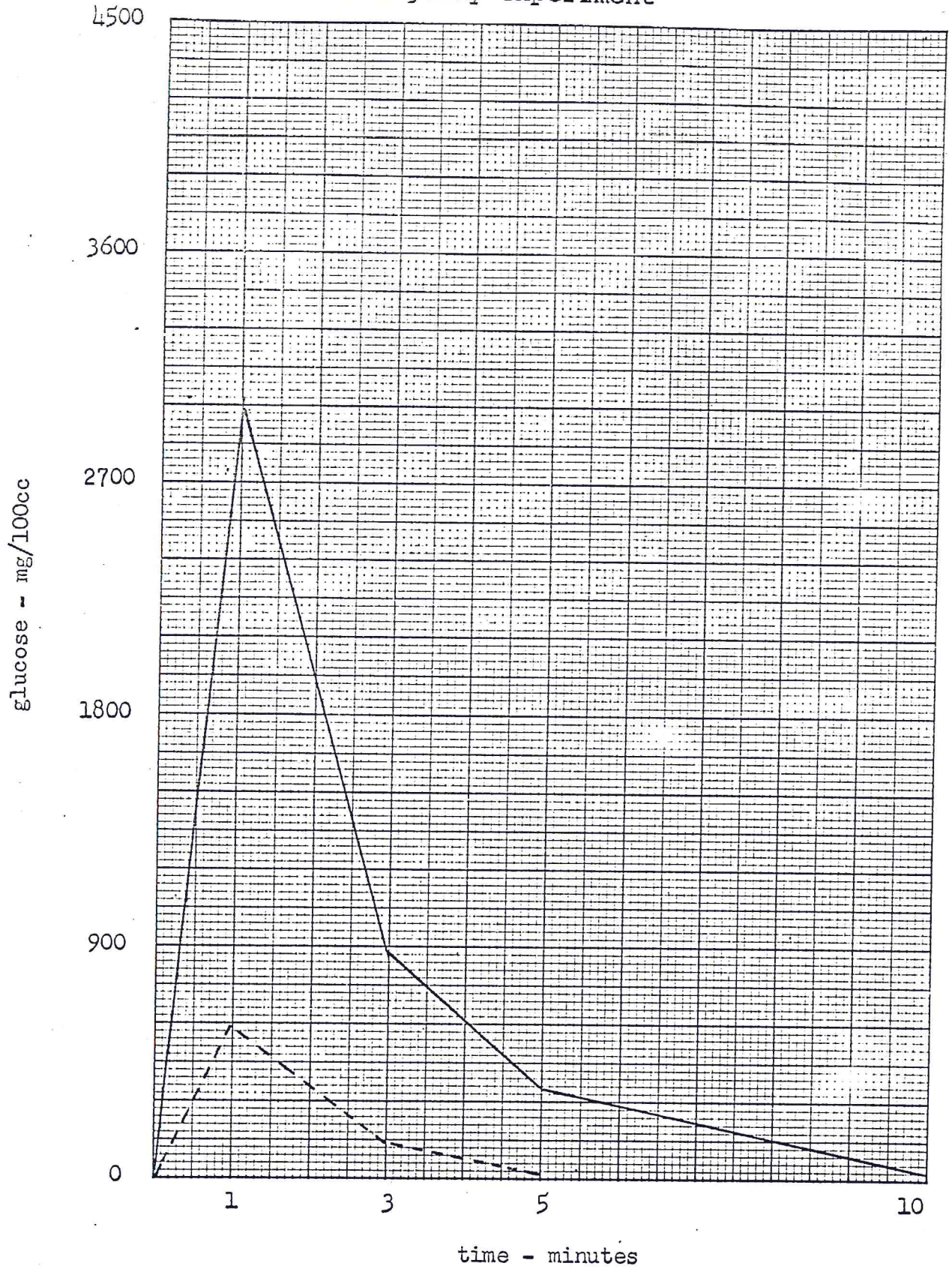
Subject D2

Time glucose concentration graph of the bread and jelly experiment



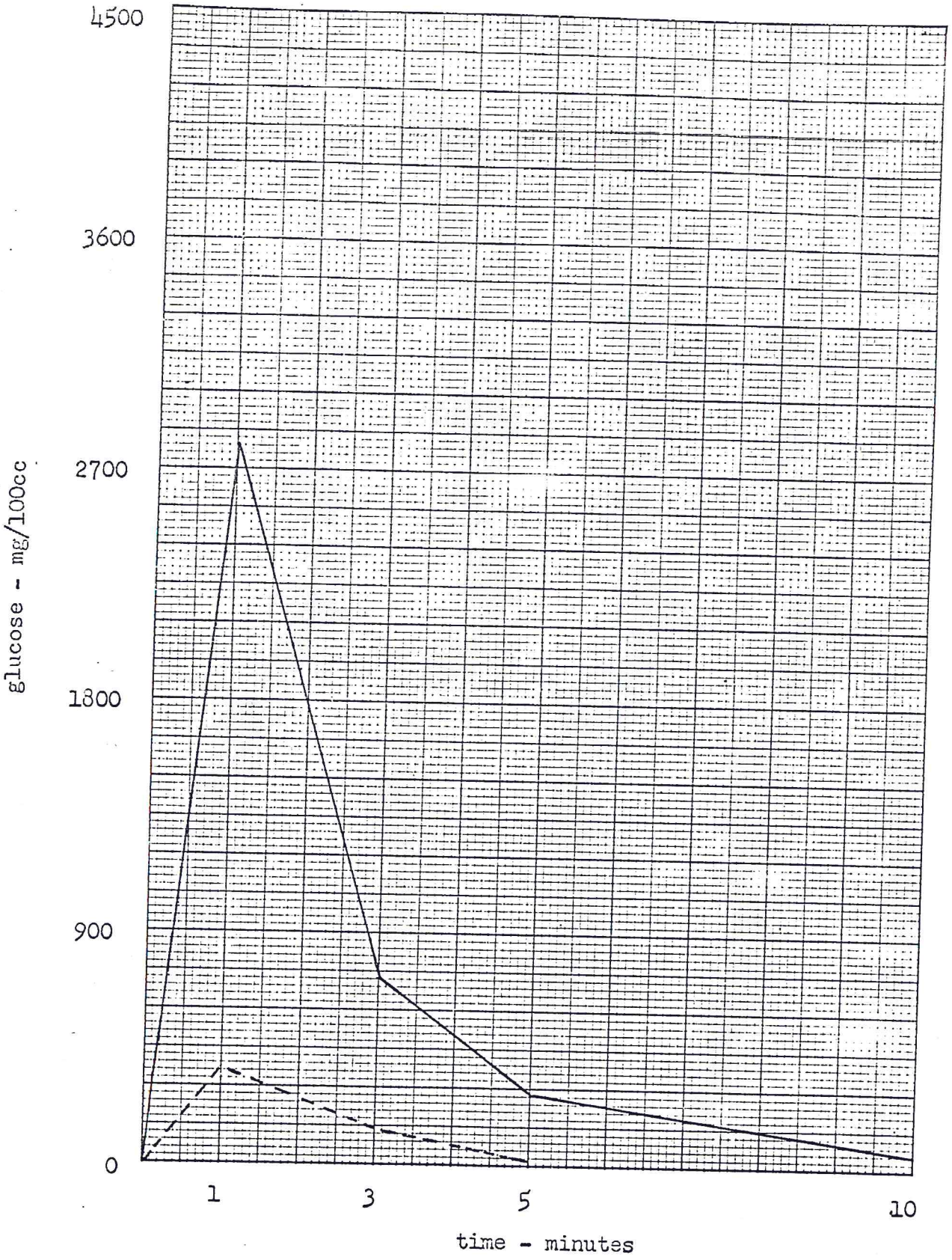
Subject E2

Time glucose concentration graph of the bread and jelly experiment



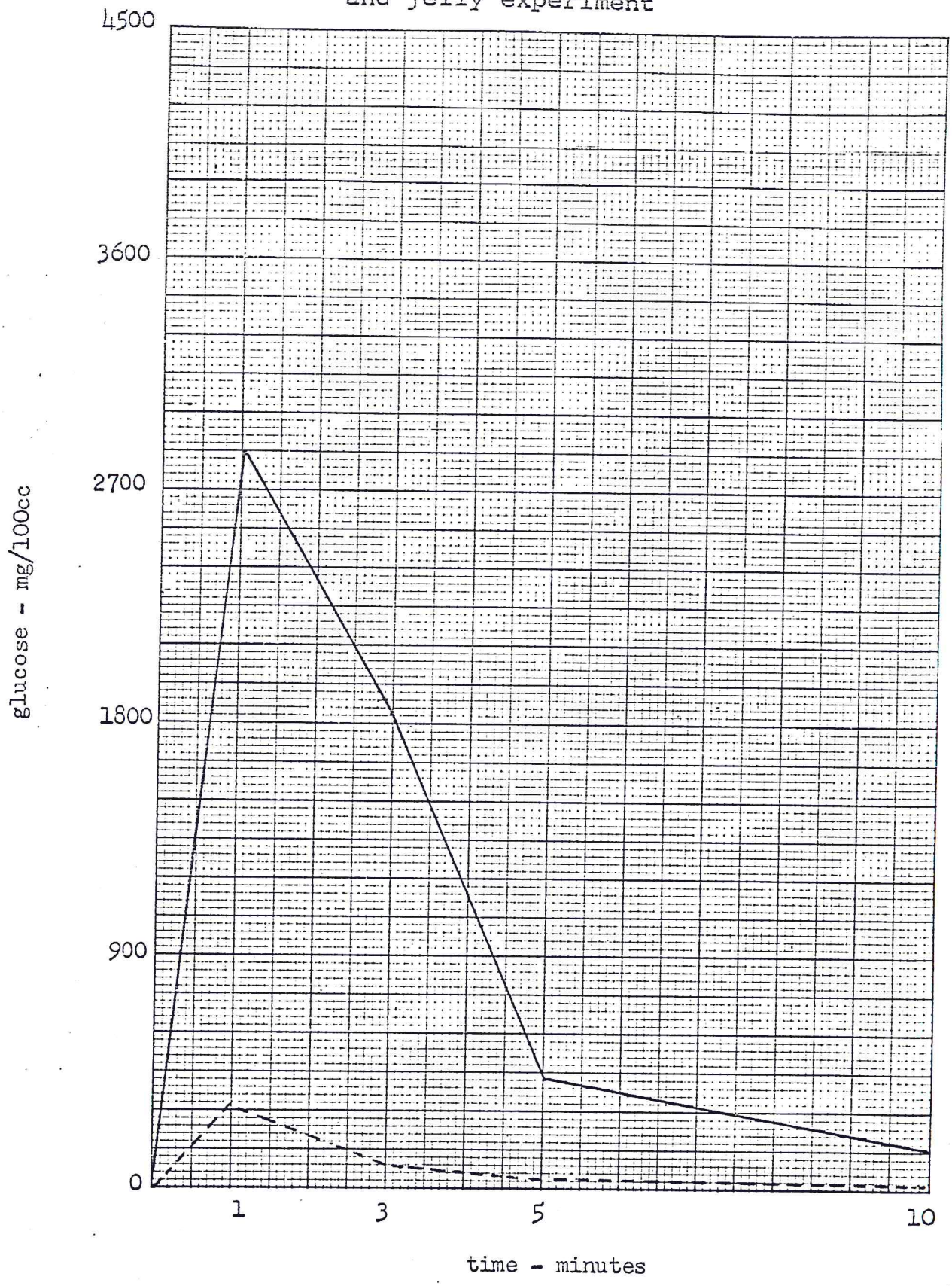
Subject F2

Time glucose concentration graph of the bread and jelly experiment



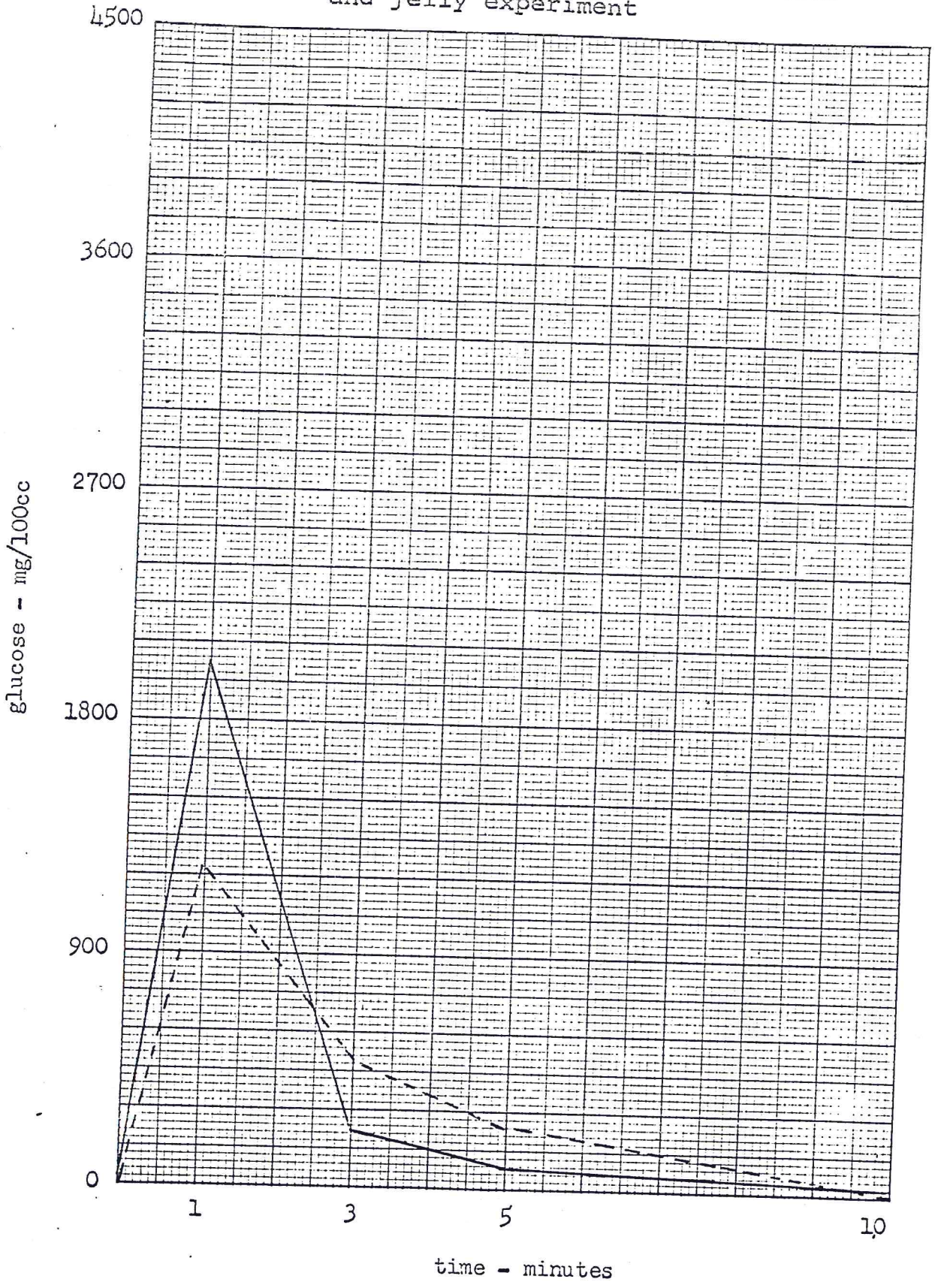
