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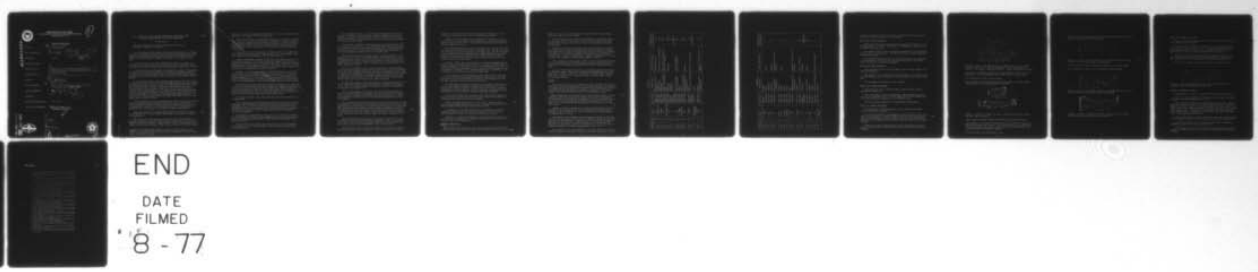

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IRIS COLOR AS A "TEST" FOR THE QUANTITATIVE ESTIMATION IN MAN /725*
OF MELANIN CONCENTRATION IN THE STRIA VASCULARIS**

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In our preceding investigations concerning the distribution of melanin in the stria vascularis in the various sections of the human cochlea, there appeared considerable individual quantitative differences which affected overall the concentration of pigment with regard to the segmental structure.

As we have said, this is also represented in man by a greater concentration of melanin at the apical tract than at the cochlear base agreeing with what is found in guinea pigs (Beck, Cherubino, Bonaccorsi, Galioto).

To this particular distribution, we attributed a possible "phylogenetic-angioprotective" functional significance through the recognized enzymatic activity of the melanin granule in the cellular respiratory oxydo-reductive processes and through the probable role of biological semiconductor (Munday, Kerkut).

In consideration then of the inversely proportional ratio in the stria vascularis, as in the skin, of the "melanin-dehydrogenase" binomial, through the diverse molecular structure of the two enzymes and through their differing utilization phase, we have attributed to this pigment the value of "energy reserve" of the cochlea. This would follow in the respiratory chain when the dehydrogenases would be inactivated by prolonged and intense acoustic stimulations favoring the metabolism of the strial epithelium and later guaranteeing the efficiency of the hemato-labyrinthine barrier. In this way, the sensorial neural epithelium would find itself protected from damage owing to acoustic shock.

In the continuation of our investigations in this field, we have experimentally observed on guinea pigs how the acoustic deficit which sets in after a prolonged exposure to industrial noise is inversely proportional to the pigmentary content of the stria vascularis which, in this animal, according to Beck, corresponds to the degree of general pigmentation.

Hence the importance of reliably knowing whether the melanin content /726
in the internal ear is proportionally equivalent to the degree of tegumental pigmentation.

With respect to this correlation, there are only a few mentions in technical literature beyond the one referred to above with clear differences appearing between the authors. Whereas Kolmer (1927) was successively confirmed by Beck (1961), Alexander (1901) wrote that the "labyrinthine pigment

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* Numbers in the right margin indicate pagination in the original text.
**Annali di Laringologia, Otologia, Rinologia, Faringologia (Torino), LXIV, Fasc. 6, 1965, pp. 725-738.

inasmuch as concerns arrangement and quantity is no way in relation to the behavior of the pigment in the skin and hair".

Confronted with these contradictory statements, we carried out experiments "in vivo" on guinea pigs (Cherubino, Bonaccorsi, Galioto) as well as on a conspicuous number of animals during the study of cochleo-vestibular microcirculation these last two years which strongly indicate that there actually exists such a quantitative relationship but not representing a general rule.

There can also be in these animals a "gene plurality" or chromosomic zonal interferences through which the pigmentation of the tegumental and hair systems do not correspond to that of the internal ear.

Indeed, in albinism, frequent in many races, the outer layer is completely white whereas "the irises" are normally pigmented with various gradations of intensity as are additionally the "stria vascularis" and the "typical seats of the posterior labyrinth" (Cherubino, Bonaccorsi, Galioto). This would allow the assumption that we are not dealing with a single gene responsible for pigmentation in these territories.

