Total hydroxymethylglutaryl CoA reductase activity in the small intestine and liver of insulin-deficient rats

Nancy L. Young,[1] Christopher D. Saudek, and Sarah A. Crawford

Department of Medicine, Cornell University Medical College, 1300 York Avenue, New York, NY 10021

Abstract We examined the effect of streptozotocin-induced diabetes on the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl CoA reductase (EC 1.1.1.34), in liver and small intestine of rats. During the acute phase of insulin deficiency (first day), food intake, plasma cholesterol, and reductase specific activity in liver all decreased. By 3 days, food intake, plasma cholesterol, and reductase activity in small intestine were all increasing. After 1 week, total reductase activity in small intestine was 2.5 times normal, whereas activity in liver remained low. Thus diabetes shifted the major site of cholesterol synthesis from the liver to the small intestine. These data support the proposal that hyperphagia by diabetic rats leads to increased input of both dietary and newly synthesized cholesterol by the small intestine into thoracic lymph and thereby contributes significantly to their hypercholesterolemia. The possibility that diabetes affected the F^--inhibitable activation of reductase in vitro was also tested. There was no evidence of an effect in small intestine, but activation of reductase in vitro was decreased by 1/3 in liver. These data suggest that, in liver, either the activity of the activator was decreased or the fraction of reductase in the active state was increased after more than 12 hr of insulin deficiency.—**Young, N. L., C. D. Saudek, and S. A. Crawford**. Total hydroxymethylglutaryl CoA reductase activity in the small intestine and liver of insulin-deficient rats. *J. Lipid Res.* 1982. **23:** 266–275.

Supplementary key words streptozotocin • diabetes • hyperphagia • hypercholesterolemia • hypertriacylglycerolemia • hyperglycemia • phosphatase

Hypercholesterolemia (1–4) and premature atherosclerosis (5, 6) are common features of diabetes mellitus in humans. In rats, insulin deficiency leads to an increase in cholesterol content of all plasma lipoproteins (7, 8). This effect is usually attributed to decreased clearance of lipoproteins (9–11) since cholesterol synthesis in liver is suppressed (12, 13). However, recent work suggests that the intestine may contribute to hypercholesterolemia in diabetic rats, since synthesis (14, 15), absorption (16, 17), and secretion of cholesterol by intestine into thoracic lymph (16) are increased.

The present study describes changes in several aspects of cholesterol homeostasis with time after induction of insulin deficiency in rats with the beta-cytotoxic drug streptozotocin. The parameters studied include activity of the rate-limiting enzyme in cholesterol synthesis (HMG-CoA reductase) in liver and small intestine; levels of insulin, glucose, and lipids in plasma; body and organ weights; and food intake. We use a new method for assaying HMG-CoA reductase in small intestine (18) that permits estimation of total enzyme activity and of the extent of activation in vitro. From the sequence of events after streptozotocin, we have considered possible causal relationships among the responses.

MATERIALS AND METHODS

Treatment of animals

Wistar rats, 200–250 g (Charles River Laboratories, Wilmington, MA) were housed with lights on from 6 PM to 6 AM, and fed Purina Formulab chow #5008 ad lib. Diabetes was induced after 2 weeks when males weighed ca. 300 g and females weighed ca. 250 g.

Streptozotocin (kindly supplied by Dr. John Dulin, Upjohn Co., Kalamazoo, MI) (25, 32.5, or 40 mg) was dissolved in 0.5 ml of 0.1 M citrate buffer, pH 6.68, and 1 ml/kg was immediately injected into a tail vein of rats anesthetized with ether. Control rats received injections of buffer only. Rats that were to be killed 1 or more days later were injected at noon ± 0.5 hr; those to be killed 4 or 12 hr later were injected at 8 AM or 12 midnight, respectively. After injection animals had free access to food and water.

Alternatively, pancreatic tissue was removed with sterile cotton swabs after abdominal incision under ether anesthesia. The control rats had an identical surgical incision with manipulation of abdominal contents.

Tissue collection

On the day of killing, rats were weighed between 9 and 10 AM, and anesthetized with ether at noon ± 0.5

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; TG, triacylglycerol.

[1] To whom reprint requests should be addressed.

hr, the middle of their dark period. Plasma samples (19), homogenates, and subcellular fractions of liver and of small intestine (18) were prepared as described previously.

Assays of plasma constituents

Glucose was measured enzymatically with a glucose analyzer (Beckman Instruments, Fullerton, CA). Triacylglycerol was measured enzymatically with a reagent kit (Dow Chemical Co., Indianapolis, IN) using glycerol standards. Cholesterol was measured enzymatically with a reagent kit (Beckman Microbics, Carlsbad, CA) using cholesterol standards (Palomar Chemicals, Carlsbad, CA). Insulin was measured by radioimmunoassay (20) using ^{125}I-labeled porcine insulin (Cambridge Nuclear Radiopharmaceutical Corp., Bellerica, MA), guinea pig anti-insulin serum (Arnel Products, Brooklyn, NY) and rat insulin standard (Novo Research Institute, Bagsvaerd, Denmark). Antibody was diluted 1/1000, and antibody and insulins were incubated at 4°C for 48 hr.

Assays of HMG-CoA reductase, HMG-CoA reductase inhibition, and HMG-CoA cleavage activities

Technical details and methods of calculation are described in the accompanying report (18). Enzyme specific activity, nmol mevalonate/(min × g tissue), expressed in liver microsomes and in small intestine homogenate was multiplied by organ weight to give total activity in each organ. Total activity was then divided by body weight to correct for weight gain in control rats and slight weight loss in diabetic rats. Activity expressed per g tissue may be converted to activity per mg protein using data for protein recovery in **Table 1.** Note that protein recovery was quite reproducible and was not affected by diabetes or sex of the rats.

Statistics

Data are expressed as means ± standard error of the mean. Ratios of means are presented ±70% confidence limits, which were estimated with Fieller's theorem (21). The Student's t test was used to determine the significance of difference between means (21). Values of P in the text and tables are for comparison of treated and control values unless stated otherwise.

RESULTS

Responses to streptozotocin dose

We first determined the dose of streptozotocin required for significant responses in parameters related to cholesterol metabolism. Streptozotocin was injected into male rats at doses of 50, 65, and 80 mg/kg and responses at 8 days were measured.

