Metabolic Regulation of Heme Catabolism and Bilirubin Production

I. HORMONAL CONTROL OF HEPATIC HEME OXYGENASE ACTIVITY

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ABSTRACT Heme oxygenase (HO), the enzyme system catalyzing the conversion of heme to bilirubin, was studied in the liver and spleen of fed, fasted, and refed rats. Fasting up to 72 hr resulted in a threefold increase in hepatic HO activity, while starvation beyond this period led to a gradual decline in enzyme activity. Refeeding of rats fasted for 48 hr depressed hepatic HO activity to basal values within 24 hr. Splenic HO was unaffected by fasting and refeeding.

Hypoglycemia induced by injections of insulin or mannose was a powerful stimulator of hepatic HO. Glucose given together with the insulin abolished the stimulatory effect of the latter. Parenteral treatment with glucagon led to a twofold, and with epinephrine to a fivefold, increase of hepatic HO activity; arginine, which releases endogenous glucagon, stimulated the enzyme fivefold. These stimulatory effects of glucagon and epinephrine could be duplicated by administration of cyclic adenosine monophosphate (AMP), while thyroxine and hydrocortisone were ineffective. Nicotinic acid, which inhibits lipolysis, failed to modify the stimulatory effect of epinephrine. None of these hormones altered HO activity in the spleen.

These findings demonstrate that the enzymatic mechanism involved in the formation of bilirubin from heme in the liver is stimulated by fasting, hypoglycemia, epinephrine, glucagon, and cyclic AMP. They further suggest that the enzyme stimulation produced by fasting may be mediated by glucagon released in response to hypoglycemia.

The possibility is considered that the enhanced HO activity in the liver may increase hepatic heme turnover and hence, bilirubin production, which may explain the rise of unconjugated serum bilirubin observed in fasting or hypoglycemic individuals.

INTRODUCTION

Clinical observations indicate that fasting leads to increased concentration of unconjugated serum bilirubin in normal individuals (2), in patients with Gilbert's syndrome (3), and in normal horses (4). Spontaneous hypoglycemia in newborn infants appears to aggravate the transient hyperbilirubinemia of the neonatal period (5, 6). In normal adults, hypoglycemia induced by infusion of mannose was found to be associated with transient unconjugated hyperbilirubinemia (7).

Although several possible mechanisms may be advanced to account for this positive relationship between fasting or hypoglycemia and the rise in serum bilirubin, a plausible explanation may be enhancement of bilirubin formation from increased heme turnover in the liver. This explanation is rendered particularly attractive because of analogous observations in hepatic porphyria, in which fasting stimulates and glucose administration represses formation of the heme precursors δ-aminolevulinic acid (8), porphobilinogen (8, 9), and protoporphyrin (10, 11) in the liver.

If increased bilirubin formation in the liver were to account for the hyperbilirubinemia of fasting, it would be necessary to demonstrate (a) that the enzymatic mechanism responsible for the conversion of heme to bilirubin is stimulated by fasting, and (b) that this

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enhanced enzyme activity increases hepatic heme catabolism which in turn, by the release of negative feedback repression (12, 13), would accelerate heme synthesis and turnover in the liver. Investigation of the first postulate was made possible by the recent identification and characterization of microsomal heme oxygenase (HO)[1] (14, 15), which catalyzes the formation of bilirubin in the liver. In the following experiments, HO activity was studied in the liver and spleen of rats which had been fasted, rendered hypoglycemic, or treated with hormones that are related to hypoglycemia and fasting.

METHODS

Animals. In all experiments, female Sprague-Dawley rats of 200–300 g weight were used, except in one instance in which male rats of comparable weight were selected to permit evaluation of the sex on the enzyme activity. Animals were maintained on standard laboratory chow and water ad lib. Fasting rats were kept in individual cages with free access to water.

Adrenalectomy or ovariectomy was performed under light ether anesthesia. Operated animals and sham-operated controls were used for the experiments 4–7 days after surgery. Adrenalectomized rats were given 0.9% aqueous saline instead of drinking water.