However, in not infrequent cases of albinism "localized to the eye", we have noted "in vivo" albinism also "of the internal ear". This would demonstrate on the other hand the unambiguity of the gene including the melanic pigmentation of the ear and of the acoustic and vestibular labyrinth and would represent a confirmation of the plurality of genes responsible for the pigmentation of the aforementioned seats and of the tegument.

We had never proposed to check the pigmentary content of the iris and of the stria vascularis in guinea pigs through the intermediate gradations of colors of the eyes inasmuch as these animals were hardly suitable for such an investigation to the extent that they passed from the absence of pigment (albinism in the eyes) to a discrete pigmentary content such as to give the iris colorations which range from brown to black.

In general, however, a less intense brown of the iris corresponds to a lighter skin.

In the flecked variety (white-chestnut, white-black) which is the one habitually used in laboratory research and which is also the most common, customarily the color of the iris has a more intense brown in animals flecked with black. The specimens with a coal-black layer have the darkest eyes and, in addition, have a very abundant content of melanin in the stria vascularis. /727

According to our observations hence, there is a correlation between the concentration of melanin in the tegumental system, iris and stria vascularis, this equivalence being quite often found in the binomial "iris-stria vascularis".

We do not know yet to what extent this may be true in other mammals since there is a lack of bibliographical data concerning investigations in this direction.

Only Alexander (1901), in a very detailed comparative study on the presence of pigment in various mammals, uses the pigmented cells of the internal ear which are morphologically similar to those of the choroid membrane but without making quantitative references concerning the pigmentary content of these seats.

This appears to emphasize the embryological correlation between the stria vascularis and the iris. These are mesenchymal formations structurally similar in organs of ectodermal derivation such as the internal ear and the eye which at a certain stage of development were penetrated by the mesenchyma which produced the local vascularization.

It is in this phase of development that occurs the migration of the melanoblasts of the neural crest with typical localizations both in the tegumental system as well as in the seats where rich vasal plexi are being developed which in various organs become suitable for the production or reabsorption of intracavital liquids (aqueous humor, vitreous humor, endolymph, perilymph, liquor).

In cats, in which the color of the iris and that of the hair system are almost always separate, there has been found in the case of heterochromism of the iris (Przibam) homolateral deafness with depigmented eyes. From the histological standpoint on the deficit side, in addition to the absence of pigment, there was present a cochlear degeneration, more plain in the "apical tract" than in that of the basal tract as has been also found by Wilson and Kane in a race of white cats with blue irises.

In many other mammals, there also exists no relationship between color of the outer layer and that of the eyes (horses, milkcows, dogs, sheep, etc.). There is nothing available concerning the relative concentration of melanin in the internal ear.

Also in human beings, the lack of correlation between pigmentation of hair and skin in general and pigmentation of the iris may be verified with a discrete incidence but attention has never been given to a comparative examination with the entity of the labyrinthic pigmentation in the same subject.

The intervening relationship between these various seats since our last formulations of the problem of the function of melanin in the acoustic labyrinth and the positive success of experimentation to which we have previously made reference loses the meaning of pure anatomo-topographical curiosity. The utility which arises from this is to be able to select, on the basis /728
of pleiotropism of one gene, subjects with considerable content in melanin of the stria vascularis also having a number of exterior phenotypical aspects.

Every observation up until now has been made on animals and we have now wished to extend the investigation to humans in order to see whether or not there exist the same relationships and whether the pigmentation of the cochlea is genetically independent of that of other seats.

This is why our study, up until now limited to the anterior labyrinth, has been formulated on the histological control of melanin in the stria

vascularis, associated with the direct macroscopic observation of color of the hair system and of the irises of individuals examined.

Indeed, it is well known that to determine the coloration of this last item, two factors compete: the pigmentation of the deep layer represented by the iris part of the retina and the melanin which can exist in the more superficial layers.

The pigment which is found in great quantities in the deep layer (retinal) gives to this epithelial layer a very black tint. However, the luminous rays which come into the iris and from which layer they are reflected in part undergo diffraction phenomena upon passing through the vascular-connective mesiris of the translucid iris. There results from this a tinting of the iris which fluctuates between light blue and slate which is the "basic color of the iris".

However, in many cases, the elements of the normal pigmented melanogenetic system, with their granular content of melanin are distributed in a varying quantity in the surface layers. In the European races, this can be confirmed with less frequency in the northern populations and with considerably more frequency in the southern populations. In addition, it is a constant fact in the black races on the other hand.

By the presence of melanic pigment in the surface layers, the basic color of the iris can be obscured or modified. When, then, in the eyes, so-called black ones, whose pigment is abundant and widespread over all the iris, the basic color is not allowed to appear.

