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Sir Archibald Garrod's "Inborn Errors of Metabolism"

III. Albinism

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"The suggestion, which we would make for the consideration of the physiologist, is that the ultimate difference between the normally pigmented individual and the albino will be found after all to be one of *structure*. It is easier to grasp the influence of a difference of gametic constitution on structure than on chemical process." *A Monograph on Albinism in Man*, Pearson, Nettleship and Usher, 1911.

INTRODUCTION

THE INCLUSION OF ALBINISM in the list of inborn errors of metabolism in 1908 must be ascribed to Garrod's intuitive genius. Certainly the evidence available to him at that time was not convincing. Nor did his concept appeal to Pearson, Nettleship and Usher (1911) who wrote the above quotation three years later in the midst of the most complete survey of albinism ever attempted. Until then the appropriate data had not yet been collected to show whether albinism was an acquired disease, an arrested development or an hereditary defect of some kind. The condition was known, of course, from ancient times, and its ready diagnosis and sharp segregation should have marked it for early study. Pearson did choose it as a test case of the applicability of Mendelianism to man. His method was a monumental compilation of all known references, pedigrees and pictures of albinism of all grades in man and animals. All work on albinism must now start with this monograph, even though the study included an enormous variety of pigmentary defects, both hereditary and acquired. It was left for the later investigators to define universal complete albinism as a separate entity among the many disturbances of pigmentation, and to prove its transmission by a single autosomal recessive gene. Only now are the mysteries of pigment cell metabolism with its additional variations through physiological control beginning to be laid bare. Contrary to the popular impression, our understanding of albinism as an inborn error of metabolism is still incomplete. Yet the hope perseveres of confirming Garrod's remarkable insight that albinism is an inherited lack of an enzymic step in pigment production.

I. CLINICAL DESCRIPTION AND DEFINITION

The striking appearance of the complete albino is familiar to almost everyone. We owe most of the clinical observations on albinism to dermatologists and opthalmolo-

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gists, who deal for the most part, with the same readily apparent external abnormalities that the layman sees. From such a viewpoint the many varieties of deficient pigmentation tend to be classed with complete albinism, even though the relationship is only assumed and superficial. This has led to oversimplification of the problem of albinism as one concerned with the simple presence or absence of pigment. With the assumption of some relation between the various types of pigment deficiency, whether in man or animals and in hair, skin or feathers, goes the single greatest barrier to the understanding of albinism. For this reason, consideration here will be limited to the simplest, most pronounced, and most unmistakeable type, complete albinism. Information derived from studies on other kinds of abnormal pigmentation will be adduced explicitly so far as possible, and only for what insight they may give into complete albinism. Some of these other varieties will be described before attempting a definition of complete albinism.

Varieties of Pigmentary Deficiency: People of all races are seen with deficient pigmentation present at birth or developing later in life. The late developing forms range from early graying of hair to localized areas of depigmented skin and hair that follow injury and inflammation (vitiligo) or that occur spontaneously over wide areas (leucoderma). While it cannot be said that heredity plays no role in the production of these deficiencies, interest is greater in those forms present from birth (or from the earliest age when they can be noted, since pigment accumulates with age). Such congenital forms include well-demarcated patches centered more or less symmetrically somewhere along the median line of head or abdomen (white locks and "spotlings"), and the quite separate piebald markings which do not have this center of symmetry. The inheritance of these is usually dominant (Gates, 1946), and they may be remarkably constant in location, if not in size throughout a pedigree. If the areas affected are sufficiently large, the condition is frequently classed as "partial albinism", which is an unfortunate term that causes confusion. It must, for example, be distinguished from "incomplete albinism," in which some small residues of pigment can be found in the affected areas of an albino.

Ocular albinism is a precisely defined anomaly which has been seen in a limited number of families, and is not associated with complete albinism even though the eye is affected in the same manner in both conditions. Ocular albinism is sex-linked. The affected males show the usual findings of the albinotic eye, with fair or normal coloration elsewhere. The heterozygous females can be distinguished regularly by a granular clumping of pigment in the periphery of the fundus (Falls, 1951, 1953; Francois & Deweer, 1952; Ohrt, 1957).

Incomplete Albinism: Less common than complete albinism are individuals who show traces of pigment. They have cream-colored or yellow hair and yellow or blue irides. Such individuals may occur more commonly among the heavily pigmented races, and have been called "xanthous" or "rufous" as well as incomplete albinos. They are unmistakably albinos, nevertheless, and they are probably only an arbitrary class of the "complete" albino (Pfandler, 1950). Examples were described by Franceschetti (1930) and Waardenburg (1932). As Pearson *et al* (1911–13) first observed, proof is never available that the universal complete albino is everywhere lacking in all pigment. The existence of this slightly pigmented group warns us not

to expect any absolute defect in the albino. Especially is this so when coupled with evidence that many albinos have some pigment at autopsy and even accumulate pigment during life. The amount may not be noticeable, but sufficient accumulation to ameliorate the visual difficulties is often expected by the practicing opthalmologist.

Universal Complete Albinism: Geoffrey Saint Hilaire in 1832 described "perfect albinism" (quoted by Pearson *et al* (1911)). He was the first to distinguish this type from "imperfect" and "partial" types:

"The skin and all the hair are milk white, sometimes yellow white. The iris and choroid, like the skin, are entirely *or almost entirely* deprived of coloring matter; so the iris is ordinarily rose or red, sometimes bluish or a pale or yellowish gray. The pupil, in place of black, is a bright red, little different from the color of fire". The italics have been added to emphasize the existence, referred to above, of undoubted albinotic individuals who possess some slight degree of pigmentation. To this description should be added the usual ocular accompaniments of defective vision, photophobia, nystagmus and ametropia (near- or far-sightedness plus astigmatism).

Albinos with some slight pigmentation and the "rufous albinos" with diffuse red pigment instead of granular black pigment are undoubtedly confused with the very fair individuals who occur among North European stocks, and with individuals having very widespread depigmentation of localized origin. An additional criterion besides that available from the superficial impression is needed to make such distinctions. Until we have one based on the knowledge of the disease mechanism, a somewhat arbitrary distinction must be accepted. In practice the distinction has usually been made on the basis of the visual defect. If this were present to any degree, the condition is classified as albinism. Such borderline cases are not too common, however. Pearson *et al*, believed there was a more or less gradual transition from complete, through incomplete, to partial albinism (white locks and piebaldism), yet they had no great difficulty in classifying 528 of their sibships as examples of complete albinism and 279 as incomplete and partial albinism. Complete albinism, recognizable superficially as an (essential) absence of pigment from the hair, eyes and skin, therefore makes up a relatively common although a purely descriptive, group of the pigmentary deficiencies in man (Fig. 1).

