THE EFFECT OF TESTOSTERONE ON THE MELANOCYTES AND MELANIN IN THE SKIN OF THE INTACT AND ORCHIDECTOMISED MALE GUINEA-PIG*

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The hormonal control of melanin formation in the skin has aroused the attention of research workers for over forty years. It is now generally accepted that endocrine factors play an important role in melanogenesis in man and lower animals but it is surprising to find that although much experimental and clinical data has been obtained it is still far from clear what action the male sex hormone has on melanin formation. Much of the obscurity probably stems from the fact that a great number of the observations have been macroscopic only and the microscopic studies have been confined to vertical skin sections. Vertical sections have the disadvantage that accurate melanocyte counts are impossible and that the appearances of the melanocytes cannot be adequately demonstrated. The present research is a controlled histochemical investigation into the effect of testosterone on the appearances of the melanocytes and melanin in the skin of the male guinea-pig using both skin sheets and vertical skin sections. It was hoped that the results would indicate what action testosterone has on pigment formation in the male guinea-pig.

MATERIALS AND METHODS

Eighteen guinea-pigs were used for the investigation and these were divided into three groups each consisting of three pure black and three pure red animals. 1) Six immature animals all weighing under 265 grammes and known to be less than 25 days old to study the effect of testosterone on the melanocytes before the onset of sexual activity. 2) Six mature animals all weighing between 440 and 573 grammes to study the effect of the hormone on melanocytes in the normal adult guinea-pig. 3) Six animals weighing between 350 and 390 grammes. which according to Deanesly and Rowlands (1) are at a period of growth when rapid sexual development occurs, were orchidectomised and one month later were treated with testosterone to study the effect of this hormone on the melanocytes in a group of animals deprived of their normal secretion of male sex hormone.

Skin samples measuring about 0.5 sq.cm. were taken from all the animals from the ear, anterior abdominal wall and sole of foot; in addition the right areola was excised. The animals were then given 2 mgms. of testosterone propionate (Organon) intramuscularly once daily for 5 days a week for a period of 2 weeks in the case of the immature animals and for a period of 4 weeks in the case of the remaining animals. All the animals were killed by a blow on the back of the neck and a further series of skin samples were removed from areas adjacent to those taken previously and the left areola was excised. The two skin samples from each region were processed as outlined below and then compared.

Most of the skin samples were left intact after excess subcutaneous fat and the deeper part of the dermis had been excised under a dissecting microscope. A few of the samples were treated with trypsin to disengage the epidermis using the "skin-splitting" technic of Billingham and Medawar (2), as modified by Szabó (3). All the samples were then processed under exactly identical conditions. The skin was fixed in 5% formol saline for 16 hrs. and after washing it in normal saline for ¾ hr. it was incubated at 37°C in a 1 in 1000 solution of L-dihydroxyphenylalanine at a pH of 7.4. After two hours the substrate was renewed and incubation continued for a further 1¾ hrs. Fixation for a further period of 16 hrs. in 10% formol saline was then carried out, followed by dehydration and clearing. The greater part of each skin sheet was mounted in Canada Balsam, the remainder was embedded in paraffin and vertical sections 6μ thick were cut. In order to identify the various layers of the epidermis a number of the vertical sections were counterstained with hematoxylin and eosin.

All the skin sheets were examined with the dermal surface uppermost except those of the anterior abdominal wall where, owing to the large number of hair roots present in the dermis, it was found more satisfactory to examine the sheets with the epidermis uppermost. The hormonal influence on the melanocytes was studied by comparing the skin samples removed before and after the

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TABLE I

Showing the effect of testosterone on the melanocyte counts in the different skin regions of the immature male guinea-pig

