SIMULATING THE ABSORPTION OF INSULIN FROM A SUBCUTANEOUS DEPOT

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ABSTRACT

The System Dynamics model described in this paper presents a new approach to the mechanisms of subcutaneous absorption of dissolved insulin. Experimental investigations have shown that the apparent absorption constant varies in time, and that this variation depends both on the volume and the concentration of the injected solution.

Our model assumes that insulin is present in the subcutaneous depot in three forms: (i) a dimeric form, (ii) a hexameric form, and (iii) an immobile form in which the molecules are bound in the tissue. The model describes how diffusion and absorption gradually reduce the insulin concentrations and thereby shift the balance between the three forms according to usual laws of chemical kinetics. By assuming that only dimeric molecules can penetrate the capillary wall, we have found that the model can fully account for the observed variations in the absorption rate. At the same time the model can be used to determine at least 5 parameters characterizing the involved processes: the diffusion constant for insulin in subcutaneous tissue, the absorption constant for dimeric insulin, the equilibrium constant for the dimeric/hexameric polymerization process, the binding capacity for insulin in the tissue, and the average life time for insulin in bound state.

Combined with a simplified model of the distribution and degradation of insulin in the body, the diffusion-absorption model has been used to simulate different insulin delivery schedules, i.e. a single major injection contra dosage with infusion pump. The model has shown that a pump repetition frequency of 1-2 /hr can secure a sufficiently constant plasma insulin concentration.

INTRODUCTION

In the treatment of diabetes mellitus insulin is usually administrated subcutaneously, i.e. a dose is injected under the skin to form a depot from which the insulin is gradually released to the blood as it diffuses through the capillary walls. To delay the absorption and maintain a reasonably constant supply to the blood between subsequent injections, a significant fraction of the insulin is normally in an undissolved form, and the rate of absorption is then controlled by the dissociation rate for this compound.

The recent introduction of infusion pumps has renewed the interest in studying the much faster absorption of dissolved insulin. Besides a bolus (major dose) before each main meal the infusion pump gives small injections at a regular pace, for instance every five minutes. The aim of this insulin delivery method is to attain a near-normal blood glucose concentration throughout the day. It is assumed that an efficient and consistent control of blood glucose may prevent or at least delay the development of the late complications of the disease.

By labeling the insulin with a γ-emitting isotope (usually I-125), the amount of insulin remaining at the injection site can be determined as a function of time by external counting for instance with a scintillation detector. This is a well-known technique, and several studies [1, 2, 3, 4] have shown that the measured count rate is proportional to the amount of biologically active insulin in the underlying tissue.

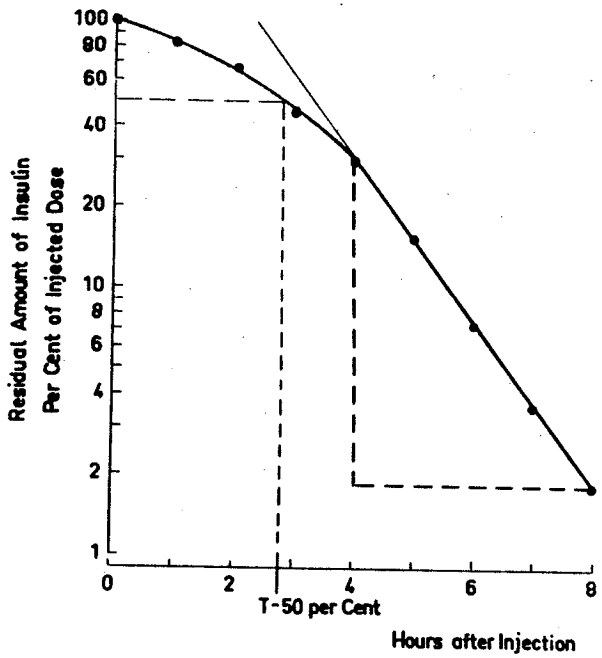

Figure 1. Absorption curve for insulin, subcutaneously injected into the femoral region. (0.3 ml, 40 IU/ml)

Figure 1 shows the absorption following a subcutaneous injection of dissolved insulin (0.3 ml, 40 IU/ml) into the thigh [1]. (Concentration is measured in International Units per ml; 1 mg of insulin equals 27 IU). A characteristic feature observed in most investigations [5] is that the relative absorption rate rises during the first hours, and then remains constant. It has been proposed [1, 6] that this time course is due to a so-called self-depression effect, i.e. to a reduction of the absorption caused by endogenously liberated compounds, presumably accompanied by a decrease in the capillary membrane area. The duration and extent of this self-depression should then be functions of the composition of the injected fluid, including its pH, the injection pressure, etc.

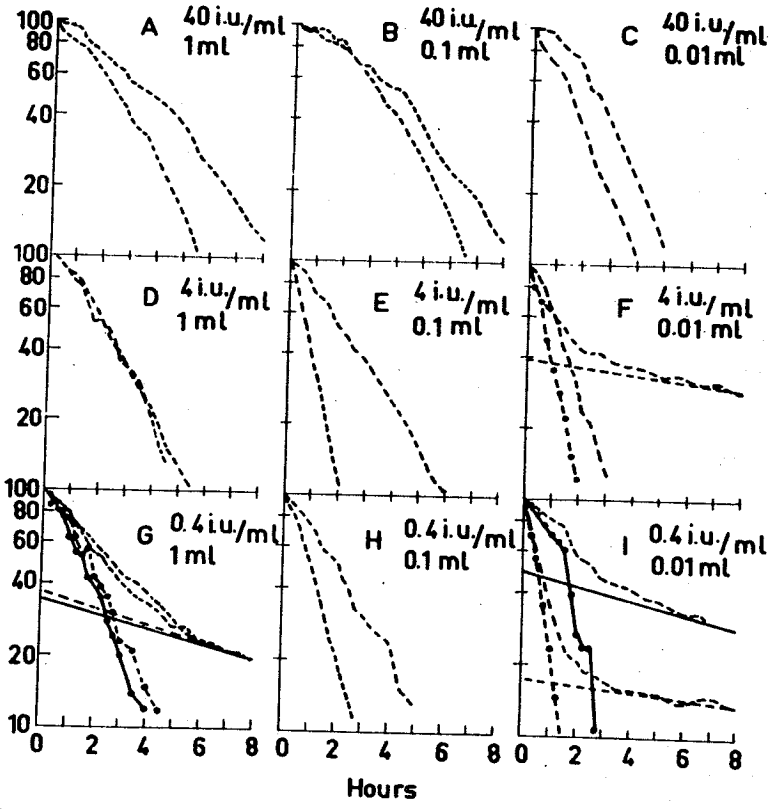

Figure 2. Absorption curves for insulin injected in various volumes and concentrations. A subtraction is made of the terminal part of the curve where this is slower (solid curve).

