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THE CONVULSIVE ACTION OF 2,5-DICARBETHOXY 3,4-DIHYDROXYTHIOPHANE (DICETOL)

N.K. DADKAR, S.K. DAMLE AND B.B. GAITONDE

Department of Pharmacology, Grant Medical College, Byculla, Bombay 8, India

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Antimetabolites have been used for some time in the treatment of malignant disorders. Farber *et al.* (1) used folic acid antagonists and, since then, a number of antimetabolites of purines and pyrimidines have been investigated. One of the important drawbacks of all such compounds is their toxicity to host cells due to non-specicity of action. An ideal drug, therefore, would be one which would have a preferential action on tumour cells. This would be possible if tumour cells were shown to have a distinct biochemical pattern different from that of the host cells. Gadekar and Sahasrabudhe (2) have put forward evidence that there is a relative preponderence of the hexose monophosphate (HMP) pathway activity in neoplastic tissue. On the basis of this suggestion, Sahasrabudhe *et al.* (3) have reported 2,5-dicarbethoxy, 3,4-dihydroxythiophane (dicetol) to be effective in blocking the HMP pathway preferentially in tumour tissue. They have also reported anticancer properties for this compound in transplanted fibrosarcoma in Swiss mice (2-4). Since the compound showed promising results in experimental cancer, it was decided to investigate some of its pharmacological actions.

Dicetol was found to produce marked pharmacodynamic effects in experimental animals. Various aspects of this problem are presented in this paper.

MATERIALS AND METHODS

All experiments were carried out with mice (Swiss strain), rats (Wistar strain) and cats. Dicetol in N/250 sodium bicarbonate (pH 8) was administered in varying doses intravenously (i.v.) in mice and rats and the effects were noted. The fluid injected did not exceed 0.2 ml. It was previously ascertained that the same volume of the solvent did not produce any effects in mice. On the basis of the effect seen, the dose causing convulsions in 50% of the animals (CD_{50}) was calculated by the method of Litchfield and Wilcoxon (5). The effects of dicetol on pentobarbital and ether induced sleeping time were also noted. Dicetol was injected in varying doses, i.v., followed 30 minutes later by pentobarbital sodium, 50 mg/kg, intraperitoneally (i.p.). In the ether hypnosis experiments, dicetol was injected i.v. in a dose of 40 mg/kg and 30 minutes later, the animals were placed individually under a beaker of 500 ml capacity and exposed for 2 minutes to 2 ml of ether.

Since dicetol caused convulsions in mice, the effect of reserpine on dicetol induced convulsions was also studied. Reserpine was administered in a dose of 2.5 mg/kg i.p. for two consecutive days and dicetol injected 24 hours after the last dose of reserpine.

Brain acetylcholine: Dicetol was administered to a group of 12 mice in a dose of 80 mg/kg i.v. Picrotoxin was used for comparison and was injected in a dose of 0.25 mg/kg i.p. to another group of 12 mice. Solvent injected animals acted as controls. All four groups of animals were sacrificed by decapitation 5 and 30 minutes after injection and the brain acetylcholine content estimated by the method of Richter and Crossland (6). Assays were performed on pooled samples consisting of the brains of two animals each.

Absorption studies: In order to study the absorption, the drug was given by intragastric tube to a group of 6 rats in a dose of 250 mg/kg dissolved in 4 ml of N/250 sodium bicarbonate. Since the drug was found not to be absorbed, it was decided to study its absorption from the duodenum. Rats were anesthetised with ether or pentobarbital sodium, 50 mg/kg i.p. Six rats were put under ether anesthesia and dicetol introduced into the second part of the duodenum. A control group received solvent. Both treated and control groups were observed for 10 days for any evidence of toxicity. Another group of 12 rats was anesthetized with pentobarbital. Dicetol was introduced into the first and second parts of the duodenum in 6 rats, acting the other 6 as controls. The effect of the drug on sleeping time was observed to assess the degree of absorption, if any. The animals were observed for 4 hours for behavioural changes and then for mortality at the end of 24 hours and again at the end of five days.

