Table I—Half-Wave Potentials of 1,2,5-Trimethyl-3-arylpyrazolium Perchlorates (II–VIII)

	Compound						
	II	III	IV	V	VI	VII	VIII
X	p-CH_3	p-OCH_3	p-OC_2H_5	p-OH	p-Cl	p-Br	m-NO_2
$E_{1/2}$	−1.75	−1.77	−1.77	−1.82	−1.63	−1.60	−0.6, −1.71

with the corresponding structural parameters. As indicated from the scattered results relating the MIC of the pyrazolium salt to the $E_{1/2}$ value (Fig. 4), the microbiological data of these compounds do not correspond with their polarographic behavior. This can be probably attributed to the existence of biological pathways other than those concerned with the reduction potential of the investigated compounds. In fact, the application of the Hammett equation in such biological fields is still limited to a small group of organic compounds.

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Comparison among Four Vehicles and Four Routes for Administering Δ^9-Tetrahydrocannabinol

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Abstract □ The effect of (−)-Δ^9-*trans*-tetrahydrocannabinol (I) on latency to hindlimb tonic extension produced by maximal electroconvulsive shock was studied in mice following injection by four routes of administration: oral, subcutaneous, intraperitoneal, and intravenous. For each route, four different vehicles were used: bovine serum albumin, polysorbate 80, polyvinylpyrrolidone, and propylene glycol. The anticonvulsant effect of I was strongest in the propylene glycol vehicle with subcutaneous and intraperitoneal routes and equal in the propylene glycol and polyvinylpyrrolidone vehicles with the oral route. With these three routes, bovine serum albumin and saline appeared to be inadequate vehicles for studying the anticonvulsant activity of I. When the intravenous route was used, anticonvulsant activity of I was found with all four vehicles, with propylene glycol and polyvinylpyrrolidone allowing the greatest effect of I; however, the propylene glycol vehicle itself also showed anticonvulsant activity. A further experiment showed that the duration of action of I following oral administration was substantially longer in the propylene glycol than in the polyvinylpyrrolidone vehicle.

Keyphrases □ Tetrahydrocannabinol—effect on hindlimb tonic extension, four vehicles and four routes of administration compared, mice □ Marijuana—comparison of four vehicles and four routes of administration, hindlimb tonic extension, mice □ Vehicles—effect on tetrahydrocannabinol anticonvulsant activity, comparison of four vehicles and four routes of administration, hindlimb tonic extension, mice

Recently, a report (1) from this laboratory described a study with mice which compared four vehicles for intraperitoneal administration of (−)-Δ^9-*trans*-tetrahydrocannabinol[1] (I), the apparent major psychoactive constituent of marijuana. The bioassay for an effect of I was latency to hindlimb tonic extension in mice following a maximal electroconvulsive shock. The most suitable vehicle for injection of I was found to be 10% propylene glycol–1% polysorbate 80[2]–0.9% saline. Other vehicles compared were 3% polyvinylpyrrolidone (2), 5% bovine serum albumin (3), and 1% polysorbate 80 (4), all in 0.9% sa-

[1] An alternative name for the same compound is Δ_1-tetrahydrocannabinol, using the pyran instead of the formal numbering system.

[2] Tween 80.

Table I—Effects of I on Latency to Convulsion (Mean ± *SE*) in Mice When Given by Four Different Routes of Admininistration in Four Vehicles and Tested 1 hr after Injection[a]

Vehicle	Route of Administration	Control (n = 12)	I, 20.0 mg/kg (n = 12)[b]
Bovine serum albumin (5%)	Oral	1.48 ± 0.07	1.54 ± 0.11
	Subcutaneous	1.42 ± 0.06	1.69 ± 0.10*
	Intraperitoneal	1.55 ± 0.09	1.68 ± 0.08
	Intravenous	1.53 ± 0.12	2.02 ± 0.17*
Polysorbate 80 (1%)	Oral	1.37 ± 0.08	1.55 ± 0.08
	Subcutaneous	1.42 ± 0.09	1.60 ± 0.09
	Intraperitoneal	1.56 ± 0.06	1.69 ± 0.10
	Intravenous	1.44 ± 0.08	2.28 ± 0.12***
Polyvinyl-pyrrolidone (3%)	Oral	1.49 ± 0.06	1.96 ± 0.12**
	Subcutaneous	1.40 ± 0.08	2.42 ± 0.22***
	Intraperitoneal	1.49 ± 0.11	2.75 ± 0.18***
	Intravenous	1.58 ± 0.06	3.48 ± 0.37***
Propylene glycol (10%)	Oral	1.28 ± 0.12	1.73 ± 0.10**
	Subcutaneous	1.46 ± 0.06	3.10 ± 0.21***
	Intraperitoneal	1.46 ± 0.06	3.38 ± 0.30***
	Intravenous	1.88 ± 0.09	3.88 ± 0.30***

[a] A maximum interval of 5 sec was recorded for mice that failed to convulse by that time (two with propylene glycol after intraperitoneal administration, four with propylene glycol after intravenous administration, three with polyvinylpyrrolidone after intravenous administration, and all in I-treated animals). [b]* $p < 0.05$ for difference from control. ** $p < 0.01$ for difference from control. *** $p < 0.001$ for difference from control.

line. The present report describes the results of a further investigation in which I was given by four different routes of administration in each of the same four vehicles.

