

bilden stellenweise im Schnitt zapfenartig aussehende Formationen, die sich radiär weiter teilen und auch dort auftreten, wo die Retina flach aufliegt (Figur 3, 4). Im Bereich der retinalen Falten kann man fingerförmige Aufspaltungen der Gebilde sehen. Die Zapfen lassen sich nur mit der Heidenhain-Färbung darstellen (HEGNER<sup>6</sup>).

*Lutra lutra* Linnaeus (Carnivora, Fissipeda, Mustelidae). Das Auge des Fischotters fällt unter anderem durch die stark ausgeprägte Irmuskulatur und das Tapetum lucidum cellulare auf. Mit gewöhnlichen Färbungen kann man in der Retina 2 Zellarten in der äusseren, 3 in der inneren Körnerschicht und 3 im Stratum ganglionate unterscheiden. Die Ganglienzellschicht ist zellreicher als bei den vorher beschriebenen Arten. Die Retinafalten (Figur 5) sind stark ausgeprägt, ubiquitär vorhanden und in der hinteren Bulbushälfte höher ausgebildet. Es sind wie beim Flusspferd und Weisswal alle Schichten der Netzhaut mit Ausnahme des Pigmentepithels an der Faltenbildung beteiligt. Im Gegensatz zu den vorher

beschriebenen Arten sind selten Gefässe am Scheitel der Falte zu sehen.

Wir können zusammenfassend sagen, dass die Retinafalten bei allen 3 untersuchten Arten ähnlich ausgebildet sind. Sie sind bei *Lutra* am stärksten ausgeprägt. Unterschiede finden sich vor allem im Auftreten der Scheitelfgefässe, die bei *Lutra* praktisch fehlen. Die Falten kommen bei Vertretern von drei Säugerordnungen (Cetacea, Ungulata und Carnivora) vor und wurden bei den Landsäugetieren bisher nicht beobachtet. Wir interpretieren sie als eine konvergente Erscheinung, als Ausdruck der morphologischen Anpassung mancher Säuger an die aquatische Lebensweise. Die genauen physiologischen Bedingungen für diese Vorrichtung kennen wir nicht. Vielleicht spielen der Wasserdruck beim Tauchen und nachfolgende Schwankungen des Augenvolumens eine Rolle. Es bleibt noch zu bemerken, dass der kanadische Biber (*Castor canadensis* Kuhl, Rodentia, Castoridae), eine dem Wasser vorzüglich angepasste Nagerart, die beschriebenen Falten der Retina nicht aufweist (PILLERI<sup>7</sup>).

**Summary.** The folds found in the retina of 3 orders of mammals, the white whale, the hippopotamus and the otter, are described. All the layers of the retina take part in the folds, with the exception of the pigmented epithelium. The folds are a morphological adaptation to aquatic life.

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Fig. 5. Retinafalten beim Fischotter, *Lutra lutra* (Färbung: HE).

<sup>6</sup> BARBARA HEGNER, Acta zool., Stockh., im Druck.

<sup>7</sup> G. PILLERI, Acta anat., Suppl. 42, 47 (1961).

## Enhanced Healing of Skin Wounds in Dogs with Systemically and Locally Administered Drugs<sup>1</sup>

Many drugs are marketed for the purpose of accelerating healing of skin wounds<sup>2</sup>. However, efficacy has not been adequately documented<sup>3</sup>. This comparative study of drugs causing a variety of physiological changes in skin was designed to reveal the more effective agents, which in turn might suggest a rational basis for future investigations of other drugs.

**Methods.** Dogs fed a commercial dry dog food<sup>4</sup> were housed individually and given several days to adapt to their new surroundings. The 23 mongrels weighed 11–29 kg. Surgery and measurement of wound tensile strength were performed aseptically under sodium pentobarbital anesthesia.

In a study of systematically administered drugs, each dog had 1 dorsal mid-line incision at the base of the neck and another over the lumbo-sacral junction. 18 dogs were divided into 3 treatment groups of 6 each, with equal numbers of each sex. 5 mg of methandrostenolone<sup>5</sup> were given orally 30 min prior to surgery and once a day for the next 3 days. Another treatment group received 500

U/kg of heparin s.c. at surgery and once each day for the next 3 days. A similar procedure was used with animals in a physiological saline control group.

In a study of locally administered drugs, 6 dorsal mid-line incision sites were chosen in the lower thoracic-lumbar region. Incision sites were separated by 2 cm and the sequence of therapy in the 5 males was controlled by Latin square design. At the time of wound tensile strength measurement, a new incision was made and a new treatment begun. The treatment regimen was as follows: 2 ml of physiological saline, copper sulfate (1%), or phenylephrine<sup>6</sup> (1%) were applied to the incision before tying

<sup>1</sup> Supported by N.I.H. General Research Support Grant. Published as departmental paper No. 553.