TABLE 1. Recovery of protein in liver microsomes and small intestine homogenate

Sex	Treatment		n	Liver Microsomes	Small Intestine Homogenate
				mg protein/g wet weight	
Male (all times)	Diabetic	$+F^-$	62	22.7 ± 0.8	141 ± 4
		$-F^-$	62	22.2 ± 0.8	146 ± 5
	Control	$+F^-$	63	23.6 ± 0.7	140 ± 3
		$-F^-$	63	23.6 ± 0.7	144 ± 4
Female (8 Days)	Diabetic	$+F^-$	5	20.2 ± 1.3	153 ± 5
		$-F^-$	8	19.5 ± 0.8	149 ± 8
	Control	$+F^-$	5	21.5 ± 1.0	164 ± 7
		$-F^-$	8	20.6 ± 0.7	151 ± 9

Diabetic rats were injected with streptozotocin at 65 mg/kg from 4 hr to 22 days previously (males), or 8 days previously (females). Controls received injections of carrier solution only at the same time. F^- was added or omitted at the time of homogenization of the organ. Data are means ± SEM. Differences between male and female, between diabetic and control, and between $+F^-$ and $-F^-$ are all insignificant. Protein was assayed by a fluorescent method described previously (19).

Growth, organ weights, and plasma constituents. At the lowest dose, growth was impaired, plasma glucose was elevated, and liver and small intestine weights as percent of body weight were increased ($P < 0.001$ each case, **Fig. 1**). As the dose was raised, there were increments in the levels of TG ($P < 0.1$) and cholesterol ($P < 0.05$) in plasma. Body weight continued to decline with higher doses, but organ weights as percent of body weight did not continue to increase.

HMG-CoA reductase activity. We estimated total HMG-CoA reductase activity in liver and small intestine to determine effects of diabetes on cholesterol synthesis by each organ. To estimate total activity, we assayed small intestine homogenate and liver microsomes, preparations which give the highest yields of enzyme activity (18). Total activity divided by body weight was decreased in liver ($P < 0.02$) and increased in small intestine ($P < 0.001$) at the lowest streptozotocin dose; incremental responses with higher doses were not significant (**Fig. 2**).

The effects on total activity relative to body weight were due mainly to changes in reductase specific activity (**Table 2**). The increases in organ weight relative to body weight (Fig. 1) tended to amplify the increase in reductase specific activity in small intestine, but tended to nullify the decrease in specific activity in liver slightly.

The increased HMG-CoA reductase specific activity

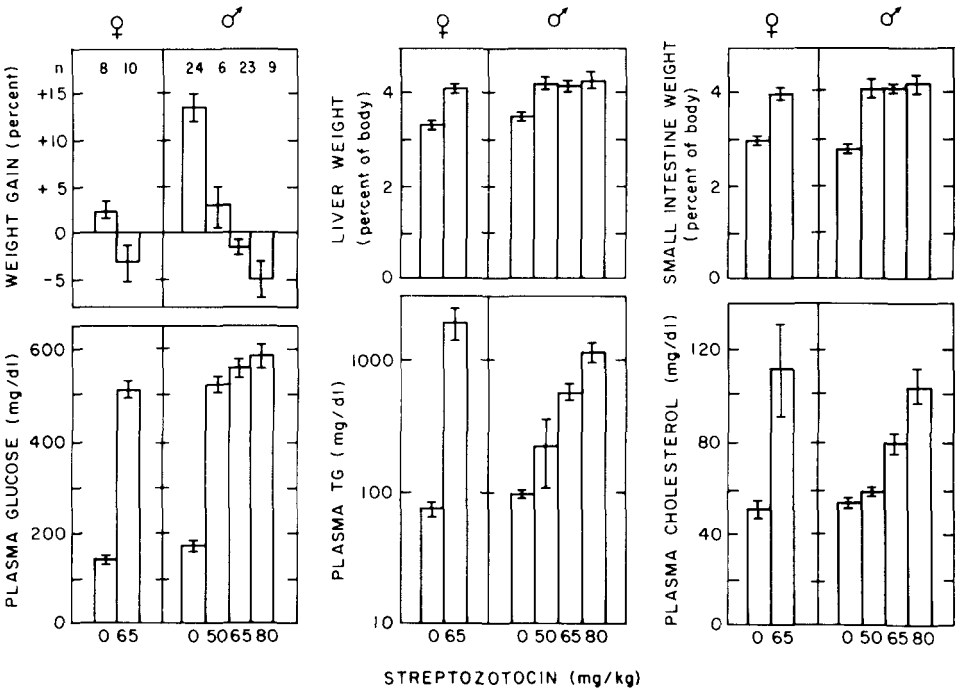

Fig. 1 Growth, organ weights, and constituents of plasma 8 days after injection of streptozotocin at various doses. Female rats weighed 259 ± 5 g and males weighed 307 ± 5 g at the time of injection. The number of rats studied (n) at each dose is shown in the top left hand panel. Plasma TG level is shown on a log scale.

in small intestine was observed in sedimentable fractions, including microsomes, as well as in homogenate (Table 2).

Factors affecting expression of HMG-CoA reductase in vitro. Data above are for reductase that was activated in vitro. To determine if diabetes affected nonactivated activity, F^- was added at the time of homogenization to inhibit activation. Nonactivated reductase activity was progressively increased in small intestine homogenate and progressively decreased in liver microsomes as streptozotocin dose was increased from 50 to 65 mg/kg (**Table 3**). Activation was calculated as the ratio of reductase activity without F^- to that with F^-. Diabetes did not affect activation of reductase in small intestine homogenate, but did decrease activation in liver microsomes (Table 3). It is apparent, then, that this decrease contributed to the decrease in activity observed in liver microsomes prepared without F^- (Table 2).