The great variety of other conditions that have been associated with albinism demands that the classification introduce some simplification. To this end, the many transitional forms and the associations with other anomalies have been omitted (see Busti-Rosner, 1956). Albinism is not necessarily associated with any other abnormalities save those referable to absent pigment. The visual defects may result entirely from the depigmentation. Despite the frequent allusions to macular degeneration and reduction of the retinal nerve layer, few of the albinotic eyes examined have had such pathological changes (Nettleship, 1906). Waardenburg (1932) and Franceschetti (1930) give later studies which have been made on albinotic eyes. Healthy, intelligent and long-lived albinos are well-known. The physician Sachs, who in 1812 described himself and his albinotic sister in a monograph ("Historia naturalis duorum leucaethiopum auctoris ipsius et sororis ejus") was one of these. There is a natural tendency to describe as separate entities albinism associated with

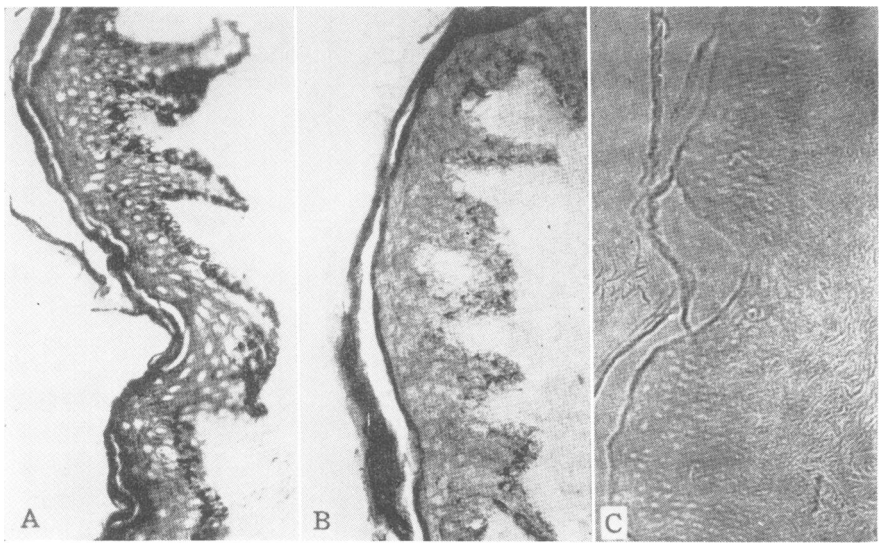

FIG. 1. Negro (A), Caucasian (B) and albino (C) human skin sections treated with Fontana silver stain. Note the relative amounts of melanin (argentophil granules) normally in the basal cells and diffused through the epidermis (from Young, 1957).

minor peculiarities in a single individual or in a single family. It has not been emphasized before that the fraternities most likely to contain albinos, i.e. those from consanguinous matings, are also prone to contain other recessive conditions, and that many of the abnormalities recorded as associated with albinism may be present on this basis. It can be stated that there are no known constant associations of albinism with other specific abnormalities and that many of the associated conditions that have been described were incidental. The possibility remains that within the descriptive group of complete albinism there exists a number of genetically distinct conditions each with its own peculiar syndrome. Only genetic analysis could at present reveal such types by their "breeding true".

Limiting our consideration to the one certain form of albinism has considerable heuristic value. Any progress made in understanding it will help with understanding the other forms. But the interplay works both ways. There was a positive value in Pearson's indiscriminate collection of information on all forms of pigment defect:— "All grades of pigmentation, all changes of pigmentation, and all local absences of pigmentation are of peculiar interest to the student of albinism. Above all, it is important to indicate any links which may occur between these classes of phenomena." (Pearson *et al*, Part I, p. 197).

II. GENETIC HISTORY

The earliest tales about albinos implied that the condition was hereditary. One of the most widespread of myths, comparable to those of Atlantis and of the Amazons, speaks of a race of albinos who remained hidden from the light during the day but at night could see to race swiftly through the forests. Linnaeus accepted Pliny's

version of these people, and they appeared in the first twelve editions of his *Systema* as *Homo nocturnus*. Confirmation of a sort was given this myth when localized areas in the world were found where the incidence of albinism was unusually high. The most famous of these, but by no means the only one, is the Caribbean area inhabited by the San Blas Indians, where an incidence of albinism of 7 per 1000 was found (Harris, 1926). In general, however, the geographic distribution reported by Pearson *et al* was fairly uniform in different climes and among different peoples and amounted to a world-wide incidence of about 1 in 20,000. The possibility that the heterozygote has some advantage in certain environments should not be overlooked as an explanation for the localized departures from this mean incidence.

With the background information provided by the myth of the albino race it may seem curious that scientific opinion should have erred so grievously for so long in recognizing the nature of albinism. Throughout the nineteenth century it was regarded as a disease acquired in humid and insalubrious regions (from which such individuals often came) or produced *in utero* by psychic shock to the mother. Such ideas remind us that the simple concepts of heredity were not always a part of common sense. Those writers before Garrod, and Pearson *et al*, who attributed albinism to a defect in the parental germ were the reverse of scientific and espoused ideas which Pearson called "metaphysical or metaphysiological." Ascoles (1871) had recorded from Sicily for other reasons data about 24 families, 5 consanguineous, containing in all 60 albinos. There were two instances of direct transmission of albinism from parent to child. On this slender evidence Garrod rested his contention of recessive inheritance of albinism.

Mendelian vs. Ancestrian: Several apt studies published shortly after Garrod's Croonian Lecture should have left little doubt about the hereditary nature of albinism. Bateson was convinced of its hereditary transmission as a recessive trait by the available fraternities which totaled 197 normals and 126 albinos, plus the high incidence of consanguinity found (Church, 1909, p. 28). The pedigrees known included that of Fig. 2, which is reproduced in the original form with shaded symbols for the normally pigmented Negroes. The normally pigmented son of an albino had in two marriages to normal women a total of fifteen offspring, four of them albinos (Mudge, p. 122 in Church, 1909). It was criticized because of the small chance that two successive marriages to heterozygotes could occur. The incidence of heterozygotes was then supposed to be about twice the incidence of albinism! Thirty-one pedigrees of recessive albinism were reported by Davenport & Davenport (1910, 1916), including three unique marriages of albino to albino, each of which resulted in one or two albino children and no normally pigmented children. This report was described by Pearson *et al*, (Part IV, Biblo No. 667) as "An attempt to demonstrate that albinism in man is a "unit character" and obeys Mendelian laws," and "his data are sparse, and his investigation from the statistical, ophthalmological and microscopical standpoints very inadequate" (Part II, p. 490). Pearson was almost able to have judgement suspended on the hereditary nature of albinism in man until he should make known the outcome of the massive project he had undertaken for the study of this disease. The nature of this work and its time in history require special comment.

