change may be antecedent to a threatened "break" in the tolerant status of the host. Obviously, more information is needed on thymic function in immunological deviations.

Summary. The LCM virus penetrated the "germfree barrier," thus reaffirming the congenital dissemination of the virus. The lesions associated with LCM infection in the gnotobiotic mice were clearly related to a hyperreactive immunologic mechanism, which induced degenerative kidney changes, depletion of cortical thymocytes, lymphoid cell infiltrations of many organs, and hypergammaglobulinemia.

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1. Traub, E., J. Exptl. Med. 63, 533 (1936).
2. Traub, E., J. Exptl. Med. 63, 847 (1936).
3. Traub, E., J. Exptl. Med. 68, 229 (1938).
4. Hotchin, J., Public Health Service Publ. No. 1378, 341 (1965).
5. Rowe, W. P., Naval Med. Res. Inst., Res. Report Project NM 005-048-14-01 12, 167 (1954).
6. Haas, V. H. and Stewart, S. E., Virology 2, 511 (1956).
7. Levey, R. H., Trainin, N., Law, L. W., Black, P. H., and Rowe, W. P., Science 142, 483 (1963).
8. Pollard, M. and Matsuzawa, T., Proc. Soc. Exptl. Biol. Med. 116, 967 (1964).
9. Pollard, M., Kajima, M., and Teah, B. A., Proc. Soc. Exptl. Biol. Med. 120, 72 (1965).
10. Kajima, M. and Pollard, M., J. Bacteriol. 90, 1448 (1965).
11. Reyniers, J. A. and Trexler, P. C., "Micrurgical and Germfree Methods," p. 114. Thomas, Springfield, Illinois, 1943.
12. Wagner, M., Ann. N. Y. Acad. Sci. 78, 89 (1959).
13. Bealmear, M. and Wilson, R., Anat. Record 154, 2 (1966).
14. Hotchin, J. and Weigand, H., J. Immunol. 86, 392 (1961).
15. Gordon, H. A. and Wostmann, B. S., Anat. Record 137, 65 (1960).
16. Helyer, B. J. and Howie, J. B., Brit. J. Haematol. 9, 119 (1963).
17. East, J. and deSousa, M. A. B., Natl. Cancer Inst. Monograph No. 22, (1965).
18. Klemperer, P., Pollack, A. D., and Baehr, G., Arch. Pathol. 32, 569 (1941).
19. Hartsough, G. R. and Gorham, J. R., Natl. Fur News 28, 10 (1956).
20. Pollard, M., Nature 213, 142 (1967).

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The Effect of Glucagon and Insulin on the Prothrombin Response to Coumarin Anticoagulants* (32794)

MURRAY WEINER AND DORIS MOSES
New York University Research Service, Goldwater Memorial Hospital, Welfare Island, New York, New York 10017

The clinical observation that poor nutrition may exaggerate the prothrombin response to coumarin anticoagulants has been readily reproduced in controlled experiments with short periods of starvation in guinea pigs (1). Further studies have indicated that deficiency in protein rather than caloric intake was a responsible factor (2). The effect is not related to an altered fate of the coumarin drug as reflected in its blood levels (1).

Recent studies by Greengard and Baker (3) point out that the administration of glucagon mimics the effect of starvation on several enzymes which play a role in tryptophan and tyrosine metabolism. The present report presents data indicating that glucagon also mimics starvation in its effect on the prothrombin response to the anticoagulant, acenocoumarin. Insulin has no significant effect on sensitivity to acenocoumarin, but blocks the hypersensitivity induced by glucagon.

Methods. Guinea pigs weighing 279–580 gm were fed with Purina guinea pig chow *ad libitum,* supplemented by fresh lettuce before

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TABLE I. Effect of Glucagon on the Prothrombin Time Response to Acenocoumarin in Guinea Pigs.

	Intraperitoneal doses days 1 and 2				Prothrombin time[a] on day 3 (sec)	
	Saline (0.4 ml)	Glucagon (10 mg/kg)	Protamin Zn insulin (1.7 units/kg)	Acenocou. (25 mg/kg)	Average	Range (N=9)
Series A	+	+	—	—	35.1	32– 38
	+	—	—	+	75.3	55– 92
	—	+	—	+	151.1	101–180
Series B	+	—	—	+	75.2	57– 97
	—	+	—	+	100.5	73–131
	—	—	+	+	73.1[b]	56– 82
	—	+	+	+	70.7	53– 96

[a] Normal untreated guinea pig plasma prothrombin time ranges from 31 to 39 sec (1,2). Prothrombin times were not monitored beyond 180 sec. Longer values in the third group of Series A were taken as 180 sec in calculating averages.

[b] Average of 6 animals. Three of the 9 died before the experiment was completed.

the study. Groups of 3 animals each were studied in parallel and the results of 3 such studies were pooled. On days 1 and 2, all animals were given intraperitoneal injections of acenocoumarin, 25 mg/kg and either saline, glucagon 10 mg/kg, or protamine zinc insulin 1.7 units/kg. Controls without acenocoumarin, and combined glucagon and insulin administration were also studied. On day 3 all animals were sacrificed as previously described (1), and the prothrombin time of plasma from cardiac blood was determined. In some studies food consumption was measured daily to determine whether any of the treatments significantly altered food intake.