The expression of reductase activity in intestinal homogenate is inhibited by unidentified factors in the homogenate, possibly including metabolites of HMG-CoA produced during the assay by cleavage enzymes (18). While inhibition is reduced by preincubation, it is not entirely eliminated (18). Thus, it is possible that the increase in reductase activity expressed with diabetes was due to a decrease in inhibitory activity. This possibility was tested and ruled out by measuring HMG-CoA cleavage and HMG-CoA reductase inhibition in intestinal homogenate. After a 30-min preincubation (when we assayed reductase), reductase inhibition was not affected by diabetes (**Table 4**). However, it is interesting to note that in small intestine homogenate from diabetic rats inhibition activity before preincubation was lower, and HMG-CoA cleavage activity was also lower both before and after preincubation.

In summary, all parameters except plasma lipids were significantly changed at 8 days after a streptozotocin dose of 50 mg/kg. A dose of 65 mg/kg was required to significantly increase plasma lipids. Total HMG-CoA reductase activity relative to body weight 8 days after injection of streptozotocin was decreased in liver and increased in small intestine. The effect in liver was due mainly to a decrease in the nonactivated level of reductase specific activity which was amplified by a decrease in F^- inhibitable activation in vitro. The effect in small intestine was not due to a decrease in reductase inhibition activity or to an increase in activation, but was due to an increase in the nonactivated level which was amplified by an increase in organ weight and a small decrease in body weight.

Comparison of males and females

The responses in females at 8 days after streptozotocin at a dose of 65 mg/kg were like those in males, and even

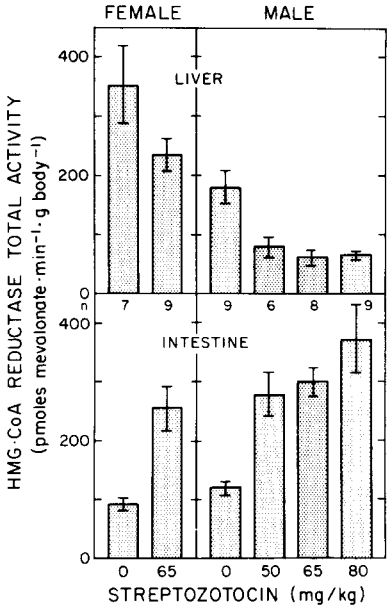

Fig. 2 Total HMG-CoA reductase activity in liver and small intestine relative to body weight 8 days after injection of streptozotocin at various doses. Body weight (g) at the time of injection in groups of rats from left to right in the figure was 246 ± 4, 251 ± 5, 295 ± 10, 324 ± 5, 301 ± 11, 323 ± 4. The number of rats (n) in each group is shown at the top of the bottom panel. Reductase activity was assayed in small intestine homogenate and in liver microsomes prepared without F^-.

more pronounced in several parameters. Thus, plasma insulin level was decreased more (**Table 5**), plasma cholesterol and TG were increased more (Fig. 1, $P < 0.05$ and $P < 0.001$, respectively, comparing males and females), and the relative increase in HMG-CoA reductase activity in small intestine was larger (Fig. 2, Table 2) in females. Reductase activity in liver was much higher in control females than in control males, and the relative decrease with diabetes was smaller in females (Fig. 2, Table 2).

Responses with time after streptozotocin injection

Data for various times from 4 hr to 3 weeks after streptozotocin at a dose of 65 mg/kg are shown in **Fig. 3** with time on a log scale to permit visualization of both early and late changes in the same graph.

Early responses associated with decreased plasma insulin and decreased food intake. Food intake was decreased during the first 2 days ($P < 0.02$). At 4 hr, plasma glucose was elevated ($P < 0.001$), and plasma cholesterol was decreased ($P < 0.005$). At 12 hr, insulin was decreased ($P < 0.05$), cholesterol remained low ($P < 0.005$), while TG was increased ($P < 0.001$). HMG-CoA reductase specific activity in liver was first decreased at 12 hr ($P < 0.001$) and minimal at 24 hr. At 24 hr there were also decreases in body weight ($P < 0.001$) and liver weight ($P < 0.001$), but plasma cholesterol had risen to nearly normal.

Late responses associated with increased food intake. At the end of the third day, food intake (Fig. 3) was normal but food intake per g body weight (not shown) was increased ($P < 0.05$). Total HMG-CoA reductase activity in intestine per g body and plasma cholesterol were also increased ($P < 0.001$ for each). With continued hyperphagia, reductase specific activity in small intestine was 57% above control at 8 days ($P < 0.005$) and then declined to 33% above control ($P < 0.05$) by 3 weeks.

TABLE 2. HMG-CoA reductase specific activity 8 days after injection of streptozotocin

			HMG-CoA Reductase Specific Activity			
			Small Intestine			Liver
Sex	Streptozotocin Dose	n	Homogenate	10,000 *g* Pellet	100,000 *g* Pellet	100,000 *g* Pellet
	mg/kg		*nmol mevalonate/(min × g tissue)*			
Female	65	9	6.2 ± 0.8 *****	2.5 ± 0.2 *******	1.3 ± 0.5[b] **	5.9 ± 0.7[c] ****
	0	7	3.2 ± 0.3[a]	0.6 ± 0.2	0.5 ± 0.2	10.8 ± 1.9[d]
Male	80	9	9.1 ± 1.5 *****	3.2 ± 1.2 *******	0.6 ± 0.2 ns	1.5 ± 0.3 ******
	65	8	6.5 ± 0.5 ******	1.7 ± 0.7 ******	0.7 ± 0.3[b] *	1.6 ± 0.3[c] ****
	50	6	6.7 ± 0.7 ******	1.1 ± 0.8 ***	0.7 ± 0.3 **	1.8 ± 0.4 ****
	0	9	4.2 ± 0.3[a]	0.7 ± 0.5	0.4 ± 0.2	4.7 ± 0.7[d]

Reductase activity was assayed in preparations without F^-.
Comparison of males and females: [a], $P < 0.05$; [b], $P < 0.02$; [c], $P < 0.001$; [d], $P < 0.01$.
Comparison of diabetic and controls: *, $P < 0.1$; **, $P < 0.05$; ***, $P < 0.025$; ****, $P < 0.02$; *****, $P < 0.01$; ******, $P < 0.005$; *******, $P < 0.001$.

