extent of further metabolism to 11-dehydrocorticosterone (21-hydroxypregn-4-ene-3, 11, 20-trione)[9] slowly declined, before finally falling to low levels again after the 4th day of culture. Analysis of the quadrant outputs of corticosterone in unstimulated cultures failed to reveal any evidence of significant circadian variation from a steady rise and fall, such fluctuations as were measured falling essentially within the limits of experimental error, the coefficient of variation of the assay procedure being 10% at these low levels of corticosterone.

We have demonstrated previously[2] that the predominant fluorogenic steroid secreted by adrenal monolayers maintained without ACTH for several weeks is 20α-dihydroprogesterone (20α-hydroxypregn-4-ene-3-one), owing principally to the decline in 21-hydroxylase activity that takes place in the absence of ACTH[3]. During the initial phase of culture examined here, however, the fluorogenic steroid secreted by both ACTH-stimulated and unstimulated cultures was accounted for as corticosterone when medium extracts were subjected to Sephadex LH-20 column chromatography.

The present experiments have therefore failed to reveal any significant circadian variation in the corticosterone output of either stimulated or unstimulated adrenal monolayer cultures. This finding is in marked contrast to results obtained with organ-cultured hamster[5,6] and lemming[7] adrenal glands, where circadian rhythms of corticosteroid secretion often exceeded 40% of the mean output in amplitude and persisted for over a week in ACTH-stimulated and unstimulated cultures. The present results do not exclude the possibility of unsynchronized rhythmicity in the individual cells in the non-proliferating confluent monolayers, but there was no evidence of any rhythm of diminishing amplitude, such as might be expected to result from a gradual desynchronization of individual cellular rhythms.

The absence of rhythmicity in monolayer cultures and its apparent persistence in organ cultures suggests that organized tissue structure is important for its maintenance in vitro, although a species difference between adrenal rhythms in the rat and in the hamster and lemming can not be ruled out. The significance of endogenous adrenal rhythmicity in vivo, however, is doubtful. It has been shown that deafferentation of the medial basal hypothalamus in the rat results in both a high pituitary ACTH content[10] and high steady levels of corticosterone secretion[11], similar results being obtained with suprachiasmatic lesions[12]. These experiments indicate that variations in corticotrophin releasing factor synthesis[13] resulting in variations in ACTH secretion[14] are responsible for at least the greater part of the circadian variations in steroid secretion by the rat adrenal cortex in vivo. Such a mechanism is consistent with our failure to detect circadian variations in the steroid output of monolayer-cultured rat adrenal cells. Thus the significance and origin of the endogenous rhythms of steroid secretion in organ-cultured adrenals remain to be determined.

Résumé. La stéroïdogénèse par les cellules surrénaliennes du rat adulte (zones fasciculaire et réticulaire), cultivées en couche monocellulaire a été examinée dans le but de trouver les rythmes circadiens, semblables à ceux qui ont été observés dans la sécrétion de corticosteroïdes des glandes surrénales en culture organotypique. On n'a pas observé de rythmes de sécrétion, ni dans les cultures stimulées par ACTH ni dans les cultures non stimulées, ce qui indiquerait que si le cortex surrénalien possède en effet une fonction rythmique intrinsèque, cela doit dépendre de la structure histotypique du tissu glandulaire.

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Certain Features of Hypophyseal Intermediate Lobe Materials as Seen in Tissue Culture[1]

In an attempt to purify, characterize and determine the function of bovine hypophyseal intermediate lobe materials i.e., intermediate lobe tissue and intraglandular colloid, it was learned that materials characterized at one point in time may, within 24 to 48 h, show different characteristics. Since these materials are so highly active, and if meaningful data are to be secured from ongoing chromatographic and immunological studies, it was felt that some time relationship be established between the breakdown of intermediate lobe tissue, the ultimate formation of intraglandular colloid[2], and the elaboration of various substances during the breakdown period. Tissue cultures were utilized to secure this information and this communication, in part, is based on these findings.

