CHRONIC TOXICITY STUDIES ON FOOD COLOURS. V. OBSERVATIONS ON THE TOXICITY OF BRILLIANT BLUE FCF, GUINEA GREEN B AND BENZYL VIOLET 4B IN RATS

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The food colours Brilliant Blue FCF, Guinea Green B and Benzyl Violet 4B were fed to rats at concentrations of 0·03, 0·3 and 3 per cent of the diet for 75 weeks. Thirty rats were used at each feeding level. Brilliant Blue and Benzyl Violet had no adverse effects on growth at any of the levels used. With Guinea Green there was an initial depression of growth at the two higher levels accompanied by a decreased food efficiency and, in one group, a lower food consumption. There was an increase in mortality in female rats fed 3 per cent of all three colours. With Brilliant Blue, indications were that this increase was not related to treatment. In the groups fed 3 per cent Guinea Green and 3 per cent Benzyl Violet there was a total of eight malignant tumours. Five of these were derived from the epidermis. Further investigation of these two colours is required.

This report is one of a series covering the food colours permitted for use in Canada. Reports on eight other colours have been published (Allmark, Grice and Lu, 1955; Allmark, Grice and Mannell, 1956; Mannell, Grice, Lu and Allmark, 1958). Since the work began, four of the colours tested have been deleted from the Canadian list of permitted food colours. These are Oil Yellow OB, Oil Yellow AB, Oil Red XO and Orange SS.

In this experiment the effects of feeding Brilliant Blue, Guinea Green and Benzyl Violet on growth, food consumption, food efficiency, and mortality in rats were investigated. Histopathological and haematological studies were also done.

Methods

Details of the methods used have been given by Mannell and others (1958). The colours were fed at concentrations of 0·03, 0·3 and 3·0 per cent. Fifteen rats of each sex were included in each test group and in the control group, 300 rats in all. They were of Wistar origin and ranged from 30 to 37 days of age at the start of the experiment. Weekly records were kept of food consumption and food efficiency during the first year, while body weight was recorded weekly until the experiment was terminated at 75 weeks. Red blood cell counts, haemoglobin estimations and haematocrit readings were done at intervals throughout the test.

Rats that died on test were examined to determine, if possible, the cause of death. At the end of the experiment, the surviving rats were killed and a gross examination was made of all organs and tissues. A detailed examination was made of haematoxylin-eosin stained paraffin sections from five representative male and female animals of each of the groups. The organs studied were, lung, heart, liver, spleen, thyroid, pancreas, stomach, small intestine, kidney, urinary bladder, adrenal, testis, prostate, coagulating gland, ovary, uterus and thymus. In addition, all tissues in which any gross pathological change was observed, and all tumours, were examined histologically.

Results

Growth, Food Consumption, Food Efficiency

Brilliant Blue. This colour had no adverse effect on growth of either males or females at any of the levels fed. In fact, from the tenth to the twenty-sixth week, the males fed 0·3 per cent were significantly heavier than the control group (Table I). The females at this feeding level had a larger mean body weight than the controls after 5 and 10 weeks on test.

