

# EFFECT OF HYDROCORTISONE ON THE SKIN OF THE DEVELOPING CHICK EMBRYO

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## ABSTRACT

Hydrocortisone treatment of chick embryos caused acceleration of epidermis thickening and keratinization, decrease in dermal cell number and increase of dermal collagenous fibres. The hormone also caused a marked increase of some protein fractions with low mobility and also decreased acid and alkaline phosphatase activity.

## INTRODUCTION

Avian skin was described in detail (Fell, 1962; Mottet and Jensen, 1968 and Demarchez *et al.*, 1984) and feather development was extensively studied histologically (Fell, 1962; Wessells, 1965; Garber, 1967; Garber and Moscona, 1967; Bellairs, 1971; Haake *et al.*, 1984 and Mayerson and Fallon, 1985) and ultrastructurally (Kischer, 1963; Kallman *et al.* 1967; Wessells and Evans, 1986 and Haake and Sawyer, 1982).

Hydrocortisone was found to accelerate skin keratinization *in vivo* (Stuart *et al.*, 1972 and Demarchez *et al.*, 1984) and *in vitro* (Fell, 1962; Sugimoto and Endo, 1969; Sugimoto *et al.*, 1974 and Kojima *et al.*, 1976) and also to inhibit normal feather development *in vivo* (Stuart *et al.*, 1972 and Demarchez *et al.*, 1984) or *in vitro* (Fell, 1962; Sugimoto and Endo, 1969; Sugimoto *et al.*, 1974; Kojima *et al.*, 1976).

Electrophoretic studies of solubilized epidermal proteins revealed two distinct protein groups, SCME<sub>p</sub>A and SCME<sub>p</sub>B, in which the former was selectively accumulated during hydrocortisone-induced keratinization *in vitro* (Sugimoto *et al.*, 1974; Kojima *et al.*, 1976).

Johnson and Bevelander (1947) reported that alkaline phosphatase was present in the mesoderm throughout the development of the feather and appeared in the ectoderm only at later stages. Alkaline phosphatase detected in the cells of developing feather pulp was more reactive than that of the epidermal cells (Hinsch, 1960).

The present work investigates the effect of hydrocortisone on (a) embryonic chick skin and feather, and (b) on soluble protein content and acid and alkaline phosphatase activity during skin development.

## **MATERIALS AND METHODS**

Fertilized eggs of the Egyptian domestic fowl, *Gallus domesticus*, were incubated at 38°C and about 70% relative humidity and turned 2 to 4 times daily. The embryos were staged according to Hamburger and Hamilton (1951) criteria.

After 6 days of incubation, a single dose of 0.1 mg hydrocortisone 21-sodium succinate (Sigma) in 0.1 ml of 10% sterile Tyrode's solution was deposited on the chorioallantoic membrane. A control egg group was similarly injected with 0.1 ml of sterile Tyrode's solution and a third group was untreated.

The dorsal skins 10-, 12- and 15-day control and hydrocortisone-treated chick embryos were examined.

### **Histology**

The dorsal skin was fixed in aqueous Bouin's fluid, dehydrated through a graded ethanol series, embedded in paraffin wax and sectioned at 7  $\mu$  -thick. Sections were mounted and stained with Harris haematoxylin and eosin.

### **Protein content and enzyme activity**

The dorsal skin and attached feather were rinsed three times in 5 mM tris-glycine buffer, weighed and homogenized in tris-glycine buffer (0.1 g wet tissue/ml buffer). The homogenates were centrifuged at 10,000 rpm and the supernatant was stored at -20°C.

Total protein content of skin extract was estimated as described by Kingsley *et al.* (1953) and acid and alkaline phosphatase activities as described by Kind and King (1954). 7% polyacrylamide gels were prepared according to the method of Davis (1964) with the following modifications: The separating gels (150 mm long, 180 mm wide and 1 mm thick) were poured as slabs and the gels pre-electrophoresed at 20 mA for 30 min. The current was raised to 40 mA for 3 h and stopped when the bromophenol blue dye reached the gel bottom. For protein fixation, the gels were immersed in 20% trichloroacetic acid for 20 min, washed, stained in comassie brilliant blue and destained in 7% (v/v) acetic acid.

Alkaline phosphatase activity was visualized after incubation at 37°C for 60 min in a stain buffer mixture of 50 mg Na $\alpha$ -nitrophenyl phosphate disodium salt (Sigma), 50 mg fast blue RR, 123 mg MgSO<sub>4</sub> · 7H<sub>2</sub>O and 100 ml (0.06M) borate buffer, pH 9.6. For acid phosphatase, the stain mixture consisted of 100 mg Na $\alpha$ -nitrophenyl phosphate disodium salt, 100 ml of 0.05 M acetate buffer, pH 5, and 100 mg Fast garnet GBC salt (Sigma) and the gels were incubated at 25°C for 60 min.

