Organ Culture of the Rat Thyroid Gland

Following the first successful study of the ultrastructure of organ cultured thyroid glands by PETROVIC and PORTE[1,2], organ culture was also used light microscope radioautography of the thyroid[3]. It was decided to repeat these experiments using the improved techniques for organ culture described by BAKER and NEAL[4,5]. If this were successful it would provide a suitable model for the study of protein synthesis and secretory mechanisms using the technique of ultrastructural radioautography[6].

Materials and methods. Thyroid glands were dissected aseptically from adult rats of the Birmingham strain. They were cut into small pieces (ca. 1–2 mm³) in preparation for organ culture[4,5]. The explants, supported on stainless steel grids covered with lens tissue, were maintained in plastic petri dishes containing Eagle's minimal essential medium supplemented with calf serum (20% v/v), glutamine and antibiotics. No attempt was made to add essential precursors of protein and hormone synthesis, and the serum contained 6 IU TSH/ml (radioimmunoassay carried out by Dr. L. WEIDE, University of Uppsala, Sweden). Racks containing 4 petri dishes were placed in modified 'Kilner' preserving jars[4,5] and were gassed with 5% CO_2 in air at a pressure of 0.703 kg/cm². The high gas pressure was maintained in the Kilner jar for the duration of culture since this was found to virtually eliminate necrotic changes which otherwise characterize explanted tissue. The fragments of thyroid gland were cultured at 37 °C for 4 to 8 days, after which they were fixed in buffered 1% osmium tetroxide prior to embedding in Araldite. Thin sections cut on a Porter Blum ultramicrotome were examined with a Siemens Elmiskop 1A electron microscope.

Results. The histological appearance of the organ cultured thyroid gland (Figure 1) is essentially similar to that described by NADLER et al.[6] for tissue fixed immediately after removal from the animal. However, the cells appear active and their microvilli are increased in size and number (Figures 2 and 3). There is also an increase in the amount of collagen in the perifollicular space (Figure 4). The amount of colloid within the follicles decreases in organ culture and it contains some cellular debris (Figure 1).

The follicular cells contain larger mitochondria and, while precise counts were not made, the mitochondria appear to be increased in number (Figure 3). The Golgi zone is also enlarged and contains numerous small vesicles (Figures 4 and 5). Colloid droplets within the

1 A. PETROVIC and A. PORTE, C. r. Séanc. Soc. Biol. *155*, 1848 (1961).
2 A. PORTE and A. PETROVIC, C. r. Séanc. Soc. Biol. *155*, 1701 (1961).
3 P. WHUR and A. MERSCOVICS, Anat. Rec. *160*, 450 (1968).
4 T. G. BAKER and P. NEAL, Biophysik *6*, 39 (1969).
5 T. G. BAKER and P. NEAL, in *Oogenesis* (Eds. J. D. BIGGERS and A. W. SCHUETZ; University Park Press, Baltimore 1972).
6 N. J. NADLER, B. A. YOUNG, C. P. LEBLOND and A. MITMAKER, Endocrinology *74*, 334 (1964).

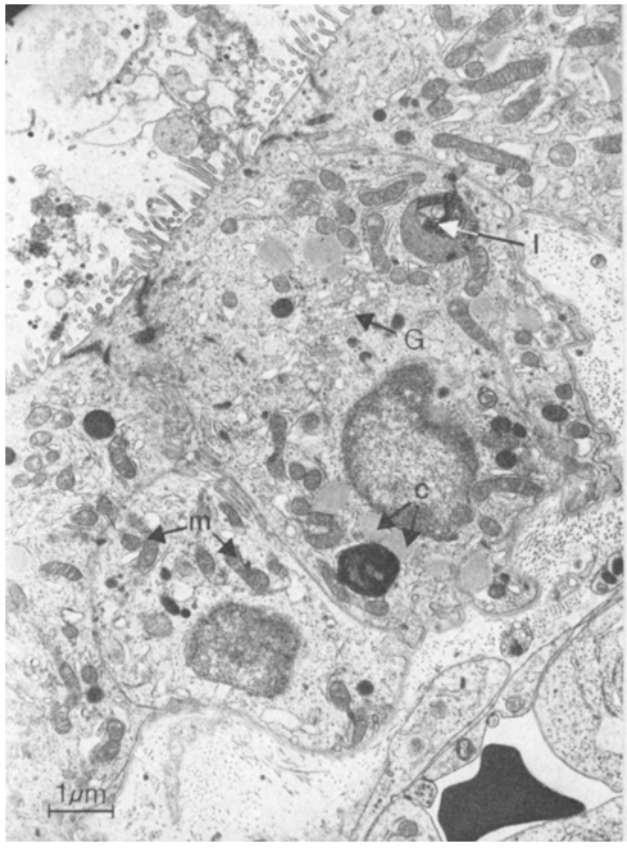

Fig. 1. Ultramicrograph from a piece of thyroid cultured for 4 days. Note the activity of the follicular cells and the debris in the colloid space. G, Golgi zone; l, lysosome; c, colloid droplets; m, mitochondria. ×10,000.

