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Chronic toxicity of brilliant blue FCF, blue VRS, and green S in rats*

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Groups of twenty rats were given weekly subcutaneous injections of one of the food colours Brilliant Blue FCF, Blue VRS, and Green S. The dose, 20 mg in isotonic saline, was given for 45 weeks after which the rats were observed for an additional 26 weeks. There was ulceration and abscess formation at the site of injection in rats given Blue VRS. The other two colours produced no noticeable local or systemic effects. No subcutaneous fibrosarcomas were seen in any of the rats. Two rats given Blue VRS developed rhabdomyosarcomas in the area of the injection site. This finding is being investigated.

BRILLIANT Blue FCF (C.I. 1924, No. 671), Light Green SF Yellowish (C.I 1924, No. 670) and Fast Green FCF (C.I 1956, No. 42053), three food colours permitted for use in Canada, have been reported to produce fibrosarcomas in rats when given by subcutaneous injection (Nelson & Hagan, 1953). Two colours that have been suggested as alternatives to the ones mentioned are Blue VRS (C.I 1924, No. 672) and Green S (C.I 1924, No. 737). These are on the permitted list of food colours in Great Britain and some other countries. These two colours, as well as Brilliant Blue FCF, were studied using the procedures described by Nelson & Hagan (1953).

Methods

Eighty rats, originally of the Wistar strain, were divided into four groups when they were 5 weeks of age. There were ten males and ten females in each group. The animals were housed in colony cages, ten rats to a cage and given regular laboratory chow and water *ad libitum*.

The rats were given weekly subcutaneous injections of 0·5 ml of one of the following: (1) Isotonic saline (control group). (2) 4% Brilliant Blue in isotonic saline. (3) 4% Green S† in isotonic saline. (4) 4% Blue VRS‡ in isotonic saline.

Thus each rat in groups 2, 3 and 4 received 20 mg of colour per week. The injection site, on the back, was clipped regularly. The rats were examined weekly when injected and were weighed every other week. Forty-five injections were given for a total dose of 900 mg of colour. The experiment was terminated at 71 weeks, i.e. 26 weeks after the last injection. Rats that died during the test were examined to determine cause of death. At the end of the experiment the survivors were killed and gross examination was made of the tissues and organs. Portions of skin, fascia and muscle from the area of injection and other grossly

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* The sixth of a series on the Toxicity of Food Colours.

† Manufactured by Imperial Chemical Industries Ltd., England.

‡ Manufactured by L. J. Pointing & Son, Ltd., England.

abnormal tissues were embedded in Paraplast* after formalin fixation, and stained by haematoxylin, phloxine and saffron, and by Mallory's phosphotungstic acid haematoxylin technique.

Results

MORTALITY

The % mortality at intervals during the test is given in Table 1. Although there appeared to be differences among the groups, pathological examination indicated that almost all the deaths were due to respiratory infections. None of the deaths were attributed directly to the effects of the injection of food colour.

TABLE 1. PER CENT MORTALITY IN RATS GIVEN SUBCUTANEOUS INJECTIONS OF FOOD COLOURS

Colour	No. of weeks on test					
	12	24	33	44	59	71
None (Control)	0	5	5	15	45	60
Brilliant Blue FCF	0	10	15	15	45	55
Green S	5	30	45	55	85	90
Blue VRS	0	5	10	45	80	85

PATHOLOGY

Gross examination of the skin was made throughout the study and material was available for histologic examination from those animals dying of pneumonia before termination of the test. The skin reaction was most marked in male rats injected with Blue VRS. There was ulceration and abscess formation within 3 weeks of the beginning of treatment in some animals. Focal alopecia and dermatitis developed in other rats on this colour, followed by abscess formation and ulceration. There were irregular exacerbations and remissions of these skin conditions during the experiment. Ultimately there was pachydermia as a result of the chronic sclerodermatitis and focal cicatrisation. There were no similar skin lesions in any of the other three groups.

Tumours were observed in two female rats at the site of injection of Blue VRS. The tumours were similar in size and appearance. One measured 3·5 cm by 2 cm and the other 3 cm by 2 cm. They were roughly round and flattened. The skin overlying the tumours was ulcerated and there was crater formation in both. The skin was adherent near the rim of ulceration but peripherally it could be separated by blunt dissection. In both animals attachment to the underlying fascia and muscles was firm and there appeared to be invasion of these structures by the tumour. One mass was soft, smooth and friable in some areas and dense and firm in others. It was pink to white in colour. The other tumour was firm or rubbery except near the area of ulceration and was pink to grey-white in colour.

Histologically the skin lesions progressed from the areas of normal

* Manufactured by Biological Research Inc.