The means, standard deviations and standard errors were calculated for the skin protein content and acid and alkaline phosphatase activities in control and hydrocortisone-treated groups and the data were compared using the Student's t-test.

## RESULTS

### A-Histology

#### 1-10-day chick embryo (4 days following hydrocortisone treatment)

##### Dorsal skin

The epidermis (E) of 10-day control (untreated and Tyrode'-treated) chick embryos consists of two layers; a basal layer of short columnar cells with spherical nuclei and a superficial layer of flattened peridermal cells (P) with oval nuclei (Fig. 1a). The epidermal cells rest on a delicate basement membrane (BM) of fibrous connective tissue. The dermis (D) consists of numerous collagenous fibres and spindle-shaped dermal cells containing oval nuclei (Plate 1a).

In treated embryos, the epidermis consists of two or three cell layers. The dermis contains fewer dermal cells and the large intercellular spaces are filled with numerous and larger bundles of collagenous fibres (Plate 1b).

##### Developing feather

The protruding feathers of control (untreated and Tyrode's-treated) embryos possess barb ridges (BR) especially at the terminal part. The central part, the dermal pulp (DP), contains spindle-shaped cells and richly vascular connective tissue. This protruding part is enveloped within a thin layer of flattened cells beneath which numerous pigment cells occur (Fig. 1a).

In treated embryos, the developing feathers contain fewer pigment cells and the barb ridges are less distinct than those of the control embryos (Fig. 1b).

## **2-12-day chick embryo ( 6 days following hydrocortisone treatment )**

### **Dorsal skin**

The epidermis of control chick embryos consists of two or three cell layers with no sign of keratinization. The periderm is still formed of a single layer of flattened cells with oval nuclei. The dermis consists of large spindle-shaped cells and collagenous fibres ( Plate 1c ).

In treated chick embryos, the epidermis consists of three to six cell layers and the periderm of one or two layers of flattened keratinized cells. The dermal layer contains few small cells and an abundant network of collagenous fibres ( Plate 1d ).

### **Developing feather**

The protruding feathers of control embryos are surrounded by a thick keratinized sheath ( FS ). The dermal pulp is filled with dermal cells, few collagenous fibres and few blood vessels. The barb ridges are highly distinct and heavily pigmented. Pigments are black branched spots occur in branched melanophores scattered at the feather periphery and beneath the sheath ( Plate 1c ).

In treated embryos, the barb ridges are less distinct than in the feathers of control embryos. Pigmentation is poor and pigment cells are usually rounded spots. the small dermal pulp contains dense cells and abundant collagenous fibres ( Plate 1d ).

## **3-15-day chick embryo ( 9 days following hydrocortisone treatment )**

### **Dorsal skin**

The epidermis of control embryos consists of two or three cell layers and the periderm of one or two layers of flattened cells with oval nuclei. The dermis possesses spindle-shaped dermal cells and collagenous fibres. The dermal cell axes are usually parallel to the epidermal layer. The collagenous fibres are abundant near the basement membrane and become gradually less dense toward the deeper dermis ( Plate 1e ).

In treated embryos, the dorsal skin consists of 3 to 6 cell layers. Virtually, no peridermal cells are seen on the epidermis and the outer part is formed of a thick keratin layer. In the dermis, there are few small cells each with a small pyknotic nucleus. The collagenous fibres are abundant and randomly distributed in the dermis ( Plate 1f ).

### **Developing feather**

The dorsal feather of control embryos consists of barb ridges in which barb cortical (BCC), barb medullary (BMC) and barbule (BBC) cells are well differentiated. The protruding feather part is enveloped within a thick keratin sheath (Plate 1e).

In treated embryos, feathers are entirely absent from the mid-dorsal surface (Plate 1f) while abortive feathers are usually recognized in the lateral dorsal skin.

### **B-Protein content**

The protein content of the skin extract is similar in both control groups. In 10-day control embryos, the protein content is  $8.09 \pm 0.014$  mg protein/g. The protein content of skin extract of treated embryos is higher ( $p < 0.02$ ) measuring  $8.16 \pm 0.017$  mg protein/g. The electrophoretic protein pattern of control and treated skin extracts revealed 10 anodal migrating bands. However, in treated embryos the protein fraction intensity in bands 3, 5, 6, 7, 8 and 9 is higher while the intensity of other bands is lower than those of the corresponding bands in the controls (Table I, Fig. 1 a and b).

After 12 days of incubation, the dorsal skin extract of control embryos contains  $8.66 \pm 0.016$  mg protein/g. In treated embryos, the protein content is much higher ( $p < 0.001$ ) than that of the controls measuring  $10.51 \pm 0.191$  mg protein/g. Electrophoretically, the protein fraction intensity in bands 3, 7, 8 and 9 is higher while that of the other bands is lower than those of the corresponding bands in the controls (Table I, Fig. 1 c and d).