TABLE I

Body weight of rats fed brilliant blue, guinea green, or benzyl violet

Treatment	Dosage (per cent of diet)	Weight, g. (Mean ± S.E.)							
		Initial	3 weeks	5 weeks	10 weeks	16 weeks	26 weeks	52 weeks	75 weeks
Males									
Control		72 ± 3	149 ± 4	184 ± 5	234 ± 4	268 ± 7	308 ± 10	332 ± 16	285 ± 23
Brilliant Blue	0·03	70 ± 3	150 ± 3	187 ± 4	242 ± 5	279 ± 7	321 ± 8	331 ± 16	325 ± 19
	0·3	70 ± 3	151 ± 4	192 ± 4	†259 ± 5	†300 ± 5	*341 ± 7	346 ± 9	320 ± 14
	3·0	69 ± 2	148 ± 4	185 ± 4	242 ± 6	280 ± 8	321 ± 8	336 ± 12	322 ± 9
Guinea Green	0·03	71 ± 3	146 ± 3	186 ± 3	247 ± 6	*289 ± 7	331 ± 7	360 ± 12	*359 ± 17
	0·3	71 ± 3	*134 ± 4	*171 ± 5	230 ± 6	270 ± 6	316 ± 8	367 ± 8	*348 ± 15
	3·0	72 ± 3	139 ± 6	175 ± 4	234 ± 6	271 ± 9	317 ± 11	342 ± 12	320 ± 17
Benzyl Violet	0·03	72 ± 3	148 ± 3	183 ± 2	244 ± 3	*287 ± 4	330 ± 5	357 ± 13	†397 ± 12
	0·3	72 ± 3	149 ± 4	191 ± 5	*252 ± 5	†293 ± 6	334 ± 8	352 ± 10	332 ± 15
	3·0	70 ± 3	142 ± 2	180 ± 3	238 ± 4	277 ± 5	322 ± 10	*376 ± 12	*367 ± 28
Females									
Control		62 ± 2	114 ± 2	127 ± 2	155 ± 3	177 ± 3	201 ± 3	223 ± 3	212 ± 7
Brilliant Blue	0·03	63 ± 3	114 ± 2	128 ± 3	154 ± 3	175 ± 4	202 ± 6	216 ± 7	212 ± 11
	0·3	65 ± 2	116 ± 2	†135 ± 2	*164 ± 3	183 ± 3	209 ± 4	221 ± 6	232 ± 5
	3·0	64 ± 2	114 ± 2	†135 ± 2	161 ± 3	179 ± 4	202 ± 4	217 ± 7	216 ± 10
Guinea Green	0·03	64 ± 2	114 ± 2	130 ± 2	156 ± 3	177 ± 3	203 ± 4	224 ± 5	225 ± 8
	0·3	64 ± 2	*108 ± 1	126 ± 2	155 ± 2	175 ± 3	198 ± 3	228 ± 5	*230 ± 5
	3·0	64 ± 2	*108 ± 2	125 ± 2	151 ± 3	*169 ± 3	194 ± 3	214 ± 4	210 ± 7
Benzyl Violet	0·03	64 ± 2	115 ± 2	*134 ± 2	159 ± 3	182 ± 3	201 ± 4	226 ± 4	216 ± 10
	0·3	64 ± 2	113 ± 2	132 ± 2	156 ± 3	175 ± 3	202 ± 4	228 ± 4	235 ± 8
	3·0	65 ± 2	113 ± 3	*134 ± 2	161 ± 3	178 ± 3	201 ± 3	223 ± 4	243 ± 26

* P less than 0·05. † P less than 0·01.

Food consumption was not reduced by the addition of Brilliant Blue to the diet up to the 3 per cent level (Table II). In the group of male rats fed 0·3 per cent of the colour a significant increase in food consumption occurred from the tenth to the twenty-sixth week. This coincided with the increase in weight for this group noted above.

Food efficiency was also increased in this same group for the same period (Table III). In no group fed Brilliant Blue was there a diminution of food efficiency at any time during the experiment.

Guinea Green. Male rats fed Guinea Green at 0·3 per cent in the diet had a smaller mean weight than their controls for the first 5 weeks of the test (Table I). By the tenth week the weight of this group was the same as the control value and at the end of the experiment had surpassed it. Food efficiency values followed a similar pattern (Table III). At the 3 per cent level, food efficiency was significantly reduced at 3 and 5 weeks (Table III), but the weight was not significantly affected (Table I). After the tenth week the only effect observed in the males on 3 per cent Guinea Green was an increase in food consumption at 16 and 26 weeks (Table II).

