settling basin for much of the pollutants from the Twin Cities area which lies 40 miles (64 km) up-river. Midge larvae (Diptera: Tendipedidae) which are tolerant of low oxygen concentrations presently occupy much of the lake bottom in lieu of burrowing mayflies. Adult midges constitute more of a nuisance than do mayflies in this area. The lack of current in the lake probably allows an oxygen deficiency to develop in the thin layer of water at the mud-water interface where burrowing mayflies obtain their respiratory water. Such an oxygen deficiency has been suggested to be a limiting factor for benthic invertebrates in similar habitats (*9*). Mass emergences of *H. bilineata* or *P. vittigera* have not been recorded from the lake. One mass emergence of *H. limbata* has been recorded.

Many industries add wastes to the Mississippi River in the St. Louis area (*10*). As of December 1962, raw domestic sewage entered the Mississippi River or its tributaries in the St. Louis area from the following population centers: Keokuk, Iowa; Alton, Illinois; St. Louis, Missouri; East St. Louis, Illinois; Jefferson City and Cape Girardeau, Missouri; and Cairo, Illinois. Most of these cities now have treatment plants in progress or in the planning stage.

No mass emergences of *H. limbata* and *P. vittigera* and only one emergence of *H. bilineata* have been reported below St. Louis. It seems very unlikely that St. Louis is merely the southern limit of the range of the three species in the Mississippi River, because *H. bilineata* and *P. vittigera* have been collected as far south as Florida (*11*) while *H. limbata* has been collected as far south as central Texas (*12*) and southern Mississippi (*13*).

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Insulin Action in Alloxan Diabetes Modified by Actinomycin D

Abstract. *Insulin corrects the disturbances in lipid and carbohydrate metabolism in rats made diabetic with alloxan. However, the concomitant administration of actinomycin D with insulin prevents the repair of enzymatic defects in the syntheses of total fatty acids by adipose tissue, of monounsaturated fatty acids by liver microsomes, and of hepatic glycogen. The hypoglycemic action of insulin in diabetes is not modified by actinomycin injections. These findings indicate that a fundamental mechanism of action of insulin is the induction of enzyme synthesis through stimulating the renewal of cellular RNA.*

Since the observations of Stetten and Boxer (*1*) it has been recognized that a major metabolic disturbance in diabetes is a suppression of the biosynthesis of fatty acid from acetate (*2*). We have described another impairment in lipid metabolism in diabetes characterized by a striking depression of the biosynthesis of monounsaturated fatty acids from either acetate or saturated fatty acids (*3*). In mammalian cells, olefin synthesis is principally localized to a microsomal enzyme system which is dependent upon molecular oxygen and reduced pyridine nucleotide (DPNH or TPNH) (*4*). In diabetes, microsomal desaturation of long-chain saturated fatty acids virtually ceases, but the defect is readily repaired by the administration of insulin (5). We now report the effect of actinomycin D, administered with insulin to rats made diabetic with alloxan, on the synthesis of olefins by liver microsomes, on the total fatty acid synthesis in adipose tissue, on the formation of liver glycogen, and on the blood-sugar concentration. The evidence indicates that insulin induces enzymes concerned with the synthesis of saturated and monounsaturated fatty acids and of glycogen by stimulating the renewal of cellular RNA. The hypoglycemic action of insulin is not related to new enzyme synthesis.

Male Wistar rats (150 g) of the Chester Beatty colony were divided into four experimental groups: (i) normal, (ii) untreated diabetic, (iii) insulin-treated diabetic, and (iv) insulin plus actinomycin-treated diabetic. All animals received food as desired, and the drinking water contained 5 percent dextrose. The diabetes, produced by subcutaneous injection of alloxan at least 14 days prior to experimentation, was stable as indicated by a persistent glycosuria (4 plus) and hyperglycemia (300 mg per 100 milliliters of blood or higher). Therapy with glucagon-free insulin was given in three doses of five units each subcutaneously during the 26 hours immediately prior to the experiment. Actinomycin D in doses of 5 to 25 μg per 100 grams of body weight was injected intraperitoneally ½ hour before each insulin injection.

Blood sugar was determined by the glucose oxidase method (*6*). Liver glycogen was measured by the phenol sulfuric acid method (*7*). The biosynthesis of total fatty acid was measured in vitro in the epididymal adipose tissue with acetate-1-C^{14} as substrate (*8*). Liver microsomes were obtained by differential centrifugation. Olefin synthesis was determined by the conversion of stearic acid-1-C^{14} to oleic acid-1-C^{14} during a 20-minute incubation at 37°C in 3 ml of

Table 1. The conversion of stearic acid to oleic acid by liver microsomes of animals treated with insulin and actinomycin. Additions and incubation are described in the text. Equal amounts of microsomes were used in each experiment—4 absorbancy units at 260 mμ representing microsomes from approximately 20 mg (wet weight) of liver. One-tenth milliliter of supernatant after centrifugation at 100,000*g* of extract from liver of untreated diabetic rats was used except where otherwise indicated. Incubation was carried out in oxygen for 20 minutes at 37°C; stearic acid-1-C^{14} (40,000 count/min) was added (3.0 mμmole). Results are expressed as the mean and standard deviation where four or more experiments were performed; in the others, the individual results are given. The dosage of actinomycin is in micrograms per 100 g of body weight for each injection.