<sup>2</sup> H. B. HALEY, in *The Healing of Wounds* (Ed. M. B. WILLIAMSON; McGraw-Hill Book Co., 1957), p. 181.

<sup>3</sup> E. D. HOWES, in *La Cicatrisation* (Centre National de la Recherche Scientifique, Paris 1965), p. 209.

<sup>4</sup> Wayne Tail Wagger, Allied Mills Inc., Chicago, Ill.

<sup>5</sup> Dianabol, CIBA Pharmaceutical Co., Summit, N.J.

<sup>6</sup> Neo-Synephrine, Winthrop Laboratories, 90 Park Ave., New York, N.Y.

the sutures. Drugs were injected subcutaneously on the next day in the same amount; to minimize trauma, the injection was made through intact skin 2 cm lateral and ventral to the incision, with the needle tip resting under the incision. On the 2nd and 3rd days after surgery, 2 ml were applied on the skin's surface. The vitamin C preparation<sup>7</sup> was given similarly, except that 2 ml (200 mg calcium ascorbate) were given initially, followed by 1 ml injection on the next day and 1 ml applied topically on the 2nd and 3rd days. Fibrinogen powder<sup>8</sup> (5 g) was applied to the incision only at the time of surgery. Ethyl alcohol, 70%, was applied in the incision initially (1 ml), followed by a 1 ml s.c. injection on the next day and 1 ml applied to the surface on the next 2 days.

In all tests, the incisions were 3 cm long and were closed with 6 dermal sutures<sup>9</sup> (0.3 mm diameter). The incision edges were approximated loosely. The wound was bandaged with gauze and tape which completely encircled the body. Immediately after surgery, 300,000 U of long-acting penicillin<sup>10</sup> were injected i.m.

Wound tensile strengths were measured on the 4th day after surgery, with the dogs under general anesthesia and in sternal recumbency. A safety pin (4 cm long) was inserted s.c. parallel to and 2 cm lateral to each side of the incision, via a 20-gauge, sharp hypodermic needle. The pins were connected via 30-gauge wire (50 cm long) (parallel to the floor) to an anchor point on one side of the dog and to a pulley and weight pan on the other side. Interposed between the pins and connecting wire were metal rings which permitted a uniform pull on the safety pins. After sutures were cut and gently removed, the weights were gradually added to the pan until the incision ruptured. Although differing in some details, a similar procedure for survival tests has been advocated by previous workers<sup>11</sup>. Following measurement, the incision was trimmed and re-sutured for the dog's comfort and health.

**Results.** The data indicate enhanced wound healing by systemically administered drugs, statistically signifi-

cant with methandrostenolone, at both locations (Figure 1). Analysis of variance revealed a treatment significance due to methandrostenolone ( $F = 10.3$ ,  $P < 0.01$ ). Treatment vs. sex significance was due mainly to methandrostenolone's action on males ( $F = 3.84$ ,  $P < 0.05$ ). Region vs. sex significance ( $F = 5.52$ ,  $P < 0.05$ ) was due primarily to the high-neck low-lumbar values in males and the low-neck high-lumbar values in females.

3 of the topically administered drugs improved healing considerably (Figure 2). Analysis of variance indicated a high degree of significance ( $F = 7.04$ ,  $P < 0.005$ ) and Hartley's test<sup>12</sup> revealed that alcohol, fibrinogen, and vitamin C data were significant at the 5% level or better.

**Discussion.** The results add to the list of other substances which have been reported to enhance the healing of skin wounds, such as testosterone<sup>13,14</sup>, estradiol<sup>15</sup>, antibiotics<sup>3</sup>, certain organic sulphur compounds<sup>16</sup>, and bovine tracheal cartilage<sup>17</sup>. The reported data on the value of histamine releasers are conflicting<sup>18</sup>.

The improved wound healing by the androgen, methandrostenolone, confirms that reported in rats and mice with testosterone<sup>13,14</sup>. The marked sex differences suggest that sex hormones are quite important in wound healing and should be studied further. Pre-existing differences in endogenous androgen levels may account for some of the region and sex differences that were noted.

