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## Measurement of glucose in diabetic subjects using noninvasive transdermal extraction

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Results from the Diabetes Care and Complications Trial show that tight blood glucose control significantly reduces the long-term complications of diabetes mellitus<sup>1</sup>. In that study, frequent self-testing of glucose and insulin administration resulted in a significant reduction in long-term complications. This protocol, however, also resulted in a threefold increase in the frequency of hypoglycaemic incidents. Currently, self-testing requires a drop of blood for each measurement. The pain and inconvenience of self-testing, along with the fear and danger of hypoglycaemia has led to poor patient acceptance of a tight control regimen, despite the clear long-term advantages. A

continuously worn, noninvasive method to periodically measure glucose would provide a convenient and comfortable means of frequent self-testing<sup>2,3</sup>. A continuously worn device could also alert the user of low glucose levels, thereby reducing the incidence of hypoglycaemia<sup>4,5</sup>. Guy *et al.* demonstrated a noninvasive method to transport glucose through the skin using low-level electrical current<sup>6,7</sup>. To provide a quantitative measurement, the flux of glucose extracted across the skin must correlate with serum glucose in a predictive manner. The results presented here show a quantitative relationship between serum and transdermally extracted glucose in diabetics.

A comparison of venous serum glucose and extracted glucose flux indicates that there is close tracking after an equilibration period in both the hypoglycaemic (Fig. 1a) and hyperglycaemic (Fig. 1b) range. This indicates that the extracted and serum measurements track over a glucose concentration range from 50 to 400 mg dl<sup>-1</sup>. In four of five subjects, the extracted glucose flux tracked serum glucose after the first 60 minutes, whereas in one subject tracking was not achieved until two hours. The length of this period is most likely due to the time required for skin hydration, equilibration of the skin with the extraction solution buffer, or stabilization of the electrical properties of the skin.

The results in Fig. 1a and b also show that the extracted glucose flux profile is shifted to a later time relative to the serum profile, suggesting a time lag between the two measurements. An estimate of time lag was obtained from a series of linear regression analyses of serum glucose against glucose flux, where each regression was determined with successive shifts in the sampling interval between the readings. In other words, the serum glucose

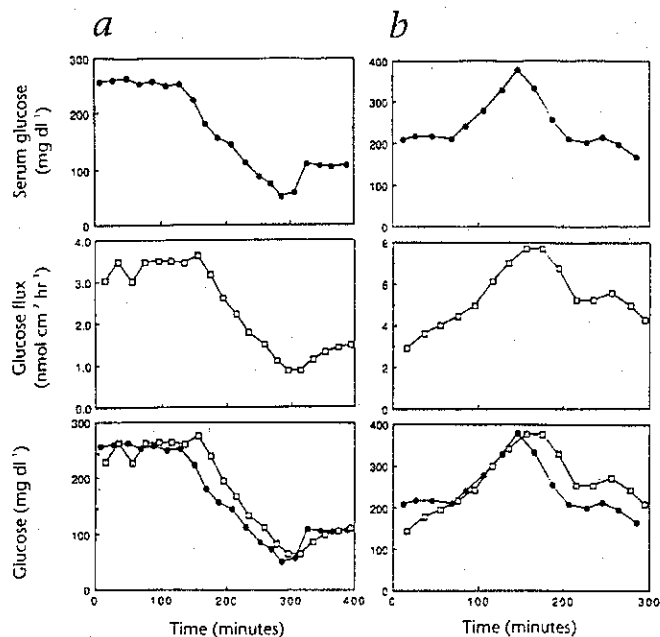


Fig. 1 Serum glucose (●, top plot) and extracted glucose flux (□, middle plot) profiles. Extracted flux is reported as the average of the two sample chambers unless otherwise noted. The bottom plot shows a direct comparison of the glucose level calculated from the transdermally extracted glucose flux and the serum glucose. *a*, Hypoglycaemic response of subject 4 (type 1): i.v. insulin was administered at 120 minutes and i.v. glucose was administered at 280 minutes. *b*, Hyperglycaemic response of subject 2 (type 2): Oral glucose was administered at 60 minutes and i.v. insulin was administered at 120 minutes.

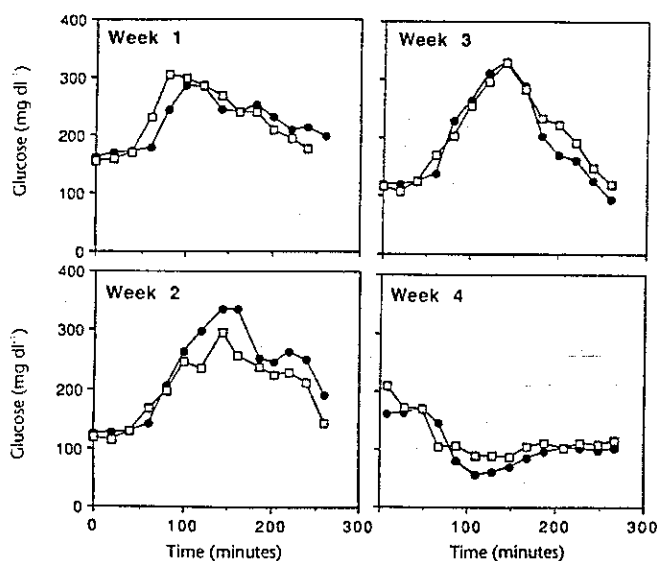


Fig. 2 Comparison of serum glucose (●) and glucose calculated from the transdermally extracted glucose flux (□) from subject 3 (type 2) on four different days. The extraction data are uniformly shifted by an extraction period to match the time of the previous serum glucose measurement. Hyperglycaemia was induced in sessions 1–3 and hypoglycaemia on session 4. A single fingerprick calibration at the one hour time point (as evidenced by the overlap of the two curves) was used to calculate equivalent serum glucose from the transdermally extracted glucose.

values were analysed and compared with the extracted readings obtained during the overlapping time interval, and then with the values obtained one, two or three sampling intervals later. In this way, the time lag yielding the largest correlation coefficient was determined as the 'best fit'. The best fit time lag ranged from zero to three sample periods, with an average and mode of one sample interval for all evaluations; that is, the serum value from the midpoint of one interval is correlated with the average flux of the next 20-minute collection interval. This delay is caused by both the time required for the analyte to travel through the skin and the 20-minute collection interval.

Results obtained for the same subject on four separate days illustrate the tracking in successive measurements (Fig. 2). The extracted glucose results shown in Fig. 2 were all shifted forward by one collection period to match the time of the previous serum glucose measurement. A linear regression was performed with these data for all times beyond 60 minutes. The results show an average correlation coefficient ( $r$ ) of 0.91 for the four separate determinations. A similar analysis was performed for all 12 evaluations with the values for  $r$ , and the slope and intercept are summarized in Table 1. The results show an average correlation coefficient of 0.89, demonstrating the close correlation between the extracted flux and plasma serum glucose values.

The results in Table 1 show that the intercept is generally close to zero, with 8 out of 12 studies showing a zero intercept ( $P < 0.05$ ). Additionally, the time lags are generally close to one sample period. Hence, to further simplify the analysis the glucose flux was divided by the serum glucose for each point, again assuming a one-sample-period time lag and omitting the data from the first hour. The results (Table 1) show that the ratio differed at most by a factor of two among all subjects tested with an average (standard error) of 4.12 (0.27)  $\mu\text{m hour}^{-1}$ . It is interesting that the ratio obtained for the one-parameter fit shows less variability than the slope obtained with the two-parameter fit.

