CHRONIC TOXICITY STUDIES ON FOOD COLOURS

PART IV. OBSERVATIONS ON THE TOXICITY OF TARTRAZINE, AMARANTH AND SUNSET YELLOW IN RATS

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Received July 14, 1958

Tartrazine, amaranth and sunset yellow fed at concentrations of 0·03, 0·3, and 1·5 per cent of the diet for 64 weeks, did not increase mortality in male or female rats. Amaranth, at a level of 1·5 per cent, caused a significant decrease in growth rate in female, but not male, rats. This was attributed to an effect on food utilisation rather than on food consumption. Female rats fed amaranth at 0·3 and 1·5 per cent concentrations showed an increase in the weight of the liver. At the higher concentration there was also an increase in kidney weight. Histopathological studies were made on the lung, heart, liver, spleen, thyroid, pancreas, stomach, small intestine, kidney, urinary bladder, adrenal, testis, prostate, coagulating gland, ovary, uterus, and thymus. None of the changes observed were considered to be due to the ingestion of the food colours. There was no significant difference in tumour incidence between the control animals and the rats receiving the colours.

PROVISION is made in the Regulations under the Food and Drugs Act in Canada for the use of 15 coal tar colours in foods. Of the 15, amaranth, tartrazine and sunset yellow account for some 75 per cent of the total amount used. Because of this relatively high level of use and the renewed interest in their toxicity, it was decided to include them in our studies even though considerable toxicological data have been presented previously, especially for amaranth and tartrazine. Summaries of these data have been compiled[1].

As described for other food colours in earlier papers in this series[2–4], the effects of oral administration of amaranth, tartrazine, and sunset yellow on mortality, growth, food consumption, food efficiency, and organ weights were investigated. Histopathological, haematological and other studies were also done.

METHODS

The colours were added to the normal laboratory diet to give concentrations of 0·03, 0·3, and 1·5 per cent for each dye. In the lower concentrations, alphacel (a non-nutritive cellulose) was added so that the level of dye plus alphacel was 1·5 per cent. The control diet contained 1·5 per cent alphacel. The rats were between 6 and 7 weeks of age at the start of the experiment and were distributed to give similar initial mean body weights among groups. All animals were kept in individual cages and were given free access to food and water. Groups of 15 males and 15 females were assigned to each level of each food colour, and 15 rats of each sex were given the control diet. Body weight, food consumption and food

efficiency data were recorded weekly. Necropsies were performed on the rats that died during the test. After 26 weeks on test, five rats of each group receiving 1·5 per cent colour were killed for histological examination. Haemoglobin estimations were done using a slight modification of the pyridine-haemochromogen method of Rimington[5].

At the end of the experiment, electrocardiograms and electroencephalograms were recorded from six rats of each sex on the control diet and three

TABLE I

CUMULATIVE NUMBER OF DEATHS OF RATS FED TARTRAZINE, SUNSET YELLOW AND AMARANTH

Treatment	Conc. of colour (per cent in diet)	No. rats on test	Time in weeks on test												
			16	20	24	28	32	36	40	44	48	52	56	60	64
Males															
Control ..		15	0	0	0	0	0	1	1	1	1	1	2	3	4
Tartrazine ..	0·03	15	0	0	0	0	0	0	0	0	1	1	1	1	1
	0·3	15	0	0	0	1	1	3	3	3	3	3	3	3	3
	1·5	15	0	0	0	*5	5	5	5	5	5	5	5	5	6
Sunset yellow	0·03	15	0	0	0	0	0	0	0	0	0	1	1	1	1
	0·3	15	0	0	0	0	0	0	0	0	1	2	2	3	3
	1·5	15	0	0	0	*5	5	5	5	5	5	5	5	5	6
Amaranth ..	0·03	15	0	0	0	0	1	1	2	2	3	3	4	4	5
	0·3	15	0	0	0	1	1	1	1	1	2	2	3	4	4
	1·5	15	0	0	0	*5	5	5	5	5	5	5	5	5	6
Females															
Control ..		15	0	0	1	1	1	1	2	2	3	3	3	3	5
Tartrazine ..	0·03	15	0	0	0	0	0	0	1	1	1	1	1	2	2
	0·3	15	0	0	0	0	0	0	0	0	0	0	0	0	1
	1·5	15	0	0	0	*5	5	5	5	5	5	5	5	5	5
Sunset yellow	0·03	15	0	0	0	0	0	0	0	0	0	0	0	0	0
	0·3	15	0	0	0	0	0	0	0	0	0	0	1	1	2
	1·5	15	0	0	0	*5	5	5	5	6	6	6	6	6	6
Amaranth ..	0·03	15	0	0	0	0	0	0	0	0	0	0	0	0	1
	0·3	15	0	0	0	0	0	0	1	1	1	2	2	2	2
	1·5	15	1	1	1	*5	5	5	5	5	5	5	6	6	6

