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The melanocytes and melanin in human abdominal wall skin: a survey made at different ages in both sexes and during pregnancy

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Numerous clinical observers have suggested that sex hormones can influence melanogenesis in human skin, but conclusive proof of this is lacking. Our interest in this problem was stimulated by the well-known phenomenon of increased pigmentation of the skin which occurs in certain areas of the body in pregnancy and by the suggestion frequently made by authors that the incidence and rate of growth of melanomas is related to the sex hormones. It is known, for example, that the growth of benign and malignant melanomas is accelerated at about the time of puberty in both sexes (Pack, 1948; Spitz, 1948; Raven, 1953) and that growth and spread of malignant melanomas takes place more rapidly during pregnancy (Pack & Scharnagel, 1951; Cade, 1957). The experiments reported in the literature attempting to substantiate a melanocyte–sex hormone relationship fall short of the ideal in that a great number of the observations have been macroscopic only and the microscopical 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. In a number of previous experiments on guinea-pigs using both skin sheets and vertical skin sections and a carefully controlled histochemical technique, we have been able to show that, in the female, ovariectomy produced marked inhibition of melanogenesis, while the administration of oestrogen and to a lesser extent progesterone produced the converse effect (Bischitz & Snell, 1960; Snell & Bischitz, 1960*b*). In the case of the male guinea-pig, orchidectomy resulted in increased melanogenesis, while the administration of testosterone produced changes which were not clear-cut; although certain inhibitory influences were observed particularly in the anterior abdominal wall skin (Snell & Bischitz, 1959; Bischitz & Snell, 1959*b*). The present investigation on human abdominal wall skin is a continuation of this work. It is hoped that a survey of the numbers of melanocytes present together with a study of their morphological characteristics and the appearances of the melanin, at different ages in the two sexes and during pregnancy, might provide information about the pattern of melanocyte activity in the human subject, at different periods of sexual activity.

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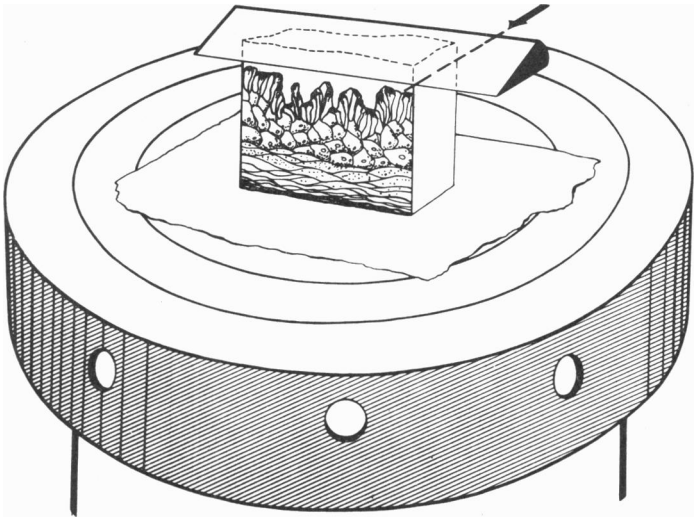
MATERIALS AND METHODS

The present investigation, which is essentially a comparative study of numerous skin samples, has been confined to skin removed from one region, since it has been shown that, in human skin, the population density of melanocytes and their morphological characteristics differ from one region of the body to another (Szabó, 1954; Staricco & Pinkus, 1957). Anterior abdominal wall skin has been chosen since it is a region from which it is possible to obtain a large number of samples. The skin has been taken from Caucasian patients undergoing treatment for conditions which do not directly involve the skin.

After each skin sample was removed, it was immediately wrapped in a muslin bag moistened with isotonic saline and placed over crushed ice in a thermos flask. The flask was then stored overnight in a refrigerator at 4° C. Pieces of skin measuring



Text-fig. 1. Diagram illustrating the method used to remove the greater part of the dermis from the epidermis. By this means, the skin sample was made sufficiently thin to enable the melanocytes to be viewed through the epidermis.

about 0·5 cm. in diameter were then treated as follows. Excess subcutaneous fat and the deeper part of the dermis were excised under a dissecting microscope. The skin was then fixed in 5% formol saline for 6 hr. 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 for 16 hr. The skin was then fixed for a further period of 24 hr. in 10% formol saline. Most of the remaining dermis was now removed by fixing the skin sample epidermis downwards on the head of a freezing microtome and shaving off the dermis with the microtome knife (Text-fig. 1).

This was followed by dehydration and clearing in chloroform. The greater part of each skin sheet was then mounted epidermal surface uppermost in Depex; 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 methylene blue, neutral red or haematoxylin and eosin.

The appearances of the melanocytes in the skin samples were studied by assessing the number and size of the melanocytes and the amount, colour 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 colour of the free melanin present, i.e. that melanin which was situated outside the melanocytes. The melanocytes were counted in ten areas chosen at random, each measuring 0·07 mm.^{2}. The counts were carried out using a graticule fitted in the eyepiece of a microscope and a magnification of $\times 353$ was used. In order that an accurate comparison could be made between the skin samples removed from different individuals, all the samples were processed histochemically under identical conditions.

For the purposes of the investigation, the skin samples were separated into groups according to the sex and age of the patient from whom they were removed. The age groups are as follows: (1) birth up to 12 years, (2) 20–59 years, and (3) 60 years and over. In the case of the female, the menstrual history of each patient was obtained and an additional group of specimens removed during pregnancy was included.

RESULTS

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

Male skin sheets

The skin samples showed well-formed epidermal ridges and dermal papillae (Pl. 1, fig. 1). These together formed a characteristic pattern which took the form of wide circular areas, produced by the dermal papillae and separated from one another by the narrow epidermal ridges (Pl. 1, fig. 2). It was interesting to note that in the older age groups the ridges and papillae were less well marked (Pl. 1, fig. 5). In all the skin samples, the melanocytes were found to be mainly concentrated on the summit and sides of the epidermal ridges.

