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Template Activity of Liver Chromatin Increased by *in Vivo* Administration of Insulin*

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Abstract. The hypothesis that insulin may serve as a derepressor of genetic information in the liver of diabetic rats has been tested by comparing the template activity for RNA synthesis of chromatin from liver of insulin-treated diabetic rats to that of chromatin from liver of insulin-deficient diabetic rats. The template activity of the chromatins of insulin-treated diabetic rats is found to be 28 per cent greater than that of chromatin from liver of diabetic rats not treated with insulin. Time course studies show that the template activity of liver chromatin of rats injected with a single dose of insulin reaches a peak at two hours, which is some hours before the appearance of a typical insulin-induced liver enzyme, glucokinase. We conclude that insulin derepresses genetic material of the diabetic liver genome that is repressed in the absence of insulin.

It is known that when diabetic rats are supplied with insulin, the activities of several liver enzymes increase and that such increase is dependent on both RNA and protein synthesis.[1] Among the hepatic enzymes whose activities are thus controlled by insulin level are glucokinase, phosphofructokinase, pyruvatekinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and malic enzyme. The fact that the insulin-induced appearance of these enzymes requires RNA synthesis implies that insulin in the diabetic liver causes derepression of previously repressed genetic material. We show below that insulin treatment does increase the proportion of the diabetic genome which is available for transcription.

Materials and Methods. Male Sprague-Dawley rats of 150–250 gm were used. Insulin-depleted rats were prepared by a single intravenous injection of 2% alloxan monohydride, 40 mg/100 gm of body weight.[2] Blood glucose determinations were made with the alkaline ferricyanide method as adapted to the Technicon auto-analyzer. The alloxan-diabetic animals had blood glucose concentrations in excess of 400 mg/100 ml, and were used 1 week to 1 month post-alloxan.

At the appropriate time, each rat was decapitated and exsanguinated. Each liver was removed, cut into small pieces, and each piece allowed to drop directly into a container of liquid nitrogen. The harvest of liver tissue from each group of animals was stored at −80°C.

For one set of experiments the livers from 20 normal rats, 20 alloxan-diabetic rats, and 20 insulin-treated rats were harvested. The group of 20 insulin-treated, alloxan-diabetic rats had been treated with insulin in such a manner as to insure a long-lasting insulin effect, i.e., injected with four units of insulin‡ intraperitoneally and four units of protamine zinc insulin subcutaneously, each 24 hr before harvest of their livers.

For a time-course study, 20 alloxan-diabetic rats were injected subcutaneously with four units of insulin each. At 1, 2, 4, 8, and 16 hr after the insulin injections, the livers of five rats were harvested. The livers of five untreated alloxan-diabetic rats were harvested for 0 hr of insulin treatment.

Sheared liver chromatin was prepared from frozen liver tissue by the method of Marushige and Bonner.[3] The chromatin was transcribed by the F_4 fraction of *E. coli* RNA polymerase (Chamberlin and Berg,[4] prepared as described by Bonner *et al.*[5]). The RNA polymerase had a DNA dependency greater than 200, and had 2460 units of activity per milligram. Rate of DNA-dependent synthesis of RNA was used to determine the template activity of the liver chromatins.[5] Calculations of V_{max} were made utilizing a computer program for the Michaelis-Menten equation prepared by Brutlag and Cleland.[6]

Results. Rates of RNA synthesis supported by liver chromatin from normal, alloxan-diabetic, and 24-hour insulin-treated diabetic rats as template are identical in the absence of added *E. coli* RNA polymerase, as shown in Table 1.

TABLE 1. *DNA-dependent synthesis of RNA with rat liver chromatin as template and no added E. coli RNA polymerase.*

Source of DNA: chromatin from:	$\mu\mu$M ^{14}C-AMP into RNA
Normal	23 ± 2.8
Alloxan-diabetic	25 ± 2.1
24-hr insulin-treated	26 ± 0.9

Values are means ±SEM for four determinations. Differences between any two pairs of means are not significant by *t* test, $P = 7.10$.
One OD_{260} of chromatin per 0.25-ml reaction mixture used throughout.

Rate of RNA synthesis is, however, increased 15-fold or more in the presence of added *E. coli* RNA polymerase (Figs. 1, 2, 3).

The data of Figures 1 and 2 show that rate of RNA synthesis per unit DNA is 28 per cent greater when chromatin from 24-hour insulin-treated diabetic rats is used as template than when chromatin from alloxan-diabetic rats is used as template. The chromatin of insulin-depleted rats is less template active than that of normal rats. In complementary fashion, the chromatin of insulin-treated rats is more template active than that of normal rats.

RNase activity present in liver chromatin of insulin-deficient rats is of the same low level as that of the liver chromatin of the insulin-treated rats (Table 2).

Our time-course studies show that the template activity of liver chromatin of rats injected subcutaneously with four units of insulin two hours before the livers are removed is greater than that of nontreated rats (Fig. 3). By eight hours after a single subcutaneous injection of four units of insulin, the template activity of liver chromatin is not significantly different from that of insulin-depleted rats.

