EFFECTS OF IRRADIATION AND STARVATION ON THE REGULATION OF RAT LIVER ENZYMES*

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Communicated by Shields Warren, August 25, 1966

The amounts of specific enzymes in animal tissues change in a variety of physiological states. Damage to the systems responsible for the synthesis of macromolecules may interfere with this form of metabolic adaptation. Previous studies showed that the elevation of rat liver tryptophan pyrrolase¹ or tyrosine transaminase² by cortisone *in vivo* is prevented by actinomycin,³ whereas this inhibitor of RNA synthesis does not interfere with the elevation of tryptophan pyrrolase by its substrate³ or the elevation of tyrosine transaminase by pyridoxine.⁴ These observations led to the general hypothesis that accumulation of enzyme in response to hormones involves an increase in the number of specific RNA templates, whereas accumulation in response to cofactor or substrate occurs by the function of an unchanged number of synthetic sites.⁵ Indeed, in all cases so far tested⁶⁻⁸ where hormones *in vivo* increased the amount of specific proteins, actinomycin was inhibitory.

Actinomycin represents a group of "radiomimetic" agents which, like radiation, affect the most rapidly growing normal tissues,⁹ are carcinostatic,¹⁰ and enhance the effect of radiation in man¹¹ and animals.^{12, 13} One purpose of the present study was to test whether the effect of ionizing radiation on the two kinds of accumulation of enzymes *in vivo* resembles that of actinomycin. The results indicate that irradiation influences the processes of enzyme accumulation in a completely different way than does actinomycin.

Methods.—An inbred strain of rats (NEDH), or Sprague-Dawley rats (SD) obtained from the Charles River Breeding Laboratories, of 180–250 gm body weight were used 5–8 days after bilateral adrenalectomy. Hydrocortisone (2.5 mg per 100 gm body weight) as the acetate (HCA) or the hemisuccinate (HCS), or 100 mg L-tryptophan or α -methyl-DL-tryptophan per 100 gm body weight, were given by intraperitoneal injection. The livers were assayed for tryptophan pyrrolase after prior incubation as described by Knox *et al.*¹⁴ Tyrosine transaminase was assayed by the enol-borate method as previously described.¹⁵ The enzyme activities are expressed as μ moles of product (kynurenine or p-hydroxyphenylpyruvate) formed per hr per gm wet weight of liver at 25°C.

Total body X-irradiation of 250 kv was applied at 70-cm distance and at a rate of 16.4 r per min through a Thoreus II filter with a half-layer equivalent to 2.95 mm copper. Four rats in a compartmented, circular cage of wire were irradiated simultaneously in the same field. The rats received no food after irradiation but were given saline to drink. Unirradiated controls were either fed or starved for a similar period.

Results.—The response of liver tryptophan pyrrolase was studied in rats irradiated 24 or 48 hr earlier and killed 5 or 11 hr after injection with hydrocortisone.

TABLE 1
RESPONSE OF LIVER TRYPTOPHAN PYRROLASE TO HYDROCORTISONE IN FED, IRRADIATED,
AND STARVED RATS

Sex	Strain	Hydrocortisone salt	Time (hr)	Tryptophan Pyrrolase Activity (units/gm liver $\pm \sigma$)		
				Fed	Starved	Irradiated (and starved)
M	NEDH	None	—	4.4 $\pm$ 0.5 (4)	—	4.2 $\pm$ 0.5 (6) ^a
M	NEDH	HCA	5	15.9 $\pm$ 2.0 (4)	—	15.4 $\pm$ 2.5 (6) ^a
M	NEDH	HCA	11	18.7 $\pm$ 5.0 (4)	—	17.9 $\pm$ 4.2 (4) ^a
F	NEDH	HCS	5	13.8 $\pm$ 1.7 (7)	13.4 $\pm$ 3.5 (7)	11.8 $\pm$ 1.0 (7) ^b
F	NEDH	HCS	5	—	—	14.2 $\pm$ 4.5 (6) ^c
M	SD	None	5	3.0 $\pm$ 0.4 (7)	3.2 $\pm$ 0.2 (5)	3.8 $\pm$ 0.3 (5) ^a
M	SD	HCS	5	24.8 $\pm$ 2.8 (4)	17.7 $\pm$ 2.4 (6)	16.9 $\pm$ 2.1 (5) ^b

The activity of tryptophan pyrrolase was measured in each liver of adrenalectomized rats 5 or 11 hr after the administration of hydrocortisone hemisuccinate (HCS) or acetate (HCA). The superscripts indicate the amount and time (hr before injection) of irradiation given (^a = 800 r, 24 hr; ^b = 800 r, 48 hr; ^c = 1600 r, 24 hr). The time after irradiation was the same as the duration of starvation of groups of rats in the same horizontal line. The values are means $\pm \sigma$; the number of rats is given in parentheses.

