CHRONIC TOXICITY STUDIES ON FOOD COLOURS

PART I. OBSERVATIONS ON THE TOXICITY OF FD&C YELLOW NO. 3 (OIL YELLOW AB) AND FD&C YELLOW NO. 4 (OIL YELLOW OB) IN RATS

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BADGER *et al.*[1] and Sugiura[2] reported that tumours were not produced in mice or rats when Yellow AB and OB colours were applied to the skin or incorporated in the diet for periods of treatment longer than one year in some instances. Where relatively large doses were administered the mortality was high, but no mention was made of the possible cause of death. In a recent report issued by the British Ministry of Food it was mentioned that these colours and some others are suspected to have harmful effects[3].

The experiments here reported were made in order to determine the effects of the oral administration of Oil Yellow AB and Oil Yellow OB on growth, food consumption, food efficiency, blood values, and on the pathology of a number of the organs.

METHODS

The food colours were incorporated in the laboratory diet in the following concentrations: 0·03, 1·5, and 3·0 per cent. To the basic diet (Fox Breeder Meal, Toronto Elevators, Ltd.) was added a supplement, which contained ground Fox Breeder Meal 38·4 per cent., Haliver oil with viosterol (60,000 I.U. Vitamin A and 10,000 I.U. Vitamin D per g.) 1·6 per cent. and crude casein 60·0 per cent., in the following proportions: ground Fox Breeder Meal 92·0 per cent., supplement 5·0 per cent. and alphacel plus colour 3·0 per cent. Each colour was added to the basic diet in the dry form and incorporated by means of a blender. The rats, in groups of 25 males and 25 females, were approximately 5 to 6 weeks of age at the beginning of the experiment. The three concentrations of each food colour were assigned at random. The animals were kept in groups of 12 or 13 rats to a cage and were given free access to their respective diets and water. Their weights and food consumption were recorded weekly. For a more accurate evaluation of food consumption it would have been preferable to put one rat only in a cage, but this was not possible. Post-mortem examinations were made on the rats which died but, in many cases due to advanced autolytic changes it was not possible to determine the cause of death. All other surviving animals were killed at the end of the experiments and post-mortem examinations made.

RESULTS AND DISCUSSION

The effect on Growth Rate, Food Consumption and Food Efficiency. Growth, food consumption and food efficiency curves of the experimental

groups receiving 0·03 per cent. of the colours in the diet and the control groups are shown in Figures 1 and 2. The growth curves for the experimental groups did not deviate appreciably from the controls during the

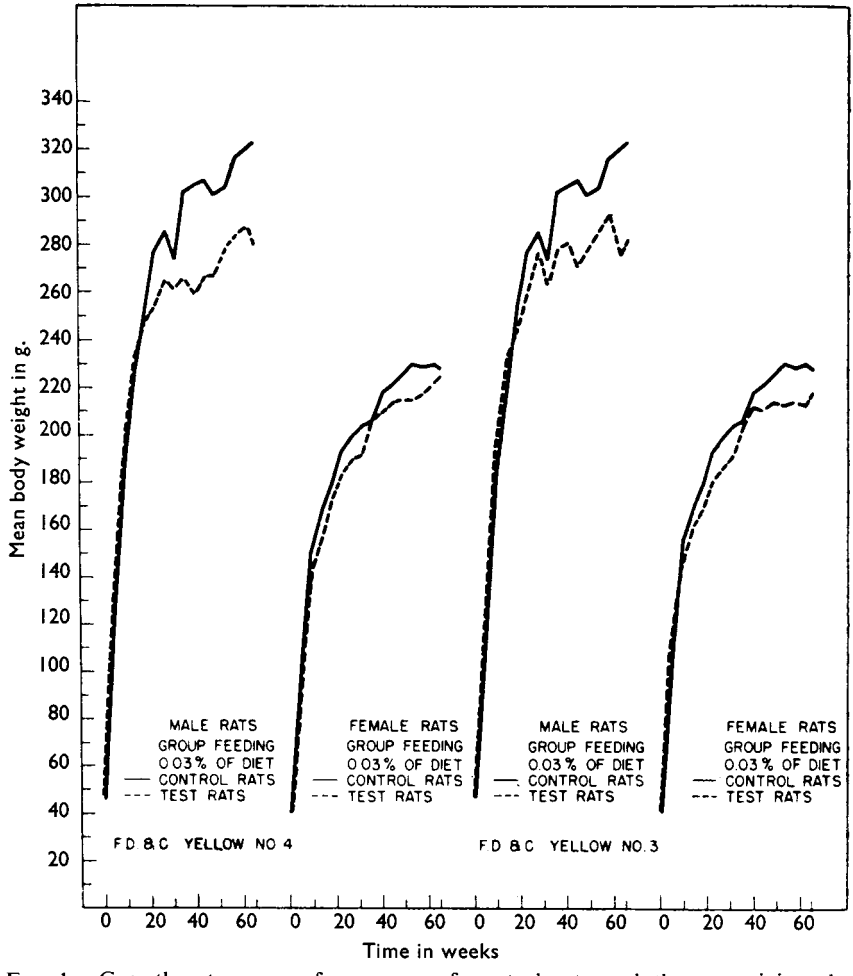

FIG. 1. Growth rate curves for groups of control rats and those receiving the colours.

