THE INDUCTION OF TUMOURS OF THE SUBCUTANEOUS TISSUES, LIVER AND INTESTINE IN THE MOUSE BY CERTAIN DYESTUFFS AND THEIR INTERMEDIATES

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THE purpose of the experiments about to be described was to test a number of amines and azo-dyes for carcinogenic activity in the mouse, a species which has been comparatively little used for the investigation of this type of compound. The chemicals chosen were 2 dyes (auramine and magenta), the manufacture of which constitutes an industrial hazard according to Case and Pearson (1954); 6 dyes formerly or at present in use as food colorants (carmoisine, rhodamine B, sunset yellow, ponceau 2R, xylylazo-2-naphthol and *o*-tolylazo-2-naphthol); 3 dyestuffs intermediates (benzidine, 1-naphthylamine and 2-naphthylamine); and 2 ortho hydroxy amines related to known metabolites of aromatic amines (3 : 3′-dihydroxybenzidine hydrochloride and 0-methyl-2-amino-1-naphthol hydrochloride).

The tumours induced by the chemicals were of three types: subcutaneous sarcomas, hepatomas and intestinal polyps and carcinomas.

Spontaneous intestinal tumours in the mouse have been described occasionally (Table I). Murray (1905, 1908) found 2 adenocarcinomas in the small intestine invading the wall of the gut and expanding under the peritoneum. The drawings of these tumours clearly indicate their nature. Slye, Holmes and Wells (1917) described a squamous carcinoma of the rectum arising in the prolapsed organ, the only intestinal tumour observed in 16,500 *post-mortem* examinations on mice. Slye (1924) mentioned that 2 squamous carcinomas of the rectum had been seen at *post-mortem* in 40,370 mice and one of the duodenum in 1600 mice. Strong (1941) stated that strain A mice were liable to develop spontaneous tumours of the caecum.

Bonser and Jull (unpublished observation) found two caecal polyps in a female albino stock mouse, bought from a dealer but having no relation to the mice used in the present experiments, and treated for 136 weeks by means of subcutaneous injections of arachis oil. These tumours were composed of irregular intestinal glands, some of which were cystic and dipped through the muscularis mucosae into the subepithelial tissues, there evoking a mild inflammatory reaction. They also observed a caecal carcinoma in a female mouse of the same stock in which a paraffin wax pellet had remained implanted in the bladder for 40 weeks with negative result. The mouse was thus 52–56 weeks of age. This tumour was an anaplastic bowel carcinoma, invading the muscular wall as far as the serosa and permeating subserosal and mesenteric lymphatic vessels. Embedded in the tumour were cystic spaces lined by mucus-secreting columnar epithelium similar to those seen in the polyps, but situated at all levels in the muscular wall and also beneath the serosa. From this appearance it was decided that the tumour arose in a benign polyp.

Tumours have been induced in the mouse intestinal tract by a variety of chemicals (Table II). Badger, Cook, Hewett, Kennaway, Kennaway, Martin and Robinson (1940) described a carcinoma of the small intestine in one of 10 mice receiving 6-methyl-3 : 4-benzphenanthrene by mouth for 394 days. Lorenz and Stewart (1940) described local and metastasising carcinomas of the small intestine in mice of the A strain receiving 1 : 2 : 5 : 6-dibenzanthracene and of the A backcross receiving 20-methylcholanthrene in an olive oil emulsion in the drinking water. Later (1941) these authors found similar tumours in mice of other strains treated with these chemicals, together with one carcinoma of the caecum and a few in the upper part of the colon. White and Stewart (1942) observed small intestinal polyps and carcinomas and also haemangio-endotheliomas in the intestinal wall and other abdominal sites in mice of the C3H and C strains receiving methylcholanthrene in the diet. The photographs and morbid anatomical descriptions of these tumours are extremely lucid. More males than females were treated and only one tumour was stated to have occurred in a female. Foulds (1947) described a small intestinal carcinoma with a secondary deposit in a regional lymph node in one male out of 25 receiving 2-acetylaminofluorene in the diet.

Thus it would appear that spontaneous benign and malignant tumours of the intestine occur but rarely in the mouse. The sex incidence is not known. Induced tumours resemble the spontaneous ones in site and structure and have been more commonly described in males.

Source of mice.—The mice were brought from a local dealer, who has now gone out of business. They were divided into groups of 30, comprising equal numbers of each sex, and were kept 5 in a box. Where the mortality was high in the early weeks of the experiment, another 30 mice were subsequently added. They were fed on rat cake, supplemented by cod liver oil and marmite and were given water *ad lib.* All mice used in the experiment subsequent to April 1953 were vaccinated on the tail with calf lymph as a protection against ectromelia.

Administration of the chemicals.—At first, a standard technique was adopted of subcutaneous injection twice weekly of a solution or suspension of the chemical

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EXPLANATION OF PLATES

FIG. 1.—Mouse H 285, male. Forty-six weeks from start of treatment with benzeneazo-2-anthrol. Vasoformative sarcoma or haemangio-endothelioma of back muscles. A fragment of the destroyed vertebral body is seen top middle. × 150.

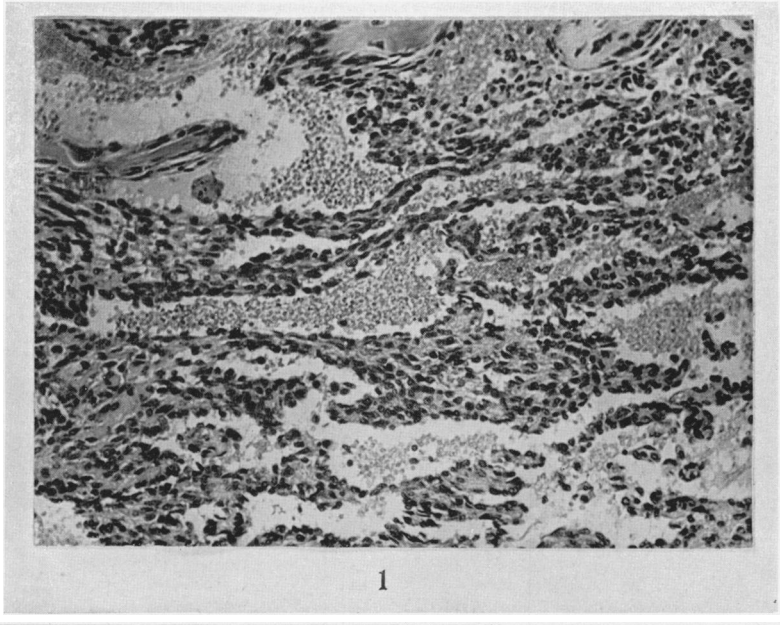
FIG. 2.—Mouse H 127, male. Seventy weeks from start of treatment with arachis oil. Benign early polyp of caecum, showing regular proliferating glands lined by tall epithelium with hyperchromatic nuclei and granular cytoplasm, secreting little mucus, situated superficial to the muscularis mucosae. There is inflammation and fibrosis of the stroma. × 102.