No. of Animal	Color	Ear		Anterior Abdominal Wall		Areola	
		Control	Experimental	Control	Experimental	Control	Experimental
2	Black	1300	1480	398	430	1008	1118
4	Black	1500	1458	385	463	763	715
5	Black	1373	1415	413	388	940	1290
11	Red	1135	1228	213	220	665	608
12	Red	985	1225	123	133	675	550
13	Red	1420	1320	110	258	443	330
Mean difference in counts per sq. mm.....		−69		−42		−20	
Standard deviation.....		129		62		182	
Probability...		>0.2		>0.1		>0.7	

testosterone therapy in each of the animals. The appearances of the melanocytes in the skin samples were studied by assessing the number and size of the melanocytes and the amount, color and position of the melanin within the cells. The length, width and complexity of the dendritic processes were also noted and an attempt was made to assess the amount and color of the free melanin present, i.e., that melanin which is situated outside the melanocytes. The skin sheets of the sole of the foot were examined only in the red animals since the very high concentration of melanin present in the skin of the black animals in this region prevented their accurate assessment.

The melanocytes were counted by projecting the image of a skin area measuring 0.04 sq. mm. on to a ground glass screen marked out in the form of a grid. Ten randomly chosen areas were counted at a magnification of 375. Black skin was less easy to count than red because it possessed more and darker free melanin granules which tended to obscure the melanocytes. In the sole of the foot melanocyte counts were found to be impracticable owing to the melanocytes being superimposed one upon the other on the sides of the very steep dermal papillae.

RESULTS

The melanocytes of guinea-pig skin occur in two sites, the hair follicles and the basal layer of the surface epidermis. In the present work our study has been confined to the melanocytes and melanin of the surface epidermis.

Effect of testosterone on the melanocytes and melanin in the immature male guinea-pig

In the skin of the ear no definite changes were noted. In the anterior abdominal wall the amount of free melanin was increased in four of the animals and was unchanged in the remaining two. The other results for this region were inconsistent; for example the size of the melanocytes, the length, complexity and width of the dendritic processes and the amount of melanin within the cells was reduced in three of the animals whereas in the remaining animals there was either no change or there was a tendency for the reverse changes to occur. In the areola the amount of melanin in the cell bodies and the depth of colour of the melanocytes was reduced in the majority of the animals but the remaining changes which occurred in this region were inconsistent. In the sole of the foot the amount of melanin in the basal layer of the epidermis was increased.

The melanocyte counts for each of the regions are shown in Table I. It is seen that there is no significant difference between the cell counts of the specimens removed before and after the testosterone treatment in each skin region.

Effect of testosterone on the melanocytes and melanin in the mature male guinea-pig

In the skin of the ear the length, complexity and width of the dendritic processes in 4 of the animals was reduced and in three of these the amount of melanin within the dendritic processes was reduced but in the fourth it was increased. In 4 of the animals which included 3 of those showing the dendritic changes the amount and depth of colour of the free melanin was increased. In the anterior abdominal wall although a number of changes were produced these were found to be inconsistent. In the areola the amount of free melanin was increased in 4 animals, reduced in 1 and was unchanged in the remaining animal. There was a tendency for the cell size to increase in 3 of the animals. No further consistent changes were noted. In the sole of the foot (Figs. 1 & 2) the amount of melanin in the basal layer of the epidermis was increased in all of the animals and this increase was seen to be greater than that found in the immature animals.

FIG. 1. Vertical section of skin of sole of foot of black intact mature male guinea-pig before testosterone treatment. Shows melanocytes to be situated mainly in the epidermal ridges and to be in the basal layer of the epidermis. Free melanin is seen to be scattered throughout the remaining layers of the epidermis. Full thickness skin preparation treated with Dopa reagent. No counterstain. × 180.