After 15 days of incubation, the total protein of dorsal skin extract of control embryos is  $10.31 \pm 0.027$  mg protein/g. In treated embryos, the protein content, measuring  $13.27 \pm 0.112$  mg protein/g, is much higher ( $p < 0.001$ ) than that of the controls. The protein fractions of treated embryo skin extract in bands 5, 7, 8 and 9 are more intense and other bands are less intense than the corresponding bands of the controls (Table I). At this stage of development, a new fraction (x-band) appears between bands 6 and 7 in the skin extract of the controls and treated embryos representing 1.2 and 2.4 % of the protein fractions, respectively (Table I, Fig. 1 e and f).

**Table 1**  
Protein content ( % ) of the different fractions in the dorsal skin extract of control and treated chick embryos.

Fraction	10-day chick embryos		12-day chick embryos		15-day chick embryos	
	Control	Treated	Control	Treated	Control	Treated
1	6.6	5.2	1.5	1.1	2.7	0.8
2	5.8	3.4	5.1	4.7	4.1	2.3
3	1.6	2.2	7.6	8.3	6.7	5.4
4	10.7	10.5	10.4	9.6	7.9	7.6
5	1.1	1.4	1.9	1.8	1.1	4.1
6	15.6	17.1	13.2	7.2	13.4	11.6
x-band	-	-	-	-	1.2	2.4
7	23.2	24.6	21.8	25.6	19.8	26.3
8	1.9	2.3	1.4	1.7	2.2	2.3
9	19.2	20.1	22.7	25.9	22.2	25.7
10	14.3	13.2	14.4	14.1	18.7	11.5

### C-Acid phosphatase activity

The acid phosphatase activity is similar in both control groups. In 10-day chick embryos, the activity of acid phosphatase in skin extract of controls is equivalent to  $0.094 \pm 0.002$  King and Armstrong units/g. The acid phosphatase activity in treated embryos, equivalent to  $0.062 \pm 0.003$  King and Armstrong units/g, is significantly lower ( $p > 0.001$ ) than that of the controls. Acid phosphatase was separated into 4 distinct bands in both control and treated embryos following electrophoresis. In treated embryos, the intensity of the first and third bands is lower while the intensity of other bands is higher than those of the corresponding bands of the controls ( Table 2, Fig. 2 a and b ).

After 12 days of incubation, acid phosphatase activity of control skin extract is equivalent to  $0.125 \pm 0.003$  King and Armstrong units/g. The enzyme activity of skin extract of treated embryos equivalent to  $0.089 \pm 0.002$  King and Armstrong units/g, is much lower ( $p < 0.001$ ) than that of the controls. Electrophoretic pattern reveals the lower of the first and third bands and higher intensity of the other bands than the corresponding bands of the controls ( Table 2, Fig. 2 c and d ).

The acid phosphatase activity of control skin extract increased ( $P < 0.001$ ) after 15 days of incubation, equivalent to  $0.181 \pm 0.004$  King and Armstrong units/g. In treated extract, acid phosphatase activity, equivalent to  $0.112 \pm 0.002$  King and Armstrong units/g, is much lower ( $p < 0.001$ ) than that of the controls. Electrophorograms of acid phosphatase activities show that the intensity of the first and third bands is lower while that of the second and fourth bands is higher than the corresponding bands of the controls ( Table 2, Fig. 2 e and f).

Table 2

Acid phosphatase activity (%) of the different fractions in the dorsal skin extract of control and treated embryos.

Fraction	10-day chick embryos		12-day chick embryos		15-day chick embryos	
	Control	Treated	Control	Treated	Control	Treated
1	4.3	4.2	1.3	1.2	2.4	2.1
2	17.2	17.3	14.9	17.2	12.3	17.7
3	47.4	41.1	48.6	42.5	49.7	42.4
4	31.1	37.4	35.2	39.1	35.6	37.8

#### D-Alkaline phosphatase activity

Alkaline phosphatase was similar in skin extract of both control groups. Alkaline phosphatase activity of control skin extract is equivalent to  $0.024 \pm 0.0014$  King and Armstrong units/g after 10 days of incubation. In treated embryos, the alkaline phosphatase activity, equivalent to  $0.018 \pm 0.0016$  King and Armstrong units/g, is lower ( $p < 0.05$ ) than that of the controls. Three distinct bands of alkaline phosphatase activity were detected in the skin extract of control and treated embryos. The intensity of the first and second bands is lower while that of the third band is higher in treated embryos than the corresponding bands of the controls ( Table III, Fig. 3 a and b ).