Fasting and refeeding. Groups of rats were fasted for various periods of time up to 120 hr before sacrifice for enzyme assay. In all refeeding experiments, the fasting was terminated after 48 hr, and the animals were refed orally or by i.p. injections for 24 hr before being killed. For oral refeeding, the rats were offered laboratory chow ad lib and drinking water containing 5% glucose. Intraperitoneal refeeding of fasted animals was performed by two individual injections of 10% glucose in water, given 24 and 8 hr before sacrifice. The glucose doses were chosen to provide 0.5 g/kg per hr over this 24 hr refeeding period.

Induced hypoglycemia. Hypoglycemia was induced by repeated i.p. injections of insulin or mannose. Insulin (24 IU/mg, Sigma Chemical Co., St. Louis, Mo.) was given to fed rats in a dose of 1 or 12 IU per 100 g body weight 7 and 5 hr before sacrifice. In some experiments, 2 g of glucose per 100 g weight in 10% aqueous solution was administered together with the insulin.

Mannose in 10% aqueous solution was injected i.p. in fed animals 24 and 8 hr before sacrifice. The doses were selected to provide 0.5 g/kg per hr of mannose for the 24 hr period before the animals were killed. In another group of rats, this regimen of mannose administration followed a 48 hr period of fasting.

Four rats fed with laboratory chow were in addition offered drinking water containing 5% glucose for 48 hr before sacrifice.

Treatment with hormones and related compounds. The following hormones were given i.p. in two individual injections to fed animals 7 and 5 hr before sacrifice, unless otherwise stated. Doses of each injection are listed per 100 g body weight: epinephrine (adrenaline chloride, Parke Davis & Co., Detroit, Mich.), 0.1 mg; glucagon (Eli Lilly & Co., Indianapolis, Ind.), 1 mg; hydrocortisone (hydrocortisone acetate, Merck, Sharpe & Dohme, West Point, Pa.), 10 mg; thyroxine (Na-levothyroxine, Synthroide, Flint Chemical Co., San Antonio, Texas), 10 mg; cyclic AMP (adenosine 3′,5′-cyclic monophosphoric acid, Sigma Chemical Co., St. Louis, Mo.), 1.25 mg; or dibutyryl cyclic AMP (N_6O_2-dibutyryl adenosine 3′,5′-cyclic monophosphoric acid, Sigma Chem. Co.), 0.25 mg. In some animals a 10% aqueous glucose solution was injected together with the epinephrine or glucagon, calculated to provide 0.5 g/kg per hr of glucose for the 7 hr period before sacrifice. In 9 rats, epinephrine and glucagon were given simultaneously in the doses and at the times listed above. Epinephrine or glucagon in the doses listed were also administered to rats that had been fasted for 48, 72, or 92 hr. The animals received two individual injections 7 and 5 hr before sacrifice while the food continued to be withheld.

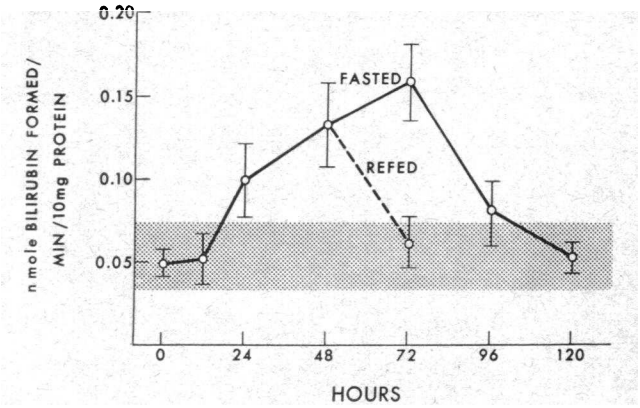

FIGURE 1 Hepatic heme oxygenase activity in rats fasted up to 120 hr and in rats fasted for 48 hr and then refed for 24 hr with laboratory chow and 5% glucose in drinking water. Enzyme values are given as the mean and standard deviation. The normal range of the enzyme activity in fed animals is indicated by the shaded area.

