or whole blood. The required virucidal dosage of HN2 is below 500 mg/l of blood or plasma if the pH is at or near 7.0. The end products of decomposition of the added HN2 are essentially nontoxic if the pH of the system is not too low. No evidence of antigenic or other toxic reactions has been produced in two dogs and two humans receiving repeated injections of treated plasma. Human red blood cells withstand the application of virucidal dosages of HN2 without hemolysis and with only a slight increase in fragility. Complement, immune bodies, phosphatase and fibrinogen are only slightly affected by sterilizing dosages of HN2. Prothrombin time is markedly prolonged by HN2.

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Timed Intravenous Infusion of Metrazol and Strychnine for Testing Anticonvulsant Drugs.*

MARSHALL J. ORLOFF, HARRY L. WILLIAMS, AND CARL C. PFEIFFER.

From the Department of Pharmacology, University of Illinois College of Medicine, Chicago, Ill.

The validity of the use of metrazol for the evaluation of anticonvulsant drugs has been well established.[1,2] When given to animals, subconvulsant doses of metrazol will elicit electro-encephalographic patterns similar to those found in patients with petit mal.[3] The marked antagonism between metrazol and 3,5,5-trimethyloxazolidine-2,4-dione (Tridione) and the success of this drug in the treatment of petit mal epilepsy further substantiate the validity of the metrazol antagonism test as a method for screening anti-epileptic compounds.

The present metrazol test method employs the subcutaneous route of administration whereby an effective dose of the anticonvulsant is injected into a group of animals, followed, after time is allowed for absorption, by a subcutaneous dose (usually 90 mg/kg) of metrazol known to be convulsant in the non-protected animal. If the animals fail to have seizures, a second group is given a higher dose of metrazol. This procedure is repeated until a dose is attained which produces typical convulsions. In this way the degree of protection (or metrazol antagonism) furnished by a drug in question may be ascertained.

For several years we have used a timed intravenous infusion of metrazol in mice, and have found it to have some distinct advantages over the previous procedure. A similar procedure has been evolved for the measurement of strychnine antagonism by anticonvulsant drugs.

Method. The apparatus employed consists of a cone-shaped mouse holder made of plexiglass and attached to a metal base. The plexi-glass makes it possible to clearly observe the reactions of the animal within the holder. A slit, through which the tail of the mouse is pulled, extends the length of the cone at its top. Attached to the base is a small electric light connected in series through a transformer to a synchronous electrical timer. The synchronous timer is set so that the electric bulb lights every 10 seconds. The reflection of the light is transmitted throughout the transparent holder and thus is readily noted by the observer.

The mouse is pulled into the holder by drawing its tail through the slit in the cone, and the injection is made into a tail vein, using a 27 gauge needle. A 0.5% solution of metrazol or a 0.01% solution of strychnine sulfate is employed in all tests, and injected

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1 Everett, G. M., and Richards, R. K., *J. Pharm. and Exp. Therap.*, 1944, **81**, 402.

2 Goodman, L. S., Toman, J. E. P., and Swinyard, E. A., *Am. J. Med.*, 1946, **1**, 213.

3 Goodman, L. S., Toman, J. E. P., and Swinyard, E. A., *Am. J. Med.*, 1946, **1**, 219.

at the rate of 0.05 cc every 10 seconds.

The reactions of the mouse to the timed intravenous infusion of metrazol follow a definite pattern. Three "signs" of reaction follow each other in strict sequence as the concentration of metrazol is increased in the blood stream. The first of these is the "first twitch," a sharp single twitching of the animal's entire body. This is followed very shortly by a "pseudoconvulsion," a series of clonic movements usually accompanied by an audible squeak. Very often the mouse pulls his head down under his body. The "pseudoconvulsion" is followed by alternate clonic movements and resting phases. The third sign, or "persistent convulsion," appears at the highest concentration of metrazol, and is the final end-point of the injection. The "persistent convulsion" consists of a tonic flexor component followed by a usually lethal tonic extensor component.

The doses of a 0.5% solution of metrazol at which the above-mentioned reactions occur were determined in control animals. Results appear for groups made up of mice weighing 15-17 g, and another series weighing 18-20 g (Table I). It will be noted that the thresholds of the mice weighing 15-17 g are lower, but that the mg/kg dosage is approximately the same.