Figure 2 displays absorption curves for a wide range of volumes and concentrations of insulin solutions [1]. From this

figure we observe:

(1) For the same concentration, the initial phase of slow absorption is shortened when decreasing the injection volume.

(2) For a constant injection volume, the initial absorption rate increases with decreasing concentrations.

(3) With small doses of injected insulin (small volumes and/or concentrations), a tail develops on the absorption curve.

The volume and concentration effects have been observed by other investigators [7]. We do not know of other reports on insulin absorption curves with tails. This is presumably explained by the fact that such small volume / low concentration studies are seldom performed.

A self-depression theory can hardly explain both the volume and concentration effects on the initial absorption course in detail. Such an explanation also includes a significant ad-hoc element as it doesn't lent itself to a detailed formal description. We have therefore tried to formulate an absorption model based only upon 'classic physio-chemical principles', i.e. diffusion, mass balances etc. It should be stressed, however, that we cannot exclude the possibility of self-depression being a factor of importance.

BASIC ABSORPTION MODEL

The model to be presented here is based on the following assumptions:

(1) Dissolved insulin contains several multimeric forms, mainly hexameric and dimeric insulin.

(2) No significant degradation of insulin takes place in the depot.

(3) Insulin molecules move in the tissue according to the usual diffusion equation.

(4) The depot is cleared by means of absorption of only dimeric molecules into the capillaries.

(5) Insulin can be locally bound to cell surfaces or to protein molecules in the intercellular fluid.

(6) All water from the solution is bound in the tissue immediately after the injection so that the insulin molecules are located just around the injection site. Consequently, convection does not contribute to the dispersion of the molecules except during the first, few minutes.

We thus think of insulin as being on either hexameric or dimeric form, controlled by the equilibrium constant Q :

$$CHI = Q \cdot CDI^3$$

Here, CHI and CDI denote concentrations of hexameric and dimeric insulin, respectively. A reduction in the total concentration will shift the balance towards the dimeric form. Provided only dimeric molecules can penetrate the capillary wall, this explains at least qualitatively the initial time course of absorption and

its dependence on the injected insulin concentration. Spreading of the depot by diffusion also reduces the total insulin concentration. As we shall see, the volume effect can therefore also be explained by the existence of a hexameric-dimeric balance. (Small injected volumes spread relatively more than bigger ones, the diffusion constant being the same in both cases).

According to figure 2, the volume effect is only significant when decreasing the injection volume below 0.1 ml. This suggests that diffusion expands the depot volume by approximately 0.1 ml during the first hour or two. If diffusion in the subcutaneous tissue is basically lateral, the expansion volume can be estimated by

$$\Delta V = \pi \cdot R_d^2 \cdot W$$

where $R_d = 2 \cdot \sqrt{D \cdot t}$ is the diffusion distance. D is here the diffusion constant and t the elapsed time. The cylinder height W is a characteristic dimension of the injected volume or the medium in which diffusion takes place. Setting W = 0.5 cm, we get

$$D = \frac{\Delta V}{4\pi \cdot W \cdot t} = \frac{0.1 cm^3}{4\pi \cdot 0.5 cm \cdot 60 min} = 2 \cdot 10^{-4}\ cm^2/min\ ,$$

which is about twice the diffusion constant of insulin in water [8]: $D_{H_2O} = 0.9 \cdot 10^{-4}\ cm^2/min$.

During the last part of the absorption course (4-8 hours on figure 1) the insulin concentration is so small that nearly all insulin is on dimeric form. Consequently, the absorption constant B for dimeric insulin in subcutaneous tissue is given by the terminal slope of the absorption curve :

$$B = \frac{1}{240 min} \log \frac{30}{2} = 1.2 \cdot 10^{-2}/min\ .$$

The absorption constant is assumed to be directly proportional to the capillary pore area per unit volume of tissue.

In the hexameric-dimeric balance, production of dimeric insulin is given by

$$\frac{dCDI}{dt} = k_1 \cdot CHI - k_2 \cdot CDI^3 = P \cdot (CHI - Q \cdot CDI^3)$$

with k_1, k_2 and P being rate constants. In the quasi stationary equilibrium established immediately after the injection we have

$$Q = \frac{CHI}{CDI^3} = \frac{(1-d) \cdot ICO}{(d \cdot ICO)^3}$$

where ICO is initial total insulin concentration and d is initial fraction of dimeric insulin. From the experimental results in figure 1, we can estimate d as the ratio between the initial and terminal slopes of the absorption curve. This gives d = 0.25 or for the equilibrium constant

$$Q = 0.023\ ml^2/IU^2\ .$$

There is no indication that the transformation rate of hexameric to dimeric should be a limiting factor. We therefore assume the hexameric-dimeric balance to be near equilibrium during the entire absorption course. This requires a large rate constant P compared to the absorption constant B. We have chosen

$$P = 0.5/min.$$

The tail phenomenon observed in figure 2 is ascribed to temporary binding of insulin in the tissue. Since the binding capacity per volume of tissue (BC) is small compared to the insulin concentration of normal therapeutic solutions, tails will not occur until more than eight hours after the injection unless very small volumes or concentrations are used. Capture and binding of the molecules is probably a relatively rapid process, governed by the concentration of free, dimeric insulin CDI. Once bound, the molecules are released at a rate determined by the average life time in bound state T.

