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Plasma β-endorphin Levels in Patients with Polycystic Ovarian Disease

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Abstract—**In order to determine endocrinologic changes by obesity in patients with polycystic ovarian disease (PCOD) and to assess the involvement of peripheral β-endorphin in the pathogeneis of PCOD, levels of plasma β-enorphin, gonadotropin, androgen and prolactin were measured by radioimmunoassay. Thirty-one PCOD patients (21 normal weight, 10 overweight) and 27 normally ovulating women, 20 normal weight, 7 overweight (normal controls) were studied. There were no significant differences in the plasma gonadotropin, androgen, and prolactin levels between the overweight and normal weight group in PCOD patients and normal controls. No significant difference in plasma β-endorphin levels was found between normal weight controls and normal weight patients. Plasma β-endorphin levels were similar in overweight controls and patients. These values were significantly higher than those in normal weight controls and patients respectively. There was a significant correlation between plasma β-endorphin levels and the percentage of ideal body weight in patients with PCOD and normal controls. These data suggest that peripheral opioid abnormality in PCOD may be a phenomenon associated with obesity and not related to the pathogenesis of disease.**

Key words: *Peripheral β-endorphin, Polycystic ovarian disease, Obesity.*

INTRODUCTION

The most characteristic picture in women with polycystic ovarian disease (PCOD) is an abnormality of gonadotropin secretion, i.e., an increased serum luteinizing hormone (LH) and a normal or decreased serum follicle stimulating hormone (FSH) level, resulting in an increased LH/FSH ratio (Rebar *et al.*, 1976; Baird *et al.*, 1977). Several studies have shown that the endorphins, a group of endogenous opioids, may play an important role in the release of gonadotropin (Quigley and Yen, 1980; Blankstein *et al.*, 1981; Ropert *et al.*, 1981). Cumming *et al.* (1984) found the failure of LH respond to opiate antagonist, naloxone in PCOD patients, suggesting a lack of opioid inhibition of gonadotropin-releasing hormone (GnRH), and proposed that an abnormality of central opioid activity might be responsible for the increased gonadotropin secretion in PCOD patients. On the other hand, several investigators report that plasma β-endorphin levels are elevated in PCOD patients (Given *et al.*, 1980; Aleem and McIntosh, 1984; Wortsman *et al.*, 1984), but most of their patients were obese. Recently, an elevated levels of plasma β-endorphins also have been observed both in obese adults and

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children (Genazzani *et al.*, 1986; Facchinetti *et al.*, 1986, 1987; Giugliano *et al.*, 1988)

Therefore it is not clear whether this elevation in plasma β-endorphin levels in PCOD patients is associated with obesity or involved in the pathogenesis of disease. This study was undertaken to compare levels of various hormones including β-endorphin in the peripheral blood between overweight and normal weight PCOD patients and to assess the relationship of peripheral β-endorphin levels with body weight.

MATERIALS AND METHODS

1. Subjects

We studied 31 women, 20 to 30 years of age, with PCOD, 21 normal weight (mean weight ± S.E : 98.91 ± 2.01% of ideal body weight) and 10 overweight (127.68 ± 2.65%). The criteria for diognosing PCOD were amenorrhea or oligomenorrhea with or without hirsutism, anovulation determined by basal body temperature chart and/or endometrial biopsy, high LH basal levels with the LH:FSH ratio>2, and characteristic findings of polycystic pattern in transvaginal ultrasonography. Ideal body weights were determined from the standards of Korean women (Park, 1987). Overweight was defined as body weight greater than 120% of ideal body weight. Normal weight patients were within 20% of their ideal body weight. For controls, 27 normally ovulating women, 20 normal weight (98.70 ± 1.49%) and 7 overweight (126.58 ± 1.60%) were also included. All subjects were free of systemic and other endocrine diseases.