Addition	No. of experiments	Conversion (%)
Normal control		
None	6	30.7 ± 3.6
Untreated diabetic		
None	6	2.6 ± 1.5
Insulin-treated diabetic		
None	6	33.4 ± 8.9
Supernatant from insulin and actinomycin-treated diabetic	2	28.2; 30.4
Actinomycin in vitro	2	36.6; 38.5
Insulin and actinomycin-treated diabetic		
4 μg of actinomycin	2	46.4; 51.8
10 μg of actinomycin	1	12.5
15 μg of actinomycin	2	9.8; 4.3
20 μg of actinomycin	4	6.1 + 2.4
25 μg of actinomycin	2	4.0; 5.0

0.15*M* KCl containing 4μM adenosine triphosphate, 0.2μM coenzyme A (CoA), 2.5μM DPNH, 10μM α-glycerol phosphate, 15μM magnesium chloride, 4.5μM glutathione, 3μM sodium cyanide, 125μM sodium fluoride, 1μM nicotinamide, and 125μM phosphate buffer (*p*H 7) (*5*). The separation of the fatty acids as their methyl esters and the collection of individual fatty acids for radioactivity determinations were done by gas liquid chromatography (*5*) with Apiezon L on Chromosorb W packing in a Perkin-Elmer gas chromatograph model 800. All measurements of radioactivity were made by liquid scintillation spectrometry.

Table 1 summarizes the experiments on the microsomal desaturation of stearic acid to oleic acid in vitro. Microsomes from the liver of normal control rats converted approximately 31 percent of the substrate stearic acid. The result agrees well with our earlier experiments (*5*). Liver microsomes from untreated diabetic rats had virtually lost the capacity to desaturate stearic acid, but, 26 hours after insulin therapy was begun in the intact animal, the microsomal synthesis of oleic from stearic acid was restored to normal.

The addition of supernatant from liver homogenates of rats treated with insulin and actinomycin, or the addition of 5 μg of actinomycin to the incubation mixture, did not impair olefin synthesis. This demonstrates that actinomycin does not have a direct toxic effect on this microsomal enzymatic function. The injection of actinomycin concomitantly with insulin, however, in doses of 10 to 25 μg per 100 g of body weight prevented the insulin repair of the microsomal enzyme deficiency in diabetic rats.

When actinomycin was administered together with insulin therapy, the restoration by insulin of fatty acid synthesis in the fat from diabetic rats was blocked (Table 2).

Two other biochemical disturbances in diabetes which are corrected by insulin therapy were studied to determine whether actinomycin modified the response. Actinomycin had no effect on the hypoglycemic action of insulin (Table 3). The restoration of liver glycogen by insulin in the diabetic rats was completely prevented by actinomycin. These observations on liver glycogen confirm the report of Salas *et al.* (*9*).

Wieland *et al.* have shown that acetylcarboxylase, which catalyzes the reaction of acetyl CoA with CO_2 to form malonyl CoA, is markedly depressed in diabetes (*10*). Our evidence indicates that this rate-limiting step in fatty acid biosynthesis from two carbon fragments was corrected by insulin; the repair was blocked by the concomitant administration of actinomycin. The microsomal oxygenase necessary for monoene synthesis from saturated fatty acid was depressed in diabetes and corrected by insulin therapy. This insulin-effected repair was also prevented by actinomycin. Finally, the insulin-mediated increase of glycogen synthetase activity which restored the depressed levels of liver glycogen to normal in the diabetic rat was nullified by the coincident injection of actinomycin.

Actinomycin selectively inhibits the DNA-directed synthesis of RNA (*11*). Our results support the hypothesis that a biologically important mechanism of action of insulin is the induction of new enzyme synthesis by stimulating the renewal of cellular RNA. Wool has demonstrated that insulin enhances RNA synthesis in muscle (*12*), and he has also reported that the hormone leads to an increase in the synthesis of messenger RNA (*13*). The observed correction by insulin of the enzymatic defects in lipid biosynthesis and glycogen formation in diabetes is

Table 2. The incorporation in vitro of acetate into total lipids of adipose tissue from diabetic rats treated with insulin and actinomycin. Epididymal fat was incubated for 2 hours in Krebs-Ringer bicarbonate with 3 percent bovine albumin, 50 μmole glucose, and 20 μmole acetate containing acetate 1-C^{14} (5 μc) in a total volume of 4 ml. Five units of insulin were injected subcutaneously at 8- to 12-hour intervals for three doses during 26 hours prior to the experiment, and actinomycin (10 to 25 μg per 100 grams of body weight) was injected intraperitoneally at the same time where indicated. The results are the mean and range in four separate experiments, expressed in μmole acetate incorporated per gram per hour.