To be clinically useful, glucose measurements must be quantitatively reproducible and accurate. One criterion for establishing clinical accuracy is the Error Grid Analysis<sup>8</sup>. The analysis divides the comparison between the reference serum glucose and the measured glucose (serum glucose as calculated from transdermal extraction in these experiments) into five zones. Values in zones A and B are clinically acceptable, whereas values in zones C, D, and E lead to clinically significant errors. We have developed a calibration method by dividing the serum glucose value during the third sample period (one hour, to allow the skin to equilibrate) by the extracted glucose flux taken one sample period later (to account for the time lag). This ratio was then used to calculate serum glucose from glucose flux for all subsequent measurements. The calculated glucose results obtained with the data from all five subjects (twelve studies), are plotted in an Error Grid Analysis in Fig. 3. These results show that only four of 140 data points (3%) are outside of the A and B region. Using a single-point calibration from day 1 for subsequent data on all days for a given subject resulted in 95% of the data falling within the A and B region. Both results are similar to those obtained with commercial blood glucose monitors<sup>9</sup>.

Another criterion for clinical accuracy is the mean absolute error between the reference and calculated glucose value defined in these experiments by the following equation: Mean absolute error = [absolute value (serum glucose – calculated glucose)/serum glucose]  $\times$  100%. The results obtained here show a mean absolute error of 12.9% for all measurements ( $n = 140$ ), a value comparable to currently available self-testing glucose monitors<sup>9</sup>. These results suggest that extracted glucose accurately and reproducibly reflects

serum glucose values.

In conclusion, correlation between serum glucose and extracted glucose flux was firmly established for five subjects under a variety of conditions. Quantitative measurement was possible through the use of a single-point calibration analysis. These results demonstrate a painless and bloodless method for extracting glucose through the skin. The extraction technique potentially provides a noninvasive means of continuously monitoring serum glucose in diabetics, leading to the possibility of significantly enhanced health care.

## Methods

Three male and two female subjects from 22 to 58 years of age, diagnosed with either type 1 or 2 diabetes mellitus for between 5 and 15 years and having a body weight within -30% to +50% of ideal as defined by the Metropolitan Life Insurance tables, participated in the study. Subjects at medical risk or those with a skin disease on the forearms were excluded from the study. Written consent was obtained from all volunteers after they were informed of the nature and possible consequences of the study. The protocol was approved by an institutional review board. Three subjects were tested in three successive weekly sessions under a hyperglycaemic protocol, and three subjects (one repeat subject) were tested under a hypoglycaemic protocol.

Subjects fasted for at least 8 hours and omitted any insulin dose

Table 1 Relationship between serum glucose and iontophoretic flux

Subject	Number of data points	<i>r</i>	LINEAR REGRESSION		Average ratio of flux to serum glucose ( $\mu\text{m hr}^{-1}$ )	
			Slope ( $\mu\text{m hr}^{-1}$ )	intercept ( $\text{nmol cm}^{-2} \text{hr}^{-1}$ )		
<i>Hyperglycaemic protocol</i>						
Subject 1	Week 1	12	0.98	5.55	-0.60 <sup>c</sup>	5.14
	Week 2	12	0.89	3.15	1.36 <sup>c</sup>	4.11
	Week 3 <sup>a</sup>	12	0.93	4.20	0.59 <sup>c</sup>	4.59
Subject 2	Week 1 <sup>b</sup>	9	0.67	3.43	2.26 <sup>c</sup>	4.74
	Week 2 <sup>a,b</sup>	9	0.98	2.09	0.91	3.10
	Week 3 <sup>a,b</sup>	9	0.92	1.78	2.78	3.68
Subject 3	Week 1	12	0.79	6.28	-0.96 <sup>c</sup>	5.37
	Week 2	12	0.93	3.34	1.37 <sup>c</sup>	4.38
	Week 3	12	0.96	2.49	0.84 <sup>c</sup>	3.30
<i>Hypoglycaemic protocol</i>						
	Subject 3 <sup>a</sup>	12	0.83	1.81	1.01	3.70
	Subject 4	17	0.99	2.46	0.02 <sup>c</sup>	2.48
	Subject 5	12	0.96	2.26	1.38	4.79
Average			0.89	3.24	0.91	4.12

Regressions and ratios were calculated using an equilibration period and a one sample period time lag.

<sup>a</sup>Data from a single collection chamber rather than average of two chambers. <sup>b</sup>Subject required two hour equilibration period. <sup>c</sup>Intercept not significantly different from zero ( $P = 0.05$ ).

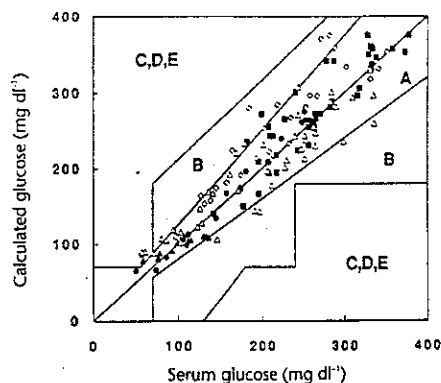


Fig. 3 Error Grid Analysis for all five subjects, 12 experiments ( $n = 140$ ) with calculations as describe in the text and in Table 1. Subject 1 (■), subject 2 (◇), subject 3 (△), subject 4 (●), subject 5 (▲).

immediately before the study. To induce hyperglycaemia, the subjects were administered 100 g of glucose (Glucose Tolerance Beverage, Stephens Scientific, Riverdale, New Jersey) orally after 1 hour of baseline sampling. Once the blood glucose reached 300  $\text{mg dl}^{-1}$ , insulin (Humulin regular, Lilly, Indianapolis, Indiana) was administered intravenously (i.v.) in 5- to 20-U bolus doses as required to bring serum glucose to normal levels. To induce hypoglycaemia, insulin was administered i.v. to achieve a target blood glucose of 40–60  $\text{mg dl}^{-1}$ . Dextrose (Astra Pharmaceutical, Westboro, Massachusetts) was then administered i.v. if the subject's blood glucose went below 40  $\text{mg dl}^{-1}$ , or if subject was below 60  $\text{mg dl}^{-1}$  for longer than 60 minutes.

Two adhesive extraction devices, each containing a Ag/AgCl electrode and an aqueous collection reservoir accessible through a syringe port were applied to the volar surface of the forearm of the subject. Each reservoir had a collection surface area of 2.85  $\text{cm}^2$  and a volume of 0.4 ml. The extraction solution was a 1:9 (vol/vol) mixture of 5% sodium bicarbonate, USP (as listed in the US Pharmacopeia), for injection (Baxter) and 0.45% sodium chloride in water for injection, USP (Baxter) with a pH between 8.2 and 8.8. This buffer was introduced into and removed from the collection reservoirs through the port, using a 1-ml syringe. A current of 0.32  $\text{mA cm}^{-2}$  was applied for 15 minutes across the two electrodes using a Phoresor II (Iomed, Salt Lake City, Utah) commercial iontophoretic power supply. The electrical polarity was reversed halfway through each 15-min cycle. Previous work has indicated that polarity reversal enhanced transdermally extracted glucose transport. The extraction period was followed by a 5-min interval during which the buffer was removed from each reservoir. The samples were placed in tared vials

and weighed, and then frozen until subsequent analysis. Each collection reservoir was then refilled with fresh buffer. The 15-min current application and 5-min sampling procedure was repeated continuously over the duration of the experiment (generally 5 h).