"A Monograph on Albinism in Man" was published in six quarto volumes in 1911

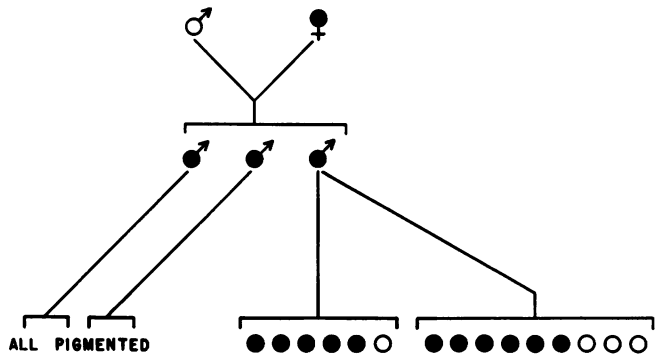

FIG. 2. Farabee's early pedigree of albinism, cited by Mudge (1909). Since the incidence of heterozygotes was then assumed to be about one hundredth the probable incidence, only convinced Mendelians found support for recessive transmission in this pedigree.

and 1913 by Pearson, Nettleship and Usher. Pearson was the director of the Francis Galton Eugenics Laboratory and of the Biometric Laboratory, and Professor of Applied Mathematics at University College, London. He initiated the project, and later induced the two physicians interested in the subject because of their ophthalmological studies to join him in its execution. This was the time of testing of Mendelism, and with the rumblings from the battles on evolution still audible, those who were not for Mendelism were judged to be against it.

Pearson's own words (November, 1908) made clear his position: "There is no definite proof of Mendelism applying to any living form at present" (Church, 1909, p. 155). His major effort was to apply exact methods to vital statistics, because "in nine cases out of ten Mendelians had not an elementary knowledge of how to deal with numbers, yet that knowledge was fundamental even to demonstrate Mendelism" (ibid., p. 131). He had denied that coat-color in horses or eye-color of man followed Mendelian principles, and the first demonstrations that they did were coupled with ironic comments on the fledgling science of statistics: "Professor Pearson has assured us that Biometry is destined to convert Biology into an exact science and to effect the salvation of biologists from the alleged perdition of theory, hypothesis, ramdom speculation, and vague terminology into which they had drifted before the biometrical angel had outspread her wings and come to earth to guard them and to lead their erring footsteps along a better road" (Mudge, p. 126, in Church, 1909). There was a reasonable suspicion that Pearson's biometry veiled an attempt to perpetuate Galton's hypothesis of heredity acting through continuous and fluctuating variations instead of the discontinuous variations called mutations: "every example of a mutation which occurs in Nature is a brick in the edifice of Mendelism and is a clod of earth dug out of the grave that is preparing for the Biometrician" (Mudge, p. 115 in Church, 1909).

The monograph on albinism appears to represent either the grave of some erroneous ideas, or laudable work killed by bad planning and naive biology. The wealth of material compiled on complete and incomplete albinism, on the various spotted conditions called "partial albinism", and on acquired leucoderma is at first over-

whelming. All known references, pedigrees and pictures bolstered by commissioned photographs, are reproduced in atlas volumes designed to accompany each of the three volumes of text. But the insistence that all biological phenomena involving color are related warns the reader. The orderly presentation of the history, geographical distribution, and varieties begun in Part I (1911) breaks down in Part II (1913). The detailed examinations of the structure of skin, eye, and hair in man is crowded by a welter of detail on animal albinism. Disjointed appendices by the authors and "distinguished specialists" appear on such subjects as the history and breeding of light colored Pekinese dogs, a report on the penis and scrotum of a South African Native with white glans penis, and the observations on the color of Norwegian Variable Hares throughout the seasons. The text of Part IV (1913) contains the individual case reports of all individuals shown in the pedigrees of the Part IV atlas volume. There are 654 numbered pedigrees, plus A to Z and AA to DD in extra plates (total 684), as well as pedigrees of the Pekinese spaniels. The preface to Part II says: "Part III. involving a general account of previous work on albinism in animals, a discussion of the vital statistics of albinism in man, and of the relation of albinism to other pathological states as well as the final reduction of our statistics of heredity of albinism in man will we hope to be ready towards the end of this year." Part III has never appeared.

The final two pages of text in the monograph, ones concluding a report on the muzzle and nose indices of albino dogs that was undoubtedly written by Pearson, must be taken as the summary of the monograph. After reiterating that the diversity of albinotic types (hereditary and acquired!) cannot be fitted into simple Mendelian theory, a conclusion of a sort is reached: "As we have seen in the course of this work albinism is a graded character, and we have every reason to believe that both in man and dogs separate grades are hereditary. Further than this we should hesitate to go at the present stage of our experimental work. Mendelism is at present the mode—no other conception of heredity can even obtain a hearing. Yet one of the present writers at least believes that a reaction must shortly set in, and that the views of Galton will again come by their own" (p. 490, Part III).

Recessive Inheritance: Citations which ascribe conclusions about the mode of inheritance of albinism to Pearson *et al*, are misleading. The apparent recessivity of complete albinism and the dominance of white spotting are in one place played off against one another to the discredit of Mendelism. The "monograph" remains a fascinating collection of items, a source book for works still unwritten on several different pigment diseases and phenomena. But the conclusions of the work were drawn eighteen years later by Hogben (1931). After a critical sifting of the pedigrees and statistical treatment of that data, he found 29 per cent of complete albino sibs in affected families. This fraction was somewhat above the expectation of 25 per cent for an autosomal recessive condition, perhaps only because the ascertainment had favored those families with unusually high proportions of albinos. The incidence of consanguinity in this and other studies ranged between 20 and 50 per cent. The earlier conclusions of Davenport and others that albinism was a recessive hereditary condition do not lessen the importance of Hogben's study. The value of the adequate statistical treatment of data as championed by Pearson can be shown in the

comparison of Hogben's conclusions with those of Magnus (1922). The latter found a total of 174 albinos in Norway, making an incidence of 1 per 10,000. Fifty families, 21 of them consanguineous, contained 108 of the albinos and 181 normal sibs. Lacking statistical methods he was forced to a descriptive analysis of individual families, and to a conclusion that albinism was both recessive and dominant in different families.

Subsequent studies have confirmed that complete albinism fits a recessive mode of inheritance quite precisely. After correction, the number of affected sibs in unselected families closely approaches 25 per cent (Sanders, 1938). A number of identical twins with perfect concordance in affected families have been reported (Wakefield & Dellinger, 1936; McCrackin, 1937; Hanhart, 1953). A number of concordant and discordant twins had been cited by Garrod. The possibility that heterozygotes can be detected clinically and that complete albinism is "incompletely recessive" (Waardenburg, 1947) has not yet been generally accepted. In some families a peculiar translucence of the iris on transillumination, independent of the degree of pigmentation, has been seen in the heterozygotes. Such studies are to be welcomed as possibly providing some indication of the number of different genes which may produce albinism in a recessive manner (Hanhart, 1953).

Alleles and Loci: There is a real possibility that complete albinism, though its transmission is uniformly recessive, can be produced by a series of allelic as well as non-allelic genes. The most impressive indication of different alleles is the report of two normally pigmented infants with good vision born from the union of two complete albinos (Trevor-Roper, 1952). The variations met in the degree of residual pigmentation, as well as the quality of color present in rufous and xanthous albinos, suggest the existence of multiple genes for albinism like those known to occur in guinea pigs (Wright, 1942). The invariable association of other abnormalities or characters with albinism in certain families, if this could be demonstrated, would provide another indication of a variety of genetic types of albinism. A different biochemical exploration might then be required for each. But until such a time, or until an extra biochemical explanation is in hand, complete albinism must be dealt with as a single entity.

