Plasma Glucose Turnover in Humans as Studied with C^{14} Glucose

Influence of Insulin and Tolbutamide

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Much evidence has been accumulated showing that insulin either from functioning pancreatic islet cells, or from continued insulin administration, is requisite for the effective action of the oral hypoglycemic drug, tolbutamide.[1-4] Further, it has been suggested that this agent potentiates the secretion of insulin,[5,6] and it has been reported that both insulin and tolbutamide suppress the output of glucose from the liver.[7-9] In spite of this evidence, the theory that tolbutamide exerts its action through potentiation of insulin secretion has been in serious doubt because of the many negative reports concerning a peripheral action for the drug.[10-12] Further, dissimilarities in intermediary metabolism following the administration of insulin and tolbutamide have been documented.[13]

In order to test the similarity of action of these two compounds on the supply of glucose to the circulation and in an effort to describe further the action of these two drugs on the kinetics of plasma glucose, we have studied both the immediate and prolonged effect of insulin and tolbutamide on the turnover of plasma glucose.

METHODS AND MATERIALS

One hundred microcuries of evenly labeled C^{14} glucose were heat sterilized in 0.9 per cent NaCl solution at a concentration of 0.033 moles/liter.

Blood samples were obtained through indwelling Cournand or Rochester plastic needles in heparinized syringes. Plasma was separated immediately from the whole blood by centrifugation. The plasma proteins were precipitated according to the method of Nelson and Somogyi,[14] and the plasma glucose concentration was determined from aliquots of the plasma protein free filtrate by the method of Folin and Wu.[15] Glucose was isolated from the plasma protein free filtrate as the glucose osazone according to a method described previously.[16] The glucose osazone was assayed colorimetrically in alcohol solution, and an aliquot of this solution was precipitated in water on an aluminum planchet, dried and counted with an end-window Geiger counter. Appropriate mass corrections were made on each sample.

Selection of subjects. Subjects were selected from the medical and orthopedic wards of the hospital on the basis of normal liver function and normal kidney function. The nondiabetic group had no history of diabetes, and the glucose tolerance was normal.

Diabetic subjects were selected on the basis of diabetic history and abnormal glucose tolerance tests. Blood sugar levels of these diabetic subjects could be adequately controlled with diet. None of the diabetic subjects required insulin to prevent the appearance of ketoacidosis.

Experimental design. All subjects were studied first with insulin and then, after a three- to five-week interval, with tolbutamide. Experiments were begun at 9:00 a.m. and concluded by 3:00 p.m. of the same day. Subjects received a light meal at 9:00 p.m. the day preceding the experiment in order to maintain the nutritional state as constant as possible. No food was allowed during these experiments.

At zero time the subject received intravenously 90 μc of sterile C^{14} glucose. Following this, at least three blood samples were withdrawn at approximately half-hour intervals. These samples were immediately centrifuged, and the concentration and specific activity (counts per minute/milligram) of the plasma glucose were determined to establish a predrug turnover curve. Insulin (0.05 units per kilogram intravenously and 0.15 units per kilogram subcutaneously) or tolbutamide (0.025 gm. per kilogram intravenously and 0.065 gm. per kilogram orally) was administered as the test drug. These doses were found to bring about approximately the same reduction of plasma glucose. The dual routes of administration were selected to achieve a rapid initial fall in the plasma glucose as well as maintenance of reduced plasma glucose concentrations for protracted

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periods of time. Blood samples were withdrawn at eight- to fifteen-minute intervals for the first hour following drug administration, then at forty-five- to sixty-minute intervals until the experiments were terminated. These samples were immediately centrifuged, and the concentration and s.a. of the plasma glucose were determined in order to estimate the effects of drug administration on the entry and removal rates of plasma glucose.

