Metabolic Studies Following the Oral Ingestion of Different Doses of Glucose

Harald Förster, M.D., Manfred Haslbeck, M.D., and Hellmut Mehnert, M.D., Munich

SUMMARY

Oral glucose tolerance tests were performed with variable glucose loads (30 gm., 50 gm., 100 gm., 200 gm., 300 gm.) in groups of healthy subjects. The concentrations of blood glucose, serum insulin and free fatty acids were measured in venous blood. Blood glucose concentration was also determined in capillary and arterial blood. The differences in glucose concentrations between capillary and venous blood were as high as 40 mg./100 ml. The magnitude of the differences was related both to the time of determination and to the glucose load. One hundred and twenty minutes after beginning the tests there were little differences between the glucose concentration in capillary blood and in venous blood after doses of 30 or 50 gm. glucose. With larger doses of glucose higher values than the pretest values were still found in capillary blood but not in venous blood at the same time and also 180 min. after beginning the tests. Also, in contrast to the findings with doses of 30 or 50 gm. glucose, serum insulin levels were still elevated at 120 minutes after the higher glucose loads. The high values of serum insulin (70 to 90 μU./ml.) and the concentration differences between capillary and venous blood are explained best by the secretion of biologically active insulin because of a continual intestinal absorption of glucose. This confirms the assumption that the secretion of insulin is obviously not only stimulated by the actual blood glucose concentration but also by intestinal absorption of glucose. This mechanism is important in the oral glucose tolerance test because only 120 minutes after a load of 100 gm. glucose there is a continuous insulin secretion. DIABETES *21*:1102-08, November, 1972.

The oral glucose tolerance test is generally held to be the simplest and most reliable method for the diagnosis of latent diabetes mellitus. But it is surprising that different workers use different amounts of glucose for the test: Doses range from a minimum of approximately 50 gm. to a maximum of approximately 150 gm., for individuals with excessive body weights receiving 1.75 gm./kg. Differences are also made in judging the results, and the withdrawal of blood may be different in that capillary blood or venous blood may be used. The most appropriate conditions for the study of the conduct of oral glucose tolerance tests are those obtained in healthy subjects. Investigations in nondiabetic persons are preferable since only in this way can comparable basal conditions be expected with feedback control for regulation of blood glucose concentration being intact.

The aim of the present study was to investigate the concentration of glucose in capillary, venous and arterial blood in relation to serum insulin concentration following oral ingestion of different doses of glucose. Attempts were made also to estimate the course of insulin secretion in relation to the intestinal absorption of glucose.

METHODS

Oral loads of 30, 50, 100, 200 or 300 gm. of glucose were given to groups of male volunteers twenty-two to twenty-eight years old who had no family history of diabetes. The glucose was dissolved in 400 ml. of tap water. All individuals were nondiabetic and their body weights were under 110 per cent of desirable body weight.

The subjects were kept on a standard diet of at least 300 gm. carbohydrate daily for at least three days and fasted overnight before the test. A polyethylene cannula was inserted in the cubital vein. In addition, glucose determinations were made on capillary blood samples drawn from the warm fingertips. In some instances blood was withdrawn from the brachial artery.

Glucose was analyzed with hexokinase/glucose-6-phosphate dehydrogenase[1] in fresh blood deproteinized with 3.3 per cent perchloric acid. Free fatty acids in blood plasma were estimated by Duncombe's method.[2]

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From the Institut für vegetative Physiologie, Universität, Frankfurt, Germany (BRD), the Third Department of Internal Medicine, Krankenhaus Schwabing, and the Forschergruppe Diabetes, München, Germany.

Address reprint requests to: Harald Förster, M.D., Institut für vegetative Physiologie, University of Frankfurt, Germany 6000.

Immunoreactive insulin was measured in the blood plasma by a modification of the method of Hales and Randle,[3] using insulin I-125. The insulin binding reagent was obtained from the Radiochemical Center, Amersham, England.

