Discussion

Our experiments indicate that heparin fails to prevent the occurrence of thrombosis in veins damaged by chemical irritation. Even when achieving almost continuous prolongation of clotting-time, with heparin given intramuscularly every four hours, no significant inhibition of thrombosis was obtained. This failure might be explained in part by the fact that we used discontinuous administration, either intravenously or intramuscularly, as suggested by Swedish workers (Crafoord, 1939; Jorpes, 1950), and in no rabbit could we be sure that the clotting-time was always above normal. Our results would seem to support those obtained in the original experiments with heparin, in which good effects were achieved only when the drug was given by continuous transfusion (Murray *et al.*, 1937).

On the other hand, ethyl biscoumacetate was found to be highly successful in preventing thrombosis. Similarly successful results were obtained with ethyl biscoumacetate using the arteries of dogs (Reinis, 1950), and also with dicoumarol (Dale and Jaques, 1942; Richards and Cortell, 1942; Thill *et al.*, 1943). The doses of ethyl biscoumacetate used by us were only slightly (two to three times) greater than those used in man, and they produced a comparable prolongation of prothrombin time, which differs from the findings of Wright *et al.* (1953), who found it necessary to give much larger doses of ethyl biscoumacetate (more than 20 times the human dose) to achieve comparable effects.

While experimental results in rabbits cannot be applied directly to man, we feel that the unexpected differences found between the two drugs makes it desirable that their comparative value in the prevention of thrombosis in man should be critically assessed.

Summary

A comparison was made between the actions of heparin and ethyl biscoumacetate in preventing thrombosis in chemically induced thrombophlebitis in rabbits. Ethyl biscoumacetate was strikingly successful when given in doses comparable to those used in man, whereas under the same conditions heparin did not significantly reduce the incidence of thrombosis.

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An Ehrlich-von Behring Centenary postage stamp was issued in March by the West German Government. It is a 10-pfennig stamp with the heads of Ehrlich and von Behring facing right on a mid-green background, the names of the two scientists and the dates "14 und 15 Maerz 1854." The stamp has now been included in the Ehrlich exhibition arranged by the Wellcome Historical Medical Museum at 183, Euston Road, London, N.W.1.

THE NUMBER OF MELANOCYTES IN HUMAN EPIDERMIS

BY

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[WITH SPECIAL PLATE]

Melanomas of skin (cf. Raven, 1950; Pack *et al.*, 1953; Clark and MacDonald, 1953; Wright *et al.*, 1953) have a characteristic regional frequency distribution. Melanomas are more frequent per unit area on the face, neck, feet, and external genitalia than on other regions of the body. It is now almost universally believed that, in cellular origin, melanomas of skin arise from pigmentary dendritic cells, "melanocytes."

There are alternative (but not mutually) exclusive reasons why the frequency of melanomas should vary from one part of the body to another: (a) the individual melanocytes have different degrees of susceptibility to malignant change, or are more exposed in certain areas to stimuli predisposing them towards this transformation; or (b) the likelihood of a melanocyte's undergoing malignant transformation is fairly constant throughout the body, and the observed differences of frequency are mainly due to differences of population density. Wright *et al.* (1953) have already suggested that there may be a greater concentration of "melanoblasts or precursors" in the skin of lower limbs, head, and neck than of upper limbs. Their opinion was based on observations of the duration of sun-tanning and not on statistical cellular counts.

The present investigation therefore represents a first attempt to estimate the frequency distribution of melanocytes in different regions of the human body. It was founded upon the use of Bloch's "dopa reaction" in conjunction with the "skin-splitting" technique of Billingham and Medawar (1951), which causes the epidermis to separate cleanly from the corium by enzymatic fission. By using these methods, counts of melanocytes were carried out by the methods described in full by Billingham and Medawar (1953). These authors have already shown that there are about twice as many melanocytes per mm.² in guinea-pig's ear skin than in trunk skin, but both showed great variation between individuals. There was no correlation between melanocyte concentration and differences of colour.

This report contains only a first series of estimations, relating chiefly to the melanocytes of the skin of the thigh and arms.