Nicotinic acid (Matheson, Coleman & Bell, East Rutherford, N. J.) was administered i.p. in two individual injections in a dose of 10 mg per 100 g body weight 5 and 3 hr before sacrifice. In some instances 0.1 mg epinephrine per 100 g body weight was given together with the nicotinic acid. Control animals received epinephrine alone at 5 and 3 hr. Four rats were injected with arginine (Sigma Chem. Co.), in a dose of 50 mg per 100 g body weight 5 and 3 hr before killing. Control animals received 1 mg glucagon per 100 g body weight at the same schedule. Groups of adrenalectomized or ovariectomized rats were fasted for periods of 48–72 hr. In all instances, control animals received i.p. injections of 0.9% aqueous saline instead of the listed compounds.

Assay of heme oxygenase. Animals were decapitated, and the liver and spleen rapidly removed, blotted free of blood, weighed, and placed on ice. Tissue homogenates were prepared in 0.25 M sucrose, using a motor-driven Potter-Elvehjem homogenizer with a Tephlon pestle (Arthur H. Thomas Co., Philadelphia, Pa.). The liver was homogenized in 2 vol, and the spleen in 4 vol, of 0.25 M sucrose. The homogenates were centrifuged at 20,000 *g* in a refrigerated Sorvall centrifuge, and the supernatant was used for the enzyme assay (14).

The standard incubation mixture (total volume 3 ml) contained 8–11 mg protein with liver and 4–6 mg protein

[1] *Abbreviations used in this paper:* AMP, adenosine monophosphate; FFA, free fatty acid; HO, heme oxygenase.

TABLE I

Hepatic Heme Oxygenase Activity: Effect of Hypoglycemia

Treatment*	No. of animals	Enzyme activity‡
Saline	6	0.05 ±0.02
Insulin, 1 IU	4	0.12 ±0.02
Insulin, 12 IU	4	0.34 ±0.04
Insulin, 12 IU plus glucose	4	0.13 ±0.03
Insulin, 1 IU plus glucose	5	0.06 ±0.02
Mannose	5	0.33 ±0.05
Fasting plus mannose	4	0.36 ±0.04

* For experimental details, see text.

‡ nmole bilirubin formed per min per 10 mg protein ±SD.

with spleen. The standard assay of Tenhunen, Marver, and Schmid (14) was used, and the HO activity was expressed as nanomoles bilirubin formed per minute per 10 mg protein. Protein was determined by the method of Lowry, Rosebrough, Farr, and Randall (16).

RESULTS

Fasting and refeeding. In fed rats, HO activity in the liver and spleen was comparable to values published previously (17) (Fig. 1). On fasting, hepatic HO activity increased progressively, reaching approximately 3 times the control value after 72 hr (Fig. 1). Starvation beyond 72 hr resulted in a gradual decline in enzyme activity. After a 48 hr fast, refeeding for 24 hr with laboratory chow and 5% glucose in drinking water depressed hepatic HO activity to basal values (Fig. 1); this decline was statistically significant ($P < 0.05$) (18). Splenic HO was not affected by fasting or refeeding. Glucose given by i.p. injections to rats fasted for 48 hr lowered the enzyme value in the liver to the level found in fed controls. In contrast, glucose in drinking water given to animals fed with laboratory chow had no effect on HO activity.

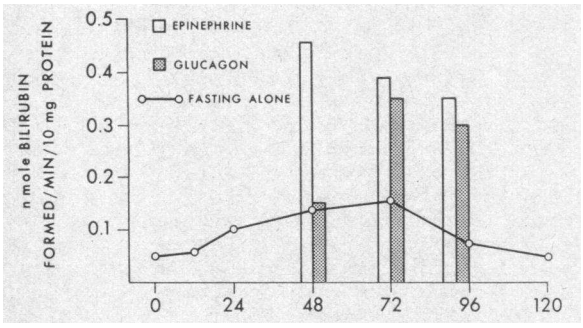

FIGURE 2 Hepatic heme oxygenase activity in rats treated with epinephrine or glucagon after fasting for 48, 72, or 92 hr. Enzyme activity in untreated fasted rats is indicated by the continuous line.

Response of hepatic HO activity to hypoglycemic agents. Hypoglycemia induced with two different doses of insulin resulted in a two- and sevenfold increase in hepatic HO activity (Table I). This stimulatory effect of insulin was attenuated when 2 g of glucose were given per 12 IU of insulin and was abolished when the ratio of glucose administered was increased to 2 g/IU of insulin (Table I). Treatment with mannose, which in man has a profound hypoglycemic effect (7), also increased hepatic HO activity (Table I).