Mean values are shown with the standard error of the mean. Statistical analysis of the data was performed by Student's *t* test.[4]

RESULTS

1. The concentration of glucose in venous blood

The venous glucose concentrations of thirteen subjects measured at the beginning of the experiment ranged from 82 to 88 mg./100 ml. (table 1). After oral administration of glucose the concentration rose, and the highest values were obtained between twenty and thirty minutes. The maximum increase in venous blood glucose concentration following ingestion of 30 gm. of glucose amounted to 31 mg./100 ml. Ingestion of 50 to 300 gm. of glucose yielded a maximum increment in venous blood glucose concentration ranging from 46 to 51 mg./100 ml. These changes in the glucose concentration are almost identical and independent of the glucose dosage, the lowest dosage of 30 gm. excepted. The venous blood glucose concentration returned to normal between sixty and ninety minutes after glucose administration. Between 90 and 120 minutes after 30 gm. of glucose the glucose concentration in venous blood was less than the fasting values. The same was true for the values between 90 and 120 minutes after 50 gm. of glucose ($p < 0.05$). However, following an oral load of 100 gm. of glucose, the glucose concentration was less than the fasting value ($p < 0.05$) only after 180 minutes. Nevertheless, these small differences in the glucose concentration in venous blood were independent of ingested doses of glucose. The glucose concentration in cubital vein blood declined to below fasting values only in the late phase of glucose absorption.

2. The glucose concentration in capillary blood

Similar to the glucose concentration in venous blood, no significant differences in concentration in capillary blood in the fasting subjects of different groups could be observed. This indicates that the peripheral glucose uptake (measured in the forearm) in the fasting period was minimum.

Maximum concentrations of glucose in capillary blood were obtained also between twenty and thirty minutes after glucose ingestion (figure 1). The greatest rise was 46 mg./100 ml. after 30 gm. of glucose, 73 mg./100 ml. after 50 gm., 70 mg./100 ml. after 100 mg., 75 mg./100 ml. after 200 gm. and 97 mg./100 ml. after oral administration of 300 gm. of glucose. There were significant deviations ($P < 0.05$ or less) with the lowest and the highest doses of glucose (30 gm. and 300 gm.), whereas the increases were of the same magnitude following the other loads of glucose.

The mean concentration of glucose in capillary blood returned to baseline level ninety minutes after ingestion of 30 or 50 gm. of glucose. With 100 gm. of glucose the return was not seen even after three hours. After the ingestion of 50 gm. of glucose the mean glucose concentration in capillary blood (measured after 150 minutes) was significantly lower than the fasting values.

3. The glucose concentration in arterial blood

Glucose concentration in arterial blood was measured in nine subjects following oral ingestion of 100 gm. of glucose. This concentration was compared with those determined simultaneously in venous and capillary blood (see table 2). It can be seen that the concentration in arterial blood was almost identical to that in capillary blood. The differences amounted to only 2 to 6 mg./

TABLE 1

Glucose concentration in venous blood following oral ingestion of different glucose loads. (The other experimental values are shown in the figures.) Glucose was analyzed with hexokinase/glucose-6-phosphate dehydrogenase (x + S.E.M.).

Glucose load	Minutes after ingestion					
	0	10	15	20	30	45
30 gm.	84.6 ± 4.3	108.0 ± 5.1	114.9 ± 7.1	115.2 ± 8.5	114.6 ± 8.9	103.6 ± 9.2
50 gm.	86.9 ± 2.8	114.5 ± 7.0	125.2 ± 6.3	132.8 ± 5.9	118.0 ± 5.2	104.2 ± 6.3
100 gm.	85.8 ± 1.7	119.3 ± 5.2	122.0 ± 3.1	133.2 ± 8.3	136.6 ± 9.1	124.2 ± 9.3
200 gm.	82.0 ± 2.7	117.3 ± 9.5	129.4 ± 10.4	129.6 ± 12.5	120.0 ± 12.2	99.3 ± 9.8
300 gm.	88.4 ± 3.0	122.5 ± 6.6	130.2 ± 6.5	137.0 ± 6.0	130.4 ± 6.3	114.5 ± 8.9

60	90	120	150	180
89.0 ± 9.5	76.3 ± 7.5	77.6 ± 2.3	81.4 ± 2.9	83.8 ± 2.3
90.6 ± 5.7	75.1 ± 3.9	72.0 ± 2.5	73.1 ± 2.5	75.9 ± 3.6
101.6 ± 8.1	94.4 ± 6.9	91.2 ± 5.4	84.2 ± 5.7	71.7 ± 6.2
90.0 ± 9.5	87.4 ± 4.9	84.3 ± 5.6	83.9 ± 4.6	79.6 ± 3.4
94.1 ± 5.9	86.1 ± 3.4	87.6 ± 7.4	90.6 ± 4.9	83.5 ± 4.4