Subject G2

Time glucose concentration graph of the bread and jelly experiment



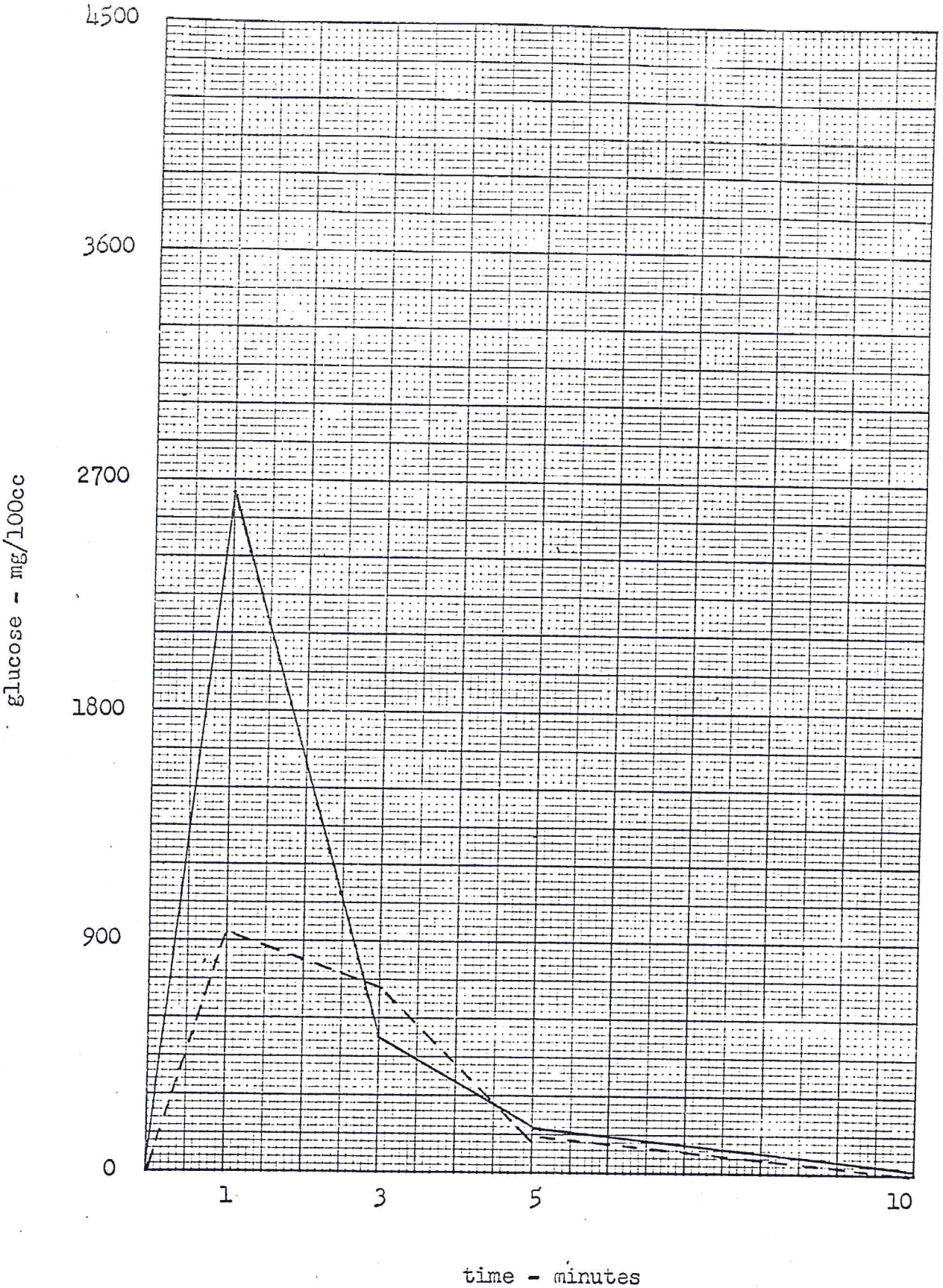
Subject H2

Time glucose concentration graph of the bread and jelly experiment



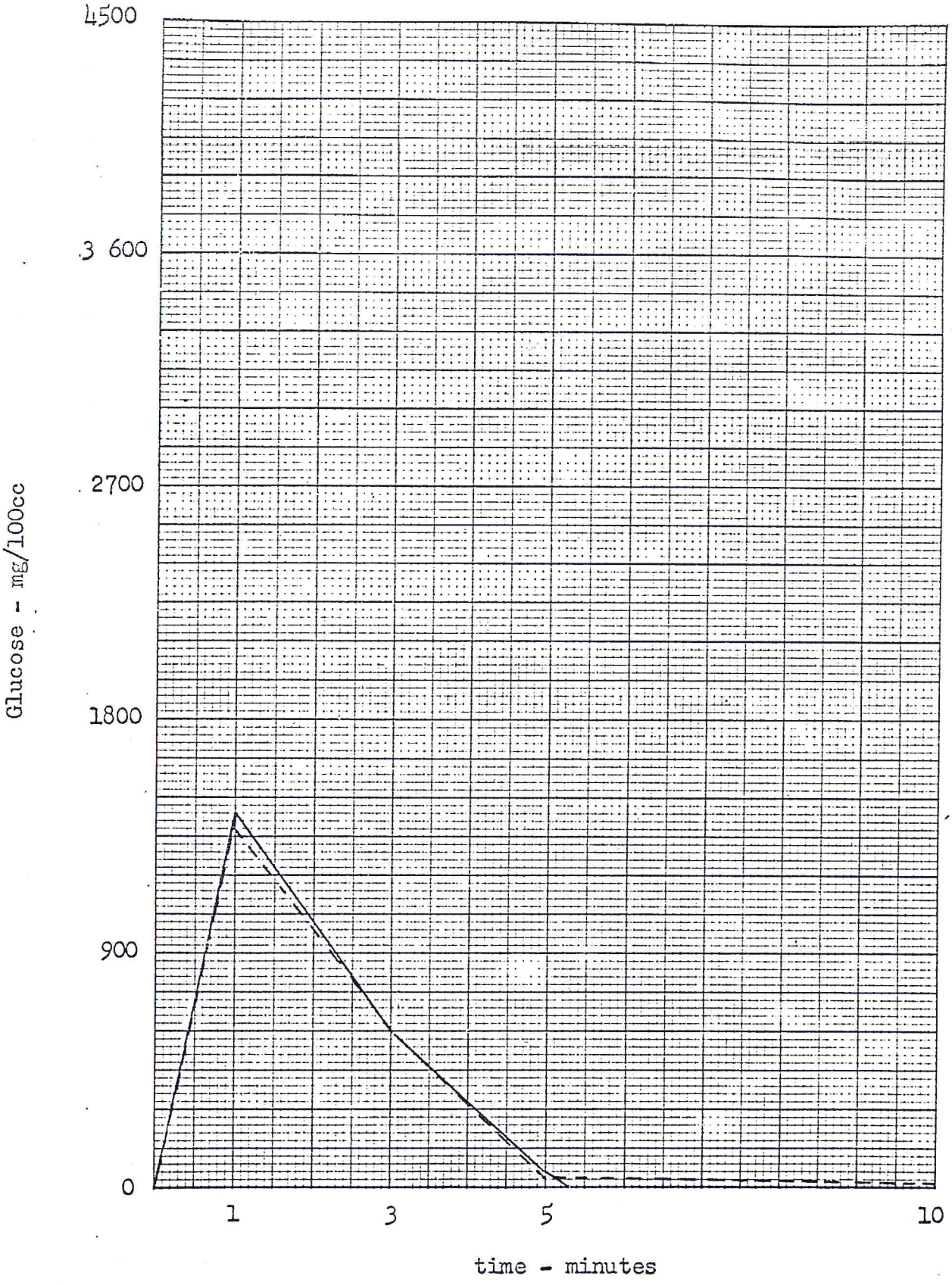
Subject I2

Time glucose concentration graph of the bread and jelly experiment



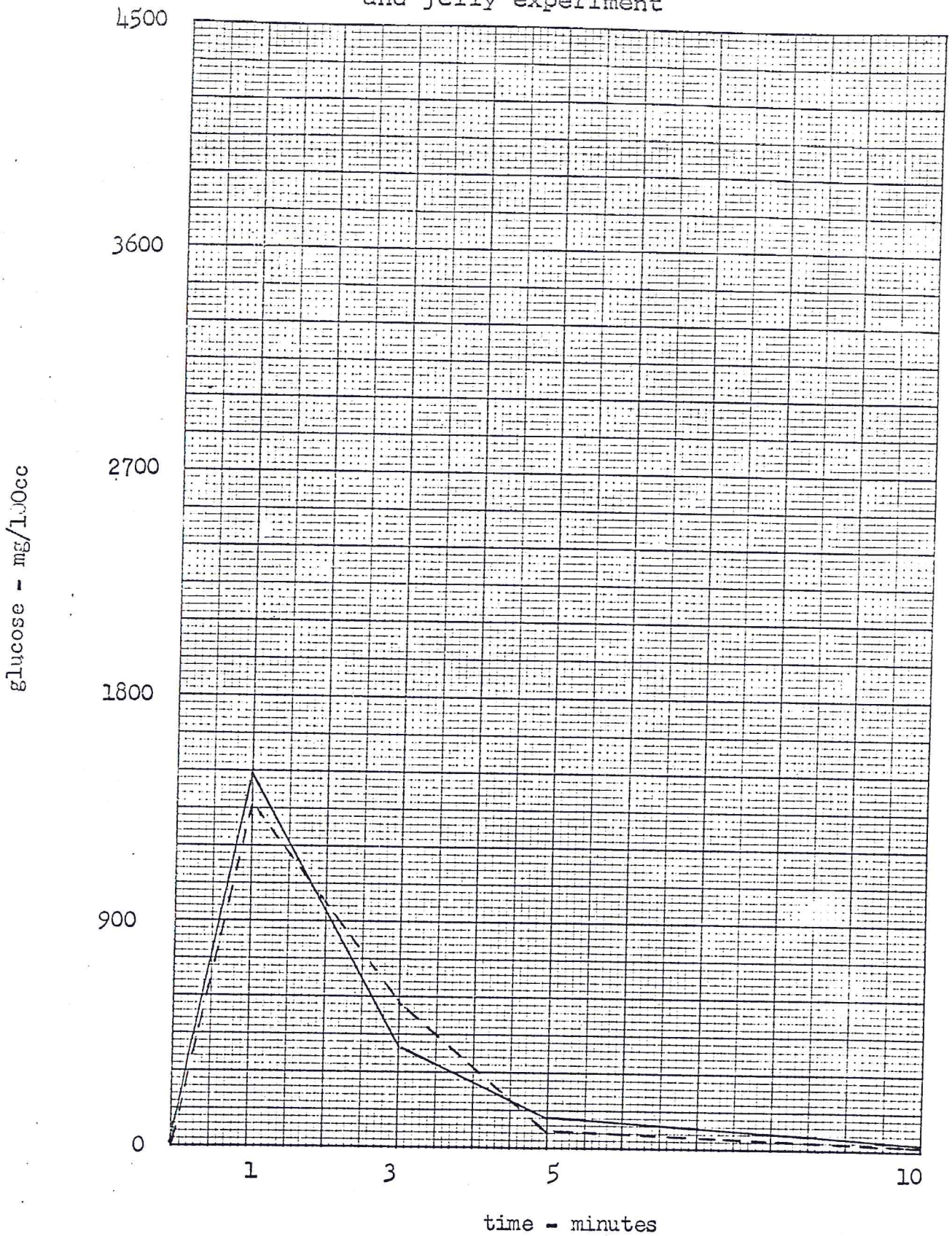