By quantitative variations of melanin in the vascular-connective mesiris, it was possible to obtain extremely varied combinations of color. In such cases, rather frequent in light-colored eyes, owing to irregular arrangements of the melanocytes, they can form spots scattered throughout the surface of the iris giving a streaked appearance (Chiarugi).

On the whole, these cells scattered in the melanogenetic system and united in their prolongations form in the dark irises a fixed network in continuation with that of the choroid membrane.

Lauber and Munch considered it to be a "type of diffuse cellular muscle" having demonstrated that the surface of these cells result in direct relationship with abundant slender nervous fibers. /729

In albinism, on the other hand, there is a decrease or congenital lacking of pigment in the iris as well as the latter's retinal layer (with pigment also lacking in the choroid membrane and retina).

The iris is, under these conditions, almost transparent and has a red tint owing to its richness in blood vessels and owing to the reflection from the back of the apigmented eye.

PERSONAL CONTRIBUTION

The research was carried out on 40 petrous pyramids from human beings of the white race (18 men and 22 women using six-month fetuses as well as →

adults up to 52 years of age) taken not longer than 25 hours following death and fixed in a Lilly formula.

The specimens were cataloged using information concerning the age of the subjects, color of iris, color of hair as well as pubic hair which was necessary in order not to incur an error of valuation in the event of dyed hair.

There then followed decalcification in aqueous solution of 5% nitric acid changed every 24 hours, immersion from 24 to 48 hours in aluminum-potassium sulfate, washing in running water from 24 to 48 hours, inclusion in a mixture of celluloid and paraffin, microsection to 12-15 micron, staining with a 1% silver impregnation at ambient temperature for 24 hours, contrast with carmalum for 5 min (Lignac method: Part One).

This staining was always used in order to avoid the excessive blackening of the melanin (as is confirmed with the stains of Masson, Gomori, Achucaro-Del Rio Hortega) which excessively masks the endocellular structures.

As test of differential destaining of the other pigments, we used for a number of sections a solution of potassium permanganate and water (the Hueck test). However, there was no solubility in formaldehyde, alkalis, weak acids, alcohol, xylol, chloroform, benzol, ethylic ether, acetone and in carbon sulfide.

RESULTS

Given the character of this research, we shall not dwell on the description of the fine histology but will just make reference to the quantity of melanin present, the number and morphology of the melanocytes, their relationship with the capillaries, the degree of maturity of the pigment grains, their presence and their entity in the epithelial cells.

From observation of the preparations, whereas there results a lack of agreement between the intensity of pigmentation of the hair system and the concentration of melanin and melanocytes in the stria vascularis, the correlation is striking between the content of melanin in the iris and that in the stria vascularis although limited to the middle age of life (Table A-B). /732

Indeed, in fetuses, limited to the three cases observed by us and in children in the first months of life, we encountered no melanin and no melanocytes could be seen in the stria vascularis only finding presence of pigmentation of the iris.

The melanin in the elements of the melanogenetic system of the stria vascularis of the subjects controlled by us appears towards the fifteenth month of life and increases in intensity up to a certain period which on the basis of our reports is at about the 60th year. Beyond this time, there are not appreciable significant variations even in the case of considerable evidences of marginal depigmentation of the iris.

Before passing on to considerations of a general character, we shall present a number of histological reports on the stria vascularis (II tract)

TABLE A

Cases	Sex	Age	Eye Color	Hair Color	Pubic Hair Color	Melanin in St. Vascul.
A 1	M	81	Light gray	White (ex black)	White (ex black)	++
B 2	M	Fetus 6 mo.	Light gray	Chestnut		--
C 3	F	58	Dark gray	Black (dyed)	Blonde (part. achromachia)	+++
D 4	F	1 month	Light blue	Chestnut		--
E 5	F	74	Dark gray	Black (adv. achrom.)	Black (adv. achromachia)	+++
F 6	F	76	Gray-green	White (ex chestnut)	White (ex chestnut)	+
G 7	F	Born dead/term	Light blue	Black		--
H 8	F	43	Dark brown	Chestnut	Chestnut	++++
I 9	M	1	Chestnut	Chestnut		--
L 10	F	3	Chestnut (streaked)	Chestnut		++ (streaked)
M 11	M	75	Light gray	White (ex blonde)	White (ex blonde)	++
N 12	M	82	Light gray	Gray (ex black)	Gray (ex black)	++
O 13	F	1 month	Gray-green	Black		--
P 14	M	15 months	Gray-green	Black		+-
Q 15	F	80	Light gray	Gray (ex chestnut)		++
R 16	M	60	Light brown	Chestnut (partial achromachia)	Chestnut	+++
S 17	M	11 days	Light gray	Black		--
T 18	F	47	Chestnut (streaked)	Gray (ex lt chestnut)	Gray (ex lt chestnut)	+++ (streaked)
U 19	M	Born dead (7 months)	Chestnut	Black		--
V 20	F	1 day	Light blue	Blonde		--
Z 21	F	62	Light gray	Chestnut (partial achromachia)	Chestnut (partial achromachia)	++