2. Collection of samples

All blood samples were collected between 9:00 AM and 11:00 AM randomly from amenorrheic patients and on menstrual cycle day 2 to 5 from menstruating women. Blood was collected in polypropylene tubes containing heparin, immediately centrifuged at 4℃ for 15 minutes at 2,500 g, and the plasma stored at −20℃ until assay.

3. Radioimmunoassays

LH and FSH was assayed by a double antibody radioimmunoassay method with kits from Amersham. Prolactin (PRL), testosterone (T), and dehydroepiandrosterone sulfate (DHEAS) assay were performed using radioimmunoassay kits purchased from Abbott Laboratories (North Chicago, IL), Diagnostic Products Corporation (Los Angeles, CA), and Sereno Diagnostics (Braintree, MA), respectively.

Determinations of β-endorphin were performed after affinity gel extraction using radioimmunoassay kits from the Immuno Nuclear Corporation (Stillwater, MN). In brief, standards and unknown samples were extracted with anti β-endorphin sepharose complex in chromatography column and the absorbed β-endorphin was eluted from sepharose particle with 0.025 ml HCL. The mixture of sepharose-HCl extract (200 μl) and 1% bovine serum albumin borate buffer (100 μl) were incubated at 4℃ for 18-hours with rabbit β-endorphin antisera (100 μl), followed by an 18 hour incubation with I^{125}β-endorphin (100 μl) and an additional 20 minutes with precipitating goat antirabbit serum (500 μl). The β-endorphin antiserum used was 100% crossreactive, with human β-endorphin, below 5% with β-lipotropin, and did not crossreact (<0.01%) with α-endorphin, leucine enkephalin, methionine enkephalin, adrenocorticotropic hormone (ACTH) or α-melanocyte stimulating hormone. Current β-endorphin assay sensitivity is 1 fmol/ml.

All samples were mesured in duplicate in the same assay for each hormone. The intrassasy variations were 7.1% for LH, 6.0% for FSH, 6.0% for T, 7.9% for DHEAS, 4.0% for PRL, and 6.5% for β-endorphin.

4. Stastical analysis

All results are expressed as the mean ± standard error (SE). Data analysis was performed using student t-test and correlation coefficients.

RESULTS

Various plasma hormone levels (Mean ± SE) in 20 normal weight and 7 overweight controls, and 10 overweight and 21 normal weight patients with PCOD are summarized in Table 1. When compared to the levels found in the normal weight controls, normal weight patients with PCOD had plasma concentrations that were significantly higher

Table 1. Plasma LH, FSH, T, DHEAS, PRL levels and LH/FSH ratio in normal weight and overweight ovulatory women, and overweight and normal weight PCOD patients (Mean ± S.E.)

	Normal Ovulatory Women		PCOD Patinents	
	Normal Weight (n=20)	Overweight (n=7)	Normal Weight (n=21)	Overweight (n=10)
LH (mIU/ml)	12.01 ± 1.21[a]	12.87 ± 1.31[c]	33.92 ± 2.16[e]	35.43 ± 1.78[e]
FSH (mIU/ml)	12.27 ± 0.94	11.50 ± 1.01	11.99 ± 0.73	11.81 ± 0.86
LH/FSH ratio	0.98 ± 0.20[b]	1.12 ± 00.23[f]	2.93 ± 0.18[f]	3.09 ± 0.225[f]
T (ng/ml)	0.43 ± 0.04[c]	0.44 ± 0.03[d]	0.66 ± 0.07	0.69 ± 0.10[d]
DHEAS (ng/dl)	1342.00 ± 230.17[d]	1401.00 ± 221.27[h]	2083.08 ± 215.53[g]	2130.00 ± 232.98[h]
PRL (ng/ml)	10.27 ± 1.20	10.51 ± 1.05	11.00 ± 1.24	10.35 ± 1.18