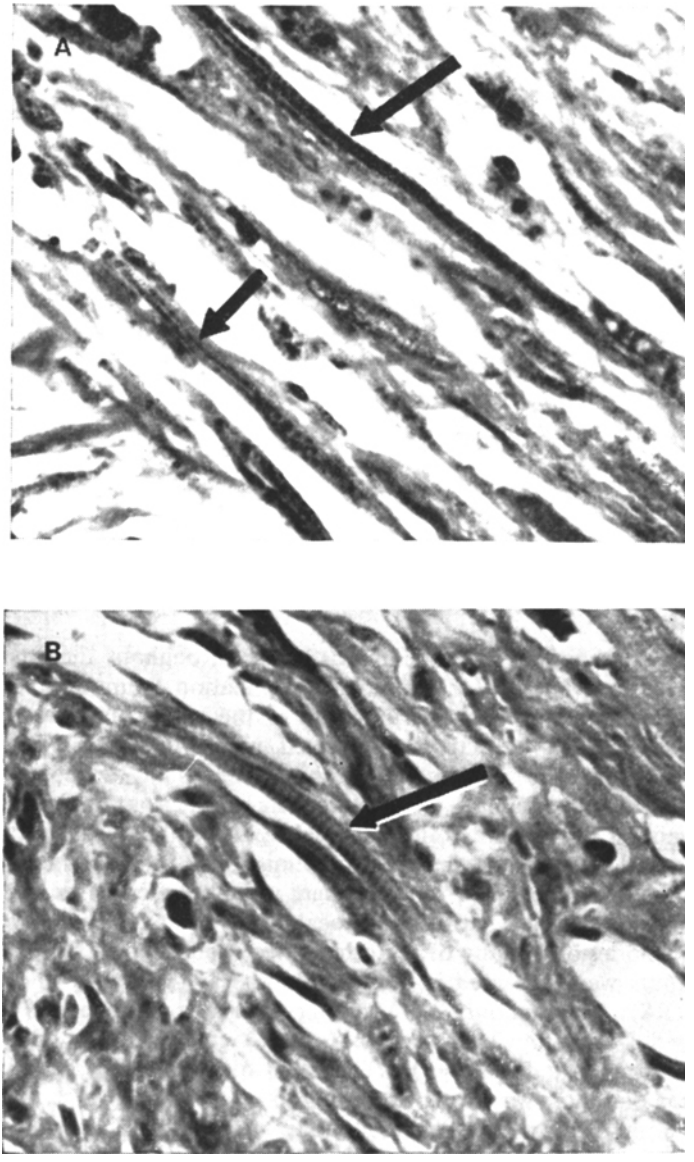

FIG. 1. Sections taken from tumours found at injection site in two rats treated with Blue VRS.

A. Arrows point to rhabdomyoblasts. Cross striations are plainly visible. One elongated cell runs diagonally through centre of field. Mallory's phosphotungstic acid haematoxylin. × 320.

B. Cross striations are visible in one cell (Arrow). Cellular pleomorphism is marked. Mallory's phosphotungstic acid haematoxylin. × 160.

epidermis to pachydermic areas that were characterised by a fibro-granulomatous reaction surrounding areas of ulceration or abscess formation. The latter were located principally in the dermis and were composed of a dense granular necrotic core surrounded by large numbers of leucocytes, predominantly polymorphonuclear neutrophiles, and macrophages and plasma cells. Dense collagen bands that encapsulated the abscesses blended with collagen bundles of surrounding dermis.

The histologic pattern of the two tumours was similar (Fig 1A and B). There was a haphazard arrangement of bands and bundles of cells. These varied greatly in shape and size from round to elliptical to strap shaped. Some cells were short and stubby while the cytoplasm of others extended across the high power field. There was great variation in shape and size of nuclei but oval shapes predominated and many had indentations. Nuclear chromatin was stippled and roughly granular. Most cells contained several nucleoli. Mitotic figures and bizarre giant cells were numerous. Cross striations were clearly exhibited in both tumours (Fig 1). These features led to the diagnosis of rhabdomyosarcoma.

The only other tumours found were a benign uterine polyp and a haemangioendothelioma of the epididymus. Both were in control rats.

Discussion

The object of this experiment was to confirm the work of Nelson & Hagan (1953) with Brilliant Blue and at the same time to see if similar results could be obtained with Blue VRS and Green S, two other triphenyl methane colours. The rats given Blue VRS tolerated the injections poorly and for this reason the treatment was discontinued after 45 weeks. We were thus not able to duplicate the conditions of the experiment of Nelson & Hagan who gave injections of Brilliant Blue for as long as 94–99 weeks. They reported, however, that tumours appeared after 40–45 weeks treatment and it was thought that by observing our animals throughout their life-span some tumours might be found.

This did not occur with rats given Brilliant Blue. It was necessary to end our experiment after 71 weeks because of high mortality from respiratory disease and poor condition of the survivors. Up to this time, however, no indication of fibrosarcoma was seen in any of the rats of any group.

The presence of rhabdomyosarcoma at the injection site, observed in two rats given Blue VRS, has not, to our knowledge, been reported in any of the numerous studies on subcutaneous injection of dyes. Investigation of this finding is planned.

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Reference

Nelson, A. A. and Hagan, E. C. (1953). *Fed. Proc.*, **12**, 397.