Previous investigations[2–7], established an activity period for intermediate lobe materials, from 1 to 3 days, based on the response of cells of mesodermal origin, in various organs, to an injection of these materials. Mesodermal cells are induced to differentiate and proliferate along red blood cell lines.

Gel filtration studies of intermediate lobe materials demonstrate the existance of 4 separate substances in fractions of intermediate lobe materials, and for convenience are labeled WB_1–WB_4. All of these fractions activate cells of mesodermal origin to various degrees. At this time it has not been determined whether these fractions are breakdown products of a specific intermediate lobe cell or a combination of products from profilerating intermediate lobe cells. Nevertheless, tissue culture techniques have added some basic knowledge to this study.

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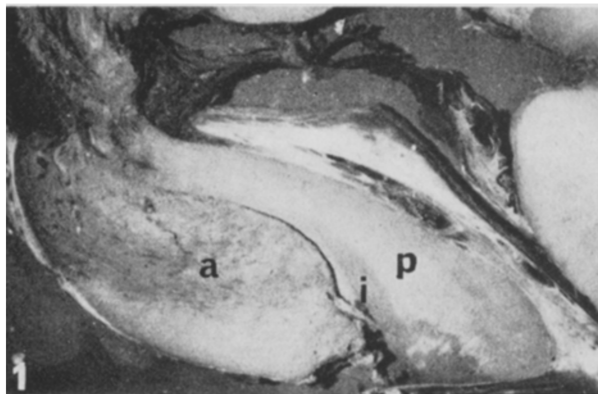
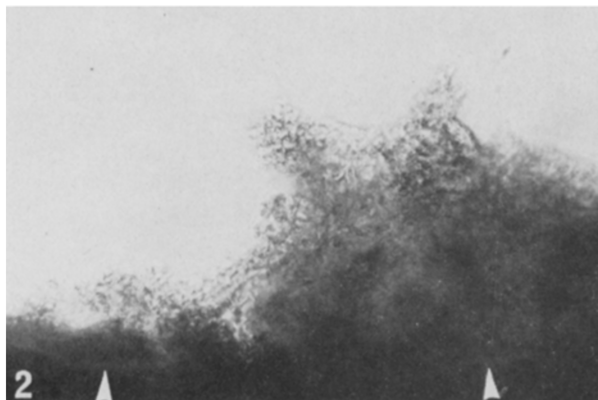
Primary cultures were obtained by mincing and trypsinizing (0.25% trypsin – Difco 1:250) duck embryonic tissue in phosphate-buffered saline (PBS) at room temperature following procedures reported elsewhere[8]. The growth media was made up of Eagle's minimal essential medium supplemented with 10% fetal calf serum (Grand Island Biological Company) and antibiotics (penicillin G potassium, 250 IU, and streptomycin sulfate 100 µg per ml of medium). Non-active marginal zone intermediate lobe tissue was cultured in a leighton tube, for 3 to 5 days at 37 °C.

Since the gland is enclosed in a dense fibrous capsule of dura, sterility can be maintained by utilizing proper techniques. Intermediate lobe tissue can be harvested by making a mid-sagittal cut through the whole gland (Figure 1) exposing the 3 lobes. In non-active glands, the intermediate lobe closely approximates the anterior lobe, the residual luman is almost entirely closed and is devoid of colloid. The narrow band of intermediate lobe tissue is darker in color than the posterior lobe to which it is intimately attached.





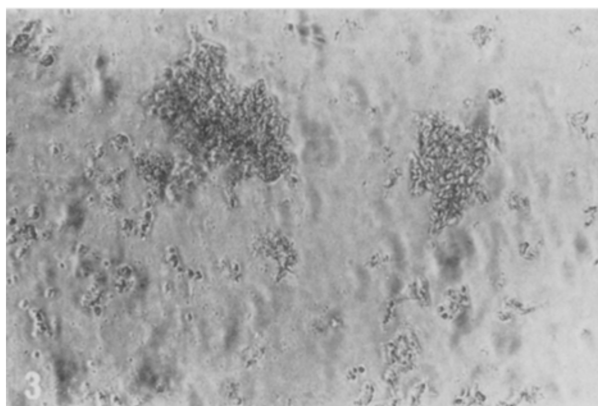


Fig. 1. Gross mid-sagittal section through the bovine hypophysis showing the anterior, intermediate and posterior partes. × 14.