FIG. 3.—Mouse H 69, female. Sixty-five weeks from start of treatment with *o*-tolylazo-2-napthol. Benign polyp of ileum, composed of irregular proliferating glands which are beginning to invade the muscular coat (top centre). There is fibrosis of the stroma. × 40.

FIG. 4.—Mouse H 702, female. Seventy-six weeks from start of treatment with 0-methyl-2-amino-1-naphthol. Benign polyp of caecum, with cystic glands penetrating the muscular coat and provoking a zone of inflammation in the serosa. × 26.

FIG. 5.—Mouse H 379, female. Eighty-four weeks from start of treatment with fresh oily solution of 2-naphthylamine (B.D.H.). Malignant polyp of caecum, composed of irregular glands lined by tall or cubical epithelium, with hyperchromatic nuclei, granular cytoplasm, and little mucus secretion, though some of the glands are cystic. The line of the base is irregular and the muscularis mucosae is partially destroyed. × 102.

FIG. 6.—Mouse H 97, male. Sixty-two weeks from start of treatment with *o*-tolylazo-2-naphthol. Anaplastic carcinoma invading the muscular wall as far as the serosa . Tumour emboli present in subserosal lymphatic vessels. Two cystic glands in muscle resembling those seen in benign polyps. × 30.



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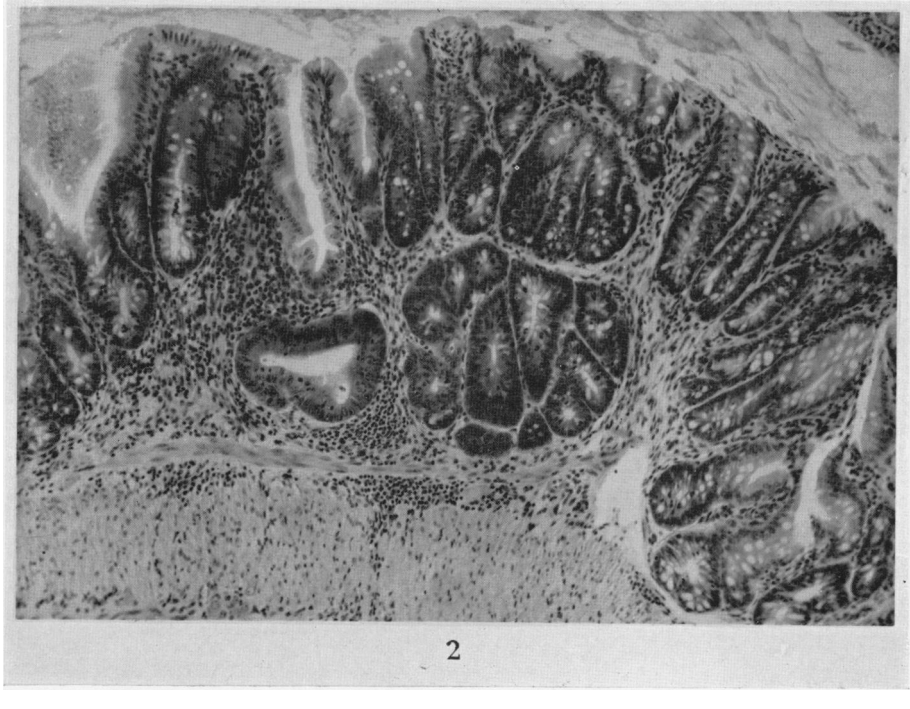

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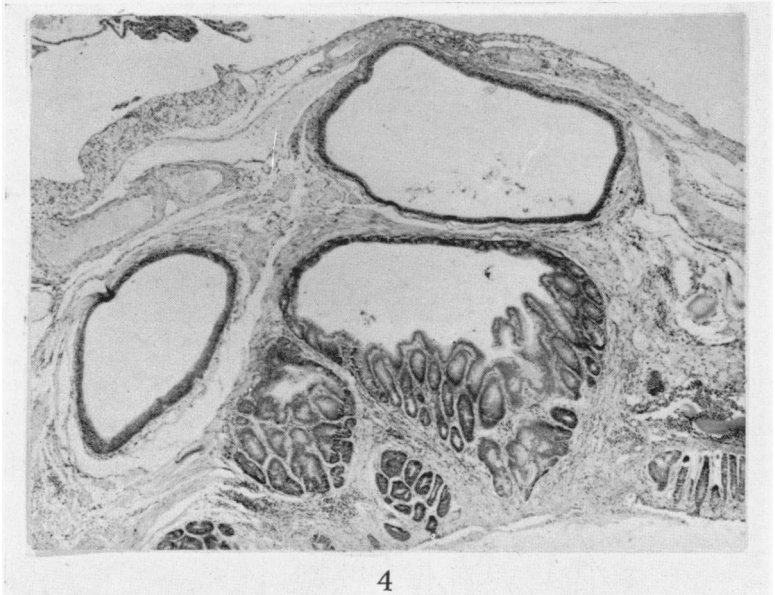

