the formation of repressor for the biosynthesis of drug-metabolizing enzymes could not be excluded.

On the other hand, Gurtoo *et al.* (11) have recently reported that the treatment with aflatoxin modified the apparent Michaelis constant (K_m) for benzpyrien hydroxylase. Since the alteration of K_m may nullify the inductive effect of phenobarbital in the present investigation, a possible alteration of apparent K_m for aminopyrine N-demethylation and pentobarbital oxidation by aflatoxin should be investigated.

Further studies on the both possibilities are now under investigation for elucidating the mechanism of the induction of drug-metabolizing activities of liver microsomes by phenobarbital and will be published in detail in elsewhere.

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METRAZOL TEST FOR RAPID SCREENING OF ANTICONVULSANTS

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In spite of the availability of several tests no single screening test is capable of picking up all the active anticonvulsant compounds. In routine screening, therefore, one is obliged to include at least one threshold raising test and one pattern modifying test. Antagonism of metrazol induced clonic seizures and supramaximal electroshock seizure by drugs effective in petit mal and grand mal epilepsy respectively have been employed as screening tests discovering new drugs for these disorders. Goodman *et al.* (1) and Chen *et al.* (2) have reported maximal tonic seizure following intravenous administration of metrazol in mice and they (1) have also suggested that anticonvulsant tests utilizing tonic extensor seizure whether evoked by metrazol or electroshock yield similar information. Intravenous administration of gradually increasing doses of metrazol in mice produces clonic convulsions followed by terminal tonic extension and death (8). We observed that metrazol could produce these three stages in mice when given subcutaneously in a dose of 100 mg/kg and diphenylhydantoin blocked the tonic phase of metrazol seizures (3); such observation has been also mentioned by Toman (4) but detailed investigation is not available (5). The present investigation was, therefore, undertaken to find out if anticonvulsants of various types could be picked up by a single metrazol test and whether these anticonvulsants produced differential effects.

Materials and methods: All experiments were conducted on mice of either sex weighing 20–25 g at room temperature $26 \pm 1^\circ\text{C}$. A fixed dose of 100 mg/kg of metrazol was injected subcutaneously in 25 mice to record the pattern of seizures.

The effects of the graded doses of the sodium salts of trimethadione, diphenylhydantoin and phenobarbitone were studied on the responses of metrazol 100 mg/kg, s.c. For comparison activity of the drugs in metrazol seizure threshold (MST), (metrazol 80 mg/kg, s.c.), and supramaximal electroshock seizure (SMES), (48 mA, 0.2 sec), pattern tests were studied according to the method of Swinyard *et al.* (6). Metrazol (100 or 80 mg/kg, s.c.) or electrical challenge was given to the groups of treated mice (10–15 in each group) at the time of the peak effect of the drugs. PD₅₀ (protective dose in 50 per cent of mice) with 95 per cent fiducial limits of these drugs were calculated by probit analysis. Approximate LD₅₀ and TD₅₀ values of these drugs were also obtained.

Results and discussion: Metrazol, in a fixed dose of 100 mg/kg, s.c., consistently produced clonic convulsions followed by terminal tonic extension and death within 15 minutes on an average. A perusal of the results, which has been summarized in Table 1, shows that diphenylhydantoin has blocked only the tonic phase in the metrazol test and has been active in SMES test. It may be noted that its PD₅₀ in the two tests is identical. Trimethadione and phenobarbitone prevent all the phases (clonic, tonic convulsions and death) in metrazol test and are also active in MST and SMES tests. In the case of phenobarbitone also the PD₅₀ values against tonic convulsion in metrazol and SMES tests do not differ significantly. The regression lines of the various drugs for blocking the tonic phase in metrazol test and the activity in SMES test are parallel with a common slope of 6.0 ± 0.1 and thereby suggesting a similar mechanism of action. This is further supported by the fact that PD₅₀ values of diphenylhydantoin and phenobarbitone in metrazol and SMES tests are identical. The compounds effective against clonic convulsions in metrazol and MST tests similarly have parallel regression lines thereby suggesting again a common mechanism of action. To see if diphenylhydantoin could block the other phases of metrazol convulsions in larger doses, very heavy doses (up to LD₅₀) of the drug were administered but they were ineffective. Diphenylhydantoin thus specifically blocked only the tonic phase of the experimentally induced convulsions.

