tions (usually 0.02–0.06 ml) was added to the bathing medium. Every 10–15 min, another small volume of the test solution was added to the bathing medium to produce a step-wise increase in the concentration of the test compounds, until the heart went into a systolic arrest.

The results are summarized in the Table. As can be seen from this table, despite the absence of 3β-hydroxy group, 3-deoxydigitoxigenin produced almost as strong a cardiotonic action as digitoxigenin in this preparation. Very recently, ZÜRCHER et al.[5] have reported that 3-deoxydigitoxigenin could induce a marked inhibition of a transport ATPase prepared from the heart muscle of the guinea-pig, a finding which is in good accordance with ours.

Actions of digitoxigenin and 3-deoxydigitoxigenin on the isolated frog's heart (STRAUB's preparation)

Concentration (g/ml)	10^{-8}	3×10^{-8}	10^{-7}	3×10^{-7}
Digitoxigenin	—	—	+	×
	—	+	+	×
	—	+	+	×
	—	+	+	×
	—	+	+	×
3-Deoxydigitoxigenin	—	—	+	×
	—	—	+	×
	—	—	+	×
	—	+	+	×
	—	+	+	×

—, no effect; +, improvement of contractility without a tendency to systolic arrest; ×, systolic arrest.

In a previous communication[3], we reported that 14-deoxy-14βH-uzarigenin retains cardiotonic activity, despite the absence of 14β-OH. Thus, it is now clear that, against the long-standing belief on the structure-activity relationship of the cardiotonic steroids, neither 3β-OH nor 14β-OH is indispensable for the cardiotonic action of the compounds.

A preliminary report of the present study was read at the 42nd Annual Meeting of the Japanese Pharmacological Society held in Tokyo on the 2nd of April, 1969[6].

Zusammenfassung. Die Herzwirksamkeit von 3-Deoxydigitoxigenin am isolierten Froschherzen wurde geprüft. Im Gegensatz zur bisherigen Auffassung (3β-Hydroxygruppe notwendig für kardiotonische Wirkung der Digitalis-Verbindungen) zeigte 3-Deoxydigitoxigenin eine starke kardiotonische Aktivität.

K. TAKEDA[7], T. SHIGEI[7] and S. IMAI[8]

Department of Pharmacology, Institute for Cardiovascular Diseases, and Faculty of Medicine, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo (Japan), 18 February 1970.

[5] W. ZÜRCHER, E. WEISS-BERG and CH. TAMM, Helv. chim. Acta *52*, 2449 (1969).
[6] T. SHIGEI, K. TAKEDA, S. MINESHITA, S. IMAI and M. OKADA, Folia pharm. jap. *65*, 29 § (1969), in Japanese.
[7] Department of Pharmacology, Institute for Cardiovascular Diseases, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo (Japan).
[8] Department of Pharmacology, Faculty of Medicine, Tokyo Medical and Dental University, Tokyo (Japan).

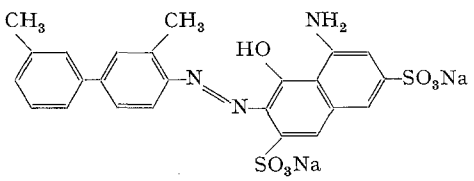
The Red Impurity in Trypan Blue

Dyes such as trypan blue, Evans blue and Niagara blue 2B, prepared by coupling benzidine or a derivative with an aminonaphtholdisulphonic acid, were introduced into biology as vital stains and later used in the measurement of blood volume. Publications on these biological uses contain numerous references to complications arising from the invariable presence of contaminating red compounds in samples of the dyes. The first systematic study, of the red impurity in trypan blue, was made by KELLY[1], whose paper also includes a summary of the earlier literature. Further interest in the red impurities followed the discovery that trypan blue was both teratogenic[2] and carcinogenic[3], and subsequent reports of variable responses to different commercial samples of trypan blue led to suggestions that the red impurity might be the active principle of the whole dye. Experiments on fractions isolated from commercial trypan blue suggest that this is not so: the teratogenic activity of trypan blue is probably due to its major blue component[4,5], whereas the carcinogenic activity appears to reside in a further 'purple' impurity present in some samples[6]. However, it is not certain whether the red impurity contributes to the teratogenic or carcinogenic potency of the whole dye. An investigation of this question requires more of the compound than can conveniently be prepared by extraction from the whole dye. We have therefore determined the structure of the red impurities of Niagara blue 2B (Colour Index no. 22610) and trypan blue (Colour Index no. 23850) with a view to preparing synthetic samples in quantity for metabolic and toxicological studies.

A small quantity of the red impurity of Niagara blue 2B, extracted from the whole dye as described by BECK and LLOYD[4], was reduced with dithionite, acidified and the decolourized solution passed through a column of Dowex 1 (chloride form) to remove anionic materials. The eluate gave an absorption spectrum in 0.1 *N* HCl and 0.1 *N* NaOH identical with that of 4-aminobiphenyl[7]. Paper electrophoresis of the reduction products by the method of LLOYD and BECK[8] was consistent with these

[1] J. W. KELLY, Stain Technol. *33*, 79 (1958).
[2] J. GILLMAN, C. GILBERT, T. GILLMAN and I. SPENCE, S. Afr. J. med. Sci. *13*, 47 (1948).
[3] J. GILLMAN, T. GILLMAN and C. GILBERT, S. Afr. J. med. Sci. *14*, 21 (1949).
[4] F. BECK and J. B. LLOYD, J. Embryol. exp. Morph. *11*, 175 (1963).
[5] A. N. BARBER and J. C. GEER, J. Embryol. exp. Morph. *12*, 1 (1964).
[6] J. DIJKSTRA and J. GILLMAN, Nature *191*, 803 (1961).
[7] A. R. KATRITZKY and P. SIMMONS, J. chem. Soc. *1960*, 4901.
[8] J. B. LLOYD and F. BECK, Stain Technol. *39*, 7 (1964).

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.

J. B. LLOYD and F. E. FIELD

Department of Biochemistry, University College, Cardiff (Wales), 23 February 1970.

[9] R. WEISE, Z. wiss. Mikrosk. *54*, 398 (1937).

[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

[1] This work was supported by a grant from the National Research Council of Canada to D. J. MCCALLION.

[2] C. H. WADDINGTON, J. exp. Biol. *10*, 38 (1933).

[3] G. L. WOODSIDE, J. exp. Zool. *75*, 259 (1937).

[4] J. GALLERA and J. CASTRO-CORREIA, C. r. Soc. Biol., Paris *154*, 1728 (1960).

[5] L. PASTERNAK and D. J. MCCALLION, Can. J. Zool. *40*, 585 (1962).

[6] L. VAKAET, C. r. Soc. Biol., Paris *159*, 232 (1965).

[7] A. LEIKOLA and D. J. MCCALLION, Can. J. Zool. *46*, 205 (1968).

[8] J. R. VISWANATH, A. LEIKOLA and S. TOIVONEN, Experientia *25*, 38 (1969).

[9] J. GALLERA, Bull. Ass. Anat. *125*, 632 (1965).

[10] M. A. WAHEED and D. J. MCCALLION, Ann. Zool. Fenn. *7*, 67 (1970).

[11] M. A. WAHEED and L. MULHERKAR, J. Embryol. exp. Morph. *17*, 161 (1967).

[12] D. A. T. NEW, J. Embryol. exp. Morph. *3*, 326 (1955).