III. PIGMENT PHYSIOLOGY

The casual reader may ask, "Are the pigment cells in albinism absent or just unable to make pigment?" It is obvious that colored materials of the body should early attract investigators, but it is unexpected that such a question could not be answered until now. In 1940, Meirowsky, who had been an active investigator of the melanotic pigment of the skin since the turn of the century, published "A Critical Review of Pigment Research in the Last Hundred Years." He attempted to list "what results can be looked upon as secured and from what points future research must be restarted." He considered largely elementary questions such as the identity of the cells forming pigment, the movement of pigment in the tissues and the general nature of its biochemical formation. These items are essential for the precise definition of the defect in albinism. Six of his nine fundamental questions would today be answered differently. There is still a need for ascertaining the correctness of our general con-

clusions, especially those based on textbook statements originating long before 1940. Only secondarily should we explore the specialized detail of this fascinating field. At the risk of being doctrinaire, some decision has been taken here on the correctness, or at least the relevance to albinism of the more popular aspects of pigment research. This is safer to do for the present subject than is often the case, since many different aspects of the pigment cell problem have been competently reviewed, and the reader can readily inform himself about doubtful points. Five excellent sources of information are Lorincz' review in Rothman's (1954) "Physiology and Biochemistry of the Skin", the published conferences on "Pigment Cell Growth" (Gordon, 1953) and "The Biology of Melanomas" (Gordon *et al*, 1948), and the reviews by Lerner and Fitzpatrick (1950, 1953).

Physical and Chemical Nature of Melanin: The primary concern in the study of albinism is the presence or absence of granules of brownish black pigment. Some explanation is therefore required for the variety of shades and tints of color normally seen in the body. Ignoring for the moment the yellowness of fat, the redness of blood, and other pigments which are not lacking in albinos (indeed these tints shining through the albino tissues give him his peculiar spectral quality), there remain a great variety of color effects referable solely to the sombre particles of melanin in a tissue. These are colors arising from physical structure and not from pigmentation *per se*. A single mechanism which gives rise by light scattering to the blue of the eyes or the blueness of deeply inbedded skin pigments, both called Tyndall blues after the discoverer of this phenomenon, will explain much of what is seen in normally pigmented individuals and what is missing in albinos.

Blue-scattering of light is of wide occurrence in nature, and accounts for the blue of the sky as well as of eyes. When light strikes microscopically divided, unordered particles greater than 1 μ in diameter, all wavelengths are reflected, and the material appears white. Much of the chalky character of the albino's skin is produced by a rather inefficient reflection of this type from the various cytological structures. There is superimposed on this a suffused pink from the hemoglobin showing through the translucent tissues. If particles are present less than 0.7 μ in diameter (and the melanin granules are smaller than this) only the short, blue light rays are reflected and the object appears blue. The longer redder rays pass through and are usually lost. If viewed by transmitted light such an object appears red (or yellow or brown), since only these longer rays come through. An iris with insufficient pigment to give it a hazel or brown color will show to the external observer only the Tyndall blue of the selectively reflected short rays of light, provided that the red light passing on through is absorbed and not reflected from the fundus. In the same way, a vein or a pigment deposit deep in the skin of a fair person will absorb the penetrating red rays and take on a bluish cast from the short rays reflected by the melanin granules in the skin overlying the object. The biological colors produced by this and related physical means, such as light interference, acting on the number, size and depth of successive layers of reflecting particles of pigment, are described in fascinating detail by Fox (1953). Since the breath-taking color displays of the perineum of certain baboons (male drill) and of the neck of turkey gobblers, for example, are produced simply by the arrangement of dark pigment particles, it is at least possible that a

variety of subtle tints in the eyes, skin and hair of man would disappear in the absence of similar undistinguished dark granules.

The color of normal skin has been attributed to five primary pigments on the basis of spectrophotometric reflectance measurements by Edwards and Duntley (1939) and the effects of these pigments are modified by the physical distribution patterns as discussed above. The pigments were oxy- and reduced hemoglobin, carotene, melanin and melanoid. Only the last two are missing from albinos. They were distinguished by their slightly differing general absorption of light in the violet end of the spectrum. The separate existence of melanoid as a pigment is uncertain, since it may only be melanin in the process of being cast off in the cornified skin. It is said to be formed independently of melanin, however, in the skin of eunuchs given testosterone (Edwards, Hamilton, Duntley & Hubert, 1941). The most convincing evidence from these studies that albinism might be considered purely as the absence of the single pigment, melanin (and its possible derivative, melanoid), is that no qualitative pigmentary differences were demonstrated between people of the same or different racial types. The clearly evident differences in skin color of people depended chiefly on the quantitative differences in the amounts of melanin present. These general conclusions have been confirmed by similar studies of others (Goldzieher *et al*, 1951).

At this point it is necessary to state that the term "melanin" still has no precise definition. It is merely the "dark pigment" localized in certain cells with its maximum absorption in the ultraviolet region. The modern usage which extends this histological definition to cover the brown or black polymers derived from the oxidation of tyrosine or dihydroxyphenyl compounds is based on an assumption which will be evaluated in due course. For the moment the soundest procedure will be to consider it purely as a cytological substance.

Melanin is seen in brown or black granules, usually located intracellularly. It reduces silver and gold salts and osmium tetroxide, which are often used to intensify its color, and it can be bleached to a light tan by strong oxidizing agents such as hydrogen peroxide or potassium permanganate. The melanin granules are specific structures which have been isolated by differential centrifugation of homogenized colored human skin, beef eyes and mouse melanomas (Mason, Kahler, MacCardle & Dalton, 1947). Electron microscopy of these preparations revealed the granules to be about 0.3 μ in diameter, and to have a uniform rounded or short rod shape more or less characteristic for each of these sources. These particles from the mouse melanoma are in chemical combination with at least one protein, a pseudoglobulin with specific antigenic properties, which can be progressively digested off by pancreatin to leave an increasingly high content of sulfur-amino acid bound to the pigment (Greenstein *et al*, 1940). This presumably indicates the means of binding of the pigment to the protein. More recently it has been unequivocally demonstrated that the pigmented particles from the mouse melanoma are analogous to the mitochondria of other cells. They consist of an elaborate array of chemical and enzymic constituents in addition to the pigment (Woods *et al*, 1950).

The identification of the pigment granules of the mouse melanoma cells as pigmented mitochondria is of great importance for the interpretation of studies of the

chemical composition of "melanin." Until it is obtained as a less complex chemical combination, it can be assumed that all samples of natural "melanin" represent part of the structure of the cell and not a simple chemical substance. The chemical analyses which have been reported, usually of material that had been extracted in alkali and precipitated with acid and that was still conjugated with protein, therefore, should not be expected to shed much light on the chemical nature of melanin. Indeed, almost nothing is known of the chemical nature of natural melanins.