The melanocyte counts are set out in Table 1, and they were statistically analysed as shown in Table 2. On comparing the mean log. cell counts of groups 1 and 2, the difference was found to be $0{\cdot}233 \pm 0{\cdot}041$, $P < 0{\cdot}001$. A similar comparison between groups 2 and 3 showed a difference of $0{\cdot}031 \pm 0{\cdot}065$, $P > 0{\cdot}6$. It was thus seen that the melanocyte counts for the skin of young individuals, i.e. group 1, were significantly higher than those of older individuals, i.e. groups 2 and 3, and that those of the skin of group 2 were not significantly higher than those in the oldest group.

The melanocytes (Pl. 1, figs. 1, 3, 4) were seen as ovoid cells which showed considerable variations in size within each section. The dendritic processes which extended out laterally were relatively short and of a simple type, few possessing secondary branches. Some of the dendrites possessed fusiform swellings along their

Table 1. *The melanocyte counts in male human abdominal wall skin at different ages*

No. of specimen	Age	Melanocyte count per mm.^{2}
	Melanocyte counts 0–12 years (group 1)	
10918	$\frac{3}{52}$	1732
10948	$\frac{1}{12}$	1217
10895	$\frac{1}{12}$	1425
9942	$\frac{3}{12}$	1507
11003	$\frac{8}{12}$	2200
10949	$\frac{9}{12}$	1669
10753	$\frac{11}{12}$	1397
10924	$1\frac{2}{12}$	1482
10906	$1\frac{5}{12}$	1794
10963	$1\frac{6}{12}$	1264
10938	$1\frac{6}{12}$	1685
10897	$1\frac{10}{12}$	877
10956	$1\frac{11}{12}$	1404
10910	$1\frac{11}{12}$	1950
10920	2	1154
10945	$2\frac{3}{12}$	1576
10892	$2\frac{7}{12}$	740
10866	$2\frac{10}{12}$	1260
15935	3	1088
10898	3	1700
15335	4	1243
452613	$4\frac{6}{12}$	1716
16162	5	1468
450117	6	905
15839	9	1793
10905	9	1794
513	10	1178
15287	12	1030
	Melanocyte counts 20–59 years (group 2)	
371647	28	767
00202	33	1123
4552	43	625
394095	44	1274
318377	44	905
6836	48	660
2163	49	898
384025	49	1092
558	56	650
62888	56	618
379818	57	644
	Melanocyte counts 60 years and over (group 3)	
11993	60	1000
6447	61	678
389437	63	945
9318	64	1173
373832	64	811
5087	68	615
5488	75	385

length and occasionally terminal swellings could be seen. The number of dendrites arising from the cell body varied from 2 to 5, 4 being the commonest number. The melanin both inside and outside the melanocytes varied from a dark brown to a golden colour. The free melanin was small in amount and was mainly concentrated in the vicinity of the melanocytes.

The general appearances of the melanocytes and melanin were similar in the different age groups, but certain quantitative differences were apparent (Pl. 1, figs. 1, 3, 5). Thus, in the older age groups, the melanocytes tended to be smaller and the dendrites were considerably shorter. Less melanin was present both within and outside the melanocytes.

Table 2. *The statistical analysis of melanocyte counts in male human abdominal wall skin for three age groups*

Age	Mean number of melanocytes per mm.^{2} (range)	Mean log. cell count and standard error	Corresponding geometric mean
Group 1. 0–12 years	740–2200	$3{\cdot}14 \pm 0{\cdot}021$	1393
Group 2. 20–59 years	618–1274	$2{\cdot}91 \pm 0{\cdot}035$	815
Group 3. 60 years and over	385–1173	$2{\cdot}88 \pm 0{\cdot}061$	759
Over-all	385–2200	$3{\cdot}05 \pm 0{\cdot}025$	1117

Male vertical skin sections

The epidermis of the anterior abdominal wall skin consisted of 4–8 layers of cells in addition to the stratum corneum. In the youngest age group, well formed wide dermal papillae separated from one another by narrower epidermal ridges were present. In the older age groups it was noted that the papillae and ridges were less well marked. The melanocytes were found to be concentrated in the epidermal ridges and only a few were situated over the dermal papillae (Pl. 1, fig. 6). They were seen to lie at the junction of the epidermis and dermis, between the basal parts of adjacent epidermal cells of the basal layer. An occasional melanocyte was found to lie at a slightly deeper level and appeared to be hanging down from the epidermis by its dendritic processes into the underlying dermis. The melanocytes seen in profile possessed ovoid or round bodies but the detailed morphology was seen to very much better advantage in the skin sheets. The dendrites extended out both laterally and upwards between the deeper cells of the epidermis. Moderate numbers of what appeared to be free melanin granules were seen in the ordinary epidermal cells of the basal layer but some of these were thought in fact to lie within the dendritic processes of the melanocytes. In the deeper cells of the stratum spinosum the free melanin granules were arranged around the periphery of the cytoplasm, but in the more superficial cells of this region only a few granules were seen. In the stratum granulosum, stratum lucidum and stratum corneum only an occasional melanin granule was present.

A comparison of the sections from the different age groups showed that the melanocytes gradually became smaller and that they contained less melanin as ageing progressed; the amount of free melanin also diminished and in the sections of skin removed from very old patients practically no free melanin could be seen.

Table 3. *The melanocyte counts in female human abdominal wall skin at different ages and during pregnancy*

No. of specimen	Age	Melanocyte count per mm.^{2}
	Melanocyte counts 0–12 years (group 1)	
10878	$\frac{4}{12}$	1370
336776	$1\frac{5}{12}$	2137
11017	$2\frac{6}{12}$	1264
11118	$3\frac{8}{12}$	1888
14900	$4\frac{9}{12}$	1294
44668	7	959
15704	7	1388
450061	9	1404
452775	9	1513
	Melanocyte counts 20–59 years (group 2)	
13253	20	758
866	31	815
8068	32	618
2653	36	1048
388330	36	764
3929	39	1010
422658	46	452
8532	48	1068
361069	49	493
416894	52	644
379571	53	811
8949	55	770
1184	57	655
	Melanocyte counts 60 years and over (group 3)	
4953	62	583
6734	67	475
322838	71	698
304840	72	603
5485	74	578
389485	82	499
59786	88	1050
	Melanocyte counts during pregnancy	
9052	26	1805
22977	27	983
1332	29	1014
200	29	1357
18156	31	980
300	37	874
2172	38	945
12366	39	705

Female skin sheets

The pattern of the epidermal ridges and the dermal papillae and the position of the melanocytes was similar to that seen in the male skin sheets. The melanocyte counts are set out in Table 3, and they were statistically analysed as shown in Table 4. A comparison of the mean log. cell counts of groups 1 and 2 showed a difference of $0{\cdot}288 \pm 0{\cdot}048$, $P < 0{\cdot}001$. A similar comparison between groups 2 and 3 showed a difference of $0{\cdot}075 \pm 0{\cdot}054$, $P > 0{\cdot}1$. It was thus seen, as in the male, that the melanocyte counts for the skin of young individuals, i.e. group 1, were significantly higher than those of older individuals, i.e. groups 2 and 3, and that those for the skin of group 2 were not significantly higher than those in the oldest group.