Subject J2

Time glucose concentration graph of the bread and jelly experiment



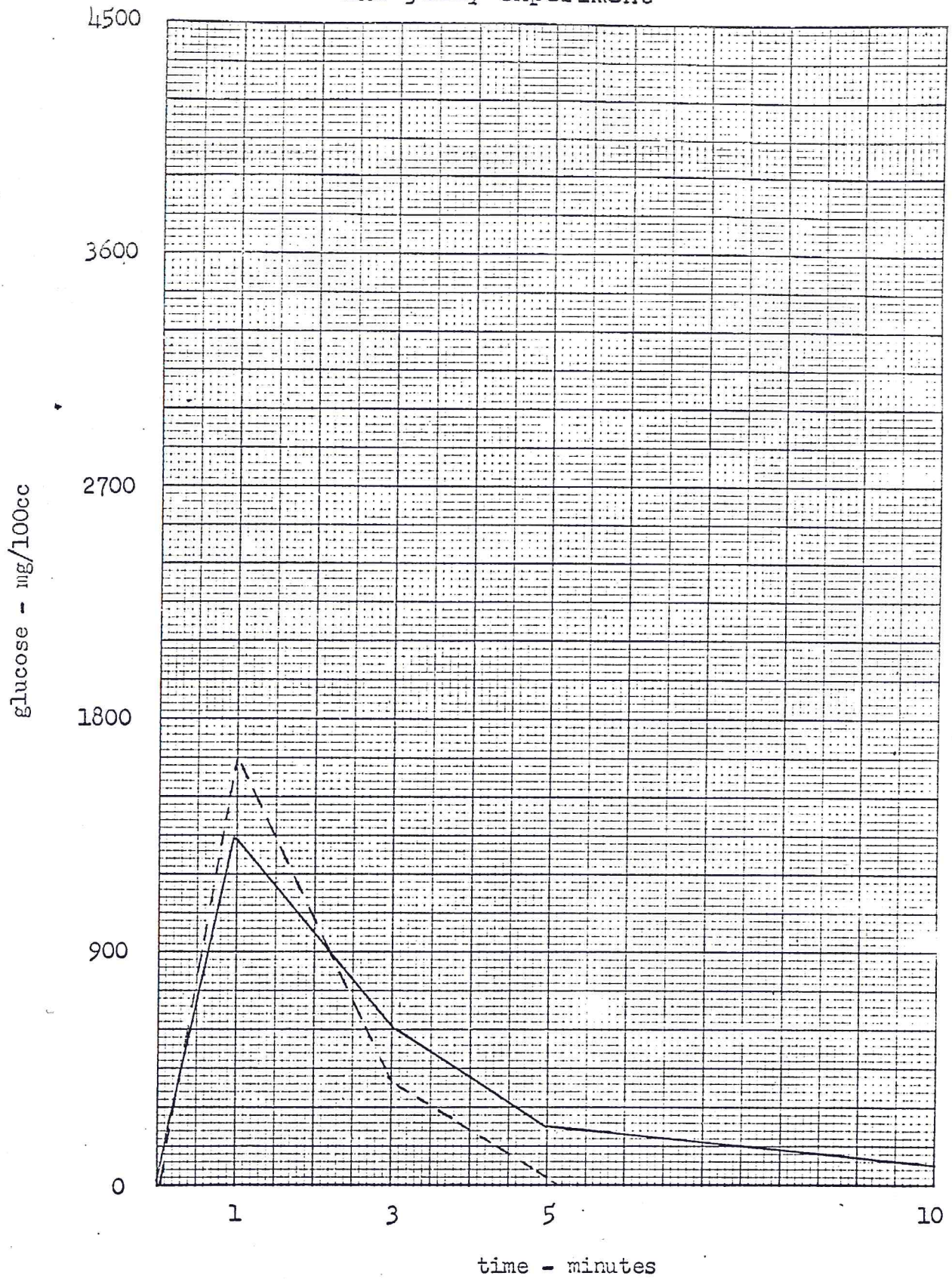
Subject K2

Time glucose concentration graph of the bread and jelly experiment



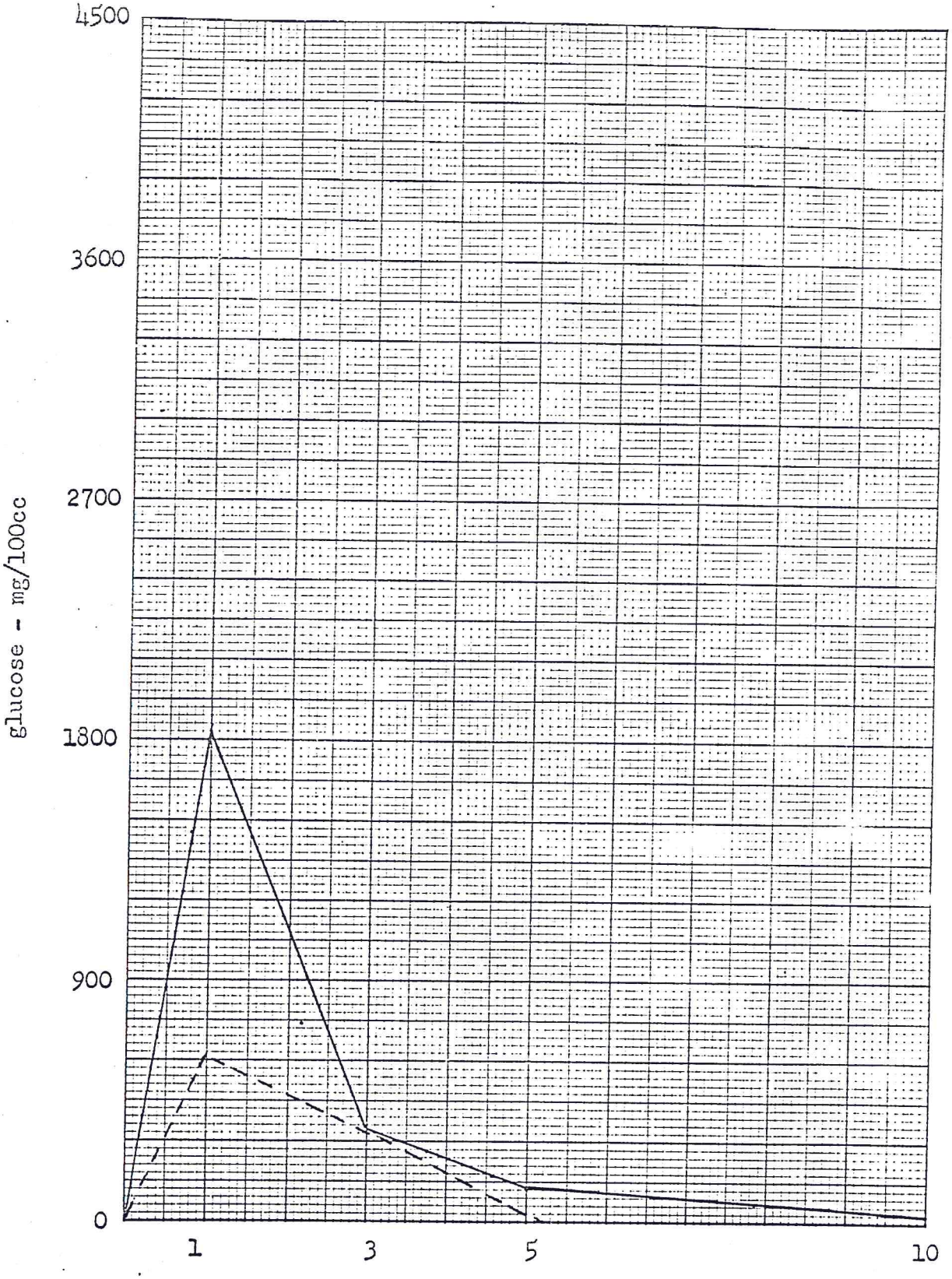
Subject L2

Time glucose concentration graph of the bread and jelly experiment



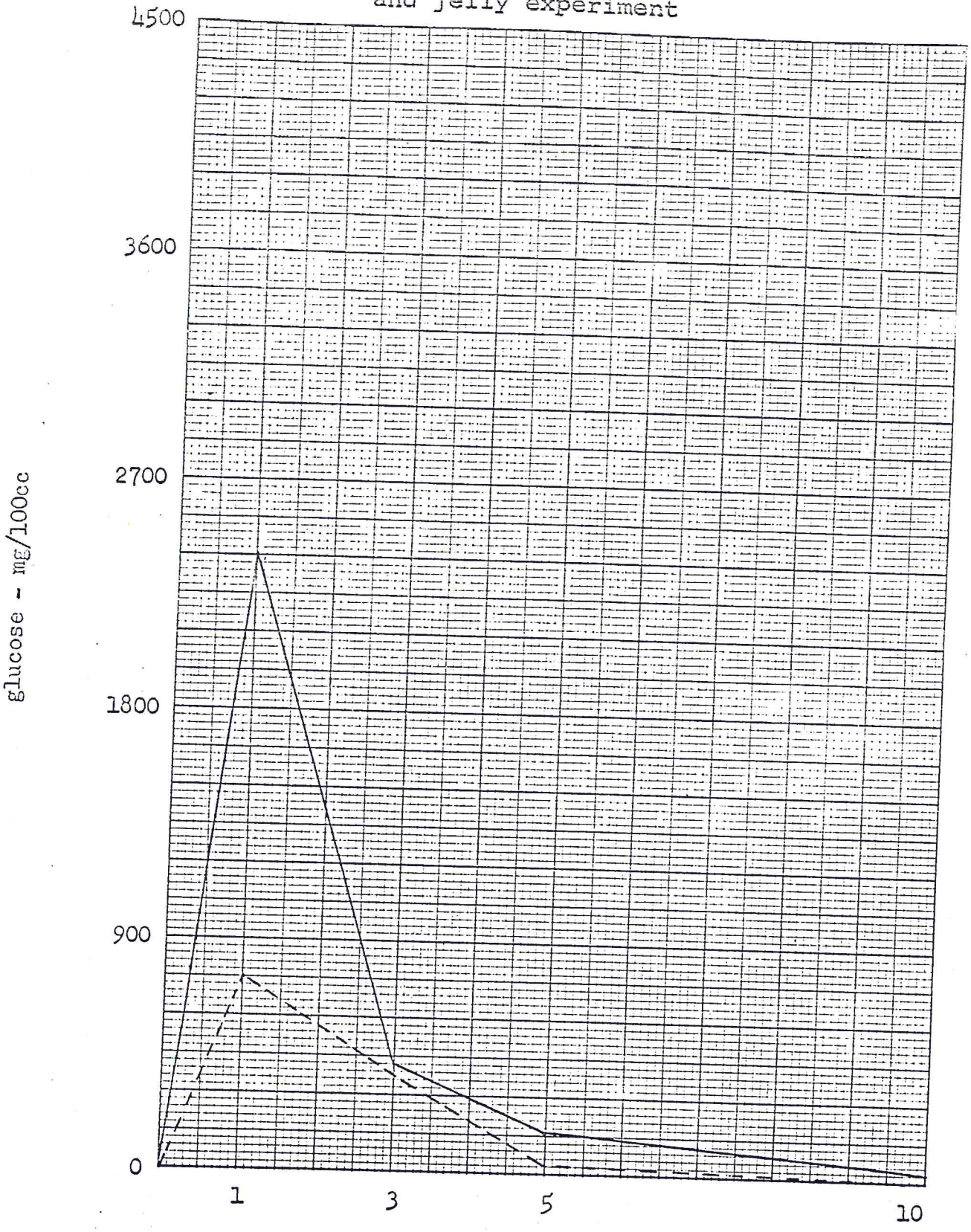
Subject M2

