The present observations revealed that verapamil was a strong antagonist for Ca influx in guinea-pig stomach, since the calculated pA_2 value for Ca contracture was 7.92. The mechanism of PG-induced contraction in gastrointestinal smooth muscle was considered to depend on an increase of Ca ion permeability through the muscle membrane, since PGE_1 potentiates Ca contracture in guinea-pig stomach[5] and increases spike discharge in guinea-pig ileum[12] and taenia coli[13], and PG-induced contraction is related to the extracellular Ca ion in rabbit duodenum[13].

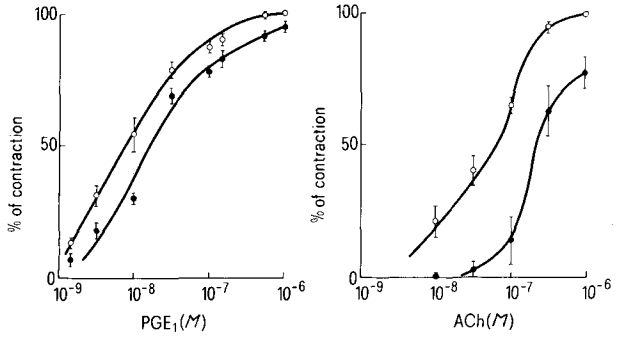

Fig. 3. Effect of verapamil on the contractile responses to PGE_1 (1.5×10^{-9} to 10^{-6} M) and ACh (10^{-8} to 10^{-6} M) in longitudinal smooth muscle of guinea-pig stomach. ○—○, control; ×—×, verapamil 10^{-5} M. Each contractile response to PGE_1 (10^{-6} M) and ACh (10^{-6} M) was taken as 100%, respectively. Each point is the mean of 5 experiments and vertical bar is ± S. E. of mean.

In the present experiments, however, verapamil (10^{-5} M) which produced marked suppression of high K-induced contractures, showed only small reduction of PGE_1 (1.5×10^{-7} M)-induced contraction (figure 2). Figure 3 showed the effect of verapamil (10^{-5} M) on dose-response curves of PGE_1 and acetylcholine (ACh), indicating that verapamil caused more significant reduction of ACh-induced contraction than that of PGE_1. The calculated pA_2 values for PGE_1 and ACh were 5.06 and 5.33, respectively.

The findings in the present study suggest that the stimulating mechanism of PGE_1 in smooth muscle of guinea-pig stomach may mainly depend on a release of bound Ca in the cell and partly depend on a Ca influx from the extracellular origin, and ACh-induced contraction may also depend on 2 Ca origins, in which the influx Ca will be more available than in PGE_1.

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Examination of the potential mutagenicity of hair dye constituents using the micronucleus test[1]

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Summary. 12 compounds which are constituents of hair dyes or chemically related aromatic amines, aminophenols, their nitroderivatives and aromatic hydroxyderivatives were examined for evidence of mutagenic potential by means of the micronucleus test. None of the compounds tested caused an increase in the incidence of micronucleated erythrocytes after oral dosing.

There has been increasing concern that some constituents of hair dyes, mainly aromatic amines, aminophenols, their nitroderivatives and hydroxyderivatives may be mutagens and possible carcinogens. Although some epidemiological evidence is available[2–4], much of the concern is based on the results of in vitro studies using microorganisms[5,6], cultures of human lymphocytes[5], Chinese hamster cells[7] and a mouse cell line[8], and on tumorigenicity studies[9]. However, other studies[10–12] have failed to show long-term toxicity, carcinogenic or teratogenic effects. The percutaneous absorption of the compounds has been studied in animals[13] and man[14].

The experimental work reported here was designed to determine the effects of the compounds on the genetic material of mammalian somatic cells when administered orally at the maximum tolerated dose, by means of the micronucleus test developed by Schmid et al.[15–18].

Materials and methods. The compounds examined were: p-phenylenediamine, 4-methoxy-m-phenylenediamine, 4-nitro-o-phenylenediamine, 2-nitro-p-phenylenediamine, p-aminophenol, m-aminophenol, 2-amino-4-nitrophenol, toluene-2, 5-diaminesulphate, resorcinol, 4-chlororesorcinol, 4-amino-2-hydroxytoluene, 1-naphthol.