After 12 days of incubation, the alkaline phosphatase activity in control skin extract is equivalent to  $0.046 \pm 0.0031$  King and Armstrong units/g. In treated embryos, the alkaline phosphatase activity, equivalent to  $0.024 \pm 0.002$  King and Armstrong units/g, is lower ( $p < 0.001$ ) than that of the controls. In skin extract of treated embryos, the intensity of the second band is lower while that of the other bands is higher than the corresponding bands of the controls ( Table III, Fig. 3 c and d ).

Alkaline phosphatase activity of control skin extract shows a marked increase after 15 days of incubation. In control embryos, the alkaline phosphatase activity, becoming equivalent to  $0.077 \pm 0.003$  King and Armstrong units/g. In treated embryos, the alkaline phosphatase activity, equivalent to  $0.03 \pm 0.0014$  King and Armstrong units/g, is much lower ( $p < 0.001$ ) than that of the controls. In treated embryos, the intensity of the first and second bands is lower while that of the third band is higher than the corresponding bands of the controls ( Table III, Fig. 3 e and f).

Table 3

Alkaline phosphatase activity (%) of the different fractions in the dorsal skin extract of control and treated embryos.

Fraction	10-day chick embryos		12-day chick embryos		15-day chick embryos	
	Control	Treated	Control	Treated	Control	Treated
1	19.5	17.6	13.5	17.1	16.6	14.6
2	49.8	42.6	50.5	46.5	52.2	51.5
3	30.7	39.8	36.0	36.4	31.2	33.9

## DISCUSSION

The present study shows that hydrocortisone accelerates epidermis thickening and keratinization. These results agree with previous *in vitro* ( Fell, 1962; Sugimoto and Endo, 1969; Sugimoto *et al.*, 1974 and Takata *et al.*, 1981 ) and *in vivo* ( Stuart *et al.*, 1972; Demarchez *et al.*, 1984 ) observations.

Moreover, the present study shows that hydrocortisone treatment causes collagenous fibre accumulation in the chick embryo dermis a result previously reported by Demarchez *et al.* (1984) and extreme retardation of feather development followed by disappearance of middorsal feathers in 15-day embryos ( Sengel *et al.*, 1969; Stuart *et al.*, 1972; Demarchez *et al.*, 1984 ).

Early keratinization in hydrocortisone-treated embryos may inhibit feather formation since already keratinized epidermis, recombined with normal feather forming dermis cannot support normal feather development ( Sengel *et al.*, 1969 ). However, Demarchez *et al.* ( 1984 ) reported that inhibition of feather formation in hydrocortisone-treated embryos was due to precocious histological maturation of the epidermis and the uniform depositing of extracellular matrix components in the dermis. Moreover, Fell ( 1962 ) reported that hydrocortisone greatly accelerates



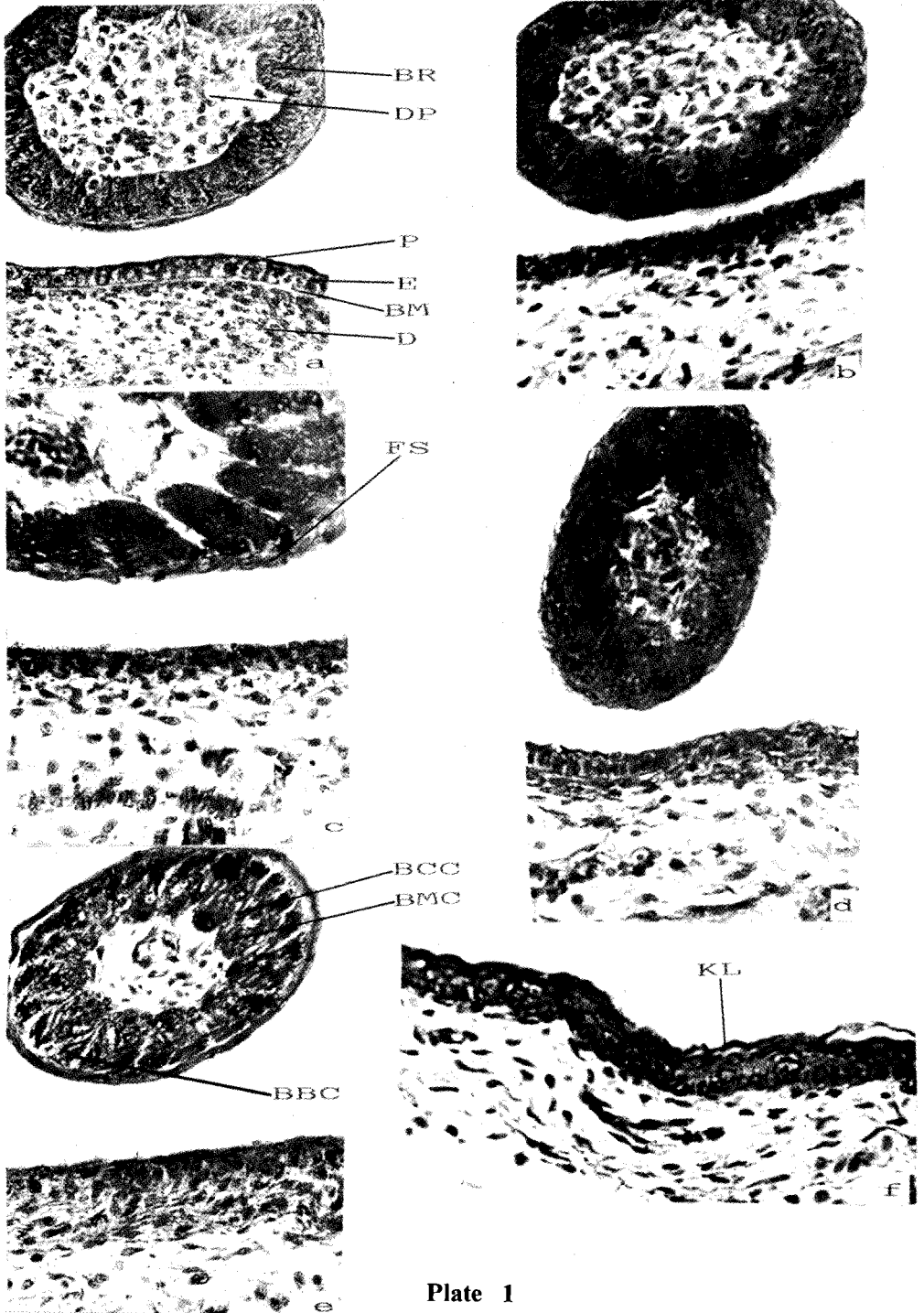
histological differentiation of feathers and arrest their elongation, hence, the feather germs aborted at an early stage.

