in incubation time such as that displayed between newly emerged males and males 12 h old (Fig. 1) leads to a two-fold difference in titre estimate. The standard curve for converting incubation time to inoculum titre has been published elsewhere[5].

A control experiment was performed in order to ensure that all flies received the same amount of inoculum. There is negligible bleeding following an injection, but because the fly exoskeleton hardens with age, old flies might have accepted less inoculum fluid than young ones. Therefore, at the same time that the flies were injected with virus, an aliquot of five males of each age was injected with randomly labelled leucine-^{14}C (0·25 μl. of specific activity 0·49 μc./ml.). These were squashed immediately on to planchets and then counted in a Nuclear Chicago gas-flow counter.

The average number of counts recovered per individual fly for each age is shown in Fig. 1. The variation in inoculum size as measured by recovered counts is too small and random to account for the observed changes in incubation time and, therefore, indicates that the age effect depends on the *Drosophila* physiology.

A consideration of the sequence of events following the injection of sigma into imagos suggested that the increase in incubation time might be related to the rate of virus multiplication. It is known that the injection of strain-*Lw* virus into young imagos is followed by the multiplication of the virus to a plateau level of 10^4–10^6 infectious units per fly. The recipient fly generally becomes CO_2-sensitive at the time that the virus titre reaches the plateau level[1,5]. The fact that the incubation period was increased in old flies suggested that the virus multiplied more slowly in old flies and reached the plateau value later.

Table 1. TIME REQUIRED FOR INJECTED FLIES TO BECOME CO_2-SENSITIVE (INCUBATION TIME)

Ageing temperature (°C)	Imaginal age at injection (days ± 0·083)	Incubation time (days)
25	0·21	7·9
25	13·00	12·1
20	0·17	7·8
20	15·00	12·6

In order to test this hypothesis, virus was injected into young and old flies and the rate of virus multiplication was measured. The procedures used were the same as reported previously[5] except that the reference-strain flies used to assay the virus were 3–7 h old. The results are presented in Table 1 and Fig. 2. The virus doubling time during log phase was indeed increased in old imagos as compared with young ones. This was true whether the flies

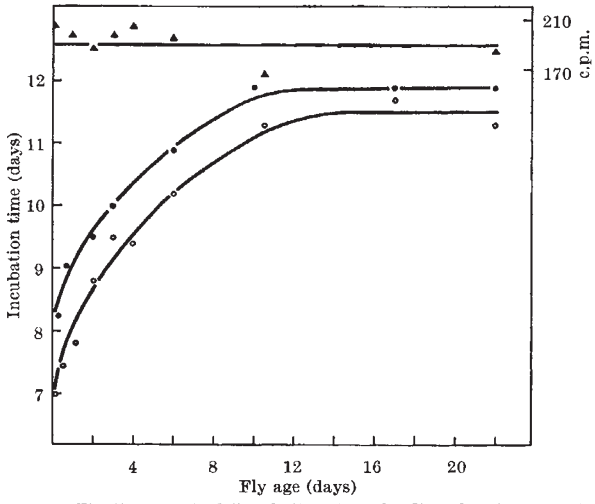

Fig. 1. The time required (incubation time) for flies of various ages to reach CO_2-sensitivity after inoculation with virus. All males (○) and females (●) received identical inocula. Fly age is measured from eclosion. Labelled leucine was injected into aliquots of males at the same time. The average number of recovered counts/fly is shown above (▲)