Table 4. *The statistical analysis of melanocyte counts in female human abdominal wall skin for three age groups*

Age	Mean number of melanocytes per mm.^{2} (range)	Mean log. cell count and standard error	Corresponding geometric mean
Group 1. 0–12 years	959–2137	$3{\cdot}16 \pm 0{\cdot}034$	1432
Group 2 20–59 years	452–1068	$2{\cdot}87 \pm 0{\cdot}032$	738
Group 3. 60 years and over	475–1050	$2{\cdot}79 \pm 0{\cdot}043$	621
Over-all	475–2137	$2{\cdot}94 \pm 0{\cdot}034$	869

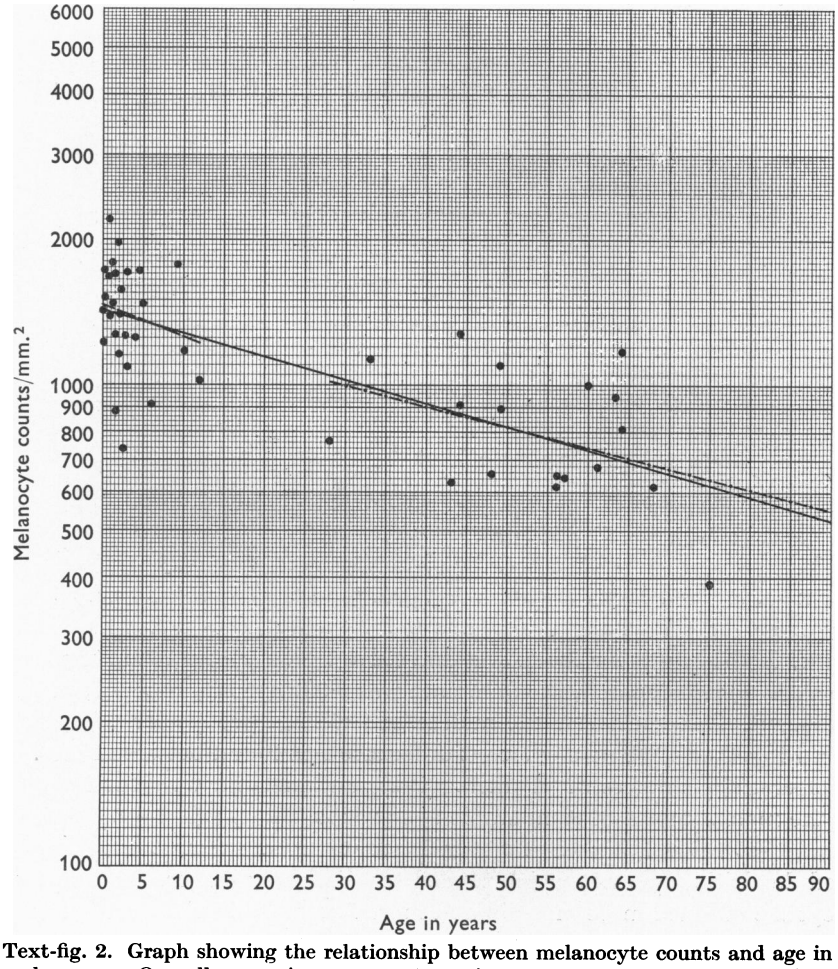
If the mean log. cell count of equivalent groups in the two sexes were compared, the difference for the young group was $0{\cdot}012 \pm 0{\cdot}042$, $P > 0{\cdot}7$, which was not significant. If the counts for the middle and old age groups were taken together and the two sexes were compared, the difference was $0{\cdot}057 \pm 0{\cdot}041$, $P > 0{\cdot}1$, which was not significant. The counts for the middle and old age groups for each sex were considered together since no significant difference was found between them.

The general appearances of the melanocytes and melanin were similar to those seen in the male samples of comparable age groups (Pl. 2, figs. 7–9). However, it was found that the cell bodies of the melanocytes in the female were larger and possessed dendritic processes which tended to be slightly longer. In addition, the melanocytes contained more melanin and more free melanin was present.

In the pregnant female, the mean number of melanocytes per mm.^{2} ranged between 705 and 1805, and the over-all mean log. cell count was $3{\cdot}02 \pm 0{\cdot}138$ standard error (geometric mean of 1042). A comparison of the general appearances of the melanocytes and melanin with those in skin specimens removed from non-pregnant women of a comparable age showed that during pregnancy the cell bodies of the melanocytes were very much larger and the dendritic processes more complex and much longer (Pl. 2, figs. 9–11). Some of the dendritic processes from adjacent melanocytes appeared to unite. There was a marked increase in the amount of melanin both within and outside the melanocytes.

Female vertical skin sections

These closely resembled the male specimens. In the skin removed from pregnant patients, the melanocytes were seen to be large and packed with melanin granules (Pl. 2, fig. 12). The dendritic processes were long and complex and insinuated themselves between the cells of the stratum spinosum.

