ACTH-induced steroid production depending upon dosage. These effects have been reported from long term (>30 min) single time interval observations. Therefore, the present study was designed to elucidate the short term temporal effects of these prostaglandin inhibitors upon basal and ACTH-stimulated glucocorticoid production by human adrenocortical tissue.

Materials and Methods. 4 adult human female adrenal glands obtained at surgery were immediately placed in cold (0–4 °C) Krebs' Ringer bicarbonate buffer, KRBGA (pH 7.4, 200 mg glucose/dl, 0.5% serum albumin fraction V). Glands were diced (2 × 3 mm) and preincubated (37 °C) in KRBGA for 45 min. These dice then were incubated (1 ml KRBGA; 37 °C; 95% O_2 + 5% CO_2) in a Dubnoff metabolic shaker for 1–32 min. The dice were exposed to indomethacin (10 µg/ml), 7-oxa-13-prostynoic acid (50 µg/ml), porcine ACTH (100 mIU/ml; chromatographically pure; 150 IU/mg) or KRBGA alone. Further, dice were incubated initially (4 min) in ACTH, indomethacin or 7-oxa-13-prostynoic acid followed by transfer to ACTH plus the appropriate test substance. Cortisol secretion into the incubation medium was quantitated by RIA[9]. Proteins were determined[10] and the data expressed as ng cortisol/mg protein, $\bar{X} \pm$ SEM. A minimum of 4 replicates were used per datum point. Data were analyzed by analysis of variance and Student's t-test. Differences were accepted as significant when $p < 0.05$.

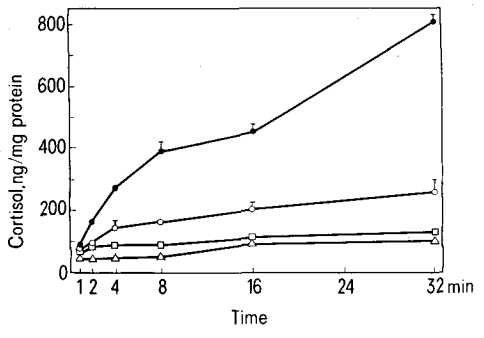

Fig. 1. Effects of preincubation (4 min) in either 7-oxa-13-prostynoic acid or indomethacin upon subsequent ACTH (100 mIU/ml) stimulated cortisol production compared to basal ACTH-stimulated cortisol production by human adrenocortical tissue. Closed circle, ACTH; open circle, KRBGA control; square, ACTH-following 7-oxa-13-prostynoic acid preincubation; triangle; ACTH-following indomethacin preincubation.

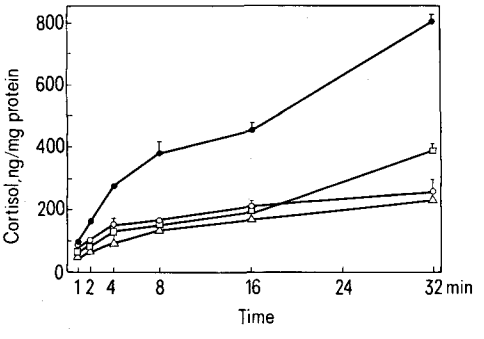

Fig. 2. Effects of 7-oxa-13-prostynoic acid or indomethacin on human adrenocortical cortisol production in response to ACTH-following preincubation (4 min) in ACTH alone (100 mIU/ml). Closed circle, ACTH; open circle, KRBGA control; square, 7-oxa-13-prostynoic acid following ACTH incubation; triangle, Indomethacin following ACTH-preincubation.

Results and Discussion. Indomethacin or 7-oxa-13-prostynoic acid alone do not significantly alter basal human adrenal cortisol production during the entire 32-min-interval studied. These results differ from those reported for feline adrenocortical cells incubated for a longer time interval[8]. However, the results are consistent with the observed inability of these inhibitors to alter basal human adrenocortical cAMP levels[2]. Preincubation (4 min) in either indomethacin or 7-oxa-13-prostynoic acid followed by transfer to a flask containing ACTH + test substance effectively blocks ACTH-stimulated steroid production for the entire 32-min-interval (figure 1). In fact, steroid production was significantly lower than the basal level during 4–32 min (figure 1). Interestingly, pretreatment with these inhibitors also depresses subsequent ACTH-stimulated cAMP levels during this interval[2]. Indomethacin pretreatment in vivo depresses steroid production following ACTH stimulation[1].