In determining the efficacy of an anticonvulsant drug, observations were made on the ability of the compound to elevate the "first twitch," "pseudoconvulsion," and "persistent convulsion" thresholds. Also, the ability of a compound to protect the animals from death and to modify the severity of the clonic convulsions was considered.

The intravenous infusion of strychnine was likewise undertaken because of the chemical similarity of phenurone (phenyl acetyl urea) to myanesin (ortho-tolyl glycerol ether) and the fact that clinically a few patients report muscular weakness as a side effect of phenurone therapy.[4] Berger and Bradley[5] have shown that myanesin protects against strychnine convulsions. From our data this test

TABLE I. Intravenous Metrazol Infusion—Mice.

Groups	No. mice	Threshold after treatment			Type of seizure	Mortality, %	Time of death, min.
		1st twitch, cc	Pseudoconvul., cc	Persistent convulsion, cc			
Normal, 15-17 g	40	0.14 ± .027 (1.00)	0.17 ± .037 (1.00)	0.45 ± .104 (1.00)	Tonic, flex.-ext.	100	Immed.
" 18-20 g	20	0.175 ± .026 (1.00)	0.23 ± .061 (1.00)	0.55 ± .181 (1.00)	" "	100	"
Tridione, 500 mg/kg 1 hr	20	0.37 ± .060 (2.64)*	0.44 ± .099 (2.57)	0.86 ± .003 (1.90)	" "	100	"
" 500 mg/kg 1½ hr	20	0.33 ± .058 (2.36)	0.40 ± .097 (2.37)	0.76 ± .109 (1.67)	" "	100	"
Phenurone, 400 mg/kg 1½ hr	20	0.32 ± .037 (2.29)	0.44 ± .067 (2.60)	0.83 ± .108 (1.83)	Clonic	30	82
Phenobarbital, 50 mg/kg 2 hr	20	0.36 ± .050 (2.55)	0.44 ± .062 (2.60)	0.85 ± .080 (1.89)	"	100	7.6
Myanesin, 300 mg/kg 10 min.	20	0.24 ± .043 (1.38)	0.32 ± .054 (1.39)	0.61 ± .088 (1.1)	"	100	27

* = Ratio: Threshold after treatment / Normal threshold of corresponding wt group.

[4] Gibbs, F. A., personal communication.

[5] Berger, F. M., and Bradley, W., *Brit. J. Pharm. and Chem.*, 1946, **1**, 269.

TABLE II.
Intravenous and Subcutaneous Metrazol in Mice.

	Intravenous							
	Control group—80 mice			Tridione 500 mg/kg I.P. 1 hr—20 mice				
	Dose mg/kg	S.D. mg/kg	S.E. mg/kg	Dose mg/kg	S.D. mg/kg	S.E. mg/kg	No. of S.D. above control threshold	Ratio: Tridione/control
First twitch	42	7	.8	111	19	4	9.9	2.6
Pseudoconvul.	51	13	1.4	131	32	7	6.2	2.6
Persistent convul. and death	134	36	4	259	38	8.5	3.5	1.9
	Subcutaneous							
	80 mice			50 mice				
CD-50	55	8	1.7	124	37	6.7	8.6	2.3
CD-95	62			178				2.9
LD-50	99	17	2.7	169	25	5.5	4.1	1.7
LD-95	121			200				1.7

may further elucidate the site of action of potent anticonvulsants.

The apparatus and technique are essentially the same as the metrazol method. A 0.01% solution of strychnine is injected at the rate of 0.05 cc every 10 seconds in all tests, after time has been allowed for absorption of the compound in question. The endpoint is the tonic extension of the animal's hind legs, *i.e.*, the peak of the strychnine convulsion.

Strychnine thresholds were determined on two control groups of mice in which the animals weighed 15-17 g, and 18-20 g respectively (Table III).

Results. The results obtained with 3 anticonvulsant drugs (sodium phenobarbital, tridione, and phenurone) and myanesin illustrate the use of the timed intravenous infusion method. These drugs were injected intraperitoneally at dosage levels which produced slight central nervous system depression. The mice were tested at the peak of drug action (usually 30 to 120 minutes). Degree of threshold elevation (Table I) is expressed as a ratio of the mean of the thresholds of the protected animals to the mean of the thresholds of the appropriate group of control animals of similar weight.

