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RADIATION EFFECTS IN SWINE.
I. Vascular Supply of the Skin and Hair

by
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The Problem:

Due to the high density of the hair coat, the skin of common laboratory animals is not suited for radiobiological experiments which are designed to simulate the responses of human skin. Densely furred skin, for example, appears incapable of the erythema response which characterizes the early radiation syndrome of human skin.

For decades, the skin of certain domestic swine has been recognized as having several features in common with that of man, including its relative nakedness. However, research reports on skin structure and function of swine contain contradictory statements regarding the sweat glands and the distribution of blood vessels in the dermis and hair follicles. In order to evaluate some aspects of the radiation response of swine skin, it has been necessary to investigate the vascular structure of the four breeds under study.

The Findings:

Histologic preparations of skin from animals receiving an intravenous injection of colloidal carbon showed the dermal ridges of the papillary

layer to be invested with a dense feltwork of capillary loops. The dermis as a whole was well vascularized. Small vessels permeated the dermal papilla and the connective tissue sheath of growing hair follicles. In contrast, the sebaceous glands and sweat glands were very sparsely vascularized.

In the transition from anagen to catagen growth stages, whether spontaneous or X-ray-induced, the hairs of swine retained a dense, functional, vascular network, confirming the hypothesis that early catagen substages precede any significant vascular shutdown.

ABSTRACT

The densely haired skin of most laboratory animals provides an excellent experimental model for certain applications, but the exclusive use of furry animals imposes some severe limitations on the information that could be derived regarding the response of skin which is less furry to a variety of traumatizing stimuli. Among readily available animals, swine have often been considered to have a cutaneous anatomy most ideally suited to research relating to human skin maladies. Four breeds of swine were used in several experiments designed to clarify some aspects of their cutaneous structure and function.

The characteristic hair coat of the domestic swine used in these studies consisted of rather coarse, evenly grouped follicles. The density of hair coat decreased rapidly with increasing age and surface area, so that the number of hairs per square centimeter on a Yorkshire animal dropped from 150 to 31 while the weight increased from 10 to 50 kilograms. The more sparsely-haired Hanford-Labco animals had only 8-10 hairs per cm^2 at maturity.

Growing hairs had a rich vascular supply to the dermal papillae and around the bases of the hair follicle. During a spontaneous or X-ray-induced transition from anagen to catagen stages of the hair growth cycle, the vessels remained functional. In contrast, the large "apocrine" sweat glands had very few associated capillaries, possibly reflecting their

very limited secretory activity.

Sections of biopsy material taken from animals injected intravenously with small particles of colloidal carbon tended to confirm the impression that the size, orientation, and distribution of vessels in the skin of the swine and human are remarkably similar. The further application of the skin of swine to problems involving radiation effects has been undertaken, and will be reported separately.

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INTRODUCTION

Several investigators in the area of research on radiation effects in skin have pointed out the need for a more appropriate experimental model (see References 1,2). Late appearing effects, in particular, have been problematic in any effort with experimental animals. The use of rodents in many laboratories has provided an extraordinary amount of data on several aspects of life-science, including the development, structure, and function of skin growths and appendages. Although research literature will undoubtedly continue to be strongly influenced by rodent-oriented programs, the exclusive use of furry animals would impose some severe limitations on the problems that could be investigated. As has been pointed out by many investigators, the densely haired skin of laboratory animals responds quite differently from glabrous or sparsely haired skin to a variety of traumatizing stimuli.

Our attempts to employ a more suitable model for investigating the acute and chronic effects of ionizing and non-ionizing radiation have set our attention on some of the structural and functional attributes of the skin and hair of swine. Just how ancient are the comparisons of the human and the pig would be very difficult to say, and whether the likenesses that have been found are comforting or distressing depends on the basis and the rationale for making the comparison.