The extracts were subsequently thawed and analysed for glucose by high-performance liquid chromatography (HPLC) with pulsed amperometric detection according to a modification of a method developed by LaCourse<sup>9</sup>. Briefly, chromatography was carried out on a Dionex CarboPac PA1 anion-exchange column by using a mobile phase of 20–25 mM NaOH. The detector was a Dionex ED40 detector with a 1.0-mm gold working electrode using a waveform pulsing from 80 to 800 to –300 mV. The HPLC error was less than  $\pm 10\%$  (relative standard deviation (r.s.d.)) and generally less than  $\pm 3\%$  (r.s.d.) as measured by repeated injection of standards throughout the chromatographic run. Verification of peak identification was established by addition of glucose oxidase to the *in vivo* extracts and noting complete disappearance of the glucose peak. The lower limit of quantification was 0.24  $\mu\text{M}$  corresponding to a glucose flux of 0.13 nmol  $\text{cm}^{-2}$  per hour.

The flux values obtained at the two collection reservoirs were averaged for each time point, and the mean values are presented. The error in sample handling was estimated from the mass of buffer collected after each extraction period. Those results show that 6.4% mean absolute error is introduced into the extracted flux values by the sampling procedure. In some cases, leakage from the collection reservoirs was noted by an error in sample recovery of greater than 10% (standard deviation), and only the non-leaking reservoir was used in the graphs and calculations.

Blood samples were taken from an antecubital vein just before current was applied for the first time (0 time) and at the midpoint of each subsequent collection session (that is, 7.5 minutes after initiation of current). The samples were sent to a clinical lab for analysis using a Beckman CX-7 serum glucose analyser. A drop of each blood sample was also analysed by the One Touch II or One Touch Basic meter to provide an immediate blood glucose measurement. Although blood samples were obtained at a specific time, the glu-

cose flux values were obtained over 15 min and hence, represent an average over that time period.

During the current application, subjects reported a mild tingling sensation, which diminished with time. Minor skin irritation, characterized by slight erythema and oedema, was observed at the test site immediately after removal of the patches. In all cases, however, erythema and oedema completely resolved before the next visit one week later. Sample patches were placed on the same general but not the same precise site in subjects who underwent successive evaluations.

#### Acknowledgements

The authors would like to thank Nooshin Azimi, Kathleen Comyns, Lewis Leung, Kamran Rafii, Mark Burnis, Michael Sekera and Gabriel Chan for their technical support of this work. Richard Guy provided invaluable scientific insight into these studies.

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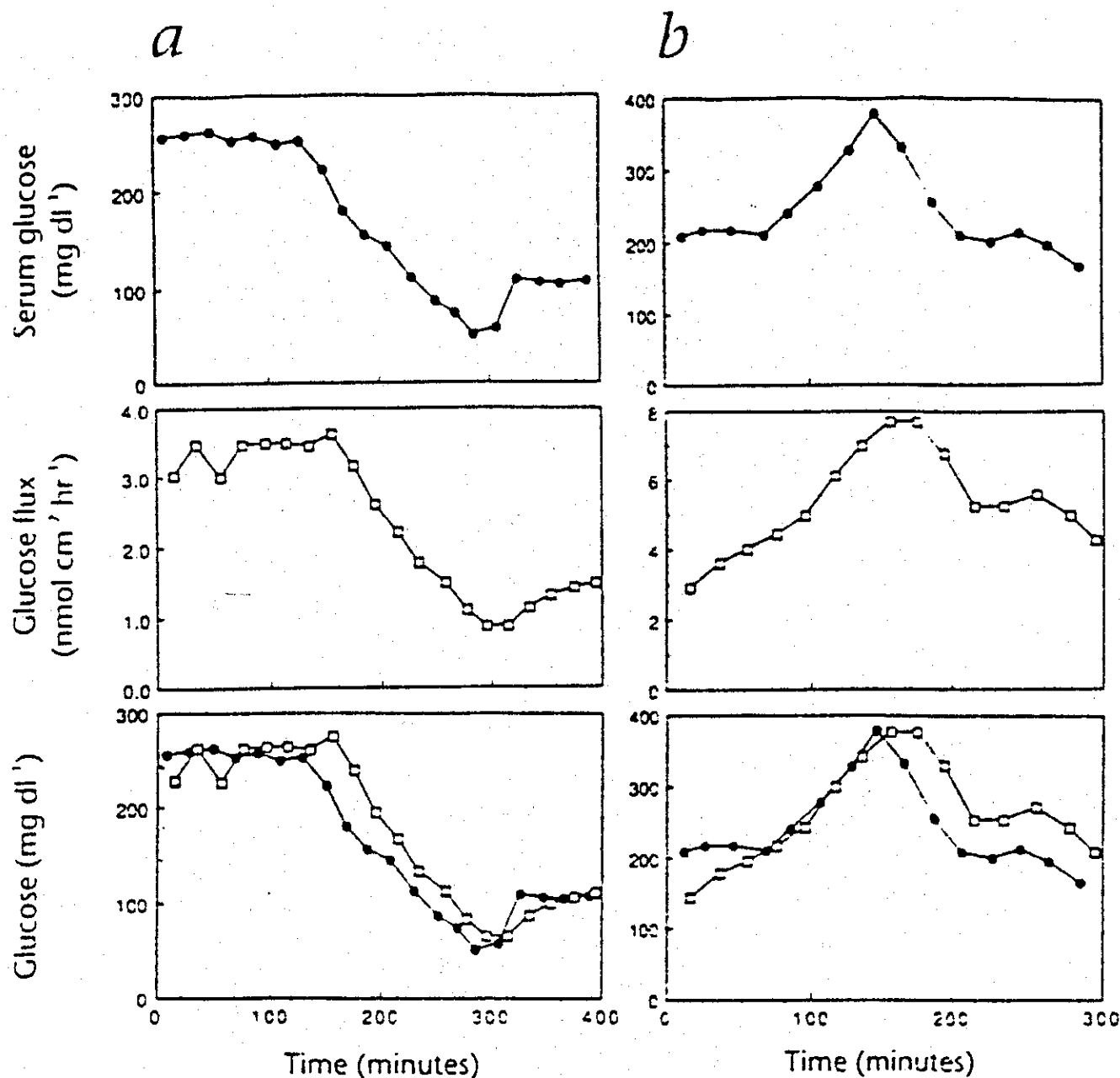


Fig. 1 Serum glucose (●, top plot) and extracted glucose flux (□, middle plot) profiles. Extracted flux is reported as the average of the two sample chambers unless otherwise noted. The bottom plot shows a direct comparison of the glucose level calculated from the transdermally extracted glucose flux and the serum glucose. *a*, Hypoglycaemic response of subject 4 (type I): i.v. insulin was administered at 120 minutes and i.v. glucose was administered at 280

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Results illustrating the extraction of glucose by one glucose sensor, these are averaged to determine the evaluation of the summation of the coefficients

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middle plot) profiles. Extracted flux is reported as the average of the two sample chambers unless otherwise noted. The bottom plot shows a direct comparison of the glucose level calculated from the transdermally extracted glucose flux and the serum glucose. *a*, Hypoglycaemic response of subject 4 (type 1): i.v. insulin was administered at 120 minutes and i.v. glucose was administered at 280 minutes. *b*, Hyperglycaemic response of subject 2 (type 2): Oral glucose was administered at 60 minutes and i.v. insulin was administered at 120 minutes.

