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A Development of a Supplemented Ham's F-10 Medium for the Maintenance of Thyroid Glands: A Method for Studying the Effect of Cortisone Acetate on I 131 Uptake

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INTRODUCTION

Early interest in thyroid-adrenal cortex interactions was stimulated in the 1930's when administration of thyroid substance was found to be associated with increased adrenal gland size (Cohen, 1935; Ingle and Kendall, 1938). Decreased thyroid function resulted in a decrease in adrenal size and activity (Leblond and Hoff, 1944; Baumann and Marine, 1945; McQuillan and Trikojus, 1946; Zarrow and Money, 1949; Melampy, Cheng, and Northrop, 1951). Deane and Greep (1947) found histological evidence of hypofunction of the zona fasiculata of the adrenal cortex, the center of glucocorticoid synthesis, following thyroidectomy. They demonstrated that the zona fasiculata of thyroidectomized animals retained the capacity to respond normally to adrenocorticotrophic hormone (ACTH) indicating a possible lack of this hormone resulting from thyroidectomy. Conn and Fajans (1953) reported that chronic hypothyroidism resulted in a decreased adrenocortical function and that adrenal hyperactivity was capable of diminishing the function of the thyroid gland. Soffer, Gabrilove, and Dorrance (1951) showed that adrenalectomy of rats followed by ACTH treatment resulted in a depression of iodine uptake by the thyroid gland indicating a suppression of thyroid stimulation by the

pituitary gland. These reports indicate that the thyroid gland and adrenal cortex may affect each other indirectly through the pituitary gland.

The injection of thyroid stimulating hormone (TSH) into hypophysectomized rats causes an increased thyroidal iodine uptake but the administration of cortisone reduces this uptake indicating a direct antagonistic action on the TSH-thyroid mechanism (Woodbury, Ghosh, and Sayers, 1951; Paschkis, et al., 1952). Halmi and Barker (1952) concluded that cortisone-induced inhibition of thyroid function was not due to an interference with the production or action of TSH. These investigators found that the histological structure of the thyroid gland revealed indications of higher thyrotrophic (TSH) stimulation following cortisone administration. They were able to show in the same experiment that cortisone promoted rather than inhibited the thyrotrophic function of the pituitary gland. These results could be explained as resulting from the stimulation of the thyroid-pituitary feedback mechanism if it could be shown that cortisone directly inhibits thyroid function.

Pituitary control of the thyroid gland was suspected in the nineteenth century when post-mortem investigation revealed the enlargement of the pituitary gland in cretins. Pituitary control was established in the 1920's with the demonstration of thyroid atrophy following hypophysectomy and thyroid restoration by a specific protein hormone extracted from the adenohypophysis (Smith, 1930). The reciprocal interaction, and effect of thyroid hormone on the

pituitary gland, was described by Aron, Van Caulaert, and Stahl (1930) and designated "negative feedback". Examples of the basic interactions of this system include the depression of TSH and thyroid atrophy caused by thyroid hormone administration, and hypertrophy induced by an increase in TSH secretion following partial thyroidectomy or when hormone synthesis is impaired by goitrogenic drugs, iodine deficiency, or congenital biochemical defects (Brown-Grant, 1967).

Vanderlaan and Greer (1950) reported that the iodine-trapping mechanism of the thyroid gland following hypophysectomy continues to function at a level which is approximately 10 per cent of its normal capacity and that full function is restored by the administration of TSH. Greer (1952) found that if the pituitary is separated from the hypothalamus, thyroid atrophy results without impairment of the iodine uptake capacity of the gland. A similar effect was observed in cortisone-injected, nephrectomized rats in that the weight of the thyroid gland decreased but the concentration of iodine per mg of thyroid tissue did not change (Yatvin, Wannemacker, and Brown, 1966). Aterman and Greenberg (1954) reported that in rats exophthalmia results following cortisone administration. Dobyns and Steelman (1953) indicated that an exophthalmos-producing factor can be separated from TSH. These reports indicate that the thyroid gland is under pituitary control through the actions of TSH, that TSH affects several physiological processes, and that cortisone may promote TSH levels.

