Immunofluorescent Localization of β-Corticotropin in the Rat Pituitary

Recent electron microscope studies indicated that a new type of granulated cell is the site of adrenocorticotropin hormone (ACTH) production in the adenohypophysis of the rat[1]. On the other hand, numerous attempts to identify a 'corticotroph' by histochemical means have yielded controversial results. While autoradiographic evidence suggested that chromophobe cells may secrete ACTH in the rat pituitary[2], the application of fluorescent antibody procedures using synthetic β^{1-24}-corticotropin as the antigen pointed to cells of the acidophil series as the source of the hormone in this species[3,4]. However, earlier studies with antisera to natural ACTH indicated that certain basophil cells represent the corticotroph in the porcine[5] and in the human pituitary[6,7].

Studies on the immunochemical properties of β-corticotropin-(1-24)-tetracosapeptide[8] led to the production of complement-fixing antibodies in the rabbit with distinct specificities towards the C-terminal portion of the synthetic peptide hormone. The antibody cross-reacted with purified ACTH (whale type), but showed no measurable specificity to the β^{25-39} portion of natural ACTH[9]. The immunofluorescent application of this well-characterized antiserum indicated what we believe is the true cellular localization of β-corticotropin in the rat pituitary.

Rabbits were immunized by the injection first into the paws and then i.p. of β^{1-24}-corticotropin (Synacthen®, CIBA) coupled[10] to rabbit serum albumin (RSA), together with complete Freund's adjuvant. The resulting complement fixing antiserum was inactivated for 30 min at 56 °C and absorbed with the uncoupled RSA-carbodiimide preparation[9]. Fluorescein isothiocyanate-labelled anti-rabbit γ-globulin from goat was obtained commercially (Microbiological Associates, Inc., Bethesda, Md.). Free fluorochrome was removed by chromatography on Sephadex G25 and subsequent dialysis, immediately before use. Immunofluorescent staining was performed by the double-layer technique (essentially according to NAIRN[11]) and the following criteria of specificity (absence of staining) were applied: fluorescent globulin alone; normal rabbit serum, followed by fluorescent globulin; rabbit anti-corticotropin serum followed by unlabelled anti-rabbit serum, followed by fluorescent globulin.

Whole pituitaries were examined from normal male albino rats (220 g body weight) from adrenalectomized rats (maintained for 5 and 7 days on 0.9% NaCl), and from rats injected s.c. with dexamethasone (1 mg/kg) and morphine HCl (10 mg/kg) 4 h prior to decapitation. The tissue was frozen in liquid nitrogen, dried at − 25 °C in a modified thermo-electric apparatus[12], and embedded in paraffin in vacuo. Methanol-fixed[5] 6 μ thick horizontal sections were subjected to fluorescence microscopy and then re-stained by a periodic acid Schiff (PAS) and Orange G procedure[13].

Strong specific fluorescence occurred in the cytoplasm of irregularly shaped polyhedral cells which, by means of processes, tended to encompass neighbouring cells or to extend to the sinusoids (Figure 1). In their gross morphology, these cells appeared to correspond to the anterior corticotrophs seen in the electron microscope[1]. In the anterior lobe, the fluorescent cells were found to be distributed mainly in the area adjacent to the pars intermedia and along the anterior surface, with the exception of the lateral wings. The intensity of fluorescent staining seemed to be higher in dexamethasone-morphin treated animals than in normal rats, and it was lowest following adrenalectomy. In the latter instance, the reactive cells tended to be enlarged.

In contrast to the pars nervosa which showed only autofluorescence, specific fluorescence was observed in the epithelial cells of the pars intermedia. When the anti-β^{1-24}-cortocotropin serum was replaced by antiserum prepared against α-MSH (melanotropin)[14], specific immunofluorescence was obtained in pars intermedia cells as well as in some of the adjacent anterior 'corticotrophs'. The present results suggest that the pars intermedia may

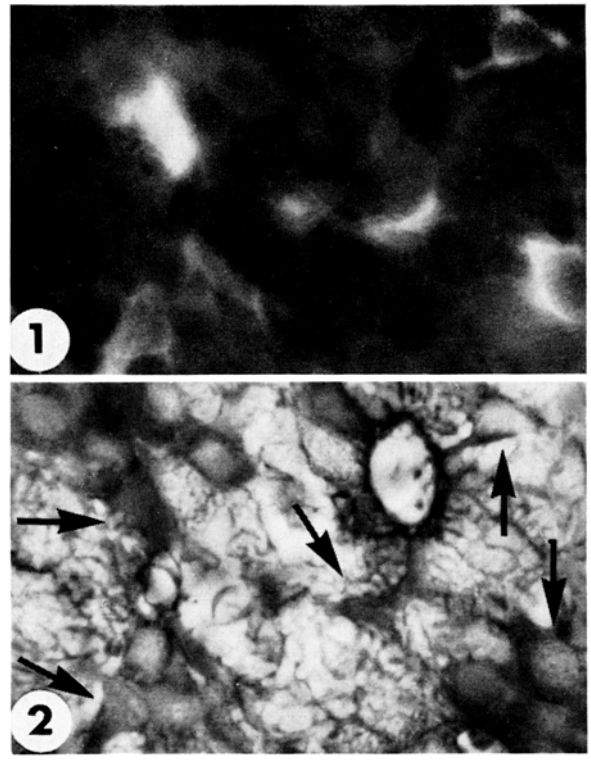

Fig. 1. Rat anterior pituitary treated with rabbit anti-β^{1-24}-corticotropin and subsequently with fluorescein-labelled anti-rabbit globulin. Specific immuno-fluorescence is confined to irregularly shaped 'corticotrophs' with process-like extensions. The animal was treated with dexamethasone and morphine.