Preincubation (4 min) in ACTH followed by transfer to ACTH + 7-oxa-13-prostynoic acid or indomethacin significantly depressed steroid release compared to ACTH alone (figure 2), however, these levels were not depressed below basal levels as observed with preincubation in the inhibitor (figure 1). At 32 min (figure 2) the cortisol output of the indomethacin group was significantly higher than that of control or 7-oxa-13-prostynoic acid treated adrenals. Interestingly, application of these inhibitors following ACTH preincubation results in a supramaximal cAMP response compared to ACTH alone. However, this nucleotide response does not result in increased corticoid output (figure 2) suggesting that prostaglandins have effects on steroid release which are not cyclic nucleotide mediated. In addition, the present findings suggest consideration of possible temporally dissimilar prostaglandin modulation of the mechanism of ACTH action in the adrenal.

9 L. B. Foster and R. T. Dunn, Clin. Chem. *20*, 365 (1974).
10 K. V. Honn and W. Chavin, Analyt. Biochem. *68*, 230 (1975).

Increased liver calcium after calcium or milk gavage in rats[1]

R. A. Meyer, Jr, and Martha H. Meyer

Department of Basic Science, School of Dentistry, Marquette University, 604 North 16th Street, Milwaukee (Wisconsin 53233, USA), 27 September 1976

Summary. For 1 or 2 h following a gavage of milk or 300 mM $CaCl_2$ (2 ml/100 g b.wt), rats had an increased liver calcium content when compared to rats receiving a deionized water gavage.

Yamaguchi et al. have reported that calcitonin administration increases the calcium content of the liver of rats both in vivo[2] and in vitro[3]. In rats, calcitonin is secreted after ingestion of a meal[4] and is useful in preventing hypercalcemia after meals of milk[5] or dissolved $CaCl_2$[6]. If the increased liver calcium is of physiological significance, then there should be uptake of calcium by the liver during times of elevated secretion of calcitonin. The liver

Table 1. Changes after milk gavage[a]

Time after gavage	Liver Ca[b] H_2O	Milk	Plasma Ca[c] H_2O	Milk	Plasma P[c] H_2O	Milk
0.5 h	3.45 ± 0.09	3.72 ± 0.05**	2.57 ± 0.02	2.61 ± 0.03	2.45 ± 0.07	2.41 ± 0.07
1.0 h	3.56 ± 0.06	3.74 ± 0.08*	2.65 ± 0.05	2.66 ± 0.05	2.45 ± 0.07	2.46 ± 0.05
2.0 h	3.60 ± 0.08	3.71 ± 0.06	2.62 ± 0.02	2.66 ± 0.05	2.37 ± 0.04	2.44 ± 0.08
4.0 h	3.69 ± 0.09	3.57 ± 0.07	2.62 ± 0.05	2.66 ± 0.04	2.53 ± 0.08	2.49 ± 0.04

[a] Mineral levels at indicated times after water or reconstituted dry milk gavage (2 ml/100 g b.wt.) to intact rats. Each value is the mean ± SE for 8 rats. Asterisks denote significant differences between the water and milk groups for the same collection time: *$p < 0.05$, **$p < 0.01$. [b] μmole/g dry weight. [c] mmole/l.

Table 2. Changes after $CaCl_2$ gavage[a]

Time after gavage	Liver Ca H_2O	$CaCl_2$	Plasma Ca H_2O	$CaCl_2$	Plasma P H_2O	$CaCl_2$
0.5 h	3.49 ± 0.09	3.65 ± 0.12	2.51 ± 0.02	2.82 ± 0.07**	2.58 ± 0.10	2.49 ± 0.05
1.0 h	3.52 ± 0.07	3.78 ± 0.08*	2.64 ± 0.05	2.92 ± 0.07**	2.61 ± 0.10	2.35 ± 0.11*
2.0 h	3.50 ± 0.10	3.85 ± 0.06**	2.58 ± 0.11	2.90 ± 0.09**	2.70 ± 0.10	2.26 ± 0.07**
4.0 h	3.56 ± 0.16	3.56 ± 0.08	2.56 ± 0.11	2.64 ± 0.03	2.73 ± 0.07	2.29 ± 0.09**

[a] Mineral levels following gavage of water or 300 mM $CaCl_2$ (2 ml/100 g b.wt) to intact rats. Each value is the mean of 7 rats. Table organization is the same as table 1.

may be important as a storage site which would prevent hypercalcemia after a meal by sequestering the excess calcium. These experiments were designed to test whether there is a change in liver calcium content during times of known calcitonin secretion. Striated muscle samples were also examined since parathyroid hormone administration alters muscle inorganic phosphate content[7].

