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ON THE PORPHYRIN NATURE OF THE FLUORESCENT "BLOOD CAKED" WHISKERS OF PANTOTHENIC ACID DEFICIENT RATS

WEANLING rats fed a diet deficient in pantothenic acid develop, in a period of four to six weeks, peculiar symptoms characterized by the accumulation of red material around the nose and on the whiskers. Because of the superficial resemblance of this red material to coagulated blood the condition has been described as "nose bleed"[1] or "blood caked" whiskers.[2] However, no thorough studies on the chemical nature of the deposited red material have come to our attention. We have investigated the nature of the red material from a chemical point of view and have endeavored to trace its origin in the pantothenic acid deficient rat.

The red deposits on the nose and whiskers of pantothenic acid deficient rats exhibited a bright red fluorescence in ultraviolet light. An ultraviolet lamp (G.E.B.H.$_4$) was used as a light source. Hemochromogen tests showed that only a minute quantity of the material is hemoglobin. A large proportion was found to be a coproporphyrin. The heavy red deposit around the mouth and on the whiskers of several pantothenic acid deficient rats was collected by washing with water slightly acidified with acetic acid. Zeile and Rau's[3] modification of H. Fischer's extraction method for porphyrins was followed. The completeness of the extraction was controlled with the aid of ultraviolet light. The red fluorescent material was completely driven into 0.54 per cent. HCl (coproporphyrin fraction) and showed after washing with chloroform the following spectrum in 25 per cent. HCl: I 551 mμ II 570 mμ III 594 mμ; the spectrum of its copper salt in pyridine was : I 565 mμ II 528 mμ. These spectra were compared with those of a sample of synthetic coproporphyrin I[4] and its copper salt respectively and proved to be identical under the experimental conditions. The spectroscopic measurements were carried out with the aid of a Zeiss pocket spectroscope, equipped with a wave-length scale.

Since it has been shown that the Harderian glands in rats exhibit a red fluorescence, and contain and secrete porphyrin[5,6] it was assumed that the fluorescent material might pass to the nose and whiskers of the rats by way of the naso-lacrimal duct. An attempt to test this hypothesis revealed a fluorescent naso-lacrimal duct in a pantothenic acid deficient animal. It therefore appeared likely that the source of the porphyrin deposit was the Harderian gland. The Harderian glands were removed from eleven weanling albino rats; to insure complete removal of fluorescent tissue the operation was performed under ultraviolet light. The animals were then placed in all-glass cages and fed a diet deficient in pantothenic acid. Seven unoperated controls subsisting on an identical diet all developed fluorescent porphyrin deposits on the nose within six weeks. The eleven operated animals developed the other known symptoms of pantothenic acid deficiency, but no fluorescent red-colored material appeared on the nose, whiskers or fur.

Three grams of the fresh Harderian glands (from 17 animals) were extracted for porphyrins. In agreement with Derrien and Turchini[5] the main fraction appeared to be protoporphyrin. However, a very small fraction could be extracted with 0.54 per cent. HCl, and this was identified spectroscopically as coproporphyrin. The spectrum in 25 per cent. HCl was : I 553 mμ II 595 mμ; the spectrum of the copper salt in pyridine was I 565 mμ II 530 mμ under the conditions of our experiments.

We therefore conclude that the red deposit around the nose and on the whiskers of pantothenic acid deficient rats is not blood but coproporphyrin and that this is derived from the Harderian gland.

After this paper was submitted for publication it was noted that Chick, Macrae and Worden[7] had attempted to characterize the reddish exudate which accumulates on rats deprived of vitamin B_2 factors. Our own independent and more exhaustive investigations confirm their observation that the material is not blood, and that it contains large amounts of porphyrin. They state that the material contains protoporphyrin. The data submitted above identify the porphyrin washed from the whiskers and fur of our rats as coproporphyrin.

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