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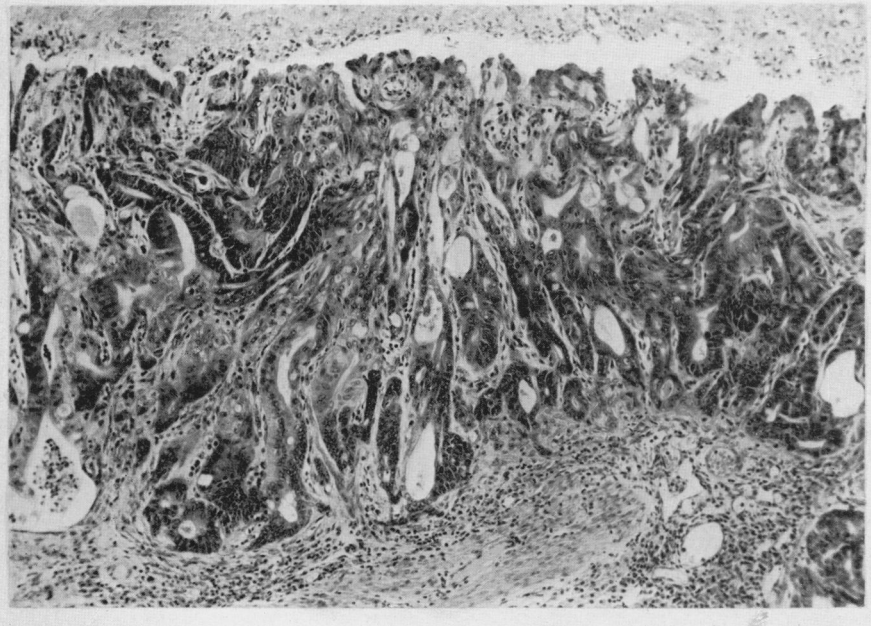

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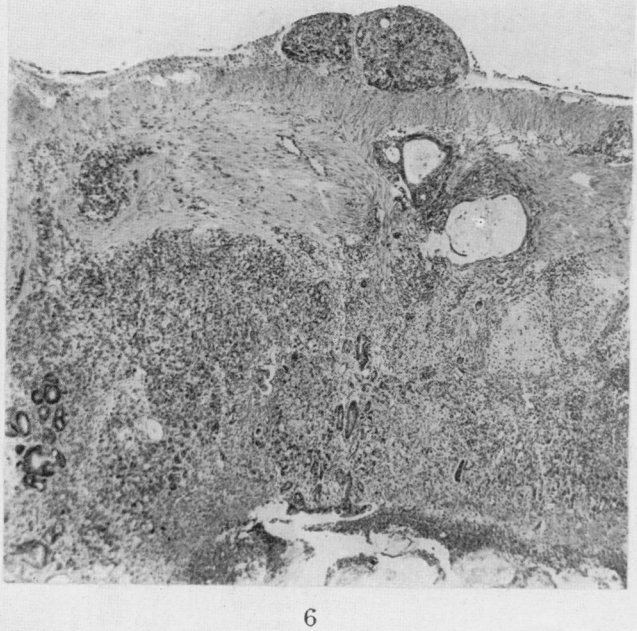

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TABLE I.—*Spontaneous Tumours of the Small and Large Intestine in the Mouse*

Author	Type of mouse	Age in weeks	Sex	Small intestine				Large intestine			
				Site	Benign	Malignant	Incidence	Site	Benign	Malignant	Incidence
Murray, 1905	Not stated	Not stated	Not stated	Not stated	—	Adeno carcinoma invading muscular wall	Not stated	—	—	—	—
1908	,,	,,	,,	,,	—	Ditto	,,	—	—	—	—
Slye, Holmes, and Wells, 1917	Not stated	116	Male	,,	—	—	,,	Prolapsed rectum	—	Squamous carcinoma	1/16,500
Slye, 1924	,,	Not stated	Not stated	Duodenum	—	Carcinoma	1/41,165	Rectum	—	Ditto	1/40.370
Bonser and Jull (unpub.)	Stock from breeder, injected with arachis oil	148	Female	—	—	—	—	Caecum (multi focal)	Adenoma with downgrowth of cystic epithelium	—	1/5
	Stock from breeder, bladder implanted with paraffin wax pellet	52–56	,,	—	—	—	—	Caecum (annular)	—	Anaplastic adeno-carcinoma with lymphatic permeation	1/881

TABLE II.—*Induced Tumours of the Small and Large Intestine in the Mouse*

Author	Type of mouse	Age in weeks	Sex	Chemical	Mode of administration	Small intestine				Large intestine			
						Site	Benign	Malignant	Incidence	Site	Benign	Malignant	Incidence
Badger *et al.*, 1940	Not stated	56	Not stated	6 Methyl-3:4-benz-phenanthrene	2–4 per cent solution in olive oil in food	Not stated	—	Carcinoma	1/10	—	—	—	——
Lorenz and Stewart, 1940	Strain A	>30	M. and F.	1, 2, 5, 6-dibenzan-thracene	Olive oil emulsion in drinking water (0·8 mg./day)	3–19 cm. from pylorus	—	Adeno-carcinoma	9/30	—	—	—	
	Strain A back cross	—	M.	20-Methylcholan-threne	Ditto	—	—	Ditto	4/10	—	—	—	—
Lorenz and Stewart, 1941	Strain C57 Brown, C57 Bl.	Not stated	Not stated	Ditto	Olive oil emulsion in drinking water	Not stated	—	,,	Not stated	Caecum. Upper part of colon	—	Adeno-carcinoma	A few
	C3H Dba	—	—	1, 2, 5, 6-dibenzan-thracene	Ditto								
White and Stewart, 1942	Strain C3H	>16	Not stated	20-Methylcholan-threne	Fatty solution incorporated in diet	8–25 cm. from pylorus	—	,, Haemangio-endothe-lioma	6*/50 11†/50.	— —	— —	— —	— —
	C	>29	,,	Ditto	Ditto	Ditto	—	Adeno-carcinoma	4‡/32	—	—	—	—
								Haemangio-endothe-lioma	21†/32	—	—	—	—
Foulds, 1947	Strain RIII	52	M.	2-Acetylamino-fluorene	In food	Not stated	—	Carcinoma	1/53	—	—	—	—
Miller and Pybus, 1956	Hybrid NBT × CBA	86	F.	20-Methylcholan-threne	Descendents of mice injected subcutaneously with 1 mg. in 0·1 c.c. sesame oil	—	—	—	—	Rectum Colon	— —	Tumour ,,	— —
		56	,,	Ditto	Ditto	Not stated	—	Tumour	—	—	—	—	—

* In addition, 18 mice had precancerous lesions of the small intestine. † Lesion present in mice of both sexes.
‡ In addition, 5 mice had precancerous lesions of the small intestine.

