region of *A. tigrinum* and *A. punctatum* neurulae were removed bilaterally. In these cases melanophores, xanthophores and the dorsal fin failed to develop in the entire trunk of the embryo, while the head and tail were normally pigmented. This experiment is similar in results to a slightly different experiment by Holtfreter on Triton,[1] and affords striking evidence in favor of the ganglion crest origin of pigment cells.

*Heteroplastic transplantations:* Dorsal ectoderm of the trunk region with the adhering neural crest cells was transplanted between *A. punctatum* and *A. tigrinum* and from black axolotls to *A. punctatum*. All operations were performed on tail bud stages. Melanophores and xanthophores of the donor type appeared in all grafts from the axolotl to *A. punctatum* and from *A. tigrinum* to *A. punctatum*. The graft areas were easily distinguished from the host skin by the pigment differences which were maintained throughout larval life and after metamorphosis. In the grafts from *A. punctatum* to *A. tigrinum* melanophores of *A. punctatum* (donor) type developed in 9 out of 17 cases. In all cases they were replaced by melanophores of tigrinum (donor) type before metamorphosis. Flank or limb ectoderm and mesoderm transplanted between any of these species at tail bud stages acquires host pigmentation[2] in contrast to the results with dorsal ectoderm and neural crest cells. However, limbs transplanted to *A. punctatum* from *A. tigrinum* at older stages (stage 38, limb bud beginning to elongate) and from the axolotl at stage 34–37 develop the pigmentation as well as the size and form of the donor limb.

*Experiments involving the white axolotl:* The white axolotl develops melanophores only on the dorsal parts of the head and trunk, and none on the flank. Epidermal melanophores are not present, and the epidermal cells do not produce melanin as they do in pigmented species of Amblystoma. Flank ectoderm of *A. punctatum,* carefully cleaned of mesoderm cells, was transplanted to the flank of the white axolotl. In all cases subepidermal melanophores appeared under the grafted ectoderm. Later epidermal melanophores and xanthophores appeared in the graft region. Ectoderm from the abdomen was transplanted to the flank of the axolotl with exactly similar results. The melanophores induced by the grafted ectoderm are undoubtedly of axolotl (host) origin since they are similar in size and shape to those of the host and not to those of the donor. Furthermore flank or abdominal ectoderm of *A. punctatum* never gives rise to melanophores when explanted or transplanted to the abdomen.

[1] Holtfreter, *Roux' Archiv,* Bd. 127, 1933.

[2] Harrison, SCIENCE, Vol. 74, found similar results in limb transplantations.

It is clear that there are subepidermal cells in the white axolotl which acquire melanin and become typical melanophores when the non-pigmented axolotl epidermis is replaced by the pigmented epidermis of *A. punctatum.* Harrison (unpublished data) has found that if Amblystoma ectoderm is grafted to the limb area of *Triturus torosus* melanophores appear under the graft long before they develop in the other limb. The relationship here is the same as that between *Amblystoma punctatum* and the white axolotl.

It is suggested that in both of these cases the transplanted epidermis supplies some substance or substances to the underlying cells which they utilize in melanin formation. In the light of Bloch's experiments[3,4] the substance supplied might be dopa or dopa oxidase, which would then react with the one or the other present in the underlying cells to form melanin.

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## HYPERIMMUNE ANTIPOLIOMYELITIC HORSE SERUM[1]

WITHIN recent years various workers[2–7] have demonstrated the successful hyperimmunization of horses to the poliomyelitic virus. That a potent antiviral serum can thus be produced in a refractory animal is again indicated in the following report.

Three horses were inoculated over a period of 2½ years with 10 per cent. poliomyelitic virus filtered through a Berkefeld N candle. Four different strains of virus were used, 2 monkey passage and 2 isolated from human cases during the New York epidemic of 1931.[8] Injections were given both intracutaneously and subcutaneously at bi-weekly intervals for the first 3 months, reduced later to weekly periods with a dosage of 200 cc, given by the combined intramuscular and subcutaneous routes. Trial bleedings were taken several times and the serums eventually neutralized the virus in dilutions of at least 1–200 or 1–300. The

[3] Bloch, *Zeit. exp. Med.,* Bd. 5, 1917.

[4] Bloch, *Centralbl. f. Haut-u. Geschlects-Krankheiten.* Bd. 8, 1923.

[1] Supported by a fellowship grant from the Eli Lilly Company.

[2] M. Neustaedter and E. J. Banzhaf, *Jour. Am. Med. Assn.,* 68: 1531–1533, 1917.

[3] R. W. Fairbrother, *Brit. Jour. Exper. Path.,* 11: 43–53, 1930.