RESULTS

The time course of the concentration and s.a. of plasma glucose for three of the subjects studied is shown in figures 1-3 and 1a-3a. Figures 1-3 present the data obtained in the insulin studies while figures 1a-3a present the data obtained in the tolbutamide studies. The simultaneous and equal steady state rates of entry and removal of plasma glucose (plasma glucose turnover), and the unequal nonsteady state rates of entry and removal of plasma glucose have been calculated for each of the fourteen studies reported here. Standard equations for first order reaction kinetics[17] were employed when a constant level of plasma glucose and a logarithmic decline of the s.a.-time curve of plasma glucose were evidenced. When these criteria were not met, nonsteady state calculations as outlined by Dunn and associates,[18] and Jacobs and associates[19] were employed. The results of these calculations for the insulin studies are in table 1, and for the tolbutamide studies in table 2. The calculations are listed as applied to three phases of the studies. These phases were arbitrarily chosen for purposes of discussion and are outlined as follows: Phase 1 is the period following C^{14} glucose administration but prior to drug administration. Phase 2 begins at the time of drug administration and continues to the time the maximal acute depression in plasma glucose is observed. Phase 3 is a time period averaging two hours at the end of the studies after the acute phase of plasma glucose depression when steady state conditions are again evident.

The effect of insulin on the turnover of plasma glucose in nondiabetic and diabetic human subjects. Figures 1 and 3 illustrate a response to insulin characterized by an immediate change in the slope of the s.a.-time curve of plasma glucose to a zero value, indicating a cessation in the supply of nonlabeled glucose to the circulation. The average time duration of this zero slope, noted in four of seven subjects studied with insulin, was approximately thirty minutes. A calculation was made of the rate of removal of glucose from the circulation during this time (Phase 2). The nondiabetic

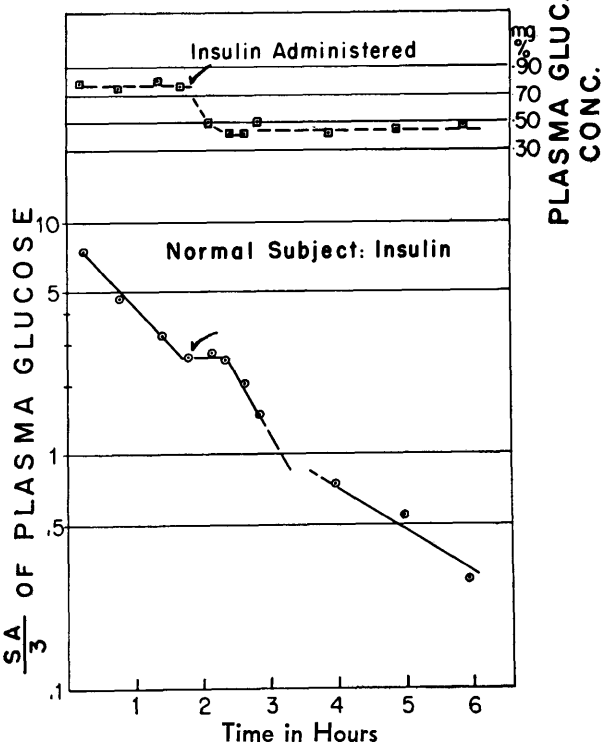

FIG. 1. Nondiabetic (normal) subject A.Z. Weight 41 kg. Insulin (two units intravenously, 4 units subcutaneously) administered at the arrow.