* Five rats killed at 26 weeks.

rats of each sex on the 1·5 per cent level of each colour, lightly anaesthetised with pentobarbitone. Short hypodermic needles served as electrodes for both the ECG and the EEG. A Grass Electroencephalograph, Model III–D, was used to record the electric potentials.

After 64 weeks on test the surviving rats were anaesthetised with ether and a cursory gross examination was made of all organs and tissues. To facilitate fixation of tissues, the right auricle was then cut open and warm physiological saline was injected into the left ventricle; when the flow of saline from the right auricle was only slightly blood tinged a 5 per cent solution of formol-saline was injected into the left ventricle. A detailed gross examination of all tissues and organs was then made. The weights of certain organs were recorded. The tissues in which any gross pathological change was observed were studied histologically.

In addition, a detailed examination was made of haematoxylin-eosin stained paraffin sections of lung, heart, liver, spleen, thyroid, pancreas,

stomach, small intestine, kidney, urinary bladder, adrenal, testis, prostate, coagulating gland, ovary, uterus, and thymus.

Results and Discussion

Mortality

The mortality of control and test rats during the course of the experiment is shown in Table I. If the figures for males and females are combined, the mortality for the control rats at the end of the experiment was 30 per cent. For all rats on tartrazine and sunset yellow the mortality was 20 per cent, and for amaranth 27 per cent. These figures include

TABLE II

MEAN BODY WEIGHT OF RATS FED TARTRAZINE, SUNSET YELLOW AND AMARANTH

Treatment	Dosage (per cent of diet)	Mean body weight (g. ± S.E.)					
		Initial	4 weeks	16 weeks	32 weeks	48 weeks	64 weeks
Males							
Control ..		129 ± 4	206 ± 4	289 ± 6	338 ± 8	364 ± 8	363 ± 17
Tartrazine ..	0·03	130 ± 3	205 ± 4	283 ± 5	330 ± 8	350 ± 10	351 ± 17
	0·3	123 ± 5	198 ± 5	283 ± 7	335 ± 6	352 ± 11	336 ± 13
	1·5	130 ± 5	202 ± 5	278 ± 7	322 ± 12	337 ± 18	336 ± 25
Sunset yellow	0·03	132 ± 4	200 ± 4	280 ± 6	320 ± 7	340 ± 13	349 ± 10
	0·3	131 ± 4	206 ± 4	293 ± 7	331 ± 11	351 ± 11	364 ± 10
	1·5	139 ± 5	208 ± 5	290 ± 6	344 ± 11	345 ± 14	362 ± 15
Amaranth ..	0·03	135 ± 3	203 ± 3	284 ± 4	344 ± 6	367 ± 11	389 ± 9
	0·3	135 ± 4	209 ± 5	293 ± 6	332 ± 9	347 ± 15	373 ± 13
	1·5	127 ± 3	198 ± 3	284 ± 5	336 ± 8	351 ± 8	359 ± 15
Females							
Control		105 ± 3	141 ± 3	185 ± 3	218 ± 4	230 ± 4	222 ± 9
Tartrazine ..	0·03	103 ± 3	139 ± 3	174 ± 3*	205 ± 5	223 ± 5	232 ± 7
	0·3	104 ± 3	140 ± 3	175 ± 4	211 ± 5	227 ± 5	224 ± 8
	1·5	106 ± 3	141 ± 3	177 ± 4	210 ± 7	209 ± 8*	209 ± 11
Sunset yellow	0·03	108 ± 2	143 ± 3	185 ± 4	217 ± 4	232 ± 4	238 ± 5
	0·3	107 ± 3	137 ± 2	180 ± 3	207 ± 3*	216 ± 2*	224 ± 7
	1·5	106 ± 3	141 ± 1	181 ± 3	206 ± 3*	230 ± 3	233 ± 6
Amaranth ..	0·03	103 ± 2	134 ± 2	175 ± 4	204 ± 3*	217 ± 3*	226 ± 8
	0·3	103 ± 3	135 ± 3	175 ± 4	207 ± 4	222 ± 4	224 ± 5
	1·5	103 ± 3	133 ± 2*	174 ± 3*	199 ± 3*	217 ± 3*	217 ± 3