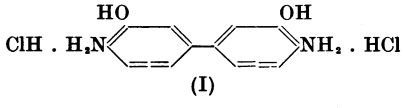
TABLE III.—*Methods of Administration and Source of Chemicals*

Chemical	Dose	Subcutaneous injection: Weekly dose per per mouse	Subcutaneous injection: Total dose per mouse at 52 wks.	Source of chemical	Number of mice at start	Whether vaccinated
Arachis oil	0·1 c.c.	0·2 c.c.	10 c.c.	Boots Ltd.	60	No
Benzidine	0·1 c.c. of 3 per cent suspension in arachis oil	6 mg.	312 mg.	British Drug Houses	30	,,
				(AnalaR)	30	Yes
(I)—						
3 : 3′-Dihydroxybenzidine hydrochloride	Ditto	6 mg.	312 mg.	Synthesised in laboratory	30	No
1-Naphthylamine	Ditto	6 mg.	312 mg.	British Drug Houses (specially purified to be free of 2-naphthylamine)	30	,,
					30	Yes
2-Naphthylamine* B.D.H.	0·1 c.c. of 3 per cent suspension in arachis oil allowed to stand	6 mg.	312 mg.	British Drug Houses (specially prepared, (Bonser, 1943))	30	No
B.D.H.	Ditto, prepared fresh	6 mg.	312 mg.	Ditto	30	Yes
R.C.H.	Ditto	6 mg.	312 mg.	Prof. Bergel (specially purified)	30	,,
(II)—						
0-Methyl-2-amino-1-naphthol hydrochloride	0·1 c.c. of 3 per cent solution in water	6 mg.	312 mg.	Synthesised in laboratory	30	,,
(III)—						
Benzeneazo-2-anthrol	0·2 c.c. of 3 per cent suspension in arachis oil	12 mg.	624 mg.	Ditto	30	,,
(IV)—						
Carmoisine	0·1 c.c. of 3 per cent suspension increased to 6 per cent after 6 months	6 mg. 12 mg.	468 mg.	Pointing, Hexham	30	No
(V)—						
Xylylazo-2-naphthol	0·1 c.c. of 3 per cent suspension in arachis oil	6 mg.	312 mg.	,, ,,	30	Yes

Chemical	Dose	Oral: Weekly dose per mouse	Oral: Total dose per mouse at 52 wks.	Source of chemical	Number of mice at start	Whether vacinated
(VI)—						
o-Tolylazo-2-naphthol	0·1 c.c. of 1·5 per cent suspension in arachis oil	3 mg.	156 mg.	Synthesised in laboratory	30	No
(VII)—						
Magenta	0·2 c.c. of 3 per cent suspension in arachis oil by stomach tube	12 mg.	624 mg.	British Drug Houses	60	Yes
(VIII)—						
Auramine	0·1 per cent in diet	14 mg.†	728 mg.	,, ,, ,,	30	,,
Xylylazo-2-naphthol	Ditto	14 mg.	728 mg.	Pointing, Hexham	30	,,
o-Tolylazo-2-napthol	Ditto	14 mg.	728 mg.	,, ,,	30	,,
(IX)—						
Rhodamine B	0·05 per cent in drinking water	17 mg.	884 mg.	,, ,,	30	,,
(X)—						
Sunset yellow	Ditto	17 mg.	884 mg.	,, ,,	30	,,
(XI)—						
Ponceau 2R	Ditto	17 mg.	884 mg.	,, ,,	30	,,

* For further details of preparation of solutions and chemicals see Bonser, Clayson, Jull and Pyrah (1956).
† It is assumed that each mouse eats approximately 2 g. of food per day and drinks 5 c.c. of water.

in arachis oil for 52 weeks. Thereafter the mice were allowed to survive as long as possible. For three reasons, this technique was varied: (*a*) to compare the effects of oral and subcutaneous administration in the case of certain chemicals which might be altered in the alimentary tract or metabolised in the liver; (*b*) to avoid inflammation of the subcutaneous tissues by irritant chemicals; and (*c*) to reduce labour. The methods of administration, dose, and source of the chemicals are given in Table III. The chemical formulae of the less well-known compounds are as follows :—



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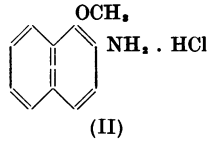

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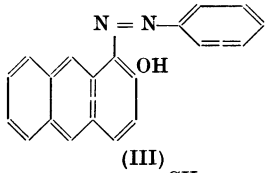

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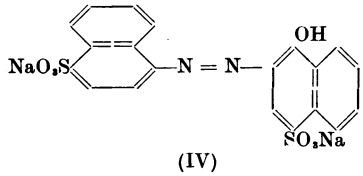

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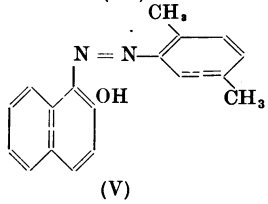

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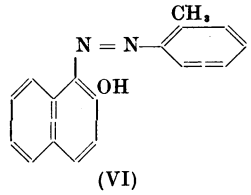

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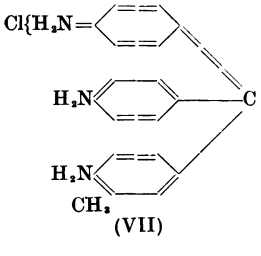

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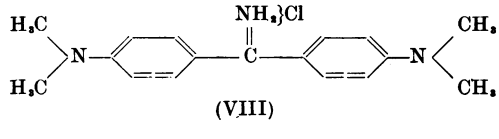

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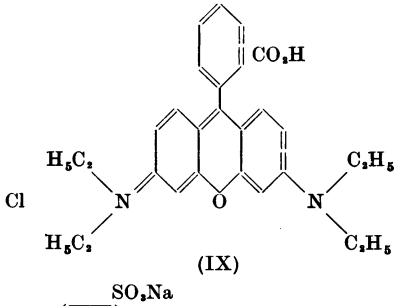

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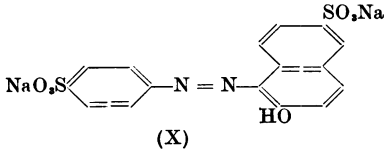

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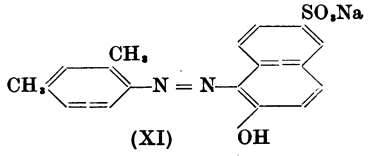

(XI)

Post-mortem *examination.*—At death a thorough examination of each mouse was made by searching for tumours in all organs. Microscopical examination of all suspicious lesions and of many apparently normal organs was undertaken.