TABLE II

FOOD CONSUMPTION OF RATS FED BRILLIANT BLUE, GUINEA GREEN, OR BENZYL VIOLET

Treatment	Dosage (per cent of diet)	Food consumption in g./rat/day (Mean ± S.E.)					
		3 weeks	5 weeks	10 weeks	16 weeks	26 weeks	52 weeks
Males							
Control		14·0 ± 0·36	14·3 ± 0·32	15·3 ± 0·26	15·7 ± 0·28	16·3 ± 0·28	17·3 ± 0·38
Brilliant Blue	0·03	14·2 ± 0·32	14·7 ± 0·26	15·8 ± 0·25	16·2 ± 0·32	16·9 ± 0·36	17·9 ± 0·37
	0·3	13·9 ± 0·27	14·6 ± 0·21	*16·0 ± 0·18	†17·0 ± 0·19	†17·3 ± 0·19	17·9 ± 0·16
	3·0	14·0 ± 0·32	†18·5 ± 0·36	15·8 ± 0·29	16·5 ± 0·31	16·8 ± 0·29	17·4 ± 0·26
Guinea Green	0·03	13·9 ± 0·21	14·5 ± 0·22	15·5 ± 0·20	16·1 ± 0·20	16·9 ± 0·16	17·8 ± 0·17
	0·3	13·3 ± 0·23	13·8 ± 0·24	15·2 ± 0·24	16·0 ± 0·25	16·6 ± 0·29	17·9 ± 0·22
	3·0	13·8 ± 0·34	14·7 ± 0·34	15·9 ± 0·31	*16·8 ± 0·31	*17·5 ± 0·33	18·2 ± 0·32
Benzyl Violet	0·03	14·6 ± 0·23	*15·2 ± 0·21	†16·3 ± 0·17	†17·0 ± 0·17	†17·6 ± 0·20	*18·3 ± 0·20
	0·3	14·5 ± 0·32	15·1 ± 0·36	*16·0 ± 0·29	*16·6 ± 0·29	17·1 ± 0·28	17·9 ± 0·24
	3·0	14·3 ± 0·22	*15·2 ± 0·24	†16·2 ± 0·16	†16·8 ± 0·17	*17·3 ± 0·25	*18·3 ± 0·17
Females							
Control		12·3 ± 0·21	12·0 ± 0·18	12·1 ± 0·14	12·2 ± 0·13	12·8 ± 0·14	13·7 ± 0·15
Brilliant Blue	0·03	12·4 ± 0·25	12·4 ± 0·28	12·4 ± 0·22	12·3 ± 0·20	13·1 ± 0·24	14·0 ± 0·25
	0·3	11·9 ± 0·15	12·1 ± 0·14	†12·6 ± 0·03	*12·7 ± 0·20	13·2 ± 0·20	13·8 ± 0·19
	3·0	11·9 ± 0·29	12·0 ± 0·24	12·3 ± 0·21	12·3 ± 0·23	12·6 ± 0·22	13·3 ± 0·21
Guinea Green	0·03	12·0 ± 0·21	12·0 ± 0·02	12·2 ± 0·18	12·2 ± 0·18	12·7 ± 0·19	13·5 ± 0·19
	0·3	11·7 ± 0·24	11·9 ± 0·04	12·4 ± 0·20	12·5 ± 0·20	13·0 ± 0·20	13·7 ± 0·18
	3·0	†10·9 ± 0·21	†11·3 ± 0·16	*11·6 ± 0·18	11·8 ± 0·17	12·3 ± 0·20	†13·0 ± 0·19
Benzyl Violet	0·03	12·1 ± 0·17	12·5 ± 0·17	*12·8 ± 0·21	*12·8 ± 0·24	13·4 ± 0·27	14·0 ± 0·21
	0·3	12·2 ± 0·23	12·2 ± 0·21	12·2 ± 0·19	12·1 ± 0·20	12·6 ± 0·23	13·4 ± 0·16
	3·0	12·7 ± 0·30	12·5 ± 0·20	*12·7 ± 0·18	12·6 ± 0·20	13·0 ± 0·24	13·3 ± 0·18

* P less than 0·05. † P less than 0·01.

Body weight of the females on Guinea Green was down at 3 weeks in the groups fed 0·3 per cent and 3 per cent of the colour, and at 16 weeks for the high level group only (Table I). Food consumption of the females on the 3 per cent level was reduced during most of the experiment (Table II). There were two instances of reduced food efficiency, at 3 weeks for the 0·3 per cent group and at 16 weeks for the 3 per cent group.