Response of hepatic HO to hormones and related compounds. As shown in Table II, epinephrine stimulated hepatic HO fivefold, while the enzyme in the spleen remained unaffected. Epinephrine given in the same dose 5 and 3 hr before assay increased hepatic HO activity threefold. Glucose given together with the epinephrine failed to modify the effect of the hormone on hepatic HO. Animals fasted for 48, 72, and 92 hr responded to epinephrine treatment with an eightfold, sevenfold, and sixfold rise in hepatic enzyme activity, relative to untreated fed animals (Fig. 2). Since epinephrine releases free fatty acid (FFA) (19), nicotinic acid, an inhibitor of lipolysis (19), was given together with the hormone 5 and 3 hr before enzyme assay. The additional treatment with this lipolytic inhibitor failed to alter the stimulatory effect of epinephrine (Table II). However, nicotinic acid alone caused a twofold increase in enzyme activity in the liver (Table II).

Glucagon injection doubled the hepatic HO activity, while splenic HO activity remained unaffected (Table II). Glucose given together with glucagon did not abolish the stimulatory effect of the hormone (Table II). In animals whose hepatic HO had been stimulated by 48 hr fasting, glucagon had no additional effect on the enzyme activity (Fig. 2). However, on prolonged starvation, glucagon given at 72 and 92 hr doubled and tripled HO activity as compared to that in untreated rats fasted for the same length of time. Arginine, which triggers the release of endogenous glucagon (20) caused a fivefold increase in hepatic HO activity (Table II). This rise in activity exceeded the enzyme stimulation obtained with individual glucagon injections at 7 and 5, or 5 and 3 hr before assay (Table II).

Epinephrine (0.1 mg/100 g rat) and glucagon (1 mg/100 g rat) given together in two i.p. injections 7 and 5 hr before enzyme assay produced additive enhancement of hepatic enzyme activity (Table II).

Injections of cyclic AMP or its less polar derivative, dibutyryl cyclic AMP, increased hepatic HO activity twofold and threefold, respectively, while splenic enzyme activity remained unaffected (Table II). Thyroxine had no effect on enzyme activity in liver and spleen.

Hepatic HO activity in adrenalectomized or ovariectomized rats, assayed 4–7 days after operation, was not altered (Table III), nor did hydrocortisone treatment affect the enzyme activity (Table II). Fasting of these operated rats had an effect comparable to that in intact animals, except that after adrenalectomy the hepatic HO activity returned to basal values 24 hr earlier than in the nonoperated controls (Table III, Fig. 1). No difference in enzyme activity was detected between male and female rats (Table III). In no instance did fasting or parenteral treatment cause alterations in enzyme activity or weight of the spleen.

DISCUSSION

The present findings demonstrate that the enzymatic mechanism catalyzing the conversion of heme to bilirubin in the liver is under hormonal control. The hormones found to exhibit this regulatory function were glucagon and epinephrine, whereas parenteral administration of hydrocortisone and thyroxine was without effect on hepatic HO activity (Table II).

Fasting and hypoglycemia in man (21) and in dogs (22) are associated with release of endogenous glucagon (23). Although no direct measurements of portal or systemic venous glucagon levels have been reported in intact rats, in isolated rat livers perfused with hypoglycemic solutions, glucagon is required for stimulation of hepatic glucose release (24). Since fasting in rats results in a moderate hypoglycemia (25–27), it was to be expected that this would evoke a stimulatory effect on hepatic HO similar to that produced by parenterally administered glucagon. Thus, on withholding of food for 3 consecutive days, the enzyme activity in the liver increased approximately three times, but returned to base line values within 24 hr of refeeding (Fig. 1). Since stimulation of HO activity induced by fasting was readily reversed by i.p. administration of glucose, it appeared likely that the stimulatory effect was regulated to hyperglucagonemia induced by a fall in blood sugar.