TABLE B

Case	Sex	Age	Eye Color	Hair Color	Pubic Hair Color	Melanin in St. Vascul.
A 22	F	17 months	Chestnut	Blonde		+-
B 23	F	45	Gray-green	Black	Black	+
C 24	M	4 days	Light gray	Chestnut		--
D 25	F	58	Light gray	Chestnut	Chestnut	++
E 26	M	62	Gray-green	Black (partial achromachia)	Black (partial achromachia)	+
F 27	M	8 days	Light gray	Blonde		--
G 28	M	65	Light blue	Black	Black	+-
H 29	F	16 days	Light gray	Black		--
I 30	M	4 months	Gray-green	Black		--
L 31	F	2 days	Light gray	Red		--
M 32	M	60	Gray-green	Chestnut (partial achromachia)	Chestnut (partial achromachia)	+
N 33	F	23 days	Light blue	Blonde		--
O 34	F	73	Light gray	Gray (ex black)	Gray (ex black)	++
P 35	M	47	Chestnut (streaked)	Chestnut	Chestnut	+++ (streaked)
Q 36	F	53	Dark gray	Black (dyed)	Brown	+++
R 37	F	2 days	Gray-green	Blonde		--
S 38	M	4	Chestnut	Blonde		+
T 39	M	82	Gray-green	White (ex chestnut)	White (ex chestnut)	+
U 40	F	Fetus 7th month	Light blue	Chestnut		--

relative to the basic colors of the iris correlated with other data such as the hair system, sex and age of the subject.

Case G. 28: Male (65 years).

Light blue eyes, hair of head and pubic region black (Figure 1). The finding, insofar as concerns the pigment, is comparable to those relative to children 15 months old.

No evidence could be found of dendritic melanocytes of the "epidermal type" at the state of maturity. The few that could be isolated generally contained pale grains.

The infrequent grains present at the state of maturity are contained in the epithelial cells and in the very few melanocytes of the "dermal type" with usually a perivasal arrangement.

Case M. 32: Male (60 years).

Eyes gray-green, hair of head and pubic region chestnut (partial achromachia) (Figure 3).

Melanocytes of the "epidermal type" whereas those of the "dermal type" and even perivasal type are found in a greater number than in the preceding case.

The intraepithelian grains are few in number.

Case Z. 21: Female (62 years).

Eyes light gray, hair of head and pubic region chestnut (partial achromachia) (Figure 4).

There are present in a more considerable number voluminous melanocytes of the "epidermal type" in various phases of maturity together with a discrete quantity of melanocytes of the "dermal type".

There are greater abundances of intraepithelial granulations.

Case C. 3: Female (58 years).

Dark gray eyes. Black hair (dyed), hair of pubic region blonde (initial achromachia) (Figure 5).

In the stria vascularis corresponding to this coloration of the iris, there are represented in a greater number both types of the melanogenetic cells in various phases of maturity with a clear prevalent perivasal arrangement of the melanocytes of the "dermal type" and some of the "epidermal type". /734

There can be seen in these sections a great many dendrites going in various directions with infraepithelial and occasionally pericapillary arrangement.

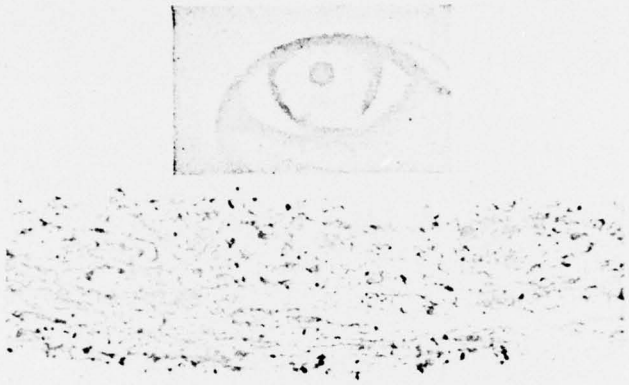

Figure 1. (Case G. 28) Male, 65 years. Light gray eyes, hair of head and pubic region black. Microphotography of the stria vascularis (II tract). 15 µ section. Magnification 800 x. Silver impregnation of Lignac.