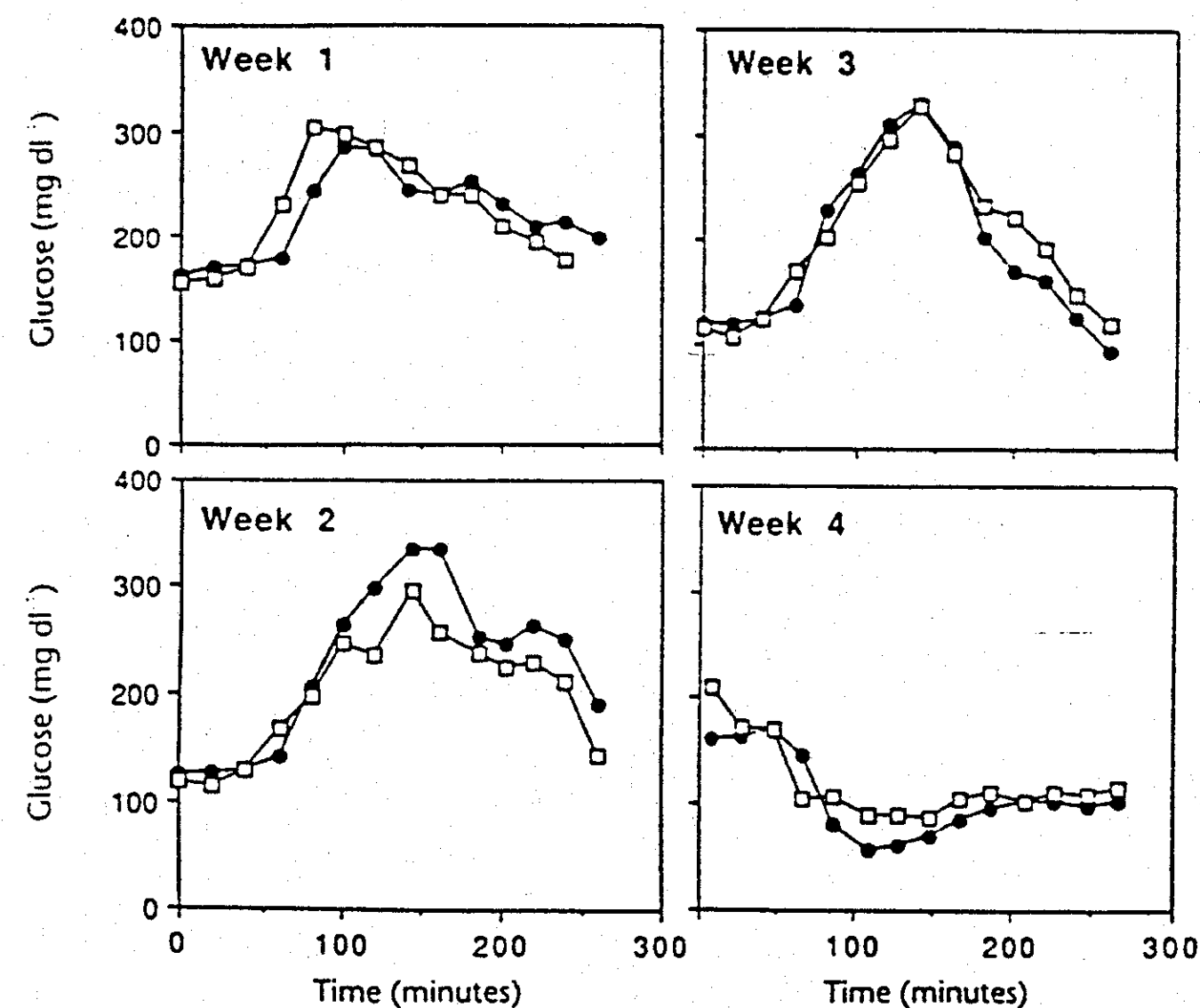
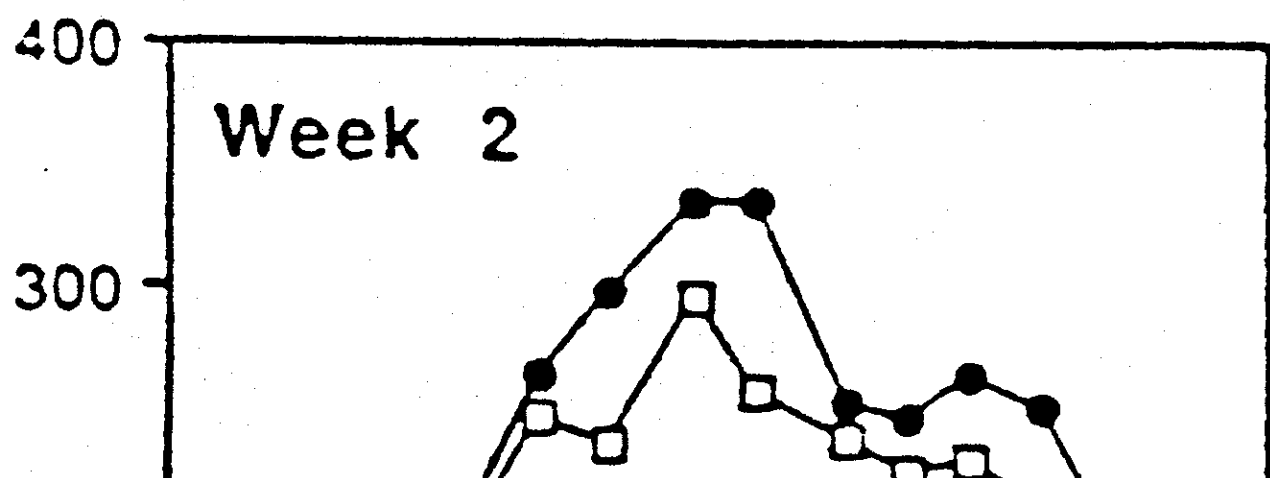
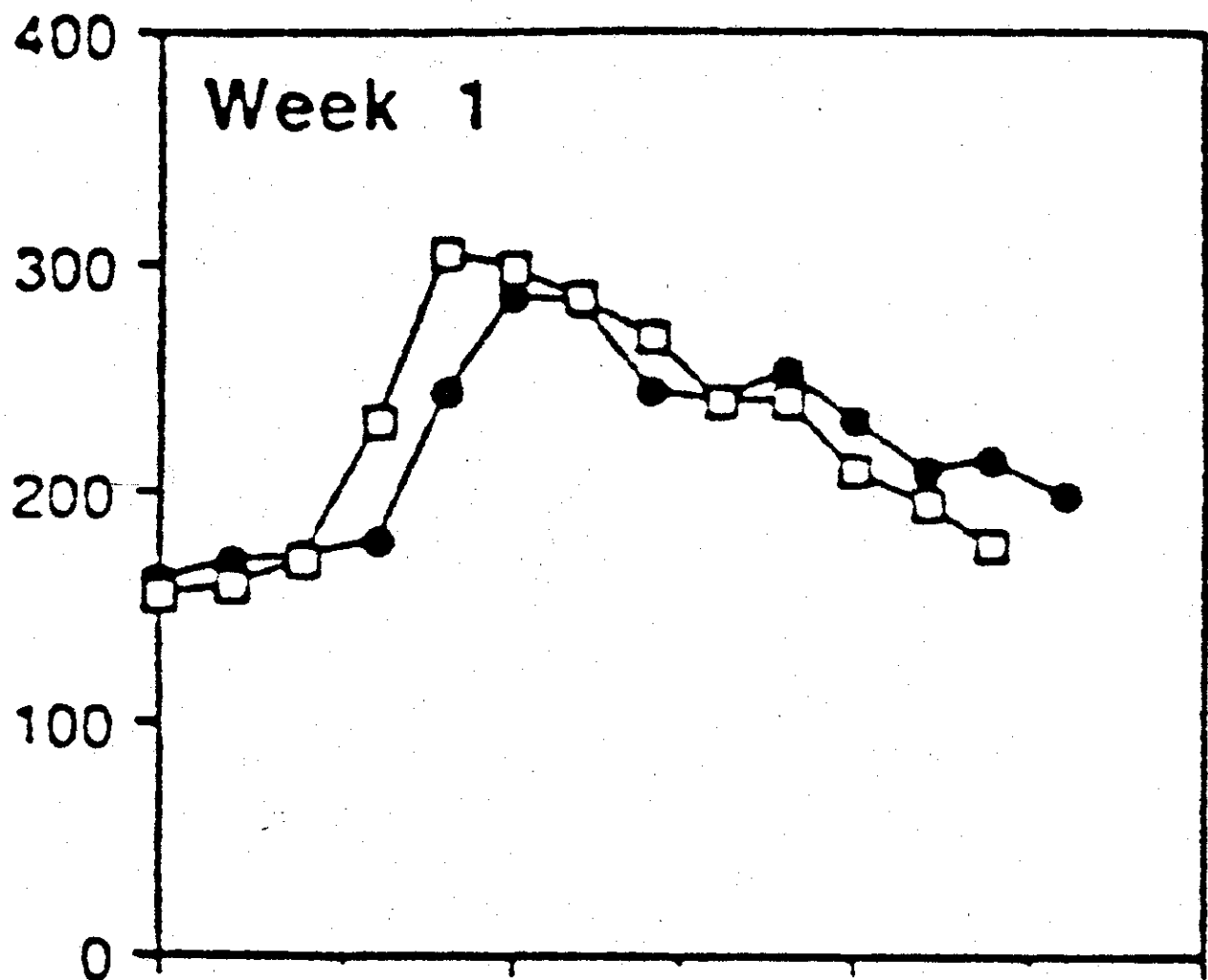


