CHAPTER IV

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Melanized leucocytes in induced depigmentation and psoralen-treated Bufo melanostictus

Wassermann[393] demonstrated the presence of melanin in the leucocytes in the blood of Amphibians and Reptiles by the highly specific ferrous iron technique of Lillie. Again, the neutrophils, some monocytes and lymphocytes have been observed to be DOPA positive[393]. More pigmented leucocytes were found in those amphibians suffering from the active skin-and subcutaneous infection than in normal animals. Wassermann[393] suggested that the leucocytes might transport enzymes capable of contributing to melanogenesis and they might gather preformed melanin and transport this to the reticulo-endothelial system where the melanin was reutilized by the lymphocytes and monocytes.

Pigmented leucocytes have also been observed in peripheral blood in normal man[393,394,395]. Again, melanin deposition has been observed in the placenta,[396] bone marrow,[397] heart, cerebellum and many other tissues of man[393]. Melanin deposition in lymph nodes has been observed in Bantu[398]. It has been suggested that melanins are circulated from the tissues where large amount of melanin are produced and carried by the mononuclear cells to deposit in the organs stated above.

Recently, from our laboratory, it has been reported that hydroquinone, a strong depigmenting agent can induce depigmentation in the skin and liver of Bufo melanostictus and psoralen, a pigmentogenic drug can induce back the pigmentation[154].

From these observations we were interested to observe the amount of leucocytes containing melanin in hydroquinone treated and psoralen treated toads.

Lillie (1957)[399] described that ferrous ion uptake was a reaction which was specific for melanin; melanins formed complexes with Fe^{++} which could be demonstrated by means of potassium ferricyanide; some part of the melanin complex formed chelate with Fe^{++} and Lillie suggested that this was o-quinhydrone configuration. We adopted this technique of Lillie to stain the melanins in the leucocytes.

Materials and Methods

Ten Indian male toads (Bufo melanostictus) were kept in a cage under tap water for 7 days and another twenty toads were kept in another cage and fed 50 µg hydroquinone/toad/day for 7 days. On the 8th day all the toads from the first cage and ten from the second cage were sacrificed. The remaining ten toads were fed 500 µg psoralen/toad/day for another 7 days. On the 16th day these toads were also sacrificed.

Blood was drawn from the hepatic portal vein in each toad and kept in siliconized sample-tube containing Na-citrate solution. The tubes were kept in rest for about 15 minutes after a shaking. Then a buffy coat of leucocytes was seen in the middle layer, red coat of erythrocytes in the lower layer and the plasma in the upper layer. The layer of leucocytes was shaken slightly to suspend the cells in the plasma; then the leucocytes in the plasma were pipetted out and transferred to a siliconized centrifuge tube and was centrifuged in very low speed for 1 min. Then the plasma (supernatant) was discarded. The leucocytes then were washed by 0.1M phosphate buffer for three times. Lastly, the leucocytes were suspended in few drops of the buffer and made then viscous by adding a small quantity of bovine serum albumin. Smear was prepared in the slides by this viscous buffer containing leucocytes. Then the slides were kept for few minutes in air for drying the smear.

Method of staining

Staining was made by the ferrous iron technique of Lillie[399]

(i) Slides were rinsed by water

(ii) Immersed for 1 hour in 2.5% ferrous sulphate ($FeSO_4$, $7H_2O$)

(iii) Washed for 20 minutes in four changes of distilled water.

(iv) Immersed for 30 minutes in 1% potassium ferricyanide in 1% acetic acid.

(v) Washed in 1% acetic acid

(vi) Dehydrated and cleared

Results

Dark-green melanin granules inside the leucocytes were observed under the compound microscope (magnification : 40 x 12.5); the background was faint green in colour. The leucocytes containing melanin granules were counted and percentage of the melanized leucocytes in each slide was calculated, which are tabulated below (Table 20, 21, 22).

Table 20. Percentage data of melanized W.B.C. in control toads

No. of toads	Percentages of melanized W.B.C.	No. of cells counted in each toad	Mean percentage ± S.D.
1.	18%	500	
2.	13%	500	
3.	16%	500	
4.	16%	500	12.1 ± 4.1
5.	12%	425	
6.	9%	700	
7.	12%	500	
8.	7%	350	
9.	6%	400	

Table 21. Percentage data of melanized W.B.C. in hydroquinone treated toads

No. of toads	Percentages of melanized W.B.C.	No. of cells counted in each toad	Mean percentage ± S.D.
1.	3%	500	
2.	7%	500	
3.	2%	500	
4.	2%	500	3.37 ± 1.99
5.	2%	1500	
6.	3%	1500	
7.	2%	300	
8.	6%	500	

Table 22. Percentage data of melanized W.B.C. in Psoralen treated toads

No. of toads	Percentages of melanized W.B.C.	No. of cells counted in each toad	Mean percentage ± S.D.
1.	8%	1500	
2.	6%	1500	
3.	5%	700	6.83 ± 2.22
4.	8%	800	
5.	4%	600	
6.	10%	400	

Discussion

It is evident from the results that the percentage of melanized leucocytes decreased in hydroquinone treated toads and increased (although not to normal level) after psoralen treatment to the hydroquinone treated toads. Chen[152] has described the effect of hydroquinone on the melanocytes in this manner : (i) Clumping of melanin granules, (ii) cytoplasmic vacuolation, (iii) pinching-off of the dendrites and (v) ultimately cell lysis. Now, the leucocytes after the low dose of Hydroquinone treatment show another type of peculiarity, - the leucocytes of these animals have been found to be more swellen than those of normal and psoralen treated animals. Again, the plasma has been found to contain numerous melanin granules. Again, among the other functions of W.B.C. one function is the transport of many substances from one organ to another organ, which is possible because these cells can undergo exocytosis and endocytosis. From all these facts it can be postulated that hydroquinone stimulates the exocytosis and psoralen stimulates the endocytosis of the leucocytes. This result of induced leucoderma may simulate that of vitiligo leucocytes.