partially account for the observed difference between infused and expired carbon-14.

About 33% of the radioactivity found in the urine was in the form of CO_2. However, identification of compounds containing the remaining radioactivity was not made. Rust *et al*(13) found radioactivity in urea of the urine of rats following injections of sodium bicarbonate-14.

The close agreement between the half-life of blood CO_2 and respiratory CO_2 calculated from the exponential part of the curve (Fig. 1 and 2) suggest that blood CO_2 data may be used in studying oxidative rates of substrates. This would eliminate the respiratory stress of the animal due to the apparatus needed to collect expired air.

The turnover rate was constant for the 3 experiments with a mean of 9.58 meq/hr/kg of body weight. The mean CO_2 space was 0.414 liter/kg body weight, which is about twice the extracellular volume. The amount of radioactivity found in the rumen would indicate that the rumen must be considered part of the CO_2 space.

Summary. The carbon dioxide pool system of sheep was evaluated by the constant infusion of sodium bicarbonate-C^{14} for a period of 4 hours. The specific activity of blood CO_2 plateaued during the final hour of infusion. Following infusion, the half-lives of blood and expired CO_2 were 32.6 and 31 minutes, respectively. Mean turnover rate, turnover time and CO_2 space were 9.58 meq/hr/kg body weight, 46.8 minutes and 41.4% of body weight, respectively. Considerable radioactivity was found in the rumen at the end of the infusion period. These observations suggest a time of at least 3 hours for complete labeling of the CO_2 pool, of which the rumen is a part, and that blood CO_2 data may be used as well as expired CO_2 data for substrate oxidation studies.

1. Annison, E. F., Lindsay, D. B., Biochem. J., 1961, v78, 777.

2. Black, A. L., Kleiber, M., Biochem. Biophys. Acta, 1957, v23, 59.

3. Kleiber, M., Smith, A. H., Black, A. L., Brown, M. A., Tolbert, B. M., J. Biol. Chem., 1952, v197, 371.

4. Sabine, J. R., Johnson, B. Connor, ibid., 1964, v239, 89.

5. Essig, H. W., Norton, H. W., Johnson, B. Connor, Proc Soc. Exp. Biol. and Med., 1961, v108, 194.

6. Morris, B., Simpson-Morgan, M. W., J. Physiol., 1963, 169, 713.

7. Gupta, J., Crump, M. H., Nichols, R. E., Am. J. Vet. Res., 1962, v23, 201.

8. Harlan, J. W., Atomlight (New England Nuclear Corp.), 1961, v19, 8.

9. Bray, G. A., Anal. Biochem., 1960, v1, 279.

10. Oppermann, R. A., Nystrom, R. F., Nelson, W. O., Brown, R. E., Intern. J. Appl. Radiation and Isotopes, 1959, v7, 38.

11. Ash, W., Dobson, A., J. Physiol., 1963, v169, 39.

12. Carroll, E. J., Hungate, R. E., Arch. Biochem. Biophys., 1955, v56, 525.

13. Rust, J. H., LeRoy, G. V., Spratt, J. L., Ho, G. B., Roth, L. J., Radiation Res., 1963, v20, 703.

Received May 10, 1965. P.S.E.B.M., 1965, v120.

Effects of Diphenylhydantoin upon the Plasma Free Fatty Acid Response Induced by Hormones.* (30490)

ANTHONY C. CHUNG† (Introduced by J. C. Houck)

Surgical Research Metabolic Laboratory, Georgetown University Hospital, Washington, D.C.

Administration of sodium bi-phenyl hydantoinate (Dilantin) to rats resulted in changes of dermal concentrations of collagen, scleroprotein and hexosamine(1,2). It was demonstrated that reductions of dermal triglyceride and phospholipid concentrations were a result of Dilantin administration(3).

* This investigation is supported in part by U.S.P.H.S. Grant HE 07395-02 and the Hartford Foundation.

† Present address: Adolescent Medicine, Children's Hospital, Washington, D.C.