* Significant at P = 0·05 or less.

those animals sacrificed at 26 weeks. If they are excluded the mortality rates are: tartrazine and sunset yellow 10 per cent, amaranth 18 per cent. Thus the dyes did not increase mortality when given at levels as high as 1·5 per cent of the diet.

Growth, Food Consumption and Food Efficiency

For results discussed in this section, significant differences between mean values were determined by Student's "*t*" test. Mean body weights at selected intervals are given in Table II. A consistent lag in growth occurred for female rats in the group fed 1·5 per cent amaranth. At the 0·03 per cent level, the amaranth-fed females showed a significantly lower body weight at 32 and 48 weeks. There were three instances where female

rats on sunset yellow had a lower mean weight than did the control group and two isolated significant decreases for the females on tartrazine.

For the male rats there were no significant differences between any of the test groups and the control group as shown in Table II. A similar sex difference was noted in an earlier study[4]. This suggests that female rats may be more sensitive to some coal-tar dyes than males where an effect on growth is concerned.

Most of the groups of females showed significant decreases in food consumption at one time or another during the experiment, as seen in Table III. There was a continuous depression in food consumption for

TABLE III

MEAN FOOD CONSUMPTION OF RATS FED TARTRAZINE, SUNSET YELLOW AND AMARANTH

Treatment	Dosage (per cent of diet)	Mean food consumption (g./rat/day ± S.E.)					
		4 weeks	8 weeks	16 weeks	32 weeks	48 weeks	64 weeks
Males							
Control ..		16·1 ± 0·28	16·5 ± 0·19	16·9 ± 0·20	17·4 ± 0·25	18·4 ± 0·25	18·6 ± 0·26
Tartrazine	0·03	16·0 ± 0·25	16·4 ± 0·28	17·2 ± 0·30	17·8 ± 0·32	18·5 ± 0·36	18·7 ± 0·38
	0·3	15·3 ± 0·29	16·1 ± 0·28	16·8 ± 0·33	17·5 ± 0·22	18·1 ± 0·30	18·3 ± 0·30
	1·5	15·3 ± 0·24*	15·9 ± 0·22*	16·5 ± 0·23	17·4 ± 0·41	18·2 ± 0·43	19·0 ± 0·39
Sunset yellow	0·03	15·7 ± 0·30	16·4 ± 0·33	17·0 ± 0·38	17·3 ± 0·41	17·9 ± 0·42	18·5 ± 0·50
	0·3	16·0 ± 0·14	16·6 ± 0·19	17·3 ± 0·31	17·8 ± 0·33	18·3 ± 0·37	18·8 ± 0·38
	1·5	15·9 ± 0·32	16·6 ± 0·32	17·2 ± 0·36	18·2 ± 0·51	18·8 ± 0·46	19·2 ± 0·54
Amaranth	0·03	16·1 ± 0·29	16·6 ± 0·35	17·1 ± 0·45	18·0 ± 0·44	18·3 ± 0·42	19·1 ± 0·44
	0·3	16·3 ± 0·32	16·5 ± 0·30	16·6 ± 0·27	17·5 ± 0·26	18·1 ± 0·27	18·8 ± 0·33
	1·5	15·6 ± 0·17	16·1 ± 0·21	16·2 ± 0·21*	17·3 ± 0·26	18·1 ± 0·33	18·8 ± 0·41
Females							
Control ..		13·4 ± 0·22	13·5 ± 0·18	13·7 ± 0·19	13·9 ± 0·20	14·6 ± 0·14	14·9 ± 0·17
Tartrazine	0·03	12·9 ± 0·31	13·1 ± 0·22	13·5 ± 0·24	13·9 ± 0·38	14·6 ± 0·29	14·9 ± 0·27
	0·3	12·7 ± 0·21*	13·1 ± 0·19	13·2 ± 0·17	13·4 ± 0·17	14·4 ± 0·14	14·7 ± 0·21
	1·5	12·6 ± 0·21*	12·8 ± 0·17*	12·6 ± 0·10*	13·0 ± 0·11*	13·9 ± 0·12*	14·2 ± 0·16*
Sunset yellow	0·03	12·9 ± 0·23	13·0 ± 0·21	13·0 ± 0·20*	13·3 ± 0·18*	14·0 ± 0·15*	14·4 ± 0·19
	0·3	12·9 ± 0·13	13·1 ± 0·17	13·1 ± 0·22*	13·1 ± 0·22*	13·6 ± 0·20*	14·1 ± 0·14*
	1·5	12·9 ± 0·26	13·3 ± 0·21	13·1 ± 0·14*	13·5 ± 0·24	14·3 ± 0·31	14·6 ± 0·34
Amaranth	0·03	12·6 ± 0·21*	12·9 ± 0·23*	13·0 ± 0·31	13·6 ± 0·42	14·5 ± 0·45	14·9 ± 0·47
	0·3	12·9 ± 0·18	13·2 ± 0·20	12·9 ± 0·16*	13·3 ± 0·21*	14·3 ± 0·22	14·8 ± 0·30
	1·5	12·7 ± 0·22	12·9 ± 0·24	12·9 ± 0·26*	13·5 ± 0·38	14·4 ± 0·34	14·7 ± 0·40