In sub-acute toxicity studies, a group of 15 rats received 200 mg/kg of dicetol orally daily. The animals were observed daily for behavioural changes, anorexia and loss of body weight. Hematological examinations were performed every 10 days. The rats were sacrificed in groups of 5 on the 10th, 20th and 30th days and the liver, kidney, brain and intestines were examined histologically.

Studies in anesthetized cats: Since the drug was found to produce convulsions in mice and rats, its effects were studied in 16 anesthetized cats. The cats were anesthetized with ether and maintained on chloralose, 80 mg/kg, i.v. Carotid artery blood pressure was recorded by a mercury manometer, respiration by a Marie's tambour and, in 3 experiments, end respiratory CO_2 was measured by an infrared CO_2 analyser (Godart). Drugs were introduced via the femoral vein. The effects of varying doses of dicetol were observed and compared with those of requisite volumes of N/250 sodium bicarbonate.

In order to establish a central site of action, the drug was administered intracerebrally in cats. In six experiments, a cannula was introduced into the lateral ventricle by the method described by Feldberg and Sherwood (7). To and fro head movements were recorded by means of a vertically fixed thread.

Drug effect was also studied in 3 experiments in which animals were made spinal by the anterior approach described by Zapro and Dipalma (8).

Studies on isolated tissue: The effects of dicetol on a section of the terminal ileum of guinea pig, suspended in oxygenated Tyrode solution at 37°C and on isolated frog rectus abdominis muscle, suspended in oxygenated frog Ringer solution at room temperature were noted. The effect of dicetol on the contractions of guinea pig ileum induced by acetylcholine (10 ng/ml of bath), histamine (20 ng/ml of bath) and barium chloride (10

μg/ml of bath) and those of frog rectus abdominis muscle induced by acetylcholine (0.2 μg/ml of bath) were also studied.

Rabbit's heart, obtained from freshly killed animals (1.5 to 2.0 kg) was perfused with oxygenated Ringer solution at 35°C (9) (Langendorff preparation). The ventricular contractions were recorded with a spring lever, on a smoked drum. The rate and force of contractions were noted and the outflow from the heart was measured to determine coronary flow. Dicetol was injected into the side arm of the aortic cannula. Increasing doses in logarithmic proportion were used.

RESULTS

The convulsant action of dicetol

Intravenous administration of dicetol in mice and rats produced severe clonic seizures. The convulsions came on immediately and invariably resulted in death. Fig. 1 shows the dose response curve obtained in mice using 10 animals per dose. The CD_{50} was found to be 52.0 mg/kg (45.5 to 57; 95% confidence limits).

The effect of dicetol on CNS depressants

There was an effective and specific antagonism between dicetol and pentobarbital. Table 1 indicates very clearly that there was a significant reduction in the pentobarbital sleeping time after the administration of dicetol, 10, 20 and 30 mg/kg, i.v. ($P<0.01$), while there was no effect on ether induced anesthesia (Table 2).

The effect of reserpine on dicetol induced convulsions

Previous reserpinization did not affect

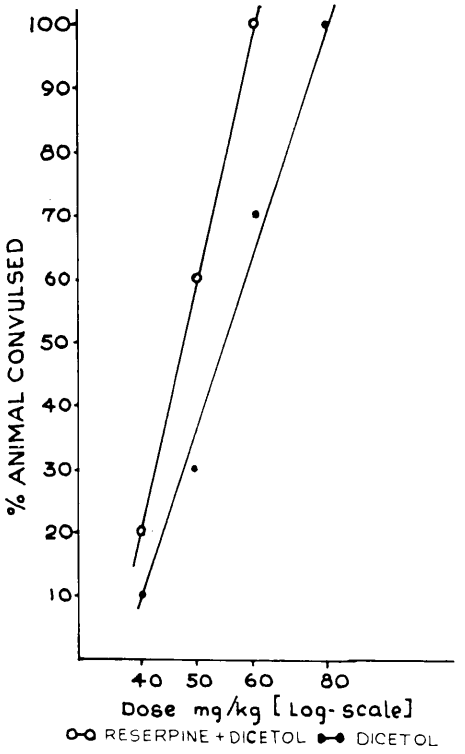

FIG. 1. Dose response relationship for dicetol. Dicetol administered i.v. in normal and reserpinized mice.