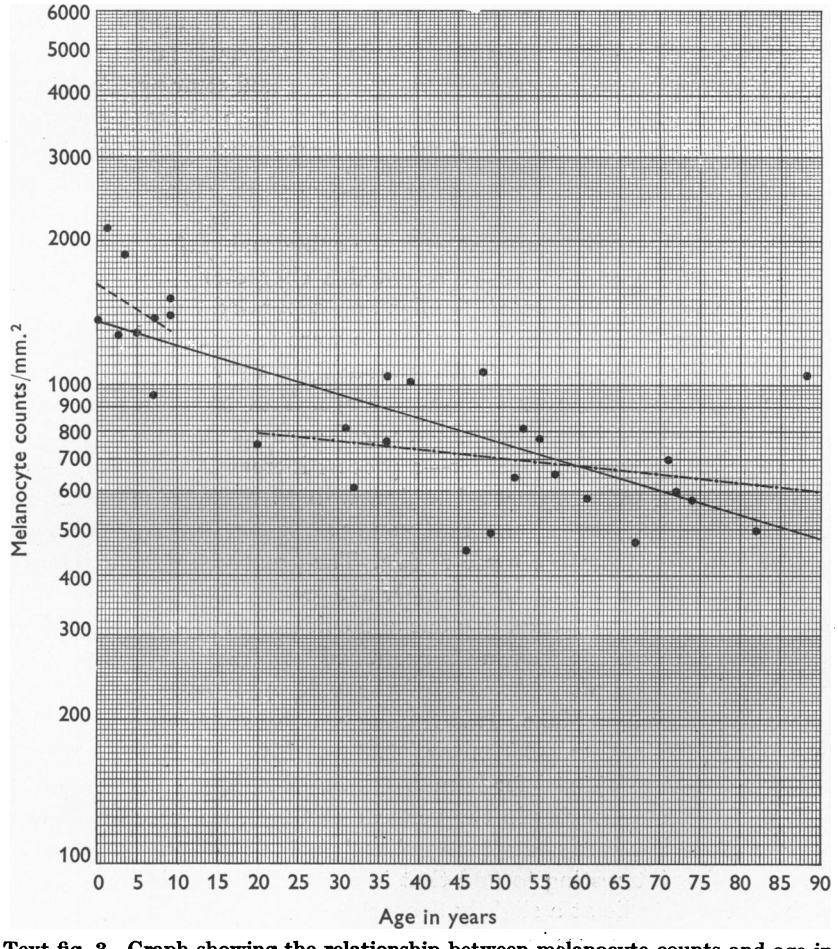


Text-fig. 2. Graph showing the relationship between melanocyte counts and age in the male. ——, Overall regression; – – –, regression on young group; –•–•– regression on middle and old groups.

When the melanocyte counts for both males and females were plotted against age, a linear relationship was found (Text-figs. 2 and 3). A regression line was fitted over all ages and the degree of association between melanocyte log. counts and age measured. This was found to be highly significant for both males and females (males r, 44 D.F. $= -0{\cdot}744$, $P < 0{\cdot}001$; females r, 27 D.F. $= -0{\cdot}746$, $P < 0{\cdot}001$). The slopes of the lines were not different and a test of the distance between the

lines showed that, when measured in relation to age, the counts of the males were not significantly different from the counts of the females, t with 73 D.F. = 1·14, $P > 0·3$. The slope of the regression line fitted for both sexes was −0·00493, which can be most easily interpreted as showing that the average melanocyte count decreased by 11% every 10 years.

Regression lines were then fitted to the data of the young group of both sexes, i.e. group 1. No correlation was found between the melanocyte counts and age for



Text-fig. 3. Graph showing the relationship between melanocyte counts and age in the female. ——, Over-all regression; – – –, regression on young group; –•–•– regression on middle and old groups.

males, r, 26 D.F. = −0·175, or for females, r, 7 D.F. = −0·352. The slopes of the lines were not significantly different, t, 33 D.F. = 0·349, $P > 0·7$, and the distance between the lines was not significant, t, 34 D.F. = 0·444, $P > 0·6$. Fitting similar regression lines to the middle and the old groups (groups 2 and 3), no correlation was found between the melanocyte counts and age. For the male, r, 16 D.F. = −0·402, and for the female, r, 18 D.F. = −0·254. The slopes for the two sexes were

compared and no significant difference was found, t, 34 D.F. = 0·947, $P > 0·3$, and the distance between the lines was not significant, t, 35 D.F. = 1·448, $P > 0·1$. This analysis therefore showed that within a specific age group it was legitimate to ignore the particular age of each individual.

DISCUSSION

In the present investigation, a detailed study of the melanocytes and melanin of human abdominal wall skin has been undertaken, using skin samples possessing both the epidermis and part of the dermis. The histochemical technique was standardized, i.e. identical quantities of the Dopa substrate were used and the same time was allowed for incubation, fixation, dehydration and clearing. The specimens were left freely suspended in the various reagents and no attempt was made to restrict shrinkage. Differences which might have been caused by unequal diffusion of the substrate into the specimens were minimised by processing small pieces of skin of approximately equal size.

When the skin sheets were examined with the epidermal surface uppermost, the epidermal ridges and dermal papillae presented a characteristic pattern of wide circular areas formed by the dermal papillae and separated from one another by the epidermal ridges. These ridges and papillae were seen to be most highly developed in the younger age groups in both sexes and were very much less marked in skin removed from patients over 60 years of age. This age difference could be partly accounted for by different degrees of shrinkage. As soon as skin is excised, the dermis starts to draw itself together—a process increased by fixation—so that folds already present are exaggerated and new ones may be created. Evans, Cowdry & Nielson (1943) have shown that young skins shrink on an average more than old ones removed from the same region. The greater shrinkage which took place in the dermis of the skin of the younger age groups may have resulted in an increase in the height of the dermal papillae and epidermal ridges in these samples.

The melanocytes in all the specimens examined were seen to be most heavily concentrated on the summit and sides of the epidermal ridges, and few were present over the dermal papillae. This finding was in agreement with those of Billingham (1948), Billingham & Medawar (1953), and Bischitz & Snell (1959*a*) for the guinea-pig, and Szabó (1954) for human skin.

The bodies of the melanocytes in the vertical skin sections were found to lie in the basal layer of the epidermis between the basal parts of the other epidermal cells of this layer. It was interesting to find that in some of the sections an occasional melanocyte appeared to lie at a slightly deeper level and was found to be hanging down by its dendritic processes into the underlying dermis. This latter observation is in agreement with that of Becker (1953) and Shukla, Karkun & Mukerji (1954), who found that some melanocytes were situated just deep to the basal layer of the epidermis.