Time glucose concentration graph of the bread and jelly experiment



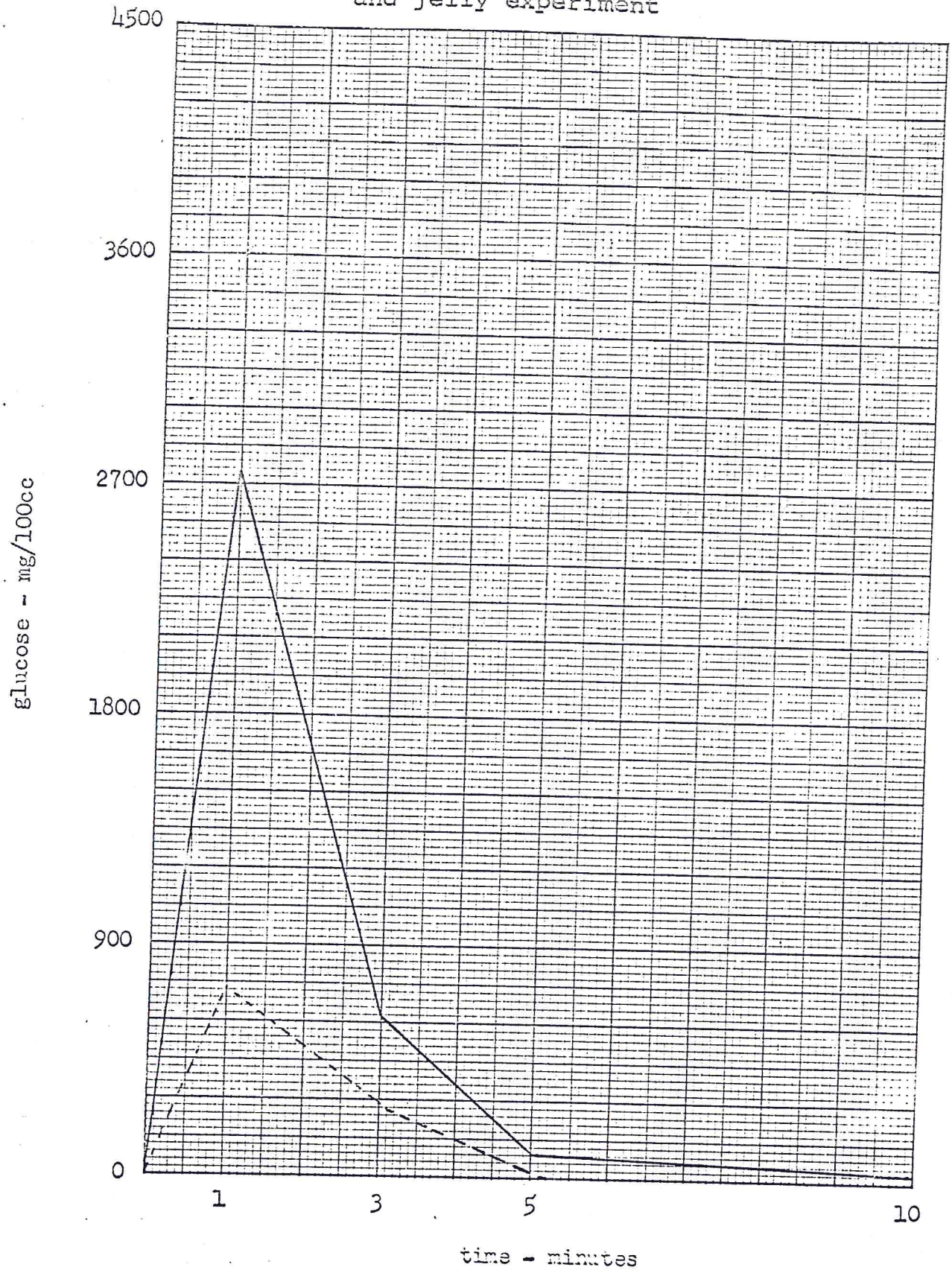
Subject N2

Time glucose concentration graph of the bread and jelly experiment



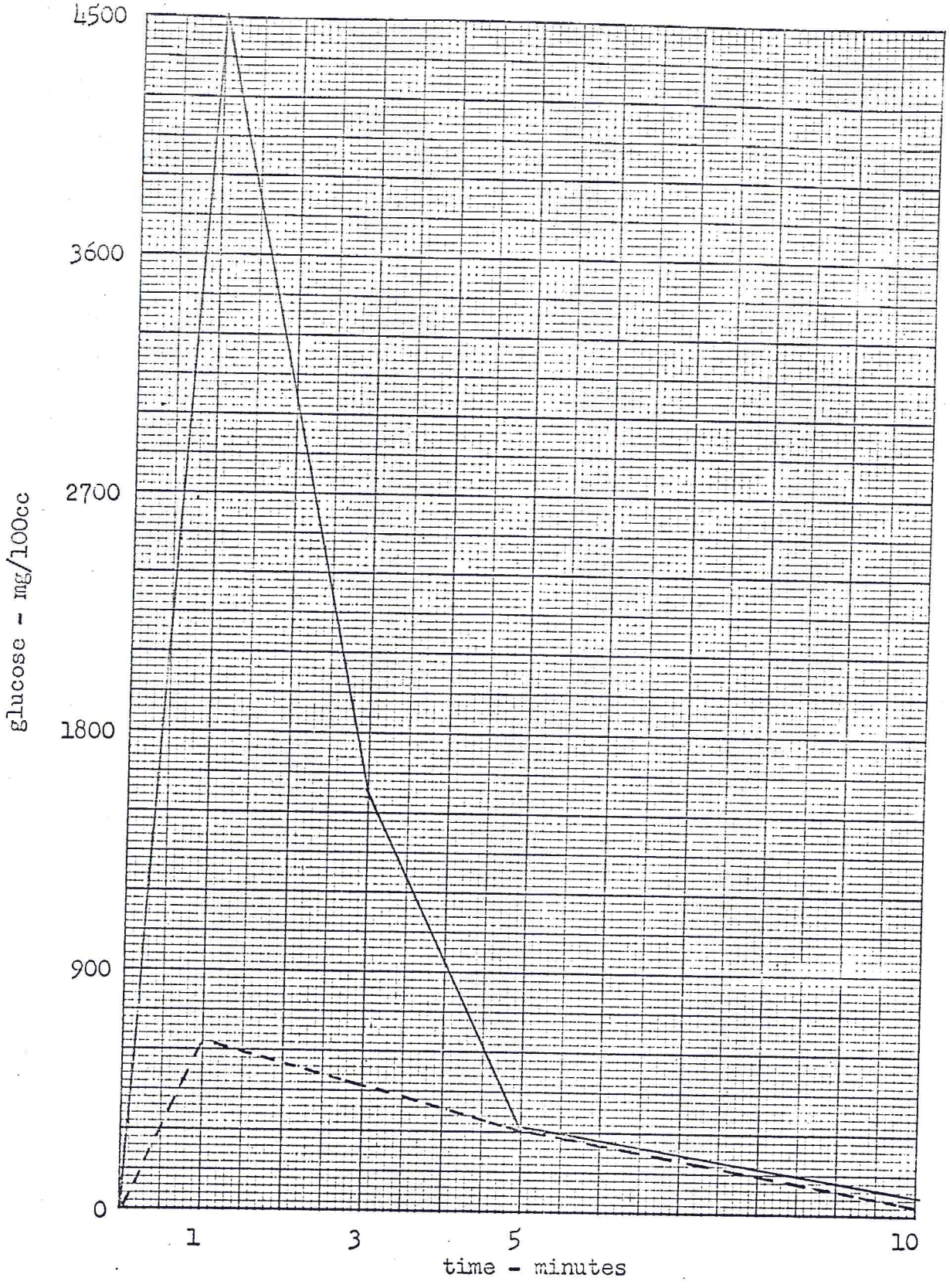
Subject 02

Time glucose concentration graph of the bread and jelly experiment



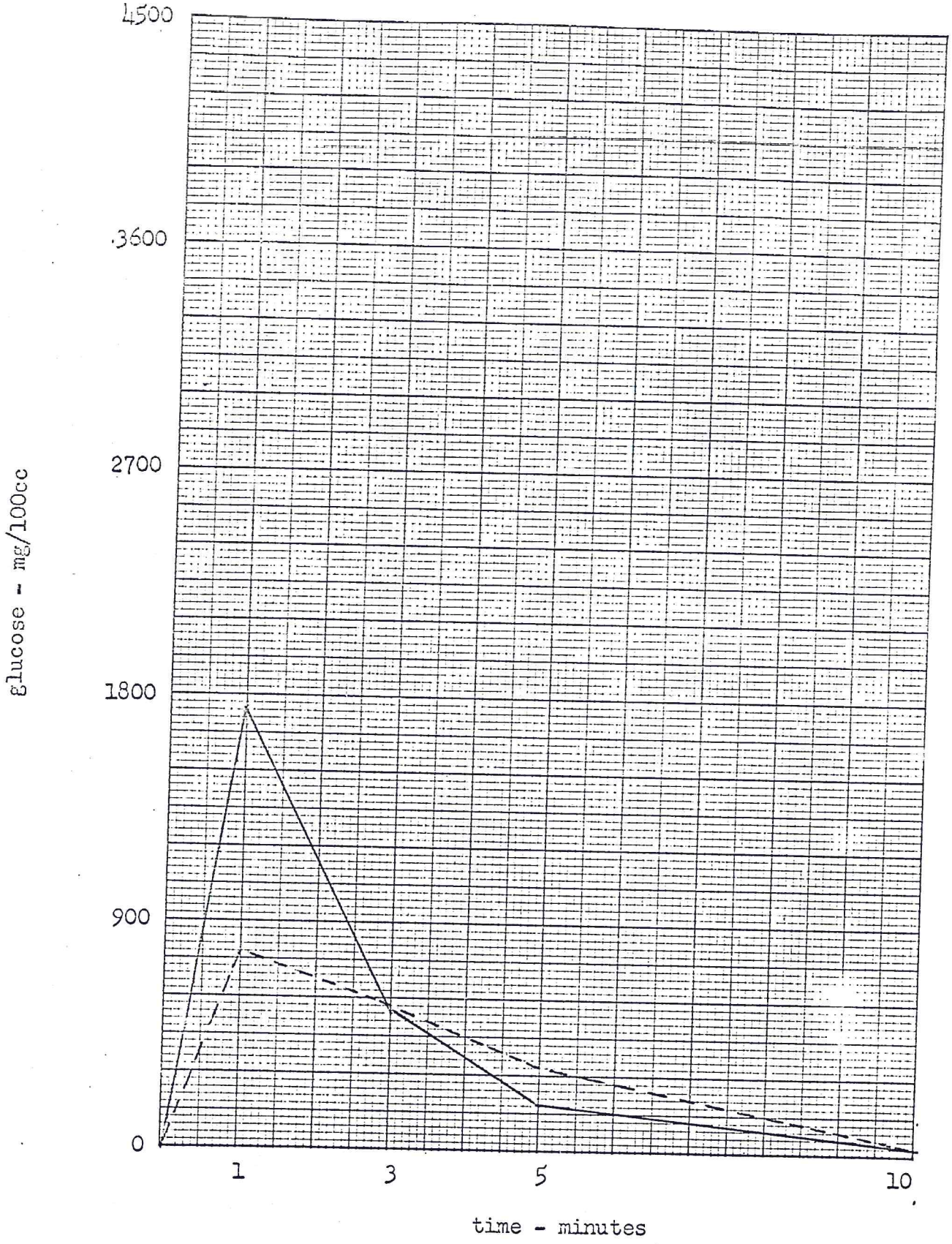
Subject A3

Time glucose concentration graphs of the caramel experiment