a,b,e,f,g: $p<0.001$; c: $p<0.01$; d,h: $p<0.05$

($p<0.001$) for LH (33.92 ± 2.16 vs 12.01 ± 1.21 mIU/ml) but not for FSH (11.99 ± 0.73 vs 12.27 ± 0.94 mIU/ml). Plasma levels of PRL (11.0 ± 1.24 vs 10.27 ± 1.20 ng/ml) showed no differences between the normal weight controls and PCOD patients. The androgen levels revealed significantly elevated concentrations of T (0.66 ± 0.77 vs 0.43 ± 0.04 ng/ml, $p<0.01$), and DHEAS (2083.08 ± 215.53 vs 1342.0 ± 230.17 ng/dl, $P<0.05$). Significant differences in the plasma levels of LH (35.43 ± 1.78 vs 12.87 ± 1.31 mIU/ml, $p<0.001$), T (0.69 ± 0.10 vs 0.44 ± 0.03 ng/ml, $p<0.001$) and DHEAS (2130.0 ± 232.98 vs 1401 ± 221.27 ng/dl, $p<0.05$) between the overweight patients and controls. In both normal ovulatory controls and PCOD patients there were no significant differences in the plasma levels of LH, FSH, T, DHEAS, PRL and LH/FSH ratio between normal weight and overweight group.

Fig. 1. depicts plasma β-endorphin levels (mean ± SE) in normal weight and overweight con-

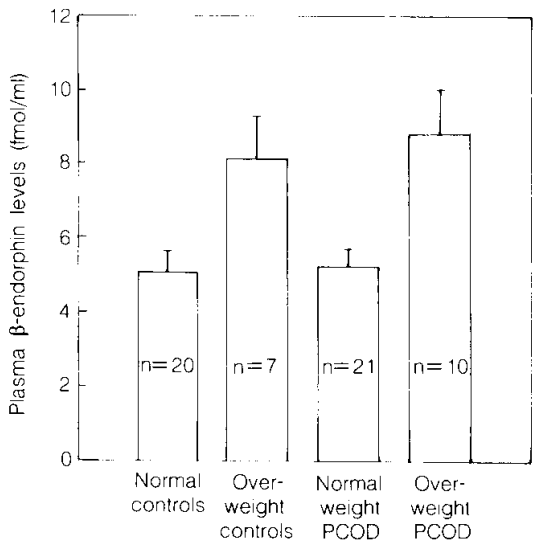

Fig. 1. Plasma β-endorphin levels (Mean+SE) in normal weight and overweight controls, and normal weight and overweight PCOD patients.

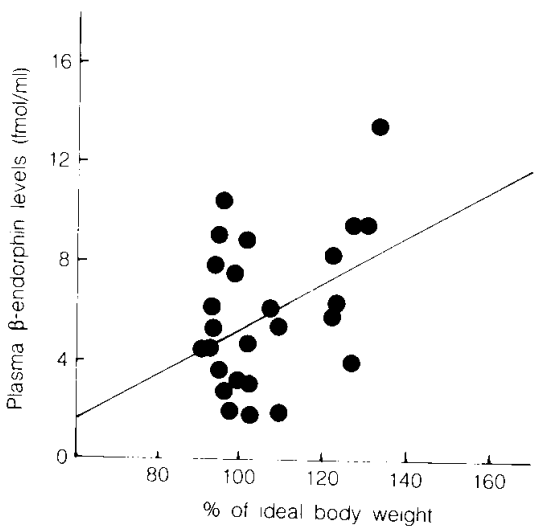

Fig. 2. Correlation between plasma β-endorphin levels and the percentage of ideal body weight in normal controls (n=27).