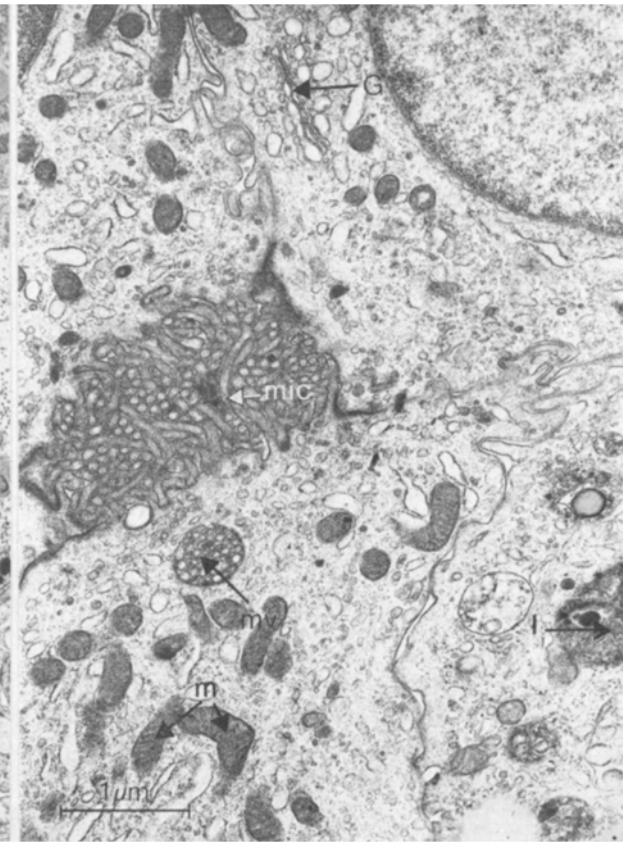

Fig. 2. Ultramicrograph from a piece of thyroid cultured for 4 days. 4 follicular cells are shown surrounding a small colloid space filled with microvilli (mic) which appear increased in size. G, Golgi zone; mv, multivesicular body; l, lysosomes; m, mitochondria. ×20,000.

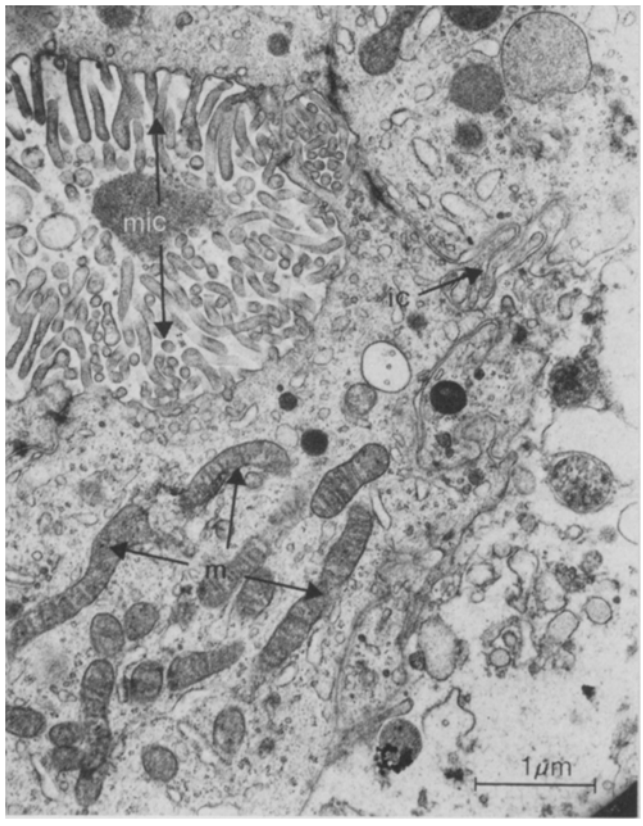

Fig. 3. Ultramicrograph from a piece of thyroid cultured for 4 days. Follicular cells are seen surrounding a colloid space filled with microvilli (mic) which appear increased in size. Intercellular channels (ic) and numerous mitochondria (m) are present. ×20,000.