The effect of glucagon on acenocoumarin plasma levels was studied by determining the drug concentration exactly 1.5 hours after a single intraperitoneal dose (25 mg/kg) as previously described (4), with or without glucagon pretreatment (10 mg/kg per day) on the previous 2 days.

Results. Effect of glucagon on the prothrombin response. Table I, Series A summarizes the pooled results of three separate experiments, each of which was performed with 9 animals (3 animals per group). In all three experiments treatment with glucagon clearly enhanced the prothrombin response to acenocoumarin. Animals treated with glucagon alone showed normal prothrombin activity. In one of the three studies daily food consumption was also determined. While there was considerable variation in the daily consumption of each animal, there were no differences between the groups.

The data in Series B again demonstrates the effect of glucagon in enhancing the response to acenocoumarin. However, this effect is eliminated by the concomitant administration of insulin. All 9 animals given glucagon in addition to insulin survived and were clinically normal. In contrast, the same dose of insulin alone caused death in 3 of the 9 animals and clinical signs of toxicity in the other 6. The surviving 6 animals showed the normal prothrombin response to acenocoumarin.

In all experiments the prothrombin time of 12.5% saline diluted plasma was determined as well as the undiluted plasma prothrombin times recorded in Table I. Without exception the 18 animals treated with glucagon and acenocoumarin showed diluted plasma prothrombin times longer than the cutoff time of 180 sec. In contrast, none of the animals treated with acenocoumarin alone, or with both insulin and glucagon in addition to acenocoumarin failed to clot in less than 180 sec.

Effect of glucagon on acenocoumarin plasma levels: The data in Table II indicate that glucagon pretreatment has no significant effect on acenocoumarin concentration in plasma 1.5 hours after dosage.

Discussion and Summary. The response to drugs such as the coumarin anticoagulants

TABLE II. Acenocoumarin Plasma Levels 1.5 Hours After Acenocoumarin. (25 mg/kg ip; 4 guinea pigs/group)

Pretreatment for 2 days	Acenocoumarin concentration (mg/liter) Average	Range
Saline (0.4 ml/day)	23.1	17.8–26.4
Glucagon (10 mg/kg per day)	24.3	18.8–34.1

may be altered by factors which either affect the physiologic disposition of the drug, or the site at which the drug acts. Clues as to the nature of the drug action may be obtained by studying the factors which influence sensitivity to the drugs without altering the fate of the drug.

The effect of Vitamin K in blocking the action of coumarin anticoagulants is well documented, and is not correlated with changes in the physiologic disposition of coumarins as reflected in plasma levels of the drug. In contrast, the effect of barbiturates is mediated through altered disposition of the coumarins (1,4). The tranquilizers chlorpromazine and reserpine exaggerate prothrombin response while not influencing plasma levels of the coumarin drug. This effect may be correlated with the action of these agents on NAD synthesis (5).

The mechanism by which starvation causes an exaggerated response to coumarins is not known. Elements of protein origin are probably involved (2). The finding of a similar effect by glucagon suggests that the metabolic fate of tyrosine and/or tryptophan may be important to prothrombin synthesis. The fact that saline diluted plasma demonstrates the glucagon effect at least as well as undiluted plasma indicates an exaggerated prothrombin deficiency rather than a superimposed heparin-like effect.

The observation that insulin has no detectable effect on sensitivity to acenocoumarin, but can block the sensitizing effect of glucagon may have clinical significance in helping to explain the "unpredictable" manner in which sensitivity to anticoagulants may be altered by an agent under one set of conditions, and not under another.

It remains to be determined whether the effect of starvation on sensitivity to coumarin anticoagulants is mediated through a stimulation of endogenous glucagon production during starvation.

1. Chenkin, T., Dayton, P. G., Weisberg, L. G., and Weiner, M., Exptl. Med. Surg. 17, 219 (1959).
2. Weiner, M. and Moses, D., Proc. Soc. Exptl. Biol. Med. 125, 277 (1967).
3. Greengard, O. and Baker, G. T., Science 154, 1461 (1966).
4. Dayton, P. G., Tarcan, Y., Chenkin, T., and Weiner, M. J., J. Clin. Invest. 40, 1797 (1961).
5. Weiner, M., Nature 212, 1599 (1966).

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Liver Insulinase Activity in Insulin Deficient Rats* (32795)

CARL R. MORGAN AND HAROLD J. WIESMAN
Department of Anatomy, Indiana University Medical Center, Indianapolis, Indiana 46202

The term "insulinase" was proposed by Mirsky and Broh-Kahn, (1) for the enzyme or enzyme system which preferentially degrades insulin. Initially the *in vitro* insulinase activity of rat liver was determined by measuring the blood sugar fall in rabbits injected with insulin solutions previously incubated with rat liver homogenates under specified conditions. Using this method, Broh-Kahn and Mirsky (2) observed a marked reduction in liver insulinase activity in fasted rats.

Insulin-^{131}I provides a much more sensitive method for quantitating the degradation of insulin (3). The insulin-^{131}I and insulin are degraded equally well and competitively (4).

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