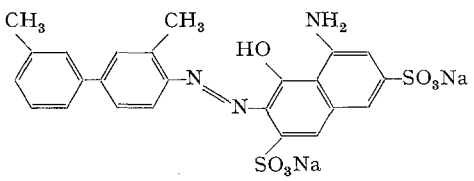
being 4-aminobiphenyl and 2,8-diamino-1-naphthol-3,6-disulphonic acid, indicating that the red impurity of Niagara blue 2B is 8-amino-2-(4′-biphenylazo)-1-naphthol-3,6-disulphonic acid (I). This compound was then synthesized, by coupling diazotized 4-aminobiphenyl with 8-amino-1-naphthol-3,6-disulphonic acid (H-Acid) in alkaline solution, and found to be identical in absorption spectrum and chromatographic behaviour with the red impurity of Niagara blue 2B. It presumably arises as a by-product in the synthesis of Niagara blue 2B, through H-Acid coupling at one but reducing the second diazonium group of a tetrazotized benzidine. A parallel study with the red impurity of trypan blue shows that this is (II), the 3,3′-dimethyl analogue of (I) (see formula). Other possible structures for the red impurity of trypan blue were considered, such as (II) *para*-substituted in the biphenyl moiety by OH (suggested by WEISE[9]), NH_2 or Cl. These could arise by incomplete tetrazotization of benzidine or by incomplete coupling followed by substitution of the remaining diazonium group. Each of these possibilities was eliminated by synthesis of the appropriate biphenyls and of the monoazo dyes resulting from their alkaline coupling with H-acid[10].



Red Impurity of Trypan Blue (II)

Zusammenfassung. Isolierung und Strukturaufklärung der roten Komponente des teratogenen und karzinogenen Farbstoffs Trypanblau: 8-amino-2-(3′,3″-Dimethylbiphenyl-4′azo)-naphth-1-ol-3,6-Disulphonsäure.

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[10] We thank Tenovus for a grant in aid of this work.

Induction by Killed Post-Nodal Fragments of the Definitive Primitive Streak in the Chick[1]

It has been well established that the living Hensen's node of the chick blastoderm induces neural differentiation when grafted under competent chick ectoderm (WADDINGTON[2], WOODSIDE[3], GALLERA and CASTRO-CORREIA[4], PASTERNAK and MCCALLION[5], and VAKAET[6]). It has also been clearly established that Hensen's node retains its inductive capacity after it has been killed with 70% alcohol or heat (WADDINGTON[2] and LEIKOLA and MCCALLION[7]). Furthermore, killed Hensen's node also exerts an inductive influence on competent amphibian ectoderm (VISWANATH, LEIKOLA and TOIVONEN[8]).

The maximum inductive capacity of the definitive primitive streak is located in Hensen's node. This quality of the streak diminishes rapidly towards the posterior end of the streak (GALLERA[9]). It is well known from a large number of studies that fragments of the definitive primitive streak taken more than 0.8 mm behind the node, when implanted into young host embryos, are incorporated into the host embryo and exert no inductive influence on the epiblast of the host. At best, these tissues only contribute to the peripheral mesoblast of the host (GALLERA[9] and WAHEED and MCCALLION[10]). It has been shown, however, that under certain conditions of pretreatment with such substances as cysteine or lithium chloride posterior fragments of the definitive primitive streak acquire some inductive capacity (WAHEED and MULHERKAR[11] and WAHEED and MCCALLION[10]). Under these conditions the implanted fragment retains its integrity, proliferates and differentiates mesoderm and often chorda and exerts some neuralizing influence on the host ectoderm.

The present study was undertaken in order to discover whether killed fragments of the posterior part of the definitive streak could exert any inductive action on competent ectoderm. Under these conditions the fragments would retain their integrity but would not proliferate or differentiate.

The embryos used in these experiments were obtained from White Leghorn eggs supplied by a commercial hatchery. The eggs were incubated for 14–16 h at 39 °C. Small fragments of the primitive streak, taken 1.0 mm or more posterior to the node of definitive primitive streak blastoderms, killed in 70% alcohol and thoroughly rinsed in normal saline solution were used as implants. Blastoderms somewhat younger than the definitive streak stage were explanted and cultured at 39 °C according to the method of NEW[12]. The blastoderms were briefly exposed to UV-irradiation in order to retard their rate of development, and the implants were inserted between the epiblast and the hypoblast at the periphery of the embryos. The purpose of the irradiation was to prevent the isolation of the implant from the epiblast by the outgrowth of lateral mesoderm (LEIKOLA and MCCALLION[7]). The embryos were examined after 24–30 h in culture. They were photographed as lightly stained whole mounts. Subsequently, the embryos were embedded in