In primary screening, compounds are generally tested for anticonvulsant and other biological activities

TABLE 1. Anticonvulsant activity of phenobarbitone, trimethadione and diphenylhydantoin using supramaximal electroshock seizures (SMES), metrazol and metrazol seizure threshold (MST) tests in mice.

Tests	PD ₅₀ (95% fiducial limits), mg/kg, oral		
	Phenobarbitone sodium	Trimethadione	Diphenylhydantoin sodium
SMES test (48 mA, 0.2 sec)	18.7* (15.2–22.2)	864.0* (727–1022)	12.1* (9.2–14.9)
Metrazol test (100 mg/kg, s.c.)	Clonic 38.2** (33.0–45.6)	571.4** (507–647)	No protection
	Tonic 20.7* (17.2–24.6)	498.0* (436.3–526.0)	12.0* (9.7–14.1)
	Death 26.8	459.4	No protection
MST test (80 mg/kg, s.o.)	25.3*** (18.5–31.6)	465.4*** (356.0–586.0)	No protection
Approx. LD ₅₀ (mg/kg/oral)	300	2500	200
Approx. TD ₅₀ (mg/kg/oral)	50	700	110

* Regression lines for these had a common slope of 6.0 ± 0.1

** Common slope 5.29 ± 1.3

*** Common slope 6.32 ± 1.2

at 1/5 LD₅₀ dose. It is evident from the results contained in Table I that all the drugs, whether used against petit mal or grand mal epilepsy, have shown activity within this dose range and it is unlikely that an active compound would be missed by metrazol test. Mention may also be made that a potent anticonvulsant compound having diphenylhydantoin type of activity has been picked up using metrazol (100 mg/kg, s.c.) test (3). A routine use of the metrazol test for rapid screening of potential anticonvulsant compounds is, therefore, suggested.

Summary and conclusions: Metrazol, 100 mg/kg, s.c., consistently produced clonic convulsions followed by terminal tonic extension and death in mice. Effects of phenobarbitone, trimethadione and diphenylhydantoin on these responses of metrazol have been studied and compared with their activity in metrazol seizure threshold (MST) and supramaximal electroshock seizure (SMES) pattern tests. Diphenylhydantoin specifically blocked the extensor phase of metrazol-induced convulsions while trimethadione and phenobarbitone blocked all the phases. Parallel regression lines with respect to the blockade of the extensor tonic phase of metrazol seizure and SMES, and that of the clonic phase in metrazol and MST tests suggest a common mechanism of action. Further, results indicate that metrazol (100 mg/kg, s.c.) test is sensitive enough to pick up active compounds of either diphenylhydantoin or trimethadione type.

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DOUBLE ISOTOPE DERIVATIVE METHOD FOR THE DETERMINATION OF NORADRENALINE IN RAT BRAIN

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This paper describes the double isotope derivative method for the determination of noradrenaline in brain tissue using *p*-toluenesulfonyl chloride-³⁵S (tosyl chloride-³⁵S). The basis of this method is the label of noradrenaline by esterification with tosyl chloride-³⁵S.

Materials and methods: Tosyl chloride-³⁵S was synthesized and purified in our laboratory (1-3). Noradrenaline in brain tissue was extracted according to the method of Shore and Olin (4). Wistar strain male rats weighing approximately 200 g were sacrificed by decapitation and brains were quickly removed and placed on ice. Approximately one gram of brain was homogenized with two volumes of 0.01 N HCl. The homogenate was transferred into a 100 ml glass-stoppered flask containing 1.5×10^9 dpm of *dl*-noradrenaline-³H (2, 120 mC/mM) and 1.7 g of solid sodium chloride. Fifteen ml of *n*-butanol was added to the flask and shaken continuously for one hour at room temperature. The mixture was centrifuged and a 10 ml aliquot