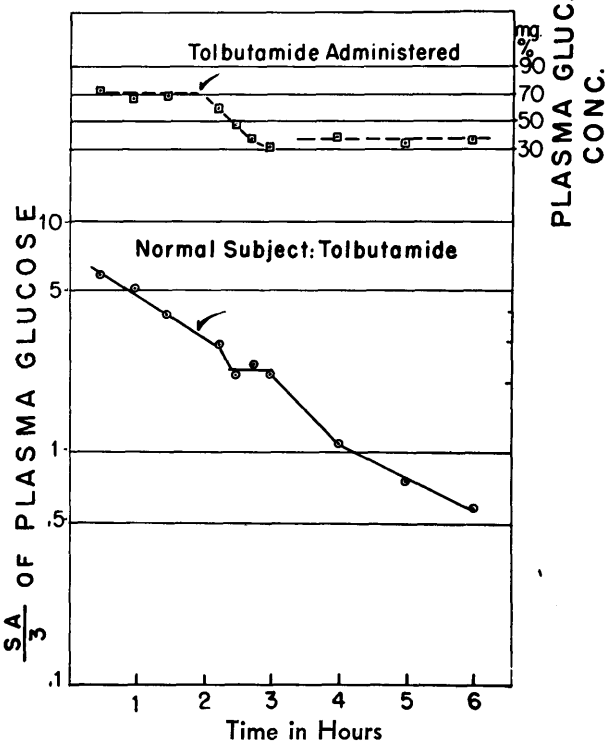

FIG. 1a. Nondiabetic (normal) subject A.Z. Weight 41 kg. Tolbutamide (1.0 gm. intravenously, 2.5 gm. orally) administered at the arrow.

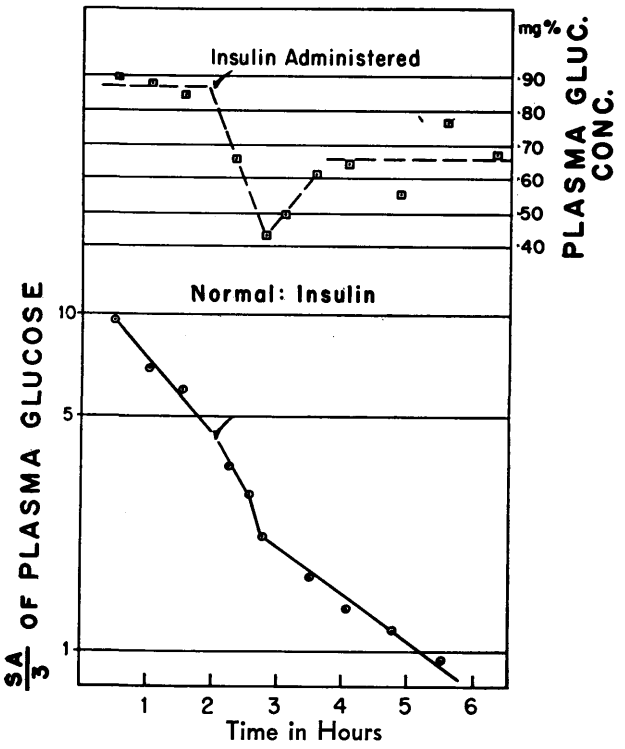

FIG. 2. Nondiabetic (normal) subject D.T. Weight 66 kg. Insulin (3.3 units intravenously, 10.0 units subcutaneously) administered at the arrow.

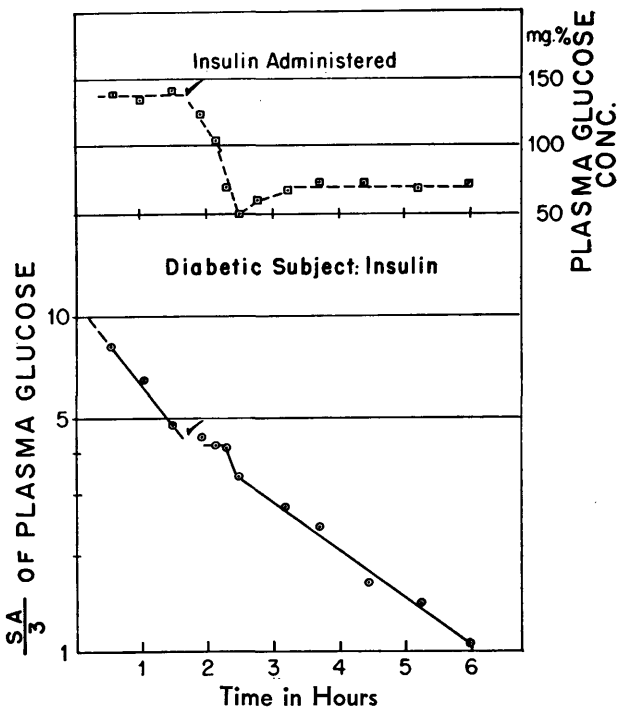

FIG. 3. Diabetic subject F.D. Weight 71 kg. Insulin (3.5 units intravenously, 11.0 units subcutaneously) administered at the arrow.