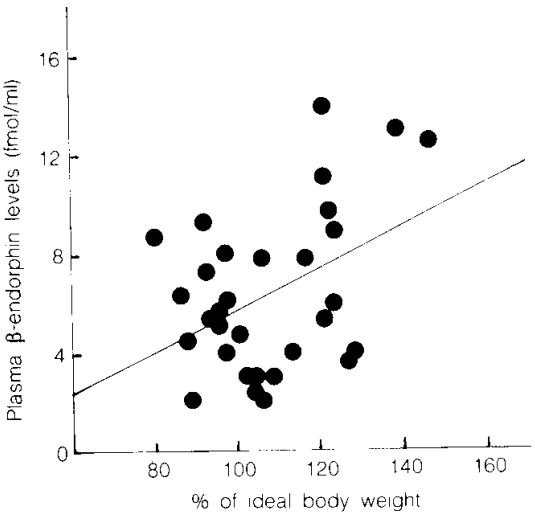

Fig. 3. Correlation between plasma β-endorphin levels and the percentage of ideal body weight in PCOD patients (n=31).

trols, and overweight and normal weight patients with PCOD. The mean concentrations of plasma β-endorphin in overweight patient with PCOD (8.82 ± 1.21 fmol/ml.) were similar to those found in overweight controls (8.14 ± 3.10 fmol/ml). There was no significant difference in the mean plasma β-endorphin levels between normal weight controls and patients (5.09 ± 0.58 vs 5.25 ± 0.50 fmol/ml). Plasma β-endorphin levels in overweight controls and patients were significant higher than those in normal weight controls and patients respectively ($p<0.02$). As is shown in Fig. 2 and Fig. 3, a significant correlation was noted between percentage (x) of ideal body weight and plasma β-endorphin levels (y) in normal ovulatory controls ($y=0.093x-3.961$, $r=0.419$, $p<0.03$, $n=27$) and patients with PCOD ($y=0.084x-2.708$, $r=0.422$, $P<0.02$, $n=31$). There was no significant relationship of plasma β-endorphin levels with plasma LH, FSH, T, DHEAS, PRL levels and LH/FSH ratio.

DISCUSSION

Circulating gonadotropin concentrations are "inappropriate" with erratic and elevated LH and relatively constant and normal or low FSH levels in PCOD patients (Rebar *et al.*, 1976; Baird *et al.*, 1977). Obesity frequently observed in PCOD patients may cause or enhance hyperandrogenism, increasing the peripheral conversion of androstenedione to estrone in adipose tissue and decreasing sex hormone binding globulin levels, and affect inappropriate gonadotropin secretion. Indeed, Plymate *et al.* (1981) found that plasma LH levels are higher in the obese PCOD patients than in normal weight PCOD patients. In this study there was no difference in plasma gonadotropin levels between the overweight and normal weight patients. Our data is in agreement with findings of Petraglia *et al.* (1985).

Several investigators have shown that β-endorphin levels in peripheral blood are elevated in patients with PCOD compared to normal controls (Givens *et al.*, 1980; Aleen and McIntosh, 1984; Wortsman *et al.*, 1984). In this study, elevated plasma β-endorphin levels were found not in normal weight PCOD patients but in the overweight controls and PCOD patients. We also confirmed the observations of others (Givens *et al.*, 1980; Aleem and McIntosh, 1984) that plasma β-endorphin levels significantly correlate with body weight. On the other hand, Margules *et al.* (1978) found that both genetically obese mice and rats have elevated plasma and pituitary levels of β-endorphin. Similar findings have been reported in the peripheral blood of obese children and adults (Genazzani *et al.*, 1986; Facchinetti *et al.*, 1986, 1987; Giugliano *et al.*, 1988). Our data in conjunction with these findings indicate that peripheral opioid abnormality in PCOD may be a concomitant phenomenon associated with obesity and not related to the pathogenesis of disease.

The physiological significance of elevated β-endorphin levels in obese patients with PCOD is conjectural at this time. It has been shown that β-endorphin stimulates the secretion and glucagon from the pancreas (Reid and Yen, 1981). Shoupe and Lobo (1984) demonstrated that hyperinsulinemia is present in obese women with PCOD. Thus the elevation of peripheral β-endorphin might be responsible for the obesity of PCOD by altering the insulin response to food and glucose.

