Correlation Between Mesenchymal Composition and Epidermal Differentiation in Chick Embryonic Skin

Several studies have demonstrated that chick epidermal differentiation depends on epithelio-mesenchymal interactions[1–3], and some data support the possibility that mesenchymal ground substance may be involved in these processes[4]. In previous research, we have shown that epidermis of embryonic skin explants develops in different ways according to its nutrition: it undergoes differentiation if maintained in a medium containing chicken serum, and does not differentiate in medium lacking chicken serum[5]. Such a system enables us to investigate the existing correlation between mesenchymal composition changes and epidermal differentiation. Current experiments therefore deal with histochemical analysis of dermal ground substance both in vitro (in differentiating and non-differentiating explants) and in vivo (in developing skin), and with the biochemical determination of some dermal intercellular components (uronic acids, sialic acids and hexosamines) in vitro.

Material and methods. For histochemical analysis, fragments from the thigh regions of 6-, 10- and 14-day White Leghorn embryos were used. Explants were derived from the same regions of 6-day chick embryos. Skin areas of the same size, removed under sterile conditions, rinsed in Tyrode's and carefully dissected, were placed in culture dishes on the vitelline membrane, according to WOLFF[6]. Two sets of experiments were performed using chicken serum (CS) or embryo extract (E) nutrient medium as previously described[5]. Cultures were incubated at 37 °C. Tissues were fixed in Bouin's fluid and routine histological procedures were followed. Serial sections (8–10 μm) were stained for morphological examination with hematoxylin-eosin and for histochemical analysis with PAS before and after chemical hydrolysis for sialic acid according to QUINTARELLI et al.[7], Toluidine bleu at 2.5 pH in Walpole's buffer, associated Alcian yellow GXS and Alcian bleu 8GX at $MgCl_2$ different concentration (namely A y 0.2 *M* and A b 0.025 *M*) before and after testicular hyaluronidase digestion (Ialovis Vister, 1 mg/ml in phosphate buffer pH 7 for 6 h at 37 °C)[8]. By means of this procedure, based on critical electrolyte concentration method (SCOTT and DORLING[9]), hyaluronic acid (HA) and condroitinsulphuric acids (CSA) are differently stained depending on $MgCl_2$ molarity concentration.

For biochemical analysis, about 40 cultures for each experiment were pooled, sonicated for 1 min at 21 Hz (MSE instrument, Model 411) in order to have the maximum solubility. On the aliquots, proteins were determined by the method of LOWRY et al.[10], sialic acid (as N-acetyl-neuraminic acid, NANA) by MIETTINEN and TAKKI-LUKKAINEN's modification of SVENNERHOLM's method[11], uronic acids by BITTER and MUIR's method[12] and total hexosamines by CESSI and PILIEGO's[13] method after hydrolysis with 1 *N* HCl at 100 °C for 4 h. HA and CSA were separated according to SCOTT's procedure[14] by means of fractional precipitation with cethilpiridiniumchloride in different salt concentration and evaluated as hexosamines content.

Results and discussion. Histological examination of skin explants confirms the previous results[5]; whithin 6 days, epidermis keratinizes only in the CS maintained cultures. By the 2nd in vitro maintenance, histochemical pattern of skin mesenchyme is nearly the same in the 2 series of cultures and closely corresponds to that of 6-day embryo dermal skin (Table I). On the other hand, remarkable differences can be observed as development proceeds. 6-day CS supplemented cultures as well as 14-day embryo skin are more PAS-positive, no stained in 2.5 pH Toluidine bleu, more stained by A b and less by A y in comparison with E cultures. These data indicate a greater accumulation of glycoproteins (GP) than glycosoaminoglycans (GAG) and a prevalent relative concentration of HA in respect to CSA. Biochemical findings agree with the histochemical observations. At the end of in vitro maintenance, a major content of NANA and hexosamines

1 C. B. MCLOUGHLIN, J. Embryol. exp. Morph. *9*, 370 (1961).
2 N. K. WESSELLS, Devel. Biol. *4*, 87 (1962).
3 J. W. DODSON, J. Embryol. exp. Morph. *17*, 83 (1967).
4 G. PANE, E. BECCHETTI and P. CARINCI, Int. J. Biochem. *5*, 579 (1974).
5 P. CARINCI and L. SIMONELLI, Experientia *28*, 443 (1972).
6 E. WOLFF, Devel. Biol. *3*, 767 (1961).
7 G. QUINTARELLI, S. T. SUIKI, Y. HASHIMOTO and W. PIGMAN, J. Histochem. Cytochem. *9*, 176 (1961).
8 P. CARINCI and G. ZANIBONI, Boll Soc. ital. Biol. sper. *44*, 670 (1968).
9 J. E. SCOTT and J. DORLING, Histochemie *5*, 221 (1965).
10 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
11 T. MIETTINEN and E. TAKKI-LUKKAINEN, Acta chem. scand. *13*, 856 (1959).
12 E. BITTER and H. H. MUIR, Analyt. Biochem. *4*, 330 (1962).
13 C. CESSI and F. PILIEGO, Biochem. J. *77*, 508 (1960).
14 J. E. SCOTT, in *Methods in Biochemical Analysis* (Ed. D. Glick; Interscience Publisher, New York 1960), vol. 8, p. 145.