TABLE 2
ELEVATION OF TRYPTOPHAN PYRROLASE BY SUBSTRATE IN FED, STARVED, AND
IRRADIATED RATS

Administered substance and time (hr)	Tryptophan Pyrrolase Activity (units/gm liver $\pm \sigma$)		
	Fed	Starved (48 hr)	Irradiated (and starved) (800 r, 48 hr before)
None	3.9 $\pm$ 1.0 (4)	2.8 $\pm$ 0.4 (4)	3.6 $\pm$ 1.0 (4)
Trp, 3.5	16.5 $\pm$ 1.0 (8)	—	7.0 $\pm$ 1.6 (9)
Trp, 3.5	—	—	7.4 $\pm$ 2.3 (7)*
α -MT, 7	14.9 $\pm$ 2.3 (7)	—	7.3 $\pm$ 1.5 (5)
α -MT, 5	14.6 $\pm$ 2.6 (8)	7.8 $\pm$ 0.4 (9)	8.0 $\pm$ 2.8 (6)
α -MT, 5	—	9.4 $\pm$ 1.4 (7)†	3.6 $\pm$ 1.7 (9)‡, †

Tryptophan (Trp) or α -methyltryptophan (α -MT) was administered to adrenalectomized female, NEDH rats 3.5–7 hr before assay. The rats were irradiated with 800 r and starved for 48 hr except as indicated by footnotes.

* Treated 24 hr after 800 r.

† *P* value of difference = <0.001.

‡ Treated 24 hr after 1600 r.

In fed, adrenalectomized rats this response consists of a severalfold rise in the enzyme level. Table 1 shows that irradiation did not significantly alter this response in a variety of situations. However, for rats allowed access to food, irradiation reduced the daily food intake from 15 to 3–4 gm per day. There were no differences in any of the measurements in this study between rats that were starved or that were allowed access to food after irradiation, but there were differences between fed and starved rats that were not irradiated. To control this variable, the irradiated rats received no food and were compared with unirradiated starved as well as fed rats. Starvation itself had no effect on the elevation of tryptophan pyrrolase by hydrocortisone in NEDH rats. Starvation caused a small decrease of the hydrocortisone effect in SD rats. This indicated that the slightly lower response of tryptophan pyrrolase in the SD strain after irradiation was merely due to the starvation. Thus, the lack of any effect of 800–1600 r irradiation on the response of tryptophan pyrrolase to hydrocortisone treatment with either the acetate or the hemisuccinate was apparent in both strains, both sexes, and at both 5 and 11 hr after treatment.

The elevation of tryptophan pyrrolase by substrate-type compounds gave a different picture (Table 2): Irradiation (with starvation) reduced the response to L-tryptophan or α -methyltryptophan. However, starvation alone significantly lowered the response to α -methyltryptophan, and only the decrease below the level in the starved controls can be attributed to the irradiation. The last line of Table 2

TABLE 3

COMPARISON OF PLASMA LEVELS, CONJUGATION OF TRYPTOPHAN PYRROLASE, AND TOXICITY OF L-TRYPTOPHAN AND α -METHYL-DL-TRYPTOPHAN IN ADRENALECTOMIZED RATS

Treatment	Plasma level at 1 hr (μ g/ml)	Tryptophan Pyrrolase (at 5 hr) Total (units/gm liver)	Degree of conjugation (%)	Died at 5 hr (total)
<i>Tryptophan injected</i>				
Fed	592 (3)	15.8 (3)	92	5/13
Irradiated (48 hr after 800 r)	627 (3)	8.2 (3)	93	10/12
<i>α-MT injected</i>				
Fed	834 (10)	14.9 (8)	—	0/11
Starved (24 hr)	678 (8)	9.4 (7)	—	3/16
Irradiated (24 hr after 1600 r)	852 (6)	3.6 (9)	—	1/10

Averaged values for the number of rats given in parentheses are tabled for plasma levels of the compound given 1 hr after the injection of L-tryptophan or α -methyltryptophan, the tryptophan pyrrolase level after 5 hr and its degree of conjugation in liver, and the number of rats dying within 5 hr out of the total treated.

shows that 1600 r, but not 800 r, significantly reduced the response to α -methyltryptophan below that in rats that were only starved. There was, in fact, no elevation of the tryptophan pyrrolase in 5 hr after treatment with α -methyltryptophan in the rats given 1600 r.