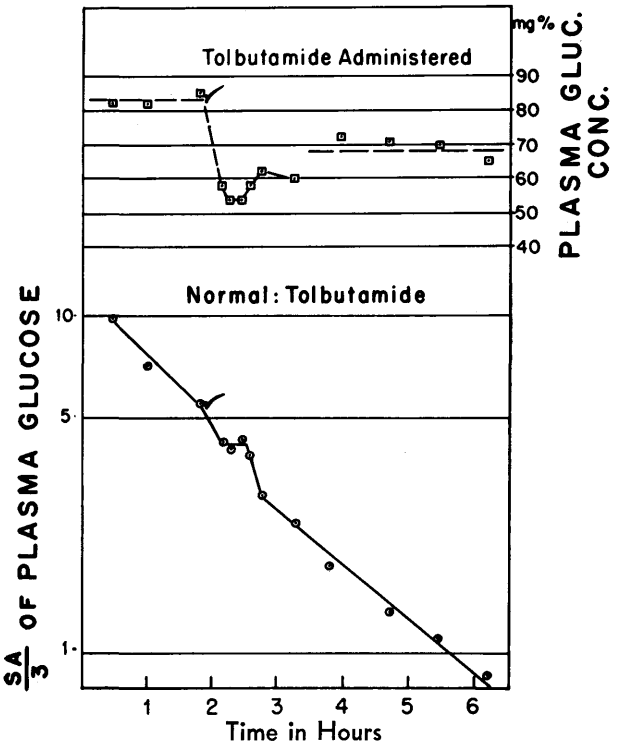

FIG. 2a. Nondiabetic (normal) subject D.T. Weight 66 kg. Tolbutamide (1.65 gm. intravenously, 4.5 gm. orally) administered at the arrow.

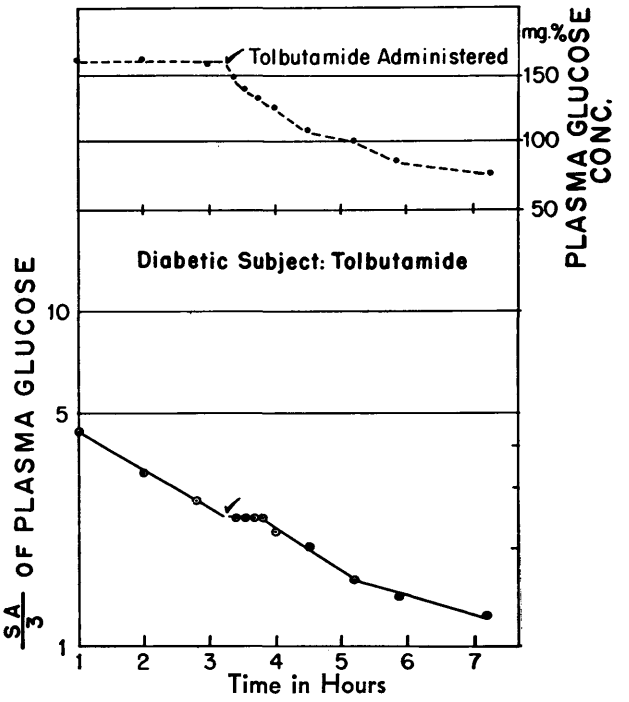

FIG. 3a. Diabetic subject F.D. Weight 71 kg. Tolbutamide (1.77 gm. intravenously, 4.5 gm. orally) administered at the arrow.

TABLE 1

Insulin studies

Subject	State	Weight kg.	Phase 1 Gluc. conc. mg. per cent	Phase 1 T.R.* mg./kg./hr.	Phase 2 E.R.† mg./kg./hr.	Phase 2 R.R.‡ mg./kg./hr.	Phase 3 Gluc. conc. mg. per cent	Phase 3 T.R. mg./kg./hr.
F.N.	Nondiabetic	70	79	108	67	327	37	42
J.J.	"	69	96	91	86	260	48	61
A.Z.	"	41	77	146	0	244	46	56
D.T.	"	66	88	114	111	329	67	54
J.F.	"	72	68	106	0	260	57	42
F.D.	Diabetic	71	139	206	0	294	72	64
C.E.	"	59	95	144	0	163	49	42

*T.R.: Turnover rate—the simultaneous and equal rates of entry and removal of plasma glucose.
†E.R.: Nonsteady state entry rate of plasma glucose.
‡R.R.: Nonsteady state removal rate of plasma glucose.

