present experiments it has been shown that normal RNA can equally produce its effect when administered to the host 5 days before the injection of the antigen, in this case rat RBC, indicating that it probably operates by depressing the immunological system rather than blocking the antigen itself (either RBC or kidney cells), which agrees with the results of ASHLEY et al.[3] and of AXELROD and MEI LOWE[4]. Previous authors have studied the inhibitory effect of normal RNA on the mechanism of immunity associated with tissue transplantation, the experiments herein described show that normal RNA also inhibits the humoral immunological system. This effect of normal RNA depressing both types of immunological response is difficult to explain; it acts even when the same RNA (immune RNA) carries immunological information. It can be concluded that: (a) normal spleen RNA acts as a biological inhibitor on the humoral side of the immune mechanism as well as on homograft rejection, and (b) RNA from sensitized spleen has two fractions: (1) a small one, carrying the information for the immunological response, which works in a different way from that of messenger RNA because no circulating antibodies are detected in the present conditions before the antigenic stimulus, and (2) a large one, which apparently acts depressing immunity as normal RNA[9,10].

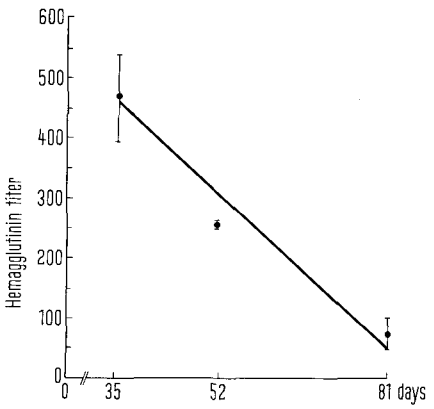

Fig. 2. Abscissa: Days elapsed from immunization to RNA extraction in donors of immune RNA. Ordinate: Hemagglutinin titer in mice receiving these same batches of immune RNA. The vertical lines represent the standard error of the mean.

Resumen. Se inmunizaron ratones BALB con glóbulos rojos de ratas de una línea endocriada. A estos ratones se les extrajo el ARN del bazo y se inyectó a otro grupo de animales de la misma cepa. Además, se inyectó el ARN proveniente de bazo de ratón normal a un segundo grupo de animales. Cinco días después de la inyección del ARN normal e inmune, ambos grupos, y un tercero, testigo, recibieron una inyección de glóbulos rojos de rata.

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In vitro Study of C (Parafollicular) Cells of Dog Thyroid in Organ Culture

The presence of C, or parafollicular, cells in the mammalian thyroid has been investigated by various authors[1–4]. It has recently been shown[5,6] that the main function of these cells is the storage and secretion of calcitonin, the serum calcium lowering hormone[7]. As a response to hypercalcemia, C cells react in vivo by losing argyrophilia[3] and metachromasia[8] and by discharging the secretory granules[3,9].

The technique of in vitro organ culture has already been applied by numerous authors[10,11] to the study of follicular cells of normal and pathological thyroids, but, so far, no attention has been paid to the presence and behaviour of C cells.

We have cultured dog thyroids (from a total of 15 animals) in a chemically defined tissue culture medium (T.C. 199 'Wellcome') for 36–120 h, in atmosphere of 95% O_2 and 5% CO_2, using the method of CHEN[12].

To detect the presence of C cells, tissue blocks have subsequently been either (a) fixed in formol-Ca and cryostat sections stained with a silver method[13] for argyrophilic cells, or (b) fixed in glutaraldehyde- picric acid-acetate fixative and the sections stained with toluidine blue after mild acid hydrolysis[8], or (c) fixed in 3.12%

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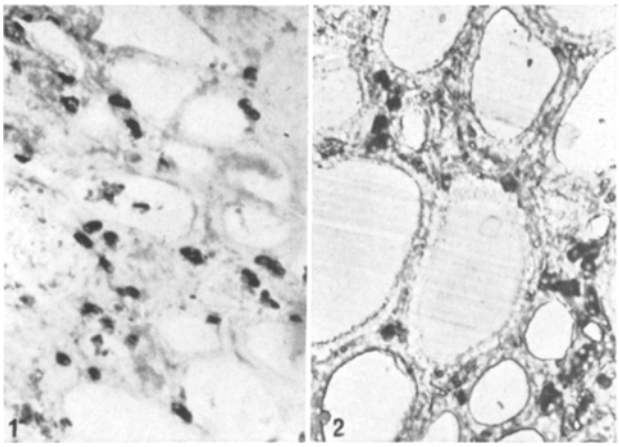

Fig. 1 and 2. C (parafollicular) cells of dog thyroid, cultured in T.C. 199 in organotypic culture for 36 h. (1) Silver stain for argyrophilic cells × 170. (2) Toluidine blue metachromatic staining after mild acid hydrolisis. × 180.