The net binding rate can thus be written as

$$\frac{dABI}{dt} = S \cdot CDI \cdot (BC \cdot V - ABI) - ABI/T$$

where ABI is the amount of bound insulin, S is a rate constant, and V is volume. In general, only the product S·BC can be estimated from the absorption curves, but provided S is large (fast capture), we can find BC by extrapolating the slow, terminal part of the curve back to t = 0 hours. From figure 2(G) we obtain

$$BC \simeq \frac{35}{100} \cdot 0.4 \text{ IU/cm}^3 = 0.15 \text{ IU/cm}^3.$$

The reciprocal average life time 1/T is given by the slope of the tail. From the experimental results in figure 2 , we deduce

$$1/T = \frac{1}{8 \cdot 60\text{min}} \log \frac{40}{30} = 1.0 \cdot 10^{-3}/\text{min}.$$

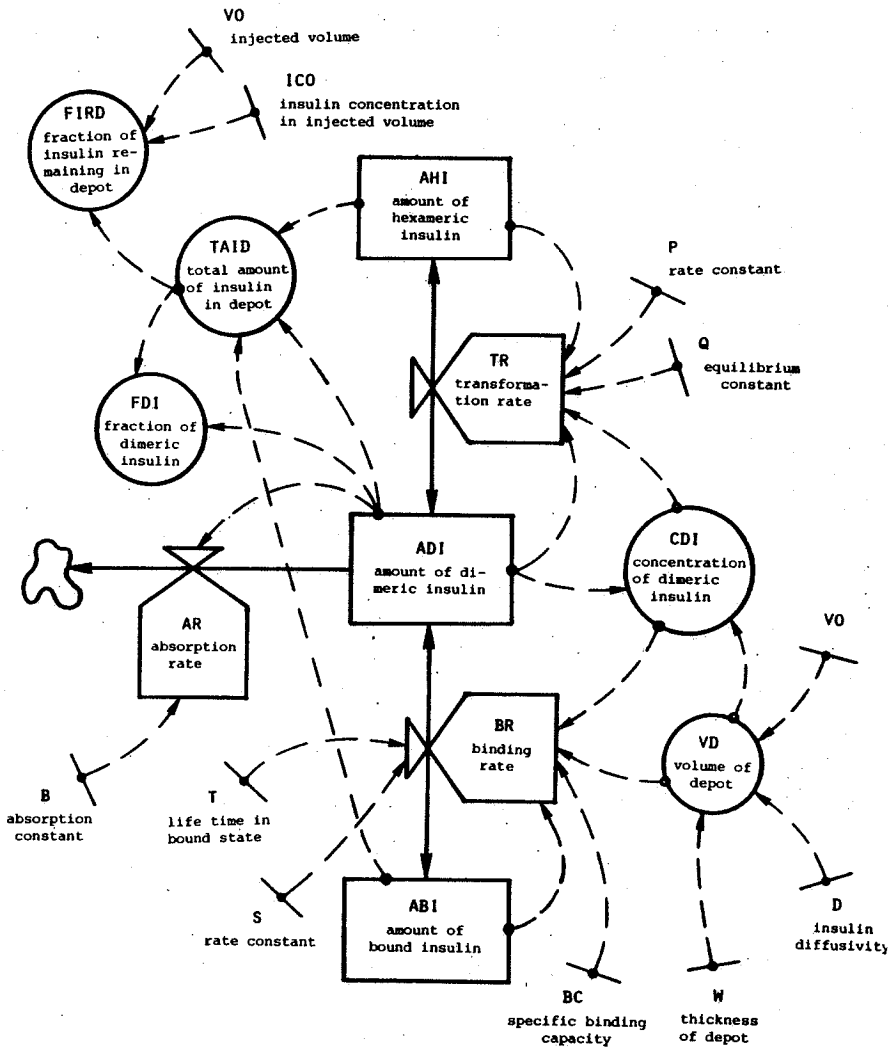

Figure 3. Flow-diagram for the basic insulin absorption model.

Figure 3 shows the System Dynamics flow-diagram for a model of the subcutaneous absorption process. Diffusion of the mole-

cules is here described approximately by letting the volume of the depot VD expand according to

$$VD = VO \cdot \sqrt{1 + \left(\frac{4\pi \cdot D \cdot W \cdot t}{VO}\right)^2} \quad .$$

VO is injected volume. Furthermore, we consider the molecules to be uniformly distributed within VD.

Injection of a certain dose is simulated by assigning appropriate initial values to the amounts of hexameric and dimeric insulin, AHI and ADI, respectively. The amount of bound insulin ABI should be 0 at start. In appendix A we provide the entire DYNAMO program.

In order to study the influence of the different effects separately, we have ignored binding of insulin by setting BC = 0. Figures 4 and 5 display the responses to different values of injected volume and concentration. Next, we have studied the binding mechanism. Figure 6 shows how the model reacts to different values of the rate constant S. By comparing the curves to those of figure 2, we find that S should be approximately 0.05 ml/(IU·min).

Finally, in figure 7 and 8, we show a complete simulation corresponding to the experimental results in figure 1. It is seen, how the fraction of dimeric insulin increases during the first six hours and then declines, due to binding. In the above coarse estimates of the parameters D, B, Q, BC etc., we considered the effects of polymerization, absorption and binding

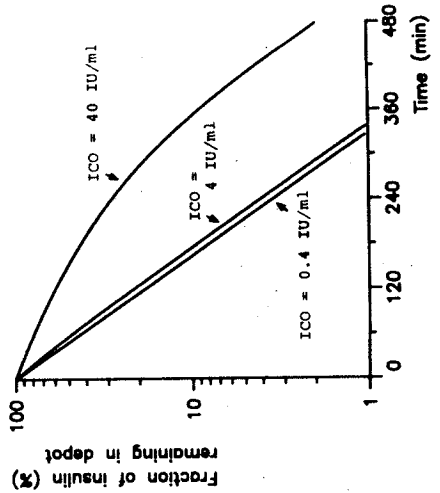

Figure 5. Simulation results using various concentrations. VO = 1.0 ml. Other constants have values as in figure 4.