TABLE 1. The effect of dicetol on pentobarbital sleeping time in mice. Pentobarbital sodium, 50 mg/kg, i.p., 30 minutes after dicetol.

Treatment	Dose mg/kg, i.v.	Sleeping time (min) Mean ± S.E.
N/250 Sodium bicarbonate	1 ml/100 g	91.0 ± 0.80
Dicetol	10	79.3 ± 0.66*
Dicetol	20	61.6 ± 1.20*
Dicetol	30	34.0 ± 1.00*

Number of animals in each group : 10

* indicates statistically significant, $P<0.01$

TABLE 2. The effect of dicetol on ether induced anesthesia in mice.

Treatment	Dose mg/kg, i.v.	Sleeping time (sec) mean ±S.E.
N/250 Sodium bicarbonate	1 ml/100 g	105 ± 7.1
Dicetol	40	90 ± 9.1

Number of animals in each group : 10

the convulsant action of dicetol significantly. Fig. 1 shows the dose response curves for dicetol in animals with and without reserpine treatment. The CD_{50} for reserpinized animals was found to be 47.5 mg/kg (42 to 52.5; 95% confidence limits). This change in the CD_{50} was not statistically significant.

The effects of dicetol and picrotoxin on brain acetylcholine

Fig. 2 shows the changes in brain acetylcholine content after the administration of dicetol and picrotoxin. Dicetol in a dose of 80 mg/kg produced severe convulsions immediately after administration, which lasted for over 30 minutes. However, there was no change in brain acetylcholine content at 5 and 30 minutes after the administration of dicetol. On the other hand, picrotoxin caused a significant reduction in brain acetylcholine content at the end of 30 minutes ($P<0.001$).

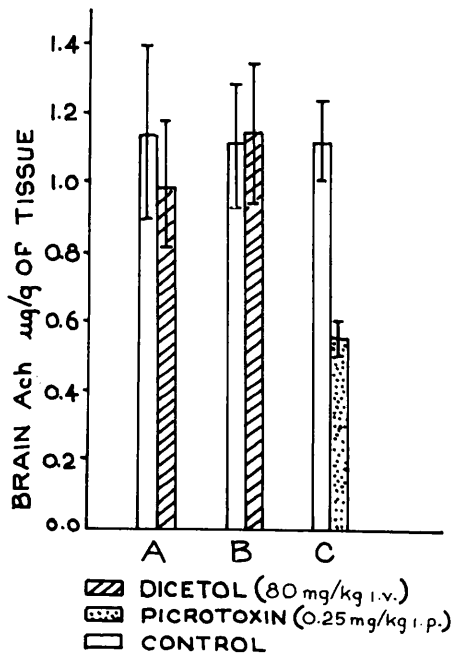

FIG. 2. Brain acetylcholine content in mice.
A : 5 minutes after dicetol, 80 mg/kg, i.v.
B : 30 minutes after dicetol, 80 mg/kg, i.v.
C : 30 minutes after picrotoxin, 0.25 mg/kg, i.p.
Vertical bars indicate standard deviation
Number of samples in each group : 6

The absorption of dicetol

Since dicetol was found to be a powerful convulsant by the intravenous route, the drug was administered orally to rats. In all the animals tested, a dose of 250 mg/kg, i.e., about 5 times the CD_{50} by the intravenous route, failed to produce any effects. Further, in these rats, the pentobarbital sleeping time was not affected. All the animals recovered completely and were normal over the period of observation, viz., 10 days.