It is a likely assumption that melanins are irregular polymers of benzenoid units, but it has not yet been possible chemically to degrade any naturally occurring melanins to some identifiable fragments which would identify the monomeric units of the polymer. The elaborate chemical work on the structure of melanins, reviewed authoritatively by Mason (1953), has been based exclusively on artifactual pigments produced *in vitro* by chemical or enzymic oxidation of phenols. One of the best known of these is the melanin formed by tyrosinase oxidation of 3,4-dihydroxyphenylalanine (dopa), for which it is virtually certain that the polymerizing unit is indole-5,6-dihydroxyquinone. Certain restrictions are known to limit to about three the variety of structures that may occur within the polymer. However, seventeen other naturally occurring phenolic substances which can be oxidized to melanins were listed in the above review. This could give "many different kinds of natural melanins, and this variety may be multiplied by the kinds of large molecules to which melanins are naturally attached and by the different modes of polymerization and conjugation possible, as well as by the different states of oxidation reductions of which melanins are capable. The structures of these naturally occurring substances, which have not been investigated from this point of view may then be quite different from anything already proposed." (Mason, 1953). Fig. 3 gives the structure of some known intermediates in the *in vitro* formation of dopa melanin, and one of the possible repeating units within this artificial melanin. In view of the above discussion these structures should only be considered to illustrate a general phenomenon, since they bear no necessary relation to the formation or structure of natural melanin in the tissues. Not only is the general structure of natural melanin in doubt, but so is the identity of the precursor from which it is formed.

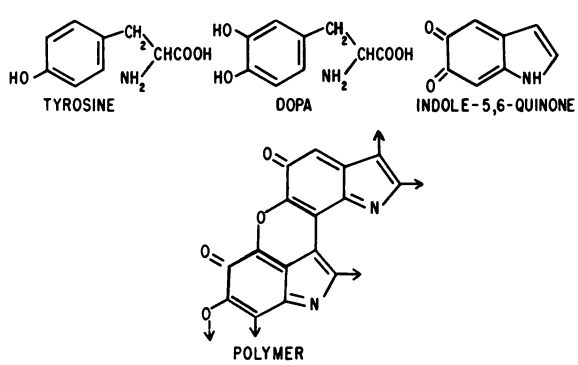

FIG. 3. Intermediates in the formation of dopa melanin *in vitro*. The polymer shown is one of several possible types (from Mason, 1953).

Embryology and Histology of the Pigment Cell: One of the two signal advances in pigment research in recent years has been the proof that there are specialized mammalian pigment cells distributed throughout the integument which arise from the neural crest (Rawles, 1948). The bulk of melanin pigment is located in dendritic melanocytes arrayed among and just below the basal cells of the epidermis and in the hair bulbs. Other pigment is transferred into the growing hair shaft. There are also melanocytes and melanin in the oral mucosa, pia mater and certain other surface membranes. Although pigment particles are found in other structures in lower animals, and even in man altered particles can be found in the keratinized layer of skin, deeper in the derma and in local lymph nodes, the source in man can be accepted as the melanocyte. This specialized cell does not arise autonomously in the surface membranes but migrates there under the control of unknown orienting factors from the neural crest at an early period in embryological differentiation. Statements that the pigment cell is a "nerve" cell in origin are deceptive, since the neural crest is merely tissue *beside* the neural tube proper.

The evidence for the origin of the cells does not depend upon uncertain cytological criteria for recognizing early, unpigmented or melanoblast stages of an elusive, migratory cell. By transplantation of mouse embryo fragments into the coelomic cavity of the chick embryo where development continued, it was shown by a variety of experiments that pigment developed only in those fragments containing neural crest or its migratory cells, and that fragments from which such cells were excluded would develop structurally normal skin and hair that was entirely pigment free (Rawles, 1953). Absence of melanocytes is therefore one possible cause for albinism. Failure of the cells to reach their proper destinations is a very reasonable explanation for the symmetrical patterns of depigmentation. Localized exclusion or destruction of melanocytes may explain certain types of piebaldness, as it has explained the colorless hair growing from an area of regenerated epidermis after freezing or irradiation injury (Taylor, 1949).

The melanocyte is a branched, light colored, "clear cell," staining less intensely than the surrounding basal cells, and from these it can also be differentiated by the lack of tonofibrillae and intercellular bridges. These criteria are hardly adequate for its identification in albino skin. Only when it contains melanin is it readily distinguished. Only pre-existing melanin can be darkened by silver impregnation. The dopa reaction gives gray to black staining even of the unpigmented dendritic cells when they are active, as they are immediately preceding as well as during actual melanin formation and after exposure of the skin to irradiation of various sorts. Since the dopa reaction may recede after pigment has been formed, it is possible to find cells which contain many melanin granules that give only a weak dopa reaction, and strong reactions in cells which have not yet accumulated pigment. Since dopa can also be oxidized non-specifically, especially by real or pseudo-peroxidases plus traces of peroxide, many non-melanocytes can give this reaction under certain conditions. The usefulness of the dopa reaction as a stain for the pigment forming melanocytes has been bolstered by the indisputable fact that albino skin does not give the reaction. This is of no value to the present question. Neither the silver stain which depends on the presence of pre-formed melanin, nor the dopa reaction which corre-

lates in a general way with the ability to form pigment, help us to decide if melanocytes (without pigment) are present in albinos.

Albino (Amelanotic) Melanocytes: Gold impregnation, introduced by Langerhans in 1868, has the happy facility of blackening both melanocytes and the very similar non-pigmented cells which can be found in equal numbers in albino skin. These are illustrated in Fig. 4. Some unknown property of the cell, perhaps connected with but surviving independently of its pigment forming ability, distinguishes by reaction with gold the colorless melanocyte from its surrounding colorless epidermal cells (Becker *et al*, 1952).

Further evidence for the existence of (amelanotic) melanocytes in the albino can be found in a second characteristic process of these cells, their occasional transformation into highly malignant melanomas. The literature often refers to the susceptibility of albinos to various forms of skin cancer, which is not unlikely in view of their lack of any pigmentary protection from sun light. But until recently no one had found any melanomas among albinos (Shapiro, Keen, Cohen & Murray, 1953). The first recorded case of malignant melanoma in a complete universal albino has now been described (Young, 1957). No pigment was found throughout the body or in the primary and metastatic sites of the melanoma, and both silver and dopa stains were negative. Yet the characteristic architecture of the cell, the junctional activity and the pattern of growth of the lesions identified these cells with those usually bearing pigment in a normal individual. Such a pigment-less melanoma in an albino must be distinguished from the so-called amelanotic melanomas frequently encountered in normally pigmented individuals. These are rarely, if ever, devoid of pigment (Fitzpatrick & Lerner, 1954). They are rich in tyrosinase which can be demonstrated