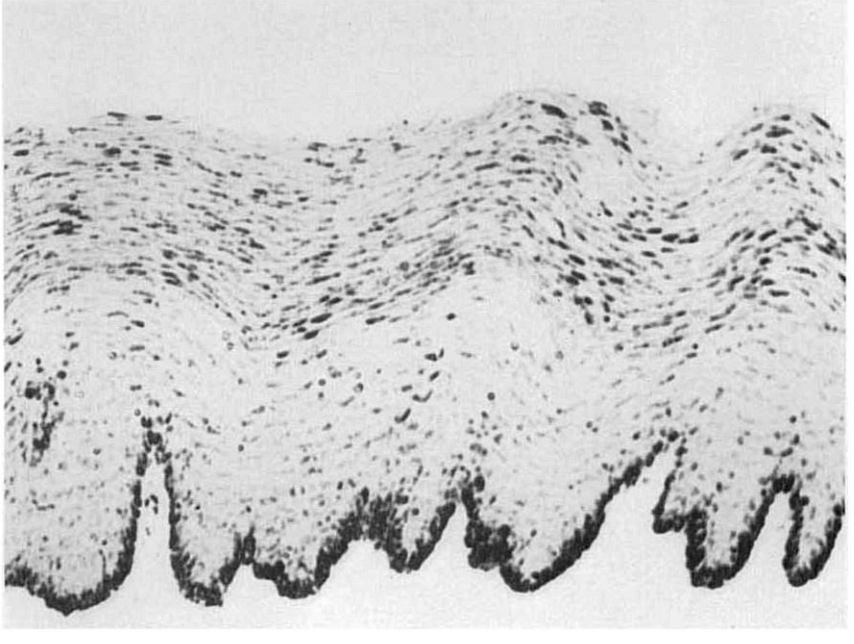

FIG. 2. Vertical section of skin of sole of foot of same animal seen in Fig. 1 after one month of testosterone treatment. Shows a great increase in the amount of melanin in all the layers of the epidermis. Full thickness skin preparation treated with Dopa reagent. No counterstain. × 180.

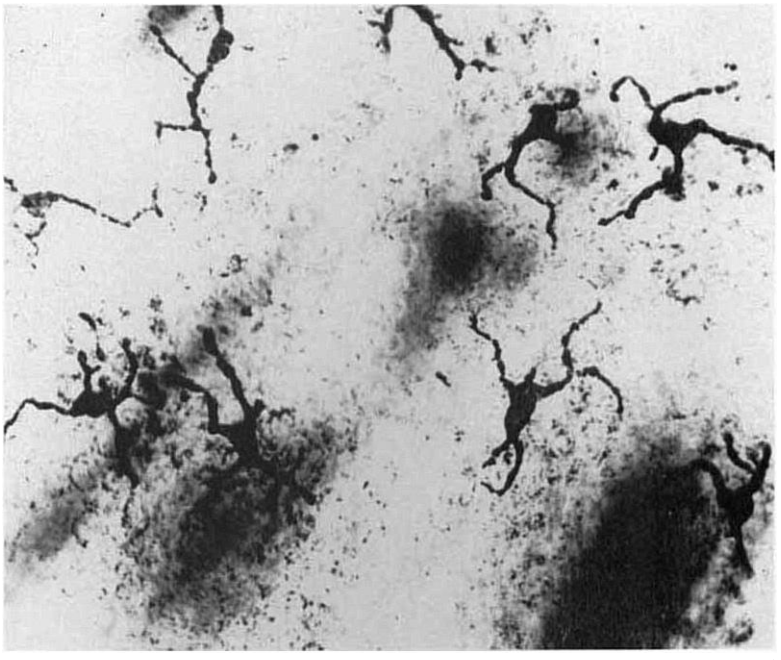

FIG. 3. Skin sheet of anterior abdominal wall of red orchidectomised male guinea-pig before testosterone treatment. Shows melanocytes with relatively large cell bodies and possessing long branching dendritic processes. Note that most of the melanocytes are filled with melanin. Full thickness skin preparation treated with Dopa reagent. No counterstain. × 400.