Subacute toxicity

Two hundred mg/kg of dicetol administered orally failed to produce any changes in food intake, weight or behavioural patterns in rats. There was no change in the hematological patterns. Histological examination of the liver, intestines, brain and kidney revealed no changes.

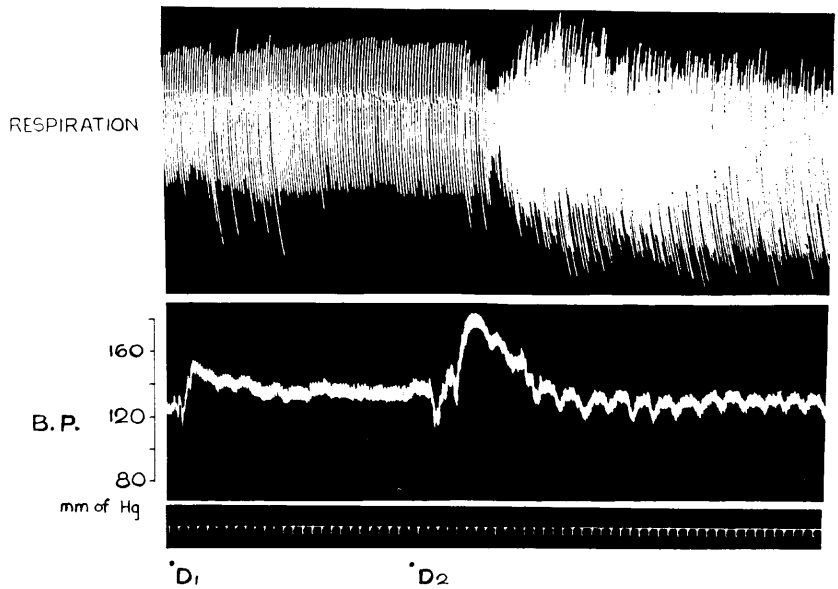

FIG. 3. Effect of dicetol on blood pressure and respiration in cat.
Cat 3.2 kg, anesthesia : Chloralose, 80 mg/kg, i.v.
Upper tracing : Respiration
Lower tracing : Blood pressure
At D_1, dicetol 20 mg/kg, i.v.
At D_2, dicetol 40 mg/kg, i.v.
Time interval : 30 seconds

The effects of dicetol in anesthetized cats

Dicetol produced a sustained rise in the blood pressure in doses ranging from 10 to 40 mg/kg. A dose of 40 mg/kg produced severe clonic convulsions. Associated with the rise in blood pressure, there was a marked tachypnoea, resulting in lowered alveolar pCO_2, as evident from the end expiratory CO_2 measurements. With 40 mg/kg of dicetol, the end expiratory pCO_2 fell from 4.6% to 3%, while with 20 mg/kg, it fell to 3.8%. Fig. 3 illustrates the effects of dicetol on blood pressure and respiration. Dicetol, 20 mg/kg i.v., produced violent head movements (Fig. 4). The effect lasted for a short time and could be reproduced by repeating the injection, thereby showing that there was no tachyphylaxis.

Intracerebroventricular (ICV) administration of dicetol in a dose of 5 mg produced a similar rise in blood pressure, but there were no head movements, as were observed after intravenous administration (Fig. 5). There was marked twitching of the facial muscles as well as the muscles of the neck. The ICV administration of 0.2 ml of the solvent, N/250 sodium bicarbonate, did not produce any effects. It was further noted that the head movements produced by the intravenous administration of dicetol could be terminated by the ICV administration of 400 μg of pentobarbital (Fig. 6).

In spinal animals, the effects of dicetol on the blood pressure were preserved, while the clonic convulsions gave way to those of the tetanic type.