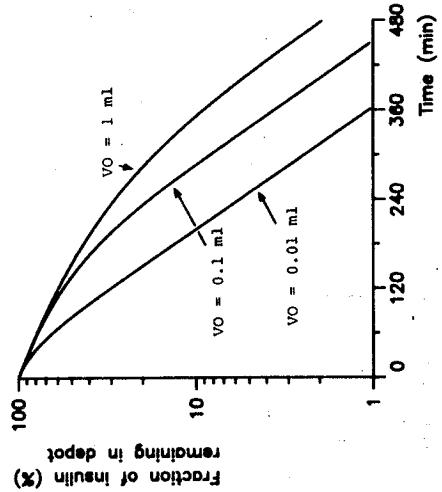

Figure 4. Simulation results for the basic model showing the volume effect. Notice, that the binding mechanism is neglected. (ICO = 40 IU/ml, $D = 2.0 \cdot 10^{-4}$ cm^2/min, $B = 1.4 \cdot 10^{-2}$/min, $Q = 0.023$ ml^2/IU^2, P = 0.5/min, W = 0.5 cm, BC = 0 IU/cm^3)

to be completely separable, which, of course, does not hold. To fit the response of the model it has therefore been necessary to adjust some of the parameters slightly. The new values are listed on figure 7.

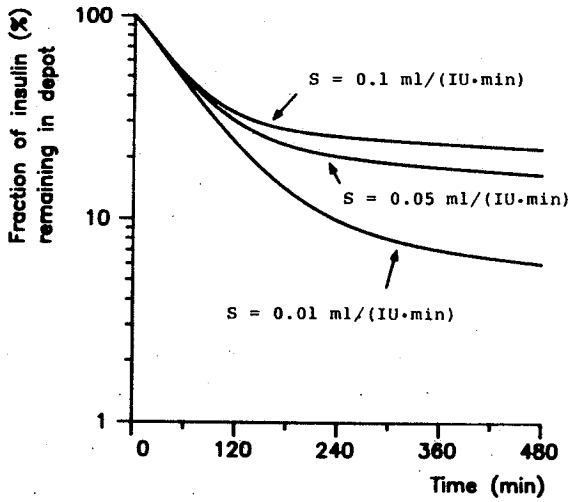

Figure 6. The binding effect. Simulations are done with BC = 0.15 IU/cm^3 and T = 1000 min. (VO = 0.01 ml, ICO = 4 IU/ml)

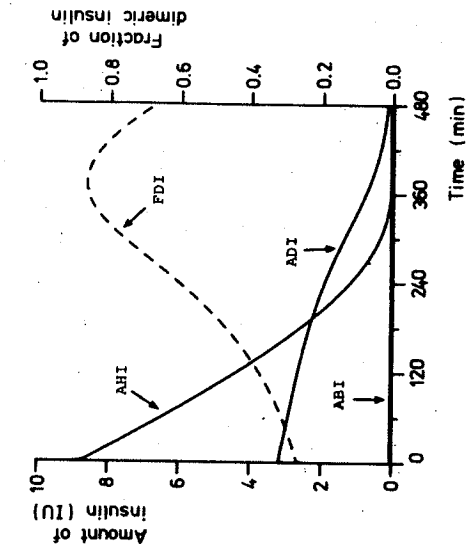

Figure 8. Same simulation as in figure 7, showing how absorption shifts the balance between hexameric, dimeric, and bound insulin.

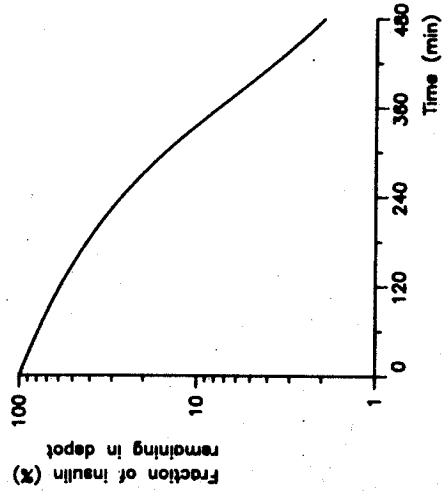

Figure 7. Simulation of a 0.3 ml injection, ICO = 40 IU/ml. To obtain a curve similar to that of figure 1, we have used D = $1.6 \cdot 10^{-4}$ cm^2/min, B = $1.2 \cdot 10^{-2}$/min, Q = 0.023 ml^2/IU^2, P = 0.3/min, BC = 0.1 IU/cm^3, T = 1000 min, S = $1.0 \cdot 10^{-3}$ml/(IU min), and W = 0.5 cm.

DIFFUSION-ABSORPTION MODEL

In the basic model we described diffusion by means of an expanding volume of uniform molecular distribution. We shall now turn to a more realistic model, in which the tissue is divided into a number of rings, centred around the injection site, see figure 9.

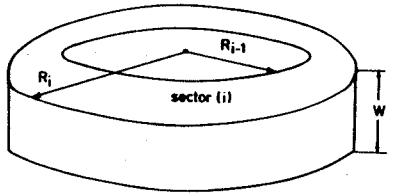

Figure 9. Geometry of a ring in the subcutaneous depot. In the SD-model we use 13 such rings.

To each ring we attach levels, rates etc. corresponding to those of the basic model, see figure 10. In order to describe the diffusion process, we replace VD of the basic model by the time invariant sector volume SV_i and introduce two new rates:

diffusion rate of dimeric insulin:
$$DIFRD_i = DDIM \cdot \frac{AREA_{(i-1,i)}}{(R_i - R_{i-2})/2} \cdot (CDI_{i-1} - CDI_i)$$

diffusin rate of hexameric insulin:
$$DIFRH_i = DHEX \cdot \frac{AREA_{(i-1,i)}}{(R_i - R_{i-2})/2} \cdot (CHI_{i-1} - CHI_i)$$

$AREA_{(i-1,i)}$ denotes the cylinder area between the i'th and the i-1'st ring and $(R_i - R_{i-2})/2$ is the average diffusion distance between the same two rings. The number and sizes of rings are chosen so that the injection volume is made up of three to six of the innermost rings and the outermost ring has a radius larger