* Significant at P = 0·05 or less.

the group fed 1·5 per cent tartrazine from the fourth week to the end of the test. However, this did not produce any significant decrease in body weight except at the 48 week interval. For sunset yellow and amaranth the differences appeared more randomly distributed. In general the groups on sunset yellow showed reduced food consumption from the sixteenth week onward while most of the differences in the rats receiving amaranth occurred within the first 16 weeks. With the exception of tartrazine these decreased food consumption values showed no correlation with the concentration of colour given in the diet. For the male rats there were only three instances where a significant drop in food consumption occurred. These were for the group fed 1·5 per cent tartrazine at 4 and 8 weeks, and for the high level amaranth group at 16 weeks.

The mean cumulative food efficiency is shown in Table IV. It appears that amaranth at 1·5 per cent in the diet interfered with the utilisation of food for growth in female rats. Although the decrease in food efficiency was not significant at 4 weeks there was a significant decrease from the eighth to the thirty-second week. In the latter half of the experiment, when food efficiency is low for all groups, this effect was not apparent.

TABLE IV

MEAN CUMULATIVE FOOD EFFICIENCY OF RATS FED TARTRAZINE, SUNSET YELLOW AND AMARANTH

Treatment	Dosage (per cent of diet)	Mean food efficiency (g. gained/100 g. food consumed ± S.E.)				
		4 weeks	8 weeks	16 weeks	32 weeks	64 weeks
Males						
Control		16·7 ± 0·65	11·8 ± 0·42	8·4 ± 0·22	5·4 ± 0·21	2·8 ± 0·18
Tartrazine	0·03	16·3 ± 0·46	10·9 ± 0·35	7·9 ± 0·19	5·0 ± 0·18	2·6 ± 0·19
	0·3	17·0 ± 0·49	11·9 ± 0·34	8·4 ± 0·18	5·6 ± 0·19	2·6 ± 0·11
	1·5	16·3 ± 0·68	11·3 ± 0·44	8·0 ± 0·19	5·0 ± 0·30	2·5 ± 0·31
Sunset yellow ..	0·03	15·3 ± 0·46	10·9 ± 0·33	7·8 ± 0·29	4·9 ± 0·19	2·6 ± 0·10
	0·3	16·0 ± 0·82	11·6 ± 0·59	8·4 ± 0·32	5·0 ± 0·18	2·9 ± 0·10
	1·5	15·2 ± 0·48	10·8 ± 0·36	7·8 ± 0·25	5·0 ± 0·26	2·6 ± 0·13
Amaranth	0·03	14·7 ± 0·55*	10·6 ± 0·44	7·8 ± 0·26	5·2 ± 0·17	3·0 ± 0·08
	0·3	15·6 ± 0·48	11·3 ± 0·39	8·5 ± 0·25	5·0 ± 0·24	2·8 ± 0·14
	1·5	15·5 ± 0·44	11·4 ± 0·25	8·6 ± 0·25	5·4 ± 0·29	2·8 ± 0·17
Females						
Control		9·4 ± 0·34	7·3 ± 0·28	5·2 ± 0·14	3·6 ± 0·11	1·7 ± 0·12
Tartrazine	0·03	9·4 ± 0·44	6·7 ± 0·29	4·7 ± 0·12*	3·3 ± 0·12	1·9 ± 0·08
	0·3	9·6 ± 0·39	6·7 ± 0·22	4·8 ± 0·15	3·5 ± 0·11	1·8 ± 0·08
	1·5	9·4 ± 0·41	6·9 ± 0·22	4·9 ± 0·18	3·4 ± 0·17	1·6 ± 0·20
Sunset yellow ..	0·03	9·3 ± 0·19	7·4 ± 0·25	5·3 ± 0·19	3·7 ± 0·12	2·0 ± 0·07
	0·3	8·1 ± 0·46*	6·9 ± 0·23	4·9 ± 0·17	3·4 ± 0·12	1·9 ± 0·12
	1·5	8·5 ± 0·65	6·9 ± 0·31	5·0 ± 0·19	3·3 ± 0·13	1·9 ± 0·05
Amaranth	0·03	8·4 ± 0·44	6·8 ± 0·23	4·9 ± 0·20	3·3 ± 0·11	1·9 ± 0·13
	0·3	8·5 ± 0·33	6·5 ± 0·29	4·9 ± 0·19	3·5 ± 0·13	1·9 ± 0·10
	1·5	8·2 ± 0·50	6·5 ± 0·25*	4·8 ± 0·11*	3·0 ± 0·07*	1·7 ± 0·06