Similar effects of Dilantin upon the concentration of rat liver and aorta lipids were also described(4). The present report deals with the effects of Dilantin administration alone and together with various hormones upon the concentration of plasma free acid (FFA) in rats.

Methods. Forty-eight male Wistar rats, weighing 250-320 g, were divided into 2 groups of 24 rats each. One group received intraperitoneally 75 mg/kg of Dilantin suspended in isotonic saline; the other group received an equal volume of isotonic saline alone to serve as control. Two hours after Dilantin or saline injection, 6 animals each from both the Dilantin-treated and saline control groups received 75 mg/kg (Upjohn Co.) of growth hormone subcutaneously. Three hours after Dilantin or saline injection, 6 animals from each group received 1 mg/kg of epinephrine in oil (Parke-Davis) subcutaneously and 6 other animals from each group received 0.2 mg/kg of glucagon (Eli Lilly crystalline glucagon, Lot no. 258-234B-167-1) intraperitoneally. Four hours after Dilantin or saline injection, all animals were sacrificed. Thus, 6 animals from both the Dilantin-treated and the control group had not received any hormone. Blood samples were obtained 4 hours after Dilantin or saline injection by heart puncture while the animals were under chloroform anaesthesia. Heparin was used as an anticoagulant. Blood tubes were put in ice and centrifuged in a refrigerated centrifuge (10°C) at 600 *g* for 30 minutes. Plasma free fatty acid (FFA) was determined according to Dole(5).

In the second experiment, 24 Wistar male rats weighing 260-310 g were fasted for 20 hours. They were divided into 2 groups of 12 animals each. One group of animals received intraperitoneally 75 mg/kg of Dilantin suspended in isotonic saline; the other group received an equal volume of isotonic saline alone to serve as control. Three hours after Dilantin or saline injection, 6 animals each from both Dilantin-treated and saline control groups received 1 IU/kg insulin (Eli Lilly) intramuscularly. All animals were sacrificed 4 hrs after the Dilantin or saline injection. Blood plasma samples were obtained and FFA determined as mentioned above.

TABLE I. Effect of Dilantin upon Alteration in Rat Plasma Free Fatty Acid Levels (μmole/l)† Induced by Hormones.

Hormone	Control	Dilantin
Normal	309 ± 45.3	429 ± 37.0
Epinephrine	*569 ± 60.7	*939 ± 69.8
Glucagon	312 ± 46.0	*576 ± 72.0
Growth hormone	*594 ±219	*756 ±148

Underlined: Significantly different from appropriate control ($P < .05$).
* Significantly different from normal ($P < .05$).
† Mean ± standard deviation.

For the third experiment, 18 Wistar male rats, weighing 245-315 g, were divided into 3 groups of 6 rats each. Groups 1, 2 and 3 received 15 mg/kg, 45 mg/kg and 75 mg/kg of Dilantin respectively. The concentrations of Dilantin suspended in isotonic saline were different, but the volumes injected intraperitoneally were similar. Three hours after Dilantin injection all animals received 1 mg/kg of epinephrine subcutaneously. They were sacrificed 1 hour later. Plasma FFA was determined as mentioned previously.

Results and discussion. Intraperitoneal administration of Dilantin caused an increase in plasma FFA concentration in both fed and fasted rats. The normal (control) values for fed and fasted rats were 309 ± 45.3 and 429 ± 43.5 μmole/l, whereas the values for Dilantin-treated rats were 429 ± 37 and 525 ± 42.9 μmole/l respectively (Tables I and II). The doses of Dilantin used in these studies were approximately the same as used in previous studies on rat skin, liver and aorta(1,2,3,4).

The combined effects of Dilantin and hormones upon rat plasma FFA concentrations are shown in Table I. It will be noted that

TABLE II. Effect of Dilantin and Insulin upon Plasma Free Fatty Acid Concentration (μmole/l)† in Fasted Rats.

	Control	Dilantin
Normal	429 ± 43.5	525 ± 42.9
Insulin	*307 ± 27.7	*255 ± 77.3

Underlined: Significantly different from appropriate control ($P < .05$).
* Significantly different from normal ($P < .05$).
† Mean ± standard deviation.