[4] A. Pettit and B. Erber, *Bull. de l'Acad. de méd.,* 107: 455–459, 1932.

[5] E. R. Weyer, W. H. Park and E. J. Banzhaf, *Jour. Exper. Med.,* 53: 553–566, 1931.

[6] E. W. Schultz and L. P. Gebhardt, *Proc. Soc. Exper. Biol. and Med.,* 31: 260–261, 1933.

[7] M. Schaeffer, *Proc. Soc. Exper. Biol. and Med.,* 31: 1232–1234, 1934.

[8] One strain of virus was kindly supplied by the laboratory of the New York City Department of Health and the other (F1) by Dr. John Paul, of Yale University.

serum of 1 animal was invariably more potent than the others, neutralizing when diluted 1–400. Since equal parts of serum and virus suspension were used, the final dilutions were always double the original amounts.

At the end of 2½ months of immunization, a large bleeding was obtained from the animals and the pooled serum was sent to the Eli Lilly Company for concentration. Two lots of the refined material neutralized *in vitro* from 16 to 32 minimum infective doses of the potent monkey passage strain of virus obtained from Dr. M. Brodie, of the New York City Health Department, in a dilution of at least 1–1,000 to 1–2,000 but not 1–5,000, while the original unconcentrated pooled serum was potent only to a 1–500 dilution and not a 1–1,000. The strength was thus increased and not lost during the refining process. Intraperitoneal inoculation of 20 cc of the pooled concentrated serum into a monkey failed to give any reaction.

Neutralization also occurred when using a virus recently isolated during the 1934 outbreak of poliomyelitis in northern California. Cord removed at autopsy from a human case produced typical poliomyelitis in a monkey after eight days incubation and has since been carried through five passages in monkeys. The second generation virus was potent in a dilution of 1–400 of the 5 per cent. suspension.

The following experiments were performed to test the strength of the concentrated serum when used both therapeutically and prophylactically. 0.005 cc of a 5 per cent. suspension of virus (0.00125 cc M. I. D.) was inoculated intracerebrally into 4 monkeys. Three animals then received 25 cc of the concentrated serum intramuscularly 4, 5 and 6 days, respectively, after the infecting dose and during the prodromal period. The fourth received no serum. All 4 animals developed paralysis and succumbed to typical poliomyelitis. Only 1 was given a second dose of serum but without effect.

The concentrated serum was then tried prophylactically, the virus being administered intracerebrally in 1 series and intranasally in 2 others.

In Series I, 3 animals were given 10 cc and 3 others 20 cc of concentrated horse serum intramuscularly. 0.005 cc of virus were inoculated intracerebrally into 2 monkeys, respectively, 1, 2 and 9 days later. One control animal was used. Only 2 of the 7 monkeys survived, 1 receiving 10 cc of serum 24 hours previous to infection and another receiving 20 cc 2 days beforehand. Although the other 2 monkeys given 20 cc of serum succumbed to the disease, yet the incubation period was greatly prolonged, being 15 days, as compared to 8 days for the control animal. The serum apparently could be of some effect even after the drastic method of infection directly into the central nervous system.

In Series II, the same number of animals was given similar amounts of concentrated serum intramuscularly. Ten per cent. virus was administered by intranasal instillation according to the method of Schultz and Gebhardt[9] at 1, 2 and 7-day intervals. Two control monkeys were included. Two animals receiving the 10 cc amounts of serum died of intercurrent infections. Of the others, 1 control acquired the disease, while all the remaining animals survived.

In Series III, 3 monkeys received 20 cc of concentrated horse serum into the muscles and 3 others 25 cc each of unconcentrated immune sheep serum. Respectively, 1, 2 and 7 days later one of each group was given a 20 per cent. suspension of poliomyelitic virus by intranasal instillation, according to the aforementioned method. Three control monkeys were included. Two of the latter succumbed to the disease, as did also the treated animal receiving sheep serum 1 week previous to the virus. The incubation was prolonged to 19 days, however. All the others and 1 control survived. It seemed apparent that the serums were of some value when inoculated prior to the administration of the virus by the less severe intranasal route.

In summary—a potent hyperimmune antipoliomyelitic horse serum has been developed which may be increased in strength by concentration as demonstrated by the *in vitro* neutralization test. The concentrated serum was without effect if given therapeutically during the prodromal stage after intracerebral injection of monkeys, but was apparently of value if administered prophylactically. This was demonstrated mainly after intranasal infection and only in part after intracerebral inoculation of monkeys.

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[9] E. W. Schultz and L. P. Gebhardt, *Proc. Soc. Exper. Biol. and Med.*, 30: 1010–1012, 1933.

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