* Significant at P = 0·05 or less.

Two other differences in food efficiency for female rats were noted (see Table IV) but it is difficult to explain the significance of these results. In the food efficiency data for males there was only one result that showed a significant difference from the control levels. This was at 4 weeks for rats on 0·03 per cent amaranth.

Electrocardiograms and Electroencephalograms

The three standard leads of ECG were recorded. The tracings were essentially normal with the exception that extrasystoles were observed in two rats (one male rat on tartrazine and one female rat on sunset yellow, see Fig. 1 A). No significant deviation of the electrical axis was observed in any rat. It may be noted, however, that the auriculo-ventricular conduction was slower in all the rats, ranging from 55 to 60 msec., as compared to the reported figure of 24 to 55 msec.[6–10] This might be related to the age of the rats, since Hundley and others[7] reported that the P–R interval increased with age in their rats. The P, R and T waves had

voltages two to three times higher than the values reported in the references cited. This may be due to a more sensitive response obtained with the recording machine used. The tracing of an exemplary ECG is presented in Figure 1 B. The mean heart rates and their standard errors of the control rats were 373 $\pm$ 13 beats per minute for males and 383 $\pm$ 13 for females. The figures for the treated rats were similar to those of the controls.

A bipolar EEG (frontal-occipital) was recorded from these rats, with the left ear grounded. All the tracings appeared normal. A typical EEG is shown in Figure 1 C.

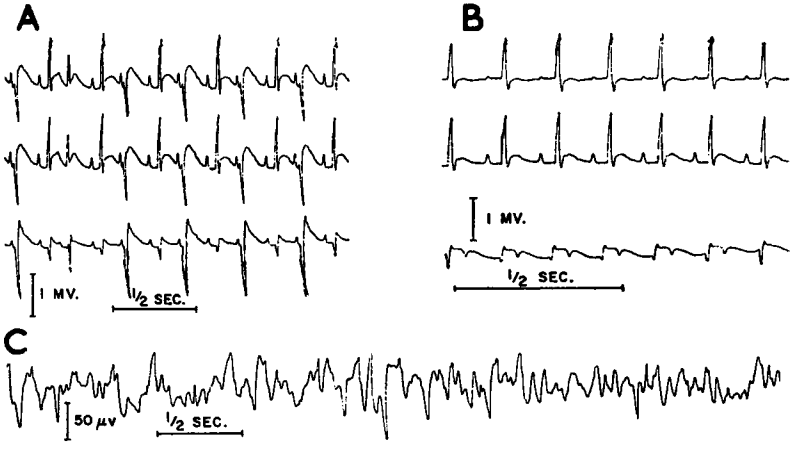

FIG. 1. A. The ECG of a female rat on 1·5 per cent sunset yellow, showing extrasystoles (septal).