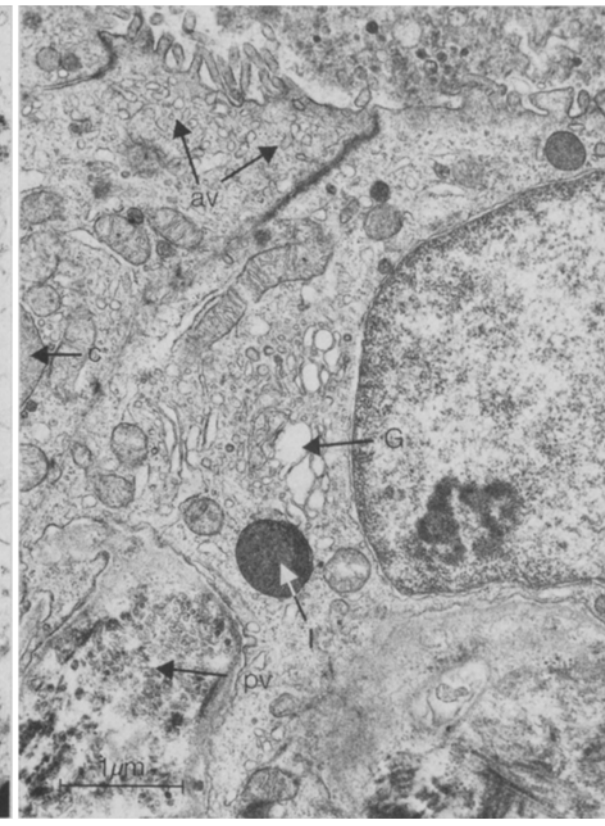

Fig. 4. Ultramicrograph from a piece of thyroid cultured for 8 days. Follicular cells can be seen containing an enlarged Golgi zone (G), a colloid droplet (c) and several lysosomes (l). Numerous apical vesicles are present (av). Collagen fibres are present in the perivascular space (pv). ×20,000.

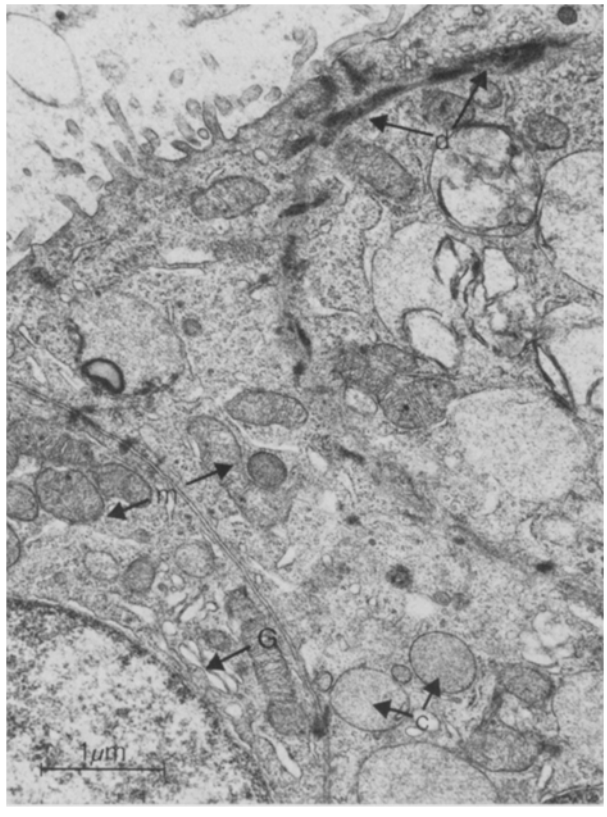

Fig. 5. Ultramicrograph from a piece of thyroid cultured for 8 days. Numerous colloid droplets (c) are present. G, Golgi zone; m, mitochondria; d, desmosomes. ×20,000.

cells are more abundant (Figure 5) which may indicate resorption of colloid from the follicles (Figure 2). Zymogen-like granules and lysosomes are evident, features which would also indicate the resorption of colloid (Figures 3 and 4). No 'light' cells[7] (also called parafollicular cells) were seen. Intercellular channels, apical vesicles and desmosomes were also noted (Figures 3 and 5).