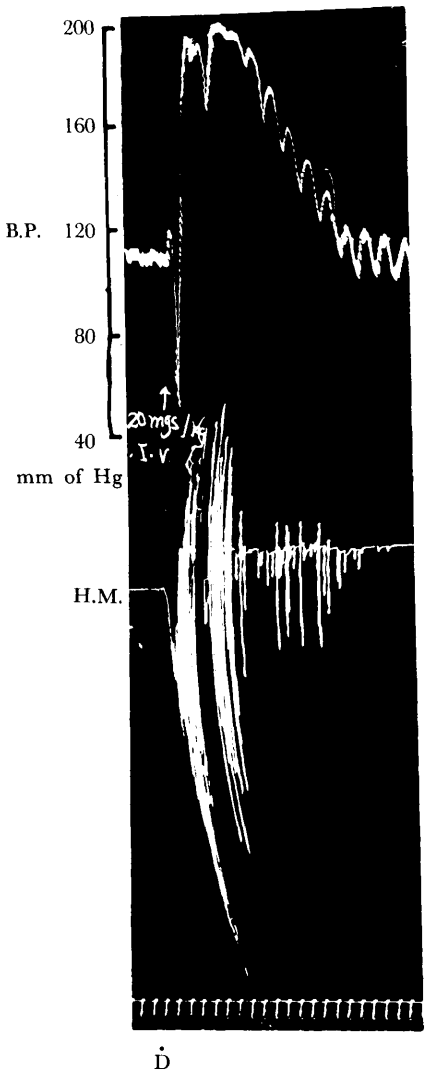

FIG. 4. Dicetol induced head movements in cat.
Cat 3.0 kg, anesthesia : Chloralose, 80 mg/kg, i.v.
Upper tracing : Blood pressure
Lower tracing : Head movements
At D, dicetol 20 mg/kg, i.v.
Time interval : 30 seconds.

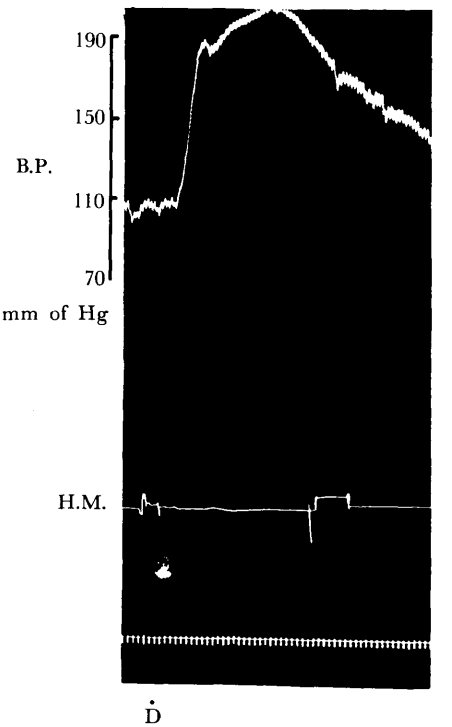

FIG. 5. Effect of ICV dicetol in cat.
Cat 3.3 kg, anesthesia : Chloralose, 80 mg/kg, i.v.
Upper tracing : Blood pressure
Lower tracing : Head movements
At D, dicetol, 5 mg ICV
Time interval : 10 seconds

The effect of dicetol on isolated tissue

Dicetol in a dose of 100 μg/ml of bath failed to produce any action on guinea pig ileum or frog rectus abdominis muscle, nor did it affect the contractions of these preparations induced by acetylcholine, histamine and barium chloride.

However, 400 μg of dicetol produced a marked inhibition of the ventricular contractions of isolated rabbit heart. There was a slowing of the rate and a decrease in the coronary flow. N/250 sodium bicarbonate did not produce any effect (Fig. 7).