A similar feedback mechanism has been shown to exist between the adrenal cortex and the pituitary gland. ACTH from the pituitary gland when perfused through isolated adrenal glands has been found to promote the production of corticosteroids (Hayano, Dorman, and Yamada, 1951; Hechter, Solomon, and Caspi, 1953; Jeanloz, et al., 1953; Levy, et al., 1953. Sayers (1950) found that the rate of ACTH release appeared to be related to the blood titer of circulating adrenal cortical hormones since exogenous glucocorticoids, as in the case of cortisone, will effectively block all forms of ACTH release. Gemzell, et al., (1951) found that adrenalectomy resulted in an increase of up to 30 times normal levels of ACTH in body fluids. Adrenal atrophy, comparable to that observed following hypophysectomy, has been shown to result from the administration of adrenal cortical extract, cortisone, or hydrocortisone (Sayers and Sayers, 1948; Gershberg, et al., 1950; Gaunt, et al., 1953; Winter, Hollings, and Stebbins, 1953; Kass and Finland, 1953). These reports indicate that adrenosteroid production is maintained by ACTH and that the level of ACTH is regulated by blood titers of adrenosteroids.

Recent investigations have revealed that the hypothalamus is involved in ACTH and TSH secretion. Electrical stimulation of the posterior hypothalamus has been shown to be associated with ACTH release (de Groot and Harris, 1950; Porter, 1954; Endroczi, Kovacs, and Lissack, 1956; Endroczi and Lissak, 1960;

Suzuki, et al., 1960). Excitation of rostral hypothalamic areas has also been shown to affect the pituitary-adrenal system (Hume, 1952; Mason, 1958; Snyder and D'Angelo, 1963). Increased TSH levels have been demonstrated following electrical stimulation of the anterior hypothalamus and anterior portion of the median eminence (Harris and Woods, 1958; Campbell, et al., 1960; D'Angelo and Snyder, 1963). D'Angelo, Snyder, and Groden (1964) demonstrated that electrical stimulation of the preoptic, suprachiasmatic, and anterior hypothalamic areas induced cytological activation of pars distalis basophils, reduced TSH stores, increased plasma TSH concentrations 4-to 5-fold, and resulted in the histological stimulation of the thyroid gland. These same investigators observed similar results following electrical stimulation of the posterior median eminence and arculate nuclear regions of the tuberal hypothalamus. They found that the mere presence of electrodes in the hypothalamus without electrical stimulation caused adrenal hypertrophy and elevated plasma corticosteroid levels. Stimulation of the rostral or caudal hypothalamus induced further adrenal enlargement. Redgate (1970) demonstrated that plasma ACTH rose following electrical stimulation at sites in the amygdaloid septal complex matching the rapidity of the response to hemorrhage or sciatic nerve stimulation. Redgate also found that ACTH release was delayed 5- 10 min following stimulation of the medullary reticular formation, medial midbrain structures, lemnisal system, and posterior lateral hypothalamus. These

results indicate that neural responses play a role in regulating ACTH release from the pituitary gland. Chemical stimulation of TSH production (propylthiouracil administration) resulted in reducing the adrenal response to anterior hypothalamic electrical stimulation indicating an antagonism between thyroid and adrenal cortex stimulation (D'Angelo, Snyder, and Groden 1964).

Sakis and Guillemin (1965) found that the pituitary gland when induced to secrete TSH by thyrotrophic releasing factor (TRF) concomitantly secretes less ACTH in response to stress and when ACTH secretion is inhibited by dexamethasone and Nembutal higher TSH secretion is induced by TRF. It was concluded that when the secretion of ACTH or TSH is inhibited or not stimulated, the other is facilitated or enhanced in response to its pertinent stimulus except when corticotrophic releasing factor (CRF) is preferentially secreted by the hypothalamus at the expense of TRF upon exposure to stress.

Numerous studies have indicated that cortisone affects the thyroid gland by depressing the rate of radioactive iodine uptake. The depression effect has been demonstrated *in vivo* in rats (Money, *et al.*, 1950; Perry, 1951; Money, *et al.* 1951; Van Middlesworth and Berry, 1951; Woodbury, Ghosh, and Sayers, 1951; Paschkis, *et al.*, 1952; Albert, Tenny, and Ford, 1952; Vezar and Vidovic, 1953; Ingbar, 1953; Ingbar and Chandler, 1953; Epstein, *et al.*, 1953; Woodbury, *et al.*, 1954; Skebel'skaya, 1958) in rabbits (Myant, 1953)

and in man (Hill, et al., 1950; Hardy, Riegal, and Erisman, 1950; Frederickson, 1951; Frederickson, Forsham, and Thorn, 1952; Kuhl and Ziff, 1952; Berson and Yalow, 1952; Zingg and Perry, 1953). Various mechanisms have been suggested as an explanation for the depression of radioactive iodine collection. Pituitary suppression of TSH secretion, extra-thyroidal utilization of TSH, or increased diuresis and excretion of iodine have been suggested as factors which could possibly affect radioactive iodine uptake (Shellabarger, 1954).