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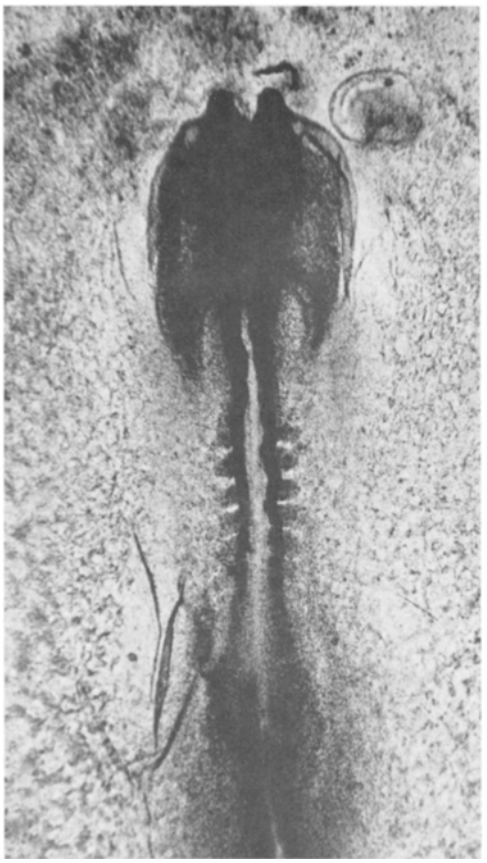

Fig. 1. Chick embryo cultured by NEW's method[12] for 24 h following brief exposure to UV-irradiation at middle streak stage. Implant may be seen at upper right.

Fig. 2. Cross-section of an embryo similar to that in Figure 1, showing implant (G) and induced neural-like tissue (I).

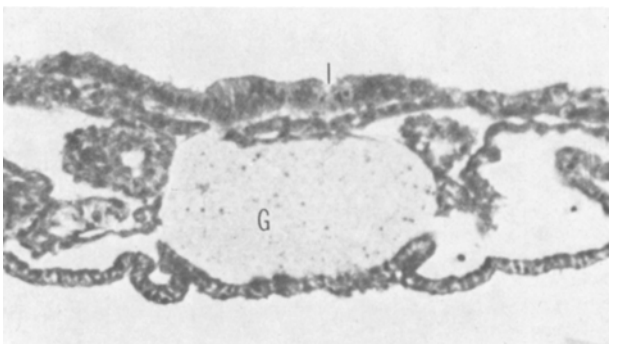

Fig. 3. Cross section of an embryo, showing implant (G) and a weak but positive reaction (I) of the host tissue to the implant.

paraffin, sectioned, mounted on slides and stained in H and E. 41 such preparations were made and studied. In addition 70 similar preparations with unirradiated host embryos were studied.

In all cases in which the host embryos were not irradiated the implants of killed primitive streak were rapidly surrounded by mesoderm and effectively isolated from the ectoderm and no inductions were found. The irradiated embryos survived and were apparently little affected by the irradiation (Figure 1). In all cases the implant could be found and identified (Figure 2). In 15 of the 41 cases studied there was a clearly positive reaction of the ectoderm to the implant with some palisading of the ectodermal cells suggesting poorly organized neuralization (Figure 2). In none of these instances did there occur a structure that could be described as a neural plate. In 18 cases the ectodermal reaction was positive but weak (Figure 3). In the remaining 8 cases the implant had no effect on the host embryo ectoderm. In a number of cases the implant seemed to have a slight organizing effect upon the host hypoblast, in which the cells resembled those of the gut endoderm.

The results of these experiments indicate that posterior fragments of the definitive streak killed in alcohol remain intact when implanted beneath host epiblast, and exert a weak neuralizing induction upon it. The inductions obtained are not as clearly neural as those obtained by MCCALLION and LEIKOLA[13] with a variety of heterogenous inductors. They more nearly resemble the neuroid masses obtained in ectoderm after a limited contact (4 h) with a heterogenous inductor (LEIKOLA and MCCALLION[14]). Similarly, better and more clearly neural inductions were obtained with similar fragments of posterior streak pretreated with lithium salts (WAHEED and MCCALLION[10]). However, under these conditions the fragments proliferated quite large amounts of mesoderm. The normal fate of the cells of the posterior part of the definitive streak is to be dissipated into the posterior end of the embryo. Living implants are similarly dissipated in the host embryo and exert no inductive influence.

Résumé. Des greffons prélevés dans la moitié postérieure de la ligne primitive achevée des jeunes blastodermes d'embryons de poulet, tués dans l'alcool à 70%, furent introduits sous l'ectoblaste des hôtes de même âge. Dans 15 sur 41 cas, le greffon a exercé une action inductrice sur l'ectoblaste. Dans 18 autres cas, l'action du greffon fut faible, voire nulle. Les inductions provoquées par les greffons rendent le tissu semblable à un tissu neural pauvrement organisé.

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