

Effect of adrenalectomy on the diurnal variation of hepatic cholesterogenesis in the rat

Peter E. Hickman,* Brian J. Horton, and John R. Sabine

Department of Animal Physiology, Waite Agricultural Research Institute, University of Adelaide, Adelaide, S.A., Australia

Abstract The diurnal variation in cholesterol synthesis exhibited by rat liver has been examined in fed, fasted, and adrenalectomized animals.

Fasting for 3 days caused a lowering of the rate of synthesis but did not abolish the diurnal rhythm. Adrenalectomy abolished the diurnal variation, and caused synthesis to remain at a uniformly high level. We suggest that corticosterone may play an essential role in the daily rhythm of cholesterogenesis.

Supplementary key words fasting · corticosterone · thyroid hormones

ALTHOUGH many mammalian circadian rhythms have been recognized and described in the past, it was only as recent as 1969 that the first reports appeared of a regular daily fluctuation in the rate of cholesterol synthesis by the livers of rats (1, 2) and mice (3).

Hepatic cholesterol synthesis has long been known to be under feedback control by dietary cholesterol (4) and to be affected by fasting (5) and by manipulations in which the level of bile salts in the intestine is altered (6). Recent evidence has reemphasized the importance of hormones in cholesterogenic regulation. Synthesis is stimulated by both noradrenalin (7) and thyroid hormones (8–10).

In a previous paper (11) we reported the effects of diet upon the daily rhythm in cholesterogenesis. In this paper we report extensions to our dietary studies, and the effect of adrenalectomy on the diurnal rhythm.

Abbreviations: β -HMG CoA, β -hydroxy- β -methylglutaryl coenzyme A.

* Present address: Department of Biochemistry, School of General Studies, Australian National University, Canberra, A.C.T., Australia.

MATERIALS AND METHODS

Animals, diets, and housing conditions

We used only adult, male rats (hooded Wistar) approximately 3 months old, bred and supplied by this university's Central Animal House. All animals were housed in small groups in wire cages in a windowless but well ventilated room (21–26°C) with a strict lighting schedule of 12 hr of light and 12 hr of darkness (lights on at 0600, off at 1800 hr). Unless otherwise stated all rats were offered food (Charlicks' M164 mouse cubes) and water ad lib. All animals were accustomed to these conditions for at least 2 wk prior to any experiment.

Adrenalectomy

Bilateral adrenalectomies were performed under Nembutal anesthesia 10–15 days prior to the particular experiment. Adrenalectomized animals were maintained following surgery on 1% NaCl. After each adrenalectomy experiment the carcasses were examined, and any animals in which remaining adrenal tissue was found were excluded.

Measurement of cholesterogenesis

Cholesterol synthesis was measured, *in vitro*, by incubating liver slices with either [1-¹⁴C]acetate or [2-¹⁴C]-mevalonate (both from the Radiochemical Centre, Amersham, England) and subsequently isolating and counting ¹⁴C-labeled cholesterol (as total digitonin-precipitable sterols) according to methods previously described (12).

RESULTS

Normal diurnal rhythm

Fig. 1 shows the diurnal rhythm of cholesterol synthesis in liver slices from rats killed at 3-hr intervals over

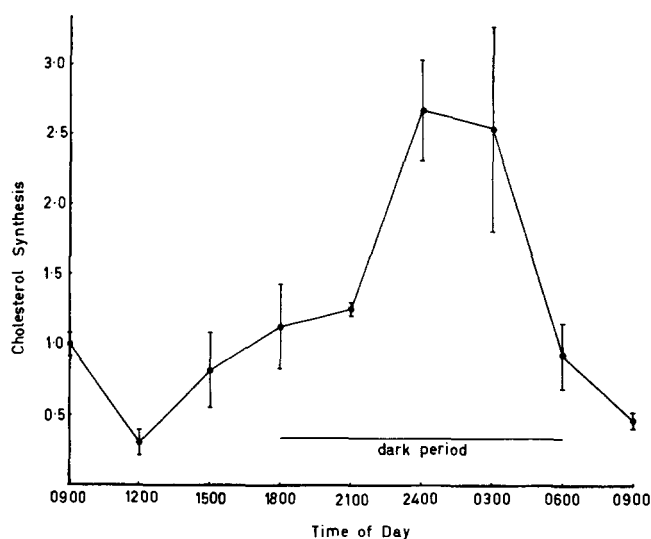


FIG. 1. Diurnal variation in liver cholesterol synthesis. Duplicate 200-mg samples of liver slices from each of three animals were incubated for 2 hr at 37°C in 2 ml of bicarbonate buffer (pH 7.3) with 4 μ moles of [1- 14 C]acetate. Cholesterol synthesis is expressed as percentage recovery of 14 C in total digitonin-precipitable sterols. Mean values \pm SEM are shown.

a 24-hr period (as previously reported, Ref. 11). The synthetic rate at midnight (2400) was six times that at midday (1200).

