The present results indicate the presence of a seasonal rhythm of immunoreactive α-melanocyte stimulating hormone (α-MSH) in 20- to 40-year-old subjects of skin type I (light color of skin and eyes, red hair, no tanning after sun exposure) and skin type II (light color of skin, eyes, and hair, rare tanning) with raised levels of α-MSH in summer and low levels in winter. With increasing age of the investigated subjects, the seasonal rhythm seems to be lost. In subjects with skin type III (light skin, brown eyes and black hair, strong pigmentation after sun exposure) α-MSH shows only insignificant variations over the whole year. A seasonal rhythm of ACTH could not be demonstrated. A diurnal rhythm could be seen for ACTH, but not for α-MSH.

To summarize, one can suppose that the seasonal rhythm of α-MSH is controlled by a varying UV exposure of the integument which is different over the whole year. J Invest Dermatol 86:454–456, 1986

**TEST SUBJECTS AND METHODS**

The study was carried out in 68 Central Europeans of both sexes (40 females, 28 males) aged 20–95 years (x = 44.4 years). The test subjects were classified in accordance with age and their skin type (type I: light eye color, red hair, no tanning on exposure to sunlight; type II: light skin and eye color, moderate tanning on exposure to sunlight; type III: light skin, brown eyes and black hair, intense pigmentation on exposure to sunlight).

The older test subjects (over 70 years of age) were residents in old peoples' homes, and the remainder were working. All test subjects were living in Germany during the period of the trial and were exposed to the usual sunlight.

The blood samples were taken in summer (August) 1982, in autumn (October) 1982, in winter (January) 1983, and in spring (April 1983); they were taken at half-hourly intervals (period 7 AM to 5 PM) to determine a diurnal rhythm of the immunoreactive α-MSH in 15 test subjects with various skin types and aged between 20 and 40 years. The blood was collected in EDTA-coated tubes, centrifuged, and immediately stored at −40°C until the measurements of α-MSH and ACTH were done. The commercially available radioimmunoassay for α-MSH (Biermann, Bad Nauheim) contains rabbit α-MSH antibodies in rabbit serum and 125I-labeled α-MSH. The antibody complex is precipitated by means of a rabbit antiserum (goat) as well as carrier rabbit serum and polyethylene glycol.

The α-MSH antibodies were tested with regard to their cross-reaction with some peptides. On addition of the 1000-fold concentration of the highest α-MSH standard (1250 pg/ml), the crossreaction with ACTH is 1–24.4%. No cross-reaction was observed with the other peptides investigated (ACTH 1–39, β-MSH, β-LPH, β-endorphine, met-enkephalin, leu-enkephalin, parathormone, somatotropichormone, calcitonin, prolactin, human growth hormone, bombesin, neurotensin). The recovery rate of α-MSH in application of 50–400 pg/ml is 90–120%. The limit of detection by the assay is 2 pg/ml. As an improvement of the prescribed method for α-MSH measurements, the rabbit α-MSH antiserum was diluted 1:2 with bovine serum albumin borate buffer (0.1 M, pH 8.4), so that the ratio B/B0 (= percentage binding of the sample compared to the zero standard as maximum binding) with 60 pg α-MSH/ml was roughly 80%, whereas it was roughly 50% at 200 pg/ml. All values measured were within the linear range.

**ALPHA-MELANOCYTE STIMULATING HORMONE (α-MSH)**

Alpha-melanocyte stimulating hormone (α-MSH) is a tridecapeptide with a molecular weight of 1663. The amino acid sequence of α-MSH is identical with the section 1–13 of the ACTH molecule. The biologic relatedness of the 2 peptide hormones, which is especially striking dermatologically although the melanogenic effect is different, is explained by this partial molecular identity. Although α-MSH induces multifarious central and peripheral effects in quite different animal species [1–5], its role and its topical occurrence in humans are still largely unclear [6]. α-MSH is regarded as a typical hormone due to its hypothalamohypophyseal [6,7] origin, its presence in the blood, and because of its effects on peripheral tissue [3]. In the periphery, the effect of α-MSH on the melanophores of lower vertebrates has been investigated especially thoroughly [8]. Furthermore, the stimulatory effect on melanocytes [2,9] and sebaceous glands in the skin [2,4,5] is also known in mammals. Finally, it could be demonstrated that this peptide hormone increases the pigment synthesis of melanoma cells in vitro via activation of tyrosinase. The growth of these tumor cells can be both stimulated and inhibited [10,11] (concentration-dependent) by α-MSH.

In humans, there are indications that the plasma levels of α-MSH which can be detected by means of radioimmunoassay are not constant, but are subject to various regulatory factors. These include the degree of pigmentation of the integument (as a genetically coded individual factor) as well as environmental factors such as exposure of the skin to UV radiation [12].

In the present study, we have investigated the question as to whether other regulatory mechanisms act besides the above factors. In particular, we aimed to establish whether diurnal or seasonal variations of the immunoreactive α-MSH and ACTH levels can be measured in the plasma.
of the curve with regard to these variations in the antibody concentrations of the samples. The intraassay variance is 15% in this test system.

ACTH 1–39 was likewise measured radioimmunologically (Biermann, Bad Nauheim). The statistical significances were calculated in accordance with the Student's t-test.

RESULTS

We were unable to detect any sex-specific difference for the proteohormones α-MSH and ACTH by means of radioimmunoassay. In the different age groups, much lower basal values (Table I) are shown for α-MSH (in contrast to ACTH) for the 60-year-olds as compared to those of 30-year-olds (difference: 30.1%). However, these differences are just as significant as the distinct seasonal variations for α-MSH (Table II). A difference of 19.1 pg (29.7%) between summer and winter can be calculated with a statistical significance from \( p < 0.001 \).