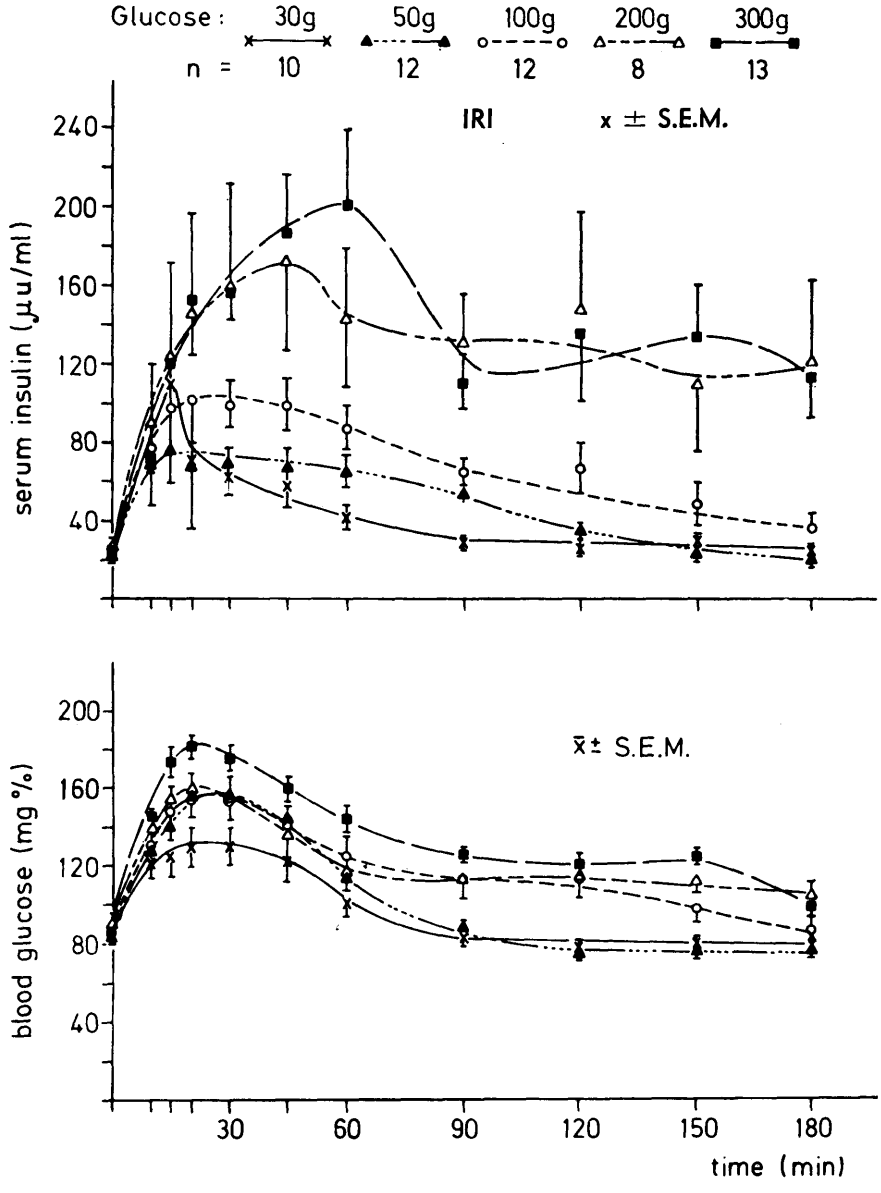

FIGURE 1

Changes in concentration of serum insulin (IRI) and capillary blood glucose following oral ingestion of different glucose doses.

100 ml. In most instances glucose concentration in arterial blood was higher than in capillary blood. In evaluating the results it appears justified to regard capillary blood glucose concentration as identical with that of arterial blood.