TABLE 3. Nonactivated HMG-CoA reductase and its activation in vitro 8 days after streptozotocin injection in males

Streptozotocin Dose	n	HMG-CoA Reductase: Nonactivated Specific Activity — Small Intestine Homogenate	HMG-CoA Reductase: Nonactivated Specific Activity — Liver Microsomes	HMG-CoA Reductase: Activation — Small Intestine Homogenate	HMG-CoA Reductase: Activation — Liver Microsomes
mg/kg		*nmol mevalonate/(min × g tissue)*		*fold*	
65	9	1.74 ± 0.12 ******	0.35 ± 0.08 ******	3.8 ± 0.2	4.1 ± 0.5 ***
50	6	1.72 ± 0.17 ***	0.39 ± 0.10 **	3.8 ± 0.1	4.9 ± 0.3 ns
0	9	1.17 ± 0.13	0.84 ± 0.14	3.8 ± 0.2	5.9 ± 0.5

Liver microsomes and intestinal homogenate from each rat were prepared with and without F^- to obtain unactivated and activated HMG-CoA reductase, respectively. The ratio of activated to unactivated activity is the fold activation in vitro. ns, $P > 0.1$; **, $P < 0.05$; ***, $P < 0.025$; ******, $P < 0.005$.

However, intestine weight continued to increase after 1 week, thereby sustaining at 2.5-fold elevation of total reductase activity per g body during the last 2 weeks, ($P < 0.001$ for each). Although reductase activity in liver remained much below normal, the pattern of change after 1 day was remarkably similar to that in small intestine. Thus, there were increases in reductase specific activity ($P < 0.05$ at 8 days, $P < 0.1$ at 22 days) and in liver weight ($P < 0.001$ at 8 and 22 days) that led to a sustained 2.3-fold elevation in total reductase activity in liver per g body weight during the last 2 weeks ($P < 0.02$) compared to the minimum at 24 hr. During this period plasma insulin remained low and plasma glucose remained high.

Activation of reductase in vitro. The ratio of activation in diabetics to that in controls is shown in **Fig. 4.** Activation of reductase in small intestine homogenate was not significantly affected by insulin deficiency from 4 hr to 22 days, averaging 3.85 ± 0.13 in controls and 3.85 ± 0.11 in diabetics. In contrast, activation of reductase in hepatic microsomes was lower in diabetics after 1 day, averaging 5.4 ± 0.5 compared to 7.4 ± 0.4 in controls ($P < 0.005$, groups from 1 to 22 days combined). Reductase activity in hepatic microsomes measured after activation (Fig. 3) decreased by 12 hr, half a day before the decrease in activation observed at 24 hr (Fig. 4).

Effects of insulin and pancreatectomy

The possibility that some of the responses in rats treated with streptozotocin were due not to insulin deficiency but to other toxic effects of the drug was ruled out by their reversal with exogenous insulin and their duplication with pancreatectomy (**Table 6**). Daily injections of protamine-zinc insulin normalized food intake,

TABLE 4. HMG-CoA reductase inhibition and HMG-CoA cleavage activity in intestinal homogenate from males 8 days after streptozotocin

Streptozotocin Dose	n	HMG-CoA Reductase Inhibition[a]: 0 min preincubation +F^-	HMG-CoA Reductase Inhibition[a]: 0 min preincubation −F^-	HMG-CoA Reductase Inhibition[a]: 30 min preincubation +F^-	HMG-CoA Reductase Inhibition[a]: 30 min preincubation −F^-	HMG-CoA Cleavage Activity[b]: 0 min preincubation +F^-	HMG-CoA Cleavage Activity[b]: 0 min preincubation −F^-	HMG-CoA Cleavage Activity[b]: 30 min preincubation +F^-	HMG-CoA Cleavage Activity[b]: 30 min preincubation −F^-
mg/kg				*%*				*nmol/(min × g tissue)*	
65	5	74 ± 3 ***	81 ± 1	10 ± 7 ns	19 ± 3	129 ± 24 *******	144 ± 33	19 ± 2 *	20 ± 2
50	6	77 ± 3 *	81 ± 4	17 ± 7	23 ± 10 ns	152 ± 24 ns	151 ± 24	22 ± 3 ns	25 ± 4
0	5	85 ± 2	81 ± 6	17 ± 7	19 ± 6	179 ± 28	213 ± 41	26 ± 4	30 ± 8

[a] Inhibition of reductase activity by intestinal homogenate was estimated by assaying reductase activity in liver microsomes with and without intestinal homogenate as described previously (19). Intestinal homogenate was prepared with (+F^-) and without (−F^-) 50 mM NaF and incubated for 0 or 30 min before assaying for reductase inhibition activity.

[b] HMG-CoA cleavage activity in the reductase assay was measured as described in Methods. Data for +F^- and −F^- were combined for tests of significance of difference from control.

ns, $P > 0.1$; *, $P < 0.1$; ***, $P < 0.025$; and *******, $P < 0.001$.

TABLE 5. Decrease in plasma insulin level 8 days after streptozotocin injection in males and females

Streptozotocin Dose	Males		Females	
	n	Plasma Insulin	n	Plasma Insulin
mg/kg		*μU/ml*		*μU/ml*
65	9	40 ± 9 ($P < 0.02$)	6	8 ± 3 ($P < 0.005$)
0	9	85 ± 12	5	46 ± 9

weight gain, plasma TG level, reductase specific activity in liver and small intestine, and total reductase activity in liver at 22 days after streptozotocin. Although intestinal weight, total reductase activity in intestine per g body, and plasma cholesterol were closer to control than to diabetic values, they remained slightly but significantly elevated. One successively pancreatectomized rat showed responses 45 days later that were qualitatively the same as those in rats 22 days after streptozotocin.

DISCUSSION

We have used a newly developed assay of total HMG-CoA reductase activity in small intestine (18) to explore factors which might contribute to the hypercholesterolemia of rats made insulin-deficient with streptozotocin. The most striking finding is that plasma cholesterol and reductase activity in small intestine both increase only after 3 days of insulin deficiency when the rats begin eating more chow (Fig. 3). This finding suggests that hyperphagia by diabetic rats contributes to hypercholesterolemia by increasing reductase activity in the intestine and by increasing dietary cholesterol. This possibility is supported by several additional observations. First, before hyperphagia starts, reductase activity in small intestine is unchanged, while plasma cholesterol and reductase activity in liver are both significantly lower than normal (Fig. 3). Second, when chronically diabetic rats are permitted to eat only normal amounts of chow for 1–3 weeks, reductase activity in intestine and in liver and plasma cholesterol are all normal.[2] Third, others have reported that cholesterol synthesis by intestinal slices in vitro (15) and secretion of endogenous cholesterol by intestine into thoracic lymph in vivo (16) are increased in chronically diabetic rats eating ad lib.