EXPERIMENTAL

Two experiments were conducted on 576 male albino mice[3] weighing 17–27 g. In the first experiment, 384 mice were divided equally among the four vehicle conditions: 5% bovine serum albumin–0.9% saline, 1% polysorbate 80–0.9% saline, 3% polyvinylpyrrolidone–0.9% saline, and 10% propylene glycol–1% polysorbate 80–0.9% saline. The preparation of each vehicle has already been described in detail (1). The volume of fluid administered was 0.1 ml/10 g (10 mg/kg), containing I in a concentration of 2.0 mg/ml (20.0 mg/kg). These four vehicle groups were subdivided into groups administered the fluid by the oral, subcutaneous, intraperitoneal, or intravenous route. Half of the animals were injected with the vehicle alone (control) and the other half with I added to the vehicle. All animals were housed in the laboratory in groups of 12 for 24 hr before use and divided at the time of administration into six vehicle and six drug-treated animals with the same vehicle and route. At 1 hr following administration of I or vehicle, each mouse was tested for latency (recorded by stopwatch to the nearest 0.1 sec) to tonic convulsion, *i.e.*, complete extension of the hindlimbs, following an electroshock of 50-mamp intensity, 0.2-sec duration, applied transcorneally (5). All procedures were conducted twice, 1 week apart, so 12 mice were subjected to each of the 32 different treatments (four vehicles, each with four routes, each with vehicle and I).

In the second experiment, 192 mice were divided equally among two vehicle conditions: polyvinylpyrrolidone and propylene glycol. These two groups were subdivided into groups of 16 mice each, half of which received the vehicle alone and the other half I (20.0 mg/kg) in one of the vehicles, all by the oral route. Mice in various groups were tested at 0.25, 0.5, 1, 2, 4, and 8 hr for latency to tonic convulsion after electroconvulsive shock.

RESULTS

Table I presents the mean latencies to convulsion at 1 hr after I or control fluid in four vehicles, each administered by four routes. The 12 mice under each condition represent a combination of results obtained from two groups of six tested 1 week apart; they showed no statistically significant differences from each other. Table II summarizes the effect of I shown in Table I as a function jointly of vehicle and route of administration. In the polyvinylpyrrolidone and propylene glycol vehicles, I caused a highly reliable increase in convulsion latency with all routes of administration. In the polysorbate 80 vehicle, I had a reliable effect only with the intravenous route. In the bovine serum albumin vehicle, the effect of I was small with all four routes, reaching statistical significance only with subcutaneous and intravenous administrations. Comparison among the routes shows the largest effect of I after intravenous administration and the smallest effect after oral administration; the intraperitoneal and subcutaneous routes were intermediate and similar to each other.

In the absence of I, the median latency to convulsion showed no statistically significant differences among the groups with different vehicles and routes except for the elevated value of 1.88 ± 0.09 sec for the propylene glycol intravenous group (t = 4.33, df = 7, $p < 0.01$ for the difference from the overall mean of 1.49 sec).

Figure 1 summarizes the difference between I and the vehicle in

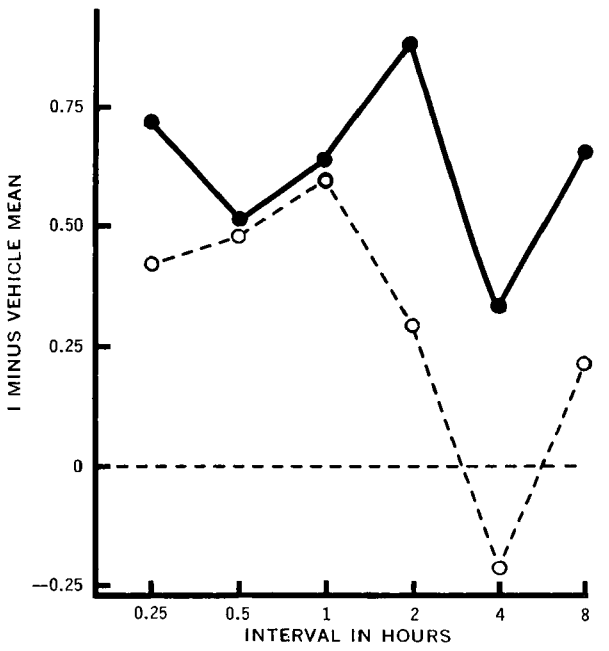

Figure 1—*Difference between the I and vehicle groups in mean latency in seconds to convulsion at intervals of 0.25–8 hr after oral administration of I (20 mg/kg) or its vehicle, showing a comparison between groups with propylene glycol* (●—●) *and polyvinylpyrrolidone* (○- - - -○) *as the vehicle.*

[3] Swiss-Webster, Hilltop Lab Animals, Inc., Scottdale, Pa.

