according to the method of NEW[10], as modified by GALLERA[4]. Hensen's node from the quail blastoderm of stage 3+ to 4 was inserted between the ectoblast and the hypoblast of the stage 3+ to 4 chick blastoderms, with the ventral side of the graft apposed to the underside of the host ectoblast at the periphery of the area pellucida (see review by GALLERA[11]). Similarly, posterior parts of the primitive streak of the quail were grafted to chick blastoderms. The embryos were maintained at 39°C in a controlled CO_2 incubator for about 30 h, to about 15–16 somites. The embryos were recovered, fixed in Zenker's fixative, serially sectioned and stained by the Feulgen technique (Schiff reaction). 40 such preparations were made with Hensen's node and 40 with posterior streak material.

Posterior parts of the quail primitive streak were apparently unsuccessful grafts or the graft disappeared. The normal fate of the cells of the posterior part of the primitive streak is to be dissipated into the extraembryonic mesoblast. Living implants are similarly dissipated in the host embryo and exert no inductive influence (WAHEED and MCCALLION[12]). Upon microscopic examination of the cultures, however, quail cells could be identified in the mesoblast of the host embryos in most cases, thus confirmning the fate of the cells of the posterior primitive streak.

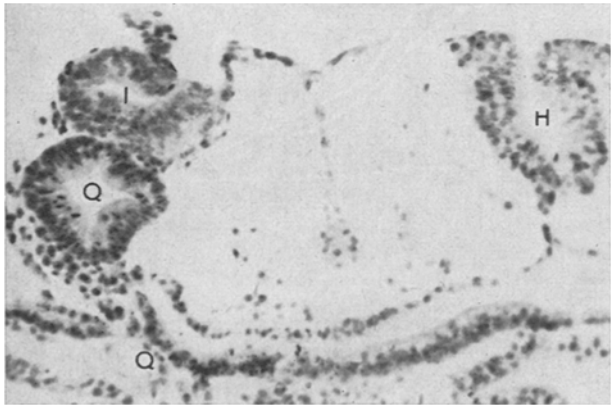

Fig. 3. Cross section of an embryo similar to that in Figure 1, showing the host axis (H), tissues derived from the graft (Q) and induced neural tissue (I). Zenker fixation, Feulger stain.

In all cases grafts of quail node to chick blastoderms were successful (Figure 1). The fate and degree of differentiation of the grafted quail Hensen's node could be clearly distinguished in microscopic sections. The graft gave rise to a neutral tube, chorda, some mesoderm, particularly associated with heart, and gut endoderm (Figures 2 and 3). This is in essential agreement with the results obtained by GALLERA[7] with chick to chick grafts. The implanted quail node also induced neural differentiation in the chick ectoblast (Figures 2 and 3) and the resulting neural tissue is obviously chick. It has already been demonstrated that inductive action is not restricted by species specificity and that chick Hensen's node induces a neural structure in the duck ectoblast (WADDINGTON[13]). The advantage of the present situation is the certainty with which quail and chick tissues can be clearly distinguished since the characteristics of the quail nucleus, following Zenker fixation and Feulgen staining, constitutes a persistent biological labelling of the grafted tissues.

Résumé. Des greffons du nœud de Hensen prélevés à des embryons de caille aux stades 3+ à 4 furent introduits sous l'ectoblaste d'embryons de poulet de mêmes stades cultivés selon la méthode de NEW[10]. Les greffons se sont différenciés en divers tissus et en même temps ont provoqéu une différentiation neurale dans l'ectoblaste des hôtes. Les cellules des greffons de la partie postérieure de la ligne primitive 5e sont dispersés dans lemésoblaste extraembryomnaire des hôtes et n'ont exercé aucune action sur l'ectoblaste.

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The Primary Tissue Culture of Rat Adult Decapsulated Adrenal Glands: Radioautographic Studies on the Metabolic Effects of β_{1-24}-ACTH

In the past radioautography was never, as far as we know, used to investigate the in vitro metabolism of primary tissue cultures of mammalian adrenal cortex, because of technical difficulties. Since these were recently overcome in our laboratory[1], we report here the metabolic changes induced in cultured rat adult adrenocortical cells by β_{1-24}-ACTH (Synacthen®, Ciba, Origgio, Italy) as revealed by radioautographic means.

Materials and methods. The adrenals taken from 10–16 adult female albino rats of Wistar SM strain were pooled and cultured as previously communicated[1]. For each experiment 32–48 separates were grown in absence of ACTH for 15 days. On the 16th day they were divided into 2 stocks: a) the first was treated with daily doses of β_{1-24}-ACTH (10 μg/ml = 1 IU ACTH/ml) given with fresh growth medium for as long as devised; b) the second stock served as control: its growth medium was also changed every day. After exposures of 24, 48, 72 and 120 h, both stocks were pulse-labelled at 37°C for 1 h. The following isotopes (all purchased from NEN Chemicals GmbH, Frankfurt/Main, Germany) were employed: a) thymidine-methyl-H^3, used at 1.0 μCi/ml; b) orotic-5-H^3 acid, used at 1.0 μCi/ml; c) L-leucine-4,5-H^3 (N) dissolved at 1.0 μCi/ml in a medium free of both leucine and serum. Both stocks were then fixed and processed for radioautography as a single batch by the coating technique of