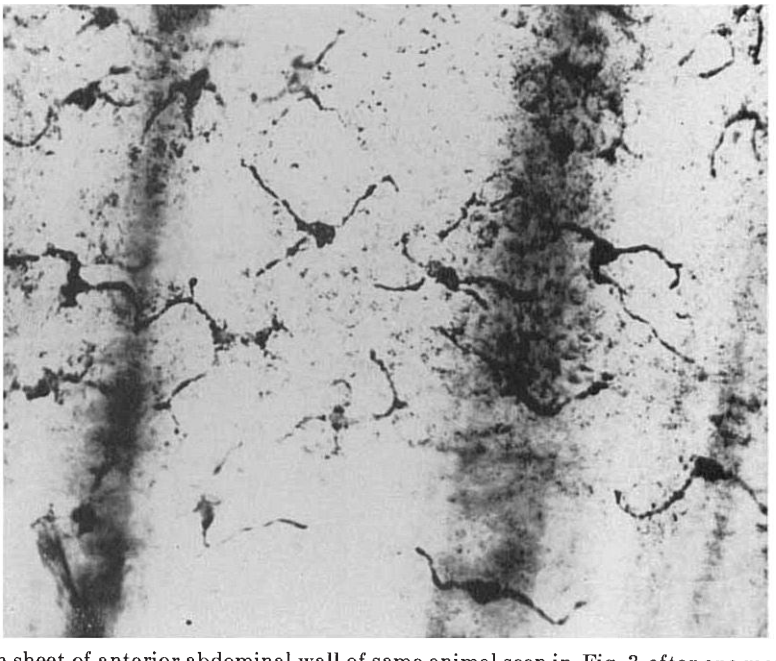

FIG. 4. Skin sheet of anterior abdominal wall of same animal seen in Fig. 3 after one month of testosterone treatment. Shows melanocytes to have smaller cell bodies and to contain less melanin. Note that in this particular animal the number of melanocytes has increased. Full thickness skin preparation treated with Dopa reagent. No counterstain. × 400.

TABLE II

Showing the effect of testosterone on the melanocyte counts in the different skin regions of the mature male guinea-pig

No. of Animal	Color	Ear		Anterior Abdominal Wall		Areola	
		Control	Experimental	Control	Experimental	Control	Experimental
141	Black	1323	893	323	285	920	890
142	Black	1210	1208	293	298	753	730
143	Black	1198	1265	208	313	905	875
110	Red	955	855	5	133	238	395
131	Red	—	—	120	130	1010	598
132	Red	1040	1058	165	88	588	528
Mean difference in counts per sq. mm.....		+89		−22		+66	
Standard deviation.....		200		80		186	
Probability...		>0.3		>0.5		>0.4	

TABLE III

Showing the effect of testosterone on the melanocyte counts in the different skin regions of the orchidectomized guinea-pigs

No. of Animal	Color	Ear		Anterior Abdominal Wall		Areola	
		Control	Experimental	Control	Experimental	Control	Experimental
125	Black	855	873	238	165	—	—
126	Black	785	845	238	238	830	793
145	Black	945	768	308	353	713	705
114	Red	818	875	280	190	818	790
115	Red	1030	988	175	193	710	630
116	Red	755	728	135	358	725	745
Mean difference in counts per sq. mm.....		+19		−21		+27	
Standard deviation.....		88		112		37	
Probability...		>0.6		>0.6		>0.1	

The melanocyte counts for each of the regions are shown in Table II. It is seen that there is no significant difference between the cell counts of the specimens removed before and after the testosterone treatment in each skin region.

Effect of testosterone on the melanocytes and melanin in the orchidectomized guinea-pigs

In the skin of the ear the size of the cell bodies was reduced in three of the animals. The length of the dendritic processes was increased in three animals which included two of those showing a reduction in cell size. Although a numbes of further changes occurred these were found to be inconsistent. In the anterior abdominal wall all six animals showed a marked reduction in the depth of color of the melanocytes and this was seen to be accompanied by a reduction in the amount of melanin within the cell bodies in 4 of the animals (Figs. 2 & 4). In 3 of the animals the size of the cell bodies was decreased. In the areola no uniform changes were produced. In the sole of the foot the amount of melanin within the basal layer of the epidermis was increased.

The melanocyte counts for each of the regions are shown in Table III. It is seen that there is no significant difference between the cell counts of the specimens removed before and after the testosterone treatment in each skin region.