α -Methyltryptophan was used in these experiments because it is less toxic to adrenalectomized rats than L-tryptophan. The latter killed most of the irradiated rats within 5 hr, while α -methyltryptophan killed only a few, even after 1600 r irradiation (Table 3). Previous studies indicated that the elevation of tryptophan pyrrolase in adrenalectomized rats by both L-tryptophan¹⁶ and α -methyltryptophan¹⁷ was associated with the conjugation *in vivo* of the tryptophan pyrrolase to the holoenzyme form. The larger part of the enzyme is normally not conjugated with its heme prosthetic group. Table 3 shows that this conjugation, measured with globin by the method of Knox and Piras,¹⁸ did occur in these experiments and its lack therefore could not be the reason for less elevation of the tryptophan pyrrolase. An alternate assay of the enzyme with mitochondria, which also measures the total enzyme,¹⁹ gave the same low activities after irradiation that are shown in the table. As a further control, the plasma levels of tryptophan and α -methyltryptophan were measured fluorimetrically²⁰ 1 hr after injection. Both were high in all animals indicating that the compounds were absorbed equally well whether or not the rats were irradiated or starved (Table 3).

Table 4 shows the effect of irradiation on the hormone and substrate types of elevation of the second enzyme, tyrosine transaminase. This enzyme was measured in the same rats described in Tables 1 and 2, plus some additional groups. Five hr after the administration of hydrocortisone, the level was substantially higher in irradiated rats of both strains than in nonirradiated, fed rats. However, starvation alone increased the response of tyrosine transaminase to hydrocortisone to the same extent in both strains of rats as did irradiation plus starvation. Thus, irradiation appears to have no effect on the tyrosine transaminase response to hydrocortisone, other than that due to the decreased food intake of irradiated rats. Table 4 also shows that α -methyltryptophan doubled the level of tyrosine transaminase in the adrenalectomized rats, as L-tryptophan has been reported to do.²¹ The level was higher in starved than fed rats after α -methyltryptophan, and significantly less in the irradiated rats than in the starved rats. The administration of pyridoxine also raised the level of tyrosine transaminase more in starved than in fed rats, and

TABLE 4
REGULATION OF LIVER TYROSINE TRANSAMINASE IN NORMAL, STARVED, AND
IRRADIATED RATS

Sex	Strain	Substances administered and time (hr)	Tyrosine Transaminase Activity (units/gm liver)		
			Fed	Starved	Irradiated (and starved)
F	NEDH	None	66.4 ± 13.0 (5)	60.0 ± 5.0 (5)	86.0 ± 14.0 (11) ^a
F	NEDH	HCS, 5	227.0 ± 40.0 (10)	515.0 ± 120.0 (7)	510.0 ± 90.0 (14) ^a 660.0 ± 28.0 (6) ^c
F	NEDH	α-MT, 5	145.0 ± 22.0 (13)	243.0 ± 89.0 (8) [*] 240.0 ± 65.0 (7) [*]	167.0 ± 55.0 (9) ^{c*} 158.0 ± 31.0 (4) ^{b*}
M	SD	None	43.0 ± 15.0 (11)	65.8 ± 6.0 (6)	58.0 ± 5.0 (3) ^a
M	SD	HCS, 5	275.0 ± 50.0 (4)	905.0 ± 105.0 (6)	851.0 ± 156.0 (5) ^b
M	SD	Pyridoxine, 4	196.0 ± 45.0 (8)	313.0 ± 39.0 (8) [†]	211.0 ± 56.0 (13) ^{a†}

Adrenalectomized rats were treated as described in Tables 1 and 2. Superscripts *a* = 800 r, 24 hr; *b* = 800 r, 48 hr; *c* = 1600 r, 24 hr. Hydrocortisone hemisuccinate (HCS—2.5 mg) and α-methyltryptophan (α-MT—100 mg per 100 gm body weight) were administered 5 hr before assay. Pyridoxine (100 mg per 100 gm) was administered twice at 2-hr intervals and livers were assayed 2 hr after the last injection.