As far as publication is concerned, one of the earliest systematic efforts at comparison was that of Thomas Hooke in his book entitled, Micrographia, published over 300 years ago. It is common knowledge that Hooke's "little boxes" in thin slices of cork received the name "cells." Something not so widely realized is the fact that Hooke made a careful comparison of the structures of various mammalian hairs, including those from his own scalp, bristles from a hog, whiskers from a cat, and various hairs from horses and deer. His detailed illustrations and his witty commentary on his observations are a treasure in the archives of anatomy. A recently released facsimile edition of his book preserves the illustrations and the commentary intact (3).

Experiments on wound healing and on the effects of external sources of radiation have accounted for many of the significant works on the skin of swine appearing in the 300 years since Hooke's time. Pinkus (4,5) reported on the tactile Haarscheiben, Brownell (6) reviewed radiation effects on the skin of swine and reported original findings on solar changes, and Hartwell (7), and Ham (8) used swine in wound healing experiments. Several other classic works are cited by Marcarian and Calhoun (9), and references to recent experimental work are found in volumes by Pekas and Bustad (10) and Bustad and McClellan (11). An annual collection of references on swine work keeps interested parties current on the subject (12). The most detailed survey of the microscopic structure of the skin of swine is probably that of a group at Michigan State University. In a series of papers, they have reported the structural detail of the skin of the pig from the fetus to the adult (13,14, and 9).

While none of these groups viewed the skin of the swine as identical with that of the human, they considered it remarkably similar to the human, and far more like the human than is the skin of any other readily available and reasonably tractable animal.

The first histochemical survey comparing swine and human skin was reported by Montagna and Yun (15). Striking differences in structure (e.g., sweat glands; friction areas), in the location of enzymes (e.g., alkaline phosphatase), etc., led them to conclude that comparisons of porcine and human skin have been vastly overrated. Furthermore, they report that the dermis of the pig is poorly vascularized in contrast to that of man, and that the pilo-sebaceous apparatuses of man are richly vascularized, but those of the pig are not. In contrast, Moritz and Henriques (16) had previously stated that, while anatomical resemblance may not necessarily denote equivalent functional similarity, the number, size, distribution and communications of the dermal blood vessels of the pig are remarkably similar to those described by Spalteholz (see Bloch et al., 17) in the human skin.

Since vascular reactions play an important part in the response to radiation exposure of skin, the work reported here was undertaken, in part, to determine the distribution of skin and hair vessels in our breeds of swine. The acute and chronic changes resulting from X-ray and ultraviolet radiation exposure, and the influence of skin pigmentation, will be reported separately.

METHODS OF STUDY

Observations reported here are based on the use of 22 swine, both females and castrate males. They represented four different backgrounds: Duroc, Landrace, Yorkshire, and Hanford-Labco "miniature."

Radiation exposures were carried out while the animals were either under pentobarbitol-sodium anesthesia or restrained in a holding device. The sources of X-rays were a Picker 280-KV Therapy Machine and a General Electric 1000-KV Generator.

At the end of the experiment period, animals were catheterized via the superior vena cava, heparinized, and infused with a concentrated carbon suspension (Pelikan C11/1431a). The material is useful as a contrast medium in light microsocpy for visualizing blood vessels (see Halpern, et al., 18).

Biopsy and autopsy materials were fixed in neutral unbuffered formalin, and processed routinely through a series of standard histological stains. Thick frozen sections were cut in a cryostat and mounted on glass slides or embedded in methacrylate plastic.

Hairs were plucked from locally- and whole-body irradiated animals at several dose levels. The hair roots are being analyzed for morphologic changes and the results will be reported separately.

Four methods were employed for estimating the hair density:

1.) Cross sections of hairs were counted in histologic sections of skin cut parallel with the surface.

2.) The stubs of hairs left behind after electrical clipping were counted on a surface area of 20 cm^2.

3.) Hair germs were counted after being plucked from an area of 20 cm^2.

4.) Casts of the skin surface were prepared by covering an area with a 3mm-thick cast of self-suring transparent rubber (General Electric "Clear Seal" R), for ease in counting hair locations at a later time.

Since the data from the four methods were in good agreement, the results are reported without distinction as to technique.