Discussion. Embryonic thyroid glands have been cultured for 4 days and the method used to study the effect of iodine metabolism[8]. The results of the present study clearly demonstrate that the ultrastructure of the rat thyroid gland can be maintained in an essentially normal condition for at least 8 days in organ culture. Some of the histological changes which occur appear to be the direct result of 1. cessation of synthetic activity by the follicle cells, and 2. resorption of colloid from the follicles. These changes would be expected since the level of TSH in the support medium (6 IU/ml) might be insufficient to maintain the synthetic activity of the gland, and essential precursors (leucine, mannose, galactose, etc.[3]) were not added. However, the presence of apical vesicles in Figure 4 would be evidence of secretion into the colloid[6]. Small quantities of amino acids are included in the formula of Eagle's medium, and it is possible that the calf serum used as a supplement to the medium may contain some important factors.

The present results confirm and extend those of PETROVIC and PORTE[1,2]. It is conceivable that the high gas pressures that we used during culture increased the partial pressure of dissolved oxygen. We were thus able to avoid the use of high concentrations of oxygen in the gas phase (e.g. 95% O_2, WHUR and MERSCOVICS[3]) since these levels may be toxic to some cells.

Our results thus clearly show that the technique of organ culture of the thyroid gland is eminently suitable for studies of biosynthetic pathways within the gland by means of ultrastructural radioautography.

Zusammenfassung. Die Ultrastruktur der Rattenschilddrüse wird bis zu 8 Tagen in Organkulturen erhalten, was für radioautografische Studien der Ultrastruktur vorteilhaft ist.

T.G. BAKER and B.A. YOUNG

Hormone Laboratory,
Department of Obstetrics and Gynaecology,
University of Edinburgh,
Edinburgh (Scotland),
and
University of Cambridge,
Department of Anatomy,
Downing Street,
Cambridge CB2 3DY (England),
6 July 1973.

[7] B. A. YOUNG and C. P. LEBLOND, Endocrinology *73*, 669 (1963).
[8] B. M. NATAF, E. M. RIVERA and I. L. CHAIKOFF, Endocrinology *76*, 35 (1965).

Ecdysone: An Antagonist of Juvenile Hormone in the Control of Cuticle Synthesis in the German Cockroach (*Blattella germanica*)

In addition to their well known morphogenetic effects, juvenile hormone (JH) and its analogues (JHA) possess the capacity to inhibit ecdysis. This effect causes death during the larval moult of all but the last larval instar of the German cockroach[1]. The length of the last larval instar can be prolonged idefinitely by continuous application of high dosages of JH (methyl 10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate; *cis/trans* mixture) or JHA (6,7-epoxy-1-(p-ethylphenoxy)-3,7-dimethyl 2-octene; *cis/trans* mixture[2])[3]. The synthesis of the new cuticle in these permanent larvae can be induced by injection of ecdysone[3]. This is the first report of antagonism between JH and ecdysone in vivo[4].

10 freshly hatched last stage larvae of *Blattella germanica* were confined in 200 cm^3 plastic cups with two 10 cm^2 paper discs each folded to form a tunnel. The JH active substance was applied in an acetone solution either to

[1] W. HANGARTNER and P. MASNER, Experientia, in press (1973).
[2] Stauffer compound No. R-20458.
[3] All compounds were kindly provided by F. Hoffmann-La Roche, Ltd. Basel.
[4] Results presented to members of the A.R.C. Unit of Invertebrate Chemistry and Physiology at Brighton (Prof. A. W. JOHNSON, Director) and Cambridge (Dr. J. E. TREHERNE, Deputy Director) on March 19 and 20, 1973.

Table I. Effect of JH and JHA on freshly emerged last stage larvae. The penultimate column contains the mean length of the last larval instar of insects which hatched (20 animals were tested in each experiment)

Treatment				No. of dead last stage larvae	No. of insects hatching to the next stage				Length of the last stage (days)	No. of permanent larvae
Substance	Application	Dosage	Frequency (day)		Perfect adults	Deformed adults	Extralarvae	Dead in old cuticle		
Acetone control	Topical	1 µl	2, 4, 6, 8, 10, 12, 14		20				16	
JH	Topical	10µg	2, 4, 6, 8, 10, 12, 14	2		1	17		21	
JH	Topical	100 µg	2, 4, 6, 8, 10, 12, 14	1			7		27	12
JHA	Topical	10 µg	2, 4, 6, 8, 10, 12, 14						> 31	20
JHA	Topical	100 µg	5, 10	2			9	9	31	
JHA	paper	100µg/cm²	permanent						> 31	20