Diurnal rhythm in fasted rats

From Fig. 1 it can be seen that the increase in synthesis began before the dark period, and thus before the animals began eating the bulk of their daily food intake. In a previous experiment (11) we observed an increase at night even in fasted rats. We have now extended these studies. Fig. 2 shows the diurnal rhythm in cholesterol synthesis in liver slices from rats during the first day of fasting, and Table 1 shows the synthesis at midday and midnight during the first, second, and third days of fasting.¹ It is evident that the occurrence of the rhythm is independent of food intake, although its magnitude is not. This is in good agreement with the results of Kandutsch and Saucier (3) and Hamprecht, Nussler, and Lynen (2).

Effect of adrenalectomy

Hamprecht et al. (2) suggested that the diurnal rhythm was perhaps hormonally controlled and, further, that either thyroid hormones or noradrenalin may be responsible. We examined this by measuring cholesterol synthesis

¹ During the second and third day of fasting, and during the later adrenalectomy experiments, the experimental animals were actually killed at 1300 and 0100, since maximum synthesis appeared to occur shortly after midnight, but for convenience we refer to these times as midday and midnight.

TABLE 1. Diurnal variation of cholesterol synthesis in fed and fasted rats^a

Days of Fast	No. of Animals	Cholesterol Synthesis	
		Midday	Midnight
0	3	0.69 \pm 0.33	2.88 \pm 0.43 ^b
1	5	0.40 \pm 0.07	1.13 \pm 0.23 ^b
2	5	0.11 \pm 0.02	0.14 \pm 0.05 ^c
3	7	0.07 \pm 0.02	0.15 \pm 0.02 ^b

^a 200 mg of liver slices were incubated for 2 hr with 4 μ moles of [1- 14 C]acetate. Values given are percentage incorporations of 14 C into total digitonin-precipitable sterols from duplicate incubations with animals killed at midday or midnight. Food was removed from all animals, except controls, at 0600. Thus, animals tested at midday and midnight during the first day of fasting were actually fasted for 6 and 18 hr, respectively. Those tested during the second and third days had been fasting for 31, 43, 55, and 67 hr. Control animals (0 days of fast) were fed ad lib.

^b Significantly different from midday values ($P < 0.02$).

^c Not significantly different from midday values.

from [1- 14 C]acetate and [2- 14 C]mevalonate at midday and midnight in liver slices from rats 10–15 days after bilateral adrenalectomy. As shown in Table 2, adrenalectomy appeared to abolish the diurnal rhythm of cholesterol synthesis from acetate and to cause synthesis to continue at a uniformly high level. Adrenalectomy did not alter synthesis from mevalonate, suggesting that the effect of the removal of the adrenal glands was on β -HMG CoA reductase, the enzyme shown by Hamprecht et al. (2) to be responsible for the diurnal rhythm. These results (i.e., from measurements at only two time points in 24 hr) do not exclude the possibility that adrenalectomy has merely shifted the rhythm and not abolished it. In this event, which seems unlikely, the overall increase in cholesterol synthesis during 24 hr would be even greater than our figures indicate.

DISCUSSION

The work of Hamprecht et al. (2) has shown that the diurnal rhythm of hepatic cholesterol synthesis is controlled through the enzyme β -HMG CoA reductase. Our results are in accordance with this, in that cholesterol synthesis from mevalonic acid showed no diurnal variation and was not noticeably affected by adrenalectomy, in contrast to cholesterol synthesis from acetate. Further, it appears that the rhythm is a manifestation of different amounts of β -HMG CoA reductase, as opposed to the differing activities of this enzyme believed to be of importance in feedback control by dietary cholesterol (13, 14). Back, Hamprecht, and Lynen (1) and Kandutsch and Saucier (3) have shown that the diurnal rhythm is abolished when animals are treated with either of the translational inhibitors cycloheximide or puromycin.