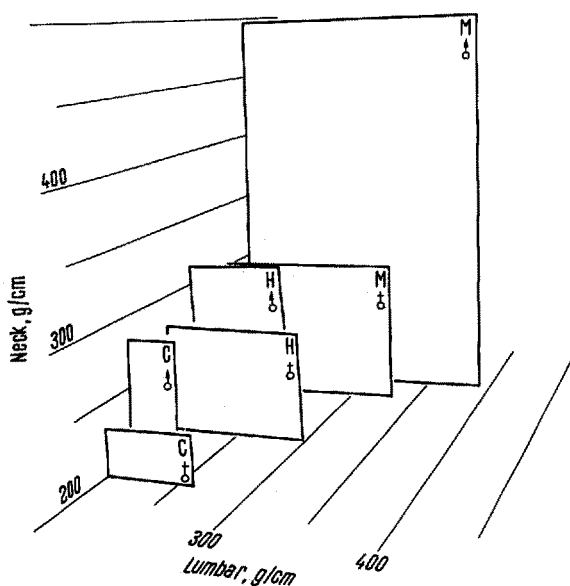


Fig. 1. Histogram illustrating neck and lumbar wound tensile strengths, which were determined for saline-injected controls (C) and dogs injected with heparin (H) or treated orally with methandrostenolone (M).

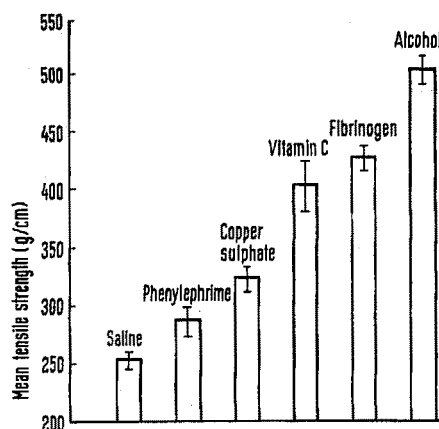


Fig. 2. Wound tensile strength data for incisions in the lumbar area which were treated topically with saline, phenylephrine, copper sulfate, vitamin C, fibrinogen, or alcohol. Deviations shown are the standard errors.

<sup>7</sup> Calcorbate, Cole Chemical Co., St. Louis, Mo.

<sup>8</sup> Fibro-AHF, kindly supplied by Merck Sharp & Dohme, West Point, Pa.

<sup>9</sup> Vetafil Bengen, Bengen and Co., Hanover, West Germany.

<sup>10</sup> Bicillin Fortified, Wyeth Laboratories, Philadelphia, Pa.

<sup>11</sup> G. DiPASQUALE and B. G. STEINER, *Proc. Soc. exp. Biol. Med.* 117, 118 (1964).

<sup>12</sup> G. W. SNEDECOR, *Statistical Methods*, 5th edn (Iowa State Univ. Press, 1956), p. 253.

<sup>13</sup> C. W. PEARCE, N. C. FOOT, G. L. JORDON JR., S. W. LAW, and G. E. WANTZ JR., *Surgery Gynec. Obstet.* 111, 274 (1960).

<sup>14</sup> V. BARBERA, L. POLLICE, and L. MAZZARELLA, *Sperimentale* 113, 105 (1963).

<sup>15</sup> O. JØRGENSEN and A. SCHMIDT, *Acta chim. Scand.* 124, 1 (1962).

<sup>16</sup> C. D. PROCTOR, in *The Healing of Wounds* (Ed. M. B. WILLIAMSON; McGraw-Hill Book Co., 1957), p. 71.

<sup>17</sup> J. F. PRUDDEN, M. L. TENEICK, D. SVAHN, and B. FRUEH, *J. surg. Res.* 4, 143 (1964).

<sup>18</sup> H. FENTON and G. B. WEST, *Br. J. Pharmac.* 20, 507 (1963).

The improvement of wound healing by vitamin C is of special interest because collagen formation in wounds in man is reported to begin only 5 or 6 days after the incision<sup>19</sup>. Thus collagen formation either starts earlier in dogs or vitamin C also acts on other cell functions involved in wound healing. The vitamin C was useful in animals that presumably had normal tissue levels, inasmuch as dogs fed an adequate diet normally synthesize all their needed vitamin C<sup>20</sup>. Possible influence of the calcium in the preparation used cannot be excluded.

Fibrinogen's effectiveness may have been due to the fibrin matrix expediting fibroplastic proliferation. Alcohol's dramatic improvement suggests that its use in an incision would not only improve antisepsis, but would also improve the healing rate.

**Zusammenfassung.** Eine neue Methode wurde entwickelt, um die Wundheilung (Zugfestigkeit) nach Verabreichung von Medikamenten objektiv beurteilen zu

können. Als Kriterium wird die Kraft bestimmt, die notwendig ist, um eine Wunde nach Entfernung der Nähte wieder aufzureissen.

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<sup>19</sup> TH. GILLMAN, in *La Cicatrisation* (Centre National de la Recherche Scientifique, Paris 1965), p. 117.