Our data extend the observations of Nakayama and Nakagawa (15), who found increased reductase specific activity in intestine of rats with insulin deficiency for 4 to 5 weeks. They measured specific activity in microsomes isolated from mucosal cells scraped from portions of the intestine, whereas we used homogenate of the whole small intestine and thus were able to estimate total reductase activity. Under our conditions, the yield of reductase activity is more than 20 times that of Nakayama and Nakagawa (15). In addition, by avoiding the time-consuming step of scraping mucosa, during which uncontrolled activation of reductase can occur, our method permits assay of the nonactivated level of reductase activity (18).

After prolonged insulin deficiency, total MHG-CoA reductase activity in the small intestine increases 2.5-fold. This is due, at first, mainly to increased specific activity; at later times, the increase is due mainly to increased organ weight. When total activity is divided by body weight, the increase is amplified somewhat in comparison with controls which continue to grow. However, the effect of the smaller body weight is relatively minor. The increase in reductase specific activity in small intestine measured after in vitro activation is not due to an increase in activation (Table 2, Fig. 4) nor to a decrease in reductase inhibition activity in the assay (Table 4) since these parameters are unaffected by diabetes.

The change in HMG-CoA reductase activity in liver with time after streptozotocin, in comparison with changes observed for other parameters (Fig. 3), suggests the following physiological sequence. Decreased plasma insulin and/or decreased food intake lead to decreased reductase activity in liver. When food intake increases, secretion of cholesterol by the small intestine increases, and there is a partial recovery of reductase activity in liver. Full recovery is prevented by the low level of insulin and/or the high level of plasma cholesterol. It is known that cholesterol in remnants of chylomicrons originating in the small intestine suppresses reductase activity in liver (22), and it has been suggested that this process is heightened in chronically diabetic rats (15) where more cholesterol is secreted by intestine into thoracic lymph (16). Clearly, this could not account for the initial decrease, but may contribute to the continued suppression.

The decrease in total reductase activity in liver of diabetic rats is due entirely to a decrease in specific activity and not to decreased liver weight (Fig. 3). The initial decrease in specific activity occurs between 4 and 12 hr. Later (by 24 hr), the in vitro activation also decreases (Table 3, Fig. 4) and thereafter decreased activation contributes in a minor way to the decreased specific activity measured after activation.

The decrease in in vitro activation of reductase in hepatic microsomes from diabetic rats could be due to a decrease in activator activity and/or to an increase in the fraction of reductase in the active form at the time of

[2] Young, N. L., C. D. Saudek, L. Walters, and J. Lapeyrolerie. Unpublished observation.