From the results presented in this paper plus those presented earlier (11), it is possible to calculate an approxi-

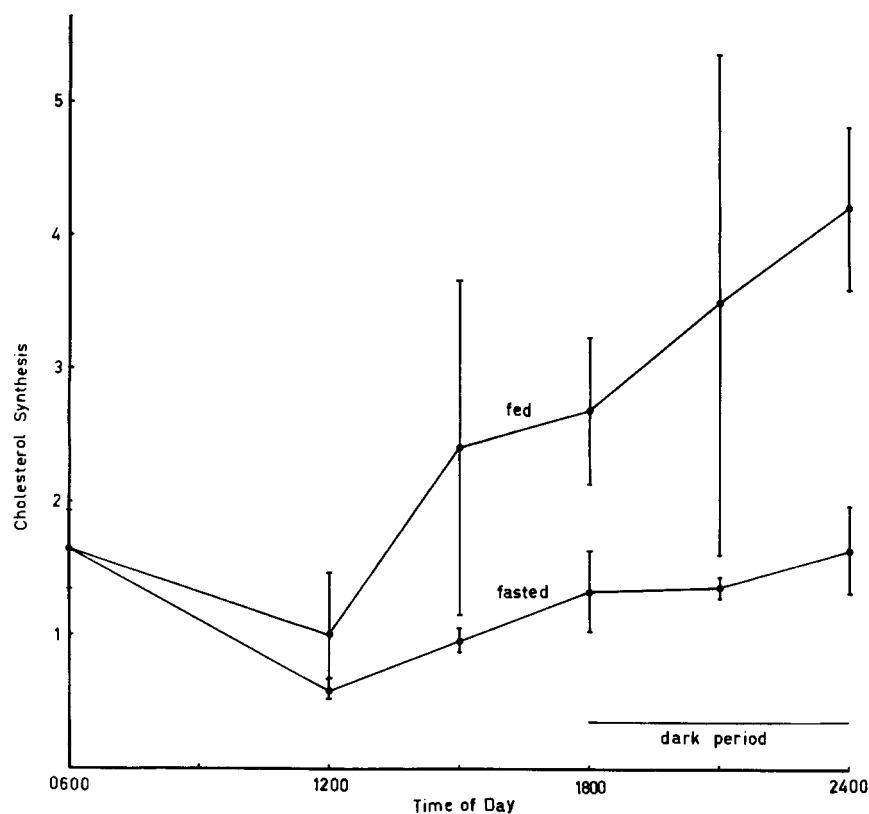


FIG. 2. Effect of fasting on the diurnal rhythm of liver cholesterol synthesis. Food was removed from the fasting animals at 0600. Other conditions are as in Fig. 1, except that points represent mean values obtained from either three (control) or five (fasting) animals.

mate value for the "effective" or "physiological" half-life of the enzyme β -HMG CoA reductase, i.e., for the excess of the rate of degradation over the rate of synthesis. This is shown in Fig. 3, where we have plotted nighttime maximum and subsequent daytime minimum values for cholesterol synthesis from nine experiments. There are, of course, three major assumptions involved in this approach: (a) that the diurnal variation in cholesterol synthesis is indeed due to variations in the amount of β -HMG CoA reductase present, (b) that the rate of

cholesterol synthesis is directly and primarily proportional to the amount of this enzyme present, and (c) that any inhibitors or activators present are not themselves subject to diurnal variation. At present these assumptions do not seem unreasonable, and hence we suggest that the "effective half-life" for this enzyme is of the order of 4–5 hr. The implication of this is that control of the net rate of degradation of β -HMG CoA reductase is an effective means by which the overall rate of cholesterol synthesis can be controlled, even on a short-term basis.

TABLE 2. Effect of adrenalectomy on the diurnal rhythm of cholesterol synthesis^a

Expt.	Treatment	Cholesterol Synthesis from			
		[1- ¹⁴ C]Acetate		[2- ¹⁴ C]Mevalonate	
		Midday	Midnight	Midday	Midnight
1	Control (4)	0.54 ± 0.07	1.73 ± 0.16 ^b		
	Adrenalectomized (4)	2.15 ± 0.84 ^d	2.50 ± 0.37 ^c		
2	Control (8)	0.83 ± 0.13	2.57 ± 0.19 ^b	5.12 ± 0.32	4.52 ± 0.31 ^c
	Adrenalectomized (5)	1.95 ± 0.53 ^d	2.40 ± 0.21 ^c	6.40 ± 0.76 ^e	5.25 ± 0.78 ^c

^a Cholesterol synthesis is expressed as the percent recovery of ¹⁴C in digitonin-precipitable sterols after incubation in duplicate of 200 mg of liver slices with 4 μ moles of either [1-¹⁴C]acetate or [2-¹⁴C]mevalonate. The figures in parentheses are the numbers of animals used at each time. Mean values \pm SEM are shown.