Central opioid activity is probably unrelated

to peripheral β-endorphin levels. Petraglia *et al.* (985) reported that a naloxone induced LH rise occurs in obese PCOD patients in a manner similiar to that in normal healthy women, whereas naloxone does not elicit the increase in plasma LH levels in normal weight PCOD patients. Recent finding suggests that the reversal of peripheral ovarian response induced by treatment for induction of ovulation may restore this impaired opioid activity in normal weight patients (Nappi *et al.*, 1989).

The source of peripheral β-endorphin in obese PCOD patients is obscure. It is well-known that β-endorphin is released simultaneously with ACTH from the anterior pituitary (Guillemin *et al.*, 1977). The demonstration of dexamethasone-resistant β-endorphin levels in obese children together with their cortisol being normally suppressed (Facchinettti *et al.*, 1987) suggests that hyperendorphinemia in these patients cannot be considered only as an overfunction of the anterior pituitary. Taking into consideration that human ovarian follicles contain β-endorphin levels 10 to 15 times higher than in the plasma (Petraglia *et al.*, 1985), that immunoreactive β-endorphin is present in theca and stromal cells as well as luteinized granulosa cells (Aleem *et al.*, 1986), and that the β-endorphin content in the follicular fluid of polycystic ovary is significantly higher than in normal ovaries (Aleem *et al.*, 1987), it may be supposed that a possible release of β-endorphin from polycystic ovaries in part contributes to the peripheral pool of β-endorphin.

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effect of this continued exposure upon melanocytes. In a previous study, we studied the effect of repeated UVR exposure for 2 weeks upon changes in the number of melanocytes by histochemical study (Kim and Youn 1988). The result was a significant increase in the population of melanocytes. In the present study, we decided to investigate the quantitative changes of melanocytes in C57 BL mice upon repeated UVR exposure for 4 weeks by histochemical study with DOPA method, and we studied the ultrastructural changes to determine the subcellular events in melanocytes.

There are various skin reactions after exposure to UVR. There are immediate reactions such as erythema and pigmentation, and chronic reactions such as photocarcinogenesis and photoaging (Gange 1987). Topical sunscreens have been used to prevent the harmful effects of UVR (Pathak 1982).

In a previous study, we studied the effect of sunscreen upon erythema (Lee *et al.* 1984; Youn and Youn 1985). Sunscreens also could be used to prevent pigmentations which are an acute reaction caused by UVR. But the influence of sunscreens upon melanocytes after continued UVR exposure is not definite (Imokawa 1986; Chun 1984). The following study was undertaken to explore the effect of UVR on epidermal melanocytes and the influence of sunsceen by histochemical and ultrastructural study.

MATERIALS AND METHODS

Materials

1. Experimental animals

The dorsal surface of ear skin of 20-25 gm, 8-10 week old C57 BL male mice was used. A total of 30 mice were used in this study. They were divided into 6 groups according to the duration of irradiation.

2. Radiation source

High pressure mercury arc (Burdick Co., USA) provided radiation of 254, 263, 297, 303, and 366 nm wavelengths (peak output at UVB).

Irradiance measurements were made with an IL 700A Research Radiometer (International Light Co., USA) fitted with a UVB sensor (SEE 240, International Light Co., USA).

3. Sunscreen

Five % para-aminobenzoic acid (PABA) cream was used for sunscreen.

Methods

1. UV exposure

The mice had both their ears irradiated daily for 5 days, from Monday to Friday, per week. One hundred mJ/cm^2 was exposed daily to the dorsal skin of the right ear (Subgroup A). Sunscreen was applied topically to the dorsal skin of the left ear 15 min before irradiation (Subgroup B).

The mice were divided into 6 groups according to the duration of irradiation as follows: Group I (3 days), Group II (1 week), Group III (2 weeks), Group IV (3 weeks), Group V (4 weeks), and Group VI (unirradiated control). UV exposure was done in the afternoon to avoid diurnal variation.

2. Skin biopsy and tissue preparation

The skin of both ears was biopsied after UV light irradiation for 3 days, 1 week, 2 weeks, 3 weeks, and 4 weeks.