RESULTS

A. The General Vascular Picture

In the pig, the large vessels entering the subcutis were seen to send branches supplying the reticular layer, the more superficial papillary layer, and the various epidermal appendages (Fig. 1). Dense accumulations of the smallest vessels were restricted to the papillary body and to the hair follicles.

One or more capillary loops were found in each dermal ridge, along the highly convoluted dermal-epidermal junction (Figs. 1-2).

In thick sections cut parallel with the surface of the skin, the distribution of the capillary loops formed a regular pattern in the inter-follicular areas (Fig. 3). Estimates of the density and distribution of the loops were made from several specimens of skin taken from the back.

The range was 60 to 75 loops per square millimeter, a figure which compares favorably with Lewis' counts of human skin (19). The distribution and density of superficial vessels in such specialized areas as the pinna, the snout, the vulva, etc., have been studied, and will be reported elsewhere.

The superficial vessels were highly reactive to radiation exposure, and an intense erythema developed under the circumstances of radiation exposure described below.

B. The Pilosebaceous Unit

The dermal papilla of the growing hair follicle contained a dense tuft of capillaries (Figs. 4,5,6). In addition, the lower portion of each hair follicle was encased in a rich network of fine vessels, which diminished rather abruptly (Figs. 5,6). In contrast, the sebaceous glands, and the sweat glands (Fig. 6) were relatively poorly vascularized.

The dermal papilla vessels, as well as those of the connective tissue sheath, remained functional after catagen had commenced (Fig. 7, 8). An experiment was undertaken to determine whether the same would be true of follicles in which catagen was artificially induced. Several skin specimens were removed from the backs of pigs which had been locally irradiated seven days previously. The radiation factors were: 280 kvp X-ray, no added filtration, target-to-skin distance 36 cm, HVL 1 mm Cu. The doses used were 350 R, 700 R, and 3500 R, each to an area measuring 6 cm by 8 cm. Histologic sections revealed that hairs were in catagen stage in the three upper dose levels, that the degree of effect was stepwise, and that it was proportional to the dose. While certain features of radiation-induced epilation were clearly distinct from spontaneous catagen, one aspect was comparable: a functional vascular supply was clearly present after normal growth (anagen) had ceased.

C. Hair Growth

When comparing such features of the hairs as color, density, distribution, and hair length, the differences resulting from age and breed characteristics were striking. Cases of opposite extremes were represented by young swine from commercial breeds, with a relatively dense pelage, and mature animals of the Hanford-Labco "miniature" variety, bearing a very sparse hair coat. Surface counts of growing hairs on two Yorkshires weighing 10 kilograms each averaged 150 per cm^2. The majority of follicles were in groups of three, and the growth rate was approximately 3 mm per week. The hair density decreased with increasing age (and surface area), so that the same regions, when body weight reached 50 kilograms, averaged 31 hairs per cm^2.

On four Hanford-Labco animals, the hair density at all ages was significantly less, exposing the skin to view. The hairs appeared to be regularly spaced, lacking the triad arrangement so obvious in commercial breeds. The apparent hair sparseness was confirmed in surface counts (Table I).

DISCUSSION

While the distribution and orientation of the components of the cutaneous vascular system are not obvious in standard histological preparations, there are, nevertheless, several useful techniques available for their demonstration. The introduction of an opaque material into the circulating blood, followed by visualization through dissection and sectioning, has proven to be useful under a wide variety of circumstances. One variant, that of employing colloidal carbon, was chosen for this project.

Two other techniques involve the induction of cutaneous hyperemia followed by staining of the lumenal contents, and the histochemical localization of enzymes in capillary walls. In papers reporting information on the vessels of porcine skin, Moritz and Henriques (16) used the former technique and Montagna and Yun (15) used the latter. Mortiz and Henriques concluded that the anatomical features of swine vessels bore a striking resemblance to those described in the human. In contrast, Montagna and Yun found the two to be dissimilar, intensifying their impatience with the "fantasy that the skin of the pig resembles more that of man than that of any other mammal." Whether the contrasting conclusions are the results of evidence gained through the use of different approaches, or different experimental material, or interpretation of data, is not clear. The observations presented in this paper would appear to support the conclusion that the cutaneous vascular anatomy, and at least certain vascular reactions, are very similar in man and in the domestic pig and that each is unlike that of most other mammals (see References 8,20).