Fig. 2. Same field as Figure 1 after restraining with PAS-Orange G. The fluorescent cells are identified as PAS-positive basophils (arrows).

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contain various ACTH peptides other than α-MSH peptides. Some degree of cross reactivity between the 2 antisera used cannot be excluded since N-terminal fragments of the tetracosapeptide including α-MSH, inhibited complement fixation of the anti-β^{1-24}-corticotropin with the antigen conjugate to a very small degree[9].

By re-staining and comparing identical fields, the fluorescing 'corticotrophs' of the pars anterior were clearly identified as PAS-positive cells of the basophil series (Figure 2). PAS-staining was diffuse, rather weak, and no granules were seen. These cells, though tinctorially similar, could be easily distinguished from the more centrally located thyrotrophs[15]. They are most probably related to the R-type mucoid cells of the human anterior pituitary which are considered to contain ACTH[7,16]. In contrast to the view taken by other investigators[3] we conclude therefore that β-corticotropin is produced by a similar, probably identical cell type in various mammalian species.

Zusammenfassung. Unter Verwendung eines spezifischen Anti-β^{1-24}-Corticotropin-Serums vom Kaninchen wurde β-Corticotropin immunhistologisch in der Rattenhypophyse nachgewiesen. Neben den Epithelien der Pars intermedia konnte kortikotropes Hormon in einem besonderen, mukoiden Zelltypus der Adenohypophyse lokalisiert werden.

R. Hess, D. Barratt
and J. Gelzer

Research Laboratories of the Pharmaceutical Department of CIBA Limited, Basel (Switzerland),
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Induction of Pigmentary Changes in the Skin of the Mongolian Gerbil by Chemical Carcinogens

Although considerable insight has been gained concerning the many unique features of skin carcinogenesis in the Syrian and Chinese hamsters, little comparable attention has been given to the related Mongolian gerbil (*Meriones unguiculatus*)[1-5]. It has been reported that 7,12-dimethylbenz(*a*)anthracene (DMBA) applied topically to the skin of gerbils produces papillomas, sarcomas and carcinomas, but further details, particularly relating to their pigmentary system, have not been published[6]. This communication demonstrates that DMBA and croton oil when applied to the hairy (trunk) skin of adult gerbils elicit striking pigmentary changes which may or may not be associated with developing neoplasms.

A total of 16 gerbils 3 months of age were divided into 4 groups each consisting of 3 males and 1 female. The experimental groups (G. 1–4) received the following treatments for periods up to 6 months: (G. 1) 1 ml of acetone applied once a week, (G. 2) 1 ml of 1% croton oil-in-acetone applied 3 times a week, (G. 3) 1 ml of 0.1% DMBA-in-acetone applied once a week, and (G. 4) 1 ml of 0.1% DMBA-in-acetone once a week for 4 weeks followed by 1 ml of 1% croton oil-in-acetone 3 times a week. Each solution was released on the shaved dorsa of the test animals from a calibrated syringe. A few animals were killed during the period of treatment, the remainder at its termination. Standard histological procedures were followed in preparing paraffin sections and whole mounts of representative skin specimens. The normal histology of the skin was determined in an additional 12 adult gerbils.

With the exception of the melanocyte system, the normal histology of the hairy (trunk) skin of the gerbil has been described elsewhere[7,8]. Our observations suggest that active melanocytes are largely restricted to the hair bulbs and occasionally the outer root sheaths of growing (anagen) hair follicles. Active melanocytes are absent from the basal layer of the thin, interfollicular trunk epidermis. The hair follicles are systematically arranged to form small groups in the dorsal skin. Variable numbers of networks of dermal melanocytes are present surrounding groups of hair follicles (Figure 1). The melanocytes which form the perifollicular networks also vary in number and as a consequence may completely or only partly encircle a particular group of follicles. The fusiform dermal melanocytes are closely applied to the sebaceous glands and connective tissue sheaths of the hair follicles. The perifollicular networks may occur singly or several adja-

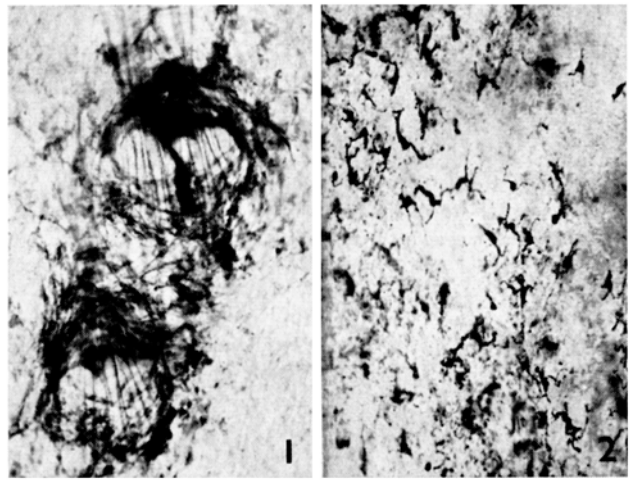

Fig. 1. Perifollicular networks of melanocytes in normal skin. × 200.
Fig. 2. Melanogenic epidermal melanocytes in DMBA-treated skin. × 200.

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