The biopsied specimen was cut into small pieces and processed for (1) histochemical study and (2) ultrastructural study.

1) Histochemical study

The whole epidermal sheet was prepared by means of a split-DOPA technique (Erickson and Montagna 1975; Kim and Youn 1988). Epidermal unfixed tissues were split after incubation with 1N NaBr solution at 37℃ for 2 hours. The split epidermis was incubated with buffered DOPA solution at 37℃ for 2 hours followed by several hours of subsequent incubation at 4℃. The buffered DOPA solution was composed of 25 ml of 0.1% DOPA solution, 6ml of 0.88% disodium hydrogen phosphate, and 2 ml of 0.9% potassium phosphate. The DOPA preparation was then fixed with 10% formalin solution and dehydrated by 70% and 99.5% alcohol solutions and xylol. The skin specimen was mounted on glass slides with the dermal-epidermal junction facing up and examined with light microscopy.

The number of melanocytes per square millimeter (No/mm^2) was determined using a light microscope (Olympus, Japan) by counting the number of melanocytes in 10 independent fields in each specimen. The significance of the results bet-

ween Subgroup A and Subgroup B was examined by Wilcoxon's matched-pairs signed ranks test. The significance of the results of the number of melanocytes through the duration of irradiation was examined by the Friedman Two-way Anova test.

2) Ultrastructural study

The specimen for electron microscopy was cut into 1×1 mm pieces, fixed with 2.5% glutaraldehyde, postfixed with 1% osmium tetroxide, dehydrated with a graded ethanol series, and embeded in EPON 812. Thin sections were cut with a diamond knife, stained with uranyl acetate and lead nitrate, and examined under an electron microscope (JOEL JEM-100, Japan). The specimens for electron microscopy with DOPA staining were prepared by the same procedures as those for usual electron microscopy, except that the duration of fixation with glutaraldehyde was 1 hour (usual: 2 hours), and the pieces were incubated in the buffered DOPA solution at 37℃ for 1 hour and in the changed fresh buffered DOPA solution at 4℃ for several hours (overnight) (Hunter 1970; Jimbow 1971).

RESULTS

The Effect of UVR on Melanocytes

1. Histochemical findings

The irradiated skin contained strongly DOPA-positive melanocytes with stout dendrites as well as a clearly increased number of melanocytes compared to the nonirradiated skin. After UVR exposure for 3 days the number of DOPA-positive melanocytes increased from 29.3 ± 24.4 to 138.9 ± 116.3 cells/mm^2. The number of DOPA-positive melanocytes increased to 361.6 ± 68.6 cells/mm^2 after 1 week, 386.6 ± 73.2 cells/mm^2 after 2 weeks, 633.1 ± 90.0 cells/mm^2 after 3 weeks, and 919.7 ± 45.9 cells/mm^2 after 4 weeks. During the 4

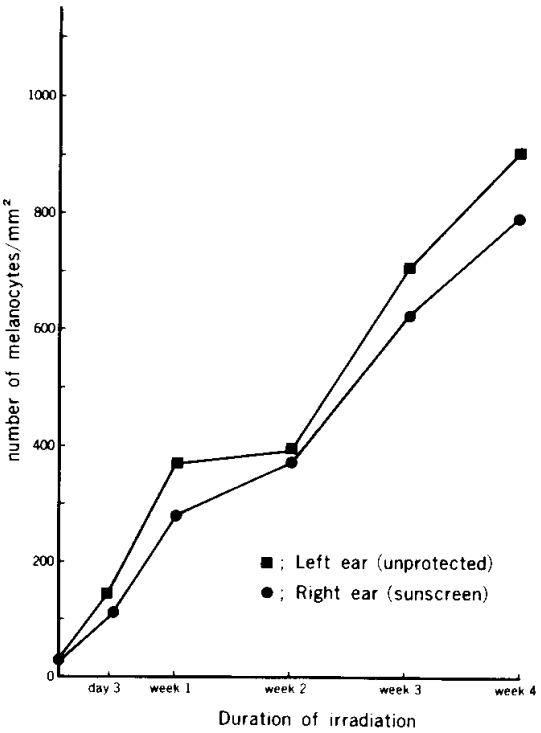

Fig. 1. The effect of repeated UVR exposure on the number of DOPA-positive epidermal melancytes