In the present study, total skin protein of chick embryos increased with development. However, total skin protein of hydrocortisone treated embryos was greater than that of control embryos and this increase may be attributed to heavily keratinized epidermis ( Sugimoto and Endo, 1969; Sugimoto *et al.*, 1974; Kojima *et al.*, 1976 ) and / or increase in the dermal collagen fibres. Electrophoretically, ten anodal migrating protein bands were separated from dorsal skin extract of the chick embryos. In addition, a new protein band ( x band ) apprares in the skin extract of 15-day control and treated chick embryos. The slow moving fractions of the skin extract of hydrocortisone-treated embryos, especially bands 7,8 and 9, increased during different stages of development. In contrast, the fast moving fractions, especially bands 1,2 and 4, of treated embryos were intensely decreased. These results confirm the previous observations that hydrocortisone-treatment directed the epidermis of the chick embryos toward keratinization through acceleration of the synthesis of epidermal structural proteins with high molecular weight ( SCMEpA ) and degradation of other proteins ( Sugimoto *et al.*, 1974 and Kojima *et al.*, 1976).

Acid and alkaline phosphatase activities gradually increased in dorsal skin extract with progress development of chick embryos. On the other hand, hydrocortisone treatment resulted in inhibition of acid and alkaline phosphatase activities. Four distinct bands of acid phosphatase have been resolved in the dorsal skin extract of the embryos. The intensity of the second and fourth bands increased while the other bands decreased in the skin extract of treated embryos as compared with the corresponding bands of control embryos.

Electrophoresis of dorsal skin extract of control and treated embryos revealed 3 distinct bands of alkaline phosphatase activity. The third band activity increased while that of other bands decreased as compared to the corresponding bands of the controls. Alkaline phosphatase activity was detected mainly in the cytoplasm of cells of the mesodermal pulp of developing feather ( Johnson and Bevelander, 1947 and Hinsch, 1960 ). Thus, in the present study it may be expected that alkaline phosphatase activity decreased owing to inhibition of feather development and / or decrease of dermal cell aggregation in the skin of hydrocortisone-treated chick embryos.