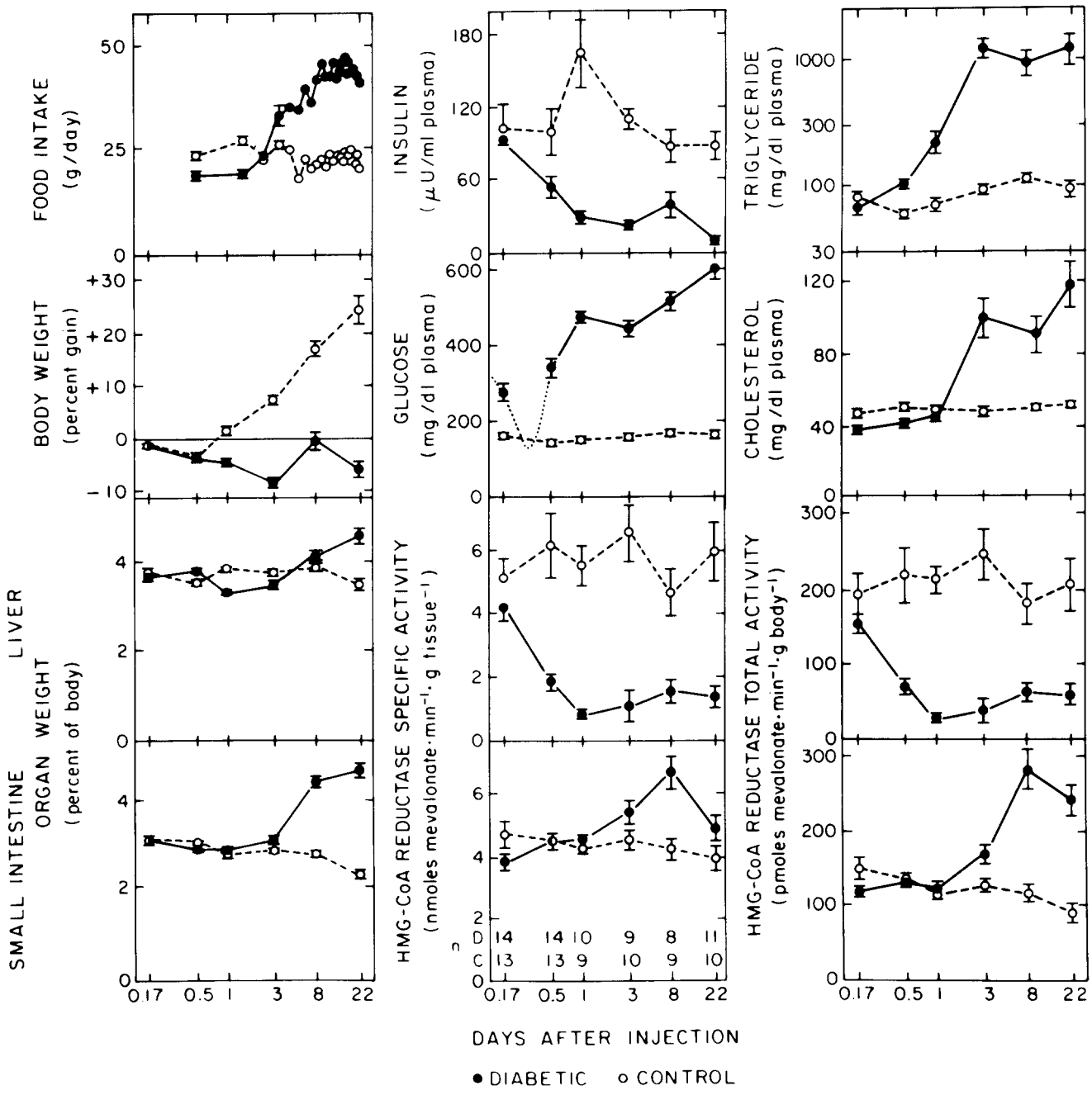

Fig. 3 Responses at various times after injection of streptozotocin. Male rats weighing 298 ± 5 were injected with streptozotocin at 65 mg/kg (●) or with buffer only (○). Daily food intake was estimated from the decrease in weight of chow in food baskets from noon on one day to noon on the next day, and is plotted at 0.5, 1.5, 2.5, etc. days after injection. The dotted line for glucose up to 12 hr is the level in serum from sequential blood samples from snipped tails. Data for plasma glucose and for all other parameters were collected from groups of rats killed at 4 or 12 hr, 1, 3, 8, or 22 days after injection, but always at noon ± 0.5 hr. The number of control (C) and of diabetic (D) rats in each group is shown in the bottom middle panel. HMG-CoA reductase activity was measured in small intestine homogenate and liver microsomes prepared without F^-. Data for small intestine are given in the row of panels at the bottom of the figure and for liver in the row above. Time and plasma TG level are shown on log scales; all other parameters are shown in linear scales.

homogenization of the liver. Previous reports (23, 24) in which this fraction was measured by activation with excess exogenous phosphatase, noted an increase in the active fraction within 2 hr after insulin treatment. This is the reverse of what might be expected from the long-term effects of insulin deficiency we observe, and implies that long and short term effects are different, that in vivo and in vitro effects are different, or that we are seeing

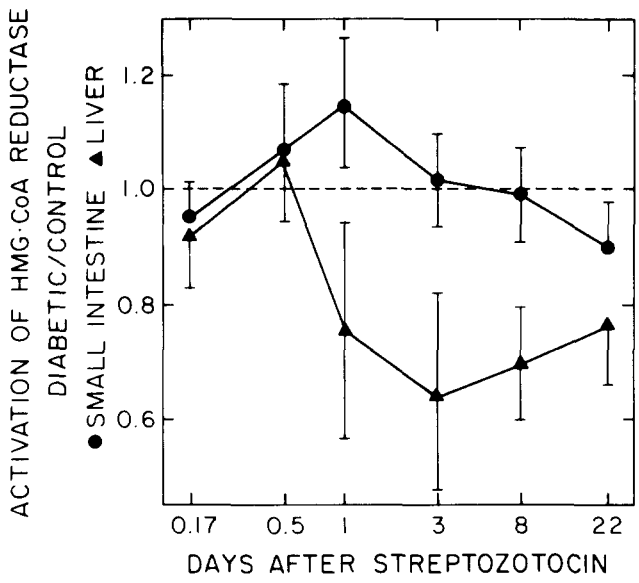

Fig. 4. In vitro activation of HMG-CoA reductase at various times after streptozotocin injection. Activation was measured as described in Table 3 for each preparation from each rat. The ratio of the mean value for activation in diabetics to the mean value in controls is shown for liver microsomes (▲) and for small intestine homogenate (●). Time is shown on a log scale. Other data for these rats are given in Fig. 3.

a decrease in activator activity rather than an increase in the active fraction.

The question of whether the activated or nonactivated level of reductase activity best represents activity in vivo remains open (18). However, we see the increase in reductase activity in small intestine and the decrease in liver regardless of whether the activated or nonactivated levels are measured.

Turley, Anderson, and Dietschy (25) have recently shown that, for female rats in vivo, the liver synthesizes twice as much cholesterol as the small intestine. We find that total reductase activity in the small intestine of female rats eating ad lib doubles with diabetes while that in liver is halved. Assuming that recovery of reductase activity is unaffected by diabetes and that reductase is the rate-limiting step in cholesterol synthesis, then diabetes with hyperphagia would shift the burden of cholesterol synthesis from the liver to the small intestine. Furthermore, the sum of synthesis in the two organs would be unchanged.

The temporal response of plasma cholesterol suggests that it is influenced at first by the decrease in reductase activity in liver and later by the increase in activity in small intestine (Fig. 3). However, other factors are undoubtedly contributing to the later hypercholesterolemia. One is increased secretion of dietary cholesterol in intestinal lymph resulting from the combined effects of increased consumption of a diet containing cholesterol and of increased fractional absorption of cholesterol by the gut (16, 17).[3] Another factor could be decreased clear-

TABLE 6. Effects of streptozotocin, streptozotocin plus insulin, and pancreatectomy

Group	n	Body Weight			Food Intake	Intestine Weight		Liver Weight		Plasma			HMG-CoA Reductase Activity			
		Initial	Gain										Specific		Total/Body	
										Glucose	TG	Chol.	Intestine	Liver	Intestine	Liver
		g	*g*	%	*g/day*	*g*	%	*g*	%	*mg/dl*	*mg/dl*	*mg/dl*	*nmol/(min × g)*		*pmol/(min × g)*	
S	11	312 ± 9	−6 ± 2		42 ± 1	14.6 ± 0.3	5.0 ± 0.2	13.4 ± 0.4	4.6 ± 0.2	581 ± 40	1504 ± 421	130 ± 18	4.9 ± 0.4	1.3 ± 0.3	242 ± 21	60 ± 14
			********		********	********	********		********	********	********	********	**	********	********	********
S + I	4	291 ± 5	30 ± 3		28 ± 2	10.7 ± 0.2	2.8 ± 0.1	13.3 ± 1.2	3.4 ± 0.2	89 ± 14	99 ± 8	64 ± 4	4.4 ± 0.5	4.4 ± 1.1	124 ± 15	148 ± 30
						******				******		***			*	
SC	10	310 ± 9	24 ± 3		24 ± 3	9.1 ± 0.3	2.4 ± 0.1	13.4 ± 0.8	3.5 ± 0.1	152 ± 10	118 ± 19	52 ± 3	3.9 ± 0.4	6.0 ± 0.9	94 ± 12	205 ± 35
P	1	111	192		—	18.8	5.8	15.8	4.9	632	198	69	8.0	0.3	466	13
PC	1	95	237		—	8.9	2.8	10.9	3.4	187	33	46	4.3	4.4	120	130