Benzyl Violet. There was no depression of growth in either males or females at any feeding level. As happened with Brilliant Blue there were instances, especially with male rats, where the test animals were heavier than the controls, at times markedly so (Table I).

As might be expected in view of the body weight results, increased food consumption was a consistent finding in males fed Benzyl Violet (Table II).

Food efficiency was lowered at the start of the test for both sexes at the 3 per cent level and for the males in the 0·03 per cent group. After 5 weeks all test groups were up to the control values (Table III).

TABLE III

CUMULATIVE FOOD EFFICIENCY IN RATS FED BRILLIANT BLUE, GUINEA GREEN OR BENZYL VIOLET

Treatment	Dosage (per cent of diet)	Food efficiency (g. gained/100 g. food consumed. Mean ± S.E.) 3 weeks	5 weeks	10 weeks	16 weeks	26 weeks	52 weeks
Males							
Control		26·0 ± 0·42	22·5 ± 0·42	15·1 ± 0·32	11·1 ± 0·23	7·9 ± 0·17	4·1 ± 0·18
Brilliant Blue	0·03	26·8 ± 0·45	22·7 ± 0·51	15·2 ± 0·39	11·5 ± 0·25	8·1 ± 0·13	4·0 ± 0·21
	0·3	27·5 ± 0·64	*24·0 ± 0·52	†16·9 ± 0·31	†12·2 ± 0·18	†8·6 ± 0·16	4·2 ± 0·12
	3·0	27·1 ± 0·71	22·8 ± 0·62	15·7 ± 0·24	11·4 ± 0·23	8·2 ± 0·17	4·4 ± 0·14
Guinea Green	0·03	25·6 ± 0·64	22·8 ± 0·55	16·2 ± 0·47	*12·0 ± 0·33	†8·5 ± 0·16	4·4 ± 0·14
	0·3	†22·8 ± 0·30	*20·9 ± 0·52	14·9 ± 0·43	11·1 ± 0·23	8·1 ± 0·14	*4·5 ± 0·11
	3·0	†23·1 ± 0·71	†19·9 ± 0·64	14·5 ± 0·50	10·5 ± 0·42	7·7 ± 0·25	4·1 ± 0·15
Benzyl Violet	0·03	*24·7 ± 0·25	*20·8 ± 0·64	15·1 ± 0·31	11·3 ± 0·24	8·0 ± 0·14	4·2 ± 0·16
	0·3	25·2 ± 0·68	22·5 ± 0·77	16·0 ± 0·37	*11·9 ± 0·22	8·4 ± 0·17	4·3 ± 0·10
	3·0	*23·8 ± 0·81	*20·6 ± 0·62	14·8 ± 0·42	11·1 ± 0·29	8·0 ± 0·24	4·6 ± 0·19
Females							
Control		20·0 ± 0·38	15·3 ± 0·45	11·0 ± 0·20	8·4 ± 0·16	6·0 ± 0·08	3·2 ± 0·05
Brilliant Blue	0·03	19·9 ± 1·12	15·5 ± 0·93	10·5 ± 0·39	8·1 ± 0·29	5·8 ± 0·21	3·0 ± 0·14
	0·3	20·5 ± 0·60	16·4 ± 0·43	11·3 ± 0·25	8·3 ± 0·16	6·0 ± 0·12	3·1 ± 0·11
	3·0	20·1 ± 0·37	†17·0 ± 0·37	11·3 ± 0·23	8·4 ± 0·19	6·0 ± 0·12	3·2 ± 0·11
Guinea Green	0·03	19·7 ± 0·62	15·6 ± 0·26	10·7 ± 0·26	8·3 ± 0·19	5·9 ± 0·11	3·2 ± 0·08
	0·3	*17·9 ± 0·66	15·1 ± 0·43	10·5 ± 0·24	8·0 ± 0·22	5·7 ± 0·15	3·3 ± 0·10
	3·0	18·9 ± 0·91	15·4 ± 0·54	10·6 ± 0·24	*7·9 ± 0·15	5·8 ± 0·12	3·2 ± 0·10
Benzyl Violet	0·03	20·3 ± 0·66	16·1 ± 0·46	10·6 ± 0·28	8·2 ± 0·18	*5·6 ± 0·13	3·3 ± 0·09
	0·3	19·2 ± 0·66	15·9 ± 0·43	10·7 ± 0·30	8·2 ± 0·19	6·0 ± 0·14	*3·4 ± 0·06
	3·0	†18·2 ± 0·49	15·7 ± 0·26	10·8 ± 0·19	8·0 ± 0·14	5·8 ± 0·13	3·2 ± 0·08

* P less than 0·05. † P less than 0·01.