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Table I. Effect of β_{1-24}-ACTH (10 µg/ml daily) on the incorporation of thymidine-methyl-H^3 by primary rat adult adrenocortical and fibroblast-like cells

Length of exposure	No. of preparations examined	Adrenocortical cells LI ± S.E.	Variation in LI (%)	Fibroblast-like cells LI ± S.E.	Variation in LI (%)
C	5	8.9 ± 1.4 (526)	—	9.5 ± 1.0 (1430)	—
24 h	5	17.6 ± 2.2 (750)	+ 97.7[c]	15.3 ± 1.9 (1137)	+ 61.0[b]
C	4	8.5 ± 0.7 (630)	—	13.2 ± 0.7 (912)	—
48 h	5	20.0 ± 1.8 (703)	+ 135.2[c]	15.8 ± 3.4 (1130)	+ 19.6[a]
C	4	9.2 ± 1.3 (717)	—	9.5 ± 2.7 (1003)	—
72 h	5	10.3 ± 2.4 (936)	+ 11.9[a]	8.4 ± 2.0 (1317)	— 11.6[a]
C	4	11.6 ± 3.2 (787)	—	19.4 ± 7.5 (857)	—
120 h	5	13.0 ± 2.7 (920)	+ 12.0[a]	15.9 ± 3.7 (1074)	— 18.1[a]

C, controls; LI, labelling index/h. The total number of cells counted in each set of preparations is indicated between parentheses. [a] P not significant; [b] $P < 0.01$; [c] $P < 0.001$.

KOPRIWA and LEBLOND[2]. The Ilford K5 Nuclear Emulsion (Ciba, Origgio) diluted $^1/_4$ with bidistilled water and melted at 50°C was used. Non-labelled cultures were coated in parallel to estimate the level of background. Positive and negative chemographic phenomena, which were looked for, were practically absent. Thymidine-labelled cells were counted under the microscope at × 1,200 and the labelling index (LI)/1 h determined. Orotate- and leucine-labelled cells were photographed and silver grains per cell counted. The means were compared by the Student's t-test (P was considered as significant only if < 0.05).

Results and discussions. The background of 'cold' radioautographs was always found to be less than 1 silver grain/100 µm² (from 0.232 to 0.941). As the data of Table I show, the number of DNA-synthesizing primary rat adult adrenocortical cells is significantly increased during the first 2 days of incubation with β_{1-24}-ACTH. This is the earliest metabolic change revealed by our experimental design, and, although transitory, it is the reason of the marked increment in number of adrenocortical cells in β_{1-24}-ACTH-treated cultures. Stimulation of DNA synthesis occurs when adrenocortical cells, as shown by their ultrastructure, are still poorly differentiated[1]. Later, when their differentiation has been completed, ACTH-treated cortical cells exhibit LI's/h close to those of controls. ACTH is known to induce an early enhancement of in vivo adrenocortical DNA synthesis in the guinea-pig[3] and a delayed one in the rat[4,5]. In contrast, an early inhibition of DNA synthesis without any significant change in uridine and leucine incorporation rates occurs in ACTH- and adenosine 3′, 5′-cyclic monophosphate (cAMP)-treated cloned mouse adrenal tumor cells[6]. Moreover, it is noteworthy that the LI's/h detected in primary 72–120 h ACTH-treated cultures are higher than those reported to occur in the various zones of the exponentially growing adrenal glands of prepubertal male rats[7]. This findings suggests that some of the yet unknown mechanisms, which in vivo repress adrenocortical cell proliferation, are lacking in vitro. The results exhibited in Table II show that since the 48th h of exposure to β_{1-24}-ACTH onwards the in vitro adrenocortical RNA synthesis is significantly enhanced. As the data in Table III demonstrate, in vitro adrenocortical gross protein synthesis is also stimulated by 48–72 h of treatment with β_{1-24}-ACTH. After 120 h, however, the protein synthesis rate of treated cells does not differ from the control levels. The in vivo long-term stimulation by

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Table II. Effect of β_{1-24}-ACTH (10 µg/ml daily) on the incorporation of orotic-5-H^3 acid by primary rat adult adrenocortical and fibroblast-like cells

Length of exposure	Adrenocortical cells Mean grain count per cell ± S.E.	% Change in grain count	Fibroblast-like cells Mean grain count per cell ± S.E.	% Change in grain count
C	88.0 ± 4.4 (40)	—	67.3 ± 3.7 (50)	—
24 h	95.6 ± 5.1 (45)	+ 8.6[a]	65.7 ± 2.1 (50)	— 2.3[a]
C	128.5 ± 5.1 (44)	—	106.1 ± 4.7 (51)	—
48 h	185.8 ± 8.7 (47)	+ 44.6[c]	135.9 ± 7.3 (49)	+ 28.0[c]
C	72.5 ± 3.0 (47)	—	73.1 ± 2.1 (51)	—
72 h	81.4 ± 3.2 (45)	+ 12.2[b]	76.6 ± 2.9 (50)	+ 4.7[a]
C	116.8 ± 4.1 (41)	—	106.7 ± 4.3 (40)	—
120 h	133.1 ± 3.9 (33)	+ 13.9[c]	108.5 ± 3.5 (42)	+ 1.6[a]

The number of cells counted for each experimental set is indicated between parentheses. C, controls. [a] P not significant; [b] $P < 0.01$; [c] $P < 0.001$.