^b Significantly different from midday values ($P < 0.01$).

^c Not significantly different from midday values.

^d Significantly different from control values ($P < 0.05$).

^e Not significantly different from control values.

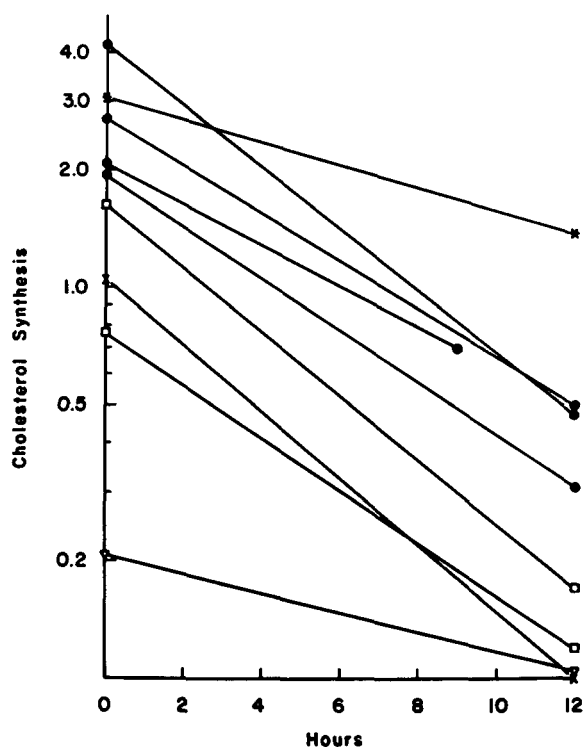


FIG. 3. "Effective" half-life of the enzyme β -HMG CoA reductase. Cholesterol synthesis, expressed as in previous figures, is plotted for nighttime maximum and subsequent daytime minimum values for nine separate experiments. Values are taken from this paper and a previous report (11). Symbols used and the corresponding values for the half-life are: ●, control, i.e., chow-fed, 3.8, 4.9, 5.7, 4.6 hr; □, fasting (first day), 3.7, 4.5 hr; ▽, fasting (second day), 12.0 hr; x, cholesterol-fed, 3.5 hr; *, cholestyramine-fed, 10.3 hr.

Our preliminary evidence² indicates that this mechanism of control may be important in other regulatory situations, including cholesterol feeding (see also discussion by Hamprecht et al. [2]). At present, because only two experiments are involved, it is not possible to attach significance to the fact that the half-life, measured in this way, was lowest in cholesterol-fed rats and well above average in animals fed cholestyramine.

Bortz (7) reported that noradrenalin stimulates cholesterol synthesis, and Hamprecht et al. (2) suggested that this hormone might control the diurnal rhythm. We tested the effect of adrenalectomy on cholesterol synthesis and found that this did indeed abolish the rhythm, with synthesis thereafter continuing at a high level. But if noradrenalin were responsible, then a uniformly low rate of synthesis should have resulted. As we observed the opposite effect, then some other adrenal hormone, which has the overall effect of limiting cholesterologenesis, must be involved. It should be noted that

²Hickman, P. E., B. J. Horton, and J. R. Sabine. Unpublished data.

our results cannot distinguish between possible mechanisms of action of this hormone; it could act either by decreasing synthesis or by increasing degradation of β -HMG CoA reductase.

In rat plasma the predominant adrenal steroid present is corticosterone (15), and several workers (16, 17) have shown that the level of this hormone undergoes a diurnal variation, with the peak concentration occurring near the end of the light period or the beginning of the dark period, i.e., perhaps 18 hr before the minimum level of liver cholesterologenesis. Fluctuations in plasma levels of corticosterone imply similar fluctuations in the level of circulating ACTH, and David-Nelson and Brodish (16) have shown a diurnal rhythm in production of corticotropin-releasing factor in the hypothalamus. The diurnal variation in plasma corticosterone can be abolished by severing the afferent nervous connections to the medial basal hypothalamus (18), or by placing the animal in continuous light (19), but so far neither of these procedures has been examined for its effects upon hepatic cholesterologenesis.