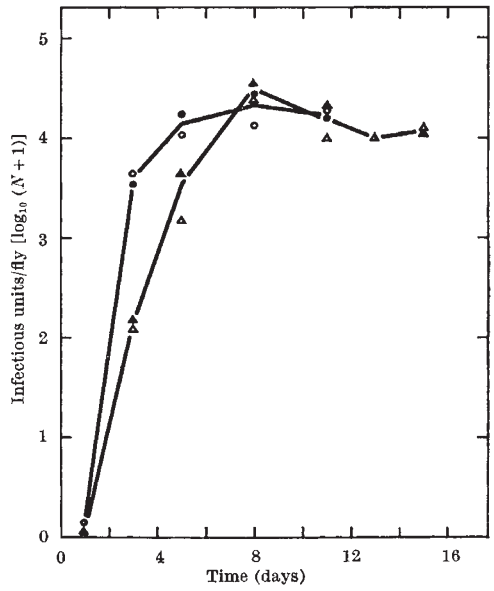

Fig. 2. Growth curves for injected virus. *N* is the average number of infectious units/fly. Virus was injected at zero time into flies aged 2 h after eclosion (circles) and into flies aged 13–15 days after eclosion (triangles). Flies had been raised and aged at 20° C (solid symbols) or at 25° C (open symbols)

had been raised and aged at 25° or 20° C. However, the increase in incubation time was greater than expected from the observed lag in virus multiplication. The virus titre plateau in old imagos was reached about two days later than in young ones, but CO_2-sensitivity was delayed for two days longer. Therefore, under these conditions, the appearance of CO_2-sensitivity is dependent on factors in addition to the virus content of the injected fly, and the absence of CO_2-sensitivity does not preclude a high virus titre in the fly.

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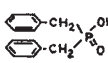
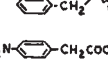
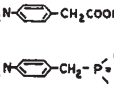
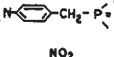
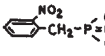
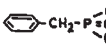
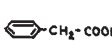
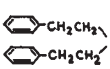
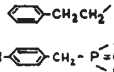
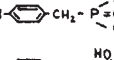
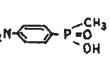
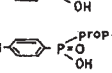
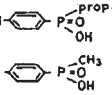
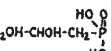
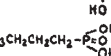
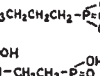
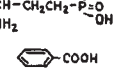
Inhibition of Encephalomyocarditis Virus Replication by Simple Phosphonic and Carboxylic Acids

IN view of our recent finding[1] that multiplication of encephalomyocarditis virus (EMC) can be inhibited by non-cytotoxic concentrations of *p*-nitrobenzylphosphonic acid, it appeared worthwhile to investigate the antiviral effect of other phosphonic acids and their carboxylic analogues. It was our hope that the investigation might give some information about the relationship between the chemical structures of the compounds and their antiviral activity, and facilitate the screening of drugs which may be useful in the chemotherapy of viral diseases. In this communication we report the effect of several simple compounds of diverse structure on the replication of EMC virus in the mouse embryo tissue culture.

The EMC virus was maintained by passage in mouse brain and tested in primary mouse embryo (ME) tissue cultures. The commercial carboxylic acids and phosphonic compounds, synthesized by one of us (P. M.) according to the methods indicated in references to Table 1, were used in this work.