Table III. Effect of β_{1-24}-ACTH (10 μg/ml daily) on the incorporation of L-Leucine-4,5-H^3 (N) by primary rat adult adrenocortical and fibroblast-like cells

Length of exposure	Adrenocortical cells Mean grain count per cell ± S.E.	% Change in grain count	Fibroblast-like cells Mean grain count per cell ± S.E.	% Change in grain count
C	70.6 ± 6.4 (30)	—	55.7 ± 3.7 (32)	—
24 h	75.6 ± 3.8 (49)	+ 7.06[a]	51.9 ± 2.3 (33)	− 6.9[a]
C	88.3 ± 3.6 (49)	—	80.3 ± 2.5 (49)	—
48 h	116.4 ± 4.5 (49)	+ 31.8[b]	97.9 ± 4.8 (48)	+ 21.8[b]
C	85.7 ± 4.3 (44)	—	88.4 ± 3.1 (50)	—
72 h	96.2 ± 2.5 (43)	+ 12.2[b]	96.4 ± 8.7 (45)	+ 8.7[b]
C	98.5 ± 2.6 (41)	—	99.0 ± 3.2 (38)	—
120 h	102.9 ± 4.2 (30)	± 4.1[a]	98.8 ± 2.8 (45)	− 0.21[a]

The number of cells counted for each experimental set is indicated between parentheses. C, controls. [a] P not significant; [b] $P < 0.001$.

ACTH[4,8,9] of the synthesis of various species of nuclear and also mitochondrial RNA's in adrenocortical cells is believed to be the main basic mechanism of the differentiative and trophic effects of the hormone, possibly mediated through cAMP[10]. The de novo synthesized RNA's encode a number of structural-enzymatic proteins of the smooth endoplasmic reticulum[11] and inner mitochondrial membranes[12], which support the whole of steroidogenic processes[13,14]. The in vitro β_{1-24}-ACTH-induced initial higher levels of adrenocortical RNA and protein synthesis probably reflect the greater cellular needs to transform into fully differentiated fasciculata-like corticosteroid-producing elements, while the later lower levels of stimulation evidenced, which at 120 h concern RNA only, may indicate the current needs to maintain such a differentiated condition, slowly undergoing hypertrophy. The inhibition of adrenocortical RNA and protein synthesis reported to take place in rats treated for 6–9 days with ACTH when there occurs a replication of adrenal DNA[5,9] does not contrast with our findings. The in vivo adult adrenocortical cells may be considered as a more synchronous population, with respect to the mitotic cell cycle, than they are in vitro, since in the animal most of them are prevented from entering the DNA synthetic or S-phase. The random asynchrony of primary adult adrenocortical cell cycles impedes a clear evidentiation of the slowed rates RNA and protein synthesis to be significantly evidenced when DNA is synthesized. Inhibition of the in vitro adrenocortical protein synthesis by ACTH has been reported[15] and ascribed to an increased tissue concentration of de novo synthesized corticosteroids[16]. Non-inhibitory[17] or stimulatory[18] effects of ACTH on the in vitro adrenal protein synthesis have been reported as well. We believe that the daily administration of β_{1-24}-ACTH with fresh growth medium prevented the rise of in vitro corticosteroid concentration to critical levels and in a more physiological way than might have been done by aminoglutethimide[18]. Finally, the non-stimulated adrenocortical protein synthesis after 120 h of exposure to β_{1-24}-ACTH might be either an incipient effect at the translational level of the increased production of corticosteroids[19,20] or a sign of decreased synthetic needs of the cortical cells. The results reported in Tables I, II and III show that in primary rat adult adrenal cultures exposed to β_{1-24}-ACTH fibroblast-like cells behave in a way distinct from that of adrenocortical cells. Our radioautographic results give some insights on the metabolism of primary adult adrenocortical cells while the β_{1-24}-ACTH-induced differentiative and, later, hypertrophic phenomena occur. They give further evidence that such in vitro cells constitute a not previously available experimental model for the study of adult adrenocortical cell physiology[21].

Riassunto. Metodi di radioautografia ottica sono stati per la prima volta applicati allo studio delle attività metaboliche di cellule di corteccia surrenale decapsulata di Ratto adulto in coltura primaria in vitro. In tali cellule, sono stati evidenziati effetti specifici di stimolo sulla sintesi di DNA, RNA e proteine dopo trattamento per 1–5 g con β_{1-24}-ACTH (10μg/ml/di). Queste variazioni dell'attività metabolica si associano ad una differenziazione e, in seguito, ipertrofia degli organuli subcellulari (reticolo endoplasmico liscio, mitocondri) in senso corticosteroidogenico.

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