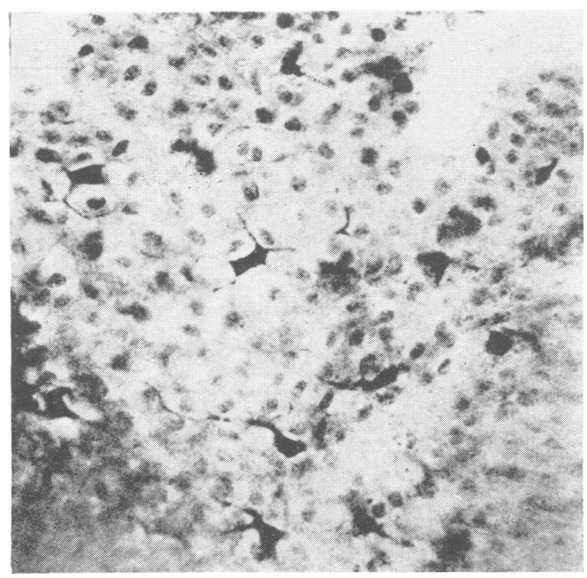

FIG. 4. Separated epidermis from albino with the melanocytes revealed by gold impregnation (from S. W. Becker, Jr., in Pigment Cell Growth, Academic Press, 1953).

simply by immersing them in tyrosine solution (Lerner & Fitzpatrick, 1953). Apparently the tumors are lightly pigmented because their growth has outstripped their tyrosine supply. In addition to this proof for melanocytes in albino man from the occurrence of a melanoma, there are hereditarily controlled melanomas that develop in the macromelanophores of certain fishes. These have also been produced in albino fish, where they appear as structurally typical tumors devoid of pigment (Gordon, 1948).

In summary, the direct visualization of melanocytes after gold impregnation and the occurrence of melanomas in albino tissues, plus the lack of evidence for any abnormality but depigmentation of the neural crest cell or its derivatives, implicate a sub-cellular defect in the melanocyte as responsible for the lack of pigment in albinism.

IV. BIOCHEMICAL MECHANISM OF MELANIN FORMATION

In view of the common assumption that tyrosinase and dopa produce melanin formation in human melanocytes, the reader should remember the absence of any real information about the chemical constitution and precursors of natural melanin. A further quotation from Mason (1953) puts the assumption in perspective: "The identities of propigments have had to be *deduced* from diphenols *coexisting with* or probably produced in the presence of phenol oxidases, or from the experimentally determinable *specificities* of these phenol oxidases. It is on such grounds that 3,4-dihydroxyphenylalanine is widely considered to be the propigment in mammalian melanocytes." On the two facts italicized above, the occurrence of a diphenol in the skin and the specificity of the phenol oxidase found there, rests whatever inferences have been drawn about the biochemical mechanism of pigment formation in mammals.

A single fact, that something was present in the pigment cells which made pigment out of a diphenol, was the basis of all thought about mammalian melanin formation. Recently a second fact has been learned. The two known facts support the commonly accepted views, but they are slender evidence supporting a colossus of theory. The first known fact was the presence in pigment cells of something like an enzyme. Specifically, this was the "dopa oxidase" reaction discovered by Bloch with the use of 3,4-dihydroxyphenylalanine added to frozen skin slices (Bloch, 1927). Melanocytes were blackened by this reaction. The much more elaborate information available about pigment formation in plants and insects, whose extracts contained an active tyrosinase that oxidized tyrosine to dopa and on to melanin, lent strength by analogy to this isolated fact known about pigmentation in mammals. The general acceptance of a similar scheme for the pigment formation in higher animals required more detailed chemical evidence that was slow in coming. Dopa, for example, was known to occur physiologically only in beans.

The original choice of dopa as a reagent developed from certain valueless clinical considerations of the pigmentation found in Addison's disease (adrenal hypofunction) and from the excretion of catechol-like compounds by patients with melanomas. These considerations directed Bloch's attention to epinephrine and catechol as possible pigment precursors. Only the chemically related dopa formed pigment in skin slices. This pragmatic basis, without evidence for the natural occurrence of dopa at the site of mammalian pigment formation or for the presence of the dopa unit in

natural melanin, was the sole reason for the provisional identification of dopa as a pigment precursor in animals.

The relevance of Bloch's work to the pigmentation problem therefore stood on what evidence there was for a specific phenol oxidase in skin which formed pigment from dopa. The histochemical usefulness of the "dopa reaction" was amply confirmed, but the existence of this "dopa oxidase" was hotly contested. Meirowsky (1940) was convinced only that a non-specific catalyst, probably not an enzyme, was present in both pigment-forming and other cells. His review gives the cogent arguments against Bloch's interpretation of the dopa reaction. Now that additional evidence supports Bloch's observations, it is of interest to see what kind of evidence for a specific enzyme was produced by (Bloch's) early use of cytochemistry. The technique consisted of immersing frozen sections of skin into a dilute solution of dopa buffered at physiological pH and noting the melanin deposition which occurred after 24 hours at room temperature. The deposition of melanin was the most intense in the regions such as the basal layer of epidermis and hair follicles, it was localized predominantly in the specialized cells he called melanoblasts (melanocytes), and it did not occur in albino skin or skin affected with the localized loss of pigment called vitiligo. He concluded that this dopa reaction was a reliable indicator of the active capacity of cells to form pigment. On this empirical observation there was general agreement provided that the slight degree of generalized darkening of the slice was ignored which resulted from the non-specific oxidation of dopa. Few of the antagonists ignored this background staining, however.

The evidence that the dopa reaction was due to an enzyme, dopa oxidase, was highly suggestive. The dopa reaction occurred only at a neutral pH range, it was suppressed by heating the slice to 100° C or otherwise denaturing the proteins, and it was inhibited by dilute solutions of hydrogen sulfide or cyanide. To these enzyme-like properties was added the unique specificity. The reaction occurred only with the natural L-isomer of dopa. Except for the background color produced by all of the easily oxidized diphenols, melanin was not formed from a large number of phenols and related compounds including tyrosine, tryptophan and the unnatural D-isomer of dopa. To this cytochemical evidence, Bloch and Schaaf (1925) added the demonstration that a similar reaction was catalyzed by extracts of new-born rabbit skin from pigmented but not from albino animals.

The inadequacies of the above explanation of the mechanism of melanogenesis are briefly enumerated, without resort to the polemical exceptions that have been taken to each item of evidence. Probably dopa was converted by enzymic catalysis to an insoluble pigment in the melanocytes of man, and this reaction was possibly absent in the albino skin. But the darkening of certain areas in skin slices or extracts caused by a substance which also darkened spontaneously in watery solution was not sure evidence for a physiological process. The specificity of the reaction for dopa hinted at an enzyme, but this fact lost weight without evidence that dopa occurred in the skin. Tyrosine was a more reasonable precursor and was supported by the analogy with the tyrosinase reactions in lower forms of life, but tyrosine was unable to act as a pigment former in mammalian skin. Against the clear cut correlation of a positive dopa reaction occurring where there was pigment formation, particularly in the

comparison of normal and albino skins, could be set a bewildering number failures of this correlation. The dopa reaction did not parallel the quantitative changes in pigmentation seen in normal skin, and this problem remains to be explained, perhaps in terms of the activation and regulation of the pigment forming enzyme *in vivo*. There was no general acceptance of the Bloch scheme of melogenesis because of (1) the inability to establish any chemical similarity between natural melanin and that formed in the dopa reaction, (2) the absence of chemical evidence for a dopa oxidase to support the cytochemical evidence, and the (3) the failure to prove the physiological occurrence of dopa as a potential pigment precursor. There is now additional evidence on the last two points.