Table 1. THE EFFECT OF VARIOUS COMPOUNDS ON THE MULTIPLICATION OF EMC VIRUS IN MOUSE TISSUE CULTURE

No.	Compound* (acid)	Ref. No.	Formula	Concentration ($\times 10^{-3}$ M)	Antiviral activity: Protection against CPE† Mean	Protection against CPE† Range	% Inhibition of virus multiplication‡ Mean	% Inhibition of virus multiplication‡ Range	Cytotoxic concentration ($\times 10^{-3}$ M)§	No. of experiments
1	2	3	4	5	6	7	8	9	10	11
1	Salicylic	—		2	> 2	> 2	99	99–99·9	> 10	10
2	Acetylsalicylic	—		2	> 2	> 2	94	85–99	> 10	5
3	*p*-Aminosalicylic	—		2	> 2	> 2	94	90–99·7	> 10	5
4	Dibenzylphosphinic	5		2	> 2	> 2	98	96–99·5	> 10	10
5	*p*-Nitrophenylacetic	—		1	> 2	> 2	97	94–99·8	2·5	5
6	*p*-Nitrobenzyl-phosphonic	3		2	1·5	1–2	90	90–98	> 20	30
7	*o*-Nitrobenzyl-phosphonic	4		2	1·5	1–2	90	85–92	> 20	3
8	Benzylphosphonic	6		5	1	0·5–1·5	88	80–96	> 20	4
9	Phenylacetic	—		5	0·5	0·5	85	80–90	> 10	2
10	*bis*(β-Phenyl-ethyl) phosphonic	4		2	1	0·5–1	78	40–95	> 10	3
11	*p*-Aminobenzyl-phosphinic	7		5	0·5	0–0·5	70	60–80	> 20	4
12	β-Phenyl-ethylphosphonic	8		5	0·25	0–0·5	40	0–80	> 20	4
13	*p*-Nitrophenyl-phosphonic	9		5	0·5	0–1	60	50–70	> 20	8
14	*p*-Aminophenyl-methylphosphinic	10		5	0	0	0	0	> 20	8
15	*p*-Aminophenyl-propylphosphinic	10		5	0	0	0	0	> 20	6
16	*p*-Nitrophenyl-methylphosphinic	10		5	0	0	30	10–50	> 20	6
17	Phenylphosphonic	11		5	0	0–0·5	16	0–50	> 20	7
18	Ethylphosphonic	12		5	0	0–0·5	23	0–70	> 20	6
19	2,3-Dihydroxy-propylphosphonic	13		5	0	0–0·5	80	80	> 20	5
20	*n*-Butylphosphonic	12		5	0	0–0·5	70	60–80	> 20	5
21	α-Amino γ-phos-phonobutyric	14		0·5	0	0	0	0	1	4
22	Benzoic	—		5	0	0–0·5	70	70–75	> 10	4
23	*p*-Nitrobenzoic	—		2	0·25	0–0·5	80	70–90	10	2
24	*p*-Aminobenzoic	—		5	0·25	0–0·5	60	40–80	> 10	2
25	Phenylpropionic	—		5	0·5	0·5–1	80	80	> 10	3
26	2,4-Dinitrophenylpro-pionic	—		2	0	0	0	0	5	2
27	*p*-Aminophenyl-phosphonic	9		5	0·25	0–0·5	75	75	> 10	2

* Sodium salts, *p*H 7·3–7·4.

† Expressed as delay (in days) of degeneration of the cell population infected with virus treated with compound in question in comparison with untreated culture.

‡ Expressed as per cent inhibition of virus yield in the medium measured in plaque-forming units/ml. 20 h after infection.

§ Minimal toxic concentrations of the compounds leading to the microscopically visible degenerative changes of cells during 48 h observation period.

The compounds prepared as 0·1 M solutions of sodium salts, *p*H 7·3–7·4, were incorporated into the fluid maintenance medium. The medium contained 2 per cent horse serum and 0·5 per cent lactalbumin hydrolysate in Hanks's saline with bicarbonate and antibiotics. The ME cultures (about 2×10^5 cells/ml.) were infected with approximately 100 plaque-forming units of virus. The antiviral activity of the compounds was determined by observing the protection of tissue cultures against the cytopathogenic effect (CPE) of the virus and by measurement of the virus yield in the medium 20 and/or 48 h after infection. For the virus titration plaque assays in serological tubes with 1 per cent methylcellulose overlay were used. The details of the methods used were the same as previously described[1].

The antiviral effects of 27 compounds tested so far are summarized in Table 1.

The results presented indicate that marked antiviral activity (more than 90 per cent inhibition of virus yield and protection against CPE for at least one day) is exhibited by two types of compounds—compounds related to salicylic acid (Nos. 1–3) and certain phosphonic and carboxylic acids with benzyl groups (Nos. 4–9). To our knowledge the antiviral activity of salicylates *in vitro* has not previously been noticed. For this reason their mode of action was investigated more in detail and the results are described in a separate communication[2].