Table 1. The number of DOPA-positive melanocytes in repeated UV irradiation

Group	Duration of Irradiation	No. of melanocytes/mm (Mean ± SD)	
		Subgroup A	Subgroup B
I	3 day	138.9 ± 116.3	110.4 ± 77.3
II	1 wk	361.6 ± 68.6	284.7 ± 117.8
III	2 wk	386.4 ± 73.2	377.9 ± 67.8
IV	3 wk	633.1 ± 90.0	600.6 ± 99.3
V	4 wk	919.7 ± 45.9	810.6* ± 34.9
VI	Control	29.3 ± 24.4	

* $P<0.05$, compared to Subgroup A

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=국 문 초 록=

자외선조사에 의한 표피 멜라닌 세포의 변화 및 일광차단제가 이에 미치는 영향
—조직화학적 및 초미세구조학적 연구—

서울대학교 의과대학 피부과학 교실

윤재일 · 이애영 · 이유신

자외선은 인체 피부에 색소반응을 위시하여 다양한 피부 반응을 일으킨다. 색소반응의 규명을 위하여 C57 BL mouse의 귀 피부에 고압수은등을 이용하여 매일 100 mJ/cm^{2}씩의 자외선을 조사하며 3일 조사 후, 1주일 조사 후, 2주일 조사 후, 3주일 조사 후, 4주일 조사 후 멜라닌 세포의 수와 형태의 변화를 조직화학적 및 전자현미경적 검사로 연구하였다. 또한 멜라닌 형성효소인 tyrosinase의 활성도를 알기 위해 DOPA-전자현미경검사도 아울러 시행하였다. 동시에 일광차단제, PABA의 효과를 알기 위하여 PABA 도포군에서의 변화를 연구하였으며 그 결과는 다음과 같다.

1. DOPA-양성 멜라닌 세포수는 비조사군에서는 표피 1평방 mm당 29.3 ± 24.4였다. 3일 조사 후는 138.9 ± 116.3로, 1주 후는 361.6 ± 68.6으로, 2주 후는 386.6 ± 73.2로, 3주 후는 633.1 ± 9.0으로 증가하였으며 4주 후는 919.7 ± 45.9로 조사기간이 길어짐에 따라 점차 그 수가 증가되었다 ($P<0.01$).
2. 멜라닌 세포는 조사기간이 증가함에 따라 점차 세포가 커지고 수지상돌기수가 증가되며, 길이가 길어졌다. 3주가 지난 후는 저배율하에서는 세포상호간의 식별이 어려울 정도로 많은 세포숫자의 증가, 심한 멜라닌 색소 증가 및 숫자가 많아지고 길어진 수지상돌기 등을 나타내었다.
3. 전자현미경 및 DOPA-전자현미경검사상 조사 3일 후에 멜라닌 소체 및 다수의 잘 발달된 골기체가 관찰되었다. 또한 골기체 주위에 멜라닌 형성효소인 tyrosinase 활성을 의미하는 DOPA 양성인 공포상 내지는 관상의 구조가 관찰되었다. 이러한 구조는 1주 후 증가되었으며 2주 후는 약간의 변성이 세포질 속에 부분적으로 나타나기 시작하여 3주 후는 세포내 구조물들의 형태가 점차 불분명해지는 소견을 나타내었다.
4. 일광차단제 도포군에서는 4주 조사 후 DOPA-양성 멜라닌 세포의 숫자가 일광차단제 비도포군에 비해 유의하게 감소된 소견을 나타내었다 ($P<0.05$). 그러나 형태학적인 변화는 관찰할 수 없었다.