DISCUSSION

The effect of testosterone on melanin pigmentation has been widely studied in both animals and man. While a few of the observations have been microscopic the majority have been macroscopic. Briefly the results may be divided into two categories: 1) Those which showed that testosterone increased the pigmentation of the skin. This has been observed by Hamilton & Hubert (4) in castrated and hypogonadal men, Hamilton (5) in women, Noble & Wurm (6) in the lores, lower mandible and buccal cavity of the night heron, Pfeiffer, Hooker and Kirschbaum (7) in the sparrow's bill, Edwards, Hamilton, Duntley & Hubert (8) in the human male castrate, Kupperman (9) in the dorso-lateral subcostal region of the male golden hamster, Wells (10) in the scrotum of the ground squirrel and Flaks (11) on the nipples of dark-coated female mice. 2) Those which showed that testosterone had no effect on skin pigmentation. This was observed by Sulman (12) on the chromatophores of the frog and Wheeler, Cawley & Curtis (13) reported that topical application of testosterone to the nipples and areolae of immature castrated

male guinea-pigs had no significant effect on pigmentation.

The results reported by Hamilton (5) and Hamilton & Hubert (4) are based solely on macroscopic observations of skin color and consequently it is not clear whether in fact the change in pigmentation found by them is due to an alteration in the melanin content of the skin or due to alteration of the other factors responsible for skin color. The work of Edwards and his co-workers (8) is based upon a spectrophotometric analysis of the skin pigments. Spoor (14) has criticized this method on the grounds that there is a variation in standards used for the calibration of the reflectance curves and also that melanin pigments absorb so strongly that their presence masks small variations in pigment content. Furthermore, Hamilton (15) concluded that the relationship of testicular secretions to integumental pigmentation is to a significant extent a vascular phenomenon, and Edwards (16), while stating that androgens cause an increase in melanin, nevertheless points out that this is usually a minor and often an uncertain change. The work on animals in which testosterone has been found to increase melanin pigmentation has been restricted to specific regions in several widely different species where according to Hamilton (15) the melanocytes may be androgen-dependent and require androgenic stimulation to assume a functional state, and, here again, a number of these studies have relied upon macroscopic observations.

The present research was designed to study the effect of testosterone on the melanocytes and melanin in the skin of the ear, anterior abdominal wall, areola and sole of the foot in pure black and pure red guinea-pigs using carefully controlled histochemical experiments. The importance of this work lies in the fact that the skin samples which were compared before and after the administration of testosterone were removed from the same region of the same animal and were processed under identical conditions. Two control experiments, which have been reported previously (Snell & Bischitz (17)) were carried out on intact male guinea-pigs and these showed that (a) initially the melanocytes and melanin are practically identical in the two skin areas of each region which are compared and (b) the operative procedure associated with the initial skin biopsy increased the melanogenic activity in the adjacent skin areas in the anterior abdominal wall and sole of foot. In the ear and areola no definite effects were produced. It is thus clear that the changes resulting from trauma must be taken into consideration when the results of testosterone therapy are assessed.

In view of our previous observation (Bischitz & Snell (18)) that the melanocytes of immature male guinea-pigs have a greater melanogenic activity then those of fully mature animals a group of immature guinea-pigs was included in the present experiment and their results were assessed separately from those of the mature animals. It was considered possible that the melanocytes of immature guinea-pigs which had not been subject to the same degree of hormonal influences might react to large doses of administered testosterone in a different manner to those of the mature animal. The inclusion of these animals seemed to be of especial interest in view of the observations of Cade (19) and others that malignant melanomas are rare before puberty in the human subject.

When the changes which are known to be produced by the unavoidable trauma associated with the skin removal are taken into consideration the effects of testosterone therapy on the melanocytes and melanin in the immature and mature guinea-pigs may be interpreted as follows: In the immature animal no uniform changes were produced in the skin of the ear. In the anterior abdominal wall the stimulating influence of trauma on melanogenesis seems to have been partially overcome by testosterone therapy suggesting that testosterone may have an inhibiting influence on melanogenesis in this region. In the areola the most consistent changes produced are a reduction in the amount of melanin within the cell bodies and a reduction in the depth of color of the melanocytes, again suggesting a slight inhibition of melanogenesis. In the sole of the foot melanogenesis has been stimulated beyond the level which could be accounted for by the effect of the operative procedure alone.

