anaphase and micro nuclei formation at telophase occured regularly in both the cell types and varied between 17–20%. In about 1.5–2% cells of both the cell types fusion nuclei were observed. Cytological observations made 1 year after tissue isolation showed about 90% preponderance of diploid metaphase. When the culture was maintained for over 12 years, divergency in ploidy of the cell population occurred. Simultaneously there was a shift from predominant diploidy to dominant tetraploidy. Regular occurrence of unequal separation of chromosomes, persistant telophase bridges and fusion nuclei indicate the way in which polyploidization might have occurred in *Althaea* tissue cells. Pre-existing polyploid cells present in the initial explant were also likely to have played a part in upward shift in ploidy level[2].

Crown gall tumour tissues were generally considered cytologically stable material[3] due to the absence of aneuploid cells both in in-vivo[4] and invitro[5] cells. Occurrence of high percentage of aneuploid cells in the present study indicates that *Althaea rosea* crown gall tissue may not be a cytologically stable material.

Zusammenfassung. Kulturen von Tumorgewebe (Crown Gall) und normalen Explantaten von *Althaea rosea* wurden nach mehr als 10jähriger Kultivierung zytologisch verglichen.

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Induction in the Chick by Quail Hensen's Node[1]

It has been well established that the living Hensen's node of the chick blastoderm induces neural differentiation when grafted under competent chick ectoderm (Waddington[2], Woodside[3], Gallera and Castro-Correia[4], Pasternak and McCallion[5], Vakaet[6]). By implanting grafts of primitive streak material in a manner that keeps structures derived from the graft quite separate from the host embryonic axis Gallera[7] was able to analyze the fate of the graft and the degree of its self differention. Similarly, he was able to evaluate the inducing capacity of the graft. These studies, however, were based on chick to chick grafts and, therefore, difficult to distinguish precisely induced and grafted tissues. Since quail cells can be clearly distinguished from chick cells in embryonic associations of tissues of the two species (LeDouarin[8]), we undertook a study of the fate and inductive capacity of Japanese quail Hensen's node in the chick embryo.

The chick embryos used in these experiments were obtained from White Leghorn eggs supplied by the Department of Poultry Science at the University of Guelph, Guelph, Ontario. The Japanese quail (*Coturnix coturnix Japonica*) embryos were obtained from the eggs of birds maintained on our own laboratories. The chick eggs were incubated at 39°C for 14–16 h to obtain stages 3+ to 4 (Hamburger and Hamilton[9]). The quail eggs were similarly incubated to obtain equivalent stages. Chick blastoderms were explanted and cultured at 39°C

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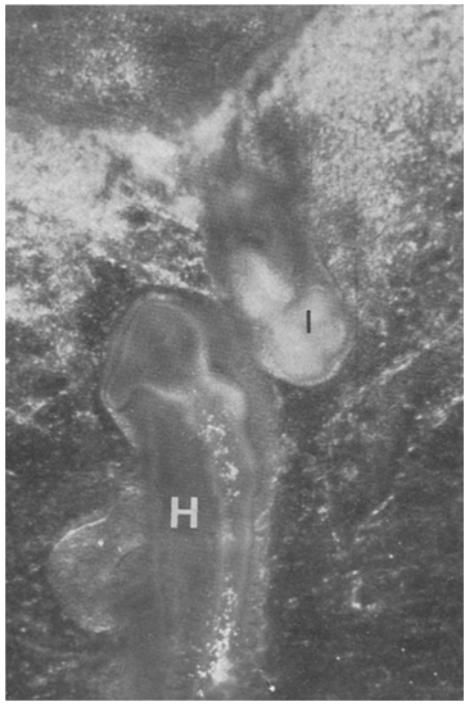

Fig. 1. Photograph of a typical chick host blastoderm (H) on which a graft of quail Hensen's node (I) had been implanted at stage 3+ to 4 showing the results 30 h later.

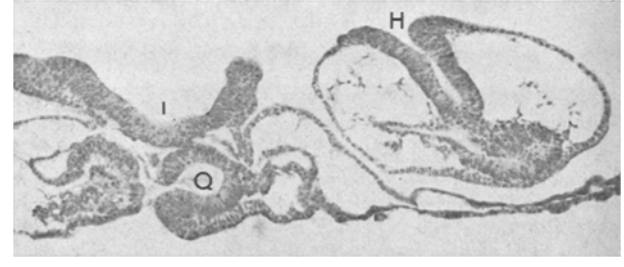

Fig. 2. Cross section of an embryo similar to that in Figure 1, showing the host axis (H), tissues derived from the graft (Q) and induced neural tissue (I). H. & E.

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.

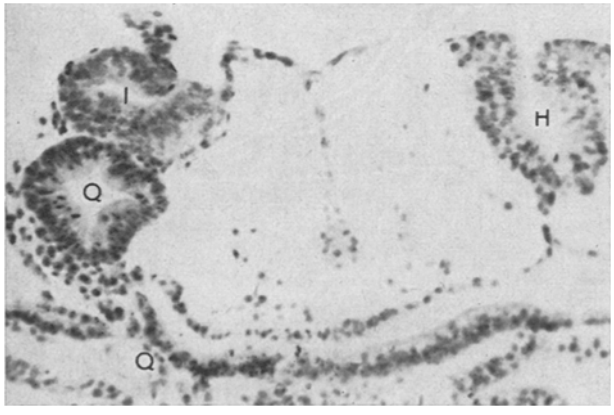

Fig. 3. Cross section of an embryo similar to that in Figure 1, showing the host axis (H), tissues derived from the graft (Q) and induced neural tissue (I). Zenker fixation, Feulger stain.

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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