the difficulty of observing the time in the T tube and by the impossibility of redispersing the precipitate by gentle means.

In Fig. 1 we see from the upper curve that within the reproducibility the number of crystals is independent of the initial concentration c when the latter is less than 0.01 M and proportional to c^{10} (the tenth power of the concentration) at $c > 0.01$ M. We do not know any other mechanism than homogeneous nucleation that can explain this phenomenon.

The second curve in Fig. 1 consists of three straight lines, corresponding to three different types of precipitation reaction. A similar curve (drawn from a compilation of different authors' data) has been published before [3], but without interpretation. Now we know that at $c < 0.0004$ M ($\log c < -3.4$) the crystals grow by a fourth-order surface reaction, and at $c > 0.0004$ M the rate determining step is diffusion through the liquid [4]. The change of growth-controlling mechanism is the cause of the change of slope of the curve.

The other elbow is situated very close to the concentration where, as deduced from the upper curve, the precipitation process changes from being induced by foreign nuclei into development from spontaneously formed nuclei.

The bottom curve has been calculated as the number of crystals formed per unit volume, divided by the induction period. In case of homogeneous nucleation the induction period is supposed to be of the same order of magnitude as the effective nucleation period, and therefore we conclude that for $c > 0.01$ M the points of the curve show the rate of nucleation. This rate turns out to be proportional to c^{18} so that, in the language of chemical kinetics, the nucleation is an eighteenth order reaction.

A more detailed analysis of these results based on the Volmer-Becker-Döring theory of nucleation [5] will be published later. It leads to the conclusion that the interface tension between barium sulfate and aqueous solution is 90 ± 10 ergs/cm², both when it is calculated from the slope and from the position of the curve.

The surface tension of barium sulfate has never been measured before. Enüstün and Turkewich [7] recently found $\gamma = 84 \pm 8$ ergs/cm² for strontium sulfate against aqueous solution, and discovered the reason why all previous attemps to measure the surface tension of barium sulfate had failed. Enüstün and Turkewich calculate the surface tension of barium sulfate from the two assumptions, (1), the nucleus contains $3 \times 3 \times 3$ unit cells, and (2), the critical supersaturation ratio is 21.5. From the present work the most probable values are (1) 18 ions or 9 molecules of $BaSO_4$ = 775 Å [3], and (2) extrapolating the tangent to the right hand end of the bottom curve on Fig. 1 until $\log (N/t) = 0$ we get the critical concentration for spontaneous nucleation, $c = 10^{-2.2}$ M. Thus $c/s = 10^{2.8} = 630$ when s = solubility of $BaSO_4 = 10^{-5}$ M. From these data, and eqn. (15) of Enüstün and Turkewich' paper. about 90 ergs/cm² is found for the surface tension, the exact value depending on the geometry assumed for the nucleus.

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Does Insulin Bind Hexokinase to Liver Mitochondria?

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During the last years excellent progress has been made about the rôle of insulin in conjunction with the transport of sugars through cell membranes. There are, however, still a lot of effects of insulin on the cell metabolism which cannot be easily explained by an altered permeability only. This is especially evident in liver tissue, where insulin has no significant effect on the transport of glucose. The metabolism of glucose in liver is nevertheless strongly dependent on the presence of insulin.

Brain tissue, on the other hand, does not seem to require insulin to any larger extent. Recently it was shown by Johnson [1] that 75 % of the hexokinase of rat brain was present in the mitochondrial fractions, and was firmly attached to the particles. This firm binding of hexokinase to mitochondria does not exist in other tissues.

Lee and Wiseman [2], injecting insulin-^{131}I intravenously in rat, found that the major part of the intracellular ^{131}I in liver was bound to mitochondria and microsomes, and could not be removed merely by washing.

This observation by Lee and Wiseman together with the circumstances that on the one hand brain tissue, with its firmly bound hexokinase, has no obvious need for insulin, while using glucose almost exclusively, whereas on the other hand liver, with a more loosely bound hexokinase, shows a strong dependency on insulin for the metabolism of glucose, led us to the assumption that insulin might form a link between hexokinase and liver mitochondria *.

Experiments with alloxan diabetic rats which had shown glucosuria and polyurea for 2—10 days were used in an attempt of getting closer to this problem. They were treated in pairs, and for each experiment rats were chosen which had been injected with alloxan at the same time, and which as far as possible showed the same degree of diabetes. One rat was injected intravenously with 100 i.u. insulin together with a subcutaneous injection of the same quantity, the other rat remaining untreated. After varying times, from 30 min to 4 h, the rats were decapitated, and liver mitochondria prepared. Of the mitochondrial suspension, in 0.25 M sucrose, one half was mixed with hexokinase. Either was kept at 0°C with careful stirring for 15 min, centrifuged, washed once with sucrose, and recentrifuged. They were then incubated in Warburg vessels with glucose as a phosphate acceptor but without further addition of hexokinase. Glutamate was used as substrate. The oxygen consumption of the mitochondria from the insulin injected and from the untreated diabetic rats is seen in Table 1. As a comparison the oxygen consumption is given, when in addition hexokinase has been added to the vessels.

Table 1. Oxygen consumption of liver mitochondria from alloxan diabetic rats with and without addition of hexokinase. *Insulin* means that the diabetic rats have been injected with insulin before decapitation, *controls* that no insulin has been given. *Insulin + hexokinase* and *controls + hexokinase* denote the mitochondria pretreated with hexokinase as described in the text. Temp. 30°C. The figures are given as mm^3 O_2/20 min.

Treatment of the rats	No hexokinase added to the vessels	Hexokinase added to the vessels
Insulin	36	159
Insulin+hexokinase	42	160
Control	39	160
Control + hexokinase	44	171

The results seen in Table 1 are typical for all experiments, whether the rats were taken shortly after the diabetic symptoms or after ten days. Neither was the time which passed between the injection of insulin and the decapitation of any importance. It might be mentioned that alloxan diabetic rats exhibited convulsions only after very large doses of insulin as compared with normal animals. This indicates that alloxan in some way destroys insulin. On this account we had to use the relatively large quantities of the hormone.

The table shows that injected insulin had no effect on the respiration capacity of liver mitochondria from diabetic rats. The mitochondrial respiration is low in both insulin injected and untreated rats, and not until hexokinase is added directly to the vessels the respiration rises to normal values in either case. No indications of a function of insulin as a link between hexokinase and liver mitochondria could thus be obtained by this method.

* This hypothesis was also put forward recently by Bessman in connection with a lecture by R.B. Fisher and reproduced in print in *The Mechanism of Action of Insulin*, Blackwell Scientific Publications Ltd. 1960.

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