* *P* value = < 0.05.

† *P* value = < 0.01.

this increase was less in irradiated rats than in the starved rats. The response of fed, NEDH rats to pyridoxine was less than that of SD rats and therefore was not tested with irradiation. Thus, irradiation appeared to inhibit the response of tyrosine transaminase to both α-methyltryptophan and pyridoxine, when compared with the appropriate starved control rats.

Table 5 compares the effect of starvation itself on the responses of the two enzymes studied. Since no significant differences were observed with 1 or 2 days of starvation, these values were combined. The level of tryptophan pyrrolase with any treatment was never higher, but was often lower, in starved than in fed rats. The level of tyrosine transaminase with all treatments was higher in starved than in fed rats. The largest effect was with hydrocortisone. Starvation enhanced the elevation of tyrosine transaminase by hydrocortisone 2.5 times in NEDH rats, and 3.5 times in SD rats. The differential effect of starvation on the accumulation of these two enzymes has not been noted previously, and the underlying mechanism requires further study.

Discussion.—Reports are varied about the effects of irradiation on enzyme synthesis in microorganisms and animals. The studies in microorganisms are complicated by the influence of the irradiation on growth in general, and this may be the reason for reports of both inhibition²² and noninhibition²³ of substrate-induced enzyme synthesis by radiation. In higher animals there are additional complications. Some of the effects reported are due to the side effects of irradiation. Like other stressful treatments, radiation stimulates the pituitary-adrenal system, and in this indirect way can influence those enzymes whose levels are controlled by the glucocorticoids. Thus, irradiation altered carbohydrate metabolism in the rat, and this was abolished by hypophysectomy.²⁴ Irradiation also raised the levels in rat liver of alanine aminotransferase²⁵ and tryptophan pyrrolase,²⁶ but adrenalectomy eliminated at least the effect on the latter enzyme. Casein hydrolysate given to intact, protein-depleted rats raises the level of serine dehydrase, and this rise was inhibited by irradiation.²⁷ Radiation also interfered with the synthesis of some enzymes during liver regeneration.²⁸ The elevation of tryptophan pyrrolase by cortisone was not inhibited,²⁹ in agreement with the present results, and the elevation of drug-metabolizing enzymes by phenobarbital was also unchanged by prior

TABLE 5
EFFECT OF STARVATION ON THE REGULATION OF THE LEVELS OF TRYPTOPHAN PYRROLASE AND TYROSINE TRANSAMINASE IN ADRENALECTOMIZED RATS

Strain	Substance administered and time (hr)	Fed		Starved		Starved/Fed TP
		TP	TT	TP	TT	
NEDH	None	3.9 ± 1.0 (4)	66.4 ± 13.0 (5)	1.9 ± 0.5 (7)	73.0 ± 20.0 (7)	0.5
NEDH	α-MT, 5	14.9 ± 2.6 (8)	145.0 ± 22.0 (13)	7.8 ± 2.0 (21)	224.0 ± 76.0 (20)	1.1
NEDH	HCS, 5	13.8 ± 1.7 (7)	227.0 ± 40.0 (10)	14.4 ± 3.4 (10)	555.0 ± 53.0 (10)	0.5
SD	None	3.0 ± 4.0 (7)	43.0 ± 15.0 (11)	3.0 ± 0.2 (6)	65.0 ± 16.0 (6)	1.0
SD	Pyridoxine, 4	3.9 ± (3)	196.0 ± 45.0 (8)	3.9 ± 0.4 (13)	265.0 ± 68.0 (21)	1.5
SD	HCS, 5	24.8 ± 2.8 (4)	275.0 ± 50.0 (4)	16.9 ± 6.0 (13)	950.0 ± 230.0 (17)	1.0
						0.7

Substances were administered, as in Table 4, to adrenalectomized rats that were fed or starved for 24–48 hr. The last 2 columns represent the ratio of the values in "starved" and "fed" rats for tryptophan pyrrolase (TP) and tyrosine transaminase (TT).

irradiation of the animals.³⁰ Radiation given after hydrocortisone enhanced the elevation of tyrosine transaminase by hydrocortisone,²⁷ an effect like that attributed here to starvation.