EXPERIMENTAL RESULTS

The mortality at the beginning of the experiment, which took 3 years to complete, was very high. Improvement was obtained in some groups by vaccination against ectromelia.

Subcutaneous sarcomas.—With one exception, these occurred in mice receiving oily subcutaneous injections of the chemicals (Table IV). The 12 subcutaneous sarcomas induced with 2-naphthylamine have already been reported and discussed (Bonser, Clayson, Jull and Pyrah, 1956) as have the three induced by subcutaneous injection of *o*-tolylazo-2-naphthol (Bonser, Clayson and Jull, 1954). Of the three tumours induced in the subcutaneous tissues with benzeneazo-2-anthrol, two were spindle-cell sarcomas of vaso-formative type (Fig. 1), which resembled those described by Andervont (1950) as haemangio-endotheliomas following subcutaneous administration of crystalline *o*-aminoazotoluene, and one was a spindle-cell sarcoma. They occurred at 34, 46 and 56 weeks in 2 males and 1 female. The three tumours induced with *o*-tolylazo-2-naphthol were spindle-cell sarcomas in females, at 46, 53 and 61 weeks respectively, as was the one tumour occurring at 56 weeks in the flank of a female mouse receiving oral auramine. The latter was not associated with parasitic infection.

Hepatomas.—A significant incidence of this type of tumour was obtained with benzidine, 2-naphthylamine, auramine and 0-methyl-2-amino-1-naphthol hydrochloride (Table IV). Single tumours were obtained with benzeneazo-2-anthrol and the injection of *o*-tolylazo-2-naphthol. Ten hepatomas occurred before and 16 after 70 weeks ; in the latter group were 6 induced by 2-naphthylamine. Those occurring after treatment with benzidine and 2-naphthylamine were large in size and sometimes multiple. Although no metastases were found, their structure frequently resembled that of malignant tumours. Those induced by auramine and 0-methyl-2-amino-1-naphthol were small and benign. It was rare for cholangiomatous areas to be present in addition to hepatomas.

Lymphomas.—This term is used to cover all the lesions arising in the reticular system. Using the classification of Dunn (1953), the two common tumours were lymphosarcoma and reticulum-cell sarcoma. The former varied greatly in its extent, and might be confined to the liver and spleen, or to the lymph glands, in an individual mouse. The latter arose usually in the abdominal cavity or in the liver and kidney. The tumours were composed of spindle cells and it was necessary to make a distinction between this type of tumour and the spindle-cell sarcomas arising locally in relation to subcutaneous injections. This was not difficult when the anatomical and microscopical appearances in each mouse were studied. This statement is in accord with the opinion of Dunn (1953).

Approximately one-third of untreated mice of this type used in this and other experiments suffer spontaneously from the disease, the females being more commonly affected than the males. Treatment by the chemicals under review did not significantly raise this incidence.

Intestinal tumours (Table V).—One year after the beginning of the experiment an intestinal carcinoma was found in the ileo-caecal region of a mouse injected

TABLE IV.—*Mortality and Incidence of Subcutaneous Sarcomas, Hepatomas and Leukaemia*

Chemical	Method of administration	Number of mice at start		Mice dying in weeks: 20–49 M.	20–49 F.	50–69 M.	50–69 F.	70–89 M.	70–89 F.	Over 90 M.	Over 90 F.	Total M.	Total F.	Lymphoma M.	Lymphoma F.
Arachis oil	Injected	60	D	1	1	1	4	6	2	1	2	9	9	1	4
Benzidine	„	60	D	1	—	3	7	2	—	0	0	6	7	1	2
			H	1	—	1	3	2	—	—	—	4	3	—	—
3 : 3′-Dihydroxybenzidine	„	30	D	4	—	3	3	1	3	0	0	8	6	0	4
1-Naphthylamine	„	60	D	—	4	2	5	3	1	0	2	5	12	1	6
2-Naphthylamine	„	90	D	13	7	6	6	1	5	1	2	21	20	3	8
			S	5	5	—	1	—	—	—	1	5	7	—	—
			H	—	—	1	—	1	2	1	2	3	4	—	—
Auramine	Oral	30	D	1	1	2	4	5	6	0	0	8	11	3	8
			S	—	—	—	1	—	—	—	—	—	1	—	—
			H	—	—	1	2	3	1	—	—	4	3	—	—
Magenta	„	60	D	3	4	2	5	2	1	0	3	7	13	2	2
			H	—	—	—	—	—	—	—	1	—	1	—	—
0-Me-2-amino-1-napthol hydrochloride	Injected	30	D	1	3	3	2	4	5	0	0	8	10	5	6
			H	—	—	—	—	2	—	—	—	2*	—	—	—
Carmoisine	„	30	D	—	3	1	3	—	5	0	0	1	11	0	7
Benzeneazo-2-anthrol	„	30	D	4	1	4	7	2	4	0	0	10	12	0	4
			S	1	1	1	—	—	—	—	—	2†	1	—	—
			H	—	—	1	—	—	—	—	—	1	—	—	—
Rhodamine B	Oral	30	D	2	1	4	2	—	1	4	1	10	5	4	3
Xylylazo-2-naphthol	„	30	D	4	4	3	4	4	2	0	0	11	10	5	8
	Injected	30	D	1	1	4	1	2	1	0	0	7	3	2	2
			H	—	—	—	—	1	—	—	—	1	—	—	—
o-Tolylazo-2-naphthol	Oral	30	D	3	1	2	5	7	2	0	2	12	10	1	5
	Injected	30	D	—	2	3	6	1	3	0	0	4	11	0	4
			S	—	1	—	2	—	—	—	—	—	3	—	—
Sunset yellow	Oral	30	D	2	—	3	6	4	4	0	0	9	10	3	6
Ponceau 2R	„	30	D	2	1	5	2	1	2	0	0	8	5	6	1

D = deaths. S = subcutaneous sarcomas. H = hepatomas.
* One liver with advanced cholangioma also.
† One squamous carcinoma of orbit in addition.