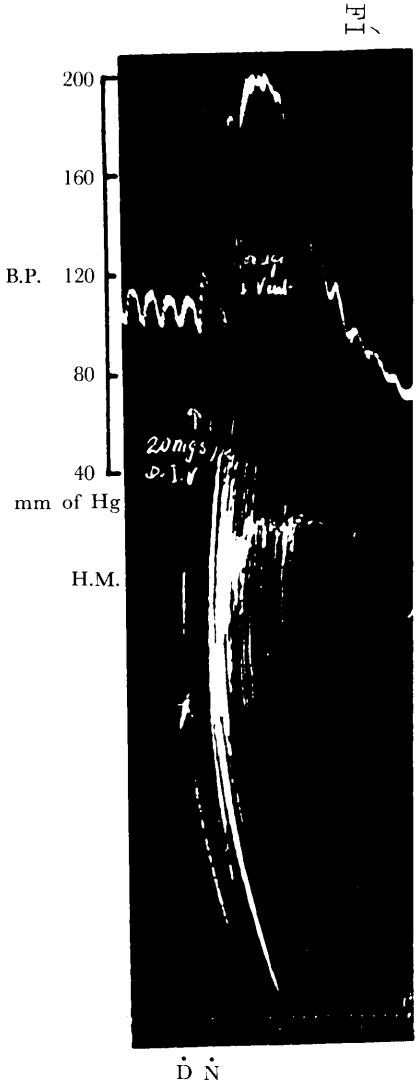

FIG. 6. Antagonism of dicetol and pentobarbital.
Cat 3.0 kg, anesthesia : Chloralose, 80 mg/kg, i.v.
Upper tracing : Blood pressure
Lower tracing : Head movements
At D, dicetol 20 mg/kg, i.v.
At N, pentobarbital sodium, 400 μg ICV
Time interval : 30 seconds

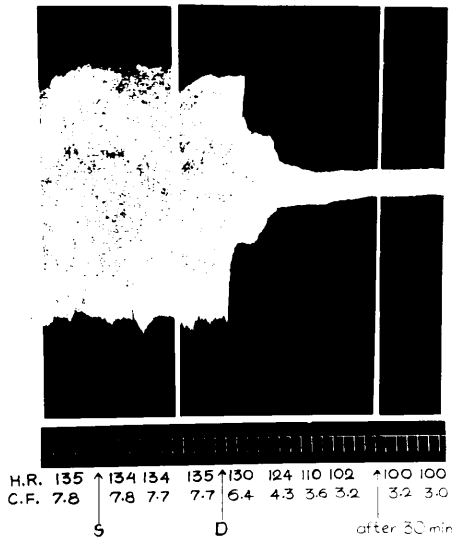

FIG. 7. Effect of dicetol on isolated rabbit heart.
At S, N/250 sodium bicarbonate
At D, dicetol, 400 μg
H.R. : Heart rate per minute
C.F. : Coronary flow, ml per minute
Time interval : 60 seconds

DISCUSSION

Dicetol is a thiophane compound and has been proposed to act as an antimetabolite to 6-phosphogluconic acid (10). 6-Phosphogluconic acid is known to exist as the *gamma* lactone. It is interesting to note, in this connection, that *gamma* butyrolactone has been demonstrated to produce sedation and anesthesia in human volunteers (11).

Dicetol antagonizes the depressant action of barbiturates on the brain. The faiure to counteract ether anesthesia may be of significance. However, in our experiments (unpublished data), we failed to antagonize ether hypnosis by the previous administration of leptazole, 40 mg/kg, i.p. It appears, therefore, that the small changes produced by ether anesthesia are not measurable. Further, in anesthetized cats, the hyperexcitable state induced by the intravenous administration of dicetol is effectively counteracted by the ICV administration

of pentobarbital. The antagonism of dicetol and barbiturate appears, therefore, to occur at the level of the diencephalon, which is the probable site of action of barbiturate in the induction of sleep.

Dicetol produces a heightened excitability of the entire neuraxis, although the primary site of action appears to be rostral. In cats, it produces respiratory stimulation accompanied by a marked fall in the end expiratory pCO_2. In spinal animals, dicetol induces twitching of skeletal muscles and at larger doses, tetanic contractions. However, the effects of dicetol are not potentiated by previous reserpinization, as in the case of other convulsants, like leptazole.