The essential role of hypoglycemia in initiating the enzyme stimulation was established by observations in fed rats treated with insulin or mannose. Insulin-induced hypoglycemia was associated with an increase in hepatic HO activity that was similar to that seen in fasted animals (Table I). This stimulatory effect of insulin was blunted or abolished when the injected insulin was "covered" with glucose administered simultaneously (Table I), which indicates that insulin by itself does not stimulate the enzyme. Mannose which in man produces severe hypoglycemia (7) was found to be another powerful stimulator of HO in rat liver (Table I).

TABLE II

Heme Oxygenase Activity: Effect of Treatment with Hormones and Related Compounds

Treatment*	No. of animals	Enzyme activity‡ Liver	Spleen
Saline	5	0.06 ±0.02	0.62 ±0.14
Epinephrine	6	0.33 ±0.04	0.57 ±0.08
Epinephrine and glucose	4	0.30 ±0.06	
Epinephrine and nicotinic acid§	3	0.33 ±0.04	
Epinephrine§	4	0.21 ±0.03	
Nicotinic acid§	3	0.15 ±0.03	
Glucagon	6	0.14 ±0.02	0.47 ±0.08
Glucagon and glucose	4	0.14 ±0.04	
Arginine§	4	0.30 ±0.04	
Glucagon§	3	0.11 ±0.02	
Epinephrine and glucagon	9	0.45 ±0.09	
Cyclic AMP	5	0.13 ±0.07	0.67 ±0.11
Dibutyryl cyclic AMP	4	0.20 ±0.03	0.64 ±0.12
Thyroxine	4	0.06 ±0.03	
Hydrocortisone	5	0.06 ±0.02	

* For experimental details, see text. All injections administered 7 and 5 hr before enzyme assay except as otherwise noted.
‡ nmole bilirubin formed per min per 10 mg protein ±SD.
§ Administered 5 and 3 hr before enzyme assay.

A decline in blood glucose concentration, rather than a fixed level of hypoglycemia, appears to be the major physiological stimulus for release of endogenous glucagon (21, 22, 28). Thus, glucagon concentrations in man rise during the initial 2–3 days of fasting but then decline as the hypoglycemia tends to become stabilized (21, 23). While glucagon concentrations in response to starvation in rats have not been measured, the blood glucose falls rapidly during the first 2–3 days of fasting (25–27) with relatively little change thereafter (26). This pattern was mirrored by the activity

TABLE III

Heme Oxygenase Activity: Effect of Endocrine Ablations

Treatment*	Fasting	No. of animals	Enzyme activity‡ Liver	Spleen
Normal females	Fed	5	0.06 ±0.02	0.62 ±0.14
Normal males	Fed	4	0.07 ±0.01	0.58 ±0.07
Adrenalectomy	Fed	4	0.05 ±0.02	0.60 ±0.10
Adrenalectomy	48 hr	5	0.13 ±0.02	
Adrenalectomy	72 hr	5	0.07 ±0.02	0.67 ±0.20
Ovariectomy	Fed	5	0.06 ±0.01	0.71 ±0.08
Ovariectomy	72 hr	4	0.20 ±0.02	0.55 ±0.05

* For experimental details, see text.
‡ nmole bilirubin formed per min per 10 mg protein ±SD.

pattern of hepatic HO, which reached peak levels after 72 hr of fasting, but then decreased as starvation continued (Fig. 1). After 48 hr of fasting, parenterally administered glucagon had little effect on the HO activity in the liver, suggesting that at this time, endogenously released hormone elicited a maximal stimulatory response of the enzyme (Fig. 2). In contrast, after prolonged starvation, glucagon given at 72 or 92 hr resulted in a marked enhancement of HO activity, presumably because at this time, glycogen had reappeared in the liver (25), hypoglycemia was less severe (26), and consequently, endogenous glucagon release was submaximal.