Normal	Diabetic	Insulin-treated diabetic	Actinomycin+ insulin-treated diabetic
1.40 (1.3–1.5)	.24 (.13–.35)	1.23 (.96–1.51)	.23 (.16–.30)

Table 3. The effect of actinomycin and insulin on blood sugar and liver glycogen. The insulin dose was 5 units, and the actinomycin dose was 10 to 25 μg per 100 g of body weight injected at 8- to 12-hour intervals for three doses where indicated. The blood sugar was determined by the glucose oxidase method, and is expressed as the mean and standard deviation of seven experiments. Glycogen was determined by the method of Montgomery. The results are expressed as the mean of four experiments. The numbers in parentheses are the range of values.

Treatment	Blood sugar (mg/100 ml)	Liver glycogen (mg/g)
Normal		
None	138 ± 17	92
Actinomycin	140 (130–151)	
Diabetic		
None	470 ± 178	36 (30–42)
Actinomycin	690 (650–725)	
Insulin	124 ± 60	125(116–134)
Insulin and actinomycin	80 ± 54	23 (7–63)

compatible with the suggestion of Wool that the hormone initiates the transcription of messages through combination with a repressor molecule which leads to the synthesis of specific enzymes. The known reactions of insulin with protamine, zinc, and certain quinones (*14*) may offer leads to the type of molecule which constitutes the hypothetical repressor. It is of interest that the induction of enzyme synthesis, presumably by the same mechanism noted in this study, has been reported for cortisone stimulation of liver tryptophane pyrrolase (*15*) and hepatic gluconeogenic enzymes (*16*), for insulin-dependent synthesis of liver glucokinase (*9*), for estrogen stimulated phospholipid and protein synthesis in the uterus (*17*), and for the calorigenic and increased growth rate actions of thyroid hormones (*18*).

The results of this study also demonstrate a separation of the hypoglycemic action of insulin and the hormonal induction of enzymes. This suggests that the transcellular transport of glucose stimulated by insulin is not dependent on the synthesis of new enzyme protein.

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Adaptation in Stretch Receptor Neurons of Crayfish

Abstract. *Two factors involved in the adaptation in stretch receptor cells of crayfish were separated and studied: (i) the decline of the rate of discharge during intracellular application of constant current and (ii) the decline of generator potential during sustained stretch. The change in generator potential with time was essentially identical in both rapidly and slowly adapting cells. The slowly adapting cells continued to discharge throughout the application of depolarizing currents, whereas the rapidly adapting cells stopped discharging while the current was applied. The different rates of adaptation are therefore attributable to the difference in the properties of the electrically excitable membranes rather than the properties which produce generator potentials.*

Two different causes for adaptation in sensory receptors have been suggested: one is some mechanism which leads to a gradual decline of the generator potential, the other, some effect related to the accommodation of electrically excitable membrane (*1*, *2*). Recently, the tendency has been to regard the first of these as the more important factor, because in many receptors the time course of decline of the generator potential agrees well with that of the decline of impulse discharge (*1*). In various vertebrate mechanoreceptors the decline of the generator potential in the face of a constant stimulus has been attributed largely to the mechanical properties of the coupling between the receptor and the stimulus (*3*).

Two stretch receptors which lie side by side in crustacea exhibit marked differences in adaptation (*4*); one is rapidly adapting, the other adapts very slowly. These different characteristics have been attributed (*5*) to differences in the time course of decay of the generator potential. Eyzaguirre and Kuffler (*5*) suggested that the differences might be due to differences in structural relations between the dendrites of the receptors and the muscle fibers to which they are attached. Krnjevic and van Gelder (*6*), however, examined the mechanical properties of the muscle fibers to which the two receptors are attached and concluded that these properties could not account for the differences in rate of adaptation of the receptors. Their findings suggested the possibility that the different rates of adaptation were due to different properties of the electrically excitable membranes of the receptor neurons.

In the study reported here, the link between generator potential and spike electrogenesis in the stretch receptor neurons was uncoupled by taking advantage of the different electrophysiological and pharmacological properties of electrically excitable and electrically inexcitable membrane components (*7*). The time courses of the decline of the generator potential as well as of the accommodative processes in spike electrogenesis were thus studied separately. The results show that the main difference between the slowly and rapidly adapting cells lies in the properties of the electrically excitable membrane component rather than in the mechanism of the generator potential.

Stretch receptors from the first to the third abdominal segments were isolated and mounted on a stretching device similar to that described by Eyzaguirre and Kuffler (*5*). The preparations were bathed in a crayfish saline medium (*8*). Two microelectrodes were introduced into a given neuron: one for recording potential, the other for applying a current. The current was monitored with a resistance inserted between the bath and ground, while the potential was recorded differentially. A feedback circuit was used to maintain the applied current constant.

Figure 1*A* shows the repetitive firing of a slowly adapting cell during the application of long-lasting constant currents of different amplitudes. Only the initial and final parts of the sequences are reproduced, and they demonstrate the capacity of the slowly adapting receptor neuron to respond with spikes for a long time when the cell is depolarized. Figure 1*B* shows results of