Male rats were killed 22 days after injection of streptozotocin at 65 mg/kg (S, S + I), or buffer (SC), or 45 days after pancreatectomy (P) or a sham operation (PC). Blood samples were obtained daily at noon from the snipped tails of rats in S + I for measurement of serum glucose. Protamine-zinc insulin was injected subcutaneously at noon at a dose adjusted to serum glucose of the previous day and averaged 14 U/kg in rats of S + I. Data for rats in S and SC are also shown in Fig. 3. HMG-CoA reductase activity was measured in small intestine homogenate and hepatic microsomes prepared without F⁻. *, $P < 0.01$; **, $P < 0.05$; ***, $P < 0.025$; ******, $P < 0.005$; ********, $P < 0.001$.

ance of plasma lipoproteins (9–11). Decreased clearance may be responsible for the rise in plasma cholesterol to a normal level at 24 hr after streptozotocin when reductase activity is low in liver and not yet elevated in intestine, and for the very large increase at 3 days when total reductase activity in intestine and food intake are only moderately elevated. However, decreased clearance alone is not sufficient to bring about hypercholesterolemia since without hyperphagia plasma cholesterol is nearly normal at 1 and 3 weeks after streptozotocin injection.[2]

Plasma TG level increases at 12 hr after streptozotocin injection when rats are eating less and plasma cholesterol is significantly low (Fig. 3). This strongly suggests that the initial elevation in plasma TG is not diet-induced, in agreement with a previous report (26), and that different mechanisms are responsible for the onsets of high TG and of high cholesterol level 2½ days later. It has been suggested that the initial elevation of plasma TG results from increased synthesis and secretion of TG by the liver in response to a high level of free fatty acids in plasma resulting from lipolysis in adipose tissue (27–29). Decreased clearance occurs later (9–11) and, in conjunction with increased secretion of intestinal lipoproteins (30) as a consequence of increased intake of dietary fat with hyperphagia (Fig. 3), could be responsible for the very high plasma TG level after 3 days. Again, decreased clearance alone is insufficient to raise plasma TG in chronic diabetics since plasma TG is nearly normal when hyperphagia is prevented.[2]

The responses to streptozotocin in female rats were similar to those in males, and in some respects even more pronounced. Diabetes was more severe, as evidenced by a larger decrease in plasma insulin level; consequently plasma lipid levels were higher. Reductase activity in liver was much higher in female controls than in male controls, in agreement with previous reports (31–33).

In humans, whole body cholesterol synthesis is estimated to be normal (34) or increased (35) when diabetes is poorly controlled. In a study of obese diabetics, cholesterol synthesis, plasma cholesterol level, and calories needed to maintain constant body weight all decreased when control of diabetes was improved with higher insulin dose (35). These changes are consistent with the hypothesis that when insulin is deficient, food intake is increased thereby leading to increased cholesterol input from diet and from synthesis in intestine and ultimately to hypercholesterolemia.

We would like to thank Stewart Zuckerbrod, Bradley Berger, Demosthenes Tambakos, and Maria Lopez for their expert technical assistance. This work was supported by USPHS grants RR00047, HL20488, and RR05396.

Manuscript received 8 December 1980 and in revised form 17 August 1981.

[3] The chow contained 0.36 mg cholesterol/g. Assuming female rats consumed 18 g chow/day and absorbed 60% of dietary cholesterol, dietary cholesterol input would normally be 4 mg/day. Diabetic rats eat at least twice as much food and absorb a greater percent of cholesterol, so dietary cholesterol input would be from 8 to 13 mg/day. Cholesterol synthesis in liver plus small intestine at mid-dark is 0.79 mg/hr. Assuming the rate at midlight is half the mid-dark value, the daily average rate would be about 14 mg/day. These preliminary estimates indicate that dietary cholesterol input, even from a relatively low cholesterol diet such as chow, is increased by diabetes from about 30% to 60–90% of the amount synthesized in liver plus small intestine. If the sum of synthesis in liver plus small intestine is unchanged by diabetes, then the increase in dietary cholesterol would increase the total input from these sources.

REFERENCES

1. Kaufmann, R. L., J. P. H. Assal, J. S. Soeldner, E. G. Wilmshurst, J. R. Lemire, R. E. Gleason, and P. White. 1975. Plasma lipid levels in diabetic children. Effect of diet restricted in cholesterol and saturated fats. *Diabetes.* **24:** 672–679.
2. Boyns, D. R., J. N. Crossley, M. E. Abrams, R. J. Jarrett, and H. Keen. 1969. Oral glucose tolerance and related factors in normal population sample. *Br. Med. J.* **1:** 595–598.
3. Medalie, J. H., C. Papier, J. B. Herman, U. Goldbourt, S. Tamir, H. N. Neufeld, and E. Riss. 1974. Diabetes mellitus among 10,000 adult men. 1. Five-year incidence of associated variables. *Isr. J. Med. Sci.* **10:** 681–697.
4. Sosenko, J. M., J. L. Breslow, O. S. Miettenen, and K. H. Gabbay. 1980. Hyperglycemia and plasma lipid levels. A prospective study of young insulin-dependent diabetic patients. *N. Engl. J. Med.* **12:** 650–654.
5. Kessler, I. I. 1971. Mortality experience of diabetic patients. A twenty-six year follow-up study. *Am. J. Med.* **51:** 715–724.
6. Garcia, M. J., P. M. McNamara, T. Gordon, and W. B. Kannell. 1974. Morbidity and mortality in diabetics in the Framingham population. Sixteen-year follow-up study. *Diabetes.* **23:** 105–111.
7. Cayen, M. N., J. Dubuc, and D. Dvornik. 1975. Effect of clofibrate on lipid metabolism in streptozotocin diabetic rats. *Proc. Soc. Exp. Biol. Med.* **148:** 752–757.
8. Bar-On, H., P. S. Roheim, and H. A. Eder. 1976. Hyperlipoproteinemia in streptozotocin-treated rats. *Diabetes.* **25:** 509–515.
9. Brown, D. F., K. Daudiss, and J. Durrant. 1967. Triglyceride metabolism in the alloxan-diabetic rat. *Diabetes.* **16:** 90–95.
10. Redgrave, T. G., and D. A. Snibson. 1977. Clearance of chylomicron triacylglycerol and cholesteryl ester from the plasma of streptozotocin-induced diabetic and hypercholesterolemic hypothyroid rats. *Metabolism.* **26:** 493–503.
11. Van Tol, A. 1977. Hypertriglyceridemia in the diabetic rat. Defective removal of serum very low density lipoproteins. *Atherosclerosis.* **26:** 117–128.
12. Clarenburg, R., and I. L. Chaikoff. 1966. Cholesterol synthesis in liver of alloxon-diabetic rat: role of diet. *Am. J. Physiol.* **210:** 37–47.
13. Lakshmanan, M. R., C. M. Nepokroeff, G. C. Ness, R. E. Dugan, and J. W. Porter. 1973. Stimulation by insulin of rat liver β-hydroxy-β-methylglutaryl coenzyme A reductase and cholesterol-synthesizing activities. *Biochem. Biophys. Res. Commun.* **50:** 704–710.
14. Clarenburg, R., and I. L. Chaikoff. 1966. Intestinal origin