Material and Methods

Human skin was obtained in the course of plastic surgical operations through the kindness of Mr. Rainsford Mowlem, at the Middlesex Hospital. The skin samples, removed as Thiersch (thin "split thickness") grafts, were incubated in buffered commercial trypsin solution to bring about the disengagement of the epidermis. The epidermis was thereupon stuck to a cover-glass, inner (dermal) side uppermost, with paraffin and rubber stopcock lubricant, fixed in 1-2% formol-saline overnight or sometimes longer (two to four days), and then incubated at 38° C., until the melanocytes turned dark, in a 1:1,000 solution of 3:4-dihydroxyphenyl-alanine adjusted by phosphate buffer to pH 7.4. After a second fixation in 4% formol-saline, followed by dehydration and cleaning, the pure epidermal sheets were permanently mounted in Canada balsam for inspection from the inner

side (Plate, Figs. 1-4). On a number of occasions very thin Thiersch grafts were impregnated with the dopa reagent without splitting: in these specimens the dermis was thin enough to be transparent. Paracarmine was sometimes used as a counterstain, to make the epidermal pattern more easily visible.

These methods are unsuitable for counting melanocytes in hair follicles.

Observations

The numerical results are set out in the accompanying Table, with information on the sex and age of the subject, the position and extent of the area counted (usually in 10

Population Densities of Melanocytes in Various Regions of the Body

Specimen No.	Sex	Age	Area Counted mm. ²	Population Density mm. ²	Notes
Thigh					
11	M	21	0.3424	790 ± 40	Fig. 2
12	F	44	0.3424	900 ± 50	
30	F	19	0.3424	1,230 ± 30	
33	F	56	0.3424	1,400 ± 64	
36	M	37	0.3424	1,850 ± 90	No epidermal pattern
44	F	73	0.3424	580 ± 44	
53	M	75	0.3424	670 ± 40	
63	F	44	0.3424	500 ± 30	
65	M	60	0.3424	900 ± 50	
68	F	53	0.3424	560 ± 40	
Overall mean: 940 ± 140 melanocytes/mm. ²					
Upper Arm					
15	F	44	0.1150	1,540 ± 130	
27	F	61	0.1712	950 ± 10	
28	M	51	0.3424	820 ± 20	
38	M	45	0.3424	1,490 ± 130	
Overall mean: 1,200 ± 180					
Forearm					
24	M	17	0.3424	820 ± 50	
45	M	15	0.1150	1,070 ± 70	
62	M	42	0.0804	1,210 ± 60	
Head and Neck					
13	M	44	0.1150	4,500 ± 100	Cheek
29	M	25	0.3424	2,050 ± 70	Cheek, normal and scar, Fig. 3
45	M	15	0.1150	3,500 ± 170	Cheek, Fig. 4
34	F	39	0.1712	2,300 ± 100	Forehead
53	M	75	0.3424	1,100 ± 100	
16	M	9	0.3424	1,280 ± 150	Behind ear, Fig. 1
55	F	44	0.1140	2,600 ± 200	Neck, freckled
Different Areas of Same Individual					
45	M	15	0.1150	1,070 ± 70 3,500 ± 170	Forearm Cheek, Fig. 4
53	M	75	0.3424	670 ± 40 1,100 ± 100 940 ± 60	Thigh Forehead Over nasal bridge
55	F	44	0.1150	2,600 ± 200	Neck, freckled
56	"	"	0.3424	1,920 ± 90	Axilla
123	M	73	0.1150	1,640 ± 80	Dorsum of hand
124	"	"	0.1150	800 ± 60	Palm

randomly chosen samples), and the mean number of melanocytes per mm.² of the epidermis in plane projection ± the standard error of the mean. The following preliminary conclusions can be drawn from the numerical and histological analyses.

(1) There are great differences between the numbers of melanocytes in corresponding areas of different human beings.

(2) There are also well-defined differences between the numbers of melanocytes in different areas of the same individual. The higher population densities were found on the ear (Fig. 1), cheek (Figs. 3 and 4), forehead and neck areas; the lower densities on the upper arm, forearm, and thigh (Fig. 2).

