

## COLLOID DROPLET FORMATION IN DOG THYROID IN VITRO

### Induction by Dibutyryl Cyclic-AMP

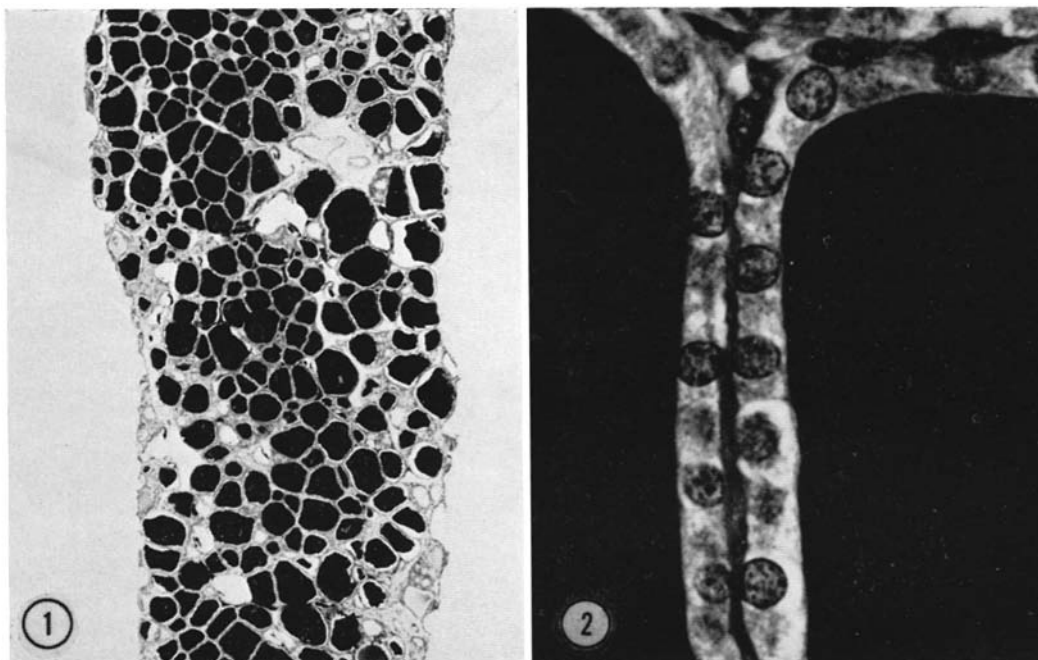
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Sutherland et al. have proposed that many hormones including thyroid-stimulating hormone (TSH) act by elevating the intracellular levels of cyclic 3'5'-adenosine monophosphate (c-AMP) (1). TSH increases the levels of c-AMP in thyroid homogenates (2) and slices (3). It is important to determine the extent to which the variety of biochemical and morphological changes produced by TSH can be duplicated by increasing the concentration of thyroidal c-AMP. Although c-AMP does not appear to stimulate thyroid tissue in vitro, dibutyryl c-AMP, a c-AMP analogue that appears to enter cells more rapidly than c-AMP and that is more resistant to enzymatic hydrolysis (4), stimulates glucose oxidation in dog slices and phospholipid metabolism in beef thyroid slices,

like TSH (5). A striking early morphological effect of TSH on the thyroid is the formation of pseudopods and colloid droplets in thyroid epithelial cells (6, 7). This paper shows that dibutyryl c-AMP, like TSH, causes the formation of pseudopods and intracellular colloid droplets when incubated with dog thyroid slices.

#### MATERIALS AND METHODS

Bovine TSH (4 I U/mg) was a gift of Dr. P. Condliffe, National Institutes of Health, Bethesda, Md. Mouse tumor TSH (1 I U/mg) was a gift of Dr. R. Bates, National Institutes of Health. Dibutyryl c-AMP was a gift of Dr. T. Posternak, Geneva, Switzerland, or was synthesized by the method of Posternak et al. (4). Acetylcholine chloride, L-epinephrine bitartrate, sero-



FIGURES 1 and 2 Cross-section of a slice of dog thyroid which had been incubated in vitro. Controls. PAS-hematoxylin. Fig. 1, incubated for 1 hr. Preservation of colloid good. Colloid missing primarily from some follicles at edge of slice.  $\times 42$ . Fig. 2, incubated for 2 hr. Note the smooth apical membranes and the absence of pseudopods and droplets.  $\times 1250$ .

tonin creatinine sulfate, and menadione (2-methyl naphthoquinone) were obtained from Mann Fine Chemicals, Inc., N.Y.; cyclic 3',5'-adenosine monophosphate and eserine sulfate, from Calbiochem, Los Angeles, Calif.; and histamine dihydrochloride and puromycin, from Nutritional Biochemicals Corporation, Cleveland, O.