Rats of the CFY strain (Sprague-Dawley descendants) weighing between 130 and 160 g were obtained from Anglia Laboratory Animals, Alconbury, Cambs., U.K., acclimatized in the laboratory for 1 week and then randomly allocated into groups each including 5 males and

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Table 1

Group	Material	Total dosage over 24 h (mg/kg)	Mortality*	Incidence of micronucleated cells per 2000 polychromatic erythrocytes per rat	
				Mean	Range
1	Vehicle control	–	0	1.8	0–5
2	p-Phenylenediamine	300	0	1.7	0–4
3	4-Methoxy-m-phenylenediamine	1000	0	2.1	0–4
4	4-Nitro-o-phenylenediamine	5000	1	1.8	0–4
5	2-Nitro-p-phenylenediamine	2000	0	1.5	1–3
6	p-Amino-phenol	3200	0	2.1	1–4
7	m-Amino-phenol	5000	3	1.9	0–4
8	2-Amino-4-nitro-phenol	5000	1	2.0	0–4
9	Toluene-2,5-diamine-sulphate	120	0	0.9	0–2
10	Resorcinol	500	0	1.6	1–2
11	4-Chlororesorcinol	600	1	2.0	1–4
12	4-Amino-2-hydroxy-toluene	8000	1	2.1	0–5
13	1-Naphthol	6000	1	2.3	1–5

* In addition to the deaths toxic symptoms including agitation and/or convulsions, and/or lethargy were observed in all animals of the groups treated with the test compounds. Orange urine was seen in animals in groups 4, 5, 7 and 12.

5 females. Each group was dosed with one of the test compounds, which were all prepared as suspensions in 0.5% (w/v) gum tragacanth containing 0.05% (w/v) sodium sulphite. One group was dosed with the vehicle only. The total dosages given were determined in preliminary studies to be close to the lethal doses, and were administered by gastric intubation as 2 equal doses separated by an interval of 24 h (table 1).

6 h after the 2nd dose the animals were killed by the i.p. injection of pentobarbitone sodium (Expiral®), the femurs dissected out and bone-marrow smears prepared. The smears were fixed in methanol, defatted in xylene and stained with Giemsa stain. The stained smears were then examined microscopically to determine the incidence of micronucleated cells per 2000 polychromatic erythrocytes per animal. The group mean counts and ranges were then compared with the values obtained with the vehicle control group and with laboratory standard values.

Results. Table 1 lists the total dosages, mortalities, group mean values and ranges of the incidence of micronucleated polychromatic erythrocytes, and table 2 shows the laboratory standard values for vehicle control groups in previous unrelated tests. The mean values and ranges obtained will all of the test compounds (table 1) were essentially similar to those obtained with the concurrent vehicle control group and did not fall outside the laboratory standard range for negative control groups (table 2).

Discussion. In this test procedure and by oral administration, none of the test compounds showed any clear evidence of mutagenic potential, although 3 of them (2-nitro-p-phenylenediamine, 4-nitro-o-phenylenediamine and 2-amino-4-nitrophenol) have previously been reported to be mutagenic in a microbiological test system[6], and the first 2 compounds caused chromosome damage in cultured mammalian cells[5,7]. Another, p-phenylenediamine, was tumorigenic when administered by the sub-dermal route[9]. The test system used in this study has been shown to be sensitive and reliable[19] and gives strongly positive results with the aromatic diamine benzidine (126 micronucleated cells per 2000 polychromatic erythrocytes) when administered s.c., and the aromatic amine, 2-aminofluorene (146 micronucleated cells per 2000 polychromatic erythrocytes) when administered orally.

The mutagenic action of benzidine is reported to be detected by the same microorganism (Salmonella typhimurium TA 1538, which is sensitive to particular frame-shift mutagens), as were the related diamine test compounds[20]. Results of this nature serve to highlight the difficulties which may be encountered in the application and interpretation of short-term mutagenicity tests. A battery of such tests must be used rather than relying on just one single type of test procedure. They also emphasize the continuing need for well-designed long-term carcinogenicity studies in animals.

Table 2. Rat micronucleus test Laboratory standard values for negative control groups

Total number of animals examined	Mean micronucleated cell count	Range of group mean counts	Range of individual counts
175	1.19	0.7–2.3	0–6

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