The dendrites of the melanocytes were seen to extend out laterally in all directions parallel to the dermal surface or upwards between the other cells of the basal layer and the deeper cells of the stratum spinosum. It was noted that in the skin of pregnant individuals some of the dendrites of adjacent melanocytes joined together to form a syncytium. The club-like expansions found on the ends of the terminal

branches of the dendrites described by Masson (1948) and Billingham (1948), and their application to the walls of other basal cells was also observed in the present work; they were not seen, however, on all the dendritic processes.

The free melanin present in all the skin sheets was mainly concentrated in the vicinity of the bodies of the melanocytes and their dendritic processes. The melanin granules tended to be more discrete than those seen in the skin of the guinea-pig (Bischitz & Snell, 1959*a*, Snell & Bischitz, 1960*a*).

The melanocyte counts of the abdominal wall skin for each sex showed wide individual variations in each age group. The figures compared favourably with those reported by other workers. Staricco & Pinkus (1957), for example, reporting on abdominal wall skin removed from fifteen individuals (5 males and 10 females) of widely differing age groups, gave figures of 400–1270 melanocytes per mm.^{2}. In the case of the males, this varied from 400 to 1273 per mm.^{2}, and in the females from 550 to 990. Szabó (1957) found that in five abdominal skin specimens, obtained from adult males whose ages ranged between 30 and 52 years, the average number of melanocytes was 800 per mm.^{2}—counts varied between 680 and 900 per mm.^{2}.

A careful comparison of the melanocytes and melanin in the skin of the two sexes revealed that their general appearances were similar; however, certain quantitative differences were found. In the female, the melanocytes possessed larger cell bodies and longer dendritic processes and more melanin was present both inside and outside the melanocytes. Similar differences were found to exist between the melanocytes of male and female guinea-pigs (Bischitz & Snell, 1959*a*, Snell & Bischitz, 1960*a*). Since the comparisons have been made on skin sections processed under identical conditions, it would seem justifiable to conclude from the differences in the size of the melanocytes and the amount of melanin present that in females the melanocytes possess a greater melanogenic activity than in the males.

A comparison of the melanocyte counts of comparable age groups in the two sexes showed that there is no significant sex difference. This finding is in agreement with that reported by Staricco & Pinkus (1957) and Szabó (1957). In the guinea-pig experiments reported previously (Snell & Bischitz, 1960*a*), the melanocyte counts in the immature and mature animals of both sexes were, in most cases, similar but the counts for the areola and anterior abdominal wall were significantly higher in the mature female.

When the results for each sex were considered separately, it was seen that the morphology of the melanocytes changed with age. The size of the cell bodies of the melanocytes, the length of the dendritic processes, the amount of melanin both inside and outside the melanocytes gradually became reduced as the age increased. These facts taken together would indicate that melanogenesis gradually becomes reduced with increasing age. It is thus seen that, in the human subject in both sexes, the activity of the melanocytes is greatest before puberty, a time when it is known that the level of the sex hormones is low. Similar changes were found in the male guinea-pig when skin of sexually immature animals was compared with that of mature animals (Bischitz & Snell, 1959*a*). In the female guinea-pig, however, the main change noted was an increase in the amount of free melanin in the mature animals as compared with the immature animals (Snell & Bischitz, 1960*a*).

Small bipolar or stellate pigment cells were observed in foetal negro skin by Zimmermann & Cornbleet (1948) and by Hu, Staricco, Pinkus & Fosnaugh (1957) in the outgrowth of normal skin explants. The latter authors concluded that these small cells represent relatively young or embryonic forms of pigment cells. They further suggested that as these cells mature they slowly increase in size, exhibit a more intense dopa reaction, and their processes elongate and arborise. The results of the present work would suggest that the pigment cells having thus matured exhibit their maximum size and most marked melanogenic activity before puberty. The melanocytes then slowly decrease in size and their melanogenic activity declines as ageing progresses, unless they are secondarily stimulated as occurs during pregnancy.

A comparison of the melanocyte counts in different age groups showed that in both sexes the numbers were statistically higher in younger than in older individuals. Szabó (1957) also noticed that there was an increased number of melanocytes in the skin of younger individuals, but he found that this was not statistically significant. This reduction in cell numbers as the age increased may have been an actual reduction or only an apparent one for, as already explained, skin removed from young and from old individuals differs in its degree of shrinkage while fixing. However, the finding in this work that melanogenesis became diminished as the age increased may be the explanation, for it is possible that some melanocytes lost their ability to form melanin completely and were, therefore, not counted.

In the skin samples removed from individuals during pregnancy, it was found that the melanocytes had larger cell bodies with very much longer and more complex dendritic processes than seen in skin removed from non-pregnant females of the same age group. The melanocytes also appeared to be darker in colour and more melanin was present both inside and outside the cells. The melanocyte counts varied between 705 and 1805 per mm.^{2}, and were found to be significantly higher than those of the non-pregnant females of group 2 (t, 19 D.F. = 2·82, $P < 0·02$). This was surprising, since most of the samples were taken from individuals in which the anterior abdominal wall skin was stretched by the gravid uterus. In a previous report on the pregnant guinea-pig (Snell & Bischitz, 1960*a*) similar changes in the melanocyte morphology were noted, with the exception that the size of the melanocytes remained unaltered. The melanocyte counts in the pregnant guinea-pig were found to be significantly higher in all the regions examined than before the onset of pregnancy.

The finding that the deeply pigmented and easily seen melanocytes in the pregnant human subject were present in greater numbers than in the non-pregnant group was probably due to the fact that amelanotic melanocytes in the non-pregnant person had been stimulated into activity and had been identified and counted in the pregnant individual. If this was true, then it would add weight to the idea that the reduction in melanocyte numbers which occurred with advancing age was only an apparent reduction and was due to the failure to recognize poorly pigmented melanocytes in the aged.

The stimulating factors responsible for the increased melanogenic activity of the melanocytes during pregnancy are not fully understood. The nipples and areolae of man and many other species become hyperpigmented during pregnancy. Nishizaki

(1929) studied skin pigmentation in pregnant women using a tintometer to grade colour changes. The results showed that pigmentation increased over the entire skin as well as in certain characteristic areas where it was especially noticeable. Increased pigmentation usually starts about the face where it frequently appears as brownish areas rather than as diffuse pigmentation (Jeghers, 1944). Other areas which have been described as showing pigmentation are the region of the linea alba; it often extends to the thigh and to the region of the anus. In addition, old scars darken and pigmented moles often appear (Hill, 1954).