In the doses employed, epinephrine was more effective as an enzyme stimulator than glucagon, both in fed and in starved rats (Table II). A possible explanation for this difference is that glucagon administered parenterally in two individual injections may not be concentrated optimally in the target tissue. This speculation is supported by the observation that arginine-stimulated release of endogenous glucagon (20, 29) caused higher HO values in the liver than did pharmacologic doses of the exogenously administered hormone (Table II). The possibility cannot be excluded, on the other hand, that the two hormones exert their stimulatory effect by means of separate mechanisms (30) and that glucagon represents the primary mediator of enzyme stimulation in response to hypoglycemia (23). Consistent with this interpretation are the findings that glucagon and epinephrine had additive effects (Table II) and that in animals fasted for 48 hr, epinephrine was strongly stimulatory whereas exogenous glucagon was not (Fig. 2). This concept is further supported by the reported observations that rats fasted for 48 hr failed to excrete increased amounts of catecholamines (31) and that very large doses of epinephrine, comparable to those used in the present experiments, were required to produce a direct effect on hepatic glycogenolysis (23).

Tissue-specific receptors have been postulated as the link between certain hormones and the "secondary hormone," cyclic AMP (30). Hormones whose action is believed to be mediated by cyclic AMP include epinephrine, glucagon, and thyroxine, but exclude steroids and insulin (32). Cyclic AMP and its dibutyryl ester effectively replaced epinephrine and glucagon as stimulators of hepatic HO (Table II). This effect appeared to be specific for hepatic parenchymal cells, as HO in the spleen was unaffected by these hormones and cyclic AMP (Table II). Moreover, preliminary observations[2] indicate that the enzyme in the Kupffer cells of the liver also is unresponsive to stimulation by fasting. This suggests that hormonal regulation of HO in the liver may involve a mechanism different from that which operates in the substrate-mediated enzyme stimulation observed in the spleen (17), macrophages (33), and renal tubular cells (34)[3].

Thyroxine and hormones whose action is not mediated by cyclic AMP were ineffective as enzyme stimulators, nor did adrenalectomy, ovariectomy, or the sex of animals affect the HO activity (Table III). Fasting stimulated hepatic HO in adrenalectomized and ovariectomized rats to the same extent as in intact animals, providing additional evidence that glucagon, rather than epinephrine, may be responsible for the starvation-induced stimulation of the enzyme. On prolonged fasting, the decline in enzyme activity began earlier in adrenalectomized animals compared to intact or ovariectomized rats (Table III). This time-limited response of adrenalectomized animals to prolonged fasting may reflect their deteriorating condition after the second day of starvation or their inability to secrete epinephrine.

Fasting (35) and treatment with epinephrine (19), glucagon (36), thyroxine (19, 37), and hydrocortisone in high doses (38) cause an elevation of FFA in the plasma. However, FFA does not appear to be related to the stimulation of HO activity in the liver, since thyroxine and hydrocortisone failed to stimulate the enzyme despite their lipolytic action. Moreover, nicotinic acid, which blocks the release of FFA induced by epinephrine (19), did not interfere with epinephrine-mediated stimulation of HO (Table II). Indeed, nicotinic acid produced a moderate enhancement of enzyme activity, an effect which remains unexplained (Table II). Finally, treatment with mannose, which reduces the plasma concentration of FFA (7), stimulated hepatic HO activity (Table I).

Our findings indicate that the enzymatic apparatus involved in the conversion of heme to bilirubin in the liver is stimulated by fasting and hypoglycemia. It does not necessarily follow, however, that this elevation of hepatic enzyme activity actually results in increased bilirubin formation in vivo. To reach this conclusion, it would be necessary to demonstrate that fasting or hypoglycemia leads to accelerated heme turnover in the liver or results in increased rates of bilirubin (39) or CO (40) production. Preliminary experiments suggest that this indeed may be the case in that, in intact rats, glucagon or epinephrine was found to enhance the rate

[2] Bissell, D. M., L. E. Hammaker, and R. Schmid. In preparation.

[3] Fasting, glucagon, or epinephrine did not result in increased red cell destruction, as shown by the normal survival of transfused rat erythrocytes labeled with carbon-14. (Bakken, A. F., M. M. Thaler, and R. Schmid, in preparation).

of ^{14}CO formation from the heme precursors glycine-2-^{14}C or δ-aminolevulinic acid-5-^{14}C (41); these studies will be reported in detail in a separate communication.