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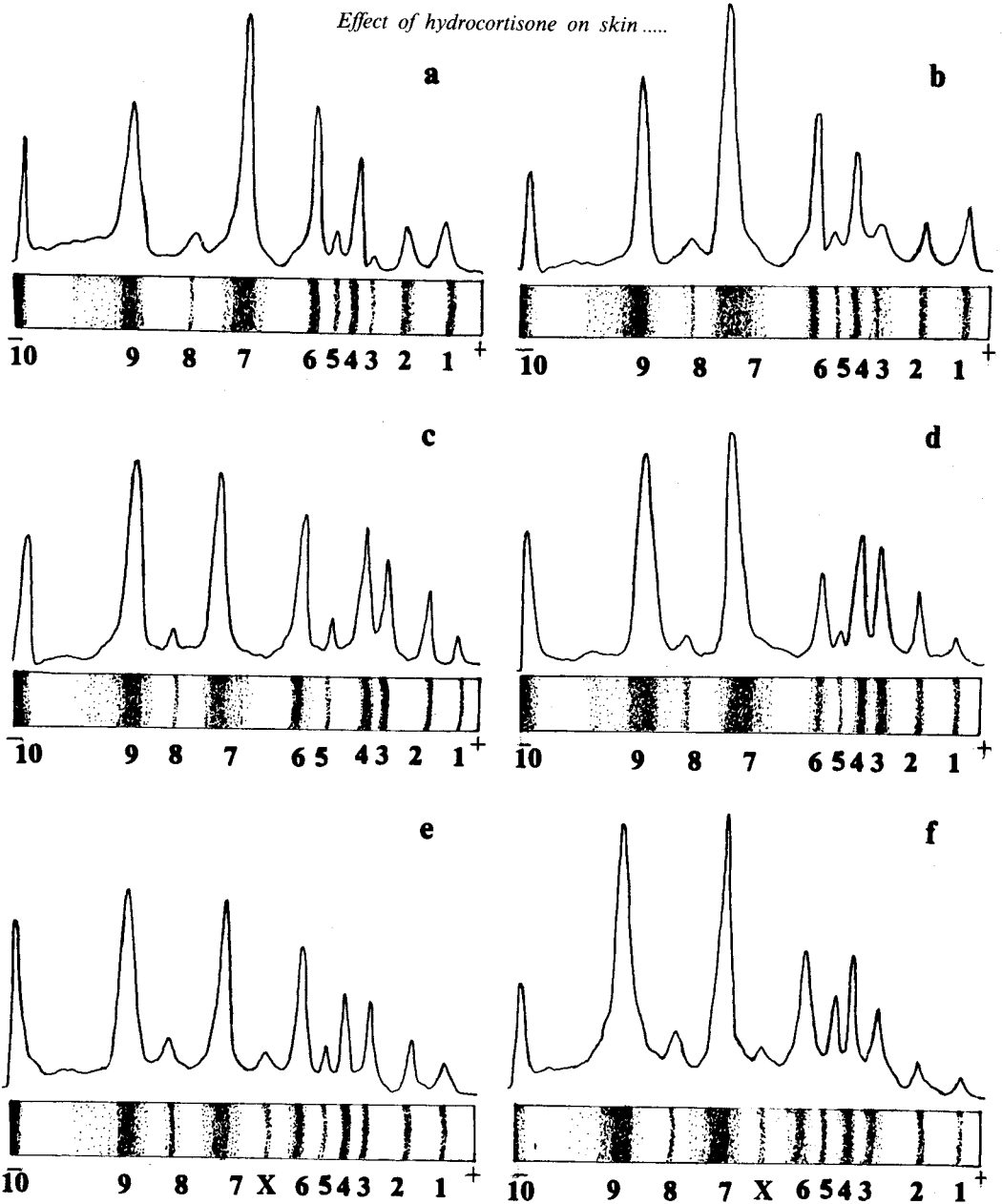