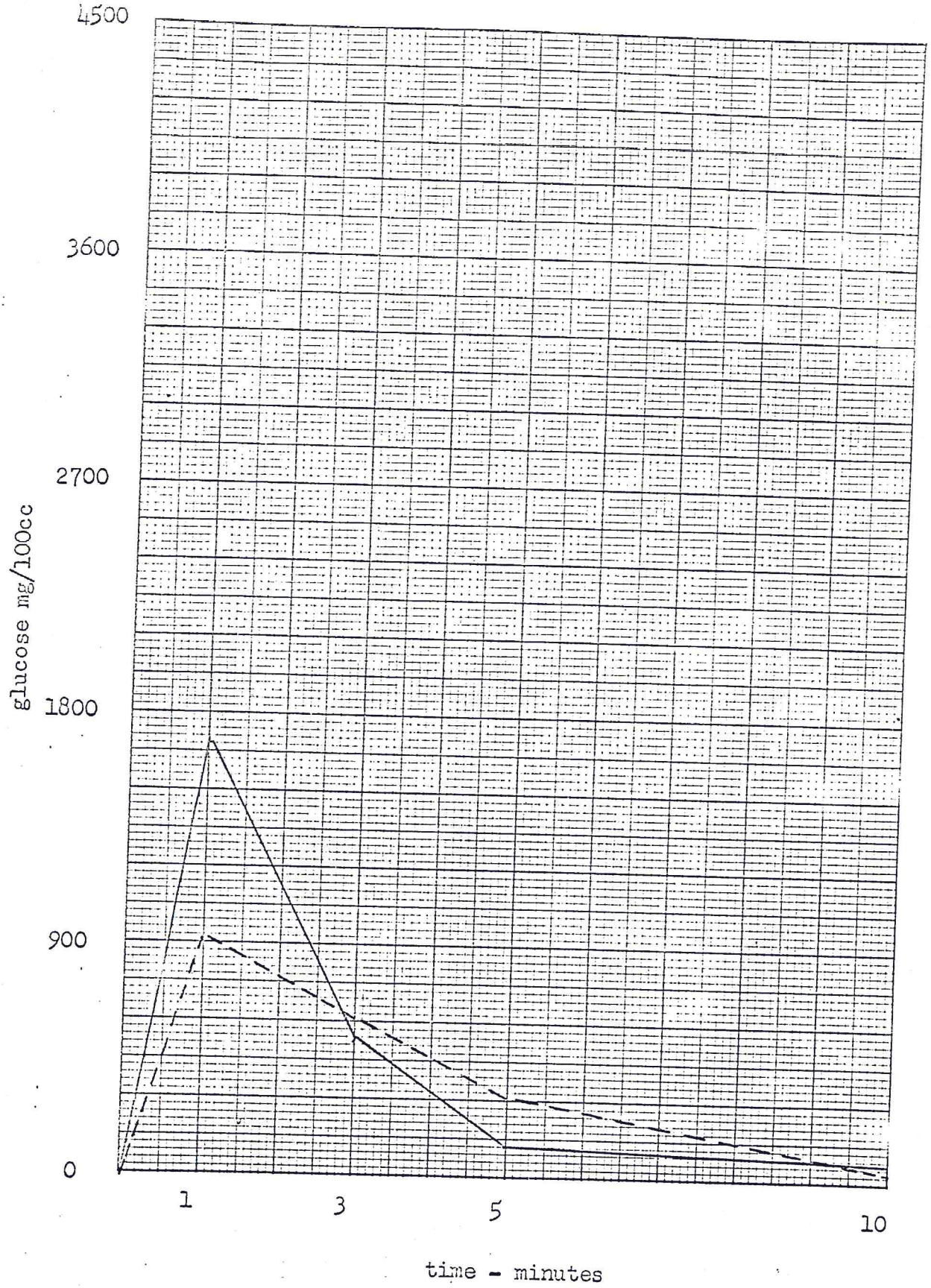
Subject B3

Time glucose concentration graphs of the caramel experiment



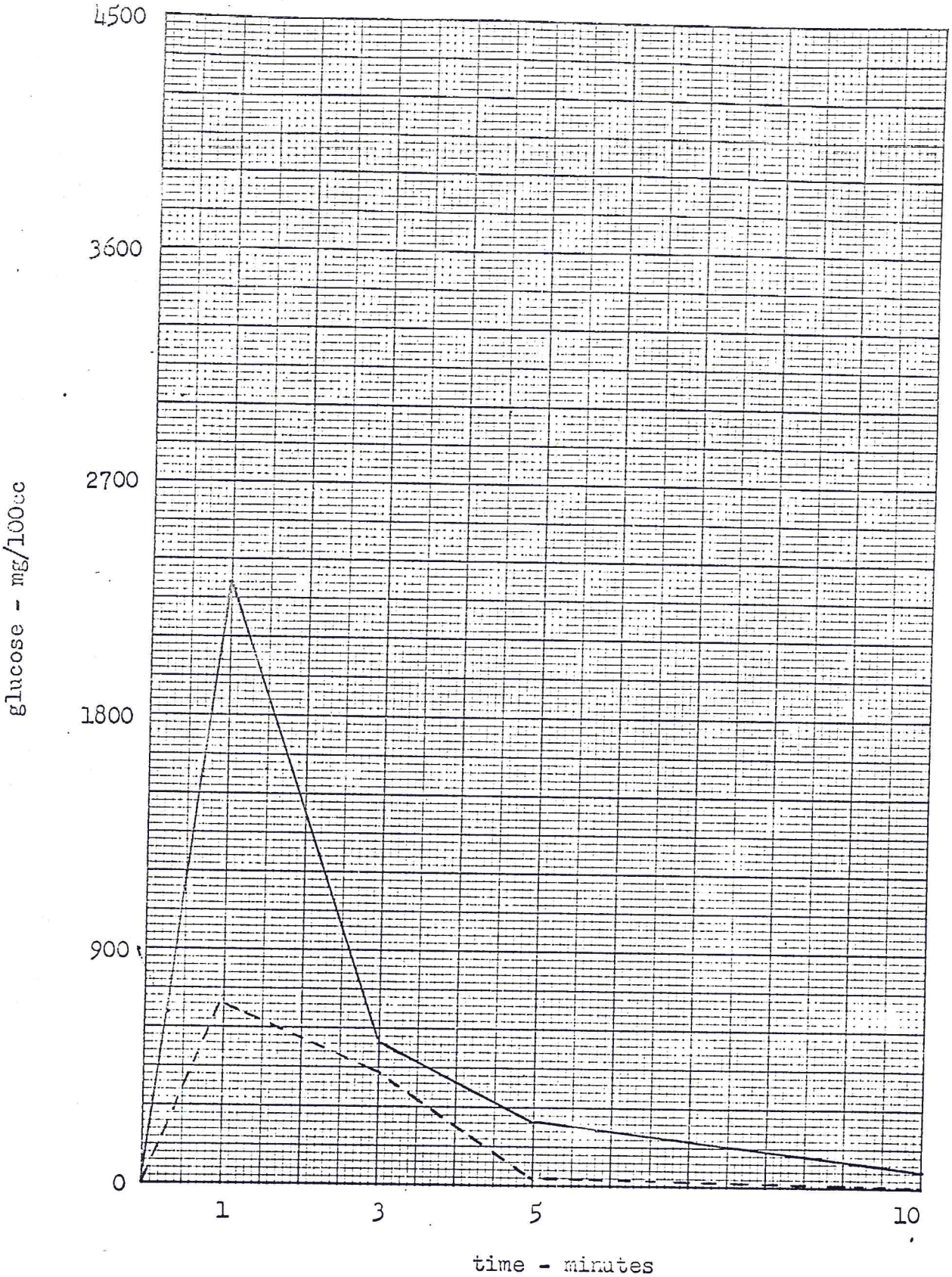
Subject 03

Time glucose concentration graphs of the caramel experiment



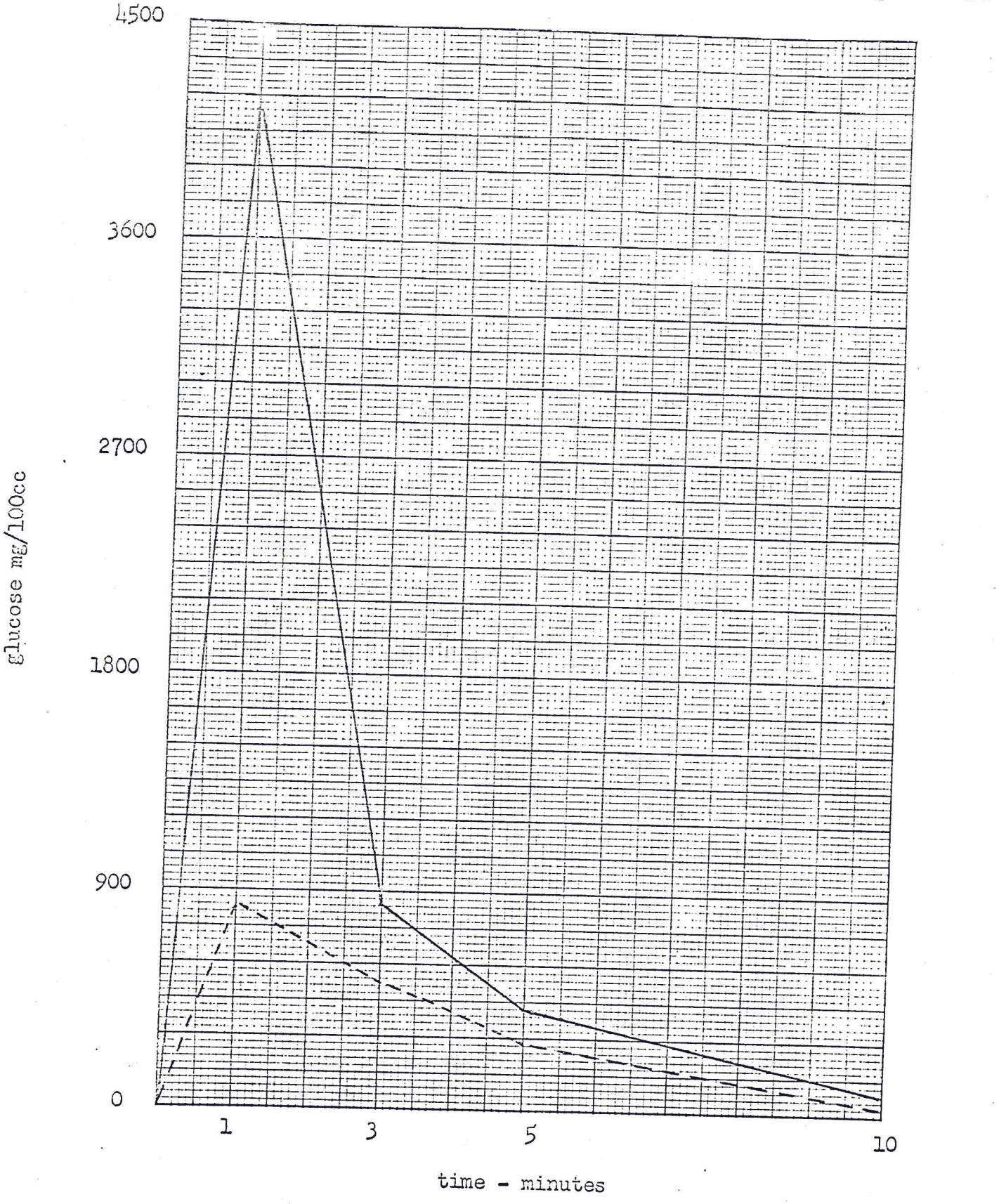
Subject D3

Time glucose concentration graphs of the caramel experiment