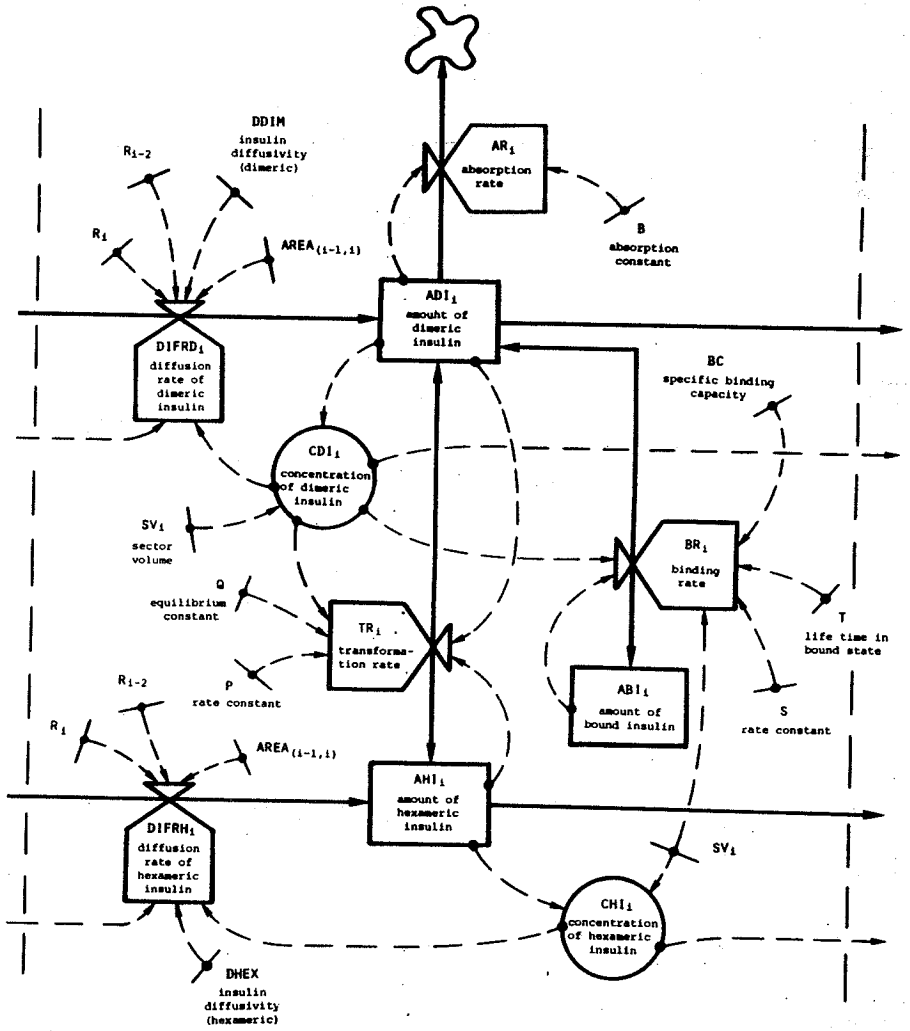

Figure 10. Flow-diagram for the i'th sector of the diffusion-absorption model.

than $2\sqrt{D \cdot t} + R_o$ where R_o is the radius of the initial depot.

Figure 11 shows a comparison between a 13-ring model

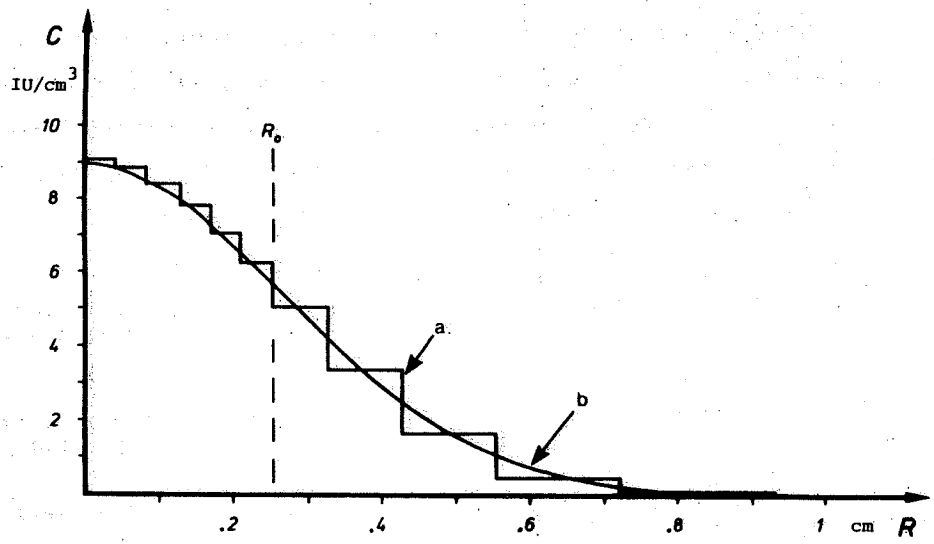

Figure 11. Concentration profiles after 180 min of diffusion, obtained by

a) the SD-model,

b) an accurate solution to the two-dimensional diffusion process.

(R_o = 0.25 cm, ICO = 20 IU/ml, VO = 0.1 ml, D = $1.5 \cdot 10^{-4}$ cm^2/min, W = 0.5 cm)

(without absorption and binding) and an exact solution to the diffusion equation in two dimensions.

With the above detailed account of spatial distribution, the diffusion-absorption model requires minor adjustments of BC and S in order to show the same responses as our basic model. Using the new values, we have simulated the experiments from figure 2. Although not quite identical, the results as presented on figure 12 exhibit very much the same dependence on volume and concentration as the original curves.