The products of various endocrine glands have been shown to exert an effect on specific target tissues. The mechanisms by which a gland exerts its effects are not clearly understood in many instances. In vivo studies may indicate an effect but determining the pathway of hormonal action remains difficult. The gland may exert a direct effect or it may affect its target indirectly by controlling the action of some other endocrine structure which affects the target in question. Development of techniques for in vitro studies of endocrine function appears promising in that in an in vitro experimental approach it should be possible to exclude results which may be obtained by hormonal interaction, glandular interaction, or other indirect effects making possible a study of the action of a single hormone on its target tissue or organ. Although in vitro studies may not reflect an exact in vivo situation, they may be used as guidelines for future experimentation.

Investigation of the thyroid-adrenal cortex relationship should be approachable through the application of tissue culture techniques. This relationship has been studied extensively in the last twenty years using *in vivo* techniques. These studies have provided contradictory results with regard to the physiological mechanism operating in the interactions between these glands.

The closed system provided by thyroid tissue culture appears to offer a method for studying the action of cortisone. A response of the thyroid gland to cortisone in *in vitro* studies may be investigated directly in an effort to determine the mechanism of the response with the exclusion of the many interactions which occur in *in vivo* studies. An *in vitro* situation would exclude pituitary variations, extrathyroidal metabolism and excretory elimination of tissue culture components, and possible unknown variables imposed by unexplored mechanisms. The information provided by the study could then be used to aid in the investigation of what appears to be conflicting interpretations of results obtained from *in vivo* studies.

A tissue culture method has been proposed by Trowell (1959) by which many organs can be maintained *in vitro* in an entirely synthetic medium and retain their normal histological appearance for about a week. The method keeps fully differentiated organs alive without either growth or dedifferentiation opening the way for experimental studies on organ physiology. Trowell found that in thyroid gland cultures the

glands apparently remained healthy and unaltered for 6 days at which time interfollicular connective tissue started to die. The follicular epithelium remained healthy for 10 days. Histological evidence indicated reduction in colloid and some follicular shrinkage resulting in the follicular epithelium becoming taller. Trowell suggested that his method may be suitable for various short-term experiments on the action of hormones and drugs. Although Trowell's culture medium gave adequate results, it was thought that Ham's F-10 medium would provide a more complete physiological environment.

Ham's F-10 medium (Ham, 1963) evolved from Fisher, Puck, and Sato's medium (Fisher, Puck, and Sato, 1959) through the development of Ham's F-7 medium (Ham, 1962). Fisher, Puck, and Sato's medium was used for the clonal growth of S-3 HeLa cells. In 1962, Ham found that this medium when, supplemented with two purified serum protein fractions, would support the growth of a diploid Chinese hamster ovary cell line CHBOC1D1. Ham altered the medium used for the S-3 HeLa cell maintainence in the following ways:

a. amino acids, vitamins, growth factors, and proteins were reduced in concentration
b. folic acid and calcium chloride were increased in concentration
c. thymidine, pyruvate, cysteine, serine, asparagine, and ferric chloride were added.

In 1963, Ham revised his F-7 medium to a medium which would support the serum-free growth of the hamster ovary line.

Nearly all the components of F-10 were omitted one at a time and added back over a wide range of concentrations in order to determine for each the minimum concentration which would permit growth, the optimum concentration, and the maximum permissible non-toxic concentration. The F-10 medium resulted. In addition to supporting the serum-free growth of the hamster ovary, Ham's F-10 medium was found to support the growth of other cell lines when supplemented with serum. Ham's F-10 medium plus serum supported the growth of CHL-1 Chinese hamster lung cells, S3-9-IV HeLa cells, HF365 diploid human line, and human white blood cells for chromosome preparations.