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REFERENCES

1. Bakken, A. F., M. M. Thaler, N. R. Pimstone, and R. Schmid. 1971. Stimulation of hepatic heme oxygenase activity by fasting and by hormones. *Gastroenterology.* **60:** 177.
2. With, T. K. 1968. *In* Bile Pigments. Chemical, Biological, and Clinical Aspects. Academic Press Inc., New York. 411.
3. Felsher, B. F., D. Rickard, and A. G. Redeker. 1970. The reciprocal relation between caloric intake and the degree of hyperbilirubinemia in Gilbert's syndrome. *N. Engl. J. Med.* **283:** 170.
4. Gronwall, R. R., and A. S. Mia. 1969. Effect of starvation on bilirubin metabolism in the horse. *Physiologist.* **12:** 241.
5. Wu, P. Y. K., P. Teilman, M. Gabler, M. Vaughan, and J. Metcoff. 1967. "Early" versus "late" feeding of low birth weight neonates: effect on serum bilirubin, and responses to glucagon and epinephrine tolerance tests. *Pediatrics.* **39:** 733.
6. Smallpeice, V., and P. A. Davies. 1964. Immediate feeding of premature infants with undiluted breast milk. *Lancet.* **2:** 1349.
7. Wood, F. S., and G. F. Cahill, Jr. 1963. Mannose utilization in man. *J. Clin. Invest.* **42:** 1300.
8. Welland, F. H., E. S. Hellman, E. M. Gaddis, A. Collins, G. W. Hunter, Jr., and D. P. Tschudy. 1964. Factors affecting the excretion of porphyrin precursors by patients with acute intermittent porphyria. I. The effect of diet. *Metabolism (Clin. Exp.).* **13:** 232.
9. Rose, J. E., E. S. Hellman, and D. P. Tschudy. 1961. Effect of diet on the induction of experimental porphyria. *Metabolism (Clin. Exp.).* **10:** 514.
10. Tschudy, D. P., F. H. Welland, A. Collins, and G. W. Hunter, Jr. 1964. The effect of carbohydrate feeding on the induction of δ-aminolevulinic acid synthetase. *Metabolism (Clin. Exp.).* **13:** 396.
11. Redeker, A. G., and R. E. Sterling. 1968. The "glucose effect" in erythropoietic porphyria. *Arch. Intern. Med.* **121:** 446.
12. Kappas, A., and S. Granick. 1968. Steroid induction of porphyrin synthesis in liver cell culture. II. The effect of heme, uridine diphosphate glucuronic acid, and inhibitors of nucleic acid and protein synthesis on the induction process. *J. Biol. Chem.* **243:** 346.
13. Marver, H. S., R. Schmid, and H. Schützel. 1968. Heme and methemoglobin: naturally occurring repressors of microsomal cytochrome. *Biochem. Biophys. Res. Commun.* **33:** 969.
14. Tenhunen, R., H. S. Marver, and R. Schmid. 1968. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. *Proc. Nat. Acad. Sci. U. S. A.* **61:** 748.
15. Tenhunen, R., H. S. Marver, and R. Schmid. 1969. Microsomal heme oxygenase: characterization of the enzyme. *J. Biol. Chem.* **244:** 6388.
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265.
17. Tenhunen, R., H. S. Marver, and R. Schmid. 1970. The enzymatic catabolism of hemoglobin: stimulation of microsomal heme oxygenase by hemin. *J. Lab. Clin. Med.* **75:** 410.
18. Snedecor, G. W., and V. W. G. Cochran. 1968. Statistical Methods. The Iowa State University Press, Ames. 6th edition. 593.
19. Eaton, R. P., D. Steinberg, and R. H. Thompson. 1965. Relationship between free fatty acid turnover and total body oxygen consumption in the euthyroid and hyperthyroid states. *J. Clin. Invest.* **44:** 247.
20. Heding, L. G. 1971. The radioimmunological determination of pancreatic and gut glucagon in plasma. *Diabetologia.* **7:** 10.
21. Marliss, E. W., T. T. Ashi, R. H. Unger, J. S. Soeldner, and G. F. Cahill, Jr. 1970. Glucagon levels and metabolic effects in fasting man. *J. Clin. Invest.* **49:** 2256.
22. Unger, R. H., A. M. Eisentraut, M. S. McCall, and L. L. Madison. 1962. Measurements of endogenous glucagon in plasma and the influence of blood glucose concentration upon its secretion. *J. Clin. Invest.* **41:** 682.
23. Sokal, J. E. 1966. Glucagon, an essential hormone. *Amer. J. Med.* **41:** 331.
24. Sokal, J. E., and B. Weintraub. 1966. Failure of the isolated liver to react to hypoglycemia. *Amer. J. Physiol.* **210:** 63.
25. Herrera, E., and N. Freinkel. 1968. Interrelationships between liver composition, plasma glucose and ketones, and hepatic acetyl-CoA and citric acid during prolonged starvation in the male rat. *Biochim. Biophys. Acta.* **170:** 244.
26. Malaisse, W. J., F. Malaisse-Lagae, and P. H. Wright. 1967. Effect of fasting upon insulin secretion in the rat. *Amer. J. Physiol.* **213:** 843.
27. Weber, G., R. L. Singhal, N. B. Stamm, and S. K. Srivastava. 1965. Hormonal induction and suppression of liver enzyme biosynthesis. *Fed. Proc.* **24:** 745.
28. Unger, R. H., A. Ohneda, E. Aguilar-Parada, and A. M. Eisentraut. 1969. The role of aminogenic glucagon secretion in blood glucose homeostasis. *J. Clin. Invest.* **48:** 810.
29. Aguilar-Parada, E., A. M. Eisentraut, and R. H. Unger. 1969. Effects of starvation on plasma pancreatic glucagon in normal man. *Diabetes.* **18:** 717.
30. Bitensky, M. W., V. Russell, and W. Roberts. 1968. Evidence for separate epinephrine and glucagon responsive adenyl cyclase systems in rat liver. *Biochem. Biophys. Res. Commun.* **31:** 706.
31. Herrera, E., R. H. Knopp, and N. Freinkel. 1969. Urinary excretion of epinephrine and norepinephrine during fasting in late pregnancy in rats. *Endocrinology.* **84:** 447.
32. Editorial. 1970. Cyclic AMP: the second messenger. *Lancet.* **2:** 1119.
33. Pimstone, N. R., R. Tenhunen, P. T. Seitz, H. S. Marver, and R. Schmid. 1971. The enzymatic degradation of hemoglobin to bile pigments by macrophages. *J. Exp. Med.* **133:** 1264.