first 20 weeks, but from the 20th week until the 65th week the test rats were retarded in their growth. However, when the test was terminated after 65 weeks the weights of the remaining rats on test were not significantly different from the controls. On the 1·5 and 3·0 per cent. diets all the rats died within 10 weeks. There was a gradual retardation of growth until death occurred. No significant differences in food consumption were found in the groups of female rats receiving 0·03 per cent. F.D.&C. Yellows No. 3 and 4 for 65 weeks compared with the controls. The amounts consumed were 12·3, 12·1 and 12·5 g. per rat per day for

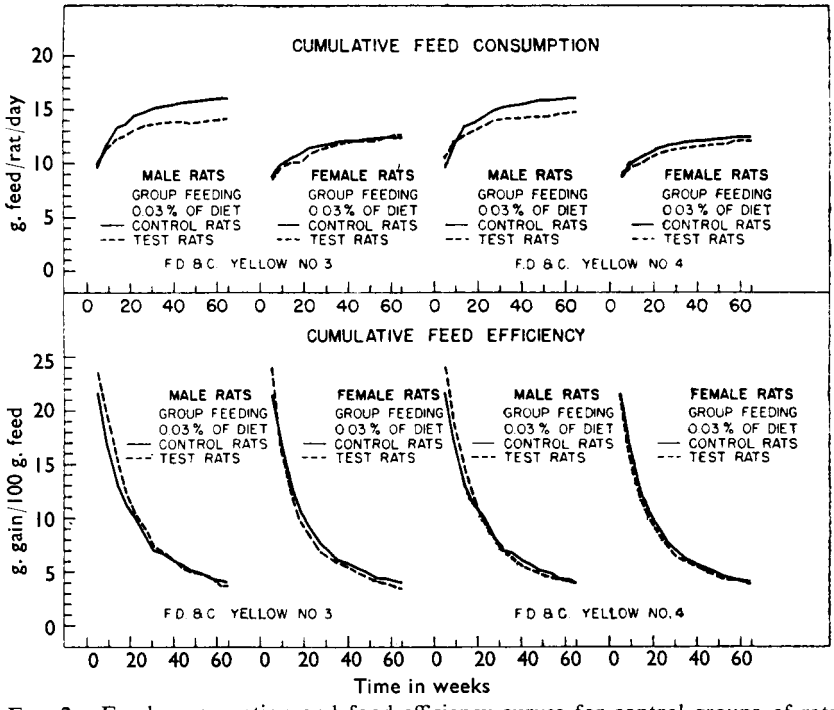

FIG. 2. Food consumption and food efficiency curves for control groups of rats and those receiving the colours.

the groups receiving the control diet and 0·03 per cent. concentrations of FD&C Yellows No. 3 and 4 respectively. The food consumption of the male rats on the same concentration of the colours was 14·7 and 14·1 g. per rat per day as compared to 16·0 g. per rat per day for the controls. The control male rats ate more than the males on test. Whether this lesser food consumption on the part of the males fed these colours in the diet is due to a dislike to the diet-colour mixture or to the toxic effects of the colours cannot be ascertained from these group feeding experiments. Another experiment in which groups of rats were force fed 5 days a week, for 20 weeks, on doses equivalent to 200 and 400 mg./kg. respectively of both colours, showed that the greatest effect on food consumption was caused by the 400 mg./kg. dose, and the food consumption of both males and females was affected. The other groups were affected to a lesser extent. It would appear then, that the food consumption is affected to some extent when the colour is consumed in the food or is given by means of stomach tube. The extent of the effect on food consumption will depend on the dose of the colour. A summary of the data on food consumption and food efficiency obtained in this experiment is shown in Table I. The weights of the rats at the termination of this experiment are recorded in Table III. On some of these doses the effect on growth could be attributed to a lower food consumption, and in others to the utilisation of the food eaten.

TABLE I

SUMMARY OF DATA ON MORTALITY, FOOD CONSUMPTION AND FOOD EFFICIENCY WHEN FOOD COLOURS WERE GIVEN BY STOMACH TUBE FOR 20 WEEKS

Dose	Sex	Mortality	Food consumption g./rat/day	Food efficiency g. gain/g. food consumed × 100
Control	M	1	13·2	5·2
	F	0	11·1	4·7
200 mg./kg./orally/daily	M	0	13·2	4·5
FD&C Yellow No. 4	F	0	10·8	3·3
400 mg./kg./orally/daily	M	3	12·0	1·3
FD&C Yellow No. 4	F	1	11·3	3·1
200 mg./kg./orally/daily	M	1	12·4	5·7
FD&C Yellow No. 3	F	0	11·1	3·7
400 mg./kg./orally/daily	M		11·2	2·6
FD&C Yellow No. 3	F	0	9·9	3·0

The cumulative feed efficiency data for the group feeding experiment is shown in Figure 2. These data when plotted in a log relationship with time and straight lines fitted to the points by the method of least squares did not show a significant difference between the lines, thus indicating that the food efficiency is not affected by these levels of the colours in the diet.