Seaman and Stahl (1956) cultivated mouse thyroid glands using a modified chamber technique (Trowell, 1952, 1954, 1955) with the alkaline fluid medium of Pikowski (1954) in a 95% O_2-5%CO_2 gaseous atmosphere. Highly differentiated follicular epithelium was maintained satisfactorily *in vitro* for a 2- 6 day period. The cultivated thyroids were exposed to I^{131} for 2- 18 hr after which radioautographs were prepared. Seaman and Stahl found that the cultured thyroid glands accumulated appreciable amounts of I^{131} in both the follicular cells and the colloid indicating physiological activity. It has not yet been determined whether or not a supplemented Ham's F-10 medium will maintain thyroid tissue.

The primary purpose of this investigation is to determine whether thyroid glands may be maintained on a supplemented Ham's F-10 medium and to determine whether this tissue culture may be used under experimental conditions to study the effects of cortisone acetate on thyroidal I^{131} uptake.

MATERIALS AND METHODS

Thyroid glands of ether killed white laboratory mice, weighing approximately 26 g apiece, (Southern Biological Company, Mackenzie, Tennessee) were grown in tissue culture by a modified raft technique (developed from Trowell, 1959) upon a supplemented Ham's F-10 medium. The culture medium was prepared by supplementing Ham's F-10 with 0.15 ug/ml KI to provide the cultures with a physiological iodide supply and to dilute radioactive iodine uptake, 1.5 mu/ml TSH to provide a physiological supply of pituitary stimulator which is essential for full function *in vivo* (Vanderlaan and Greer, 1950), and 0.5 ml 5% fetal bovine serum per ml culture medium to provide an additive nutrient supply (Paul, 1965). The preparation was then filter sterilized. The supplemented medium was used without modification for culturing tissues used as controls but was modified by the addition of a physiological concentration of a cortisone acetate (0.20 ug/ml) for culturing experimental tissues. Transfer of 3 ml of medium to sterile plastic tissue culture plates was achieved in a chemically disinfected (swabbed with 0.01M Hg_2Cl_2 and rinsed with 70% ethyl alcohol) glovebox which was kept under a slight positive pressure by air pumped through sterile cotton saturated with 70% ethyl alcohol. Autoclaved

pieces of lens paper (1.0 cm squares) were floated on the surface of the medium. The plates were then removed from the glovebox and placed in a disinfected (swabbed with 0.01M $HgCl_2$ and 70% ethyl alcohol) Model 3158 Forma anaerobic incubator (Forma Scientific Incorporated, Marietta, Ohio) at 35 C in a 95% O_2 -5% CO_2 saturated (bubbled through distilled H_2O) gas mixture. Maximum humidity was achieved by lining the incubator with sterilized paper towels soaked in distilled H_2O.

The operation of removing the thyroid glands was performed under a plastic dome covering a disinfected area which had been scrubbed with 0.01M $HgCl_2$ and 70% ethyl alcohol. Surgical instruments were kept in 95% ethyl alcohol during the operations. The instruments were rinsed in 70% ethyl alcohol before each usage. Immediately following extirpation, the interior of each lobe was exposed by pulling the tissue apart with sterile forceps in order to increase the surface area in contact with the medium. The lobes were rinsed in two changes of control culture medium at 21 C. One opened lobe was placed on the floating raft in the cortisone acetate treated medium while the other opened lobe was placed on the floating raft in the control medium containing no cortisone acetate. Time required from the killing of the mouse to placing the gland on the raft was approximately 5 min.

The cultures were provided 0.2 uC radioactive iodine (I^{131}) in 0.1 ml H_2O (Bionuclear, Houston, Texas) following 6 hours of incubation. All cultures were incubated for a 48-hr

time period at which time gross macroscopic observations were recorded. The glands after incubation were fixed in Bouin's fixative for 24 hr, dehydrated, cleared, embedded, sectioned at 7 u, stained lightly with Heidenhains' iron-hematoxylin, and counterstained with eosin Y. The dipping technique described by Gude (1968) was used to prepare radioautographs using NTB-2 nuclear track emulsion (Eastman Kodak Company, Rochester, New York). The radioautographs were developed for 6 min in Developer D-19 (Eastman Kodak Company), rinsed in distilled water, and treated 15 min in Kodak Fixer after 30 days exposure. The radioautographs were scored by counting grains per follicle cell along a line transect through the center of the gland at a magnification of 950 diameters. Tangential sectioned cells were disregarded. The average grain count per cell was computed for each gland by counting the total number of grains and dividing by the number of cells encountered. Each unlabeled slide was counted on three different occasions. Several count runs were made until the cell number reached at least 40. Grain counts from the control tissues were compared with counts from their experimental counterparts establishing a paired sample experimental design. Significance was calculated by the t-test (Steel and Torrie, 1960).