The present literature relating adrenalectomy and hepatic cholesterologenesis is particularly confusing. An important element to be considered in assessing previous work is that none of the authors concerned stated the lighting conditions under which the animals were housed, nor what relationship the time of death bore to light or food schedules, or both. But even apart from these considerations certain anomalies remain.

Perry and Bowen (20) reported that adrenalectomy caused approximately 40% reduction in the conversion of acetate to cholesterol by liver slices, whereas Willmer and Foster (21) reported a 50% increase. These latter authors used younger animals and suggested that the discrepancy in results may have been due to the differences in age, but this does not seem a satisfactory explanation. Our results, with older animals, agree with the findings of Willmer and Foster (21) in that adrenalectomy caused an increase in cholesterol synthesis from acetate by liver slices. The actual magnitude of the increase depends upon the time of day the animals are tested, with maximum effect occurring when cholesterol synthesis is normally lowest, that is, in the middle of the day.

Willmer and Foster (21) reported further that administration of several steroids, in particular deoxycorticosterone, caused an increase in hepatic cholesterologenesis, particularly in the adrenalectomized animal. This seems at variance with their observation that adrenalectomy also caused increased synthesis, but it is difficult to determine what relationship their dose of steroids bore to physiological concentrations, what effect they may have had upon normal synthesis of other adrenal hormones, and particularly what relationship the time of dosage

bore to the diurnal rhythm. It may be significant that they found that corticosterone had no effect and that the acetate forms of the hormones were less active than the alcohols.

The results of Nejad and Chaikoff (9) would seem to be more consistent with our story in that they found that corticosterone, in daily injections to hypophysectomized rats, reduced cholesterol synthesis by liver slices. Hypophysectomy should have meant less interference by other natural hormones, thus affording a more direct measure of the effect of the hormone injected than by the approach of Willmer and Foster (21). The perfused liver could perhaps give a clear-cut answer to this problem, but in the only report we know of Altman, Miller, and Bly (22) found that cortisone stimulated cholesterogenesis. In that work, however, only one experiment was reported, conditions appeared to be abnormal in that cholesterol synthesis was greater than fatty acid synthesis (an unusual circumstance), and no values were given for the "appreciable portion" of newly synthesized lipid that they reported was released into the perfusion medium.

Willmer and Foster (21) in the discussion of their work suggested that the thyroid may be an important factor in the response to adrenalectomy and adrenal hormones. Fletcher and Myant (8), Nejad and Chaikoff (9), and Guder, Nolte, and Wieland (10) have found that thyroid hormones stimulate hepatic cholesterogenesis, and there have been some, by no means consistent, reports of increased thyroid activity following adrenalectomy (see discussion by Florsheim et al. [23]). So far these results are too inconclusive for us to comment on their relationship to our own findings, but possible effects of thyroid hormones must be borne in mind in studies of this nature.

One final point should be made. The results presented in this paper, and those in previous communications from this and other laboratories concerning the diurnal variations in liver cholesterol synthesis, clearly indicate that in all future experiments in this field particular care will be needed to ensure controlled lighting conditions for the experimental animals and consistent times of assay in relation to food and lighting schedules. Indeed, much of the previous work on the control of cholesterogenesis, especially that involving hormonal regulation, may need to be reassessed in the light of our knowledge of the diurnal rhythm present.