The Effect on Mortality. During the 65 weeks the rats were on test a considerable mortality from respiratory infections resulted. For example the total mortality on the controls amounted to 54·0 per cent. The total mortality of the rats on 0·03 per cent. dietary level of FD&C Yellow No. 4 amounted to 52·0 per cent. and for the same level of FD&C Yellow No. 3 it was 20 per cent. Although it was not possible to properly diagnose the cause of death because of advanced autolysis in a number of cases, it is believed that most of these deaths were due to respiratory conditions. The rats on the 3·0 per cent. level of both colours all died by the end of the 3rd week and with the exception of one rat all the rats died on the 1·5 per cent. level of both colours by the end of the 5th week. In those rats which died on the 1·5 and 3·0 per cent. dosage levels, and on which it was possible to make autopsies soon after death, it was observed that all the tissues and organs were stained with the colour. There was an acute catarrhal gastro-enteritis; the kidneys were soft, dark and swollen. The spleen was greatly enlarged and dark. The picture was one of an acute toxæmia. Bacteriological examination was negative, that is, no pathogenic organisms were recovered from the gastro-intestinal tract, and death was attributed to the acute toxic effects of the colour.

The deaths could not be attributed to lack of food as the food consumption of the rats on the 1·5 and 3·0 per cent. levels was only slightly less than the control during their survival period. The cumulative number of deaths are shown in Table II.

The Effect on Organ Weights. In Table III are recorded the mean weights, in mg./g. of total body weight, of a number of the organs removed from the animals at the time the experiments were terminated. Although the mean weights for a number of organs have been shown to be significantly different from the control weights the differences were small in

most cases. On the 0·03 per cent. dose of FD&C Yellow No. 4 significant increases in weight of heart and liver were found in the males, while on the same dose of FD&C Yellow No. 3 in the males an increase in liver and kidney weights and a decrease in weight of testes were found. In females, with the exception of an increase in the weight of the liver, there was no appreciable change in the organ weights on the 0·03 per cent. dietary levels for both colours. For the higher doses shown in Table III, namely 200 and 400 mg./kg./rat per day, significant weight changes from the controls on a number of the organs examined were found. The rats on these dosages were killed at the end of 20 weeks.

TABLE II

CUMULATIVE NUMBER OF DEATHS

Conc. of colour in diet	Sex	No. rats on test	Time in weeks on test													
			1	3	5	10	15	20	25	30	35	40	45	50	55	60	65
FD&C Yellow No. 4																	
Control	M	25	1	6	7	8	10	11	12	13	14	14	14	14	14	16	17
	F	25	0	1	1	2	3	3	3	6	6	7	8	8	8	8	10
0·03 per cent.	M	25	0	0	0	1	2	3	5	5	5	5	7	10	13	13	13
	F	25	0	0	0	2	3	4	4	5	8	8	8	11	12	13	13
1·5 per cent.	M	25	7	20	24	25											
	F	25	11	24	25												
3·0 per cent.	M	25	13	25													
	F	25	16	25													
FD&C Yellow No. 3																	
Control	M	25	1	6	7	8	10	11	12	13	14	14	14	14	14	16	17
	F	25	0	1	1	2	3	3	3	6	6	7	8	8	8	8	10
0·03 per cent.	M	25	0	0	1	1	1	1	1	1	1	1	1	1	1	3	3
	F	25	0	0	0	1	1	3	3	5	5	5	5	7	7	7	7
1·5 per cent.	M	25	4	24	25												
	F	25	4	24	25												
3·0 per cent.	M	25	16	25													
	F	25	18	25													

Hæmatology. Hæmoglobin determinations were made at weekly intervals on groups of male and female rats, 10 rats in a group, given daily oral doses of 200 mg./kg. and 400 mg./kg. respectively of FD&C Yellow colours No. 3 and 4 for 20 weeks. A slight modification of the pyridine-hæmochromogen method of Rimington[4] was used. The combined results of these determinations on both sexes are shown graphically in Figure 3 and the mean values of the final determinations are shown in Table III. The combined blood hæmoglobin values for both sexes shows a significant decline in all the groups on both colours. However, when these values for both sexes were examined separately only the values for the males were found to be different from the controls on both dosage levels of the two colours, while in the females only the 200 and 400 mg./kg. doses of FD&C Yellow No. 3 were significantly different