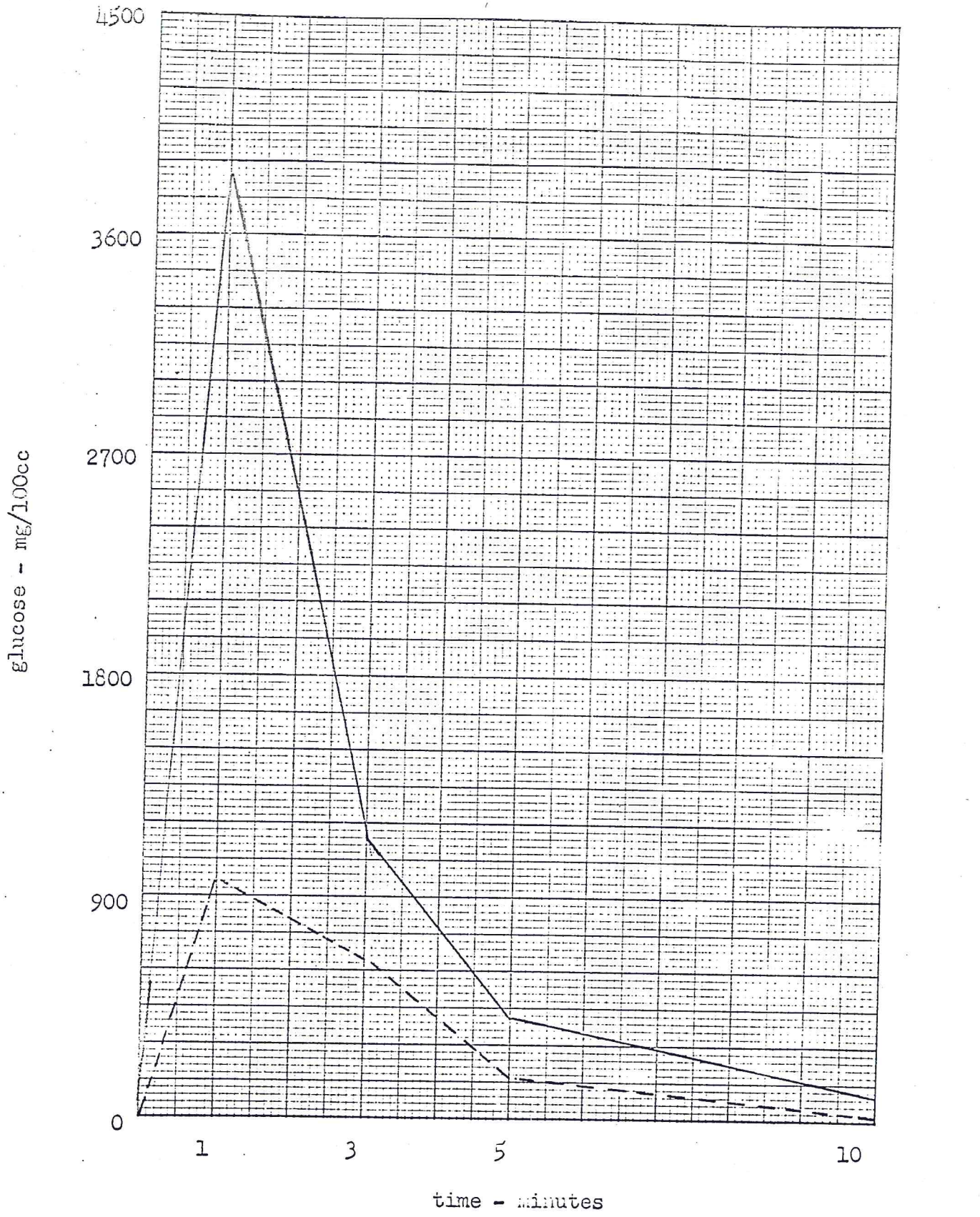
Subject E3

Time glucose concentration graphs of the caramel experiment



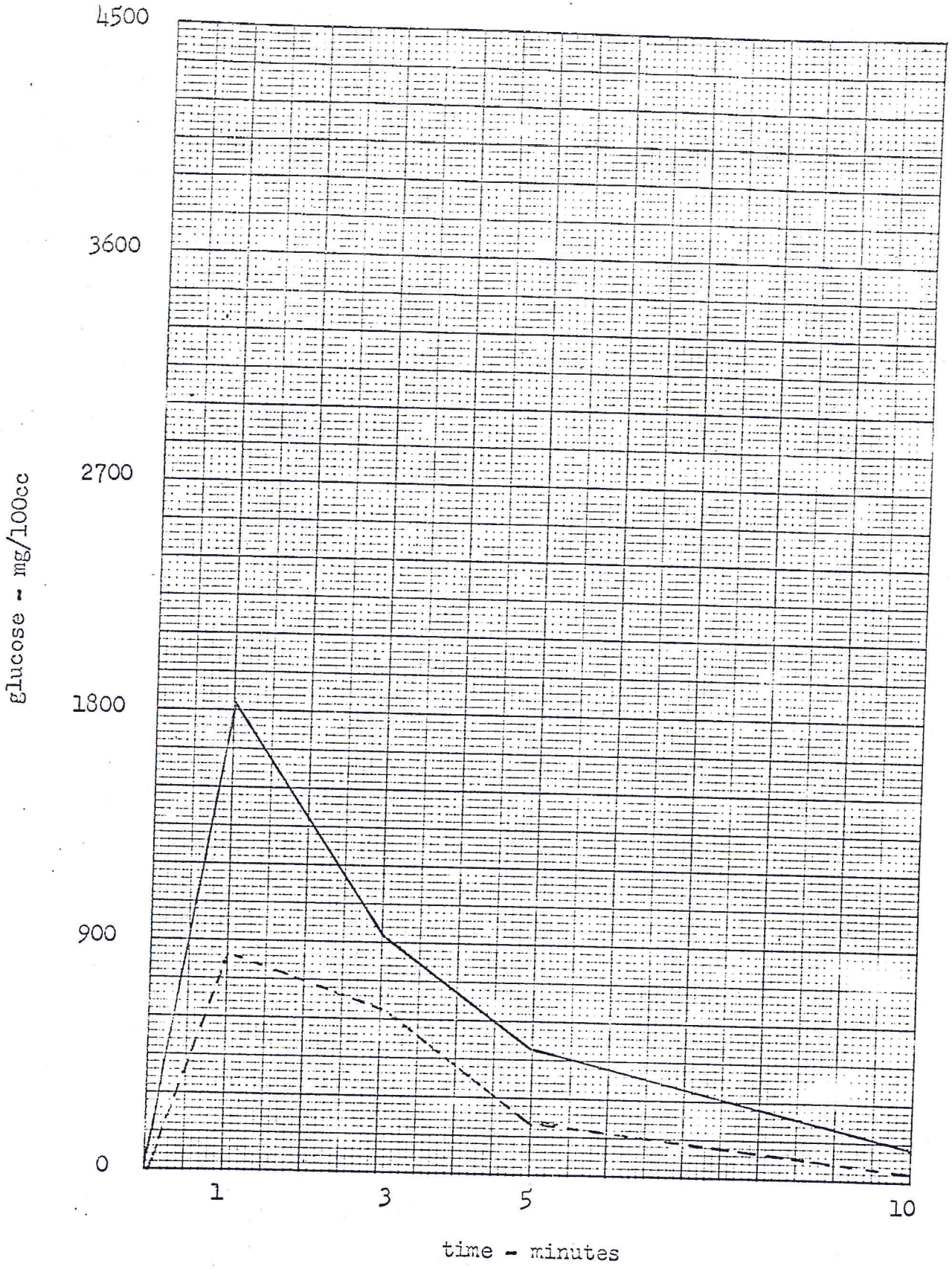
Subject F3

Time glucose concentration graphs of the caramel experiment



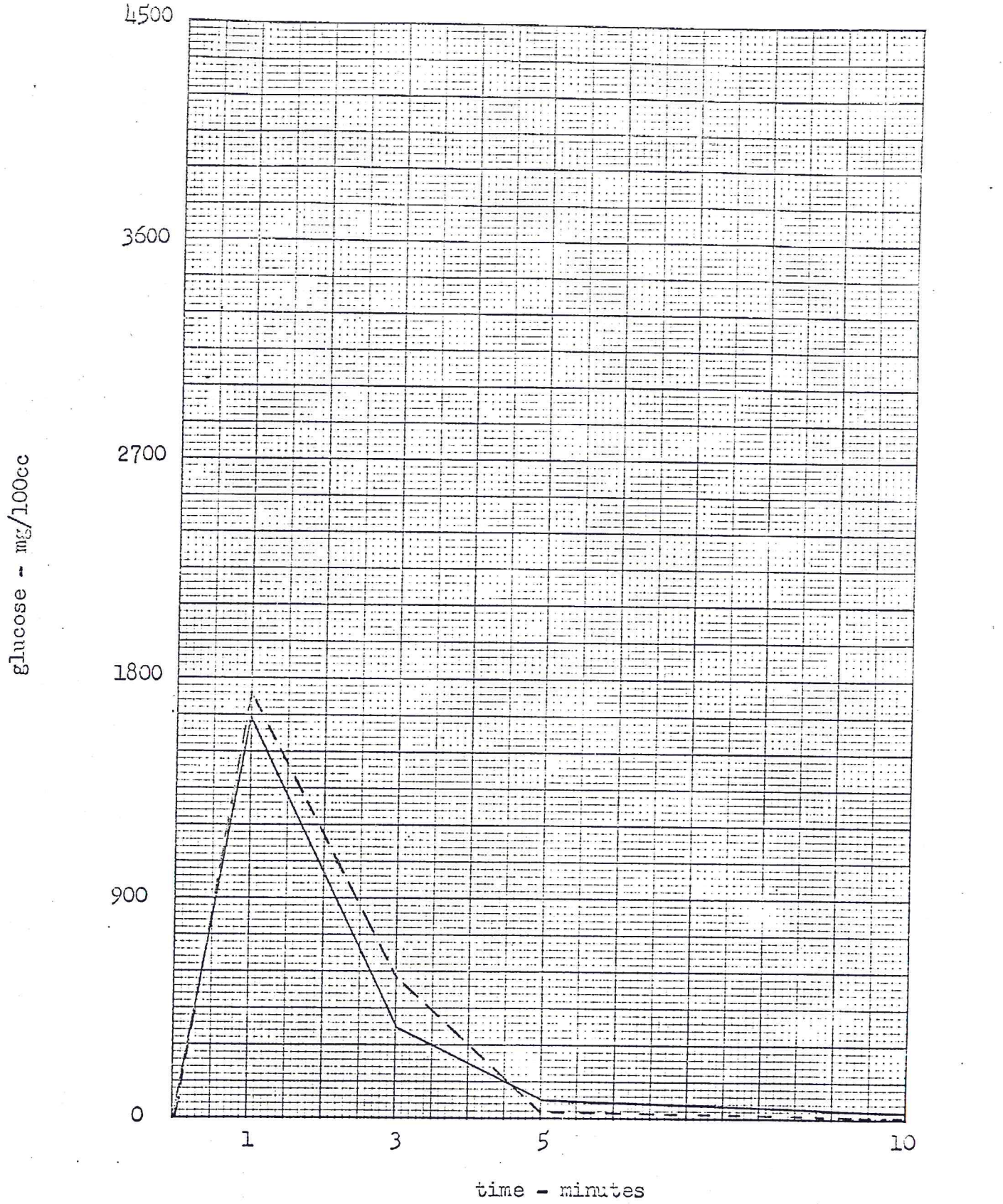
Subject G3

Time glucose concentration graphs of the caramel experiment



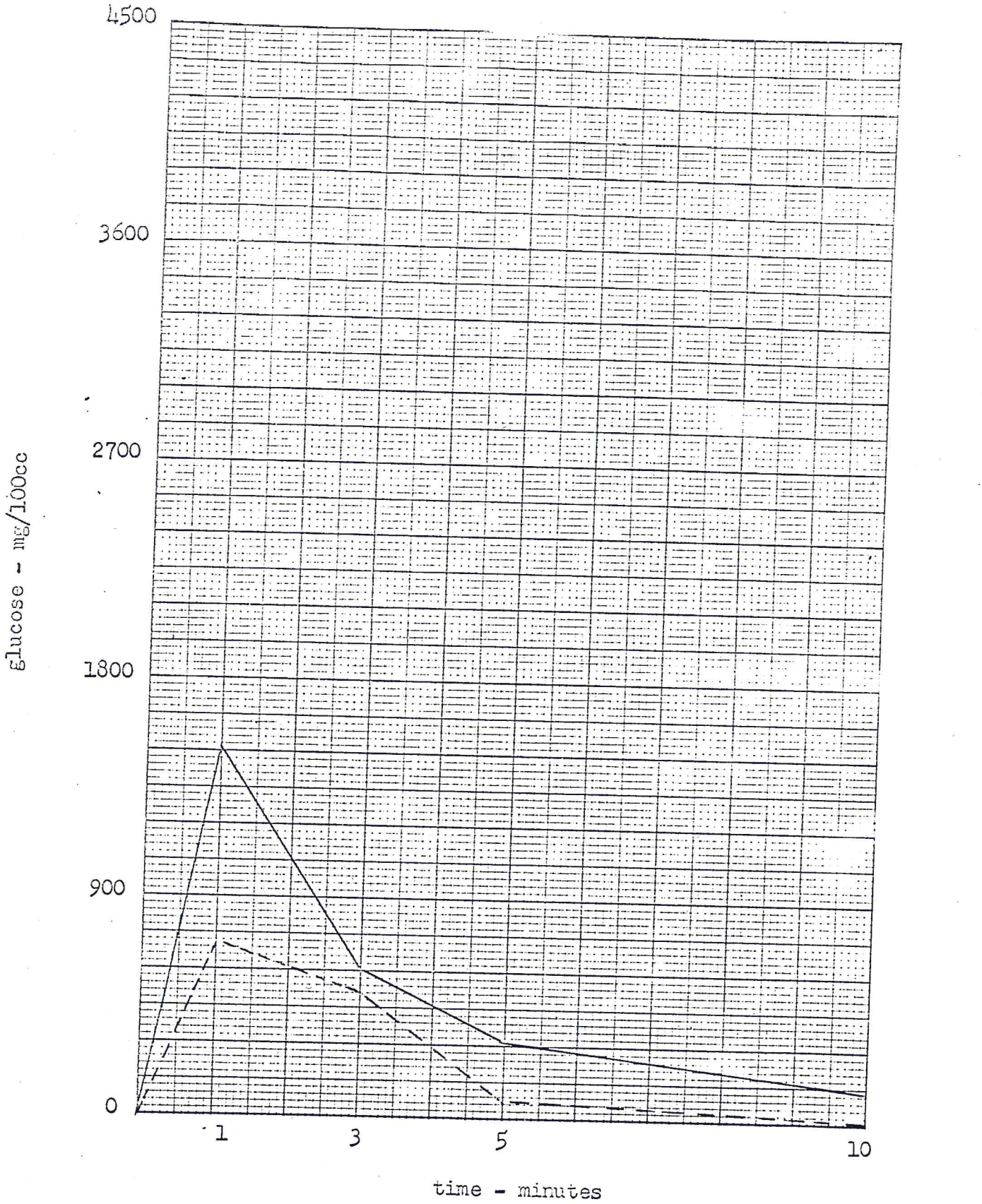
Subject H3