The significance of the benzyl grouping associated with different structures for virus inhibition emerges from the results of several investigators. Tamm *et al.*[15], who have examined the inhibition of virus replication by numerous derivatives of benzimidazole, suggested that hydroxybenzyl grouping at position 2 in the imidazole ring was of importance for the selective antiviral activity of 2-(α-hydroxybenzyl)-benzimidazole (HBB). O'Sullivan *et al.*[16] synthesized several substituted HBB derivatives with high inhibitory action on poliovirus multiplication and concluded that the 1-benzyl derivative of HBB is among the most active compounds. Loddo and Gessa[17] have recently reported that benthanidine and *o*-chlorobenthanidine, which are benzyl derivatives of guanidine, have a considerable inhibitory action on polio and vaccinia virus growth although presumably exerting their effects through a different mechanism from that of guanidine.

The work recorded here has revealed that certain simple compounds with the benzyl grouping attached to electronegative, electrophilic structures (carboxylic or phosphonic) also suppress the replication of EMC virus, whereas the compounds with the phenyl, β-phenylethyl and some other groups show little, if any, virus inhibitory action. Moreover, the dibenzyl structure was found to be far more active than mono-benzyl structure. It was also observed that the nitro group increased, whereas the amino group reduced, the antiviral effect of the compounds.

Even the most active of the compounds tested so far require a rather high concentration of 2×10^{-3} M/l. for activity and their effect diminishes rapidly at lower concentrations. On the other side, the increase of concentration from 2×10^{-3} to 5×10^{-3} was not followed by significant increase of percentage inhibition of virus yield. In case of phosphonic acids and of salicylic acid and its derivatives the high concentration necessary is counterbalanced, however, by low toxicity. For this reason the active doses were always several times lower than the toxic doses. Among the highly active compounds only *p*-nitrophenylacetic acid was found to be cytotoxic at a concentration of $2{\cdot}5 \times 10^{-3}$ M and slightly inhibited oxygen consumption and lactic acid production by mouse embryo tissue. It was, however, interesting to note that its phosphonic analogue, *p*-nitrobenzylphosphonic acid, conferred to cells rather high protection against virus infection although it was several times less toxic and did not significantly affect the tissue respiration.

In preliminary experiments *p*-nitrophenylacetic acid and dibenzylphosphonic acid gave distinct protection of mice infected with lethal doses of EMC virus. The *in vivo* activity of *p*-nitrobenzylphosphonic acid was described previously[1].

We thank Dr. A. C. Allison and Prof. Z. Skurska for their advice.

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GENETICS

Genetic Recombination with Ethylmethanesulphonate-induced Waxy Mutants in-Maize

Intracistron mapping in maize has been demonstrated by Nelson[1,2] with a technique based on differential staining reactions of starch in pollen of different genotypes. Those with the *waxy* (*wx*) locus stain reddish brown in iodine–potassium iodide solutions; whereas *non-waxy* (*Wx*) pollen stains dark blue. Therefore, in *waxy* plants genetic recombination or back-mutation at this locus is manifested by the appearance of dark blue staining pollen. The technique has a distinct advantage in genetic investigations with higher plants in that large populations of hundreds of thousands of genotypes (pollen grains) can be scored with ease.

As part of a programme in progress here on chemical mutagenesis in higher plants, the *wx* locus in maize is being used as experimental material and ethyl-methanesulphonate (EMS) as one of the chemical mutagens[3]. This communication is a preliminary report of results obtained with EMS on the induction of *wx* mutations at independent sites and the ordering of these sites within the locus. So far, nearly 50 *wx* mutations have been induced in the programme. Although each appeared independently, some of the sites may be identical, and this will be investigated in subsequent research. A number of mutants other than *waxy* have also been produced with EMS (ref. 3). Results with these, together with the data on *waxy* mutations, are providing preliminary evidence that EMS produces 'point' mutations in maize.

Results are reported here for four of the EMS-induced *waxy* mutants. These were obtained from seed treatments. Disinfected seeds were first soaked in deionized water at 27° C and bubbled continuously with oxygen for 24 h. They were then soaked in 0·05 M or 0·025 M aqueous solutions of EMS for either 2 or 3 days at 3° C. The rationale for this