4. *The differences in glucose concentrations between capillary and venous blood*

No concentration differences could be measured in the fasting state when blood was drawn from the forearm. Ten minutes after oral ingestion of glucose, concentration differences of 10 to 25 mg./100 ml. were found (figure 2). The highest differences (up to 60 mg./100 ml.) were obtained with the highest glucose dose used (300 gm.). One hundred twenty minutes after ingestion of 30 and 50 gm. of glucose, differences in concentration of less than 10 mg./100 ml. were seen. After ingestion of higher doses of glucose, concentration differences of up to 45 mg./100 ml. even 180 minutes after ingestion, i.e. at the end of the observation period, were found, however.

5. *Concentration of free fatty acids (FFA)*

Fifteen minutes after oral ingestion of glucose, a decline in the concentration of free fatty acids was seen which was independent of the ingested doses (figure 3). Sixty minutes after administration the concentration decreased more than 50 per cent. With glucose

TABLE 2

Concentration of glucose following the oral ingestion of 100 gm. glucose. Glucose determinations were performed simultaneously in venous, capillary, and arterial blood. Blood glucose was analyzed with hexokinase/glucose-6-phosphate dehydrogenase.

	0	15	30	45	60	75	90	105	120	135	150	165	180
Glucose concentration													
Venous													
n	9	9	9	8	9	9	9	8	9	9	9	7	7
x̄	90.3	124.1	154.3	142.8	118.3	93.6	94.2	103.0	92.6	91.3	85.0	87.7	83.0
S.E.M.	1.7	7.1	10.5	12.5	11.3	8.0	7.1	4.1	7.0	5.4	7.6	12.1	11.5
Capillary													
n	9	8	9	9	9	9	9	9	9	9	9	7	7
x̄	89.9	146.3	184.6	167.0	141.3	121.4	114.8	112.2	105.9	100.9	92.1	91.7	90.7
S.E.M.	1.5	8.0	10.3	12.4	10.4	7.1	4.7	6.8	8.0	4.2	7.5	13.8	14.8
Arterial													
n	9	9	9	9	9	9	9	9	9	9	9	7	7
x̄	92.2	148.4	187.0	170.9	140.3	128.0	115.9	115.3	109.3	101.6	95.3	95.7	95.9
S.E.M.	2.6	7.9	9.9	11.5	10.2	10.0	9.4	6.2	7.9	4.7	8.9	14.1	14.4

doses of 100 gm. or more, a further decline in the concentration of FFA was seen. Following ingestion of 30 gm. of glucose, a significant increase from the nadir in the FFA concentration could be observed ninety minutes after ingestion, however. The same was true for 50 gm. of glucose with FFA concentration measured 120 minutes after ingestion. In contrast to this, no increase in FFA concentration was seen within 180 minutes after ingestion of higher doses of glucose.

6. Immunologically reactive insulin (IRI)

Within ten minutes after oral glucose ingestion the IRI rose three to five times the control values (figure 1). No significant differences between the different glucose doses could be observed ten or fifteen minutes after the glucose ingestion. Thirty minutes after the ingestion of 100 gm. of glucose, IRI was significantly higher than after the ingestion of 30 or 50 gm. of glucose. In the further course of the experiments, a decline of IRI was seen after the ingestion of 30 to 100 gm. of glucose. Return to normal was attained ninety minutes after the ingestion of 30 gm. of glucose, and 120 minutes after the ingestion of 50 gm. However, following