TABLE III

COMPREHENSIVE SUMMARY OF OBSERVATIONS ON RATS FED FD&C YELLOW NO. 3 AND FD&C YELLOW NO. 4

Product	Dosage	No. weeks on test	No. rats surviving / No. rats on test	Mean body weight g. ± s.e.		Mean Hb (g. per cent) ± s.e.†	Mean organ weight, mg./g. rat ± s.e.					
				Initial	Final		Heart	Liver	Kidneys	Adrenals	Spleen	Testes
Males												
Control		65	8/25	45·6 ± 2·7	323·4 ± 20·0	17·2 ± 0·35	3·2 ± 0·11	30·3 ± 1·01	6·2 ± 0·19	0·08 ± 0·008	2·7 ± 0·26	8·6 ± 0·40
FD&C Yellow No. 4	0·03 per cent of diet	65	12/25	45·6 ± 2·6	280·4 ± 8·9	16·9 ± 0·42	3·6 ± 0·11*	36·5 ± 1·81*	7·4 ± 0·47	0·09 ± 0·004	2·4 ± 0·08	7·4 ± 0·42
FD&C Yellow No. 3	0·03 per cent. of diet	65	22/25	45·7 ± 2·7	282·1 ± 10·3	16·3 ± 0·22*	3·4 ± 0·07	35·5 ± 1·04*	6·8 ± 0·13*	0·08 ± 0·004	2·5 ± 0·07	5·1 ± 0·28*
Control		20	4/10	118·7 ± 5·7	251·0 ± 11·0	17·3 ± 0·40	3·6 ± 0·05	34·9 ± 1·55	7·6 ± 0·25	0·09 ± 0·005	2·5 ± 0·28	9·7 ± 0·38
FD&C Yellow No. 4	Oral 200 mg./kg./day	20	7/10	118·9 ± 5·0	211·6 ± 9·9*	15·3 ± 0·29*	4·3 ± 0·16*	37·8 ± 1·30	7·5 ± 0·72	0·09 ± 0·010	2·7 ± 0·02	11·1 ± 0·46
FD&C Yellow No. 4	Oral 400 mg./kg./day	20	2/10	114·2 ± 5·1	163·5 ± 1·5*	11·9 ± 0·35*	4·7 ± 0·30*	48·6 ± 0·40*	8·8 ± 0·10*	0·13 ± 0·005*	4·1 ± 0·25*	6·3 ± 2·60
FD&C Yellow No. 3	Oral 200 mg./kg./day	20	4/10	112·7 ± 3·5	221·3 ± 13·6	13·7 ± 0·24*	3·8 ± 0·09*	45·0 ± 1·16*	7·8 ± 0·15	0·10 ± 0·014	5·2 ± 0·04*	10·6 ± 0·63
FD&C Yellow No. 3	Oral 400 mg./kg./day	20	4/10	105·8 ± 6·7	152·5 ± 19·3	13·6 ± 0·35*	5·4 ± 0·44*	58·7 ± 2·91*	9·5 ± 0·65*	0·16 ± 0·024*	8·9 ± 1·08*	7·0 ± 1·76
Females												
Control		65	15/25	39·6 ± 2·4	227·9 ± 3·9	16·0 ± 0·19	4·1 ± 0·07	35·4 ± 0·84	7·0 ± 0·18	0·22 ± 0·008	3·6 ± 0·13	
FD&C Yellow No. 4	0·03 per cent. of diet	65	12/25	39·2 ± 2·2	225·2 ± 7·6	15·6 ± 0·18	4·0 ± 0·07	35·3 ± 0·70	6·8 ± 0·19	0·23 ± 0·011	3·6 ± 0·17	
FD&C Yellow No. 3	0·03 per cent. of diet	65	18/25	39·4 ± 2·2	217·9 ± 8·1	15·8 ± 0·21	4·1 ± 0·11	30·1 ± 1·02*	7·1 ± 0·24	0·19 ± 0·011	3·7 ± 0·20	
Control		20	9/10	93·0 ± 3·9	166·9 ± 6·5	15·7 ± 0·65	4·5 ± 0·41	37·5 ± 1·38	8·2 ± 0·45	0·26 ± 0·031	3·1 ± 0·33	
FD&C Yellow No. 4	Oral 200 mg./kg./day	20	5/10	91·6 ± 4·4	163·4 ± 12·8	14·9 ± 0·64	4·2 ± 0·18	40·9 ± 2·00	7·3 ± 0·22	0·19 ± 0·019	3·3 ± 0·38	
FD&C Yellow No. 4	Oral 400 mg./kg./day	20	4/10	92·2 ± 3·4	148·8 ± 1·8*	15·1 ± 0·50	4·4 ± 0·07	50·3 ± 1·26*	8·6 ± 0·21	0·19 ± 0·009	4·2 ± 0·33	
FD&C Yellow No. 3	Oral 200 mg./kg./day	20	7/10	89·6 ± 4·3	145·4 ± 5·8*	14·5 ± 0·54*	4·6 ± 0·10	47·6 ± 1·93*	8·3 ± 0·22	0·19 ± 0·012	6·9 ± 0·31*	
FD&C Yellow No. 3	Oral 400 mg./kg./day	20	7/10	94·3 ± 6·3	138·0 ± 5·2*	11·6 ± 0·70*	5·5 ± 0·20	56·0 ± 1·82	9·6 ± 0·82	0·23 ± 0·016	12·0 ± 1·01*	

* Significant at $P = 0·05$

† Determination done on 5 rats.

from the corresponding female controls. Blood hæmoglobin values were also determined on the surviving rats from the group feeding experiment. These results are also shown in Table III. With the exception of the male rats fed FD&C Yellow No. 3, 0·03 per cent. in the diet, which showed blood hæmoglobin values statistically different from the controls, the others were about the same as the controls.