TABLE 2

Tolbutamide studies

Subject	State	Weight kg.	Phase 1 Gluc. conc. mg. per cent	Phase 1 T.R. mg./kg./hr.	Phase 2 E.R. mg./kg./hr.	Phase 2 R.R. mg./kg./hr.	Phase 3 Gluc. conc. mg. per cent	Phase 3 T.R. mg./kg./hr.
F.N.	Nondiabetic	70	89	141	0	179	55	25
J.J.	"	69	99	122	0	148	77	48
A.Z.	"	41	70	69	0	110	36	31
D.T.	"	66	85	105	0	184	70	64
J.F.	"	72	66	73	0	115	45	69
F.D.	Diabetic	71	162	104	0	107	77	24
C.E.	"	59	162	116	0	112	80	38

subjects studied showed a 151 per cent increase in the rate of removal of plasma glucose following insulin administration. On the other hand the results from the diabetic subjects showed an increase averaging 31 per cent of the pre-insulin value. After the period of zero slope the s.a.-time curve of plasma glucose again declined as the concentration of plasma glucose rose slightly and then stabilized at a value approximately 50 per cent of that observed during Phase 1 in both the nondiabetic and diabetic subjects. When the glucose concentration was again relatively stable and the s.a.-time curve of plasma glucose evidenced a logarithmic decline (Phase 3), a second calculation of the turnover rate of plasma glucose was made. This calculation showed, in both the nondiabetic and diabetic subjects, that the rate of supply and removal of glucose to and from the circulation was remarkably depressed from the postabsorptive values observed in Phase 1 prior to insulin administration.

Figure 2 illustrates a quantitatively different response of the plasma glucose to insulin administration in a nondiabetic subject. Here immediately following insulin, in Phase 2, the s.a.-time curve of plasma glucose changed its slope, not to a zero value, but to an increasingly negative value. A calculation of the rate of glucose supply made during this time indicated that the rate of entry of glucose to the circulation was reduced slightly or not at all. As was demonstrated in the other insulin studies, the rate of removal of plasma glucose following insulin administration was increased. Thus the increase in the negative value of the slope of the s.a.-time curve of plasma glucose was not due to any increase in the rate of supply of glucose, but was the result of an increase in the rate of removal of plasma glucose. A calculation of the turnover rate of plasma glucose in Phase 3 demonstrated a remarkable reduction in rate as was shown in the other insulin studies.

Effect of tolbutamide on the turnover of plasma glucose in nondiabetic and diabetic human subjects. Figures 1a through 3a illustrate the response observed in the concentration and s.a. of plasma glucose before and after the administration of tolbutamide in one diabetic and two nondiabetic subjects. The insulin data from these same subjects were presented in figures 1-3. It will be noted that in each case the response of the s.a.-time curve of plasma glucose following tolbutamide administration was to change its slope to a zero value. The duration of this zero slope, observed in all the tolbutamide studies, was approximately thirty minutes. The rate of supply of nonlabeled glucose to the circulation during this time was zero. A calculation of the rate of removal of glucose from the plasma, during this

time, showed an increase in the five nondiabetic subjects studied which averaged 44 per cent greater than the rate of turnover determined in Phase 1.

In contrast to the observations in the nondiabetic subjects, the diabetic subjects studied showed no increase in the rate of removal of plasma glucose following tolbutamide administration.

A calculation of the turnover rates of plasma glucose made in Phase 3 of the tolbutamide studies showed a reduction in rate, as evidenced in the insulin studies, for both the nondiabetic and diabetic subjects.

DISCUSSION

In the nondiabetic subjects studied here the effects of insulin and tolbutamide were qualitatively similar with respect to changes in the rates of supply and removal of plasma glucose. Insulin or tolbutamide administered in amounts which brought about an approximately equal reduction in the concentration of plasma glucose caused:

1. A transient complete inhibition of the release of glucose into the plasma, a process which normally occurs at an appreciable rate in the postabsorptive state. This complete inhibition occurred consistently with tolbutamide and was observed in two of the five subjects studied with insulin.