A number of investigators have found that the occurence of convulsions, induced either pharmacologically or electrically, is associated with a lowering of cerebral acetylcholine (12). In the present study, picrotoxin produced a significant lowering of the whole brain acetylcholine content in mice, whereas dicetol failed to produce any alterations. *Gamma* butyrolactone produces an anesthesia like state in animals and is reported to cause an increase in the acetylcholine content of the brain (13). Giarman and Pepeu (14) have shown that iproniazide produces convulsions without lowering brain acetylcholine content. It appears, therefore, that changes in brain acetylcholine content may be a result of many complex biochemical changes in neuronal activity.

Sahasrabudhe *et al.* (3) have proposed that dicetol acts on the HMP pathway of glucose metabolism in cancer cells. However, Nadkarni (15) has reported that dicetol has a more generalized action on glucose metabolism in bacteria. From our results, it is evident that dicetol may have similar generalized actions on the metabolism, especially in the brain, of higher animals.

Dicetol, introduced into the stomach by gavage, or by a direct injection into the small intestine, to bypass the acid medium of the stomach, failed to produce any pharmacological actions. It may be inferred, therefore, that dicetol is not absorbed from the gastro-intestinal tract. This is further evident from the fact that chronic administration of dicetol (200 mg/kg, p.o.) did not produce any behavioural changes nor was there any evidence of histological abnormalities in the tissues examined. This may be the reason for the failure of any observable response in patients with cancer after oral administration of the drug (10).

SUMMARY

1. Dicetol was found to be a potent convulsant in mice, rats and cats, the CD_{50} in mice being 52 mg/kg.

2. The convulsant action was antagonized by barbiturate and was not potentiated by previous reserpinisation.

3. Dicetol failed to influence brain acetylcholine levels.

4. The significance of these findings is discussed.

REFERENCES

1) FARBER, S., DIAMOND, L.K., MERCER, R.D., SYLVESTER, R.F. AND WOLF, J.A.: *New Engl. J. Med.* **238**, 787 (1948)

2) GADEKAR, K.N. AND SAHSRABUDHE, M.B.: *Br. J. Cancer* **15**, 489 (1961)

3) SAHASRABUDHE, M.B., NAARURKAR, M.V., KOTNIS, L.B., TILAK, B.D. AND BHAVSAR, M.B.: *Nature, Lond.* **184**, 201 (1959)

4) SAHASRAUDHE, M.B.: *Ibid.* **182**, 163 (1958)

5) LITCHFIELD, J.T. AND WILCOXON, F.: *J. Pharmac. exp. Ther.* **96**, 99 (1949)

6) RICHTER, D. AND CROSSLAND, J.: *Am. J. Physiol.* **159**, 247 (1949)

7) FELDBERG, W. AND SHERWOOD, S.L.: *J. Physiol.* **123**, 148 (1954)

8) ZAPRO, V.J. AND DIPALMA, J.R.: *J. Pharm. Pharmac.* **16**, 427 (1964)

9) BURN, J.H.: *Practical Pharmacology*, p. 25, Blackwell Scientific Publications, Oxford (1952)

10) SAHASRABUDHE, M.B., NARURKAR, M.V. AND KOTNIS, L.B.: *Acta Union International Contre la Cancer* **20**, 221 (1964)

11) JENNY, E.H., MURPHREE, H.B., GOLDSTEIN, L, AND PFEIFFER, C.C.: *Pharmacologists* **4**, 166 (1962)

12) FELDBERG, W.: *Metabolism of the Nervous System*, Edited by RICHTER, D., p. 493, Pergamon Press, London (1957)

13) GIARMAN, N.J. AND Schmidt, K.: *Br. J. Pharmac. Chemother.* **20**, 563 (1963)

14) GIARMAN, N.J. AND PEPEU, G.: *Ibid.* **19**, 226 (1962)

15) NADKARNI, G.B.: *Biochem. Pharmac.* **13**, 191 (1964)