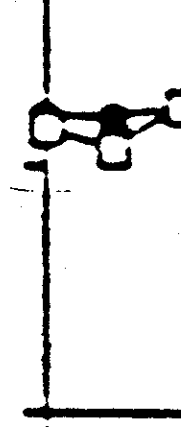
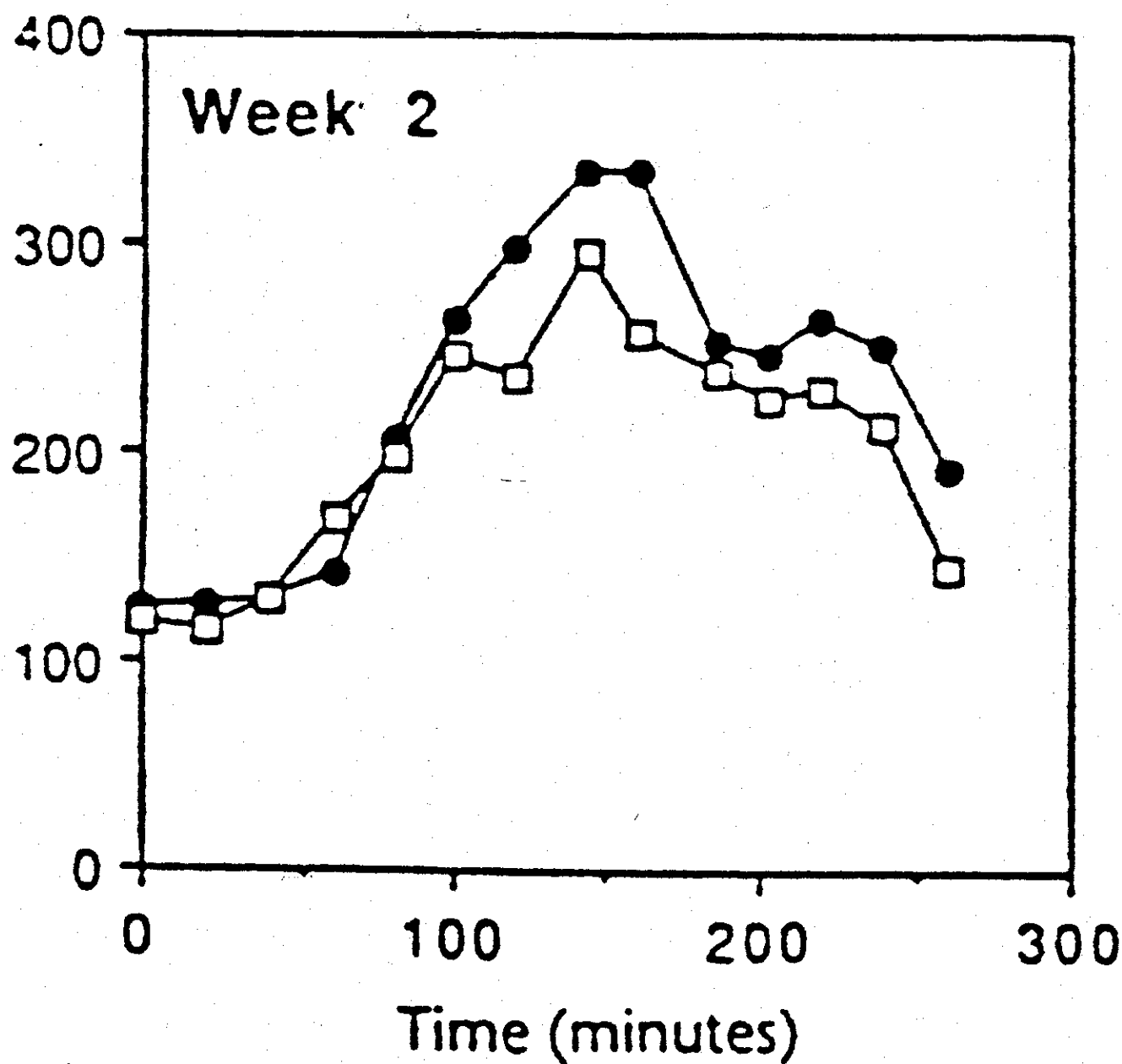
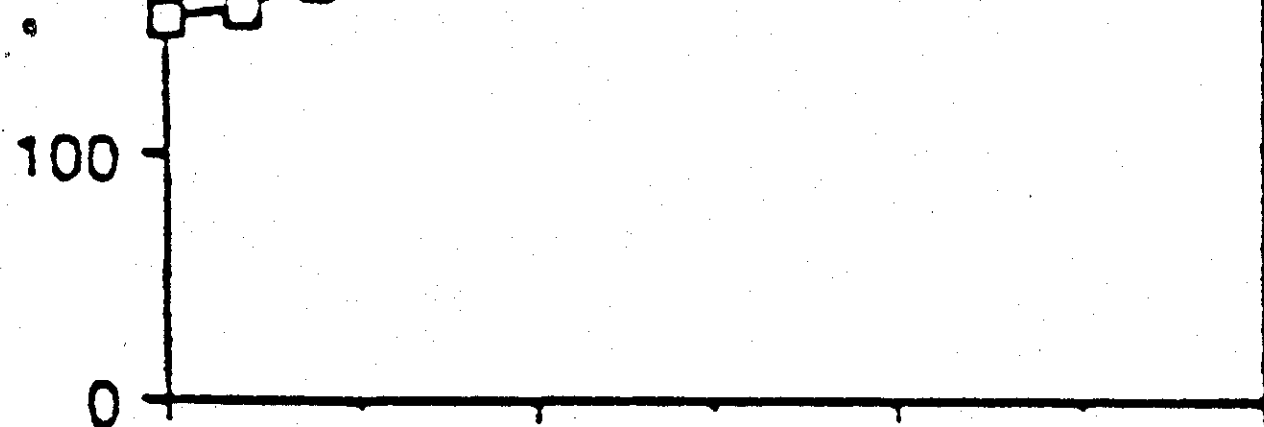
Fig. 2 Comparison of serum glucose (●) and glucose calculated from the transdermally extracted glucose flux (□) from subject 3 (type 2) on four different days. The extraction data are uniformly shifted by an extraction period to match the time of the previous

