

## Glycogen in the Epidermis of the Dolphin *Tursiops truncatus*

Glycogen, a carbohydrate storage product, can be demonstrated by histochemical staining methods in the epidermis of the foetal mammal<sup>1</sup> and can be seen in the human epidermis up to the sixth month of gestation. But in later embryonic life and in the adult, glycogen can only be found in the hair follicles and sweat glands<sup>2</sup>. Sensitive chemical procedures have detected small amounts of glycogen in the normal human epidermis<sup>3</sup>.

Although glycogen cannot be demonstrated histochemically in the normal adult epidermis, if the epidermis is injured glycogen is found in large amounts. The type of injury has no effect upon the appearance of glycogen: a hot burn in human<sup>4</sup>, cold burn with solid carbon dioxide<sup>5</sup>, X-ray<sup>6</sup>, sunburn<sup>7</sup>, or a simple surgical incision. When a portion of the epidermis is destroyed the adjacent epithelial tissue will regenerate and glycogen is particularly evident in this regenerating epithelium. The function, origin, and significance of this glycogen is obscure<sup>8</sup>. Since the glycogen containing cells of hair follicles and sweat glands may be implicated in the appearance of this carbohydrate in wound tissue, it is of interest to examine the epithelium of the dolphin, an essentially hairless mammal.

The opportunity arose to examine 3 dolphins very soon after death (1–4 h). The dolphins (*Tursiops truncatus*) were captured in the Gulf of Mexico within 12 miles off the Texas coast. They were transported to an oceanarium (Sea-Arama of Texas, Galveston, Texas) and placed with domesticated dolphins to undergo acclimatization. The animals were captured at different times and were of different sexes. The males weighed 140 lbs and about 200 lbs, the female about 220 lbs. Two of the animals died about 2 weeks after capture, but 1 male had been in captivity for several months. Death of the males was attributed to pneumonia<sup>9</sup>. All of the animals had a number of trematode parasites in the second stomach; both the males had small granulation loci in the lungs. One male had 5 unidentified tape worms, one of which was more than 15 feet in length. The trematodes in the stomach were identified as *Braunina cordiformis* (WOLF 1903). All animals had small abrasions, due to confinement. Both of the males had a small injury at the ventral anterior end of the snout; this had been caused by the animals scraping the bottom of the tank whilst retrieving fish. The female had a small abrasion on the far posterior ventral surface, presumably caused by scraping against the bottom of the tank. The wound tissue was removed, placed on ice, transferred to the laboratory and stored in a deep freezer at  $-45^{\circ}\text{C}$ . The tissue was removed from the low temperature storage and small pieces were fixed for 24 h in acetic acid-alcohol solution (1:9 acetic acid: 70% ethanol). The skin was then embedded in paraffin wax, and sections cut at  $6\ \mu$ . Sections were stained by the Periodic Acid Schiff method<sup>10</sup>, control slides were incubated in 1% diastase for 1 h at  $37^{\circ}\text{C}$  prior to staining.

Periodic Acid Schiff (PAS) positive material was found in the epithelial cells in the wound area, this material was not observed after incubation with diastase. PAS positive material was not observed in the normal epithelium removed from the dorsal surface on the animals, nor in the epidermis adjacent to the wound area.

Granular intracellular material which stains with the Periodic Acid Schiff reagent and which is removed by diastase is identified as glycogen<sup>10</sup>. It is therefore concluded that glycogen is present in the regenerating epidermis of the dolphin and that the glycogen containing cells of the hair follicles and sweat glands are not responsible for the glycogen found in mammalian wound tissue. The occurrence of glycogen would further suggest that

the carbohydrate metabolism of the epidermis of this hairless marine mammal is similar to that of the common laboratory mammals and that epidermal regeneration and wound healing are similar in the dolphin and man. Although the occurrence of glycogen in the regenerating epithelium has been reported from several mammals, there is no indication of its significance. It is an apparent anomaly that a storage product can be demonstrated in the cells of a tissue which is regenerating but not in homologous cells that are normal and apparently less active.

Several explanations of this phenomenon have been proposed. BRADFIELD, 1951<sup>5</sup> suggested that the accumulation of glycogen in wound tissue was due to the wound environment being anaerobic; SCOTHORNE et al., 1953<sup>11</sup>, suggested that glycogen was a degeneration product. WASHBURN and BLOCKER, 1954<sup>4</sup>, questioned these explanations as they found glycogen at the edge of skin explants in tissue culture; ADACHI, 1962<sup>6</sup>, has pointed out that the accumulation of glycogen must be viewed in terms of a dynamic equilibrium in a small rapidly turning-over pool and that the alteration of any one of several reactions might disturb the turnover rate and lead to accumulation of glycogen. WILLIAMS, 1967<sup>12</sup> has carried this idea further and has suggested that glycogen accumulation may be related to the resistance of the epithelial cells of a wound to Epinephrine (Adrenalin) as has been described by BULLOUGH, 1962<sup>13</sup> in relation to mitotic control in wound healing<sup>14</sup>.

**Zusammenfassung.** Das Kohlehydrat-Speicherprodukt Glykogen wurde nach Verletzungen im Regenerations-epithel des Delphins *Tursiops truncatus* gefunden, was darauf hinweist, dass Glykogen, das in der regenerierenden Epidermis anderer Säugetiere gefunden wurde, nicht vom Glykogenvorrat der Haarfollikel stammt.

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<sup>2</sup> W. MONTAGNA, *The Structure and Function of Skin* (Academic Press, New York 1962).

<sup>3</sup> K. M. HALPRIN and A. OHKAWARA, J. invest. Derm. 46, 43 (1966).

<sup>4</sup> W. W. WASHBURN and T. G. BLOCKER JR., Plastic reconstr. Surg. 14, 393 (1954).

<sup>5</sup> J. R. C. BRADFIELD, Nature 151, 40 (1951).

<sup>6</sup> K. ADACHI, J. invest. Derm. 37, 381 (1961).

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<sup>8</sup> R. K. FRIENKEL, in *The Epidermis* (Eds W. MONTAGNA and W. C. LOBITZ; Academic Press, New York 1964).

<sup>9</sup> Autopsy performed by Dr. K. G. WEISS, Department of Pathology, University of Texas Medical Branch.

<sup>10</sup> A. G. PEARSE, *Histochemistry, Theoretical and Applied* (J. A. Churchill, Ltd., London 1960).

<sup>11</sup> R. J. SCOTHORNE and A. W. SCOTHORNE, J. Anat. 87, 22 (1953).

<sup>12</sup> J. P. G. WILLIAMS, J. Cell Biol., in press.

<sup>13</sup> W. S. BULLOUGH, Biol. Rev. 37, 307 (1962).

<sup>14</sup> This investigation was supported by U.S. Navy Contract No. ONR 1598 (05).