The absence of dendritic melanocytes of the "epidermal type" at state of maturity is clear. Melanocytes of the "dermal type" are quite rare.

See description in text for more details.

The eye shown on the microphotograph does not belong to the same subject and therefore only has an indicational character.

Figure 2. (Case H. 8) Female, 43 years. Dark brown eyes, hair of head and pubic region chestnut.

Seat, section, staining, magnification identical to preceding figure.

The abundance of both types of melanocytes is clear; they are stacked together one another like a mosaic. Even the epithelial cells contain mature grains either isolated or in small groups in a quantity greater than other histological preparations examined.

For more details, see description in text.

Even in this case, the eye shown does not belong to the same subject and hence is only an indication of character.

Figure 3. (Case M. 32) Male, 60 years. Gray-green eyes, hair of head and pubic region chestnut (partial achromachia). /733

Seat, section, staining, magnification identical to preceding cases.

See description in text also for successive microphotographs.

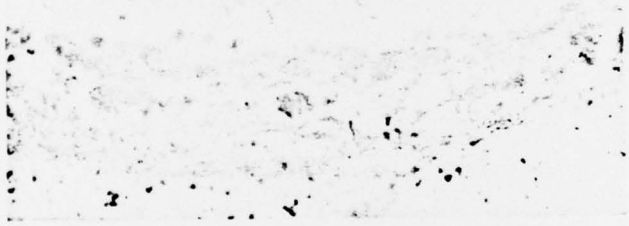

Figure 4. (Case Z. 21) Female, 62 years. Gray-light blue eyes, hair of head and pubic region chestnut (partial achromachia).

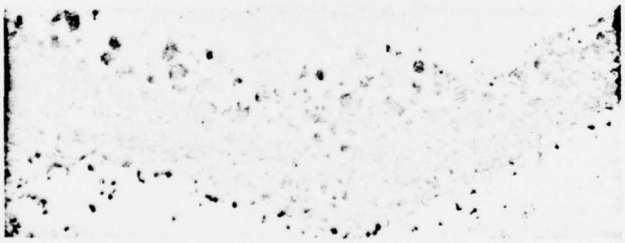

Figure 5. (Case C. 3) Female, 58 years. Dark gray eyes, black hair (dyed), pubic hair blonde (partial achromachia).

Case T. 18: Female (47 years).

Chestnut flecked (streaked) eyes. Hair of head and pubic region gray (from light chestnut) (Figure 6).

The arrangement in groups of melanocytes "of the epidermal type" is characteristic. They range from 2 to 4 in apparent syncytial union and in an advanced degree of maturity owing to being scattered with dark grains noted by the clear silvery appearance which it shows.

Alongside of these may be noted sectioned dendrites and, over the whole strial territory, a rather uniform distribution of melanocytes of the "dermal type" of which most appear with perivasal distribution.

The grains of melanin are only present in the epithelial cells.

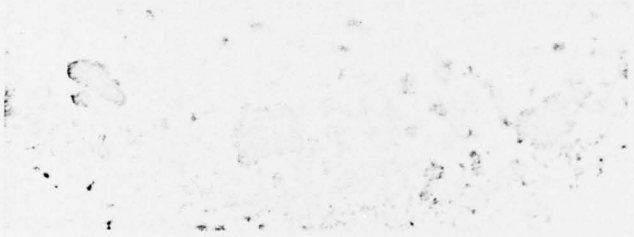

Figure 6. (Case T. 18) Female, 47 years. Gray flecked brown eyes (streaked), hair of head and pubic region gray.

Case H. 8: Female (43 years).

Dark brown eyes. Hair of head and pubic region chestnut (Figure 2).

There is a clear abundance of melanocytes of both types which appear alongside one another somewhat like a mosaic.

There can be seen elements in various degrees of activity. Commonly, there is a granular content with an intense dark coloration. However, a number of melanocytes appear to have hardly passed into the context of the tissue owing to the slight argentaffin condition of the granules of melanin which these contain. In addition, the sections of dendrites alongside these elements have a pigmentary content with the same color characteristics of the remaining cellular body.

This varying degree of maturity may either arise owing to melanocytes of the dendritic "epidermal type" or owing to those of the "dermal type".