Time glucose concentration graphs of the caramel experiment



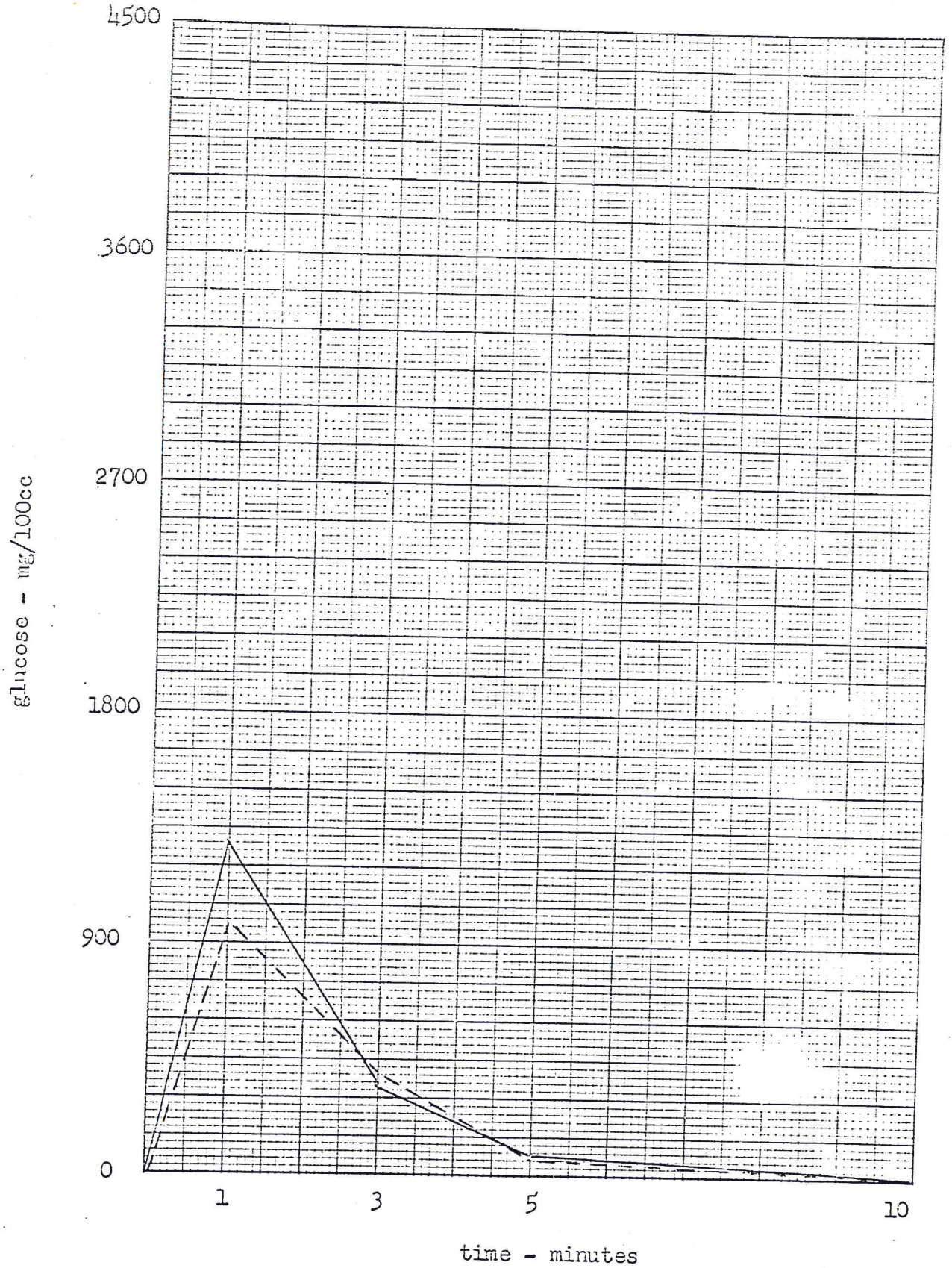
Subject F3

Time glucose concentration graphs of the caramel experiment



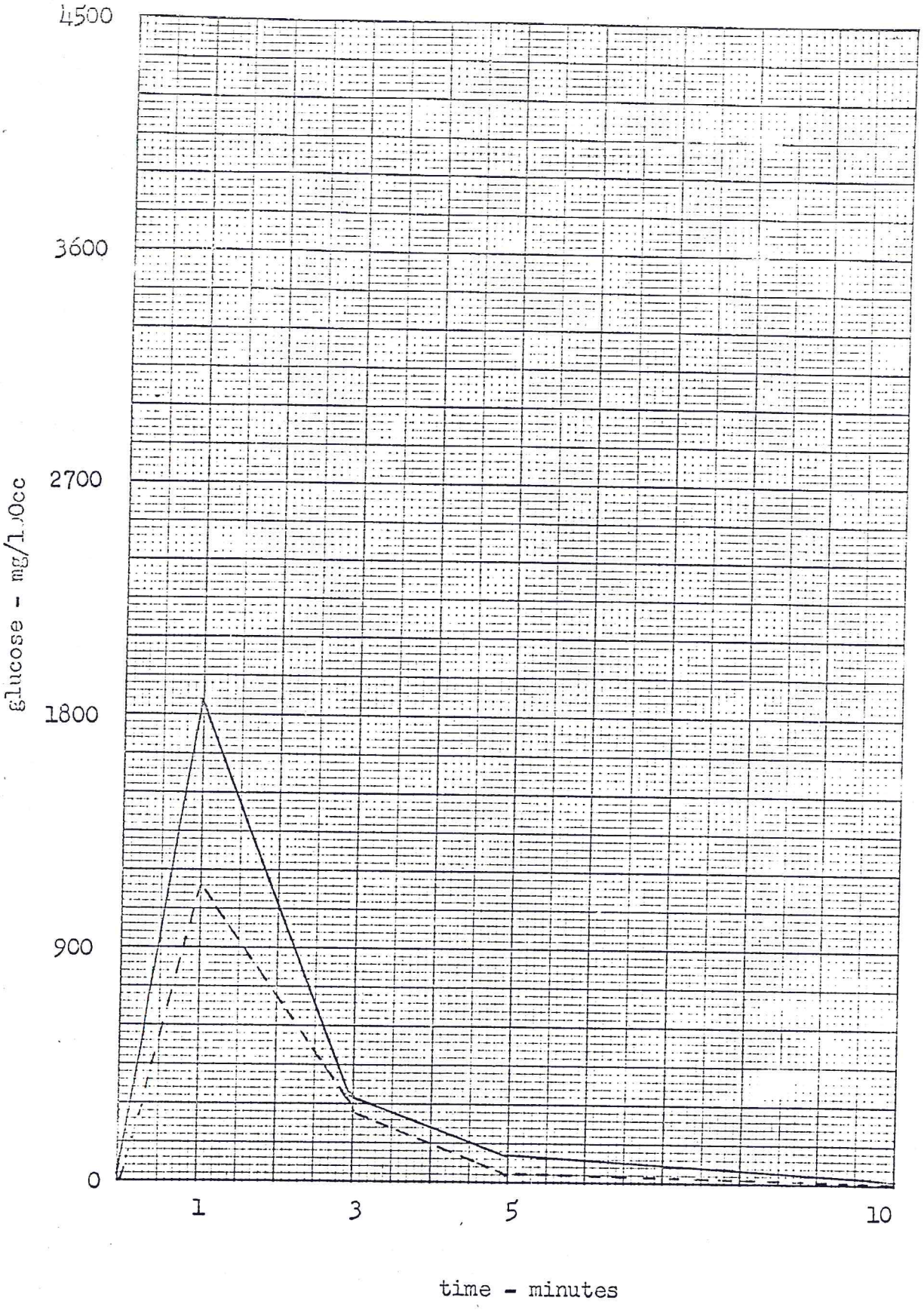
Subject J3

Time glucose concentration graphs of the caramel experiment



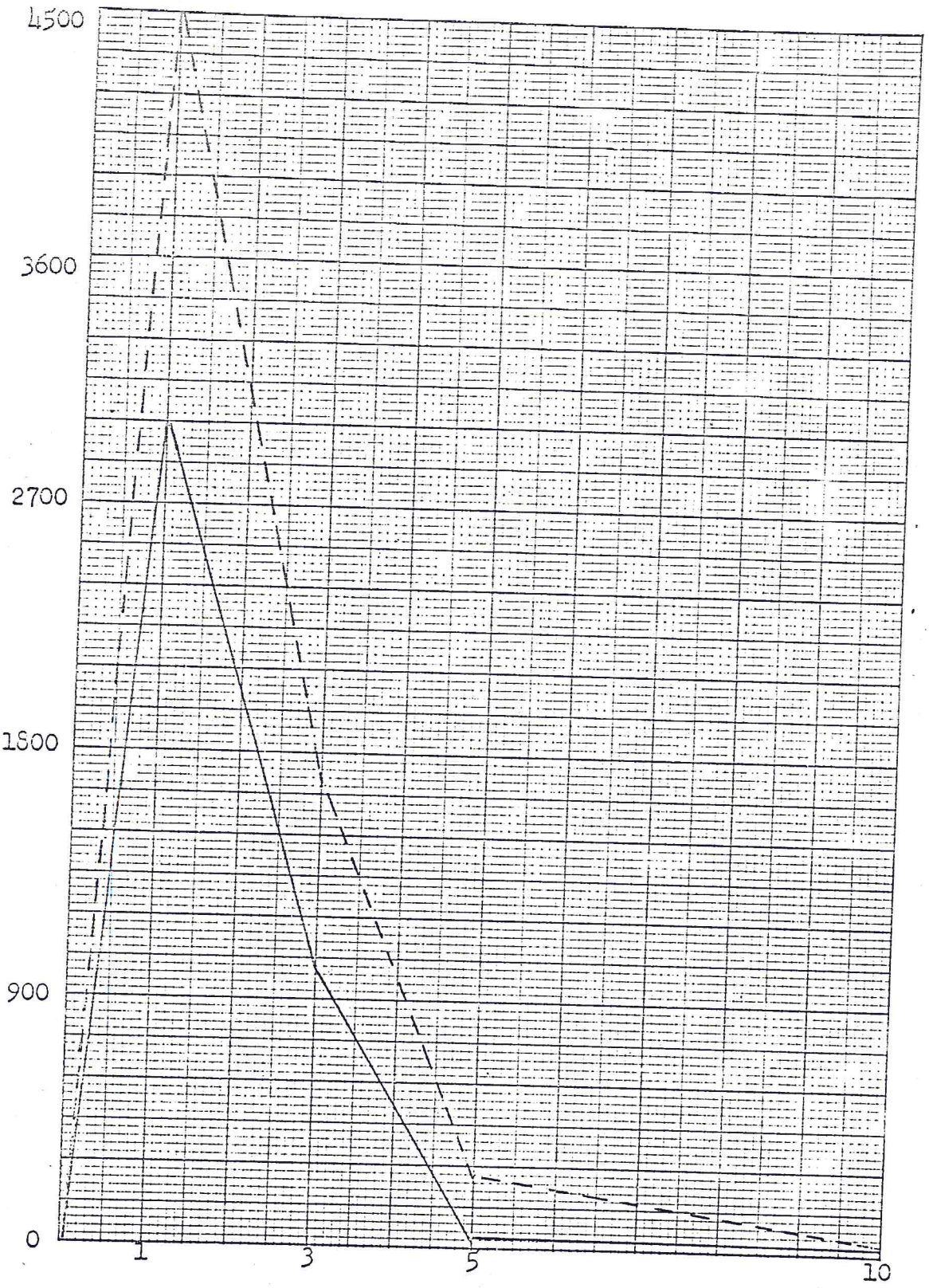
Subject K3

Time glucose concentration graphs of the caramel experiment