Mammalian Tyrosinase: The second of the two major advances in pigment cell research in recent years has been the demonstration that melanin is formed enzymically from tyrosine in mammalian skin. The over-all reaction resembles that long known to occur in lower forms of life (Raper, 1928) and the reactions, passing through dopa as an intermediate, encompass and so support the observations made by Bloch. The mammalian tyrosinase was first demonstrated unequivocally by Hogeboom and Adams (1942) in extracts of the Harding-Passey mouse melanoma. There had been earlier studies on horse melanomas that were less convincing. The tumor extracts had sufficient activity for the reaction to be followed by oxygen consumption and to permit other biochemical manipulations which left no doubt about the enzymic nature of the reaction. Only tyrosine or its ethyl ester were substrates. Purification of the enzyme beyond a certain stage was not possible, however, because it was part of the same mitochondrial unit identified with the melanin granule (Woods *et al*, 1950). Like the soluble enzymes of lower forms, the melanoma enzyme also required copper. Dopa was recognized as an essential intermediate in the reaction, being formed from tyrosine and further oxidized to melanin. Dopa acted also as a necessary cofactor for the initial oxidation of tyrosine (Lerner *et al*, 1949). It remained only to confirm these observations on melanomas with studies on the much smaller amounts of tyrosinase available in normal skin.

By the same procedures used by Bloch and incubating white human skin slices with tyrosine instead of dopa, melanin was formed in the melanocytes (Fitzpatrick *et al*, 1950). An essential difference from the earlier work was that the skin was irradiated with ultraviolet light some days before its removal in order to activate the tyrosinase. In the skin of young mice (Foster & Brown, 1957) and of guinea pig fetuses (Foster, 1956) the tyrosinase (here largely restricted to the hair bulbs) was fully active without irradiation if endogenous inhibition was overcome by iodoacetamide. Presumably the enzyme can be demonstrated only after overcoming the sulfhydryl inhibition first demonstrated by Rothman *et al* (1946). In minced skin preparations the tyrosinase activity was demonstrated to share all of the properties of the tyrosinase from melanoma (Foster & Brown, 1957). It specifically oxidized L-tyrosine to dopa and dopa to melanin, it was measured by oxygen uptake as well as by melanin formation, and it was inhibited by copper chelating reagents. In the albino mouse there was no such reaction. More complicated results were obtained with some of the allelic series at the albino locus (C) in the guinea pig, but it is premature to expect an explanation for all of the great variety of albinotic conditions in animals with this single qualitative test.

V. THE ALBINO DEFECT

The reaction of an enzyme in the mitochondria of melanocytes which normally forms melanin and conjugates it to these granules is missing in at least some albino men and animals. The melanocytes themselves are apparently present and normal except for their pigment lack. Experiments do not appear to have been done to decide whether the granules normally containing both the pigment and the enzyme are themselves missing from the melanocytes of the albino, or whether they are present but incomplete. Since these particles have many other enzyme activities, they could be readily identified. Since these other activities include the major respiratory systems of the cell, it is unlikely that the particles are missing. Even if they are shown to be present, a variety of abnormalities of these particles could still account for the inability to form pigment, and one of these possibilities is the absence or defectiveness of the tyrosinase. The elucidation of this problem will come from the biochemical analysis of the pigment forming systems in the variety of genetic strains of animals with different coat colors (Russell, Russell & Brauch, 1948). The number of such different strains, even more than the indications for genetic heterogeneity among human albinos, means that a lesion at any of several biochemical sites can cause albinism. The small number of known chemical reactions in pigment formation does not mean that the great variety of loci controlling coat color must act at these few biochemical loci. Only the bare outlines of the biochemical reactions in pigment formation have been perceived, and to this must be added the complexities of regulation. There are signs that the fifty years of complacence with an adequate theory and inadequate physiological facts about albinism is ending.

REFERENCES

ASCOLEO. 1871. *Archivo per l'Anthropologia.* I: 367 (cited by Garrod, 1908).

BECKER, S. W., JR., FITZPATRICK, T. B., & MONTGOMERY, H. 1952. Human melanogenesis: cytology and histology of pigment cells. *Arch. Derm. Syph.* 65: 511–523.

BLOCH, B. 1927. Das Pigment. In Jadassohn, *Handb. d. Haut. u. Geschlectskr.* 1: 434–451. J. Springer, Berlin.

BLOCH, B., & SCHAAF, F. 1925. Pigmentstudien. *Biochem. Zschr.* 162: 181–206.

BUSTI-ROSNER, L. 1956. Deux cas d'albinism universel incomplet (albinoidisme) d'un biotype particulier dans une souche valaisanne. *J. de Genet. hum.* 5: 197–215.

CHURCH, W. S. 1909. The influence of heredity on disease, etc. A discussion. *Proc. R. Soc. M. II,* London: Longmans, Green & Co.

DAVENPORT, C. B. 1916. Heredity of albinism. *J. Hered.* 7: 221–223.

DAVENPORT, G. C. & DAVENPORT, C. B. 1910. Heredity of skin-pigmentation in man. *Am. Natur.* 44: 641–672, 705–731.

EDWARDS, E. A., & DUNTLEY, S. Q. 1939. Pigments and colors of living human skin. *Am. J. Anat.* 65: 1–33.

EDWARDS, E. A., HAMILTON, J. B., DUNTLEY, S. Q., & HUBERT, G. 1941. Cutaneous vascular and pigmentary changes in castrate and eunuchoid men. *Endocrinology* 28: 119–128.

FALLS, H. F. 1953. Albinism. *Tr. Am. Acad. Ophth.* 324–331.

FALLS, H. F. 1951. Sex-linked ocular albinism displaying typical fundus changes in the female heterozygote. *Am. J. Ophth.* 34: 41–50.

FITZPATRICK, T. B., BECKER, S. W., LERNER, A. B., & MONTGOMERY, H. 1950. Tyrosinase in human skin. *Science* 112: 223–225.

FITZPATRICK, T. B. & LERNER, A. B. 1954. Pigment and pigment tumors: Biochemical basis of human melanin pigmentation. *Arch. Derm. Syph.* 69: 133–149.

FOSTER, M. 1956. Enzymatic studies of the physiological genetics of guinea pig coat coloration. I. Oxygen consumption studies. *Genetics* 41: 396–409.

FOSTER, M., & BROWN, S. R. 1957. The production of dopa by normal pigmented mammalian skin. *J. Biol. Chem.* 225: 247–252.

FOX, D. L. 1953. *Animal biochromes and structural colors.* Cambridge: Cambridge Univ. Press.

FRANCESCHETTI, A. 1930. Die Vererbung von Augenleiden. *Kz. Handb. d. Ophth.* I: 704–708. (Ed. by Shieck-Bruckner), J. Springer, Berlin.