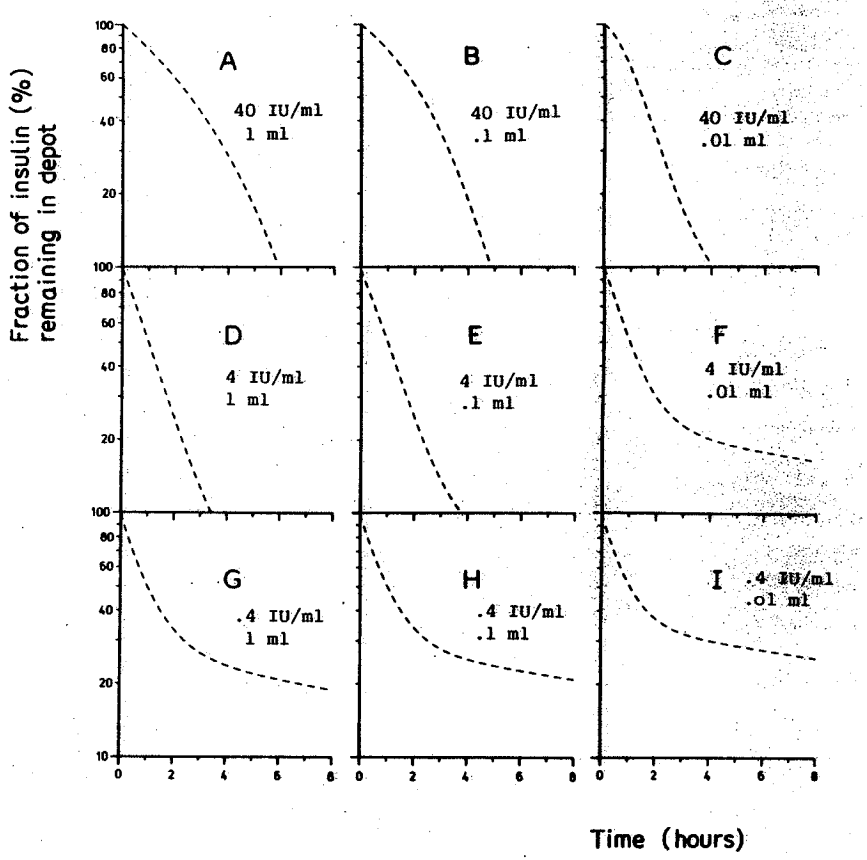

Figure 12. Simulation results obtained with the diffusion-absorption model, to be compared with figure 2.
(B = $1.1 \cdot 10^{-2}$/min, DDIM = DHEX = $2.0 \cdot 10^{-4}$ cm^2/min, Q = 0.023 ml^2/IU^2, P = 0.3 /min, BC = 0.05 IU/cm^3, T = 1000 min, S = $1.5 \cdot 10^{-3}$ ml/(IU min), W = 0.5 cm)

One should not focus too much on 'the missing tail' on figure 2(H). The investigation was carried out using only six

patients, and the conclusion should be that there is a tendency for tails to develop on the absorption curves when decreasing the injected insulin dose. We have recently initiated a new series of independent measurements to see if the above results of a slow final absorption can be reproduced and particularly to see if the tail develops on the absorption curves for higher volumes and concentrations when continued to a sufficiently small remaining insulin concentration in the tissue.

INSULIN IN PLASMA

A main function of insulin is to enable utilization of glucose as an energy source in muscle and fat cells. The insulin molecules are distributed to the different parts of the body by means of the blood system from which they diffuse into the interstitial space. Degradation of insulin takes place locally as well as in the liver. In diabetics having no endogenous insulin secretion, the concentration of insulin in plasma thus becomes a function of the subcutaneous absorption rate, the distribution volume, and the degradation rate.

In order to simulate how the plasma insulin concentration varies following a subcutaneous injection, we developed a simple distribution model, see figure 13. The model is ment to be connected with the diffusion-absorption model via the total absorption rate TAR.

The degradation rate in plasma DRP symbolizes hepatic degradation of insulin which equals about 50% of the amount of

387 insulin that passes the liver per unit time. With a liver blood flow of 1.5 l/min and a total blood volume of 5.0 l [9] we can estimate the half life of insulin in plasma HLP due to hepatic degradation:

$$HLP = \frac{5.0\ l}{0.5 \cdot 1.5\ l/min} \cdot \ln 2 = 4.5\ min$$

The insulin interchange between plasma and interstitial fluid is described by the transfer rate

$$TRPIF = K \cdot (CIP - CIIF)$$

where CIP and CIIF are concentrations of insulin in plasma and interstitial fluid, respectively, and K is a transfer constant. Clinical observations indirectly indicate a time of 10-15 min for the insulin concentrations in plasma and interstitial fluid to equalize. In the model we use

$$K = 15\ l/min\ ,$$

but in connection with subcutaneous injections this value is not critical as long as it is large compared to the absorption constant.

Insulin degrades also from the interstitial fluid but at a slower rate than in plasma. The half life in interstitial fluid HLIF is found by comparing simulation results with experimental plasma insulin curves. This points to a half life of approximately 20 min. The plasma volume PV and the interstitial fluid volume IV are related to the body weight [9]. For a 70 kg person

the standard values are

PV = 2.8 liter, and IV = 11.2 liter.

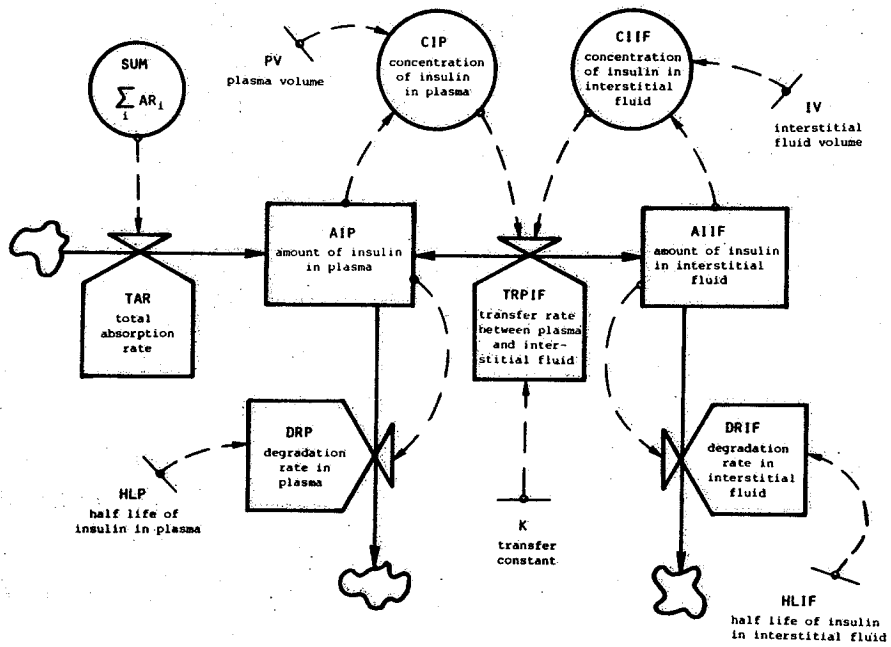

Figure 13. Flow-diagram for a simple model of insulin distribution and degradation in the body.