Block & Guldberg (1933) concluded that excess follicular hormone was responsible for the hyper-pigmentation in pregnancy. Guldberg (1934) suggested that the female sex hormones acted directly on the melanocytes, whose pigment-forming ability was increased and activated. Previous work on the guinea-pig showed that both oestrogen and, to a lesser extent, progesterone were capable of stimulating melanogenesis in both sexual and non-sexual skin regions (Bischitz & Snell, 1960; Snell & Bischitz, 1960*b*) and that during pregnancy similar changes were noted (Snell & Bischitz, 1960*a*). Johnsson & Högberg (1953) found that there was an increased excretion of the melanocyte stimulating hormone during pregnancy and they attributed the increased pigmentation to the raised level of this hormone. Snell (1962) showed in the guinea-pig that β-M.S.H. from the pituitary caused an increase in the length, width and complexity of the dendritic processes of the melanocytes, and this was accompanied by an increase in the amount of melanin present within the processes. In the anterior abdominal wall skin, the melanocyte counts were significantly raised and the amount of free melanin was increased. It is probable, however, that, in pregnancy, both oestrogen and progesterone, which are known to be circulating in increased amounts, are mainly responsible for the hyperpigmentation.

SUMMARY

1. The melanocytes and melanin have been studied in Caucasian human anterior abdominal wall skin of both sexes at different ages and during pregnancy. Both skin sheets and vertical sections have been used. The melanocytes were identified by using the Dopa reaction. The melanocytes and melanin in the hair follicles have not been examined in this investigation.

2. The melanocytes were found to be concentrated in the epidermal ridges and to lie in the basal layer of the epidermis between the basal parts of the other epidermal cells. The dendritic processes extended out laterally and upwards between the deeper cells of the epidermis.

3. The melanocyte counts showed that there were wide individual variations in each age group. No sex difference in the counts was found. In both sexes the counts showed a significant reduction as age increased.

4. The general appearances of the melanocytes and melanin were similar in the skin of the two sexes, but in the female the melanocytes possessed larger cell bodies and longer dendritic processes, and more melanin was present both inside and outside the melanocytes.

5. In both sexes, the size of the cell bodies of the melanocytes, the length of the dendritic processes, and the amount of melanin both inside and outside the melanocytes showed a tendency to become reduced as age increased.

6. During pregnancy the melanocytes were found to have larger cell bodies, with longer and more complex dendritic processes, and more melanin was present both inside and outside the melanocytes. The melanocyte counts were found to be significantly higher than those of non-pregnant individuals of the same age group.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Photomicrograph of skin sheet of anterior abdominal wall of male aged 3 years. Shows the melanocytes and melanin to be concentrated in the epidermal ridges. Note that the melanocytes vary in shape and size and that the majority contain a large amount of melanin. Treated with the Dopa reagent. No counterstain, ×403.

Fig. 2. Low-power photomicrograph of skin sheet of anterior abdominal wall of male aged 2 years and 10 months. Shows the characteristic pattern formed by the epidermal ridges and dermal papillae. The melanocytes are seen to be concentrated mainly in the epidermal ridges. Treated with the Dopa reagent. No counterstain. ×84.

Fig. 3. Photomicrograph of skin sheet of anterior abdominal wall of male aged 48 years. Shows the melanocytes and melanin concentrated in the epidermal ridges. Note that the melanocytes tend to be smaller and contain less melanin than in the younger age group (compare with fig. 1). Treated with the Dopa reagent. No counterstain, ×403.

Fig. 4. Photomicrograph of skin sheet of anterior abdominal wall of male aged 2 years. Shows a group of melanocytes in an epidermal ridge. The melanocytes contain a moderate amount of melanin. Treated with the Dopa reagent. No counterstain, ×637.

Fig. 5. Photomicrograph of skin sheet of anterior abdominal wall of male aged 68 years. Shows that the epidermal ridges and dermal papillae are not well developed and that the melanocytes contain less melanin and tend to be smaller than those seen in the younger age groups (compare with figs. 1 and 3). Treated with the Dopa reagent. No counterstain, ×403.

Fig. 6. Photomicrograph of vertical section of anterior abdominal wall skin of male aged 10 years. Shows three melanocytes lying within the basal layer of the epidermis. The cell bodies of the melanocytes contain a moderate amount of melanin but the dendritic processes appear to be short, and contain very little melanin. Only a small amount of free melanin is present in the basal cells of the epidermis. Treated with the Dopa reagent. No counterstain, ×662.

PLATE 2

Fig. 7. Photomicrograph of skin sheet of anterior abdominal wall of female aged 1 year and 5 months. Shows the melanocytes and melanin in the epidermal ridges. Note that the majority of the melanocytes appear to be very active and contain a large amount of melanin. Treated with the Dopa reagent. No counterstain, ×486.

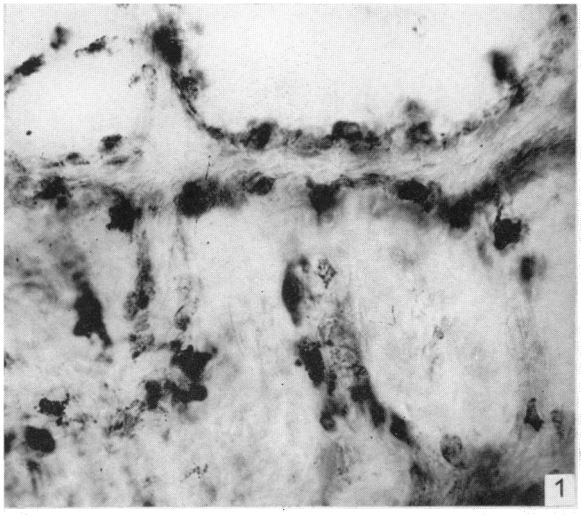
Fig. 8. Photomicrograph of skin sheet of anterior abdominal wall of female aged 7 years. Shows a group of melanocytes in an epidermal ridge. The melanocytes contain a large amount of melanin and the dendritic processes quickly pass out of focus as they leave the cell bodies of the melanocytes. Treated with the Dopa reagent. No counterstain. ×680.