Fig. 2. Proliferation of marginal zone intermediate lobe cells in culture. The arrows point to a natural division between the marginal and deep zone of intermediate lobe cells. × 500.

Fig. 3. Clumps of proliferating intermediate lobe cells within the medis. × 500.

In culture, marginal intermediate lobe cells i.e., those forming the surface of the lobe at the intraglandular lumen, become highly active within 8 to 12 h. Cellular vacuolization is the initial change observed, and within 24 to 48 h, the cells increase in diameter from 0.3–0.5 µm to 0.7–0.9 µm. This activity occurs in marginal zone cells at various loci and time intervals, developing pockets of cells which periodically detach themselves from the surface of the intermediate lobe (Figure 2).

Within 48 h, large clumps of proliferating cells break away and become isolated into groups of varying numbers of cells (Figure 3). Between 48 and 72 h, the breakdown of marginal intermediate lobe cells is complete with the resultant formation of colloid.

Microscopic sections of cultured intermediate lobe tissue stained with hematoxylin-eosin (Figures 4–7) show that vacuoles within cells in isolated groups continue to enlarge (Figures 6–7). It is significant that the breakdown of intermediate lobe tissue with the ultimate formation of colloid as observed in tissue culture, complement those reported for gross and microscopic studies of the activity of intermediate lobe materials. Indeed, whatever the nature of the cyclic behavior of the intermediate lobe, these studies indicate a breakdown in marginal intermediate lobe cells within a 72 h period. As previously reported[2–7], the marginal zone is replaced by cells within the deep zone of the lobe undergoing direct division. It is difficult to determine the replacement time. It is important to note that after the marginal zone of cells is replaced, the intermediate lobe makes direct contact with the anterior lobe[5]. It is at this juncture that blood vessels of the intermediate lobe continually communicating with those in the posterior lobe, grow into the anterior lobe, interconnecting, in this manner, all lobes of the gland. This connection is severed at the onset of the breakdown of marginal intermediate lobe cells.

In reviewing relevant literature[9–22] with reference to substances thought to be present in the intermediate lobe, no comment is made of the fact that ACTH and MSH contain little if any cysteine or cystine. Indeed, tests for cysteine and cystine in bovine intermediate lobe tissue shows the presence of these substances, although amino acid analysis shows that the substances are not present in intraglandular colloid of intermediate lobe origin. Since most tissue culture media contain either cysteine or cystine, no determination as to the presence or absence of these materials could be made from growing tissue. If it is shown that intraglandular colloid contains ACTH or MSH, the question exists as to whether there is a transport system between the intermediate lobe and other partes via intercommunicating arteries prior to the breakdown of marginal intermediate lobe cells[2], or via extracellular channels[23], managing certain substances within the gland. It is difficult to determine which cells within the marginal zone produce ACTH or MSH since all seem to be involved in this breakdown process and the formation of intraglandular colloid.

As the study of the intermediate lobe progresses, ACTH and MSH, if present for brief periods of time, would seem to be of lesser significance than other substances found in this lobe and now under investigation. It is becoming more evident that the intermediate lobe of the hypophysis should be considered as a structure of equal importance