<sup>20</sup> *Basic Guide to Canine Nutrition* (Gaines Dog Research Center, 1965), p. 37.

<sup>21</sup> The author is grateful for the laboratory assistance of Mr. ROBERT FANTON.

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## Effects of Etonitazene upon Respiratory Neurons

Administration of etonitazene<sup>1,2</sup> – a benzimidazole derivative, 1-( $\beta$ -diethyl-aminoethyl)-2-( $p$ -ethoxy benzyl-5-nitrobenzimidazole) – in suitable doses produces muscular paralysis in a wide variety of species, where the paralysis is of central origin since the stimulation of a motor nerve produces contraction of the innervated muscle(s). In preliminary experiments (unpublished), it was determined that the administration of successively higher doses of etonitazene to the squirrel monkey produced respiratory paralysis before the locomotor musculature was visibly affected. In contrast, in the dog, locomotor collapse appeared before respiratory paralysis. These observations suggested that the structures involved in the central control of the respiratory musculature, i.e. the respiratory centers, are highly sensitive to the effects of etonitazene in the monkey, and more resistant in the dog. The purpose of this study was to verify these preliminary findings with more systematic research and to determine the sensitivity of inspiratory and expiratory neurons of the 2 species to etonitazene.

**Methods.** Adult animals of either sex were used in these experiments. Squirrel monkeys (36) weighing 550–950 g were anesthetized with sodium pentobarbital (30 mg/kg), urethane (1.5 g/kg), or urethane-chloralose (800 and 35 mg/kg, respectively) administered i.p. Dogs (10) weighing 9.2–15 kg were anesthetized with sodium pentobarbital (25–30 mg/kg) i.v. A tracheotomy was performed and a jugular vein was cannulated. The animal was then placed in a stereotaxic instrument and the brain stem respiratory centers were approached, perpendicularly in the squirrel monkeys and obliquely backward at an angle of 55° with the horizontal in dogs, with a microelectrode through a small hole drilled in the top of the skull. The microelectrodes consisted of tungsten rods (1.1 mm in diameter), tapered in a reducing bath to measure less than 5  $\mu$  at the tip and subsequently coated with insulating material. The respiratory movements were recorded as either rate of

<sup>1</sup> A. HUNGER, J. KEDRLE, A. ROSSI, and K. HOFFMAN, *Experientia* 13, 400 (1957).

<sup>2</sup> P. JANSEN, *Anaesthesist* 11, 1 (1962).

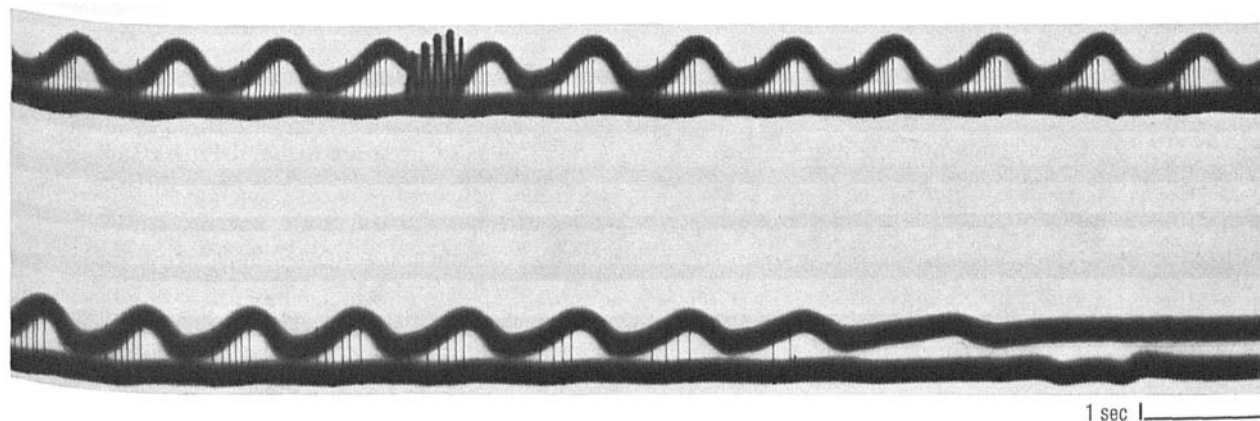


Fig. 1. Squirrel monkey. Continuous uninterrupted recording of respiration as temperature changes in the trachea (top tracing of each pair), with upward direction indicating inspiration, and the activity of an inspiratory unit (bottom). The oscillation in the top tracing indicates the time of i.v. injection of 3  $\mu$ g/kg of etonitazene.