TABLE 2. *RNase activity of rat liver chromatin preparations.*

Chromatin	RNase activity
Alloxan-diabetic	9.2 ± 2.5
24-hr insulin-treated	6.0 ± 3.3

RNase activity is expressed as per cent of ^{14}C-RNA (860 cpm in 2×10^{-8} *g*) made soluble in cold 10% trichloroacetic acid in 10 min at 37°C when incubated in a reaction mixture identical to that used for synthesis of RNA, except that the *E. coli* RNA polymerase and the nucleoside triphosphates were omitted. The values given are the means ±SEM of five determinations. There is no significant difference between the two means by *t* test, $P = >0.25$.

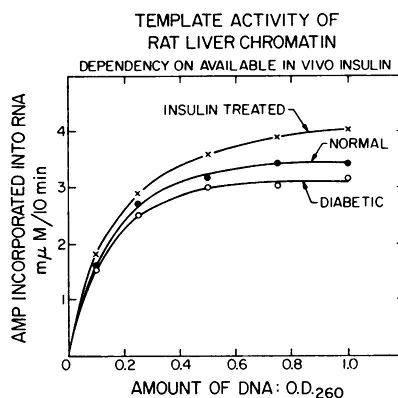

FIG. 1.—Template activity of rat liver chromatin. *E. coli* RNA polymerase was used to transcribe RNA from the DNA of rat liver chromatin of three different groups of rats. The amount of AMP incorporated into RNA was determined from the amount of ^{14}C-ATP substrate incorporated into the cold 10% trichloroacetic acid precipitate of RNA collected on membrane filters and counted in a liquid scintillation system. The mean of seven determinations is represented by each point on the curves for chromatin from diabetic and insulin-treated rats; the mean of five determinations is represented by each point on the normal rat liver chromatin curve.

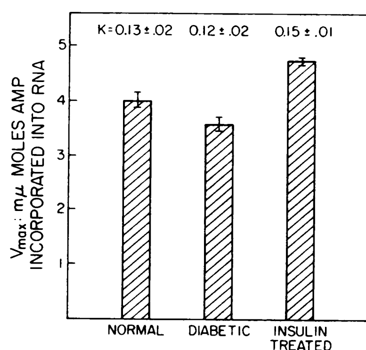

FIG. 2.—DNA-dependent synthesis of RNA by rat liver chromatins using *E. coli* RNA polymerase. V_{max}, rate of RNA synthesis at infinite template concentration, and K, template concentration required to half saturate the (constant) amount of RNA polymerase in the reaction mixture, were calculated from the data used for Fig. 1.

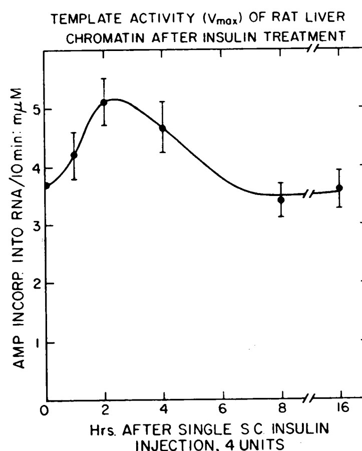

FIG. 3.—Template activity of rat liver chromatin at various times after a single subcutaneous injection of four units of insulin. Each calculated $V_{max} \pm$ SEM is based on the amount of adenosine 5'-phosphate incorporated into RNA at four different concentrations of liver chromatin DNA at each time interval.

This effect is therefore a transient one as are the other effects *in vivo* of insulin treatment.

Discussion. The increased template activity for RNA synthesis of liver chromatin from alloxan-diabetic rats treated with insulin for 24 hours is to be expected if the increased liver enzyme activities of such animals[1] is due to *de novo* enzyme synthesis. It is further of interest to know that the appearance of increased template activity precedes the appearance of increased enzymatic activities. Salas *et al.*[7] have shown that glucokinase activity is not appreciably increased three hours after a single intraperitoneal injection of four units of

insulin. After intervals of nine hours or more and with additional injections of insulin, glucokinase activity was found to be increased fourfold or more. Since chromosomal template activity is increased by two hours after a single injection of four units of insulin, and glucokinase activity is increased some hours later, the insulin effect on chromatin does precede the appearance of new enzyme activity.

It is known that the interaction of hormone with the genome is mediated in other instances by specific hormone-binding proteins (Matthysse and Phillips[8]; Maurer and Chalkley[9]) and that provision of hormone directly to isolated, repressed chromatin in the absence of the binding protein is without effect (Dahmus and Bonner[10]). Since insulin is a protein, one might wonder whether it might be its own binding protein and be able to act directly on isolated chromatin. Alas, it cannot.

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‡ The insulin was Iletin U-40, and the protamine zinc insulin was Protamine, Zn, and Iletin U-40, kindly supplied gratis by Eli Lilly Co., Indianapolis, Indiana.

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