Tridione (500 mg/kg) effectively elevates the metrazol threshold. However, it does not prevent death or modify the "persistent convulsions." Both phenobarbital (50 mg/kg) and phenurone (400 mg/kg) elevate the thresholds significantly. Phenobarbital modifies the "persistent convulsions" into clonic fits but fails to lower mortality. Phenurone modifies the "persistent convulsions" and prevents fatality in 70% of the animals. Myanesin raises the thresholds only slightly when metrazol is infused, but the lethal outcome is delayed.

For purposes of comparison, the results obtained from the application of the subcutaneous metrazol method using tridione at the same dose is presented (Table II). Control experiments were performed and it was found that 85 mg/kg of metrazol produced convulsions in 95% of the mice, severe seizures in 60%, and death in 20%. Metrazol, at a dose of 125 mg/kg subcutaneously, produced severe convulsions and death in all animals tested.

The greatest advantage of the timed intravenous infusion metrazol method is that it makes possible an accurate graded evaluation of anticonvulsant compounds requiring a minimum of time and effort. Since the concentration of metrazol within the animal's blood stream is increased at a constant rate during each *single* trial, a complete picture of the antagonistic properties of a drug may be obtained from *one* series of animals using increments in dosage of 12.5 mg/kg. The subcutaneous method, however, necessitates a

TABLE III.
Intravenous Strychnine Infusion—Mice.

Groups	No. mice	Convulsion, cc	Mortality, %	Time of death
Normals, 15-17 g	60	0.19 ± .044 (1.00)	100	Immediate
" 18-20 g	20	0.21 ± .028 (1.00)	100	"
Tridione, 500 mg/kg 1 hr	40	0.28 ± .052 (1.48)	82.5	20 min.
Phenurone, 400 mg/kg 1½ hr	20	0.28 ± .042 (1.50)	60	28 min.
Phenobarbital, 50 mg/kg 2 hr	20	0.28 ± .033 (1.50)	65	19 min.
Myanesin, 300 mg/kg 10 min.	20	0.36 ± .052 (1.70)	30	42 min.

number of series, each at a different concentration of metrazol; thus, it calls for the use of many more animals and much more time, especially with effective drugs. For example: To test the efficacy of tridione (500 mg/kg) by the timed intravenous infusion method, one group of 20 animals was used. The total procedure (including weighing and injecting the animals) required 3½ hours. Employing the subcutaneous method to test tridione (500 mg/kg), and increasing the dose of metrazol in 25 mg/kg steps for each group of mice, 50 mice were used, 10 at each dosage level. The total procedure required 15 hours when each group was tested separately. Eighty mice were used to determine control responses, LD-50 and CD-50 for statistical analysis of the data for both groups.

The timed intravenous infusion method is as accurate as the subcutaneous method. Table II shows that the protection index for tridione (Ratio: tridione/control) is approximately the same by both methods of testing.

The sensitivity of the intravenous infusion method has been demonstrated frequently in the testing of new anticonvulsant chemicals. With one drug a 30% increase in dosage caused a 36% elevation of the protection index. In another instance a 20% increase in dosage caused the ratio to increase almost 200%.

The Timed Intravenous Infusion of Strychnine. The results obtained from tests made on phenobarbital, tridione, and phenurone are presented here to illustrate the validity of strychnine infusion as a standard laboratory procedure for screening anticonvulsant compounds (Table III). All 3 compounds are effective in elevating the strychnine threshold, phenurone and phenobarbital being slightly more antagonistic than tridione. 82.5% of the mice die in the tridione tests, while phenurone and phenobarbital show greater ability to protect the animals from death. Myanesin is the most effective of all since it affords protection from death in 70% of the animals and produces the greatest rise in thresholds.

Summary. The timed intravenous infusion metrazol test makes possible an accurate, economical, and rapid evaluation of anticonvulsant compounds. It presents distinct advantages over the subcutaneous method because: (1) fewer animals are needed in order to draw a statistically significant conclusion on the drug being evaluated; (2) less time is necessary to complete the testing procedure; and (3) it is equal in accuracy to the subcutaneous method.