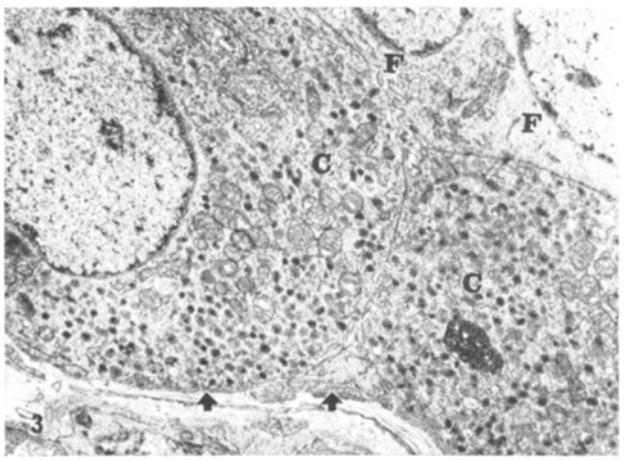

Fig. 3. Dog thyroid cultured in T.C. 199 for 36 h. 2 parafollicular cells (C) rich in secretory granules, located between the basal membrane (arrows) and follicular cells (F). × 9000.

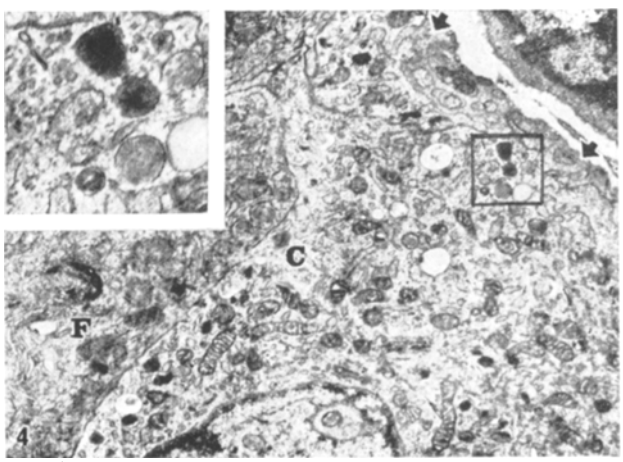

Fig. 4. Dog thyroid cultured for 36 h in T.C. 199 whose Ca level had been increased to 10.67 meq/l. The C cell contains few secretory granules and vesicles. × 12,800. Inset: × 36,000.

buffered glutaraldehyde, post-fixed in 1% OsO_4 and embedded in araldite for electron microscopical examination.

The general morphological appearance of cultured dog thyroids was similar to that described by other authors[10, 11] in human and mammalian thyroids: the organ structure was well preserved, although necrotic areas could occur in the central parts of the blocks.

With both histochemical methods (Figures 1 and 2) C cells were detected in para-follicular position, isolated or arranged in small clusters. Occasionally, some of these cells were present inside the colloid.

In electron microscopical examination (Figure 3) cultured C cells were easily recognizable because of both their classical position (inside the basal membrane, but separated from the colloid by follicular cells) and the typical secretory granules, which were numerous and well preserved.

Experiments have also been devised to test whether C cells would react in vitro to high level of calcium, as they do in vivo. A similar technique had been employed by Roth and Raisz[14] to investigate the direct influence of the Ca level of the tissue-culture medium on the morphological appearance and hormone secretion of parathyroid cells.

Dog thyroids have been cultured in T.C. 199 in which the Ca level, which is normally 3.75 meq/l, had been increased to 10.67 meq/l or decreased to 2.4 meq/l. No changes have been observed in cultures with 'low' Ca level, while the culture of thyroids in media with a 'high' Ca level resulted in a sharp loss of argyrophilic and metachromatic staining and degranulation of C cells (Figure 4).

The results show that the C cells of dog thyroid can be cultured in in vitro organotypic culture, retaining their histochemical and electron microscopical characteristics. They are not just living, but functioning: it is therefore possible to apply this technique to the study of these cells under various experimental conditions. The in vitro calcium-induced histochemical changes and degranulation suggest that the Ca level, in a similar way as in parathyroid cells[14], directly influences the reactivity of C cells, possibly by acting on the cell membranes[15].

Riassunto. Le cellule C (parafollicolari) della tiroide di cane conservano in vitro, in coltura organotipica, i loro caratteri istochimici ed ultrastrutturali. Tali cellule, cui viene attualmente attribuita la funzione di produrre calcitonina, reagiscono ad una elevazione del contenuto in calcio del terreno di coltura, con modificazioni istochimiche (perdita della argirofilia e metacromasia) e ultrastrutturali (diminuzione fino a scomparsa dei granuli di secrezione). Questi studii fanno ritenere possibile che la secrezione di calcitonina venga direttamente regolata dal livello del calcio.

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