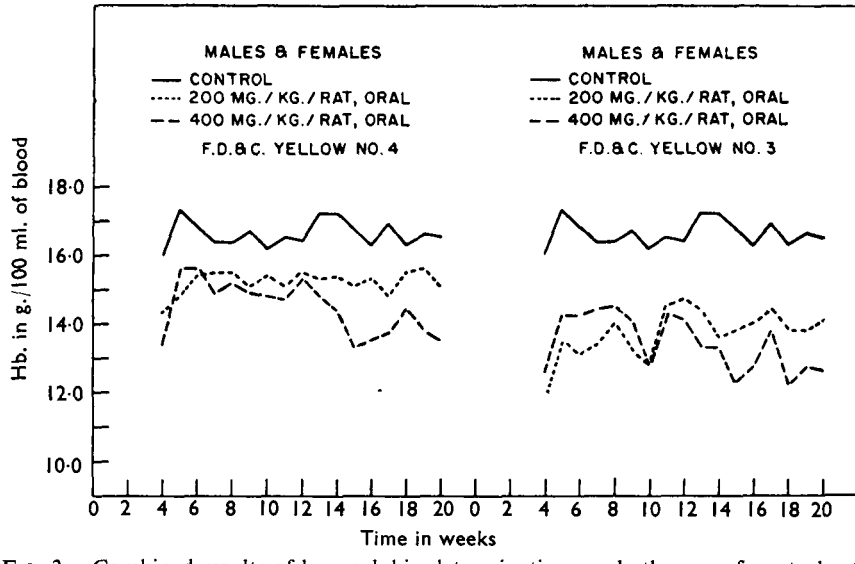

FIG. 3. Combined results of hæmoglobin determinations on both sexes of control rats and those given the two colours at different doses.

In order to obtain more information on the effect of FD&C Yellow No. 3 on the blood picture, a few rats of both sexes were given relatively high daily oral doses of this colour and determinations were made at weekly intervals for hæmoglobin, erythrocytes, hæmatocrit, reticulocytes, and total leucocytes. A summary of the mean values for the surviving rats at the conclusion of the experiment are shown in Table IV. The hæmoglobin of some of the rats before death was as low as 3 g. per 100 ml. of blood. The total erythrocyte count in some cases was below one million per cu. mm. The reticulocytes were greatly increased from a normal of 2 to 4 per cent. in control rats to 16 to 50 per cent. in the colour treated rats. The hæmatocrit values were lower in the test animals. The mean corpuscular volume in the control rats was 54·1 cubic microns. Some of the test rats had mean corpuscular volumes as high as 130 cubic microns with a mean of 85·7 and 90·0 cubic microns for the respective groups on test. The mean corpuscular hæmoglobin concentration, however, was reduced. The blood picture of these rats was therefore suggestive of a macrocytic hypochromic anæmia.

Further hæmatological findings are outlined as follows: There were poikilocytosis, anisocytosis and polychromatophilia. The occasional oxyphilic normoblast with structureless nucleus was noted. Howell

TABLE IV

SUMMARY OF HÆMATOLOGICAL FINDINGS

No. of rats	Age in weeks at start of test	Weeks on test	Dose of FD&C Yellow No. 3	Mean blood Hb g./100 ml. blood	Range	Mean RBC count millions/ mm³ blood	Range	Mean hæmatocrit ml./100 ml. blood	Range	Mean reticulocyte per cent. of total RBC	Range	Mean corpuscular volume (μ^3)	Range
5	8	12	Control	17·8	17·2–18·2	8·5	8·0–9·0	52·0	50–54	2·6	2–4	54·1	50–57
6	8	12	90 mg./orally daily	10·2	8·3–12·8	3·8	2·8–4·5	37·0	31–43	26·0	18–40	85·7	61–108
6	8	7	0·9 per cent in diet	6·8	2·3–9·8	2·5	0·9–5·6	27·0	17–42	34·0	16–50	90·0	68–130

Jolly bodies were present but not numerous. The blood serum was of a brown colour suggesting the presence of some foreign substance. However, it was not possible to detect unaltered colour on chemical examination of the serum, but the spectrophotometric curve of the serum from the treated rats was similar in many respects to a control serum which contained the added Oil Yellow AB colour, indicating that the colour is very likely present in an altered form. Giemsa and Wright stains showed the bone marrow to be megaloblastic. The total and differential white cell counts were within normal limits.