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REFERENCES

1. Back, P., B. Hamprecht, and F. Lynen. 1969. Regulation of cholesterol biosynthesis in rat liver: diurnal changes of activity and influence of bile acids. *Arch. Biochem. Biophys.* **133**: 11–21.
2. Hamprecht, B., C. Nussler, and F. Lynen. 1969. Rhythmic changes of hydroxymethylglutaryl coenzyme A reductase activity in livers of fed and fasted rats. *FEBS Lett.* **4**: 117–121.
3. Kandutsch, A. A., and S. E. Saucier. 1969. Prevention of cyclic and Triton-induced increases in hydroxymethylglutaryl coenzyme A reductase and sterol synthesis by puromycin. *J. Biol. Chem.* **244**: 2299–2305.
4. Gould, R. G. 1951. Lipid metabolism and atherosclerosis. *Amer. J. Med.* **11**: 209–227.
5. Tomkins, G. M., and I. L. Chaikoff. 1952. Cholesterol synthesis by the liver. I. Influence of fasting and of diet. *J. Biol. Chem.* **196**: 569–573.
6. Weis, H. J., and J. M. Dietschy. 1969. Failure of bile acids to control hepatic cholesterogenesis: evidence for endogenous cholesterol feedback. *J. Clin. Invest.* **48**: 2398–2408.
7. Bortz, W. M. 1968. Noradrenalin-induced increase in hepatic cholesterol synthesis and its blockade by puromycin. *Biochim. Biophys. Acta.* **152**: 619–626.
8. Fletcher, K., and N. B. Myant. 1958. Influence of the thyroid on the synthesis of cholesterol by liver and skin *in vitro*. *J. Physiol.* (London). **144**: 361–372.
9. Nejad, N. S., and I. L. Chaikoff. 1964. Effect of glucocorticoids and L-thyroxine on acetate carbon incorporation in cholesterol by livers of hypophysectomized rats. *Endocrinology.* **75**: 396–400.
10. Guder, W., I. Nolte, and O. Wieland. 1968. The influence of thyroid hormones on β -hydroxy- β -methylglutaryl coenzyme A reductase of rat liver. *Eur. J. Biochem.* **4**: 273–278.
11. Horton, B. J., P. E. Hickman, and J. R. Sabine. 1970. The effect of diet on the diurnal variation of cholesterol synthesis in rat liver. *Life Sci.* **9**: 1409–1417.
12. Sabine, J. R., S. Abraham, and I. L. Chaikoff. 1967. Control of lipid metabolism in hepatomas: insensitivity of rate of fatty acid and cholesterol synthesis by mouse hepatoma BW 7756 to fasting and to feedback control. *Cancer Res.* **27**: 793–799.
13. Siperstein, M. D., and V. M. Fagan. 1966. Feedback control of mevalonate synthesis by dietary cholesterol. *J. Biol. Chem.* **241**: 602–609.
14. Siperstein, M. D. 1970. Regulation of cholesterol biosynthesis in normal and malignant tissues. *Curr. Top. Cell. Regul.* **2**: 65–100.
15. Zenker, N., and D. E. Bernstein. 1958. The estimation of smaller amounts of corticosterone in rat plasma. *J. Biol. Chem.* **231**: 695–701.
16. David-Nelson, M. A., and A. Brodish. 1970. Evidence for a diurnal rhythm of corticotrophin-releasing factor (CRF) in the hypothalamus. *Endocrinology.* **85**: 861–866.
17. Yamamoto, M., N. D. Diebel, and E. M. Bogdanove. 1970. Radioimmunoassay of serum and pituitary LH and FSH levels in intact male rats and of serum and pituitary LH in castrated rats of both sexes—apparent absence of diurnal rhythms. *Endocrinology.* **87**: 798–806.
18. Halasz, B., M. A. Slusher, and R. A. Gorski. 1967. Adrenocorticotrophic hormone secretion in rats after partial or total deafferentation of the medial basal hypothalamus. *Neuroendocrinology.* **2**: 43–55.

19. Fiske, V. M., and S. E. Leeman. 1964. Observations on adrenal rhythmicity and associated phenomena in the rat: effect of light on adrenocortical function; maturation of the hypothalamic neurosecretory system in relation to adrenal secretion. *Ann. N.Y. Acad. Sci.* **117**: 231-240.
20. Perry, W. F., and H. F. Bowen. 1955. Effect of adrenalectomy on the incorporation of acetate into fatty acids, cholesterol and acetoacetic acid by rat liver slices. *Amer. J. Physiol.* **184**: 59-62.
21. Willmer, J. S., and T. S. Foster. 1960. The influence of adrenalectomy and individual steroid hormones upon the metabolism of acetate-1-¹⁴C by rat liver slices. II. Incorporation into cholesterol. *Can. J. Biochem. Physiol.* **38**: 1393-1397.
22. Altman, K. I., L. L. Miller, and C. G. Bly. 1951. The synergistic effect of cortisone and insulin on lipogenesis in the perfused rat liver as studied with α -C¹⁴-acetate. *Arch. Biochem. Biophys.* **31**: 329-331.
23. Florsheim, W. H., R. T. Mirise, M. A. Faircloth, and A. D. Williams. 1969. Effects of adrenalectomy on thyroid function in starved rats. *Metab. Clin. Exp.* **18**: 311-318.†