The tendency for the body hairs of the pig to be arranged in groups of three has been noted by Flatten (21), Pinkus (4), Marcarian and Calhoun (9), Winter (22), Carter (23), and others. Triads were easily demonstrable in our commercial breeds, but they were not generally in evidence in the more sparsely haired Hanford-Labco "miniature" variety. The sparseness, and the generally shorter hair, combined to produce an animal bearing a distinctly denuded appearance.

As in the human, the hairs of swine have a network of small vessels associated with the lower portion of the follicle and its dermal papilla. In fact, the hairs of the swine are consistently coarse, and their vascular anatomy undoubtedly has many features in common with the <u>large</u> hairs of most other mammals (for a review on the subject, see Reference 24). Consequently, it is particularly striking that the remainder of the pilosebaceous unit is so sparsely vascularized. The diminutive sebaceous glands of females and castrate males have few vessels, and only scattered capillaries are visible in the coils of the sweat glands (Fig. 6). It is noteworthy that the experiments of Moritz and Henriques (16), contrary to Ham's (8) assumption, indicate a lack of significant secretory activity in the sweat glands of swine.

As in the human, swine hair follicles retain a normal-appearing vascular supply to the papilla even after catagen has commenced; the bulb leaves behind a dense skein of functioning vessels. Ellis and Moretti (25) showed that, in human scalp hairs, the erosion of the lower bulb, constriction of the external sheath, etc., were evident when the blood vessels within the papilla were still patent. Through the use of the infusion technique reported here, it is clear that such vessels still have blood flow in both spontaneous (Fig. 7,8) and radiation-induced catagen.

It seems evident that skin-oriented research has benefited from the likenesses encountered when human and porcine integument are compared. Whether the similarities of swine and other mammals, or the dissimilarities of humans and pigs, seem more important, will undoubtedly depend on the uses to which each is put. The anatomical relationships may prove to be of phylogenetic interest; the practical application of swine skin as an experimental model will undoubtedly be further exploited.

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TABLE I

COMPARATIVE HAIR DENSITY

EFFECT OF AGE, BREED, BODY AREA

BREED	BODY WEIGHT (Kg.)	APPROX. HAIR LENGTH	ACTIVE HAIRS PER cm^2
Yorkshire	10	4 cm	150
Yorkshire	50	4 cm	31
Hanford-Labco Miniature	90	6 cm (Mid-dorsum)	8
Hanford-Labco Miniature	90	2 cm (Lateral Surface)	10