**Plate 1**

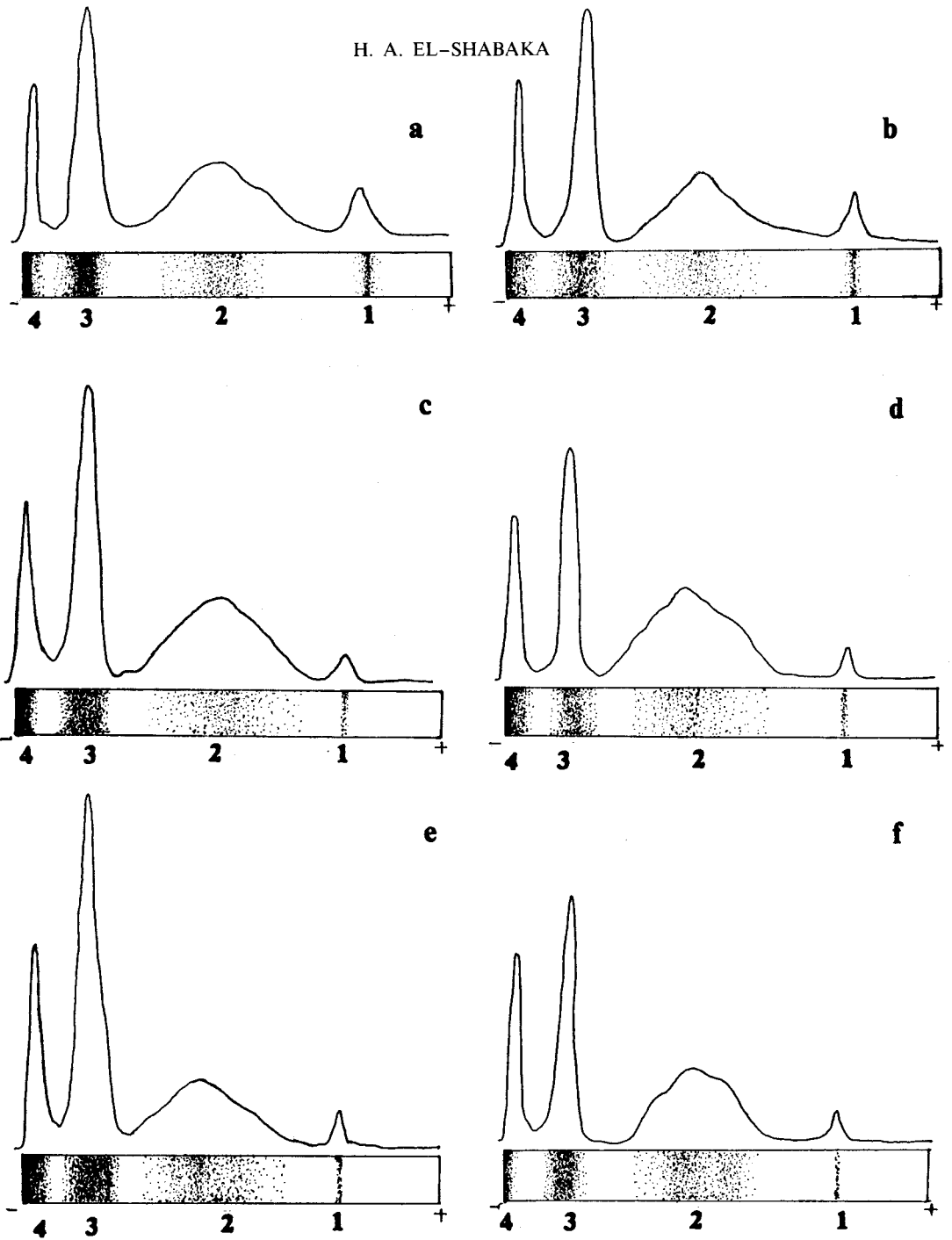
**Plate 1: V.S. of the dorsal skin and feathers at various stages of development.**

- a-V.S. of normal dorsal skin of 10-day chick embryo showing the dermal pulp ( DP ), periderm ( P ), epidermis ( E ), dermis ( D ) and newly formed barb ridges ( BR). X470.
- b-V.S. of dorsal skin of 10-day chick embryo, 4 days following hydrocortisone treatment. X550.
- c-V.S. of normal dorsal skin of 12-day chick embryo showing the thick feather sheath ( FS ) surrounding the developing feather. X640.
- d-V.S. of dorsal skin of 12-day chick embryo, 6 days following hydrocortisone treatment. X520.
- e-V.S. of normal dorsal skin of 15-day chick embryo showing the different cell types of the differentiated feather ( BBC, barbule cells; BCC, barb cortical cells; BMC, barb medullary cells ). X580.
- f-V.S. of dorsal skin of 15-day chick embryo, 9 days following hydrocortisone treatment showing the outer keratin layer. X680.