Mortality

As indicated in Table IV the mortality at the end of a year was low and the deaths were well distributed among the groups. At 75 weeks, statistical analysis (Duncan, 1953) indicated a significant increase in mortality of female rats on the 3 per cent level of each of the three colours. This significance was largely due to the very low death rate in the control group. When the mortality figures of males and females were combined there was no longer statistical significance in the results.

A summary of the necropsy findings in rats that died during the test is also given in Table IV. Sixty-six animals died of chronic respiratory disease. This disease is endemic in our rat colony and there appeared to be no connection between the colours fed and the amount of respiratory disease. Eight rats died from malignant tumours, two of renal failure, and one of starvation. Because of advanced autolysis, cause of death remained unknown in six animals.

Histopathology

The histopathological findings are summarised in Table V. Kidney changes are present to some degree in all rats of this age in our colony, Only when the pathology of the kidney was marked was any note made of the change. In the Table "nephritis" includes glomerulonephritis and nephrosis. No attempt was made to assess observed change in the

TABLE IV

MORTALITY IN RATS FED BRILLIANT BLUE, GUINEA GREEN, OR BENZYL VIOLET

Treatment	Dosage (per cent of diet)	Total number of rats on test	Number of deaths at 52 weeks: Males	52 weeks: Females	75 weeks: Males	75 weeks: Females	Causes of death: Respiratory M	Respiratory F	Tumour M	Tumour F	Other M	Other F	Unknown M	Unknown F
Control		30	2	0	6	1	6	1						
Brilliant Blue	0·03	30	1	1	7	4	6	4					1	
	0·3	30	0	0	5	5	3	5	1				1	
	3·0	30	3	2	5	6*	3	6	1		1			
Guinea Green	0·03	30	0	1	2	1	2	1						
	0·3	30	1	1	2	3	1	2				1	1	
	3·0	30	0	2	4	6*	4	1		3		1		1
Benzyl Violet	0·03	30	1	1	7	4	7	4						
	0·3	30	0	2	1	3	1	3						
	3·0	30	1	1	4	7*	3	3		3			1	1

* P less than 0·05.

TABLE V

SUMMARY OF HISTOPATHOLOGICAL FINDINGS IN RATS FED FOOD COLOURS FOR 75 WEEKS

Treatment	Control		Brilliant Blue						Guinea Green						Benzyl Violet					
Dosage (per cent of diet)			0·03		0·3		3·0		0·03		0·3		3·0		0·03		0·3		3·0	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Number of rats on test..	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Number of survivors ..	9	14	8	11	10	10	10	9	13	14	13	12	11	9	8	11	14	12	11	8
Number examined ..	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Nephritis	3	4	1	3	—	—	1	2	1	2	1	2	4	2	2	2	1	3	3	4
Hydronephrosis ..	—	6	—	6	—	7	—	6	—	12	—	9	—	8	—	4	1	13	—	5
Splenitis	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
Myocarditis	—	—	1	1	—	—	1	—	—	—	—	—	2	—	—	—	—	—	—	—
Liver cell change ..	—	—	—	—	1	1	—	1	—	—	—	—	—	1	—	—	—	—	1	2
Testicular atrophy ..	—	—	1	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—
Adrenal haemorrhage ..	—	3	—	3	—	—	1	1	—	2	—	1	—	—	1	1	—	1	—	2
Gastric hyperplasia ..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—
Tumours																				
Thyroid adenoma ..	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pituitary adenoma ..	—	1	—	—	2	—	2	1	—	1	—	1	1	—	—	2	—	1	—	—
Uterus polyp	—	4	—	4	—	3	—	1	—	4	—	5	—	—	—	—	—	1	—	—
Testis leydig tumour ..	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Mammary fibroadenoma	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Skin kerato-acanthoma	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—
Squamous cell carcinoma	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	1	—	—	1	2
Fibrosarcoma	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Kidney carcinoma ..	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Liposarcoma	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1
Lung carcinoma ..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Lymphosarcoma ..	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Malignant tumours per group	1	0	0	0	1	0	0	0	0	0	1	0	0	3	0	1	0	0	1	4