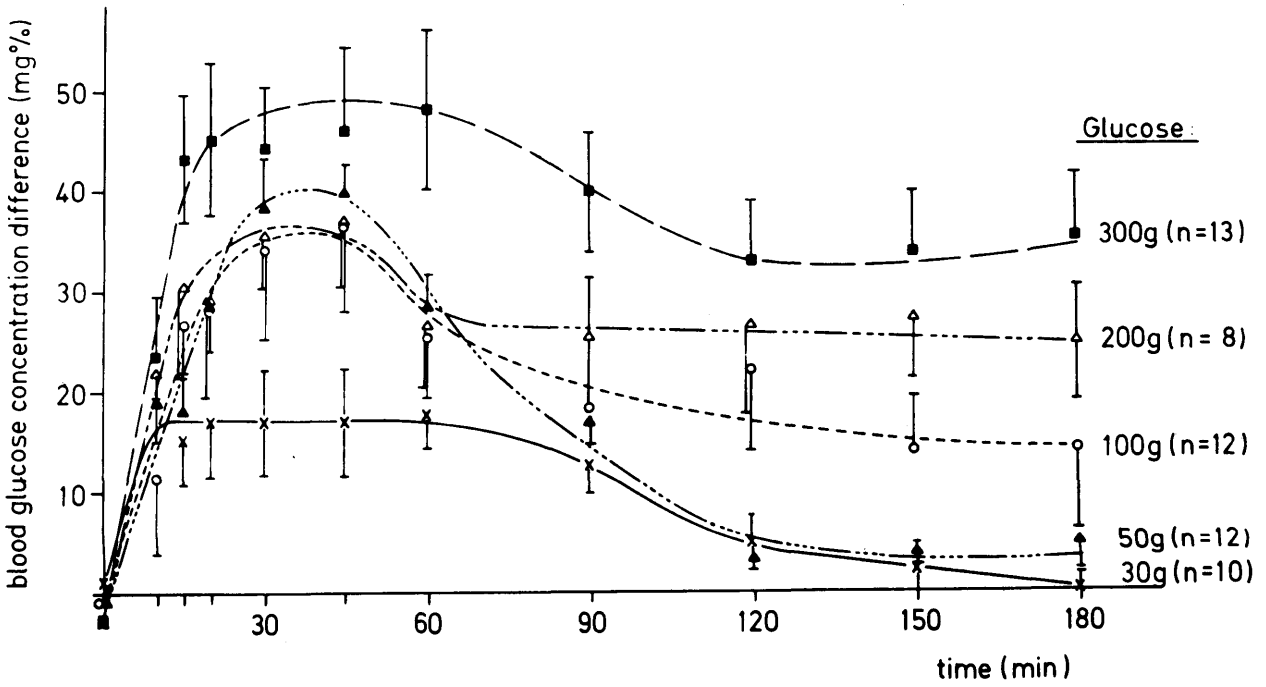

FIG. 2. Differences in glucose concentration between venous blood and capillary blood following oral ingestion of different glucose doses.