RESULTS

Of 67 pairs of the glands cultured, 15 of which were used in radioautography, it was found that eight rafts had sunk and six cultures had been contaminated at the end of 48 hr incubation resulting in the loss of 14 pairs. These 14 pairs were discarded because if the rafts sunk the cultures would not have received a full oxygen supply and if contaminated (indicated by phenol red yellowing and bacterial growth in the medium) the bacterial growth might interfere with normal cell function. The remaining 53 cultured pairs of glands appeared to be maintained in healthy condition by the medium as revealed by macroscopic observation. The glands remained light red in color throughout incubation with indications of some blood vascularization when viewed through a dissecting microscope. Histological inspection of 10 pairs of cultured glands from the final test run of 15 mice revealed little necrosis and moderate cell activity in both experimental and control groups. (Necrosis had been noticed in the interior of lobes which had not been opened in preliminary runs.) Small areas of necrosis were observed near edges of some tissues which must have been damaged when the lobes were opened up or when they were excised from the mice. In both experimentals and controls, the follicular epithelium was generally cuboidal throughout the gland although some scattered follicles exibited a squamous secretory epithelium.

The radioautographs indicated a greater iodine uptake in the peripheral follicles. Grains were noticed both in the colloid and in the secretory epithelium throughout the glands. The results presented in TABLE 1 indicate a highly significant depression effect of cortisone upon thyroidal I^{131} uptake.

TABLE 1

^{131}I IN VITRO UPTAKE IN UNTREATED AND CORTISONE-ACETATE TREATED THYROID GLANDS

Pair Number*	Untreated Thyroid	Cortisone-Treated Thyroid
1	38.5 grains/cell	11.2 grains/cell
2	33.4	15.8
3	29.2	12.4
4	27.5	12.7
5	43.3	22.2
6	41.3	19.3
7	31.3	11.4
8	39.3	12.4
9	59.3	36.4
10	52.0	34.4

*Of 15 pairs of thyroid cultures, 12 appeared to be maintained by the medium. Three pairs were lost from rafts sinking. Two other pairs were utilized in determining exposure time of the radioautographs. The t-level computed for the remaining ten pairs was greater than 5.0 which is well above t-value of the 0.01 level of probability (3.250).

DISCUSSION AND CONCLUSIONS

Macroscopic and microscopic observations revealed that Ham's F-10 medium supplemented with KI, TSH, and fetal bovine serum can support the maintainence of the thyroid gland in an apparently healthy condition. The fact that the cultures appeared healthy over a period of 48 hr coupled with a normal histological appearance indicates that thyroid glands maintained in supplemented Ham's F-10 medium remain physiologically active. The cultures generally exhibited a cuboidal epithelium indicating physiological activity according to the criteria provided by Turner (1955) although some squamous epithelium was evident indicating little activity. It has been demonstrated <u>in vivo</u> that each follicle may act independently so that one might find a variety of secretory states in the thyroid gland (Gude, 1968). Uptake of I^{131} can be used as a test of physiological activity of thyroid glands in tissue culture as Seaman and Stahl (1956) demonstrated. These investigators found I^{131} grains in the follicular cells, indicating iodine trapping, and in the colloid, indicating binding of iodine in the formation of thyroid hormone. The results (Table 1) indicate that both experimental and control groups were physiologically active establishing even further

indications of maintainence of normal thyroid glands in this culture medium. The observation that iodine uptake was greater in peripheral follicles than in more central ones may have resulted from closer proximity to iodine or TSH in the medium. Tissue in the interior would be further removed from the medium increasing the distance across which the iodine or TSH diffused possibly accounting for higher counts near the periphery than in the interior. Radioactive iodine grains were evident in both the follicular cells and the colloid.