B. The ECG of a control male rat. The heart rate was 375 beats per minute, the P-R interval, 55 msec., the QRS, 15 msec., the Q-Tc, 250 msec., the P_2, 0·23 mv., the R_2, 1·23 mv. and the T_2, 0·22 mv.

C. The EEG (frontal-occipital) of a control male rat.

Organ Weights

The mean weights (in mg./g. body weight) of some of the organs taken for histological examination are given in Table V. There were no significant weight changes in any of the organs of the male rats. There was a marked increase in liver weight for the female rats receiving 0·3 and 1·5 per cent amaranth. In the latter group this was accompanied by a significant increase in the weight of the kidneys. Such increases in organ weight may be the result of hyperactivity of the organs. There was a decrease in liver weight in two of the groups on sunset yellow and a decrease in the weight of the spleen for two groups on sunset yellow and one on amaranth. These changes were not correlated with the level of food colour in the diet and are difficult to interpret.

Pathology

A necropsy was performed on 39 rats that died during the test. Advanced autolysis precluded diagnosis in four cases. Respiratory tract

infections accounted for 28 deaths. Two animals died of starvation, one of meningitis, one of a ruptured right auricle and three as the result of neoplasms. The histopathological findings of the last three are included in Table VI.

Histopathology

Middle ear and respiratory infections have been found to be relatively numerous in older rats of the colony and in the groups under study these infections were observed in a large number of rats at autopsy (70 weeks of age). Only those changes which were considered excessive and capable of affecting the animal noticeably are listed in Table VI. There was no

TABLE V

ORGAN WEIGHTS OF RATS FED TARTRAZINE, SUNSET YELLOW AND AMARANTH

Treatment	Dosage (per cent of diet)	No. rats killed	Mean organ weight (mg./g. body weight ± S.E.)					
			Heart	Liver	Spleen	Kidneys	Gonads	Adrenals
Males								
Control ..		11	3·6 ± 0·25	27·4 ± 1·70	2·1 ± 0·08	6·9 ± 0·28	8·1 ± 0·27	0·10 ± 0·008
Tartrazine	0·03	14	3·6 ± 0·17	25·2 ± 0·56	2·1 ± 0·07	6·9 ± 0·17	8·1 ± 0·40	0·10 ± 0·007
	0·3	11	3·5 ± 0·15	24·6 ± 0·33	2·2 ± 0·06	7·0 ± 0·19	8·5 ± 0·31	0·10 ± 0·008
	1·5	8	3·6 ± 0·22	27·2 ± 1·72	2·1 ± 0·11	7·2 ± 0·27	8·5 ± 0·30	0·10 ± 0·019
Sunset yellow	0·03	14	3·4 ± 0·10	24·8 ± 0·23	2·1 ± 0·07	6·9 ± 0·16	8·3 ± 0·35	0·09 ± 0·005
	0·3	12	3·4 ± 0·10	25·3 ± 0·32	2·1 ± 0·04	7·2 ± 0·18	8·3 ± 0·28	0·09 ± 0·004
	1·5	9	3·5 ± 0·16	25·4 ± 0·81	2·2 ± 0·06	7·1 ± 0·14	8·8 ± 0·32	0·09 ± 0·005
Amaranth	0·03	10	3·2 ± 0·17	25·0 ± 0·71	2·0 ± 0·07	6·9 ± 0·25	8·0 ± 0·16	0·10 ± 0·01
	0·3	11	3·3 ± 0·11	28·5 ± 1·22	2·1 ± 0·07	6·7 ± 0·19	8·3 ± 0·35	0·08 ± 0·005
	1·5	9	3·4 ± 0·11	27·0 ± 1·31	2·1 ± 0·07	7·1 ± 0·23	8·4 ± 0·34	0·09 ± 0·005
Females								
Control ..		9	4·6 ± 0·25	30·0 ± 1·02	3·0 ± 0·12	8·4 ± 0·27	0·49 ± 0·05	0·23 ± 0·02
Tartrazine	0·03	13	4·5 ± 0·13	28·9 ± 0·68	2·8 ± 0·07	8·3 ± 0·23	0·42 ± 0·04	0·23 ± 0·01
	0·3	14	4·5 ± 0·12	28·3 ± 0·98	2·8 ± 0·08	8·3 ± 0·23	0·44 ± 0·02	0·25 ± 0·01
	1·5	10	5·0 ± 0·30	31·5 ± 2·44	2·8 ± 0·20	9·2 ± 0·52	0·50 ± 0·05	0·27 ± 0·02
Sunset yellow	0·03	15	4·3 ± 0·11	26·7 ± 0·38*	2·6 ± 0·05*	7·9 ± 0·28	0·40 ± 0·02	0·22 ± 0·01
	0·3	12	4·3 ± 0·18	25·8 ± 0·71*	2·7 ± 0·16	7·9 ± 0·20	0·43 ± 0·02	0·28 ± 0·03
	1·5	9	4·4 ± 0·14	27·5 ± 1·36	2·5 ± 0·08*	8·6 ± 0·31	0·42 ± 0·03	0·21 ± 0·01
Amaranth	0·03	13	4·1 ± 0·11	28·2 ± 1·63	2·6 ± 0·13*	8·0 ± 0·24	0·43 ± 0·03	0·24 ± 0·01
	0·3	13	4·6 ± 0·16	35·1 ± 0·79*	3·1 ± 0·08	8·8 ± 0·28	0·46 ± 0·04	0·23 ± 0·01
	1·5	9	4·9 ± 0·17	34·7 ± 0·84*	3·3 ± 0·24	9·8 ± 0·52*	0·50 ± 0·02	0·26 ± 0·02