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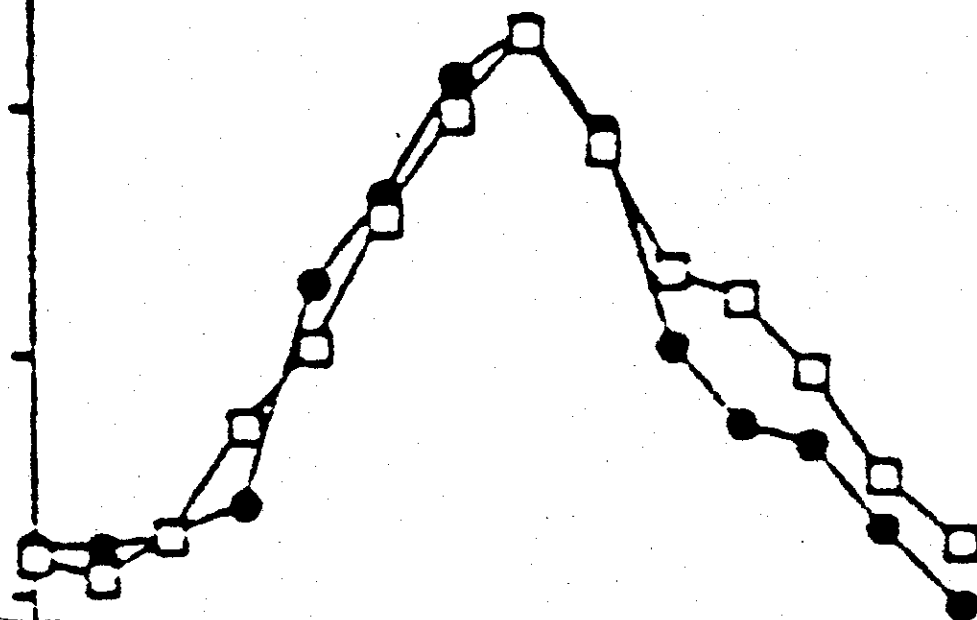




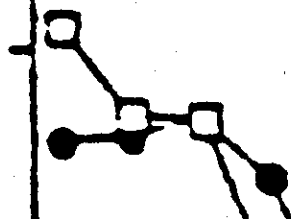
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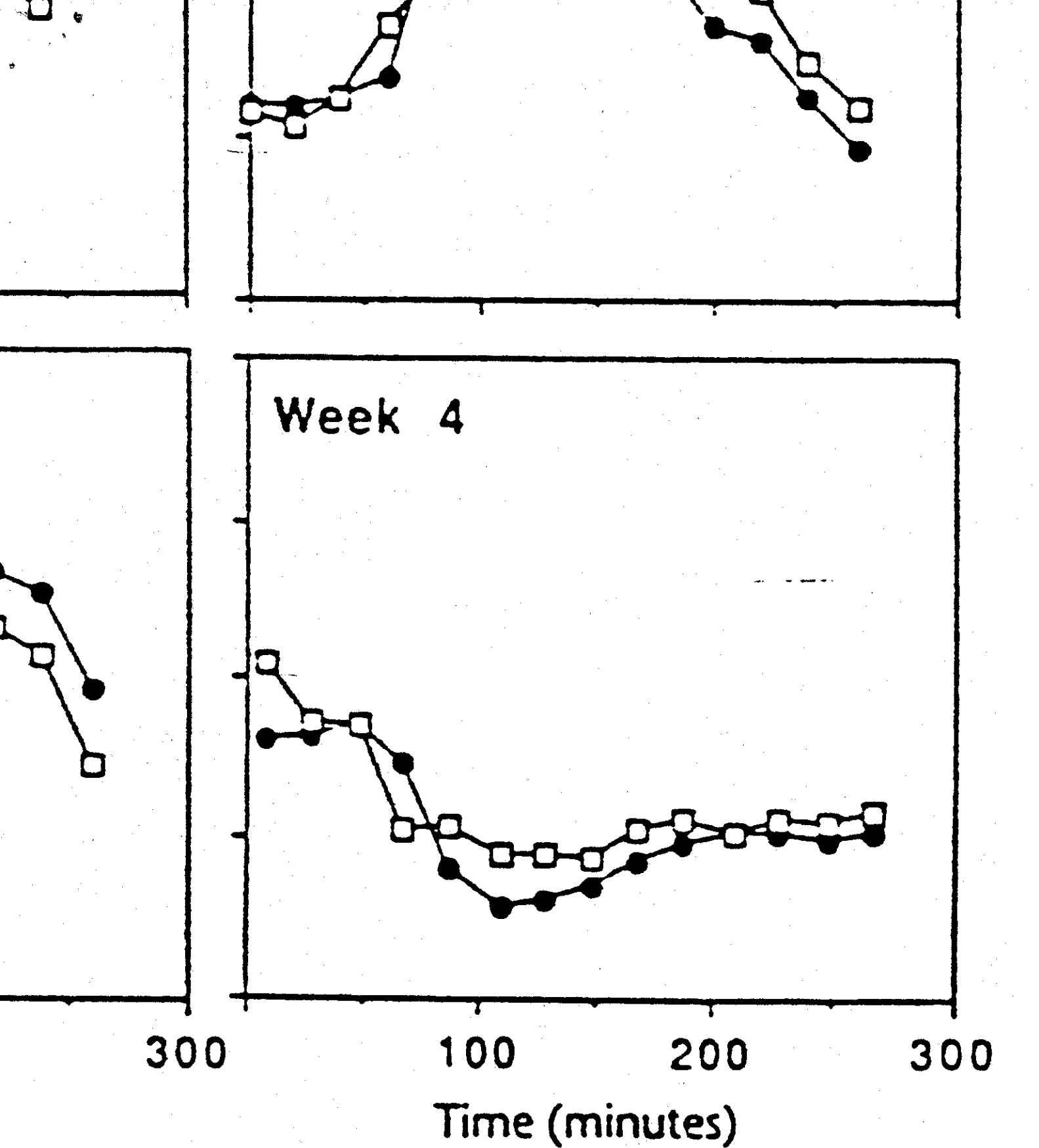