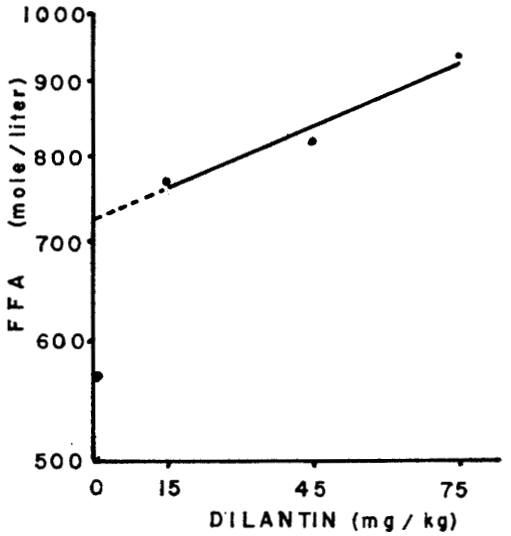

FIG. 1. Effect of Dilantin upon FFA response to epinephrine (semi-log plot).

when Dilantin was given with epinephrine, growth hormone and glucagon, the increments in FFA concentration were greater than those when these hormones were given alone. It was also observed that the plasma FFA increment caused by a combination of Dilantin and epinephrine was much greater than the sum of the increments due to epinephrine and Dilantin alone. The same observation was also true for Dilantin and glucagon. On the other hand, the plasma FFA increment due to the combined effect of growth hormone and Dilantin was very close to the sum of the increments due to growth hormone and Dilantin separately. The data suggest that Dilantin might exert a synergistic effect with epinephrine and glucagon.

The effect of Dilantin on plasma FFA was reversed when insulin was also administered. The combination of the two caused a greater decrease in FFA concentration than that caused by insulin alone (Table II).

The plasma FFA concentrations of groups of rats given 15, 45 and 75 mg/kg of Dilantin in combination with a standard dose of epinephrine (1 mg/kg) were 769 $\pm$ 117, 822 $\pm$ 103 and 939 $\pm$ 69.8 μmole/l respectively. These values were all significantly different ($P<.05$) from the control value (epinephrine alone) of 569 $\pm$ 60.7 μmole/l. When these values were plotted on a semi log scale (Fig. 1) the values fell almost on a straight line with the exception of the control value which fell far below the extrapolated line. These results demonstrated the adipokinetic action of Dilantin.

Evidence has been presented that triglyceride is mobilized from adipose tissue in the form of FFA which circulates as an albumin-FFA complex(5,6). The adipokinetic action of hormones has been reviewed by Vaughan (7) and Rudman(8). It was reported in the rat adipose tissue that when lipolysis was stimulated by epinephrine, ACTH, glucagon or TSH the rate of fatty acid esterification was also increased: but not to the same extent of lipolysis. Growth hormone showed a relatively small effect on lipolysis and no effect on esterification(9). With our present limited knowledge it is not possible meaningfully to propose a mode of action for Dilantin on lipid metabolism. In view of the apparent synergism demonstrated by Dilantin with respect to those hormones whose site of action has been shown to be the adipose tissue, it does not appear unreasonable to suggest that Dilantin increases FFA release from the adipose tissue. This proposal is not inconsistent with previous observations on loss of lipids (mainly triglyceride) from liver, aorta and skin of rats receiving Dilantin(3,4). Elucidation of the mechanism of the Dilantin effect upon the adipose tissue must await further *in vitro* studies with adipose tissue.

Summary. Dilantin administered intraperitoneally to fed and fasted rats caused a significant increase in plasma FFA. When Dilantin was given in combination with epinephrine or glucagon, a synergistic effect in increasing plasma FFA was observed. An additive but not synergistic effect was observed with growth hormone. The plasma FFA elevating effect of Dilantin was reversed when insulin was also administered to rats.