* Significant at P = 0·05 or less.

difference in incidence and severity of respiratory lesions between the control and test groups. Chronic otitis media was observed in nearly 50 per cent of the animals. The disease was evenly distributed in the various groups.

Twenty-two animals exhibited pathological change in the adrenal cortex. This change was characterised by a small focal area of haemorrhage with rarefaction of the cytoplasm of parenchymal cells and some necrosis in and surrounding the area. This particular pathology was acute in nature and was not considered to be an effect of the food colours since it was observed in two of the control animals and since there was no correlation between the incidence and the concentration of colour fed.

Changes observed in the kidneys were difficult to assess as they occur normally in aged rats in this colony. In an attempt to permit an assessment of observed change and to correlate this with the possible effects of the food colours, an index of nephrosis and glomerulonephritis was established. Nephrosis was characterised by moderate flattening of proximal convoluted tubular epithelium, tubular dilatation and hyaline

TABLE VI

SUMMARY OF HISTOPATHOLOGICAL FINDINGS IN RATS FED FOOD COLOURS FOR 64 WEEKS

Treatment	Control		Sunset yellow						Tartrazine						Amaranth						
Dosage (per cent of diet)			0·03		0·3		1·5		0·03		0·3		1·5		0·03		0·3		1·5		
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	Totals
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	300
Number of survivors	11	10	14	15	12	13	9	9	14	13	12	14	9	10	10	14	11	13	9	9	231
Number examined	11	10	8	15	12	13	9	9	14	13	12	14	9	10	10	14	11	13	9	9	225
Glomerulonephritis	4	—	2	—	6	—	5	—	5	3	4	—	3	—	3	—	7	—	5	2	49
Nephrosis	11	5	3	—	9	1	7	1	8	3	10	3	5	1	8	1	10	2	8	2	98
Hydronephrosis	—	2	—	2	—	—	—	3	—	3	—	4	—	4	—	3	—	3	—	4	28
Cystic kidney	—	—	—	—	1	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	3
Hydroureter	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1
Hydrouterus	—	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—	1	—	—	5
Pyometritis	—	—	—	—	—	3	—	—	—	—	—	1	—	—	—	—	—	—	—	—	4
Ovarian cyst	—	1	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	2	5
Testicular atrophy	1	—	—	—	1	—	—	—	1	—	2	—	—	—	—	—	—	—	—	—	5
Myocarditis	—	—	—	—	—	—	—	1	1	—	1	—	—	—	—	—	—	—	1	—	4
Periaortitis	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	2	—	1	—	4
Pneumonic abscess	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1
Pneumonia advanced	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	2
Tracheobronchitis	—	—	—	—	—	—	—	—	—	1	—	—	1	1	—	—	—	1	—	—	4
Cystic pancreatitis	—	—	—	—	—	—	—	—	—	—	1	—	—	1	—	—	1	1	—	—	4
Adrenal hemorrhage	—	2	—	1	1	4	1	—	—	3	2	4	—	—	—	2	—	—	—	2	22
Liver abscess	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	3
Tumours:																					
Uterus leiomyoma	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1	—	—	2
Uterus polyp	—	2	—	—	—	2	—	2	—	—	—	4	—	2	—	—	—	3	—	2	17
Uterus leiomyosarcoma	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Rectum neurofibroma	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1
Testis leydig tumour	—	—	1	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	3
Thyroid adenoma	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
Kidney tubular adenoma	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Skin papilloma	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Mammary fibroadenoma	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	1	—	—	—	—	4
Urinary bladder papilloma	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1
Pancreas neurogenic sarcoma	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1
Stomach adenocarcinoma	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1
Liver hamartoma (congenital anomaly)	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1