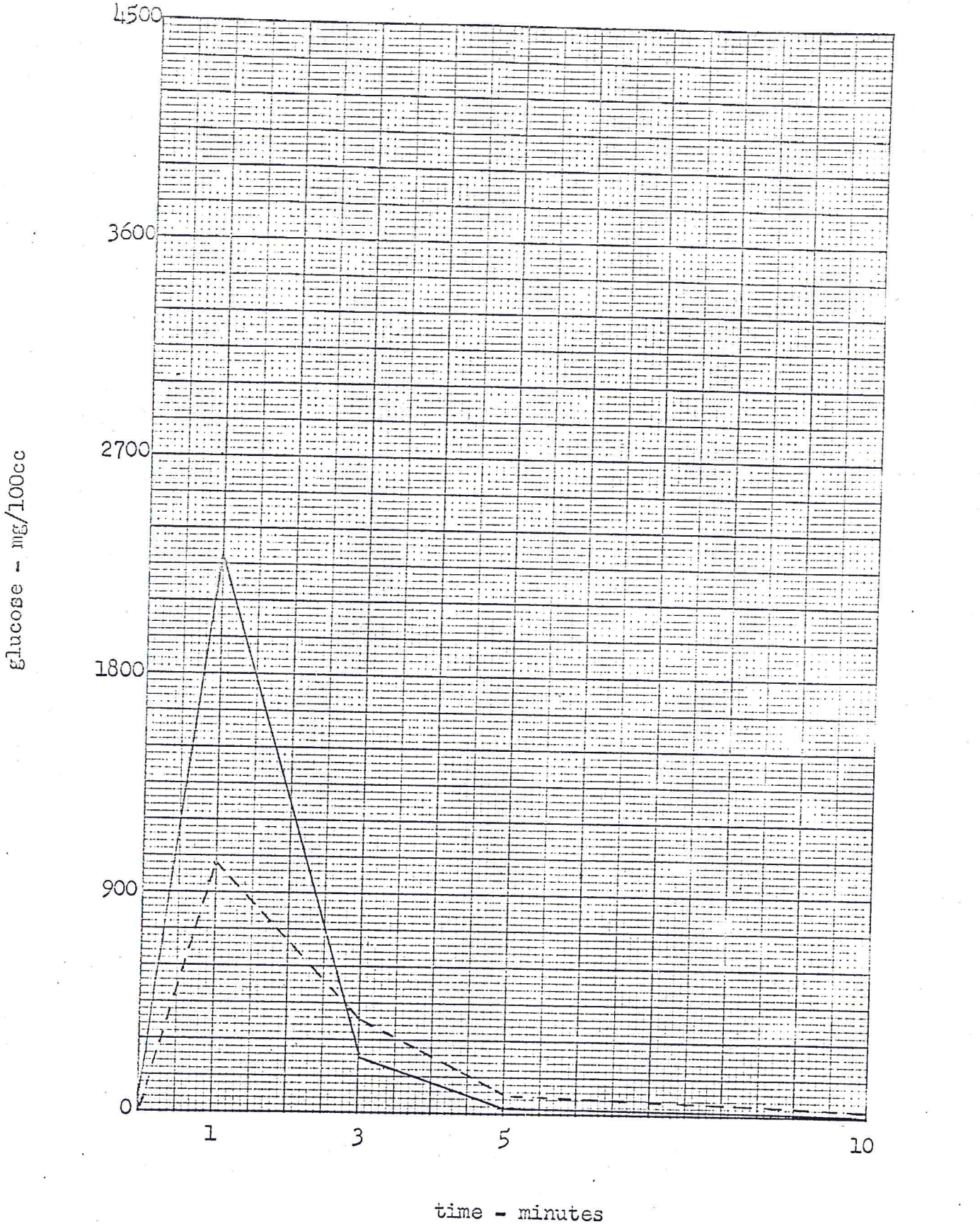
Subject L3

Time glucose concentration graphs of the caramel experiment



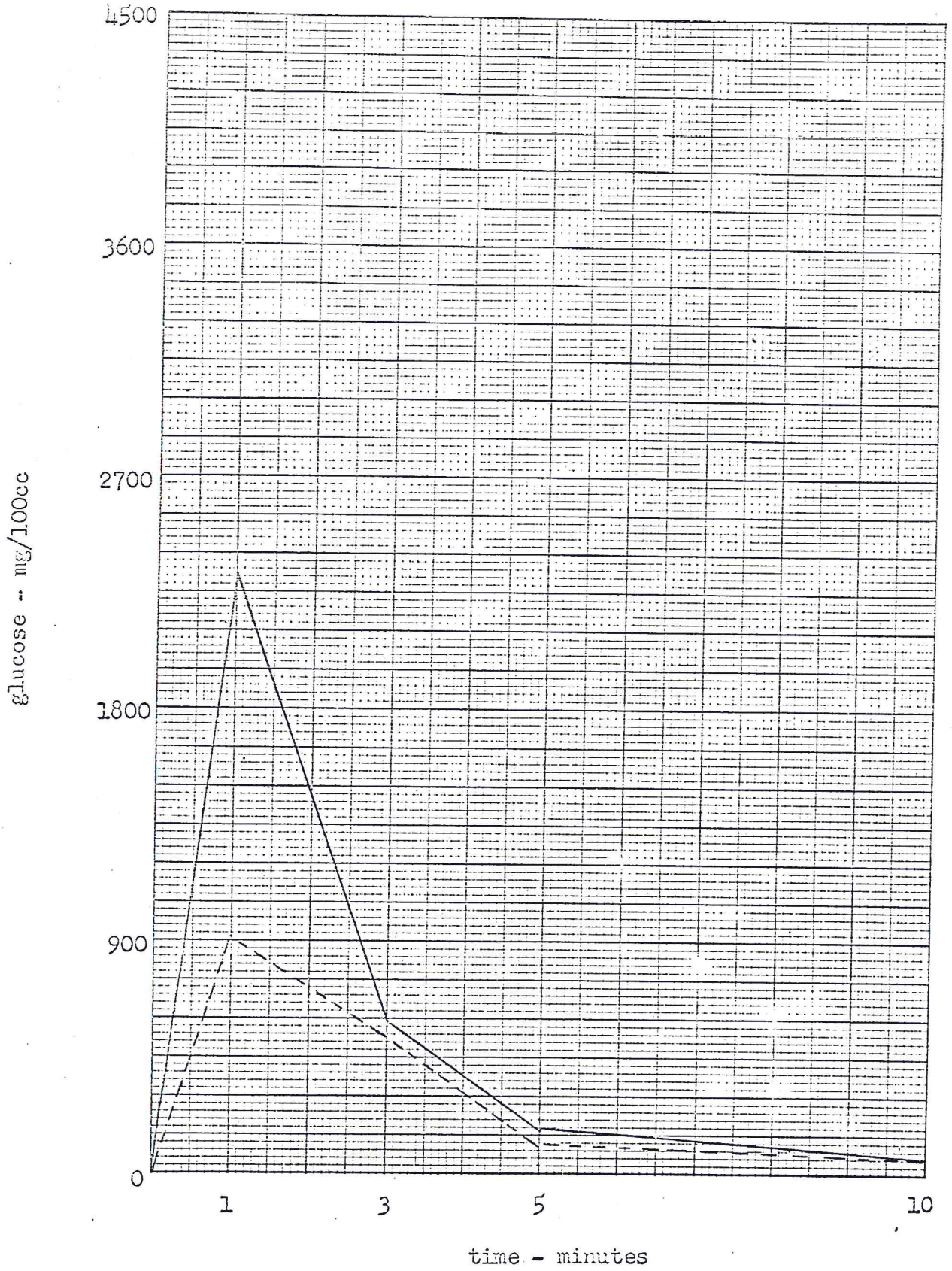
Subject M3

Time glucose concentration graphs of the caramel experiment



Subject N3

Time glucose concentration graphs of the caramel experiment



Subject 03

Time glucose concentration graphs of the caramel experiment