(3) Within any one sample of skin, melanocytes are principally concentrated in the epidermal ridges or "hills"—that is, in those areas which appear in orthodox transverse sections as inwardly directed projections of the epidermis

into the corium. Between the ridges—that is, in the areas underlain by the dermal papillae—their concentration is lower.

(4) Melanocytes vary greatly in shape. They may be fully dendritic cells, with branches projecting in many directions, or they may be almost fibroblast-like, with only two terminal processes. Although the shapes of melanocytes may be largely influenced by mechanical factors, such as the pattern and mode of packing of neighbouring Malpighian cells, there appears also to be a clear-cut dimorphism, particularly in the skin of the cheek and forehead (Fig. 4): large melanocytes with abundant cytoplasm lie among the more numerous smaller melanocytes with relatively little cytoplasm.

Comment

The results of this numerical investigation are in favour of the second of the alternative explanations proposed above to account for differences in the frequencies of melanomas between the face and neck on the one hand, and the trunk and thigh on the other—that is, the differences of frequency may be due to differences in the population densities of melanocytes. As no melanocyte counts are yet available from the foot, it is impossible to say whether a similar interpretation may not account for the high frequency of melanomas in this region; it is relevant, however, that melanocytes are very much more abundant in the sole of the guinea-pig's foot than in the skin of the ears and trunk (Billingham and Medawar, 1948, 1950).

The population densities of melanocytes in different regions of the body are, of course, correlated with differences in the thickness and mitotic activity of the epidermis as a whole. Both may be at least partly under the influence of environmental factors—for example, differences in the degree of exposure to mechanical irritation and radiation—but cell counts on infants would be necessary to determine what proportion of the difference was due merely to environmental factors. It is already known that some differences of epidermal thickness—for example, between trunk and sole-of-foot skin—are of developmental origin.

A dimorphism of melanocytes was also found by Grand *et al.* (1935) among the melanocytes of melanomas of mice and of human origin, cultured *in vitro*.

Summary

In human epidermis the population density of melanocytes (pigmentary dendritic cells) is of the order of 1,000 per mm.² in the skin of the thigh and arms, but may be twice to four times as great in the skin of the face and forehead. There is nevertheless a wide range of variation between the corresponding areas on different individuals. It is suggested that regional differences in the frequency of occurrence of melanomas may be primarily due to differences in the population density of melanocytes.

I am grateful to Mr. Rainsford Mowlem, whose co-operation, interest, and help have made the collection of specimens possible; to Professor P. B. Medawar for suggesting this line of investigation and for correcting the manuscript; and to Mr. R. E. Billingham for his many helpful suggestions. Thanks are also due to Mr. W. Brackenbury for taking the photomicrographs.

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K. L. MARKS: CONGENITAL SYPHILIS DIAGNOSED BY BONE CHANGES - 6 -

FIG. 1.—Showing marked decalcification of the submetaphyseal zone in the radius and ulna.

FIG. 2.—Showing thickening of the temporary zone of calcification in the femur.

FIG. 3.—Showing irregular decalcification at the proximal end of the tibia.

G. SZABÓ: NUMBER OF MELANOCYTES IN HUMAN EPIDERMIS - 5 -

FIG. 1.—Epidermis of skin from behind the ear (Sp. 16): dopa preparation. Note the abundance of melanocytes and the conformation of the epidermis. ($\times 95$.)

FIG. 2.—Epidermis from skin of thigh (Sp. 12): dopa preparation. Contrast with Figs. 1, 3, and 4: melanocytes are relatively sparse and the epidermal pattern much less boldly defined. ($\times 95$.)

FIG. 3.—Epidermis from the cheek (Sp. 29): dopa preparation. Note the abundance of melanocytes. ($\times 95$.)

FIG. 4.—Epidermis from skin of cheek (Sp. 45): dopa preparation counterstained with paracarmin, showing dimorphism of melanocytes into large, multiple-branched, and small forms. ($\times 390$.)