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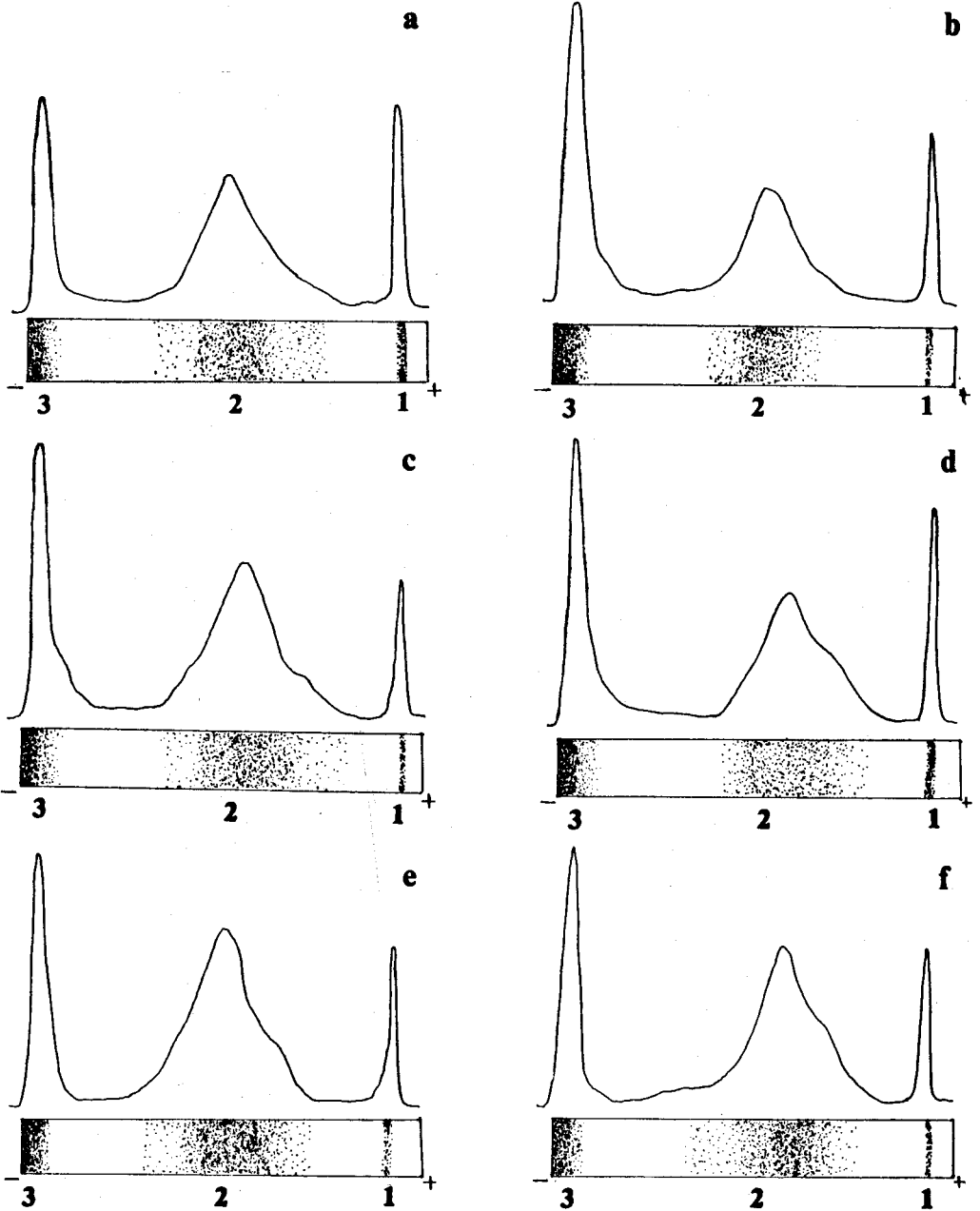


*Fig. 1* Schematic representation of disc gel electrophoresis and densitometric profiles of dorsal skin protein extract of chick embryos. a,c,d controls; b,d,f hydrocortisone-treated embryos. a,b: 10-day embryos; c,d: 12-day embryos; e,f: 15-day embryos.



*Fig. 2* Schematic representation of disc gel electrophoresis and densitometric profiles of acid phosphatase activity of dorsal skin extract of chick embryos. a,c,d controls; b,d,f hydrocortisone-treated embryos. a,b : 10-day embryos; c,d : 12-day embryos; e,f : 15-day embryos.

*Effect of Hydrocortisone on Skin....*



**Fig. 3** Schematic representation of disc gel electrophoresis and densitometric profiles of alkaline phosphatase activity of dorsal skin extract of chick embryos. a,c,d controls; b,d,f hydrocortisone-treated embryos. a,b, : 10-day embryos; c,d : 12-day embryos; e,f : 15-day embryos.

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## تأثير الهيدروكورتيزون على جلد جنين الكتكوت

حمزة أحمد الشبكه

يتناول هذا البحث دراسة تأثير الهيدروكورتيزون على جلد ونمو الريش في الكتكوت ، وقد وجد أن طبقة البشرة في الكتكوت الذي حقن بالكورتيزون كانت أكثر سمكاً وتتحول طبقاتها الخارجية إلى طبقات قرنية ، كما اتضح أيضاً أن الهيدروكورتيزون يؤدي إلى قلة عدد خلايا الأدمة مع زيادة ملحوظة في الألياف البيضاء ( الكولا جينية ) .

وقد لوحظ أن كمية البروتين الكلي في جلد الكتكوت المعالج بالكورتيزون تزداد زيادة ملحوظة عن كميته في جلد الكتكوت العادي ، وكما تم فصل بروتين الجلد باستخدام الهلام إلى حوالي عشرة خطوط واتضح أن الخطوط ذات الوزن الجزيئي الكبير تزداد نتيجة تأثير الهيدروكورتيزون .

كما وجد أن نشاط كل من أنزيمي الفوسفاتيز الحامضي والفوسفاتيز القلوي في جلد الكتكوت المعالج بالكورتيزون منخفضة ، وقد أمكن فصل انزيم الفوسفاتيز الحامضي إلى أربعة خطوط ووجد أن الخطين الأول والثالث تقل نسبتها المئوية في جلد الكتكوت المعالج بالكورتيزون ، أما انزيم الفوسفاتيز القلوي فقد فصل إلى ثلاثة خطوط ووجد أن النسبة المئوية للخطين الأول والثالث أقل في جلد الكتكوت المعالج بالكورتيزون عنها في الكتكوت غير المعالج .