1. Houck, J. C., Jacob, R. A., Maengwyn-Davies, G. D., J. Clin. Invest., 1960, v39, 1758.

2. ———, J. Invest. Dermatol., 1963, v40, 89.

3. Chung, A. C., Houck, J. C., Proc. Soc. Exp. Biol. and Med., 1962, v109, 454.

4. Chung, A. C., Duren, B. Y., Houck, J. C., ibid., 1962, v110, 788.

5. Dole, V. P., J. Clin. Invest., 1956, v35, 150.

6. Gordon, R. S., Jr., Cherkes, A., ibid., 1956, v35, 206.
7. Vaughan, M., J. Lipid Res., 1961, v2, 293.
8. Rudman, D., ibid., 1963, v4, 119.
9. Vaughan, M., Steinberg, D., ibid., 1963, v4, 193.

Received May 14, 1965. P.S.E.B.M., 1965, v120.

Influence of Neurohumors on Anterior Pituitary Lactogen Production *in vitro*.* (30491)

RICHARD R. GALA AND RALPH P. REECE
Department of Animal Sciences, New Jersey Agricultural Experiment Station, New Brunswick

In summarizing the literature on characteristics of a neurohumor, Harris(1) concluded that in all probability a neurohumor would be of low molecular weight. *In vivo* experiments suggest that neurohumors may also be involved in control of pituitary lactogen production. Epinephrine administration induces deciduomata formation(2), initiates lactation(3,4,5), retards mammary involution (3,4), and decreases pituitary lactogen content(6). Similar results have been obtained with acetylcholine(3,5) and serotonin(5,6,7). The results of some experiments(8,9) have been interpreted as indicating that oxytocin stimulated lactogen release, whereas results of lactogen assays(10,11), both *in vivo* and *in vitro,* indicate that oxytocin does not stimulate lactogen release. The investigation reported herein was conducted to determine the effect of various neurohumors on lactogen production *in vitro* and a preliminary report appeared elsewhere(12). Neurohumor is used as a general term to describe chemical entities produced by neural tissue that may act either in their immediate area of production or pass into circulation and act at some area distant to site of synthesis.

Materials and methods. Anterior pituitaries (AP) were removed aseptically from female, hooded Norway rats weighing 165 to 175 g and divided into 4 pieces. A piece from each AP was placed in each of 4 groups until a total of one AP was present per culture dish. The culture techniques were similar to those previously reported(13) and AP were cultured at 35 ± 1.0°C for 3 days. The neurohumors, serotonin, acetylcholine, epinephrine, and norepinephrine were added at a level of 1, 10, and 100 μg/ml; Pitocin and Pitressin were added to the medium at a level of 1, 10, and 100 mU/ml. Control cultures were run simultaneously with all neurohumors, with 6 to 8 culture dishes per group.

The lactogen content of the medium was determined as previously described(14),† and representative pituitary fragments from each group were examined histologically and the degree of viable tissue rated from zero to 4 (13). Differences between means were assessed statistically using Student's *t* test(15).

Results. Addition of serotonin to the medium did not alter lactogen production from that of the control medium. The lactogen content of cultured tissue, however, was significantly increased by 1 μg of serotonin but not by 10 or 100 μg. Neither acetylcholine nor norepinephrine significantly altered lactogen content of the medium, lactogen content of cultured AP tissue, or AP viability ratings. Epinephrine at 1 μg/ml increased lactogen production 43%, a significant increase, but the other 2 levels of epinephrine did not significantly alter lactogen production. Lactogen content of cultured AP tissue was significantly decreased by the 100 μg level of epinephrine but not by 1 or 10 μg. Addition of Pitressin to the medium did not alter the parameters examined. The addition of either 1 or 10 mU of Pitocin did not influence lactogen production or the lactogen content of cultured AP. Addition of 100 mU, however, significantly increased (53%) lactogen production (Table I).

Discussion. Although serotonin adminis-

* Paper of the Journal Series, New Jersey Agric. Exp. Station, Rutgers, The State University.

† The prolactin used in this study was a gift from the Endocrinology Study Section, N.I.H.