Table II—Effect of I Measured by Mean Difference in Latency between I and Vehicle Groups Shown in Table I

Route of Administration	Vehicle[a]				Average
	Bovine Serum Albumin (5%)	Polysorbate 80 (1%)	Polyvinyl-pyrrolidone (3%)	Propylene Glycol (10%)	
Oral	0.06	0.18	0.47**	0.45**	0.29
Subcutaneous	0.27*	0.18	1.02***	1.64***	0.78
Intraperitoneal	0.13	0.13	1.26***	1.92***	0.86
Intravenous	0.49*	0.84***	1.90***	2.00***	1.31
Average	0.24	0.33	1.16	1.50	0.81

[a] * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

mean convulsive latency at six time intervals after oral administration, comparing two vehicles (polyvinylpyrrolidone and propylene glycol). An analysis of variance revealed a highly reliable increase in latency caused by I, averaging all six time intervals and both vehicles ($F = 50.26$, $df = 1/168$, $p < 0.001$), and a reliably greater increase in latency when I was in the propylene glycol vehicle, averaging all time intervals ($F = 6.46$, $df = 1/168$, $p < 0.05$). Without I, the mean latencies to convulsion were closely similar for the two vehicles (1.70 for propylene glycol and 1.67 for polyvinylpyrrolidone). An analysis of variance showed no statistically significant difference between vehicles ($F = 0.20$, $df = 1/84$). The difference among time intervals approached statistical significance ($F = 1.93$, $df = 5/84$, $p < 0.10$) because at the 4-hr interval, latency was elevated (1.88 sec for the propylene glycol and 1.91 sec for the polyvinylpyrrolidone vehicle). This may account for the diminished effect of I at that time interval (Fig. 1). A more prolonged duration of effect of I with the propylene glycol vehicle was indicated by t tests showing a statistically significant difference ($p < 0.05$) between the I and control groups at each time interval with propylene glycol but only at the first three time intervals with polyvinylpyrrolidone.

DISCUSSION

The present study demonstrates that I in the 5% bovine serum albumin and 1% polysorbate 80 vehicles had weak effects after administration by the intraperitoneal route, as reported previously (1), and also after administration by the oral, subcutaneous, and intravenous routes. Even the intravenous route, which produced a statistically significant effect of I in both vehicles, was much less effective than with the other two vehicles tested.

Borgen and Davis (6) tested the effects of I on operant behavior in rats, with four vehicles and the intraperitoneal and subcutaneous routes of administration. Contrary to the present results, 1% polysorbate 80 was a satisfactory vehicle, although weaker than 10% polyvinylpyrrolidone with the subcutaneous route. They added 100% polysorbate 80 to I, followed by gradual dilution of the fluid with saline[4], similar to the method that was used for preparing a suspension of I in polysorbate 65 (7). This procedure apparently minimized the disadvantage of the insolubility of I in saline; the present procedure, as described previously (1), was to add the 1% polysorbate 80 solution to I. It is evident that the effectiveness of a suspension depends not only on the vehicle but also on the method of preparation, which should be specified in detail by the experimenter.

Other vehicles are also possible. Rosenkrantz *et al.* (8), in a comparison of vehicles, recommended the use of sesame oil with 0.4–1% polysorbate 80 or 4–5% polyvinylpyrrolidone added to it. In the present experiment, 3% polyvinylpyrrolidone and 10% propylene glycol were the most effective vehicles for I. Propylene glycol appeared to be the superior vehicle, because it resulted in a stronger effect of I at 1 hr after administration by most of the four routes (Table I) and in a more prolonged effect on I by the oral route (Table II).

Concentrations of propylene glycol greater than 10% should be avoided, especially by the intravenous route. Table I shows an anticonvulsant effect of this vehicle when administered intravenously. This finding is consistent with a report (9) that 25 and 50% concentrations of propylene glycol, when administered intraperitoneally to mice, significantly elevated the maximal tonic extensor threshold to intravenous infusion of pentylenetetrazol and strychnine. Concentrations of propylene glycol ranging from 20 to 70% did not protect against electroconvulsive shock; however, these investigators did not report on latency to hindlimb tonic extension produced by electroshock, nor did they use concentrations of propylene glycol below 20%. In the present investigations, the vehicle containing 10% propylene glycol likewise did not protect against tonic extension produced by electroshock when administered *via* any route of administration. The results of several pilot experiments[5] showed that propylene glycol in concentrations as low as 30% increased the latency to electroshock when given intraperitoneally or subcutaneously, and a similar effect was observed after oral administration of a 50% propylene glycol solution.

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