On figures 14 and 15 we show a simulation with the combined diffusion-absorption-distribution model together with experimental curves from four diabetics. All curves represent a 0.15 ml injection of 40 IU/ml insulin. The following comments should be made:

In order to fit the response of the diffusion-absorption model to the experimental results (figure 14), we have to

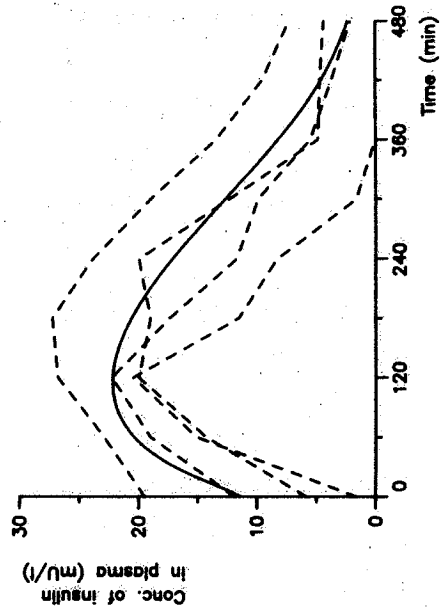

Figure 15. Plasma insulin curves corresponding to the absorption curves on figure 14.
(HLP = 4.0 min, HLIF = 20 min, PV = 2.6 l, IV = 10.4 l)

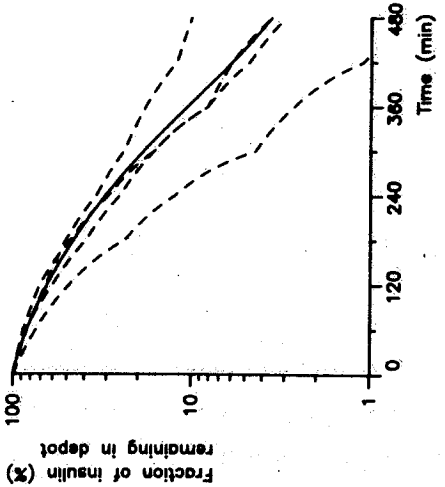

Figure 14. Simulation (solid curve) and experimental curves (dashed) showing the absorption following a 0.15 ml injection of 40 IU/ml insulin. In the diffusion-absorption model we have used Q = 0.157 ml^2/IU^2 ; all other constants are similar to those of figure 12.

postulate a higher content of hexameric insulin in the solution, i.e. a higher equilibrium constant Q, than estimated from figure 1. This may reflect an increased purification of the insulin preparations during the last twenty years as the curves on figures 1 and 2 are twenty years old while the investigation referred to on figures 14 and 15 was carried out in 1983/84.

All experimental curves on figure 15 have initial values above zero. This is due to the fact that the patients were given insulin with an infusion pump the night before the investigation. To obtain the same initial conditions in the simulation, we have specified appropriate initial values for AIP and AIIF (amount of insulin in plasma and amount of insulin in interstitial fluid).

Finally, we notice that a single-space distribution model with a distribution volume of 14 liters and an insulin half life of 11 min would perform almost as well when combined with the diffusion-absorption model. This is due to the combination of a small absorption rate and a relatively fast exchange of insulin between plasma and interstitial fluid. A single-space distribution model is worthless, however, in the case of intravenous injections, since insulin delivery can here be regarded as instantaneous, and the main delays are associated with the distribution to the interstitial fluid.

In formulating the equations and estimating the constant values for the diffusion-absorption-distribution model, we have concerned ourselves only with 'single shot' injections. Using the same model, we shall now show some simulations of how the plasma

389 insulin concentration varies when insulin is infused subcutaneously by means of a pump.

In pratice the insulin is given through a cannula taped to the skin in the abdominal region where the density of capillaries and hence the absorption constant is somewhat higher than for the thigh. A tube connects the cannula to the pump which is usually driven in pulse mode, i.e. small volumes of insulin are delivered at a certain repetition frequency.

By adding 'infusion' rates to AHI_i and ADI_i of the innermost rings, see figure 10, we can handle periodical injections in the same way as we did with single shots. Figure 16 displays simulation results for various pump frequences. At all frequences we have used a mean infusion rate of 20 IU per 24 hours, which covers about half of the bodys insulin requirements. Since most commercial pumps use insulin in a concentration of 100 IU/ml, we have chosen this value for ICO. Furthermore, we have increased the absorption constant to $B = 2.0 \cdot 10^{-2}$ /min, considering the higher absorption from the abdominal region.

Finally, we show a 'frequency characteristic' for the entire delivery-absorption system on figure 17. The curve is based on five simulations using frequences in the interval 0.5 /hr - 10.8 /hr and it shows the ripple on the plasma insulin concentration as a function of the pump repetition frequency.

Most commercial pumps are designed for frequences above 4 /hr. From figure 17 we find that a ripple value less than 1% is obtained by a repetition frequency of only 2 /hr.

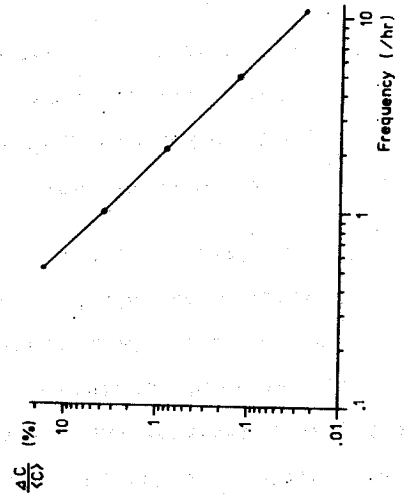

Figure 17. Frequency characteristic. ΔC is the peak to peak variation as seen on figure 16 and $\langle C \rangle$ = 16.0 mU/l is the mean concentration.