In the intact mature animals which received testosterone, in the skin of the ear there is a tendency for the length and complexity of the dendritic processes to be reduced and this is accompanied by an increase in the amount and depth of color of the free melanin. Although no uniform changes have been produced in the anterior abdominal wall it would appear that the stimulation of melanogenesis produced by trauma in this region has been at least partially counter-

acted. In the areola there was a tendency for the size of the cell bodies of the melanocytes and the amount of free melanin to increase. In the sole of the foot there was a great increase in the amount of melanin in the basal layer of the epidermis which exceeded that occurring as a result of trauma.

A further group of animals was included in the present work weighing between 350–390 gms. which, according to Deanesly & Rowlands (1), are at a period of growth when the sexual organs undergo rapid development. This group was studied because it was considered possible that the melanocytes might be more susceptible to changes in hormone level at this time. It is known for example that in the human subject the growth of benign and malignant melanomas is accelerated at about the time of puberty. (Park (20), Spitz (21), Raven (22)). In order to bring about the maximum change in the hormone level it was decided to orchidectomize these animals before commencing the administration of testosterone. Taking into consideration the changes produced by the operative trauma, the effects of testosterone therapy may be interpreted as follows: In the skin of the ear no uniform changes were produced. In the anterior abdominal wall the changes which occurred suggested that a definite reduction in melanogenic activity had taken place as seen by a reduction in the amount of melanin within the melanocytes, a reduction in cell size and a reduction in the depth of color of the melanocytes. In the areola no consistent changes were produced. In the sole of the foot there was an increase in the amount of melanin in the basal layer of the epidermis which exceeded that produced by trauma alone.

These findings are largely in agreement with those obtained in a preliminary experiment published previously (Bischitz and Snell (23)). Any minor differences which exist can be accounted for by the fact that in the preliminary investigation the animals were not separated into groups according to the stage of their development, and, since at that time we were unaware that the trauma associated with the skin biopsy stimulated melanogenesis to some extent, this factor was not taken into consideration when the results were assessed.

The majority of the results for the different groups of animals in the ear, anterior abdominal wall and areola suggest that testosterone is incapable of producing uniform changes in the melanocytes and melanin. It may be possible to produce more definite results by studying the effect of different doses of testosterone over varying periods of time. The reduction in the amount of melanin in the skin of the anterior abdominal wall which occurred particularly in the orchidectomised animals, however, was considered to be a positive finding since the operative trauma associated with skin biopsy has been found to increase the amount of melanin in this region. Furthermore, this finding is in agreement with our previous observation that orchidectomy increases the amount of melanin in the anterior abdominal wall (Snell and Bischitz (17)). The most constant effect produced by testosterone in all the animals was seen in the sole of the foot where it increased the amount of melanin in the epidermis and this change exceeded that produced by the operative trauma. A similar effect was found to occur following orchidectomy (Snell and Bischitz (17)). It would therefore appear that melanogenesis in the skin of the sole of the foot is stimulated by any change in the male hormone level.

SUMMARY

1. The effect of testosterone on the melanocytes and melanin in the skin of the ear, anterior abdominal wall, areola and sole of foot has been studied in pure black and pure red intact and orchidectomized male guinea-pigs using carefully controlled histochemical experiments. The melanocytes were identified by using the dopa reaction. The results were assessed by comparing the melanocytes and melanin in closely adjacent skin areas removed before and after testosterone treatment from each region in the same animal. In the case of the areola the right one was compared with the left. The melanocytes and melanin present in the hair follicles have not been examined in this investigation.

2. In the skin of the ear, anterior abdominal wall and areola the majority of the results for the different groups of animals suggest that testosterone is incapable of producing uniform changes in the melanocytes and melanin. However, in the anterior abdominal wall of the orchidectomized animals a definite reduction in melanogenic activity took place.

3. In all groups of animals testosterone was found to stimulate melanogenesis in the sole of the foot.

4. The significance of these findings in relation

to the observations of previous workers is discussed.