cast collection. Glomerulonephritis was characterised by interstitial nephritis with collections of lymphocytes and monocytes and by glomerular change starting with a slight thickening of capsular epithelium. Advanced changes were typical of chronic glomerulonephritis.

In establishing the index, the kidney changes were graded according to the degree or extent of nephrosis or glomerulonephritis. The data so obtained were examined by means of a rank sum test[11]. In none of the test groups was there any significant deviation from the controls.

The tumour incidence in the control group was 14 per cent and in the food colour groups it was 16 per cent. Amaranth had a tumour incidence of 11, tartrazine 19, and sunset yellow 18 per cent. Uterine polyps occur frequently in this colony of rats and accounted for approximately 50 per

cent of the neoplasms in this study. Excluding polyps, the tumour incidence was 5 per cent in 21 control animals and 9 per cent in the 204 test animals. For the individual colours the incidence was: sunset yellow 12, tartrazine 11, and amaranth 3 per cent. The differences in tumour incidence are not significant according to chi-square tests.

TABLE VII

HAEMATOLOGICAL FINDINGS IN RATS FED FOOD COLOURS FOR 64 WEEKS

Treatment	Dosage (per cent of diet)	Haemoglobin (g. per cent)	Red blood cells (X 10^6)	White blood cells
Males				
Control		18·0*	9·2**	12,490***
Tartrazine	0·03	17·7	9·8	11,770
	0·3	18·5	9·8	12,350
	1·5	18·0	9·4	12,100
Sunset yellow	0·03	18·0	9·3	13,600
	0·3	17·4	8·9	10,760
	1·5	17·6	9·0**	12,550***
Amaranth	0·03	18·3	9·7	12,900
	0·3	18·2	8·7	11,430
	1·5	18·1	9·9	12,540***
Females				
Control		17·7*	8·6**	11,920***
Tartrazine	0·03	17·6	8·5	11,440
	0·3	17·3	8·0	11,900
	1·5	18·0	9·0	10,950
Sunset yellow	0·03	17·7	9·3	11,120
	0·3	17·8*	8·9**	11,150
	1·5	17·5	8·8	†8870***
Amaranth	0·03	17·7	8·0	11,810
	0·3	17·5	8·5	11,990
	1·5	17·6	9·1	11,020***

* Readings on 9 rats. ** Readings on 8 rats. *** Two readings on each of 8 rats. † $P<0·01$.

Haematology

One week before termination of the experiment red and white blood cell counts and haemoglobin determinations were made on three animals in each group. In some groups in which the readings were suggestive of an altered blood picture extra readings were taken to ensure statistical comparison at a suitable level of confidence. The results are shown in Table VII. The only significant finding was a lowered white cell count for females on 1·5 per cent sunset yellow as compared to the controls.

TABLE VIII

DIFFERENTIAL CELL COUNTS IN THE BLOOD AND BONE MARROW OF SOME FEMALE RATS

(Each figure is the average reading for 8 rats)

	Blood per cent			Bone marrow per cent	
	Neutrophiles	Lymphocytes	Others	Myeloid	Erythroid
Control	33·1	64·8	2·1	68·0	32·0
Sunset yellow 1·5 per cent ..	29·5	68·0	2·5	70·0	30·0

Differential counts of the cells in the blood and bone marrow were made on female rats of the control and 1·5 per cent sunset yellow groups because of the lower white cell counts of the latter group. The results, as given in Table VIII, showed no significant difference between the two groups. The haematological findings suggest that these food colours did not adversely affect the blood cells of the animals.

Acknowledgements. Thanks are due to Miss Constance Cox for much help with statistical assessments and to Mrs. Elaine O'Grady and Miss C. Beliveau for technical assistance.

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