TABLE V.—*Mortality of Mice in which the Ileo-caecal Region and the Pylorus were Examined Microscopically and the Incidence of Intestinal Tumours and Pyloric Polyposis*

Chemical	Method of administration	Survivors dying at weeks: 20–49 M.	20–49 F.	50–69 M.	50–69 F.	70–89 M.	70–89 F.	Over 90 M.	Over 90 F.	Total intestinal tumours: Benign	Malignant	Total survivors M.	Total survivors F.	Mice with polyposis of stomach M.	Mice with polyposis of stomach F.
Arachis oil	Injection	—	—	—	0/3	1B/4	0/2	0/2	0/2	1	0	6	7	2	1
Benzidine	,,	—	—	0/1	0/6	0/2	—	—	—	0	0	3	6	0	0
3 : 3′-Dihydroxybenzidine	,,	—	—	1B/1	0/1	—	0/3	—	—	1	0	1	4	0	0
1-Naphthylamine	,,	—	—	0/2	0/2	0/3	—	0	1B/2	1	0	5	4	2	0
2-Naphthylamine	,,	—	—	0/5	0/3	—	1C/3	0/2	1B/2	1	1	7	8	3	3
Auramine	Oral	0	0/1	1C/2	0/2	0/3	0/3	0	0	0	1	5	6	1	1
Magenta	,,	—	—	0/1	0/2	0/2	0/1	0	0/2	0	0	3	5	1	1
0-Methyl-2-amino-1-naphthol hydrochloride	Injection	0/1	0/3	1B/1	1B/2	4B/4	2B/3	—	—	8	0	6	8	0	0
Carmoisine	,,	—	—	1B/1	0/1	—	0/4	—	—	1	0	1	5	0	2
Benzeneazo-2-anthrol	,,	—	—	0/3	0/5	0/2	0/2	—	—	0	0	5	7	0	0*
Rhodamine B	Oral	—	—	1B, 1C/3	0/1	—	—	0/4	—	1	1	7	1	2	1
Xylylazo-2-naphthol	,,	1B/4	1B/2	2B/3	0/4	0/2	—	—	—	4	0	9	6	5	0
	Injection	0/1	—	0/3	—	0/1	—	—	—	0	0	5	0	2	1
o-Tolylazo-2-naphthol	Oral	1B, 1C/3	0/1	1B/1	0/3	7B/6	0/3	0	0/2	9	1	10	9	4	1
	Injection	—	—	1C/1	1B, 1C/2	0/1	0/3	—	—	1	2	2	5	0	0
Sunset yellow	Oral	—	—	0/2	1B/5	—	—	—	—	1	0	2	5	1	1
Ponceau 2R	,,	—	—	1B/2	1B/1	—	—	1B/1	1B/2	4	0	3	3	2	1

* One squamous papilloma and one squamous carcinoma of the stomach.

Numerators represent number of mice with benign (B) or malignant (C) intestinal tumours.

with *o*-tolylazo-2-naphthol. Thereafter, the intestinal tract was searched more thoroughly for possible tumours and all suspicious areas were examined microscopically. In addition to cutting sections of the ileo-caecal region (Table V), 259 pieces of small intestine and 93 of large intestine were cut and examined.

It was decided to accept as without demonstrable action on the intestinal tract those compounds where one intestinal tumour or no tumours were present in the group. These are: arachis oil, benzidine, dihydroxybenzidine, 1-naphthylamine, auramine, magenta, carmoisine, benzeneazo-2-anthrol and sunset yellow. With these chemicals the incidence of polyps (only one of which, in an auramine mouse, was malignant) was 4 out of 31 males and 2 out of 49 females (Table V). The remaining compounds, i.e. 2-naphthylamine, 0-methyl-2-amino-1-naphthol hydrochloride, rhodamine B, xylylazo-2-naphthol, tolylazo-2-naphthol and ponceau 2R, each produced more than one intestinal tumour in the group. In total, there were 23 intestinal tumours in 49 males and 10 in 40 females (Table VI). Of these, five tumours were intestinal carcinomas.

Morbid anatomy and histology.—The site of election was the ileo-caecal junction (35 out of 39 tumours). Where the precise origin was detectable, it was usually from the wall of the caecum, but some tumours seemed to start exactly at the junction and others were multifocal, arising both at the junction and elsewhere. One benign polyp arose in the ileum at a little distance from the junction, and another in the colon near to the junction with the caecum. The site of two carcinomas was in the colon about $1\frac{1}{2}$ inches from the anus. The tumours could be detected either by observation of a thickening of the region or by the presence of small shining cysts on the serosal surface. Some of the small tumours were not detected with the naked eye.

Structure of the polyps.—These were usually sessile adenomas, raised from the mucous surface and composed of regular intestinal glands, lined by high mucus-secreting epithelium, in which the nuclei are markedly hyperchromatic (Fig. 2 and 3). Dipping of the glands through the muscularis mucosae and the muscular wall of the intestine into the mesentery was a frequent feature causing the cysts seen externally with the naked eye (Fig. 4). Other cysts were formed by oblique cutting of pockets of the intestinal lumen caused by the projecting polyp and these sometimes contained faeces or portions of tapeworm. Sometimes there was a granulomatous reaction in the mesentery, thought, to be due to the bursting of one or more of the cysts. Similar tumours were described by Miller and Pybus (1956).

