glutinins from Vi antiserum prepared with living bacilli. Further, organisms treated in this way upon injection into rabbits gave rise to a serum having a Vi titer of 1 to 2000 in tube agglutination tests. The O and H titers of the serum were each 1 to 1000. Absorption of this serum with living bacilli removed all Vi, H and O agglutinins, thus demonstrating the serological identity of Vi antigen before and after treatment with heat and alcohol. The serum prepared with organisms which had been treated with heat and alcohol was used in mouse protection tests. Its protective power was the same as that of a serum of comparable titer which was prepared with living bacilli.‡

The results show that the Vi antigen of the typhoid bacillus is not affected by absolute alcohol and that its resistance to heat is much greater than was previously supposed. This stability to heat and alcohol is also shared by the Vi antigen of *S. paratyphi* C, *S. ballerup* and certain strains of *E. coli* in which Kauffmann[10] described V-W variation. The thermolability described by other investigators is accounted for by the fact that, in previous work, aqueous suspensions were used in the study of Vi antigen.

13315 P

Melanin Formation by Deplanted Fragments of Thalamus in Amphibian Larvae.

PAUL WEISS.*

From the Department of Zoology, University of Chicago.

In the course of experiments on the functional deplantation of brain fragments into the dorsal fin of urodele amphibian larvae described in earlier communications,[1] a striking local pigmentation effect was observed, whenever thalamic grafts were used.

Donors and hosts were larvae of *Amblystoma tigrinum,* between 3 and 4 cm in length. The brain part which produced positive effects is shown as shaded area in Fig. 1. One-half of this portion, either

‡ The writer is indebted to Lieut. Geo. F. Luippold of the Army Medical School for performing the mouse protection tests.

10 Kauffmann, F., *Acta Path. Microb. Scan.*, 1941, **18**, 225.

* Aided by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

1 Weiss, Paul, PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 350; 1941, **46**, 14.

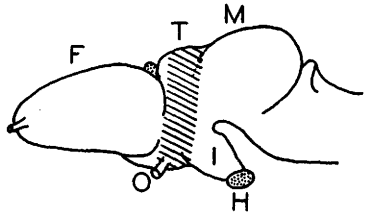


FIG. 1.
Left lateral aspect of the brain of *Amblystoma tigrinum*. F, Forebrain; T, Thalamus; M, Midbrain; I, Infundibulum; H, Hypophysis; O, Optic chiasma.

with or without an adjacent slice of midbrain, was inserted into the gelatinous connective tissue of the fin. The grafts were positively free of hypophyseal and adjoining infundibular tissue.

Within a few weeks after the implantation, the vicinity of the grafts turned deep black (Fig. 2), indicating the deposition of vast amounts of newly formed melanin. The host chromatophores were not appreciably affected. Microscopically, the new pigment appears in two forms: (a) as granules inside of richly arborized cells, which give the appearance of hypertrophied chromatophores and which are found both in the interior and along the surface of the grafts; (b) as large compact clumps interspersed among the epidermis cells of the overlying skin (Fig. 4).

Whether the melanotic cells are all of metaplastic thalamic origin, or whether some surrounding host cells have likewise been induced to form pigment, has not yet been determined. However, the fact that deplants into animals which had been deprived of their hypophysis in the embryonic stage and were consequently very lightly pigmented, gave rise to local pigment clusters, points to the graft as the main source of the melanotic cells. Whether the large intercellular pigment clumps of the host epidermis arise as diffuse interstitial pigment deposits, or represent residues of degenerated chromatophores with excessive pigment content, remains to be seen.

Of 8 grafts containing larval thalamic tissue all produced the effect. Of 118 grafts taken from extra-thalamic portions of the central nervous system (forebrain, midbrain, medulla oblongata, spinal cord, spinal ganglia) not a single one has produced any pigmentation (Figs. 3, 5). The effect is, therefore, specific for *thalamus*. It seems to emanate from the thalamic floor, since roof fragments alone fail to produce it.

A continuation of the experiments in *Triturus torosus* larvae has produced similar results, although not wholly comparable because of differences in experimental procedure. In the Triturus series the thalamus was subdivided into 3 portions, (a) roof, (b) an-