After subdividing the patients in accordance with their skin type, differences between summer and winter are significant (59.4% and 62.9%, respectively, \( p < 0.05 \)) for types I and II (Table III), but not for type III (difference: 26.9%). No differences could be detected for ACTH.

If the age factor is additionally included in the evaluation, much greater differences between winter and summer were shown by the 20- to 40-year-olds as compared to the older test subjects (Fig 1). This is underscored by a seasonal analysis of the individual results in 20- to 40-year-old test subjects with skin type I (Fig 2). Here we have found statistically significant results for α-MSH level in summer and winter with \( p < 0.001 \).

In half-hourly measurements of the 2 proteohormones, the familiar diurnal rhythm with a rise in the morning was shown by ACTH. On the other hand, α-MSH did not reveal any diurnal rhythm (Fig 3).

DISCUSSION

Our results document an unequivocal seasonal rhythm for the immunoreactive α-MSH we measured in the plasma of 20- to 40-year-old test subjects with skin types I and II. There were high values in summer and low values in winter (Fig 1). It is conceivable that these rhythmic fluctuations of α-MSH are regulated via a different integumental UV exposure in summer and winter. Likewise, light impulses which have a stimulating action via the retina and hypothalamus in summer must be considered. With increasing age of the test subjects, this rhythm is lost. This may be due to environmental influences, reduced UV exposure of older people, or altered cybernetic processes in the elderly which have not been detected so far. Finally, genetically fixed features such as the degree of cutaneous pigmentation also influence the level of the α-MSH in the plasma. We have already described this in an earlier communication [12], and this observation is confirmed once more by the results presented here.

Test subjects of skin type III display only slight, insignificant seasonal variations of the α-MSH level in the plasma. For ACTH, a seasonal rhythm could not be demonstrated. On the other hand, we were able to reproduce the familiar diurnal rhythm for ACTH in contrast to α-MSH. Little investigated so far have been the mechanisms which control the α-MSH incretion. Holzmann and coworkers [13] were able to demonstrate a rise of the α-MSH after UV irradiation of larger areas of the skin. In general, complex endocrinologic reactions are induced after ultraviolet irradiation of the whole body [14]. Recent investigations documenting the existence of α-MSH in human skin [15] indicate the so far unknown significance of this peptide hormone for the integument. Furthermore, it is known that the plasma level of α-MSH in mammals can be subject to a genetic program [16].

Finally, investigations on various mammals [3,17] indicate that the α-MSH incretion may be subject to diurnal and seasonal rhythms. However, special species-specific features would have to be considered here (e.g., seasonal coat pigmentation and depigmentations in the wassel). In female rats, rhythmic fluctuations in α-MSH secretion could be demonstrated during the sexual cycle. In contrast to the values we found in humans, diurnal

Table II. Seasonal Fluctuations in the α-MSH and ACTH Concentrations in the Plasma (pg/ml ± SD) in 68 Test Subjects

<table>
<thead>
<tr>
<th>Seasons</th>
<th>No. of Patients</th>
<th>α-MSH</th>
<th>ACTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>68</td>
<td>64.2 ± 23.7</td>
<td>32.2 ± 5.2</td>
</tr>
<tr>
<td>Summer</td>
<td>68</td>
<td>68.7 ± 24.1</td>
<td>35.0 ± 4.7</td>
</tr>
<tr>
<td>Autumn</td>
<td>68</td>
<td>59.1 ± 24.1</td>
<td>33.9 ± 5.0</td>
</tr>
<tr>
<td>Winter</td>
<td>68</td>
<td>45.1 ± 22.9</td>
<td>34.9 ± 5.2</td>
</tr>
</tbody>
</table>

Table III. Seasonal, Basal Plasma Level of α-MSH in Skin Types I–III (pg/ml ± SD)

<table>
<thead>
<tr>
<th>Skin Type</th>
<th>No. of Patients</th>
<th>Winter</th>
<th>Summer</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>33</td>
<td>43.6 ± 7.6</td>
<td>69.5 ± 4.3</td>
<td>+ 59.4</td>
</tr>
<tr>
<td>II</td>
<td>27</td>
<td>44.8 ± 9.6</td>
<td>73.0 ± 3.9</td>
<td>+ 62.9</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>42.0 ± 6.2</td>
<td>53.3 ± 8.4</td>
<td>+ 26.9</td>
</tr>
</tbody>
</table>

Figure 1. α-MSH: percentage differences of the α-MSH level in the plasma in summer in comparison to winter in test subjects of differing skin type (I–III) and ages.
fluctuations in α-MSH are also measured in these rodents. It is assumed that these are not linked to light-dark phases [8], but are subject to a genetically fixed circadian program such as has also been demonstrated for other endocrine systems [18–21]. In this connection, the effect of steroid sex hormones on the α-MSH secretion is to be mentioned. Several authors [2,5,17] have demonstrated a rise of the α-MSH concentration under the influence of progesterone and estrogens in rats. In humans, a single α-MSH injection brings about a rise of several hypophysal hormones [6].

At the present state of knowledge, it can be observed that the level of MSH in mammals is subject to multifarious influences. Beside central regulatory mechanisms such as MSH release inhibiting factor and MSH releasing factor, peripheral stimuli perceived via the integument and transmitted to the hypothalamus (UV irradiation) can probably also act in humans. In addition, there are genetically coded regulatory factors. Thus, the individual α-MSH level in the plasma is the product of a polyfactorial process in which integumental factors very probably play a role which is not to be neglected.

REFERENCES