In this preparation, there is also clear the typical perivasal arrangement of many of these last pigmented cells.

The epithelial cells also contain mature isolated granules or small groups in a quantity greater than in the other histological preparations examined.

FINAL CONSIDERATIONS /735

The reports shown refer, as has been said, to the stria vascularis in correspondence with the second cochlear tract.

The preference has been given for this particular tract of the spiral because we have seen that the latter, even in humans, shows an intermediate pigmentary content between that of the apex and that of the base of the cochlea.

This allows studying the morphology of the elements of the melanogenetic system with greater clarity as far as in cases of considerable pigmentation as is confirmed in subjects with very dark irises, the piling up of melanocytes in the apical section does not allow analyzing the other structures which have been masked by the accumulation of pigment.

In addition, the basal section hardly lends itself to this research since it is sparsely pigmented and shows little.

Hence, the stria-iris pigmentary relationship can easily be seen and almost at cochlear level.

From the histological reports of the 40 petrous pyramids examined, we have been able to establish from the data that, although they do not have an absolute value since there was a limitation in the number of subjects controlled, they allow deductions of a general character because our observations more often than not did not concern phenotypically similar individuals having about the same age.

The most important fact is that there is truly a correlation between the intensity of the coloration of the iris and the concentration of melanin in the stria vascularis. Indeed, from the findings of our research, it results that:

To the light colored irises (Figure 1), there corresponds a practically depigmented stria vascularis even in an advanced age and with black hair system;

To the dark irises (Figure 2), there corresponds a content of melanocytes and melanin which increases in quantity proportionally with an intense pigmentation of the iris.

Another fact which respects the fidelity of the distribution type of melanocytes of the "epidermal type" in the two territories is that to the flecked irises (streaked) (Figure 6) corresponding to the accumulation in the vasculo-connective-iris mesiris of melanocytes "joined in syncytium", there correspond groups of voluminous dendritic melanocytes of the "epidermal type" with a syncytial arrangement only in the stria vascularis.

This is still a morpho-constitutional sporadic pattern present at the level of all sections whereas, as has been stated in the preceding work mentioned above, "dendritic hypertrophic" melanocytes or the latter "fused together as a syncytium" are habitually reported in correspondence with the apical tract.

Still, as has been stated emphatically above, this pigmentary correlation between irises and the stria vascularis exists only in one specific /736
period of life and in subjects with normal pigmented irises whereas it has been shown to be absolute in subjects with light-colored irises whereby even late in life there has been no increase in the pigmentation of the stria vascularis.

Furthermore, it should be noted that the post mortem phaneroses of the pigment has been a phenomenon common to all the preparations except those in which the substrate was lacking for its own expression. This means in the stria vascularis corresponding to the clear irises (even at an advanced age), apart from in fetuses and in the newborn, even with normal pigmented eyes.

This confirms that the melanic pigmentation cannot occur owing to a degradation of proteins in the absence of tyrosinases.

Indeed, the histological preparations of petrous pyramids from albino guinea pigs, fixed even many hours after death, showed no argentaffin contrary to the normal pigmented ones in which this particular stain affinity is increased between certain limits proportionally to the time transpired between death and fixation.

The goal, of course, of our research was to establish in what relationships the concentration of melanin of the stria vascularis stood with the other surface pigmented formations in humans in order to have a reference index whence it would be able to catalog, with a certain approximation, constitutional types with noteworthy "melanogenetic potential set" of the stria vascularis as a function of the significance recently attributed by us to the melanin in this seat.

The results of our experimentation make therefore possible such evaluations and, although clinical research in progress is giving results which can be applied to those encountered by us in guinea pigs, still in humans psychic interferences can in part mask the effect (by the influence which the neural-hormonal system has on the capillary permeability). However, statistically, this enzymatic constitutionally variable substrate will have its effect in the orientational prophylactic criteria of social medicine.

SUMMARY /737

The research was accomplished on 40 human petrous pyramids (from 6 month old fetuses to 82 years old adult, white) comparing melanin content in the stria vascularis, with the color of the iris and hair system.

The histological findings allowed the statement that in middle-aged individuals pigment concentration in the stria vascularis is always proportional to the one in the iris; it often differs, at any age, from the concentration in the hair system.

Therefore, iris color may be considered as a "test" to estimate quantitatively, in humans, melanin concentration in the stria vascularis. On account of the recent interpretation trends on the function of the melanin granule in the cochlea, it might be considered as a useful clue in preventive medicine.

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