2. An acceleration in the removal of glucose from the plasma. This acceleration was greater with insulin than with tolbutamide. In these studies insulin increased the postabsorptive removal rate of plasma glucose in the nondiabetic subjects by an average of 151 per cent. Tolbutamide increased this rate in the nondiabetic subjects by an average of 44 per cent.

3. The release of glucose into the plasma to occur at a greatly reduced rate, when the concentration of plasma glucose was maintained at a low level by subcutaneously administered insulin or orally administered tolbutamide.

In the diabetic subjects studied here the effects of insulin and tolbutamide on the plasma glucose were similar with regard to the supply of glucose to the plasma. A transient complete inhibition of supply was observed in Phase 2 followed by a greatly reduced rate of supply in Phase 3 as compared to Phase 1.

The important difference between the action of insulin and tolbutamide in the diabetic subjects studied here was that no increase was evidenced in the rate of removal of plasma glucose following tolbutamide administration.

The effect of tolbutamide on the hepatic supply of glucose to the circulation reported here is in agreement with the results of a number of investigations recently reported in the literature.[3,7-9,19] However, that this action of tolbutamide is similar to that of insulin is in some dispute. Ashmore and associates[20] have reported that while tolbutamide depresses the output of glucose from the liver of dogs, insulin increases it. These studies employed C^{14} glucose in a manner similar to the present studies. These observations have been confirmed by Mahler and associates[21] by means of portal arterial sampling of blood glucose in dogs. The effect of insulin on the release of glucose from the liver of humans, however, has been recently reported by Jacobs and associates as being inhibitory. These studies were similar in experimental design to the present study.

Evidence for an acute peripheral action of tolbutamide similar to that of insulin, on the other hand, has not been found. Jacobs and associates[19] reported negative findings in this aspect of tolbutamide action, while the findings in the present study show an increase in the removal rate of plasma glucose following tolbutamide administration in nondiabetic subjects. The significance of this peripheral response following tolbutamide is difficult to resolve, especially in the light of the study by Jacobs. Technical differences between these two studies which might explain the variant results are as follows: (1) Plasma glucose was analyzed in the present study, whereas whole blood glucose was analyzed in the study of Jacobs. (2) The amounts and rates of drug administration differed in the two studies. In connection with the second point, two recent studies[19,22] have shown the rate and route of administration as being important in eliciting a peripheral response with exogenous insulin. Jacobs and associates were unable to obtain an increase in the rate of removal of blood glucose when they administered insulin subcutaneously, however, they did notice an effect on the supply of glucose to the circulation similar to that following tolbutamide administration. Madison and Unger reported that insulin injected intraportally fails to elicit the same response in arteriovenous glucose differences that are seen when insulin is given intravenously. A more recent report by Butterfield and associates[23] demonstrated a threshold for the uptake of glucose by the peripheral tissues. This threshold was reported to be elevated in the diabetic subject and to be lowered by insulin and two of the oral hypoglycemic compounds, tolbutamide and phenethyldiguanide. These results demonstrated a peripheral effect in the diabetic subject after some length of successful treatment with the oral hypoglycemic compounds. An acute effect of tolbutamide, in the nondiabetic subject, has been demonstrated in the current study, however, it was not possible to demonstrate this same effect in the diabetic sub-

ject. This seems reasonable since these same diabetic subjects seemed to demonstrate some degree of peripheral insulin insensitivity in these studies. It will be recalled that the nondiabetic subjects exhibited an increase in the removal rate of plasma glucose following insulin that averaged 151 per cent greater than the pre-insulin rate of turnover of plasma glucose. The diabetic subjects, however, demonstrated an increase in the rate of removal of plasma glucose following insulin of only 31 per cent greater than the pre-insulin rate of turnover of plasma glucose.