Fig. 9. Photomicrograph of skin sheet of anterior abdominal wall of female aged 49 years. Shows a number of melanocytes containing a moderate amount of melanin situated in an epidermal ridge. Treated with the Dopa reagent. No counterstain. × 525.

Fig. 10. Photomicrograph of skin sheet of anterior abdominal wall of pregnant female aged 39 years. Shows the presence of many melanocytes with large cell bodies packed full with melanin. Compare with fig. 9. Treated with the Dopa reagent. No counterstain, × 525.

Fig. 11. Photomicrograph of skin sheet of anterior abdominal wall of pregnant female aged 26 years. Shows many melanocytes distended with melanin and possessing long, branching dendritic processes. Treated with the Dopa reagent. No counterstain, × 465.

Fig. 12. Photomicrograph of vertical section of anterior abdominal wall skin of pregnant female aged 26 years. Shows a number of very active melanocytes situated in the basal layer of the epidermis. Note the long, branching dendritic processes extending up between the cells of the stratum spinosum. Treated with the Dopa reagent. No counterstain, × 898.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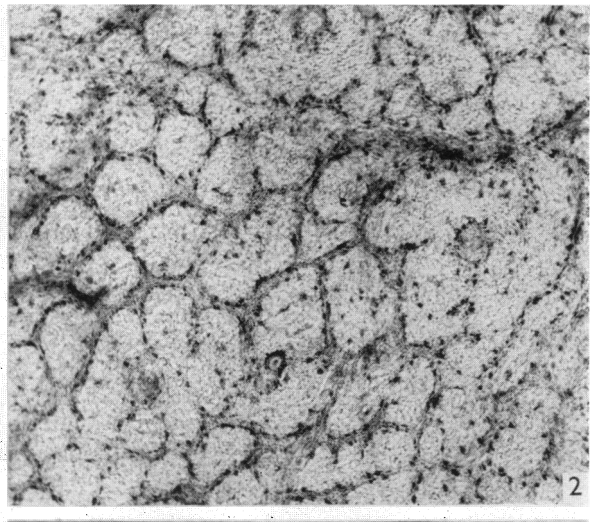