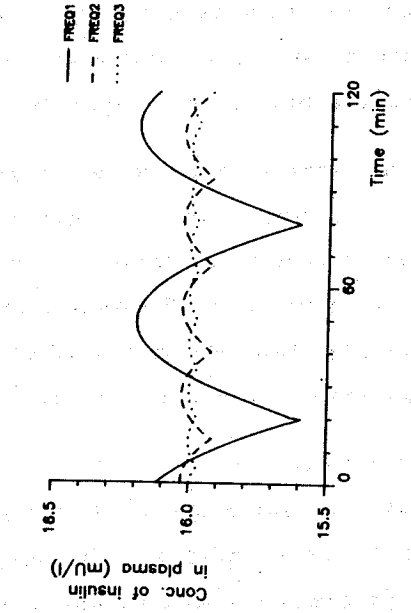

Figure 16. Plasma insulin curves for three different pump frequences : FREQ1 = 1 /hr, FREQ2 = 2.25 /hr, and FREQ3 = 4 /hr. In the simulations we have used ICO = 100 IU/ml and B = $2.0 \cdot 10^{-2}$/min; all other constants have values as in figure 14.

Many areas of medical modeling are characterized by the use of what we consider rather complicated statistical methods to fit strongly oversimplified mathematical expressions to experimental results. As an alternative approach, we have combined a set of basic physical and chemical processes such as polymerization, diffusion, binding, absorption, distribution, and degradation, and we have shown how these processes together can explain the relatively complex dynamical behaviour of the absorption system as a whole. Each of these processes can be described through simple and well-known equations of motion and many of the parameters can be obtained (or estimated) from independent experiments.

It is still possible that the slow initial absorption of insulin to some extent can be caused by self-depression. We have found, however, that the above more basic processes fully account for all major effects in the available experimental results. We have also been able to satisfactorily predict the outcome of experiments not previously performed.

A varity of new experiments have recently been initiated to test the simulation results as well as to allow extension and improvements of the model. As an example, our assumption that the absorption tail is due to local binding will be tested by saturating the tissue binding capacity with unlabeled insulin before injecting I-125 labeled insulin. The model will be improved by the inclusion of convection of insulin in the subcutaneous depot

due to the injection pressure. Convection may be significant for the size of the stationary depot produced by the infusion pump. We are also going to consider the mechanisms which regulate the blood glucose concentration in more detail.

APPENDIX A

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NOTE ********************************************************
NOTE              BASIC ABSORPTION MODEL
NOTE ********************************************************
*
C  VO=0.3  ML
C  ICO=40  IU/ML
*
NOTE       INITIAL FRACTION OF DIMERIC INSULIN
C  D=0.25
*
NOTE     FREE DIMERIC INSULIN:
NOTE
L  ADI.K=ADI.J+(DT)(TR.JK-AR.JK-BR.JK)
N  ADI=VO*ICO*D
A  CDI.K=ADI.K/VD.K
A  FDI.K=ADI.K/TAID.K
R  AR.KL=ADI.K*B
C  B=1.2E-2   /MIN
*
NOTE      HEXAMERIC INSULIN:
NOTE
L  ABI.K=ABI.J+(DT)(-TR.JK)
N  ABI=VO*ICO*(1-D)
R  TR.KL=P*(ABI.K-Q*ADI.K*CDI.K*CDI.K)
C  P=0.5   /MIN
C  Q=0.023  ML*ML/(IU*IU)
*
NOTE      BOUND INSULIN:
NOTE
L  ABI.K=ABI.J+(DT)(BR.JK)
N  ABI=0
R  BR.KL=S*CDI.K*(BC*VD.K-ABI.K)-ABI.K/T
C  S=0.05     ML/(IU*MIN)
C  T=1000     MIN
C  BC=0.15    IU/ML
*
NOTE      TOTAL AMOUNT OF INSULIN:
NOTE
A  TAID.K=ADI.K+ABI.K+ABI.K
N  TAID=TAIDO
N  TAIDO=VO*ICO
A  FIRD.K=100*TAID.K/TAIDO
*
NOTE      VOLUME OF DEPOT:
NOTE
A  VDIFF.K=3.14*4*D*TIME.K*W
A  VD.K=VO*SQRT(1+(VDIFF.K*VDIFF.K)/(VO*VO))
C  D=2.0E-4    CM*CM/MIN
C  W=0.5       CM
*
NOTE
SPEC  DT=0.1/LENGTH=480/PLTPER=12
PLOT  FIRD
RUN
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REFERENCES

1. Binder C (1969): Absorption of Injected Insulin. Acta Pharmacol. Toxicol. 27 (Suppl2).
2. Deckert T, Hansen B, Lauritzen T, Christiansen J S (1981): Subcutaneous Degradation of Insulin. Diabetologia 21.
3. Jørgensen K, Binder C (1966): ^{125}I-Insulin as a Tracer of Insulin in Different Chemical Processes. In Labeled Proteins in Tracer Studies. Donato L, Milhaud G, Sirchis J, Eds. Brussels, EURATOM.
4. Kølendorf K, Bojsen J (1982): Kinetics of Subcutaneous NPH Insulin in Diabetics. Clin. Pharmacol. Ther. 31.
5. Binder C, Lauritzen T, Faber O, Pramming S (1984): Insulin Pharmacokinetics. Diabetes Care 7(2).
6. Schou J (1971): Subcutaneous and Intramuscular Injection of Drugs. In Handbook of Experimental Pharmacology. XXVII. Concepts in Biochemical Pharmacology. Part 1. Brodie B B, Gilette J R, Eds. New York, Springer-Verlag.
7. Galloway J A, Spradlin C T, Nelson R L, Wentworth S M, Davidson J A, Swarner J L (1981): Factors Influencing the Absorption, Serum Insulin Concentration, and Blood Glucose Responses after Injections of Regular Insulin and Various Insulin Mixtures. Diabetes Care 4.
8. Creeth J M (1952): Maximum Molecular Weight of Insulin. Nature 170.
9. Scientific Tables, seventh edition. Documenta Geigy. Diem K, Lentner C, Eds. Basle, J.R. Geigy S.A.