Histopathology. Detailed examination was made on the hæmatoxylin-eosin stained paraffin sections of a number of the organs including the lung, heart, liver, spleen, thyroid, pancreas, stomach, small intestine, kidney, urinary bladder, adrenal, testes, ovaries and thymus. The number of organs in which important changes were found are shown in Table V. A brief description of the more important histological changes which were observed in those organs examined, is as follows:

FD&C Yellow No. 3, 0·03 *per cent. in the diet,* 65 *weeks*

Testes. Testicular changes were essentially of a degenerative and atrophic nature involving principally the spermatogenic cells. The testes were divided into 3 more or less arbitrary groups according to the tubular pathology. Five testes exhibited marked changes, 26 exhibited moderate changes, 11 exhibited slight changes, and 2 were normal.

In the testes in which the changes were described as marked, the majority of tubules were utterly devoid of spermatogenic cells. Sertoli cells were present but were irregular in outline, their cytoplasm appearing to merge into the cytoplasm of the walls of the tubules. The outline of the wall of the tubule was indefinite, with thin strands of cytoplasm or fibrin-like material projecting irregularly from the inner periphery towards the lumen of the tubules. Other tubules, in which degenerative changes were not as advanced, contained a lacy

TABLE V

SUMMARY OF HISTOPATHOLOGICAL FINDINGS

	Control	FD&C Yellow No. 4	FD&C Yellow No. 3	Control	FD&C Yellow No. 4	FD&C Yellow No. 3	Control	FD&C Yellow No. 4	FD&C Yellow No. 3	FD&C Yellow No. 4	FD&C Yellow No. 3	Control	FD&C Yellow No. 4	FD&C Yellow No. 3	FD&C Yellow No. 4	FD&C Yellow No. 3
Sex	Male			Female			Male			Male		Female			Female	
Dose	0·03 per cent in diet			0·03 per cent in diet			400 mg./kg./day			200 mg./kg./day		400 mg./kg./day			200 mg./kg./day	
Number of rats on test ..	25	25	25	25	25	25	10	10	10	10	10	10	10	10	10	10
Number of rats examined ..	8	12	22	15	12	18	4	3	5	4	4	8	4	6	5	7
Heart, cloudy swelling ..		1	1			1				1						
Interstitial œdema								1		1					1	
Pericarditis		1														
Lung, inflammation	6	10	17	12	11	16	4	2	5	3	4	6	2	6	5	7
Gastrointestinal, neoplasm ..			1													
enteritis-chronic fibrosing				1												
Liver, degeneration			1						3				1	5		1
Kidney, total no. showing change		3	8	5	3	10			4	1				5		1
A. Inflammations																
(i) Glomerulitis		1	8	3	3	6			4	1		1		2		
(ii) Pyelonephritis ..									1							
(iii) Interstitial nephritis ..				1		3			1	1						
B. Degenerations																
(i) Nephrosis (proximal)		1	2			1			3	1		1		2		
(distal) ..										1		1		2		
(ii) Hydronephrosis ..				2		4								3		1
C. Congenital cystic kidney		1														
D. Bladder parasites ..		5	10	2	4	8	2			2	2	3			2	3
Testes, testicular degenerations		3	22					2	3							
Adrenal																
A. Neoplasm (phæochromocytoma)						1										
B. Inflammation						1										
Spleen																
A. Hyperplasia, hyperchromic nuclei			7			4		2	4		4			6		5
B. Excessive red blood cells			5			3			2		1			6		4

fibrin-like web in which were nestled degenerating spermatogenic cells. The majority of these appeared to be undifferentiated germ cells although some were undoubtedly primary spermatocytes. In the testes described as showing moderate changes, tubules showing the above pathology were noted, but in the majority of the tubules secondary spermatocytes were present. Most of these were undergoing varying degrees of degeneration. In other tubules spermatogenesis was complete although a number of spermatozoa were undergoing degeneration. In the group in which changes were described as slight, the majority of the tubules showed complete spermatogenesis but there was a reduction in the normal number of spermatozoa. General tubular morphology appeared normal in this group although there was slight tubular atrophy. The Leydig cells in the test animals were essentially normal, although the nuclei were slightly denser and took on a more intense stain. They were somewhat more flattened than the nuclei

seen in the control animals and appeared more abundant in the testes showing moderate changes (Figs. 4, 5 and 6).

It is understandable that overlapping would occur in the three groups, that is tubules showing the features described in group 1 did occur in group 2 and tubules described as typical of group 3 did occur in group 2. It might be argued that in the tubules showing partial spermatogenesis with no cells undergoing degeneration, the processes would be one of arrest. The number of tubules exhibiting this pathology are very much in the minority and it is felt that the process is essentially a degenerative lesion of the spermatogenic cells due to a specific toxic action of the colour.