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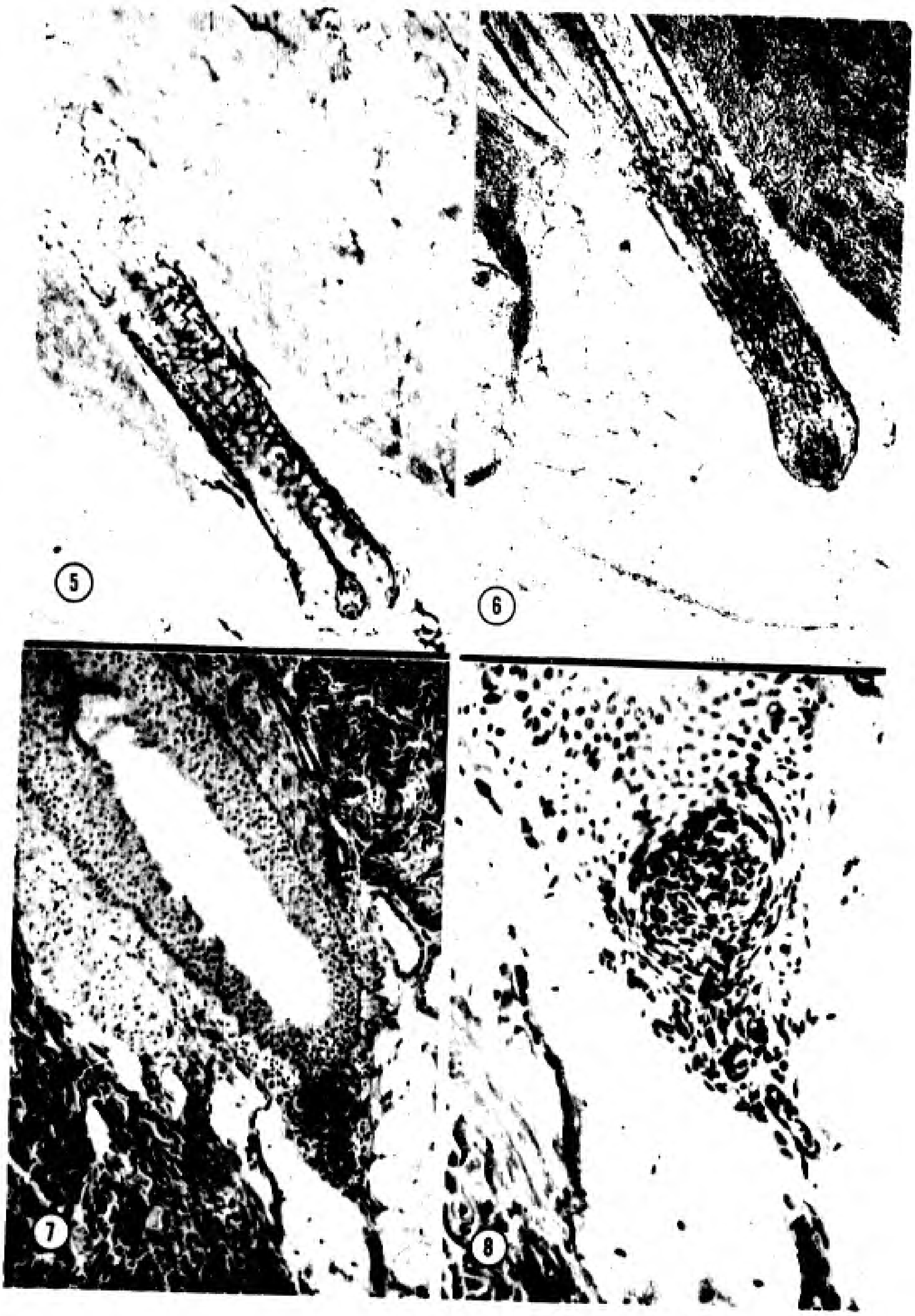
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FIGURE LEGENDS

Figure 1. Back skin from a pig injected intravenously with colloidal carbon. Capillary loops are visible in the dermal ridges, with vessels in the deeper dermis below. Frozen section, 150 microns thick; nuclear fast red stain (X 50).

Figure 2. Epidermis and papillary body from same region as Fig. 1. Superficial vessels are made prominent by carbon injection. Paraffin section, 6 microns thick; hematoxylin and eosin (X 300).

Figure 3. Thick section of back skin, cut parallel with surface, viewed from outer surface. Capillary loops are visible in each dermal ridge (X 300).

Figure 4. Lower bulb of growing hair follicle. Dermal papilla and connective tissue sheath are richly vascularized. H & E (X 300).

Figure 5. Lower half of growing hair follicle. Carbon-filled vessels invest the dermal papilla and form a plexus around base of follicle. Dermal papilla was exposed by shaving away the portion of the lower bulb facing viewer. Frozen section, 150 microns thick; no stain (X 50).

Figure 6. Thick frozen section of skin, with lower hair bulb intact. Rich vascular supply to the follicle contrasts with sparse

vascularity of adjacent coiled sweat gland. No stain (X 50).

Figure 7. Section of lower hair follicle, catagen stage. Detail of dermal papilla in Figure 8. H & E (X 120).

Figure 8. Detail of dermal papilla from Figure 7. Vascular supply to the follicle is still intact, as evidenced by presence of circulating carbon particles (X 300).

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