Structure of the carcinomas.—All but one of the 6 tumours of this type could be seen to arise in pre-existing polyps. In 2, there was no muscle invasion (Fig. 5); in 2 there was complete penetration of the muscular coat by mucus-forming irrregular cystic glandular spaces; in 2 there was anaplastic invasive growth penetrating into the mesocaecum and associated with serosal lymphatic permeation and metastasis to regional lymph nodes (Fig. 6). Adjacent to both these tumours were benign cysts of the type described above as forming the deep part of benign papillomas of the caecal region. The finding of these cysts was regarded as evidence of the previous presence of a benign tumour.

Pyloric polyposis.—This is a proliferative condition associated with overgrowth of pyloric glands, which penetrate the muscularis mucosae and the muscular coat, but do not invade lymphatic vessels nor metastasise. This condition is spontaneous in the I and other strains (Stewart and Andervont, 1938) and has

TABLE VI.—*Analysis of Benign and Malignant Tumours of the Intestine*

Compounds which produced more than one intestinal tumour				
	Intestinal tumours in :			
Chemical	Males	Females	Benign polyps	Carcinomas
2-Naphthylamine	0/7	2/8	1	1
0-Methyl-2-amino-1-naphthol hydrochloride	5/6	3/8	8	0
Rhodamine B	2/7	0/1	1	1
Xylylazo-2-naphthol—				
Injected	0/5	—	4	0
Oral	3/9	1/6		
Tolylazo-2-napthol—				
Injected	1/2	2/5	10	3
Oral	10/10	0/9		
Ponceau 2R	2/3	2/3	4	0
Total	23/49	10/40	28	5

Compounds which produced one intestinal tumour or less				
	Intestinal tumours in :			
Chemical	Males	Females	Benign polyps	Carcinomas
Arachis oil	1/6	0/7	1	0
Benzidine	0/3	0/6	0	0
3 : 3′-Dihydroxybenzidine	1/1	0/4	1	0
1-Naphthylamine	0/5	1/4	1	0
Auramine	1/5	0/6	0	1
Magenta	0/3	0/5	0	0
Carmoisine	1/1	0/5	1	0
Benzeneazo-2-anthrol	0/5	0/7	0	0
Sunset yellow	0/2	1/5	1	0
Total	4/31	2/49	5	1

recently been described by Miller and Pybus (1956) in older mice of crosses between the NBT and CBA strains. The lesion occurred in control and treated mice of the present experiment, and was more common in males than in females. It is usually non-malignant, though Miller and Pybus described one tumour which they thought was an adenocarcinoma.

Other tumours.—A squamous carcinoma of the orbit occurred at 63 weeks in a male mouse injected with an oily suspension of benzeneazo-2-anthrol. A squamous papilloma of the lower end of the oesophagus and a squamous carcinoma of the cardiac end of the forestomach also occurred in 2 females at 58 and 56 weeks respectively in the group treated wth this chemical. Occasional mammary carcinomas and granulosa-cell ovarian tumours occurred in control and other groups. One spindle-cell sarcoma of the uterus occurred at 70 weeks in the xylylazo-2-naphthol group and one osteogenic sarcoma of the jaw at 74 weeks in a female treated with sunset yellow.

DISCUSSION

Single early benign intestinal polyps were found in groups of mice treated with arachis oil, 3 : 3′-dihydroxybenzidine, 1-naphthylamine (free from 2-naphthylamine), and carmoisine. One small hepatoma was seen at 101 weeks in a female treated with magenta and an osteogenic sarcoma of the jaw occurred in a mouse treated with sunset yellow. As all these tumours might well be casual occurrences these six compounds are regarded as being without carcinogenic activity in this experiment. Case and Pearson (1954) stated that the manufacture of magenta had an " occupational hazard of causing tumour of the urinary bladder attached to it." No carcinogenic properties have been demonstrated in magenta in the present experiment, where a high dose was used but only a few mice survived to a late period.

Baker (1950) claimed that 3 : 3′-dihydroxybenzidine, recovered from the urine of industrial workers, was carcinogenic to the mouse when given subcutaneously in oily solution, producing benign and malignant tumours in the bladder and the liver. As the author stated that the chemical was not completely purified, as benign and possibly malignant hepatomas were also observed in untreated control animals and as the illustrations of the tumours are quite inconclusive, the value of the observations is doubtful.

Case, Hosker, McDonald and Pearson (1954) brought evidence to show that 1-naphthylamine was a cause of bladder tumours in industry. They thought it possible that the 2-naphthylamine content, always present in crude samples, was not the sole causative agent of the industrial tumours. The present experiment brings no further evidence that the purified compound is carcinogenic either to the bladder or to any other organ.

The following compounds were regarded as showing carcinogenic activity : benzidine, 2-naphthylamine, auramine, *o*-tolylazo-2-naphthol and benzeneazo 2-anthrol.

Benzidine was shown by Spitz, Maguigan and Dobriner (1950) to induce hepatomas; intestinal carcinomas and acoustic duct carcinomas in the rat. Baker (1953) was also able to induce these types of tumour in Slonaker rats, and he claimed 2 squamous carcinomas of the forestomach and one papilloma and 2 squamous carcinomas of the urinary bladder. Tumour induction in the dog requires a long latent period and yields only a low incidence of tumours. Spitz

(personal communication) found that the 3 longest survivors of 7 dogs given large doses of benzidine by mouth developed papillomas and carcinomas of the bladder after 7, 8 and 10 years respectively. Of 4 dogs treated similarly in Leeds, two survived for $8\frac{1}{2}$ and 9 years and had no bladder tumours at death (unpublished observation). An invasive bladder carcinoma was induced after $2\frac{1}{4}$ years in one rabbit in Leeds (unpublished observation), among 7 treated by oral administration of benzidine. Baker (1950) described 5 hepatomas—4 benign and 1 malignant —in 9 mice of Oldham stock which had received subcutaneous injections of an oily suspension of 300 mg. of benzidine base 3 times per week for 45 weeks. Five of 17 untreated mice developed 3 benign and 2 doubtfully malignant hepatomas in the same time period. The dose reported by Baker was found in the present experiment to be greatly in excess of the maximum which could be tolerated by our mice. Our tumours were confined to the liver. They occurred in both sexes between 50 and 79 weeks of treatement.