Kidney. The principal changes in the kidney involved the glomerulus. These changes were generally not marked. In most instances there was an increase in the amount of cytoplasm of the epithelium and endothelium of the glomerulus with a slight cellular increase, resulting in an increase in the density of the tuft. Even in these animals normal appearing glomeruli were relatively abundant. Hyaline change was observed in the glomerulus in two animals and was accompanied by nephrosis. In three animals the condition was one of a mild subacute glomerulitis.

Spleen. There was a hyperplasia of the white pulp, the cells of which were hyperchromatic. The white pulp did not display the reaction centre encountered in some toxic conditions. In the red pulp there was an increased number of phagocytes containing disintegrated red blood cells. The interiors of the red pulp cords were engorged with red blood cells. In general the splenic changes appeared to be of a proliferative compensatory nature.

Lungs. Chronic inflammatory changes with some degree of emphysema, atelectasis and bronchiectasis were present in the lungs of most of the test and control animals.

Neoplasm. A reticulum cell sarcoma involving principally the mesentery and infiltrating the muscular coats of the ileum and cæcum was noted in one animal and an adenoma was noted in the adrenal gland of one animal.

FD&C Yellow No. 4, 0·03 *per cent. in diet,* 65 *weeks*

There was no constant or specific change that could be attributed to the toxic effect of the colour. The testes, kidneys and spleen were essentially normal.

FD&C Yellow No. 3, 200 *mg./kg./day,* 20 *weeks*

Spleen. The histological picture was similar to that observed in the organs of rats on the FD&C Yellow No. 3 on the lower doses, which was a hyperplasia of the white pulp, an intense staining of the cells of the pulp, an increased phagocytosis of disintegrated red blood cells and an engorgement of the red pulp with red blood cells.

FD & C Yellow No. 3, 400 *mg./kg./day,* 20 *weeks*

Testes. Of the animals examined 2 were normal. The testes of 3 animals showed alterations similar to those observed in the testes of rats on the 0·03 per cent. dietary level of FD&C Yellow No. 3.

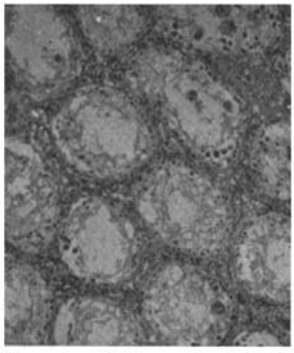

FIG. 4. Dietary level 0·03 per cent. FD & C Yellow No. 3. 65 weeks. Testes. Advanced degenerative change. Complete absence of spermatogenesis in most tubules. Tubular atrophy marked. × 210.

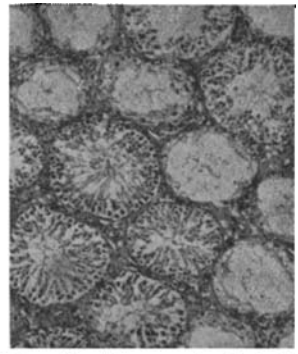

FIG. 5. Dietary level 0·03 per cent. FD & C Yellow No. 3. 65 weeks. Testes. Slight change. Some tubules show complete but reduced spermatogenesis. Occasional tubule shows complete absence of spermatogenesis. × 210.

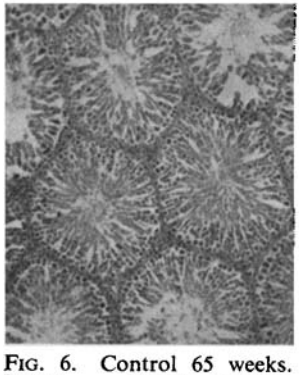

FIG. 6. Control 65 weeks. Testes. Testes normal. × 210.

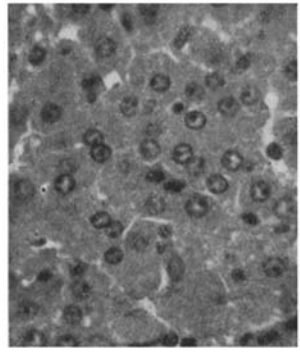

FIG. 7. Dietary level 3 per cent. FD & C Yellow No. 3. Liver. Partial compensation —partial failure—regeneration with increase nuclear ratio in a given field. Many binucleated cells, nucleoli hypertrophied and hyperchromatic. Degeneration with nuclear distortion, pyknosis, some necrosis. × 330.

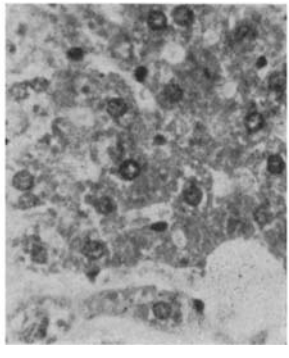

FIG. 8. Control. Liver. Liver normal. × 330.

Spleen. The Prussian blue positive pigment was excessive. There was engorgement of the red pulp with red blood cells.