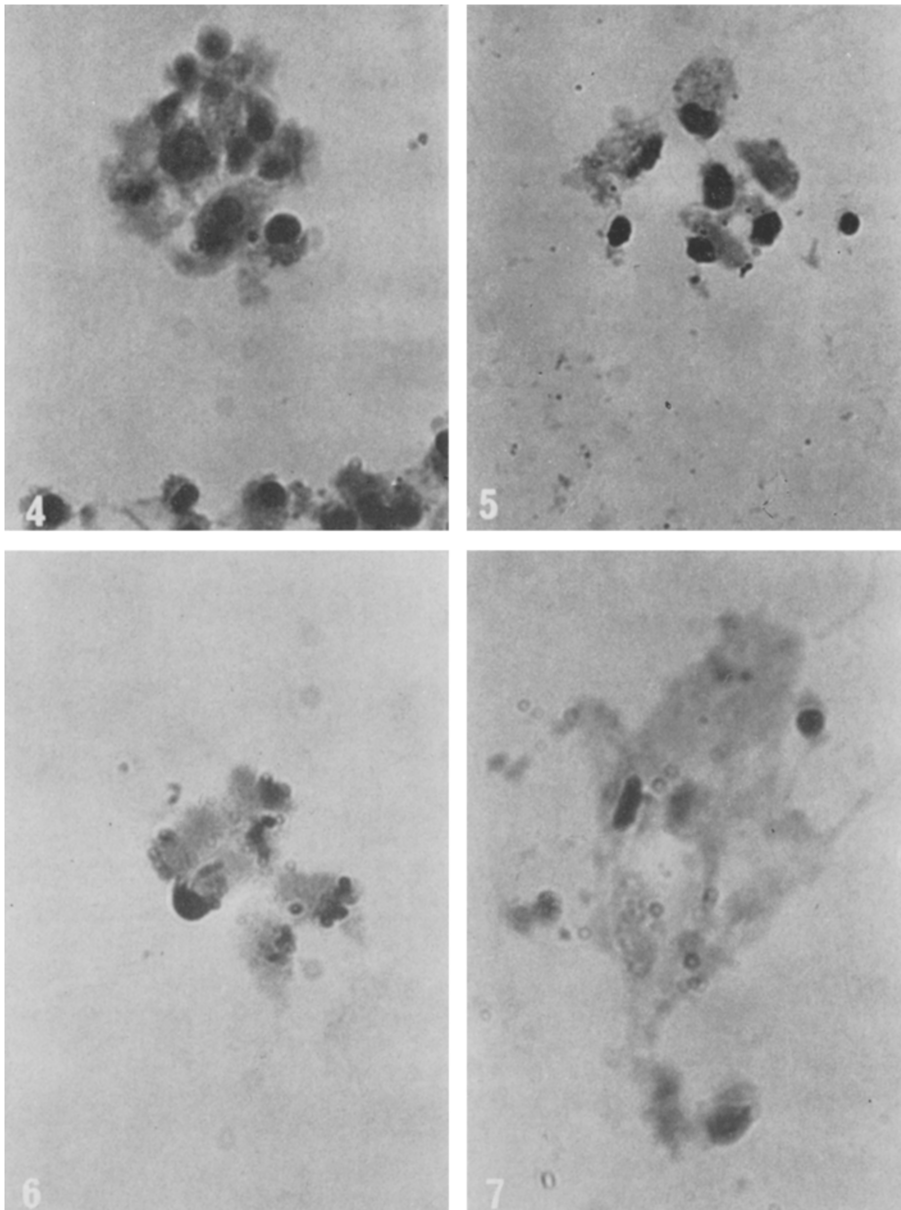


Fig. 4–7. Microscopic sections of intermediate lobe cells taken from tissue culture and stained with H. & E. showing vacuolization and the breakdown of cells ultimately forming colloid. ×1500.

as the anterior and posterior partes, and not merely as a segment of the posterior lobe[9, 11, 23–25].

Zusammenfassung. Die Isolierung einzelner Fraktionen der Hypophyse, im besonderen der Mittellappenanteile ist kompliziert und es wurden daher Kulturen von Rinderhypophysen angelegt, in welchen ACTH und MSH vorkommt. Im Zusammenhang mit dem Transportsystem wird festgestellt, dass dem Mittellappen eine gleich wichtige Funktion zukommt wie dem Vorder- oder Hinterlappen und dass jener nicht als ein Segment des Hinterlappens aufgefasst werden kann.

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