Table I. Histochemical reactivity of dermal skin of explants supplemented with chicken serum (CS) or embryo extract (E) nutritional medium and of chick embryos

	CS			E			Chick embryo		
	2[a]	4	6	2	4	6	6[b]	10	14
PAS	+	++	+++	++	++	++	+	++	+++
PAS after chemical hydrolysis	+	+−	++−	+−	+−	++	+	+−	++
Toluidine bleu pH 2.5	−	−	−	−	+−	++	−	−	−
Ay 0.2 *M* $MgCl_2$	++	+	+	++	+++	+++	++	+	+
Ab 0.025 *M* $MgCl_2$	+	++	+++	+	+	+	+	++	+++
Ay 0.2 *M* $MgCl_2$	+++	+	+	+++	+++	++	+++	++	++
Ab 0.025 *M* $MgCl_2$ after hyaluronidase	−	−	−	−	−	+−	−	−	−

[a] Days of in vitro maintenance. [b] Days of incubation.

Table II. Content of intercellular dermal components in 6-day cultured skin explants supplemented whith CS or E nutritional medium

	CS	E
NANA	0.019[a]	0.015
Uronic acids	0.021	0.025
Hexosamines	0.033	0.015
NANA/Ur ac	0.90	0.60
NANA/Hexos	0.54	0.62
Ur ac/Hexos	0.63	1.04

[a] mg/mg of proteins; each walue is the mean of 2 independent experiments each in duplicate.

and a minor amount of uronic acids is present in CS cultured explants in comparison with E cultured ones (Table II). Assuming the amount of sialic acid as an index of GP content, of uronic acids of GAG, and the amount of hexosamines as an index of both GP and GAG content, we can deduce that in CS explant GP accumulate to a greater extent than GAG. A similar situation has been previously demonstrated in vivo[4]. The concentration ratio HA/CSA in vivo (6-day 1; 10-day 1.52; 14-day 1.62) confirms the prevalent accumulation of HA.

Our results clearly demonstrate a correlation both in vivo and in vitro between the histochemical and biochemical features of dermal skin and the onset of epidermal differentiation and the dependence in vitro of mesenchyme modification on environmental factor(s) present in the chicken serum. They therefore support the suggested mechanism that environmental factor(s) can activate mesenchymal cells to produce substance(s) able to act specifically upon epidermal differentiation[5].

Riassunto. È stata evidenziata una correlazione fra caratteristiche istochimiche e composizione biochimica del mesenchima e l'instaurarsi del differenziamento dell'epitelio nella cute di pollo sia in vitro, in espianti prelevati al 6° giorno di incubazione e mantenuti in terreno nutritizio contente siero, che in vivo.

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[15] These studies were supported in part by Italian CNR grant No 74,00128, 04.

Interrelationship Between Mean Arterial Blood Pressure, Blood Flow, and Vascular Resistance in Solid Tumor Tissue of DS-Carcinosarcoma

Capillary morphometrics and blood flow measurements in implantation tumors of DS-carcinosarcoma in rat kidneys show the following results: With increasing tumor wet weight, the volume of the vascular space decreases exponentially, the reduction of the vascular bed being especially distinct during the first period of the tumor growth[1]. During tumor growth in the kidney, there results an exponential enlargement of the mean intercapillary distances about the factor 2.5–3.0, as well as a lengthening and an increase in diameter of the capillaries. The rarefaction of the terminal vascular bed with increasing tumor weight or age is attended by an exponential decrease of the total tumor blood flow, the regional microcirculation of the outer areas of the tumor showing higher blood flow values than the inner parts[2]. The present investigation attempts to analyze the interrelationship between mean arterial blood pressure, tumor blood flow, and vascular resistance in tumor tissue of DS-carcinosarcoma under standardized in vivo conditions during different stages of growth.

Materials and methods. Ascites cells of DS-carcinosarcoma are implanted into rat kidneys where they are maintained 'tissue-isolated'[3,4]. After an average of 7–12 days, the infiltrating and destructively growing tumor cells completely replace the kidney tissue, and the tumor mass is connected to the host by only a single artery and vein. Total tumor blood flow (TBF) is determined by cannulating the tumor vein by means of a burette, using a roller pump and a constant pressure buffering chamber. The operative procedure, the blood flow determination and monitoring of the mean arterial blood pressure (MABP) are described elsewhere[5].

To study the influence of the mean arterial blood pressure on total tumor blood flow under in vivo conditions, blood flow measurements in 10 animals (anesthetized with pentobarbitone sodium, 25–30 mg/kg i.p.) are performed using a bubble flow meter. Multiple measurements of the bubble passage in the burette are made not only when blood pressure values spontaneously occur in the thoracic aorta but also after changing the perfusion pressure. After decreasing the mean arterial blood pressure by controlled bleeding, or after increasing it by blood transfusion from other tumor bearing animals, we always wait for steady state conditions before blood flow is measured at a new pressure level.

Results. Under steady state conditions the dependence of tumor blood flow on mean arterial blood pressure is examined at normal respiratory gas parameters in the arterial blood. As can be seen from the representative

[1] P. Vaupel, W. Braunbeck, V. Schulz, H. Günther and G. Thews, Biblphia anat. *12*, 527 (1973).
[2] P. Vaupel, in *Funktionsanalyse biologischer Systemd* (Ed. G. Thews; Steiner, Wiesbaden 1974), vol. 1
[3] P. M. Gullino and F. H. Grantham, J. natn. Cancer Inst. *27*, 679 (1961).
[4] W. Schwarz, V. Schulz, M. Kersten, R. Wörz and P. Vaupel, Z. Krebsforsch. *75*, 161 (1971).
[5] P. Vaupel, H. Günther, J. Grote and G. Aumüller, Z. ges. exp. Med. *156*, 283 (1971).