The mechanism by which reduction of thyroidal iodine uptake in vivo is brought about has been postulated to be a result of iodide renal clearance caused by cortisone induction of iodide elimination before uptake could be achieved (Kuhl and Ziff, 1952; Ingbar, 1953; Woodbury, et al., 1954; Skebel'skaya, 1958; McHugh and Yatvin, 1969). If renal elimination is the effecting mechanism, cortisone treatment in tissue culture would not be expected to have an effect on iodine uptake. The present investigation indicates that cortisone acetate thyroid glands maintained in tissue culture by a modified Trowell technique on a supplemented Ham's F-10 medium demonstrate iodine uptake depression. Although cortisone acetate treated thyroid glands appear to remain histologically and functionally normal, iodine depression might possibly have resulted from a cortisone acetate-medium reaction resulting in the loss of a component which is necessary for iodine uptake processes or in the physical

alteration of the thyroid so that uptake is restricted. According to Shellabarger (1954), it appears likely that if such a reaction occurred, iodine uptake would be completely inhibited. Treatment of experimental thyroid glands with cortisone acetate before culturing could be used to rule out such interactions of cortisone acetate with the culture medium if the treated experimental and control glands were then cultured in the same medium. A depression of I^{131} uptake in the treated lobe without such depression in the control lobe would indicate a direct effect of cortisone acetate on I^{131} uptake by the thyroid. The possibility that cortisone acetate blocked the action of TSH (or a fraction of TSH) is unlikely. Experiments by Greer (1952) and Dobyns and Steelman (1953) indicate that TSH may be composed of two or more fractions. Hennen, Winand, and Nizet (1965) claimed that it had been impossible to separate TSH into fractions by submitting pituitary extracts to various chromatographic techniques or to enzymatic digestion. There is no conclusive evidence that the substances which promote exophthalmia, thyroid growth, or iodine uptake are separate entities. McHugh and Yatvin (1969) demonstrated that pituitary replacement following hypophysectomy resulted in an increased I^{125} uptake in cortisone treated rats indicating no TSH action interference. Yatvin, Wannemacker, and Brown (1966) reported that administration of cortisone to rats resulted in decreased thyroid size, total protein, total RNA, ratio of RNA/DNA, and H^3-leucine encorporation indicating cortisone

promotes protein catabolism in the thyroid gland. Cortisone has been shown to promote catabolism for gluconeogenesis (Welt, et al., 1952). Thyroidal I^{131} uptake may be suppressed due to the direct effects of cortisone on catabolism of this type.

If cortisone and other adrenosteroids can be shown to exhibit a direct effect on I^{131} uptake, the rise in TSH levels may result from the action of these steroids in decreasing blood titers of thyroid hormone. A fall in iodine uptake would indicate a low level of thyroid activity resulting in the stimulation of TSH release from the pituitary which correlates with the low ACTH secretion (resulting from high circulating steroid concentrations) which allows TSH to be secreted by the pituitary gland. Further investigations may reveal that the thyroid gland is not only directly controlled by TSH but also by adrenosteroids.

The technique which has been described in this study appears to be adequate for studying the direct effect of adrenocorticoids on the thyroid gland as revealed by the results described earlier obtained by treating cultured thyroid glands with cortisone acetate. It is possible with this technique to study the action of a specific hormone on the thyroid gland while excluding the multiple interactions that occur in the thyro-pituitary-adrenal axis in *in vivo* investigations.

SUMMARY

Emphasis has been placed on the use of tissue cultures as a method for studying direct hormonal actions. In an *in vivo* investigation, hormonal influences may result from indirect mechanisms which are not easily identified. These indirect mechanisms may be excluded from the closed system of an *in vitro* investigation. It has been determined that thyroid glands may be maintained on a supplemented Ham's F-10 medium and this tissue culture system may be used under experimental conditions to study the effect of cortisone acetate on thyroidal I^{131} uptake.

Thyroid-adrenal, thyroid-pituitary, adrenal cortex-pituitary and thyroid-pituitary-adrenal cortex relationships were discussed. The literature indicates that cortisone may exert an effect on all three glands mentioned. Therefore, the mechanism by which cortisone affects a particular gland may be quite complex. Tissue culture usage would appear to provide an ideal means for investigation of these *in vivo* relationships.

When thyroid glands of mice were grown in Ham's F-10 medium supplemented with TSH, KI, and fetal bovine serum in the presence of cortisone acetate, it was found that:

(1) macroscopic and microscopic observations reveal that thyroid glands can be maintained in an apparently normal condition

(2) a modified Trowell technique is suitable for thyroid gland culture

(3) cortisone acetate reduces iodine uptake in this particular system (Table 1)

(4) the method described can be used for investigation of the effects of a single hormone on a tissue or organ.

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