The tumours induced with 2-naphthylamine have already been discussed (Bonser *et al.*, 1956) and reasons given for regarding the subcutaneous sarcomas as due to the development of carcinogenic properties in oily solutions on standing. Hepatomas occurred even when the water-soluble hydrochlorides were injected and this was regarded as being due to the action of 2-naphthylamine or its metabolites on the liver. All but one occurred after 70 or more weeks of treatment, i.e. late in the experiment. In addition, one benign polyp and one carcinoma arose at the ileo-caecal junction.

The claim for carcinogenic properties in auramine lies in the 7 hepatomas (4 in males and 3 in females) occurring in 19 mice. The one carcinoma of the colon in a male mouse treated for 67 weeks is regarded as probably fortuitous and the origin of the subcutaneous sarcoma following oral administration is unexplained, though it might have been caused by the chemical.

o-Tolylazo-2-naphthol was administered by injection and feeding. One small intestinal polyp and 2 carcinomas occurred after injection (Bonser, Clayson and Jull, 1954) and 9 polyps and one carcinoma after feeding. In the latter group, only the males were affected and all the polyps but one were at the ileo-caecal junction and were of the characteristic cystic type. In the benzeneazo-2-anthrol group, were 3 subcutaneous sarcomas, 2 of vaso-formative type, usually classified by other authors as haemangio-endotheliomas (Foulds, 1930 ; White and Stewart, 1942 ; Andervont, 1950). White and Stewart suggested that their tumours occurred along the line of drainage of oral methylcholanthrene from the intestinal tract. The present tumours could either be regarded as occurring at the site of injection of the chemical or in tissues likely to be receiving lymph from the injected area. These were the only tumors of this type in the whole experiment and therefore they are regarded as being specifically related to this chemical. One squamous papilloma and one squamous carcinoma of the forestomach, and one squamous carcinoma of the orbit were also observed in the group treated with benzeneazo-2-anthrol. The significance of the last-named tumour is doubtful.

0-Methyl/-2-amino-1-naphthol hydrochloride, xzylylazo-2-naphthol, ponceau 2R and rhodamine B are chemicals of which the carcinogenic activity is in doubt. When the mice were treated with 0-methyl-2-amino-1-naphthol hydrochloride by subcutaneous injection, 2 hepatomas were found in male mice but the outstanding feature was the 8 benign polyps, all situated at the ileo-caecal junction and all diagnosed on naked-eye examination of the exterior by the presence of subserous

and intra-muscular cysts. These occurred between 55 and 78 weeks, i.e. well within the age when carcinomas had developed in other groups. Therefore, although the high incidence of benign tumours suggests that the compound is not without tumour-inducing properties, the absence of malignant change leaves its carcinogenic nature in doubt. Xylylazo-2-naphthol was administered by injection and feeding. No intestinal tumours occurred in 5 injected survivors but there was one hepatoma ; 4 benign intestinal polyps were induced when the dye was fed. These were found at the ileo-caecal junction and 3 of them showed the characteristic cysts. Four benign caecal polyps were induced in males and females with ponceau 2R.

In regard to the intestinal tumours as a whole, there was a marked predilection for the male sex (27 out of 80 males and 12 out of 89 females). There was histological evidence that carcinomas arose in polyps, but none of the polyps in mice treated with 0-methyl-2-amino-1-naphthol hydrochloride, xylylazo-2-naphthol or ponceau 2R became malignant within the period of the experiment. The preponderance of tumours at the ileo-caecal junction suggests that stasis of the intestinal contents is a factor concerned in carcinogenesis.

SUMMARY

An experiment is described in which stock albino mice, bought from a dealer, were used to test the carcinogenic properties of 14 chemicals and the vehicle of administration.

The chemicals were either : (1) dyes (auramine and magenta), the manufacture of which constitutes an industrial hazard ; (2) dyes (carmoisine, rhodamine B, sunset yellow, ponceau 2R, xylylazo-2-naphthol and tolylazo-2-naphthol) formerly or at present in use as food colorants ; (3) dyestuffs intermediates (benzidine, 1-naphthylamine and 2-naphthylamine) ; or (4) ortho hydroxyamines (3 : 3′-dihydroxybenzidine and 0-methyl-2-amino-1-naphthol hydrochloride) related to known metabolites of aromatic amines.

The chemicals were administered either by subcutaneous injection of oily solutions or suspensions, or orally in the diet, in the drinking water or by stomach tube.

The carcinogenic activity was assessed by the occurrence of subcutaneous sarcomas, hepatomas, and intestinal polyps and carcinomas. Lymphomas and pyloric polyposis occurred spontaneously and their incidence was not enhanced by the treatment.

Arachis oil, 3 : 3′-dihydroxybenzidine, 1-naphthylamine (free from 2-naphthylamine) and carmoisine induced no tumours. The one hepatoma obtained with magenta, and the one osteogenic sarcoma of the jaw with sunset yellow were regarded as fortuitous occurrences, not related to the treatment.

Benzidine, 2-naphthylamine and auramine were regarded as carcinogenic on the grounds of the occurrence of hepatomas. Two caecal polyps, one benign and one malignant, in mice treated with 2-naphthylamine and one carcinoma of the colon in an auramine-treated mouse were regarded as probably induced by the chemical. The cause of the one subcutaneous sarcoma after oral administration of auramine is unexplained. *o*-Tolylazo-2-naphthol caused 13 intestinal tumours, 10 benign and 3 malignant, in 26 mice. The degree of malignancy of the tumours in this group was greater than with any other chemical, lymph-node metastases

being present in 2 cases. There was a marked predilection for the male sex. Intestinal tumours followed both feeding and subcutaneous injection, the latter method being the cause of 3 subcutaneous sarcomas. Benzeneazo-2-anthrol injected subcutaneously in oil caused 3 subcutaneous sarcomas, 2 of which were of unusual vasoformative type, one squamous papilloma and one squamous carcinoma of the forestomach, and a squamous carcinoma of the orbit, the latter probably fortuitous.

The carcinogenicity of the remaining chemicals, i.e. xylylazo-2-naphthol, 0-methyl-2-amino-1-naphthol, ponceau 2R and rhodamine B remains in doubt, the first three because none of the intestinal polyps had progressed further than the benign stage and the last because, although one tumour was malignant, only 2 tumours were found in 8 mice.

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