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REFERENCES

1. DEANESLY, R. AND ROWLANDS, I. W.: Growth of the reproductive and endocrine organs of the guinea-pig. J. Anat., Lond., **70:** 331–338, 1936.
2. BILLINGHAM, R. E. AND MEDAWAR, P. B.: The technique of free skin grafting in mammals. J. Exper. Biol., **28:** 385–402, 1951.
3. SZABÓ, G.: A modification of the technique of "skin splitting" with trypsin. J. Path. & Bact., **70:** 545, 1955.
4. HAMILTON, J. B. AND HUBERT, G.: Photographic nature of tanning of the human skin as shown by studies of male hormone therapy. Science, **88:** 481, 1938.
5. HAMILTON, J. B.: Significance of sex hormones in tanning of the skin of women. Proc. Soc. Exper. Biol. & Med., **40:** 502–503, 1939.
6. NOBLE, G. K. AND WURM, M.: The effect of testosterone propionate on the Black-Crowned Night Heron. Endocrinol., **26:** 837–850, 1940.
7. PFEIFFER, C. A., HOOKER, C. W. AND KIRSCHBAUM, A.: Deposition of pigment in the sparrow's bill in response to direct applications as a specific and quantitative test for androgen. Endocrinol., **34:** 389–399, 1944.
8. EDWARDS, E. A., HAMILTON, J. B., DUNTLEY, S. Q. AND HUBERT, G.: Cutaneous vascular and pigmentary changes in castrate and eunuchoid men. Endocrinol., **28:** 119–128, 1941.
9. KUPPERMANN, H. S.: Hormone control of a dimorphic pigmentation area in the golden hamster. Anat. Rec., **88:** 442, 1944.
10. WELLS, L. J.: Pigmentation of the scrotum as a sensitive indicator for androgen. Anat. Rec., **91:** 305–306, 1945.
11. FLAKS, J.: The influence of testosterone propionate on pigmentation of the mammary nipples in female mice. J. Endocrinol., **5:** 259–262, 1948.
12. SULMAN, F. G.: Effect of ACTH on the frog chromatophores. Acta Endocrinol., **10:** 320–332, 1952.
13. WHEELER, C. E., CAWLEY, E. P. AND CURTIS, A. C.: The effects of topically applied hormones on growth, pigmentation and keratinisation of the nipple and areola of immature castrated male guinea-pigs. J. Invest. Dermat., **20:** 385–399, 1953.
14. SPOOR, H. J.: In discussion of paper by Edwards, E. A. 'Analysis of Skin Colour in Living Human Subjects by Spectrophotometric Means', in Pigment cell growth, p. 157, ed. Gordon M. N. Y., Acad. Press, Inc., 1953.
15. HAMILTON, J. B.: Influence of the endocrine status upon pigmentation in man and in mammals in the Biology of Melanomas, pp. 341–357, ed. Miner, R. W. ("Special publication of the New York Academy of Sciences", Vol. 4) New York: The Academy, 1948.
16. EDWARDS, E. A.: Analysis of skin colour in living human subjects by spectrophotometric means, in pigment cell growth, pp. 149–158, ed. Gordon M. N. Y., Acad. Press, Inc., 1953.
17. SNELL, R. S. AND BISCHITZ, P. G.: A study of the effect of orchidectomy on the melanocytes and melanin in the skin of the guinea-pig. Z. Zellforsch. In Press.
18. BISCHITZ, P. G. AND SNELL, R. S.: A study of the melanocytes and melanin in the skin of the male guinea-pig. J. Anat., Lond. **93:** 233–245.
19. CADE, S.: Malignant melanoma. Brit. Med. J., **1:** 119–124, 1957.
20. PACK, G. T.: 'A clinical study of pigmented nevi and melanomas', in the biology of Melanomas, pp. 52–70, ed. Miner, R. W. ("Special publication of the New York Academy of Sciences" Vol. 4) New York. The Academy, 1948.
21. SPITZ, S.: Melanomas of childhood. Amer. J. Path., **24:** 591–610, 1948.
22. RAVEN, R. W.: 'Problems concerning melanoma in man', in pigment cell growth, pp. 124–137, ed. Gordon, M. N. Y., Acad. Press, Inc., 1953.
23. BISCHITZ, P. G. AND SNELL, R. S.: Effect of testosterone and orchidectomy on the activity of the melanocytes in the skin. Nature, **181:** 187–188, 1958.