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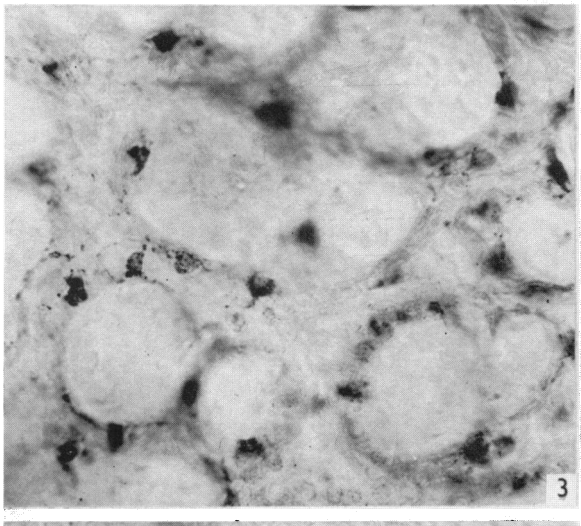
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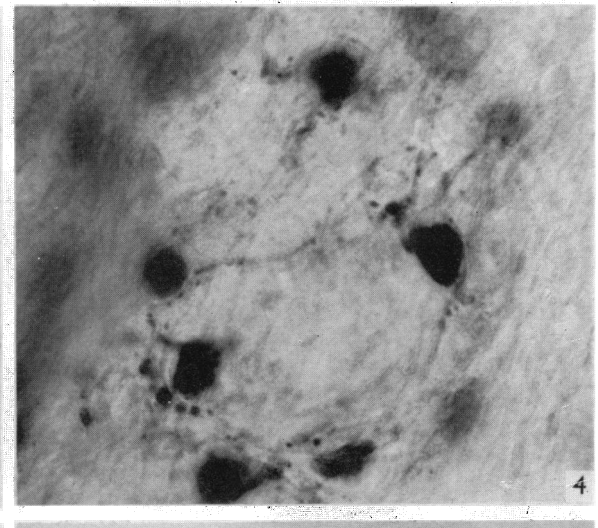
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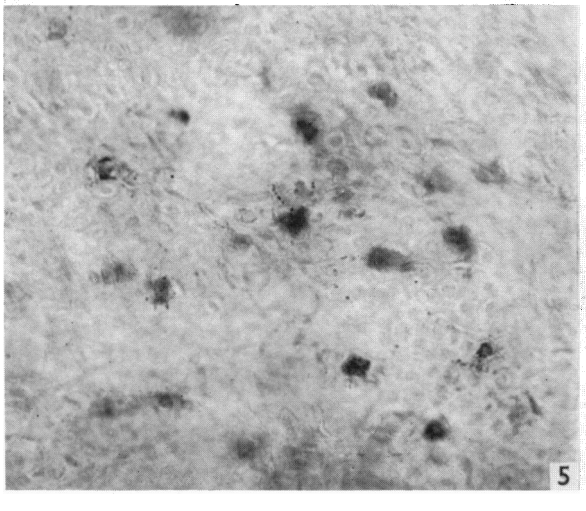
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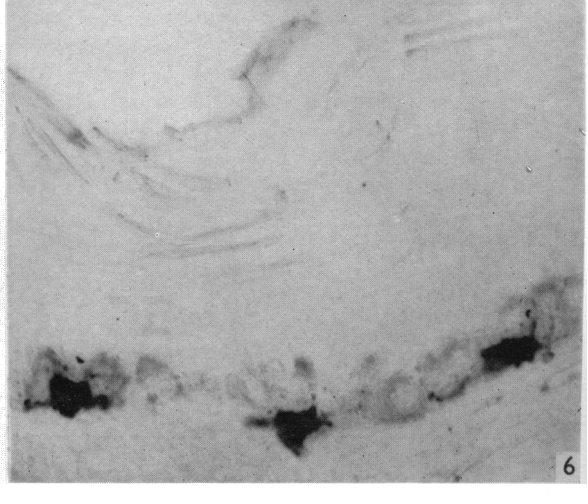
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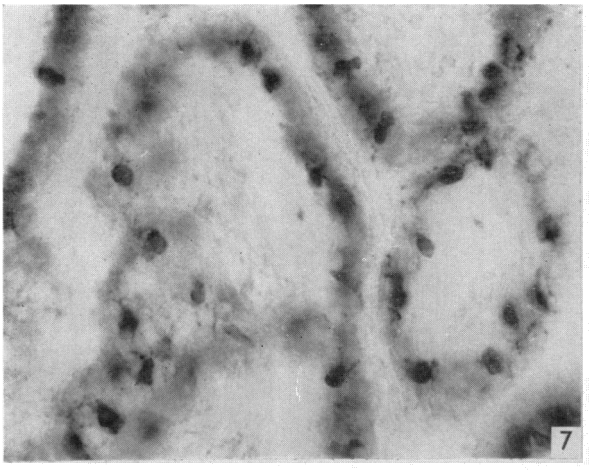
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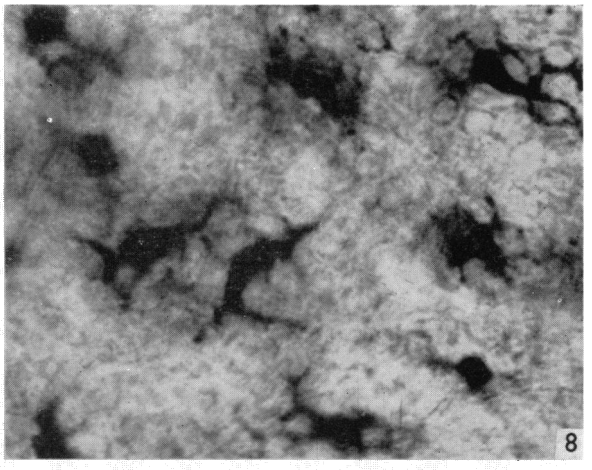
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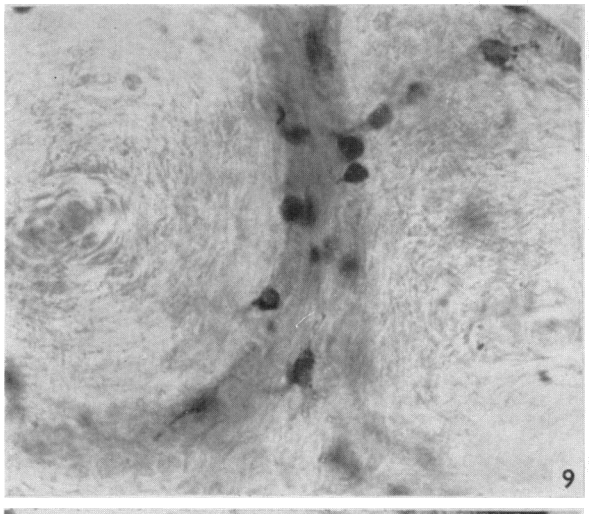
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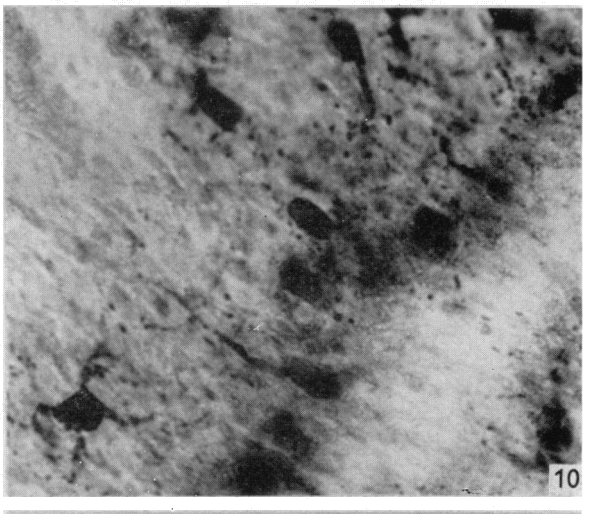
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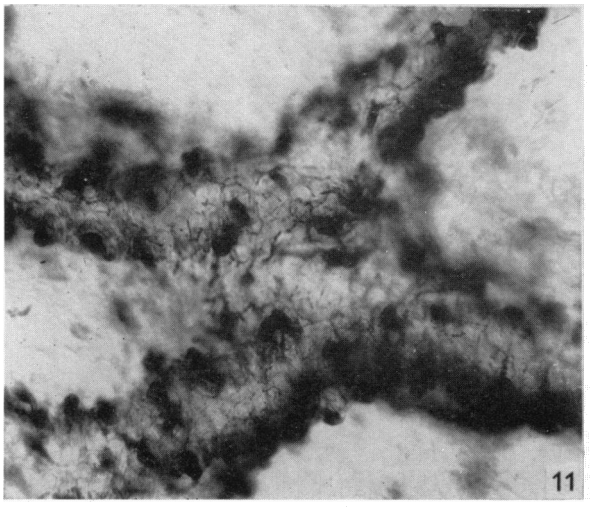
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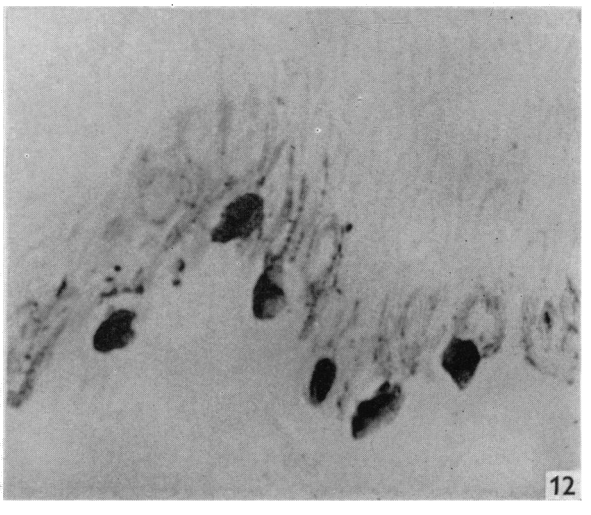
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