Thyroids were obtained from dogs anesthetized with pentobarbital and exsanguinated. The glands were chilled to  $1^{\circ}\text{C}$ , and slices were prepared with a Stadie-Riggs microtome. Slices 0.5 mm thick were used in studies of glucose-1- $^{14}\text{C}$  oxidation as previously described (8). Since thin slices showed numerous disrupted follicles, slices 1.0–2.0 mm thick were used in the histological studies. Thyroids were also obtained from young male Fisher rats weighing 125–175 g and maintained on Purina Laboratory Chow (Purina Mills, St. Louis, Mo.). The animals were anesthetized with ether, and bled from the heart; each lobe of thyroid was removed and incubated intact at  $37^{\circ}\text{C}$ .

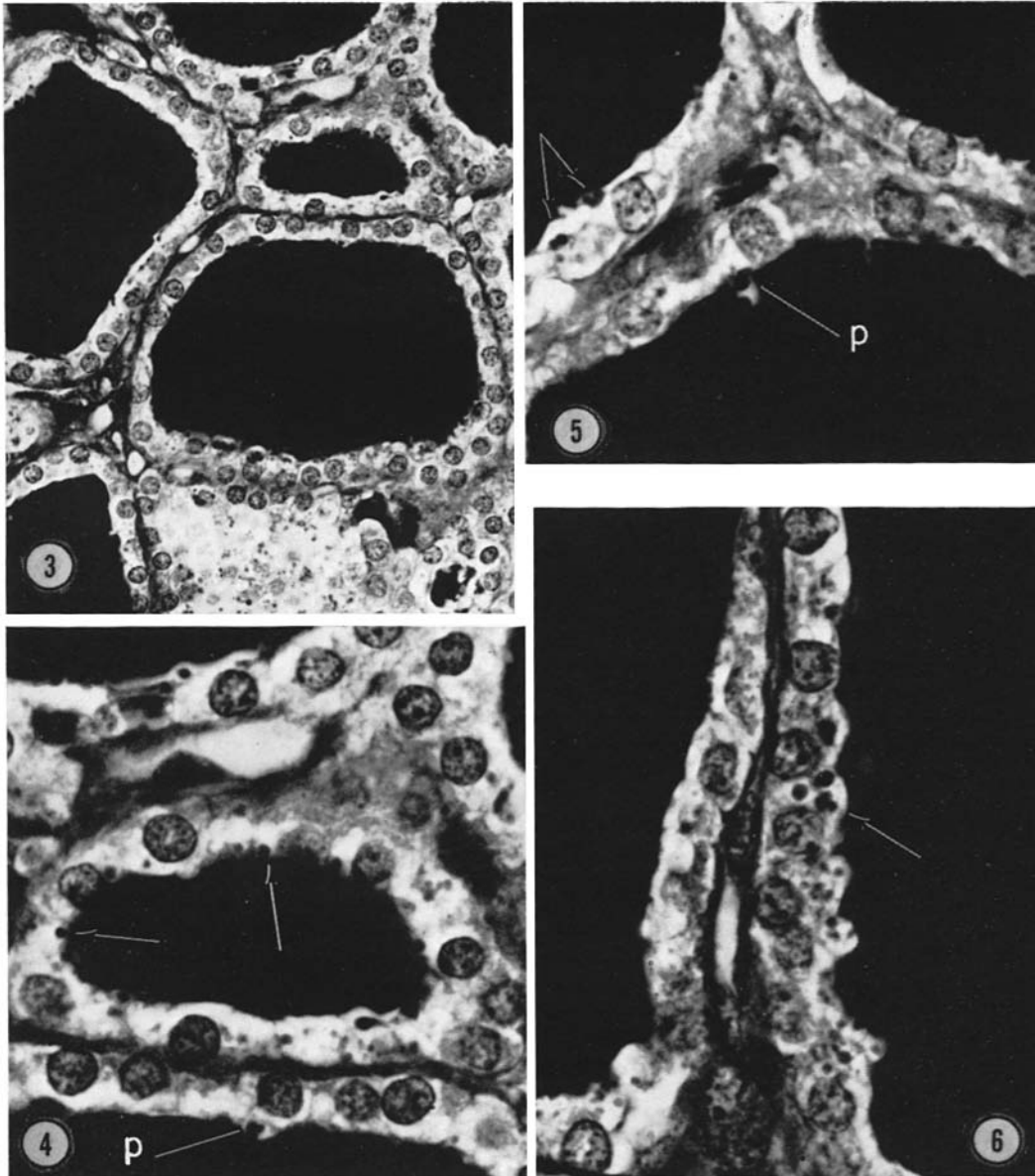
Tissues were incubated from 10 min to 2 hr at  $37^{\circ}\text{C}$  in stoppered 30-ml plastic (The Nalge Co., Inc., Rochester, N.Y.) or glass bottles containing 1 ml of Krebs-Ringer bicarbonate buffer, 5 mg albu-

min, 1 mg of glucose, and the test substance. In metabolic studies 0.5  $\mu\text{c}$  of glucose-1- $^{14}\text{C}$  was added. The pH was 7.4 and the gas phase was 95%  $\text{O}_2$ :5%  $\text{CO}_2$ . Tissue was fixed for 18 hr at  $4^{\circ}\text{C}$  in Bouin's fluid, embedded in paraffin, and stained by the periodic acid-Schiff procedure with hematoxylin counterstain.