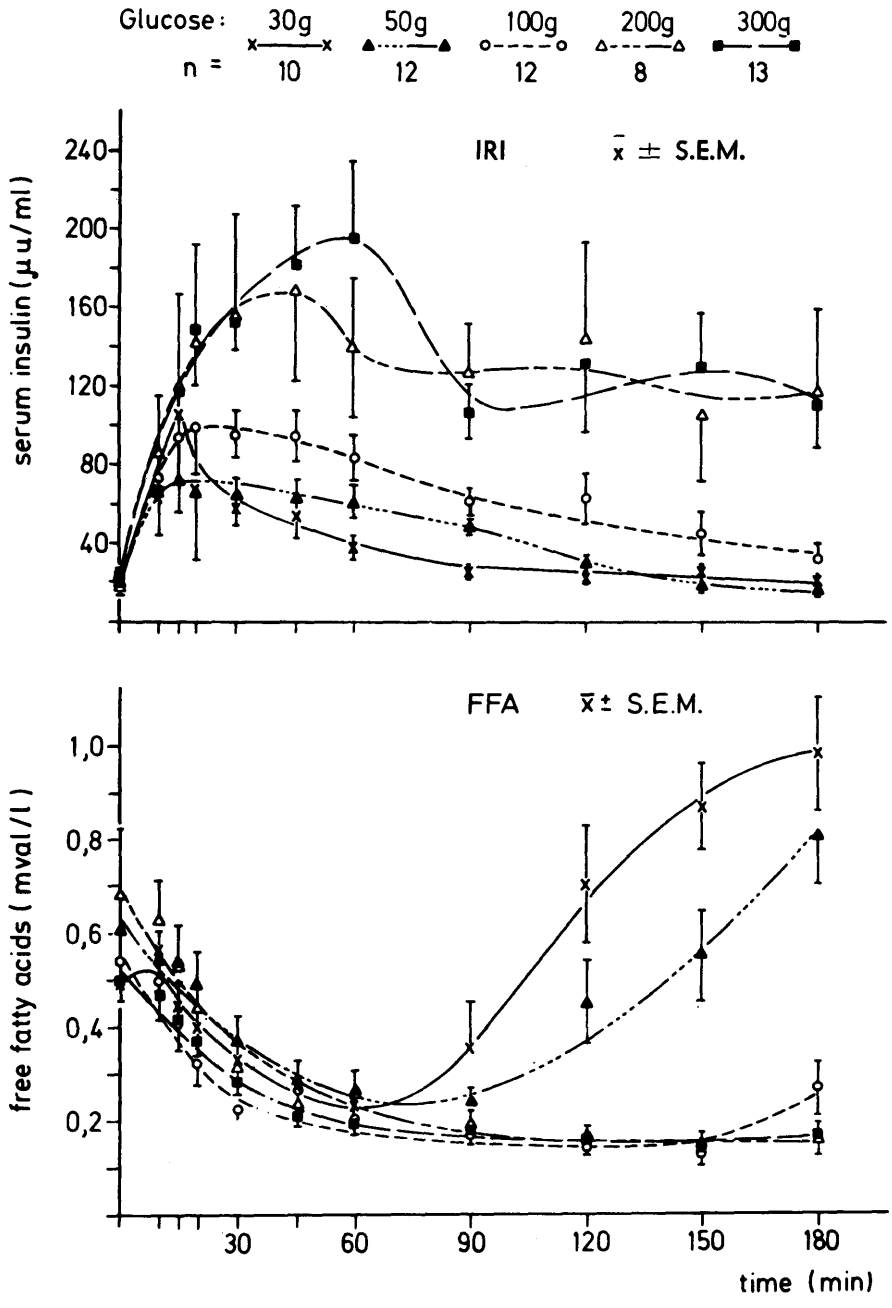

FIGURE 3

Changes in concentration of serum insulin (IRI) and free fatty acids (FFA) following oral ingestion of different glucose doses.

ingestion of 200 gm. or more of glucose, the IRI measured at 180 minutes was five times that of controls.

DISCUSSION

Concentrations of insulin and of glucose in the peripheral vein have been widely used as indices of pancreatic insulin secretion and of glucose metabolism in men following oral ingestion of glucose. It has not been taken into consideration, however, that in the postprandial period of hyperinsulinemia, glucose uptake occurs in the periphery, i.e. the glucose concentration in venous blood is not equivalent to that in arterial. It is to be expected that the difference in glucose concentrations in arterial and venous blood increases with the increasing concentration of serum insulin. Nevertheless, there have been several attempts to find a relation between glucose and insulin concentrations in venous blood.[5-7] Our previous studies of nondiabetic persons confirming earlier results[8-10] have shown that the glucose concentration in venous blood is not

related to the amount of glucose (50 to 400 gm.) administered orally. Moreover, we could not demonstrate a correlation between blood glucose and serum insulin concentration after different oral loads (50 gm., 100 gm. or 200 gm.) of glucose.[12]

In the earlier studies[11-13] we found that 120 minutes after oral ingestion of glucose, the glucose concentration in venous blood returned to baseline independently of the glucose load used. Castro et al.[14] also demonstrated that there are little differences in blood glucose concentration (venous blood) following different glucose loads (50 to 100 gm.). However, under these conditions the serum insulin levels were different depending upon the amount of glucose ingested. Similar results were published also by Perley and Kipnis.[15]

In contrast, Chandalia and Boshell[16] reported differences in blood glucose concentrations with varying glucose loads even at the two hour level. These different results might be due to the fact that the persons used for the oral tolerance tests apparently had diabetes, since blood sugar values of 156 mg./100 ml. were measured two hours following glucose ingestion.

On the other hand, ingestion of higher doses of glucose by nondiabetic persons led to concentrations of glucose in capillary blood (after 120 minutes) which were markedly higher than the fasting values (figure 1). Only with the lower amounts of glucose, i.e. 30 to 50 gm., was the glucose concentration in capillary blood at normal after 120 minutes. The normal glucose concentration in capillary blood was associated with a normal serum insulin concentration. However, the elevated glucose concentration in capillary blood following larger glucose loads was accompanied by an elevated serum insulin concentration. A significant correlation between peak capillary glucose and peak venous insulin concentrations was lacking.