While a general assessment of the biological effects of irradiation must include its stimulation of adrenal secretion and its decrease of food intake, these must be controlled in studies like the present one that attempts to distinguish the specific effects of radiation from its side effects on the physiological state of the animals. In the present experiments all the rats were adrenalectomized to avoid stimulated secretion of glucocorticoids. They were also deprived of food and compared with appropriately starved controls. In addition, the responses to treatment were tested when the immediate effects of the irradiation were over, and any permanent damage to the systems synthesizing macromolecules was established.

The results revealed an unknown difference between the two enzymes measured. Starvation alone enhanced the elevation of tyrosine transaminase by both the substrate and hormone types of regulators, especially by hydrocortisone, while the responses of tryptophan pyrrolase were unchanged or diminished in starvation. The effect of starvation was not mediated by the adrenals, since it occurred in adrenalectomized rats. Whatever the mechanism of the starvation effect, it is apparent that starved as well as adrenalectomized controls are essential for such irradiation studies as these, since the irradiated rats eat very little, and the lack of food alone alters the control of the two enzymes.

When compared with the appropriate starved controls, radiation did not affect the response of either enzyme to hydrocortisone, but it did significantly diminish the responses of both enzymes to the substrate-type regulators, α-methyltryptophan and pyridoxine. Since both enzymes in the irradiated rats can respond to the one type of regulator, but not to the other, the irradiation has specifically altered one, but not the other mechanism controlling these enzyme levels. Table 6 summarizes the effects of metabolic inhibitors on the different ways of accumulating these specific enzymes, and reveals three patterns of effects. Puromycin is the only agent which acts uniformly: it inhibits both of the ways by which the two enzymes are elevated, since each re-

TABLE 6
EFFECT OF INHIBITORS ON THE ACCUMULATION *in vivo* OF LIVER TRYPTOPHAN
PYRROLASE AND TYROSINE TRANSAMINASE

Inhibitor	Substrate-type elevation	Hormone-type elevation
Puromycin*	Inhibits	Inhibits
Actinomycin*	No effect	Inhibits
Irradiation†	Inhibits	No effect

* Results of Greengard, Smith and Acs,³ and Greengard and Gordon.⁴

† Results of this paper.

quires protein synthesis *de novo*.^{3, 4, 31} The action of actinomycin is strikingly different: it specifically inhibits the elevation of these enzymes by hormones; it does not interfere with their elevation by substrates. Irradiation appears to be a new kind of agent: a specific inhibitor of the elevations of liver enzymes by substrate-type compounds; it does not interfere with the elevations by hormones.

In addition to identifying the new kind of inhibition of enzyme accumulation that irradiation exerts, these results also distinguish the specific effect of irradiation from that of the "radiomimetic" compounds like actinomycin. Earlier morphological evidence also indicated that the intracellular damage caused by radiomimetic substances was not necessarily identical to that caused by ionizing radiation.³² The observed potentiation of the therapeutic effects of radiation by actinomycin¹¹⁻¹³ is understandable in terms of these results: the two agents act at different sites and so, when combined, they interfere additively with different mechanisms that regulate enzyme synthesis.

Summary.—The elevation of liver enzymes by the administration of various substances was studied in adrenalectomized rats after whole-body X irradiation. Because irradiation alone almost eliminates food intake, irradiated rats were starved and compared to fed and starved adrenalectomized controls. Starvation greatly enhanced (2.5- to 3.5-fold) the response of tyrosine transaminase to hydrocortisone and increased its response to α -methyltryptophan and pyridoxine. Starvation did not enhance the response of tryptophan pyrrolase to any regulators. Irradiation did not diminish the response of either enzyme to hydrocortisone, but decreased the response of both enzymes to substrate-type compounds. The increase of tryptophan pyrrolase by its substrate analogue was abolished after irradiation with 1600 r. The increase of tyrosine transaminase by α -methyltryptophan and pyridoxine was significantly inhibited by 800 r. These effects are different from that of puromycin, which inhibits both types of elevation of these enzymes, and different from actinomycin, which inhibits only the hormonal type of elevation. Irradiation, by inhibiting the substrate-type but not the hormone-type of elevation of these two enzymes, has an effect opposite to that of actinomycin.

The authors gratefully acknowledge the technical assistance of Miss Bonnie B. Spanier.

* This investigation was supported by U.S. Public Health Service grants AM 00567 and CA 07037, by Research Career Award AM-K6-2018 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, and by the U.S. Atomic Energy Commission contract AT(30-1)-901 with the New England Deaconess Hospital (NYO-901-60). An abstract of some of these findings is found in *Federation Proc.*, **25**, 285 (1966).