Kidneys. Mild tubular alterations were present in 4 animals. These were in the nature of a proximal and distal nephron nephrosis; the tubules were swollen and smudgy with a loss of epithelial elements to the lumen. There was an accompanying glomerulitis in 2 animals. Prussian blue positive pigment was present in the cytoplasm of kidney tubular epithelium.

Liver. Liver change was present in 8 animals. This was in the nature of a cloudy swelling. There was an accompanying fatty change in one animal and marked necrosis was observed in one instance. The nuclear pattern was disrupted in the affected animals with a loss of uniformity in nuclear size and increase in nuclear ratio in a given field, most cells being binucleated, that is there appeared to be some regeneration. Kupffer cells were abundant and contained large amounts of Prussian blue positive material

FD&C Yellow No. 4, 200 *mg./kg./day*, 20 *weeks*

There were no marked alterations from the normal which could be attributed to the toxic effects of the colour.

FD&C Yellow No. 4, 400 *mg/kg./day*, 20 *weeks*

Testes. Only the testes of 3 males were examined, in 1 animal the testes were normal and in the other 2 the degenerative changes were moderate. It was not possible, due to the advanced autolytic changes, to do a histopathological examination of most of the animals which died on the higher doses of the 1·5 and 3·0 per cent. dietary levels. A number of animals were killed and the findings were as follows:

Liver. The findings in the liver were similar to that found in the livers of rats on the FD&C Yellow No. 3 on the 400 mg./kg. daily dose, except the necrosis found in most livers was more marked (Figs. 7 and 8).

Thymus. The thymus generally exhibited changes of a marked involution. Medulla and cortex were indistinct. The majority of the lymphocytes were pyknotic; macrophages were prominent and phagocytosis of the degenerating lymphocytes by the macrophages was evident, but reticular network was quite distinct.

Adrenals. Congestion and necrosis of the cortex occurred in a few animals. Cloudy swelling in the zona fasciculata was noted in one instance.

Pancreas. Many glands showed a slight loss of basal basophilia with an increase in the refractile granules in the supra nuclear portion of the acini.

Kidney. The findings in the kidney were similar to that found in the kidneys of rats on the FD&C Yellow No. 3 on the 400 mg./kg. daily dose except the various conditions noted were more marked.

Spleen. The white pulp displayed toxic reaction centres. Many pyknotic nuclei were noted in the white pulp.

SUMMARY

1. FD&C Yellow colours No. 3 and 4 in concentrations of 0·03 per cent. in the diet were found to reduce the food consumption of male, but not female rats. Heart and liver weights were increased for male rats on the 0·03 per cent. level of FD&C Yellow No. 4 and kidney and liver weights were found to be slightly increased for both male and female rats on the same level of FD&C Yellow No. 3. The weights of the testes on the 0·03 per cent. dietary level of FD&C Yellow No. 3 were found to be less than the controls.

2. Daily oral doses of 200 mg./kg. and 400 mg./kg. of FD&C Yellow colours No. 3 and 4 in males caused a significant fall in hæmoglobin values, but in females only the oral doses of 200 mg./kg. and 400 mg./kg./day of FD&C Yellow No. 3 caused a significant fall in blood hæmoglobin concentration after 20 weeks. Significant weight changes in a number of organs on these levels were found at the termination of the experiment.

3. Daily oral doses of 90 mg./day/rat of FD&C Yellow No. 3 for seven weeks caused a marked decline in blood hæmoglobin concentration, total erythrocyte count, and hæmatocrit value, and an increase in reticulocyte counts. The total leucocyte and differential leucocyte counts were within normal ranges. Similar results were obtained on a dietary level of 0·9 per cent. FD&C Yellow No. 3.

4. Tissue histology of those rats surviving for 65 weeks on the 0·03 per cent. dietary levels of FD&C Yellow No. 3 revealed marked atrophy and degeneration in the testes, mild inflammatory changes in the kidney and slight hypertrophic changes in the spleen. There were no significant histological changes in the organs examined from surviving rats on FD&C Yellow No. 4 on the same dietary level. Dietary levels of 1·5 and 3·0 per cent. of both colours caused some tissue changes in the liver, thymus, adrenal, pancreas and kidney. Daily oral doses of 400 mg./kg. of FD&C Yellow No. 3 for 20 weeks caused changes in testes, spleen, kidney and liver. The only changes of significance on the 200 mg./kg. daily dose of FD&C Yellow No. 3 were in the spleen of males. The 400 mg./kg. daily dose of FD&C Yellow No. 4 caused testicular changes in 20 weeks and the 200 mg./kg. daily dose caused no changes of significance.

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REFERENCES

1. Badger, Cook, Hewett, Kennaway, Kennaway, Martin and Robinson, *Proc. Roy. Soc., Lond.*, B., 1942, **131**, 170.
2. Sugiura, *Proc. Soc. exp. Biol., N.Y.*, 1946, **61**, 301.
3. Report of Food Standards Committee on Colouring Matters in Food, British Ministry of Food, 1954.
4. Rimington, *Brit. med. J.*, 1942, **1**, 177.