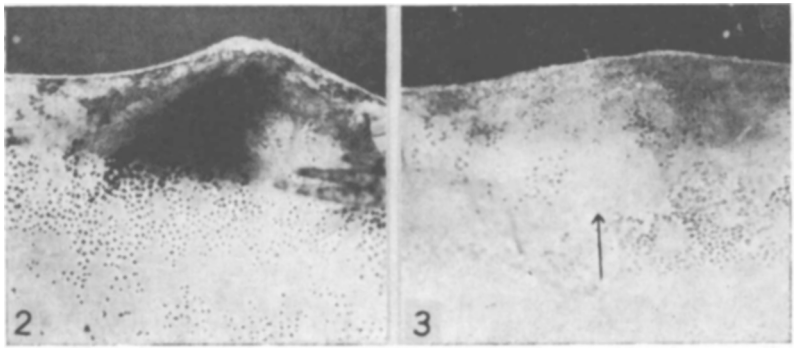
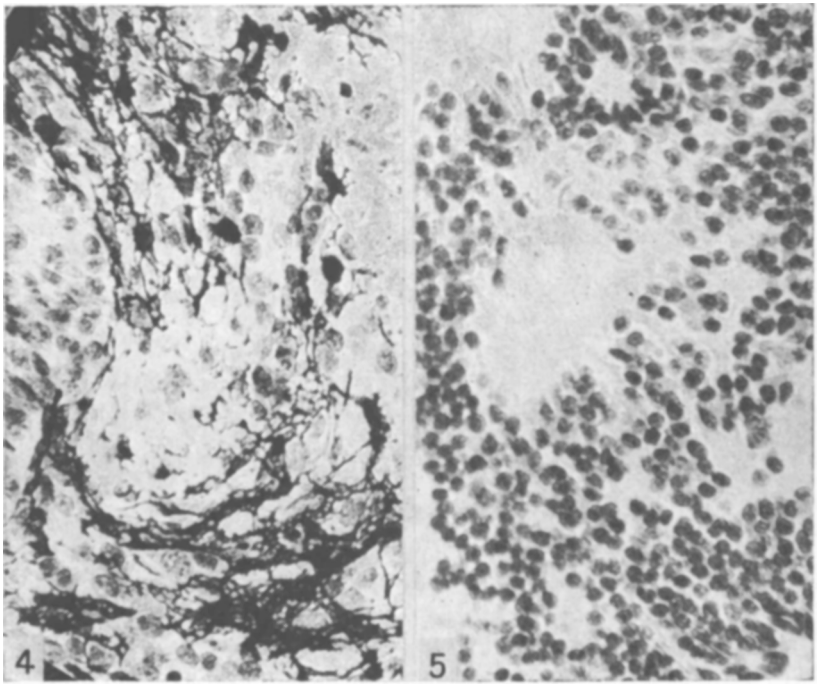


FIG. 2.
Lateral view of portion of dorsal fin containing grafted *thalamus*. Area over graft heavily pigmented. *cca.* 6 ×.

FIG. 3.
Lateral view of dorsal fin containing grafted *midbrain* (light area over arrow). No increase in pigmentation. *cca.* 6 ×.



FIGS. 4 AND 5.
Sections through a deplant consisting of both thalamus (Fig. 4) and midbrain (Fig. 5), 5 weeks after the operation. 220 ×. Masses of pigmented cells in the thalamic portion; no pigment in the midbrain region.

terior (optic and preoptic) portion of the floor, (c) infundibulum, with or without attached hypophysis. Invariably, heavy pigmenta-

tion occurred around (c). In addition, however, both epidermal and dermal host chromatophores within a radius of a few millimeters from the grafts became maximally expanded and finally confluent. This local effect appeared within a week and remained localized. Infundibular fragments without hypophysis have produced it. If pituitary was included, the whole animal, in addition, darkened progressively.

A more detailed analysis, as well as the correlation of the results with those obtained in other forms and with different methods by other workers, is planned for some later date. In conclusion, the experiments indicate triple action on pigment formation by different portions of the diencephalic-hypophyseal complex in urodele amphibians: (1) stimulation of new pigment cell formation and melanin deposition (thalamic floor), (2) local chromatophore expansion (infundibulum), (3) local and diffuse chromatophore expansion (pituitary); (2) and (3) apparently being followed by increase in total melanin content.

13316

A Method for Obtaining Gas Samples of the Total Inspired Air.

MORRIS ECKMAN AND ALVAN L. BARACH.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

In testing the efficiency of equipment used for administering gases it is important to determine the percentage of the component gases of the total inspired air. A sample of gas may be drawn from the apparatus itself during inspiration at a point near the nose, or alveolar air determinations may be obtained.

Both of these methods have limitations. In a mixture of gases, a sample taken at the beginning or end of inspiration might not be a true index of the percentage of that gas in the total inspired air. An alveolar sample is not an accurate guide of the carbon dioxide concentration in the inspired air, since changes in the volume of ventilation tend to keep the alveolar carbon dioxide concentration constant. Rather wide discrepancies in CO_2 accumulation were found in the inspired air of masks recently tested when the alveolar air samples obtained failed to reveal the significant changes found.[1]

[1] Barach, Alvan L., and Eckman, Morris, *J. Aviation Med.*, 1941, **12**, 39.