kidney and correlate this with levels of food colours fed, as was done by Mannell and others (1958). Adrenal haemorrhage, as previously described (Mannell and others, 1958), was a common finding in this group of rats. This change was not related to treatment.

With respect to neoplasms, pituitary adenomas and uterine polyps occur spontaneously in the colony. The majority of the malignant tumours was observed in rats fed 3 per cent Guinea Green or 3 per cent Benzyl Violet. Six tumours were derived from the epidermis, five of which were in female rats.

Haematology

There were slight differences noted in the results of the blood studies of the various groups but all values were within normal limits. This indicated that there were no adverse effects of these colours on the blood or blood-forming organs.

Discussion

We are aware of few published reports of toxicity studies on these three food colours. Willheim and Ivy (1953) fed Brilliant Blue and Guinea Green to rats at 4 per cent of the diet for as long as 20 months. They reported two tumours of the lymphatic tissue in nine rats fed Guinea Green and no tumours in ten rats with Brilliant Blue. Nelson and Hagan (1953) gave the same two colours to rats by subcutaneous injection for 94 to 99 weeks. With Guinea Green there was one tumour at the injection site and no distant tumours. With Brilliant Blue fibrosarcomas developed at the injection site in over 75 per cent of the animals.

In experiments in this laboratory (unpublished), no tumours were obtained with subcutaneous injections of Brilliant Blue over a 45-week period. The rats were observed for 71 weeks.

Hess and Fitzhugh (1955) studied the absorption and excretion of five triphenylmethane dyes including Brilliant Blue, Guinea Green and Benzyl Violet. When given orally to rats, the three colours were excreted almost completely in the faeces. None of the colours was found in the urine. Small amounts (less than 5 per cent) of Brilliant Blue and Guinea Green were found in the bile of dogs.

Our results indicate that Brilliant Blue and Benzyl Violet produced no deleterious effects on growth, food consumption and food efficiency in rats. With Guinea Green there was an initial depression of growth, due either to poorer food efficiency or, in one case, to reduced food consumption. In no instance did this effect last beyond the sixteenth week of feeding, and there was no effect at all at the lowest dietary level (0·03 per cent).

An increase in mortality was shown for female rats fed 3 per cent of all three colours. In the group fed Brilliant Blue the cause of death in all six animals that died was respiratory disease. This was not considered to be related to treatment. Nearly half of the deaths of females fed the other two colours at the 3 per cent level were due to tumours. This must be viewed with suspicion.

Malignant tumours were most frequent in the groups fed 3 per cent Guinea Green and 3 per cent Benzyl Violet. Six tumours were derived from the epidermis. Only one skin tumour has been observed in approximately 1,200 rats of a similar age in this colony. The incidence of skin tumours in the Guinea Green group was 13 per cent and in the rats on Benzyl Violet was 20 per cent. In addition, two females fed 3 per cent Guinea Green had benign skin lesions, which meant that nearly 25 per cent of the rats in this group developed skin tumours. From contact with the food, the fur and skin of the rats eventually became stained with the food colours. It is possible that if these two colours act as carcinogens on the skin, the action is by direct contact rather than indirectly through oral administration. This possibility is presently being investigated. The poor absorption of these colours from the intestinal tract reported by Hess and Fitzhugh (1955), might be considered as evidence favouring a direct effect on the skin.

From the results of our experiments, there appears to be no evidence to contradict the safety of Brilliant Blue as a food colour. It is felt that the status of Guinea Green and Benzyl Violet needs further clarification.

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