The capillary blood glucose values following ingestion of 300 gm. of glucose were significantly higher than those after 200 gm. of glucose, but the serum insulin concentrations did not show such differences. On the other hand, the glucose concentrations after 100 gm. and 200 gm. of glucose were almost identical, though the serum insulin concentrations were significantly different (figure 1). In all instances a relatively low increase in the capillary blood glucose concentration was followed by an increase in the serum insulin concentration. There was no linear relation between the capillary glucose concentration and the serum insulin concentration, however, and it did not appear justified to compute a coefficient of these two values.

In spite of the elevated serum insulin concentration 120 minutes after ingestion of 100 gm. or more of glucose, the glucose concentration in venous blood was normal at the time (table 1).

The high peripheral glucose utilization (figure 2) could be explained best by a continuing influx of glucose indicating that the intestinal absorption of glucose continued. Perley and Kipnis have shown that only at 180 minutes after ingestion of an oral load of 100 gm. of glucose is gastric emptying finished.[15] X-ray pictures of the stomach taken two hours after ingestion of 50 gm. or 100 gm. of glucose with addition of barium sulfate demonstrated that gastric emptying was not finished, especially following the higher glucose load.[17] The fact that glucose absorption is still continuing 120 minutes following ingestion of more than 100 gm. of glucose is supported also by the results of oral galactose tolerance tests.

The rate of intestinal absorption of galactose is similar to that of glucose. There are many indications that the mechanisms of absorption of these two sugars are identical.[18,19] But in contrast to blood glucose, blood galactose is not counterbalanced by hormonal action. Therefore, changes in the blood galactose concentration following galactose ingestion are related to the intestinal absorption of galactose. After the ingestion of 200 gm. of galactose, the blood galactose concentration rises continuously until four hours. This should also be the time needed for the intestinal absorption of this quantity of galactose.[20] The glucose absorption may be identical, but continuous glucose absorption is not reflected by a continuous rise in venous (or capillary) blood glucose concentration. However, the consequence of any rise in the blood glucose concentration is a stimulation of insulin secretion which is followed by an increase in the peripheral glucose uptake (high arteriovenous concentration differences). Therefore a return to normal of glucose concentration in venous blood can be observed in spite of the continuous intestinal glucose absorption. These conclusions are corroborated also by the results of Sisk et al. who suggested that the 100 gm. load three hour determination may possess unique diagnostic power to ascertain early chemical diabetes.[21]

The large differences between capillary and venous blood glucose concentrations (figure 2) indicate increased peripheral glucose uptake (measured in the forearm). It is very likely that this peripheral uptake is compensated for by continuous intestinal glucose absorption. On the other hand, increase in peripheral glucose uptake (high concentration differences) is most likely an in-

sulin effect. The same is true for the depression of free fatty acid levels (figure 3). Therefore, despite the nearly normal glucose concentration in venous blood 120 minutes following the ingestion of an oral load of 100 gm. or more of glucose, there are some indications for the presence of biologically active insulin.

One hundred twenty minutes after ingestion of 50 gm. of glucose, the IRI is almost normal (figure 1), there are hardly any glucose concentration differences (figure 2) and free fatty acid levels are also normal (figure 3). However, if the oral loads of glucose are increased to 200 to 300 gm. the glucose concentration differences are significant (40 mg./100 ml.). Under these conditions the IRI value reaches four times that of control values; the FFA levels are still minimum. Though the immunological determination of insulin measures the biologically inactive proinsulin as well,[22,23] the present results indicate that the elevated insulin levels are at least partly due to biologically active insulin.

The present results indicate further that for diagnostic purposes, at least in borderline cases, an oral ingestion of 50 gm. of glucose may be too low and measurements made after 120 minutes under those conditions should have only limited diagnostic value (see also reference 21). This can be recognized by the concentrations of serum insulin and FFA being already at normal and by the low differences of glucose concentrations between capillary and venous blood. We have indicated that these results are due to the termination of glucose absorption.

The purpose of a tolerance test is to provoke metabolic activity. In nondiabetic persons it appears that the induced activity has ceased 120 minutes following ingestion of 50 gm. of glucose. We should like to draw attention to the importance of the glucose dosage for the interpretation of oral glucose tolerance tests. Finally, in our opinion there are no fixed concentration differences between capillary and venous blood glucose concentrations during tolerance tests.

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