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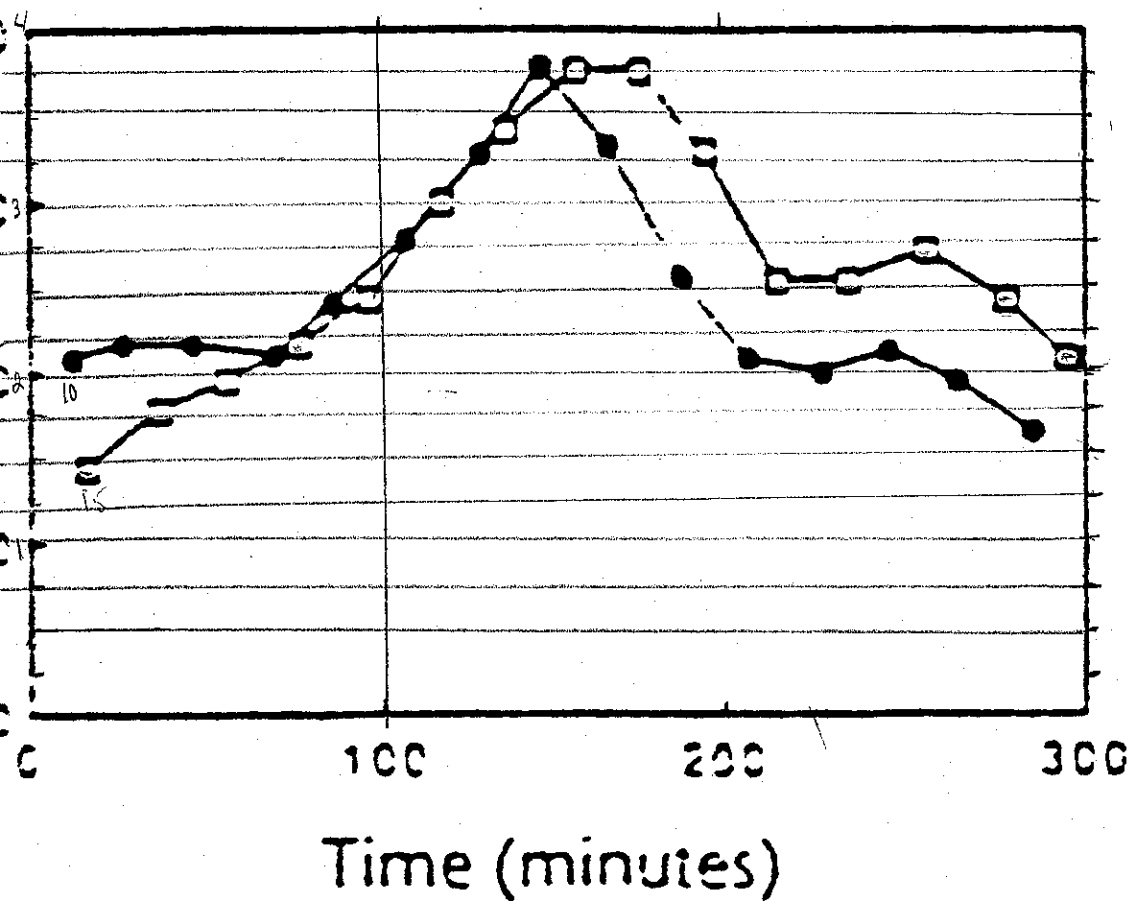
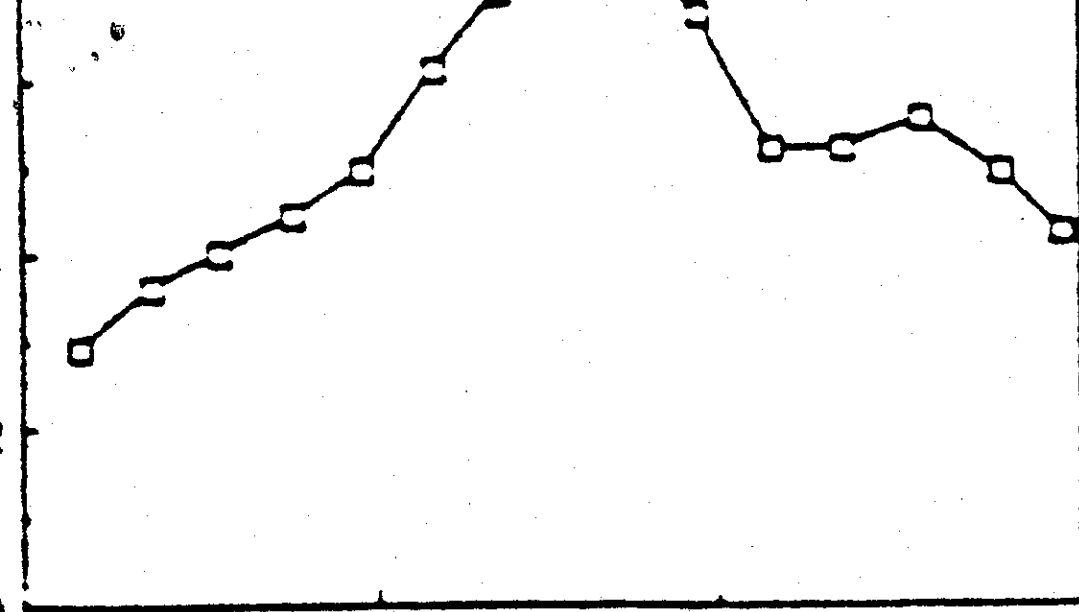