34. Pimstone, N. R., P. Engel, R. Tenhunen, P. T. Seitz, H. S. Marver, and R. Schmid. 1971. Inducible heme oxygenase in the kidney: a model for the homeostatic control of hemoglobin catabolism. *J. Clin. Invest.* **50**: 2042.
35. Owen, D. E., P. Felig, A. P. Morgan, J. Wahren, and C. F. Cahill, Jr. 1969. Liver and kidney metabolism during prolonged starvation. *J. Clin. Invest.* **48**: 574.
36. Luyckx, A. S., and P. J. Lefebvre. 1970. Arguments for a regulation of pancreatic glucagon secretion by circulating free fatty acids. *Proc. Soc. Exp. Biol. Med.* **133**: 524.
37. Deykin, D., and M. Vaughan. 1963. Release of free fatty acids by adipose tissue from rats treated with triiodothyronine or propylthiouracil. *J. Lipid Res.* **4**: 200.
38. Forbes, J., R. Rudolph, and O. Peterson. 1966. Effect of hydrocortisone feeding on the concentration of free fatty acids and other lipids of rabbit sera. *Proc. Soc. Exp. Biol. Med.* **122**: 299.
39. Schmid, R., H. S. Marver, and L. Hammaker. 1966. Enhanced formation of rapidly labeled bilirubin by phenobarbital: hepatic microsomal cytochromes as a possible source. *Biochem. Biophys. Res. Commun.* **24**: 319.
40. Landaw, S. A., E. W. Callahan, Jr., and R. Schmid. 1970. Catabolism of heme in vivo: comparison of the simultaneous production of bilirubin and carbon monoxide. *J. Clin. Invest.* **49**: 914.
41. Bakken, A. F., M. M. Thaler, and R. Schmid. 1971. Hormonal control of hepatic heme catabolism. *J. Clin. Invest.* **50**: 5 a. (Abstr.)