Many of the recent reports comparing the action of tolbutamide and insulin have emphasized the easily detectable peripheral action of insulin, i.e., an increase in the arteriovenous glucose difference[22] or an acceleration in the rate of removal of glucose from the circulation.[19] In the present study a similar acceleration in the rate of removal of plasma glucose following insulin was demonstrated. In addition we have shown that the prolonged action of insulin or tolbutamide (sustained hypoglycemia) caused a marked reduction in the rate of turnover of plasma glucose. Since the turnover rate is a measure of the simultaneous and equal rates of entry and removal of glucose to and from the circulation, it can be assumed that the rate of removal is dependent on the rate at which glucose can be supplied to the plasma in order to maintain a constant concentration of plasma glucose. Therefore a prime action of insulin or tolbutamide may be to cause the liver to exert a rate-limiting effect on the over-all metabolism of plasma glucose.

SUMMARY

The kinetics of supply and removal of plasma glucose following the administration of insulin or tolbutamide have been investigated. A comparison of the effects of these two compounds has shown that they act very similarly in lowering the plasma glucose in nondiabetic subjects. Both insulin and tolbutamide caused an inhibition of the supply of plasma glucose and an acceleration in the rate of removal of plasma glucose, immediately following their administration in nondiabetic subjects. With the prolonged action of either drug (maintenance of a depressed level of plasma glucose) it is suggested that the rate of supply of glucose to the plasma is a rate-limiting factor in the utilization of plasma glucose.

In the diabetic subject similar effects of the two drugs were noted on the supply of glucose to the plasma, i.e., an inhibition of supply. However, while a stimulation in the rate of removal of plasma glucose was noted with insulin, this was not the case with tolbutamide.

SUMMARIO IN INTERLINGUA

Le Influentia De Insulina E De Tolbutamido Super Le Metabolismo Plasmatic De Glucosa In Humanos, Studiate Per Medio De Glucosa A C^{14}

Esseva investigate le cinetica que reduce le nivello plasmatic de glucosa post le administration de insulina o tolbutamido. Un comparation del effectos de iste duo compositos ha monstrate que illos age in un maniera multo simile in tanto que illos reduce le glucosa del plasma in subjectos non-diabetic. Tanto insulina como etiam tolbutamido causava un inhibition del provision del plasma con glucosa e un acceleration del remotion de glucosa ab le plasma, immediatemente post lor administration a subjectos non-diabetic. Con le prolongation del action del un o del altere del duo drogas (mantenentia de un deprimite nivello del glucosa de plasma), il pare que un reducite alimentation del plasma con glucosa age como factor limitatori in le utilisation plasmatic de glucosa.

In subjectos diabetic, il esseva notate que le duo drogas produceva simile effectos in le alimentation del plasma con glucosa, i.e. illos inhibiva ille alimentation. Tamen, durante que un intensificate elimination de glucosa ab le plasma esseva notate in le caso de insulina, isto non esseva ver in le caso de tolbutamido.

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Sidelights on Longevity

Impressive strides in increasing the average number of remaining years of life are far-reaching:

— If a child under eighteen is orphaned today, chances are twice as great that he will lose his father instead of his mother; 2 per cent of the orphans in the United States have lost both parents; 29 per cent have lost their mothers only, and 69 per cent have lost their fathers.

— There are fewer chances today, however, of a child under eighteen becoming an orphan. As late as 1920, 16 per cent of our children had lost one or both of their parents due to death. With the marked gains in saving lives in the middle and older ages, this figure has now been reduced to 5 per cent.

— Although there are now almost ten million widowed persons (three quarters of them women) in this country, the proportion of *young* widows and widowers is going down. In 1900, H.I.F. reported, widowed persons under the age of forty-five constituted nearly one-quarter of all the widowed; by 1957 the proportion had dropped to about 6 per cent.

— Men have also benefited from reduced mortality as shown by an increase in the average length of working life expectancy. Males born in 1900 had an average working life expectancy of thirty-two years. Today this figure has increased to forty-two years.

— Gains in average length of life in the United States have steadily increased the proportion of our population in the older ages. In 1900, only 4 per cent of the population was in the age group sixty-five and over; in 1958 that figure was 9 per cent. In fifty-eight years our population over sixty-five has increased by twelve million.

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