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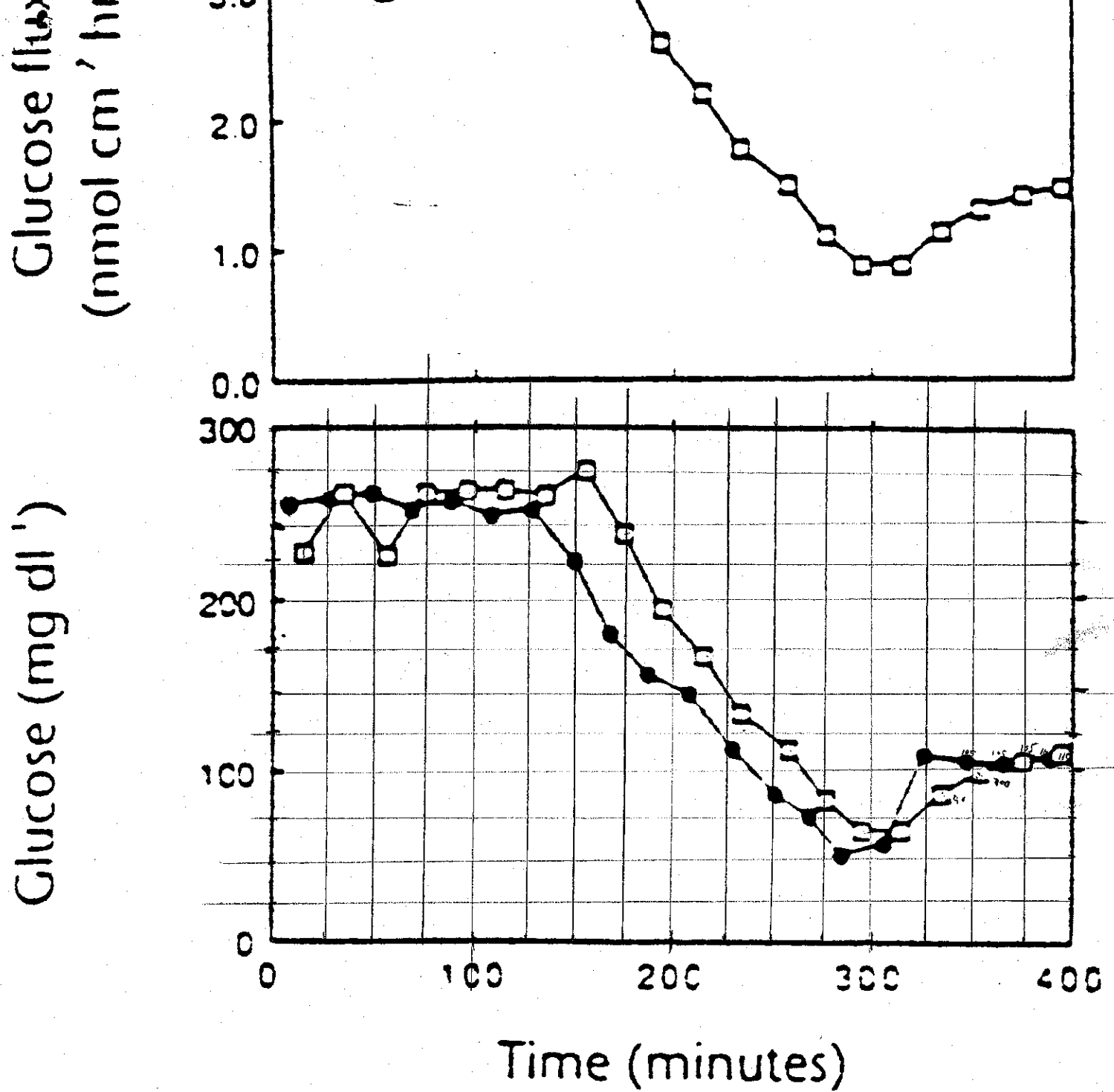


Fig. 1 Serum glucose (●, top plot) middle plot) profiles. Extracted flux is two sample chambers unless otherwise shows a direct comparison of the alu



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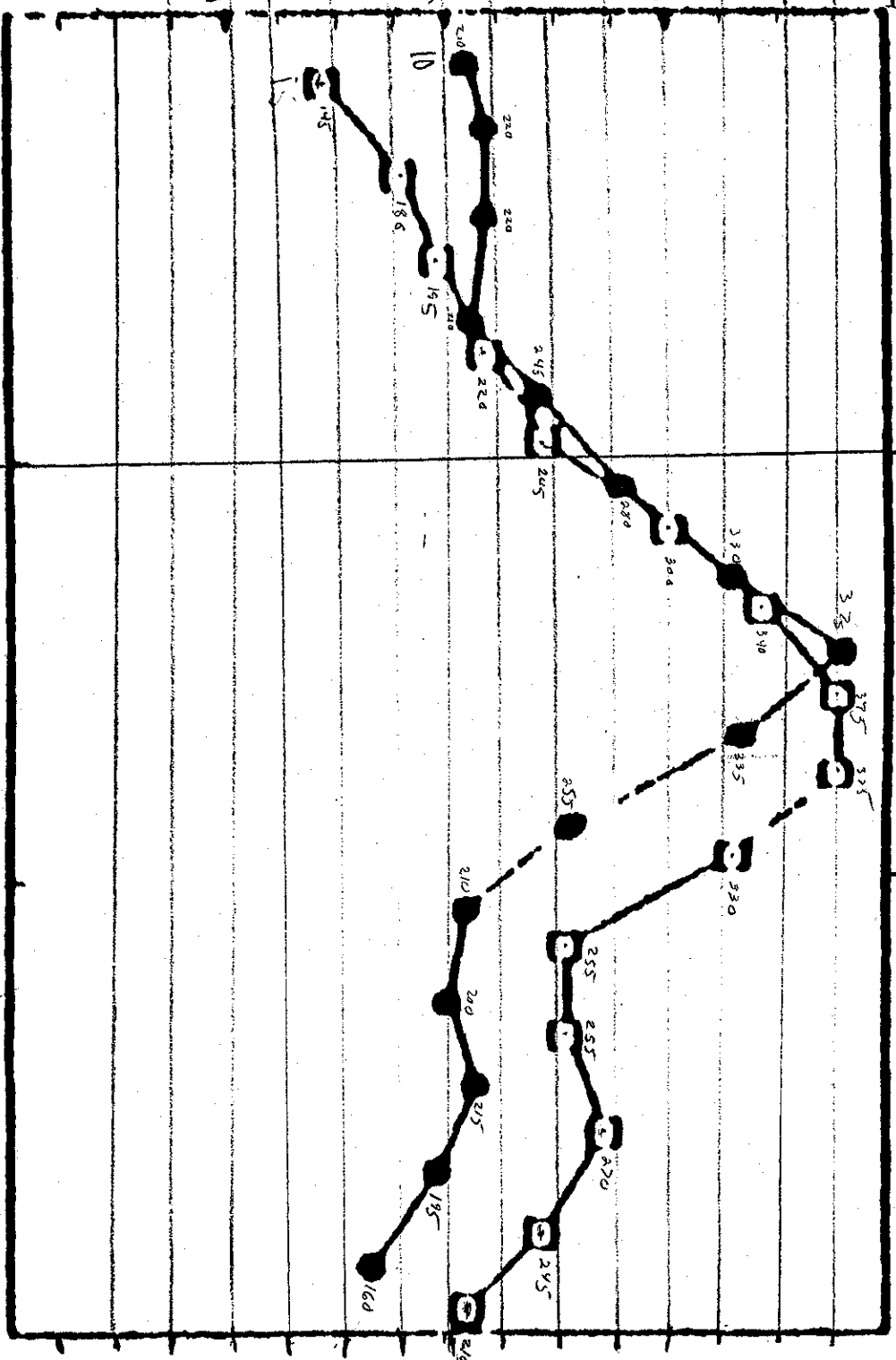
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Glucose (mg dl<sup>-1</sup>)

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