¹ Civen, M., and W. E. Knox, *J. Biol. Chem.*, **234**, 1787 (1959).

² Lin, E. C. C., and W. E. Knox, *Biochim. Biophys. Acta*, **26**, 85 (1957).

³ Greengard, O., M. A. Smith, and G. Acs, *J. Biol. Chem.*, **238**, 1548 (1963).

- ⁴ Greengard, O., and M. Gordon, *J. Biol. Chem.*, **238**, 3708 (1963).
- ⁵ Greengard, O., *Advan. Enzyme Regulation*, **1**, 61 (1963).
- ⁶ Weber, G., R. L. Singhal, and N. B. Stamm, *Science*, **142**, 390 (1963).
- ⁷ Greengard, O., M. Gordon, M. A. Smith, and G. Acs, *J. Biol. Chem.*, **239**, 2079 (1964).
- ⁸ Salas, M., E. Vinuela, and A. Sols, *J. Biol. Chem.*, **238**, 3535 (1963).
- ⁹ Philips, F. S., H. S. Schwartz, S. S. Sternberg, and C. T. C. Tan, *Ann. N.Y. Acad. Sci.*, **89**, 348 (1960).
- ¹⁰ Farber, S., in *Ciba Foundation Symposium on Amino Acids and Peptides with Antimetabolic Activity*, ed. G. E. W. Wolstenholme and C. M. O'Connor (1958), p. 138.
- ¹¹ D'Angio, G. J., S. Farber, and C. L. Maddock, *Radiology*, **73**, 175 (1959).
- ¹² Maddock, C. L., G. J. D'Angio, S. Farber, and A. H. Handler, *Ann. N.Y. Acad. Sci.*, **89**, 386 (1960).
- ¹³ D'Angio, G. J., C. L. Maddock, S. Farber, and D. L. Brown, *Cancer Res.*, **25**, 1002 (1965).
- ¹⁴ Knox, W. E., M. M. Piras, and K. Tokuyama, *J. Biol. Chem.*, **241**, 297 (1966).
- ¹⁵ Lin, E. C. C., B. M. Pitt, M. Civen, and W. E. Knox, *J. Biol. Chem.*, **233**, 668 (1958).
- ¹⁶ Greengard, O., and P. Feigelson, *J. Biol. Chem.*, **236**, 158 (1961).
- ¹⁷ Greengard, O., *Biochim. Biophys. Acta*, **85**, 492 (1964).
- ¹⁸ Knox, W. E., and M. M. Piras, *J. Biol. Chem.*, **241**, 764 (1966).
- ¹⁹ Greengard, O., N. Mendelsohn, and G. Acs, *J. Biol. Chem.*, **241**, 304 (1966).
- ²⁰ Denckla, W. D., and H. K. Dewey, *J. Lab. Clin. Med.*, in press.
- ²¹ Rosen, F., and R. J. Milholland, *J. Biol. Chem.*, **238**, 3730 (1963).
- ²² Pollard, E. C., *Science*, **146**, 927 (1964).
- ²³ Baron, L. S., S. Spiegelman, and H. Quastler, *J. Gen. Physiol.*, **36**, 631 (1953).
- ²⁴ Weber, G., and A. Cantero, *Am. J. Physiol.*, **197**, 1284 (1959).
- ²⁵ Brin, M., and R. W. McKee, *Arch. Biochem. Biophys.*, **61**, 384 (1956).
- ²⁶ Thomson, J. F., and E. T. Mikuta, *Proc. Soc. Exptl. Biol. Med.*, **85**, 29 (1954).
- ²⁷ Pitot, H. C., C. Peraino, and C. Lamar, Jr., *Science*, **150**, 901 (1965).
- ²⁸ Myers, D. K., *Can. J. Biochem. Physiol.*, **40**, 619 (1962).
- ²⁹ Thomson, J. F., and F. J. Kliptel, *Proc. Soc. Exptl. Biol. Med.*, **95**, 107 (1957).
- ³⁰ Kato, R., G. Frontino, and P. Vassanelli, *Japan. J. Pharmacol.*, **11**, 25 (1961).
- ³¹ Nemeth, A. M., and G. De La Haba, *J. Biol. Chem.*, **237**, 1190 (1962).
- ³² Warren, S., M. H. Holt, and S. C. Sommers, *Am. J. Clin. Pathol.*, **22**, 411 (1952).