## RESULTS

### *Dog Thyroids*

Morphological details characteristic of fresh tissue were well preserved during the incubation of dog slices for as long as 2 hr (Fig. 1–2). Although no intracellular droplets were observed in fresh tissue, spontaneously occurring droplets were occasionally observed after 10 min of incubation. However, droplets were not observed after 1 and 2 hr of incubation (Fig. 2). Therefore, to obtain controls in which no droplets could be detected, we added the substances to be tested after a 1 hr incubation, and the incubation was continued for an additional hour. TSH at 50 mU/ml caused the formation of typical pseudopods at apical ends of



FIGURES 3-6 Dog thyroid incubated for 1 hr in basal medium followed by 1 hr in medium containing thyroid stimulating hormone (TSH) or dibutyl cyclic AMP. Note occurrence of pseudopods and droplets. PAS-hematoxylin. Fig. 3, incubated with TSH. Note the irregularities in the apical membranes and the numerous tiny droplets in epithelial cells of follicles and in the tangential section of the follicle at the bottom of the figure. Details can be seen more clearly in Fig. 4.  $\times 500$ . Fig. 4, area from top of Fig. 3. Note pseudopod (*p*) and droplets (arrows).  $\times 1250$ . Fig. 5, incubated with dibutyl c-AMP. Note pseudopod containing droplets (*p*) and droplets (arrows).  $\times 1250$ . Fig. 6, incubated with dibutyl c-AMP. Note droplets (arrow).  $\times 1250$ .

cells and numerous droplets both in pseudopods and in the body of the cell (Figs. 3, 4). These morphological findings were duplicated by dibutyryl c-AMP at 250  $\mu\text{g}/\text{ml}$  (Fig. 5, 6). The following substances did not cause the formation of pseudopods or droplets: c-AMP at 250  $\mu\text{g}/\text{ml}$ , acetylcholine at 0.5  $\mu\text{g}/\text{ml}$  with eserine at 230  $\mu\text{g}/\text{ml}$ , epinephrine at 1  $\text{mg}/\text{ml}$ , serotonin at 1  $\text{mg}/\text{ml}$ , puromycin at 300  $\mu\text{g}/\text{ml}$ , histamine at 20  $\mu\text{g}/\text{ml}$ , and menadione at a 1:24 dilution of a saturated aqueous solution.

### Rat Thyroids

Moderate numbers of droplets were frequently seen in control rat thyroids after a 1 hr incubation. Mouse tumor TSH at 25 mU/ml always produced a striking increase in the number of droplets and in glucose-1- $^{14}\text{C}$  oxidation. In contrast dibutyryl c-AMP at 250  $\mu\text{g}/\text{ml}$  failed to produce a significant increase in droplets or in glucose-1- $^{14}\text{C}$  oxidation.

### DISCUSSION

Both dibutyryl c-AMP and TSH produced pseudopods and intracellular colloid droplets in dog thyroid slices. The droplets observed after incubation with dibutyryl c-AMP or TSH must be newly formed, since they were not present before the addition of these substances and many occurred at the apical ends of cells in configurations characteristic of newly formed droplets (6, 7).

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The finding that in the dog dibutyryl c-AMP produces pseudopods and intracellular colloid droplets, like TSH, is compatible with the hypothesis that in the dog TSH induces the formation of pseudopods and colloid droplets by elevating the level of intracellular c-AMP. The level of dibutyryl c-AMP employed was that found to give a maximal effect on glucose-1- $^{14}\text{C}$  oxidation in dog thyroid slices (5), and was also approximately equal to the level necessary to stimulate lipolysis in rat adipose tissue (9) and amylase secretion in rat salivary gland (10). The minimum level required in the dog thyroid is not known.

The pseudopod and droplet response *in vitro* is fairly specific for TSH and dibutyryl c-AMP, since it was not induced by the other substances tested. The increased glucose-1- $^{14}\text{C}$  oxidation that accompanies the addition of TSH and dibutyryl c-AMP may be necessary for the formation of pseudopods and droplets, but it is clearly not sufficient. It is also produced by epinephrine (11), serotonin (12), acetylcholine (13), and menadione (14), all of which failed to produce droplets.

Dibutyryl c-AMP at a concentration found to give a massive response in the dog thyroid did not produce an obvious response in the rat. Very little can be made of this species difference at present since the incidence of droplets in control rat thyroids is so high that a small increment would not have been detected.

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