FRANCOIS, J., & DEWEER, J. P. 1952. Albinism oculaire lie au sexe et alterations caracteristiques du fond d'oeil chez les femmes heterozygotes. *Bull. Soc. belge opht.* 102: 724–739.

GARROD, A. E. 1908. Inborn errors of metabolism. Lecture II, *Lancet* 2: 73–79.

GATES, R. R. 1946. *Human Genetics*, Macmillan, N. Y.

GOLDZIEHER, J. A., ROBERTS, J. S., RAWLS, W. B., & GOLDZIEHER, M. A. 1951. Chemical analysis of the intact skin by reflectance spectrophotometry. *Arch. Derm. Syph.* 64: 533–548.

GORDON, M. 1953. *Pigment Cell Growth.* New York: Academic Press.

GORDON, M. 1948. Effects of five primary genes on the site of melanomas in fishes and the influence of two color genes on their pigmentation. In *The Biology of Melanomas*, Spec. Pub. of N. Y. Acad. Sci. 216–268.

GREENSTEIN, J. P., TURNER, F. C., & JENRETTE, W. V. 1940. The Melanin-contained pseudoglobulin of the malignant melanoma of mice. *J. Nat. Cancer. Inst.* 1: 377–385.

HANHART, E. 1953. Eineiige Zwillingsmädchen mit konkordantem Albinismus universalis aus Ehe normalpigmentierter, ober entsprechend belasteten Vettern I. Grades in oberitalienischen Isolat. *Acta Genet. Med. Gemellol.* 2: 380–390.

HARRIS, R. G. 1926. The San Blas Indians. *Am. J. Phys. Anthrop.* 9: 17–63.

HOGBEN, L. T. 1931. The genetic analysis of familial traits. *J. Genet.* 25: 97–112.

HOGEBOOM, G. H., & ADAMS, M. H. 1942. Mammalian tyrosinase and dopa oxidase. *J. Biol. Chem.* 145: 273.

LERNER, A. B. 1953. Metabolism of phenylalanine and tyrosine. *Advances in Enzymology* 14: 73–128.

LERNER, A. B., & FITZPATRICK, T. B. 1950. Biochemistry of melanin formation. *Physiol. Rev.* 30: 91–126.

LERNER, A. B., & FITZPATRICK, T. B. 1953. The control of melanogenesis in human pigment cells, in Gordon, M.: *Pigment Cell Growth*, 319–333, New York: Academic Press.

LERNER, A. B., FITZPATRICK, T. B., CALKINS, E., & SUMMERSON, W. H. 1949. Mammalian tyrosinase: preparation and properties. *J. Biol. Chem.* 178: 185–195.

LORINCZ, A. L. 1954. Pigmentation. In Rothman, S., *Physiology and Biochemistry of the Skin.* 515–563, Chicago: Univ. Chicago Press.

MAGNUS, V., 1922. Albinism in man. *Norsk. mag. f. laegenidensk* 83: 509, abstr. *J. Am. M. Ass.* 1922, 79: 780.

MCCRACKIN, R. H. 1937. Albinism and unialbinism in twin African Negroes. *Am. J. Dis. Child.* 54: 786–794.

MASON, H. S. 1953. The structure of melanins, in Gordon, M.: *Pigment Cell Growth*, 277–303 New York: Academic Press.

MASON, H. S., KAHLER, H. MACCARDLE, R. C., & DALTON, A. J. 1947. Chemistry of melanin IV. Electron micrography of natural melanins. *Proc. Soc. Exp. Biol.* 66: 421–431.

MEIROWKSY, E. 1940. A critical review of pigment research in the last hundred years. *Brit. J. Derm. Syph.* 52: 205–217.

NETTLESHIP, E. 1906. Note on some varieties of albinism. *Tr. Ophth. Soc.*, U. K., 26: 244–250.

OHRT, V. 1957. Ocular albinism with changes typical of conductors in a Danish Family. *Acta genet.* 7: 298–301.

PEARSON, K., NETTLESHIP, E., & USHER, C. H., 1911–1913. *A Monograph on Albinism in Man.* Drapers Comp. Res. Memoirs, Series VI, VIII & IX, Parts I, II & IV, 6 vols. London: Cambridge Univ. Press.

PFANDLER, U. 1950. Quelques mutations affectant les yeux. *Bull. Acad. suisse Sc. med.* 6: 134–146.

RAPER, H. S. 1928. The aerobic oxidases. *Physiol. Rev.* 8: 245–282.

RAWLES, M. E. 1948. Origin of melanophores and their role in development of color patterns in vertebrates. *Physiol. Revs.* 28: 383–408.

RAWLES, M. E. 1953. Origin of the mammalian pigment cell etc. In *Pigment Cell Growth*, 1–15, ed. M. Gordon, New York: Academic Press.

RILEY, V. T., HESSELBACH, M. L., FIOLA, S., WOODS, M. W., & BURK, D. 1949. Application of chromatography to separation of subcellular enzymatically active granules. *Science* 109: 361–364.

ROTHMAN, S., KRYSA, H. F., & SMILJANIC, A. M., 1946. Inhibitory action of human epidermis on melanin formation. *Proc. Soc. Exp. Biol.* 62: 208–209.

RUSSELL, W. L., RUSSELL, E. S., & BRAUCH, L. R. 1948. Problems in the biochemistry and physiological genetics of pigmentation in mammals, in *Biology of Melanomas*, Gordon, *et al.*, N. Y. Acad. Sci. p. 447–453.

SANDERS, J. 1938. Die Heredität des Albinismus. *Genetica* 20: 97–120.

SHAPIRO, M. P., KEEN, P., COHEN, L., & MURRAY, J. F. 1953. Skin Cancer in the South African Bantu. *Brit. J. Cancer.* 7: 45–57.

TAYLOR, A. C. 1949. Survival of rat skin and changes in hair pigmentation following freezing. *J. Exp. Zool.* 110: 77–111.

TREVOR-ROPER, F.D. 1952. Marriage of two complete albinos with normally pigmented offspring. *Brit. J. Ophth.* 36: 107–108.

WAARDENBURG, P. J. 1947. Herkenbaarheid van Latente Overdragers van Albinismus Universalis en Albinismus Oculi. *Ned. tschr. geneesk* 91: 1863–1866.

WAARDENBURG, P. J. 1932. Das menschliche Auge und seine Erbanlagen. *Bibliogr. genet.* 7: 19–36.

WAKEFIELD, E. G., & DELLINGER, S. C. 1936. Report of identical albino twins of negro parents. *Ann. Int. M.* 9: 1149–1153.

WOODS, M., DU BUY, H., & BURK, D. 1950. Evidence for the mitochondrial nature and function of melanin granules. *Zoologica* 35: 30–31.

WRIGHT, S. 1942. The physiological genetics of coat color of the guinea pig. *Biol. Sympos.* 6: 337–355.

YOUNG, T. E. 1957. Malignant melanoma in an albino. *Arch. Path.* 64: 186–191.