of non-cholesterol sterols in chyle, plasma, and liver. *Am. J. Physiol.* **210:** 48–56.
15. Nakayama, H., and S. Nakagawa. 1977. Influence of streptozotocin diabetes on intestinal 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in the rat. *Diabetes.* **26:** 439–444.
16. Nervi, F. O., A. Gonzalez, and V. D. Valdivieso. 1974. Studies on cholesterol metabolism in the diabetic rat. *Metabolism* **23:** 495–503.
17. Thomson, A. B. R. 1980. Unidirectional flux rate of cholesterol and fatty acids into the intestine of rats with drug-induced diabetes mellitus: effect of variations in the effective resistance of the unstirred water layer and the bile acid micelle. *J. Lipid Res.* **21:** 687–698.
18. Young, N. L., C. D. Saudek, S. A. Crawford, and S. L. Zuckerbrod. 1982. Recovery and activation of hydroxymethylglutaryl coenzyme A reductase from rat small intestine. *J. Lipid Res.* **23:** 257–265.
19. Young, N. L., and V. W. Rodwell. 1977. Regulation of hydroxymethylglutaryl-CoA reductase in rat leukocytes. *J. Lipid Res.* **18:** 572–581.
20. Herbert, V., L. Kam-Seng, C. W. Gottleib and S. J. Bleicher. 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol.* **25:** 1375–1384.
21. Goldstein, A. 1964. Biostatistics, An Introductory Text. The MacMillan Co., New York, NY.
22. Nervi, F. O., H. J. Weis, and J. M. Dietschy. 1975. The kinetic characteristics of inhibition of hepatic cholesterogenesis by lipoproteins of intestinal origin. *J. Biol. Chem.* **250:** 4145–4151.
23. Nordstrom, J. L. 1976. Reversible modulation of rat liver hydroxymethylglutaryl coenzyme A reductase. Ph.D. Thesis. Purdue University, W. Lafayette, IN.
24. Ingebritsen, T. S., M. J. H. Geelen, R. A. Parker, K. J. Evenson, and D. M. Gibson. 1979. Modulation of hydroxymethylglutaryl-CoA reductase activity, reductase kinase activity, and cholesterol synthesis in rat hepatocytes in response to insulin and glucagon. *J. Biol. Chem.* **254:** 9986–9989.
25. Turley, D. S., J. M. Anderson, and J. M. Dietschy. 1981. Rates of sterol synthesis and uptake in the major organs of the rat in vivo. *J. Lipid Res.* **22:** 551–569.
26. Meir, J. M., J. D. McGarry, G. R. Faloona, R. H. Unger, and D. W. Foster. 1972. Studies of the development of diabetic ketosis in the rat. *J. Lipid Res.* **13:** 228–233.
27. Reaven, E. P., and G. M. Reaven. 1974. Mechanisms for development of diabetic hypertriglyceridemia in streptozotocin-treated rats. Effects of diet and duration of insulin deficiency. *J. Clin. Invest.* **54:** 1167–1178.
28. Woodside, W. F., and M. Heimberg. 1976. Effects of anti-insulin serum, insulin, and glucose on output of triglycerides and on ketogenesis by the perfused rat liver. *J. Biol. Chem.* **251:** 13–23.
29. Murthy, V. K., and J. C. Shipp. 1979. Synthesis and accumulation of triglycerides in liver of diabetic rats. Effects of insulin treatment. *Diabetes.* **28:** 472–478.
30. Risser, T. R., G. M. Reaven, and E. P. Reaven. 1978. Intestinal very low density lipoprotein secretion in insulin-dependent rats. *Diabetes.* **27:** 902–908.
31. Shefer, S., S. Hauser, V. Lapar, and E. H. Mosbach. 1972. HMG-CoA reductase of intestinal mucosa and liver of the rat. *J. Lipid Res.* **13:** 402–412.
32. Carlson, S. E., A. D. Mitchel, and S. Goldfarb. 1978. Sex-related differences in diurnal activities and development of hepatic microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol 7α-hydroxylase. *Biochim. Biophys. Acta* **531:** 115–124.
33. Abul-Hajj, Y. J. 1978. Stimulation by estradiol benzoate of hepatic β-hydroxy-β-methylglutaryl coenzyme A reductase in normal and ovariectomized rats. *Steroids.* **31:** 841–847.
34. Saudek, C. D., and E. L. Brach. 1978. Cholesterol metabolism in diabetes I. The effect of diabetic control on sterol balance. *Diabetes.* **27:** 1059–1064.
35. Bennion, L. J., and S. M. Grundy. 1977. Effects of diabetes mellitus on cholesterol metabolism in man. *N. Engl. J. Med.* **296:** 1365–1371.