Materials and methods. Male SPD rats, 204 g mean b.wt, from ARS/Sprague Dawley, Madison, Wisconsin, were fed Purina Laboratory Chow and tap water ad libitum until 12 h prior to the experiment when food was withdrawn and deionized water was given ad libitum. The next morning, at either 4, 2, 1 or 0.5 h prior to tissue collection, each rat received either a) a sham gavage by inserting the gavage tube into the stomach, b) a deionized water gavage of 2 ml/100 g b.wt, or c) the test meal of 2 ml/100 g b.wt of either Carnation Instant Nonfat Dry Milk Powder reconstituted by package directions with deionized water (30 mmoles/l Ca) or 300 mmoles/l $CaCl_2$. The latter meal was based on the Ca intake of rats eating 2 g/100 g b.wt Purina Laboratory Chow (1.2% Ca) per day[4]. At the time of tissue collection, each rat was anesthetized with ether, blood samples were collected from the tail vein for analysis of plasma calcium[8] and inorganic phosphate[9], samples of the liver and rectus abdominis were collected for analysis of calcium[10] and inorganic phosphate[11], and the wet weight of the whole liver was measured. The extracellular fluid (ECF) content of each organ was estimated by administering 10 μCi $Na^{35}SO_4$ i.v. 1 h prior to tissue collection and determining the perchloric acid-soluble ^{35}S ratio of plasma to each organ. The data were analyzed as a 4×3 factorial analysis of variance (4 times × 3 meals) for each variable. Significant differences between the groups were determined by Duncan's New Multiple Range test[12].

Results and discussion. Liver calcium was significantly elevated for 1–2 h following both meals (tables 1 and 2). Plasma calcium and phosphate were not significantly altered by the milk gavage (table 1), but were significantly affected by $CaCl_2$ gavage (table 2). The inorganic phosphate content of the liver and muscle, the muscle calcium content, the ECF content of liver and muscle, and the liver wet weight were not significantly affected by the test meals. The water gavage groups were not significantly different from the sham gavage groups for any of the variables tested. The lack of change in plasma calcium, liver weight and liver ECF content means that the increased liver calcium seen after the milk meal must represent increased intracellular calcium. We have not determined whether this increase is dependent upon the presence of the thyroid gland, which secretes calcitonin in mammals. This increase in liver calcium might seem to be evidence that the liver acts as a storage site for plasma calcium until the magnitude of the change is considered. The liver wet weight averaged 3.0 g/100 g b.wt for our rats, of which 30% was dry weight. The peak increase in liver calcium was about 0.3 μmoles/g dry weight for both experiments. This corresponded to an increase of 0.27 μmoles/100 g b.wt for the whole liver. This was quite small relative to the calcium content of the milk gavage, 60 μmoles/100 g b.wt, and even more calcium was given in the $CaCl_2$ gavage. This increase in liver calcium of 0.3 μmoles/g dry weight was also only about 10% of the increase reported by Yamaguchi[2] following s.c. calcitonin administration. It seems that the liver is not an important storage site for plasma calcium and that physiological stimuli of calcitonin secretion do not result in as large an increase in liver calcium as reported following injection of the hormone.

1 Supported in part by a grant from the National Science Foundation (PCM 75-23603). We are grateful to Mr John O'Brien for his technical assistance.

2 M. Yamaguchi, Y. Takei and T. Yamamoto, Endocrinology *96*, 1004 (1975).
3 M. Yamaguchi and T. Yamamoto, Chem. pharm. Bull. *24*, 159 (1976).
4 R. V. Talmage, S. H. Doppelt and C. W. Cooper, Proc. Soc. exp. Biol. Med. *149*, 855 (1975).
5 J. Lederer, F. Stein and A. M. Arnould, Ann. Endocrin. *30*, 132 (1969).
6 T. K. Gray and P. L. Munson, Science *166*, 512 (1969).
7 R. A. Meyer, Jr, and M. H. Meyer, Endocrinology *94*, 1331 (1974).
8 B. C. R. Sarkar and U. P. S. Chauhan, Analyt. Biochem. *20*, 155 (1967).
9 P. S. Chen, Jr, T. Y. Toribara and H. Warner, Analyt. Chem. *28*, 1756 (1956).
10 M. H. Meyer and R. A. Meyer, Jr, Clin. Chem. *22*, 1396 (1976).
11 B. E. Wahler and A. Wollenberger, Biochem. Z. *329*, 508 (1958).
12 R. G. D. Steel and J. H. Torrie, in: Principles and Procedures of Statistics, p. 107. Mc-Graw-Hill Book Company, New York 1960.