*Brit. J. Pharmacol.* (1953), **8**, 399.

# HAYATIN METHIODIDE: A NEW CURARIFORM DRUG

BY

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(RECEIVED JUNE 15, 1953)

While investigating the pharmacological properties of an alkaloid " hayatin " from the root of an indigenous plant, *Cissampelos pareira,* Linn., its methiodide* was found to depress respiratory movements and, on further investigation, to be a muscle relaxant. In a preliminary note (Pradhan, Ray, and Varadan, 1952), the curariform property of this compound was briefly reported. It is a colourless, crystalline substance, m.p. 281° C. (decomp.), soluble in warm water and less so in cold water. Its empirical formula is $C_{35}H_{36}O_6N.CH_3I$† (Bhattacharya, Sharma, and Dhar, 1952). In the present paper the curariform activities of hayatin methiodide and of *d*-tubocurarine chloride are compared.

## MATERIAL AND METHODS

Several species of animals were studied. A 1% solution of crystalline hayatin methiodide in distilled water and a 1% solution of *d*-tubocurarine chloride (Burroughs Wellcome and Co.) were used throughout unless otherwise stated.

*Mice.*—Thompson's inclined-screen procedure for bioassay of insulin was adapted for the quantitative evaluation of curariform activity in mice (Thompson, 1946). Hoppe (1950) compared the curariform activity of Win 2747 and *d*-tubocurarine chloride by this method. In the present study, doses of hayatin methiodide, at intervals of 0.05 mg./kg. in a volume of 0.01 ml./g. body weight, were injected subcutaneously to groups of 5 mice. The mice were placed in small stalls, 6 in. × 12 in., on a fine-mesh wire screen inclined at an angle of 50° to the horizontal plane. The dose producing typical skeletal-muscle paralysis in a mouse, and causing it to slide down the screen abruptly within half an hour after injection, was considered to be the effective dose for that animal. The percentage of mice developing paralysis at each dose level was determined. The effective dose (ED50) and its standard error were calculated by Kärber's method (Finney, 1952).

*Rabbits.*—Curariform activity of hayatin methiodide was investigated in rabbits by a quantal assay procedure (Hoppe, 1950). Suitably graded doses of the substance, in a volume of 1.0 ml./kg., were given intravenously, at the rate of 1.0 ml./5 sec., to groups of 10 rabbits. After injection the rabbits were placed in a large enclosure on the floor for detection of head-drop. The response was considered positive when the head dropped forward on the supporting surface and could not be raised in spite of a light tap on the snout of the animal. The percentage of rabbits showing head-drop at each dose level was determined. The head-drop dose (HD50) and the standard error were calculated by Kärber's method. Mean head-drop was determined for hayatin methiodide by using concentrations of 0.025 mg./ml./min. Comparison of *d*-tubocurarine chloride and hayatin methiodide was made by both a quantal assay procedure and a cross-over test.

*Cats and Dogs.*—Sciatic-gastrocnemius preparations were prepared in cats and dogs anaesthetized with quinalbarbitone or sodium phenobarbitone as described by Burn (1950). A piece of the bone into which tendo Achilles is inserted was cut through, and a stout cord tied round the tendon was passed over a pulley to a suitable weight. A light thread, also fastened to the tendon, was connected to a writing lever for recording the individual muscle contractions. The sciatic nerve was dissected, crushed at the proximal end, and stimulated by means of an electronic stimulator giving impulses of 0.001 sec. duration at the rate of 15 per min. After a control period of repeated stimulation, during which a series of contractions of uniform height was recorded, hayatin methiodide was injected intravenously in a suitable dose. When the muscle contraction and respiration had returned to normal, a further dose of the same drug, or of *d*-tubocurarine chloride, was injected. Percentage paralysis in the muscle was calculated from the diminution in the height of contraction from its original level. Muscles were stimulated directly by pulses of 0.005 sec. duration at the rate of 15 per min. Carotid blood pressure and tracheal air pressure were also recorded in these animals, and arrangements were made for artificial respiration when necessary. Diaphragmatic respiration was recorded directly by means of a light thread fixed to the peritoneal aspect of the right hemidiaphragm. Intercostal movement was recorded by a thread tied round the lateral part of the 6th, 7th, or 8th rib.

*Frog Rectus Abdominis.*—The rectus abdominis muscle was dissected from a pithed frog and was put for one hour in Ringer solution containing 1 in

* Isolated and prepared in the Medicinal Chemistry Division of the Central Drug Research Institute. Lucknow.

† Now modified to $C_{35}H_{36}O_6N.2CH_3I$.

500,000 eserine. It was then put in a 50-ml. bath already filled with eserinized Ringer solution. After four minutes this fluid was replaced by ordinary frog-Ringer solution. A dose of hayatin methiodide was added and the effect noted. A buffered solution of acetylcholine (0.005%) was then added to the same bath, 1 ml. every two minutes till the muscle contracted. The muscle was washed with ordinary frog-Ringer solution and the effect of acetylcholine alone was tested on the same muscle. Another dose of hayatin methiodide was then tried, either on the same or on a different muscle.

Toxicity was studied in mice (18–22 g.), rabbits (about 2 kg.), and dogs.

## RESULTS

### *Curariform Activity*

*Mouse Inclined-screen Method.* — Hayatin methiodide, injected subcutaneously into mice, produced restlessness within two to five minutes; the animals gradually became quiet and signs of respiratory embarrassment became apparent. Paralysis of skeletal muscles started in the hind-leg and increased till the mice lost their ability to retain their position on the wire mesh of the screen and abruptly slid down. With increasing doses the interval between the injection of the drug and the onset of paralysis diminished and the duration of paralysis of limb muscles increased. Table I shows the effect of various doses on the onset and duration of paralysis. After a while the animals were able to get up slowly from the lying down position, and after longer intervals they were able to move about with complete recovery from paralysis. Death from high doses, and also from low doses in susceptible individuals, took place from respiratory failure. In such animals twitchings of the limbs, and micturition, occurred.

TABLE I

ONSET AND DURATION OF PARALYSIS AFTER SUBCUTANEOUS INJECTION OF HAYATIN METHIODIDE TO MICE

| Dose (mg./kg.) | No. of Animals Used | No. of Animals Paralysed | Interval between Injection and Onset of Paralysis (min.) | Duration of Paralysis (min.) |
|---|---|---|---|---|
| 0·15 | 5 | 0 | — | 0 |
| 0·20 | 5 | 3 | 9±2·18 | 13±11·42 |
| 0·25 | 5 | 5 | 9±2·40 | 21± 5·47 |
| 0·30 | 5 | 5(a) | 6±1·41 | 39± 4·84 |
| 0·35 | 5 | 5(b) | 6±2·03 | 30± 9·70 |
| 0·40 | 5 | 5(c) | 3±0·90 | |

(a) One died after 43 min.
(b) ,, ,, ,, 14 ,,
(c) All died with paralysis after 23±7·8 min.

The ED50 in mice, after subcutaneous injection, was 0.195±0.0187 mg./kg., which is about half the ED50 for *d*-tubocurarine chloride obtained by Hoppe.

*Rabbit Head-drop Method.*—The head-drop potency (HD50) in rabbits by a quantal assay procedure was 0.0526±0.0009 mg./kg. for hayatin methiodide, and 0.1124±0.0113 mg./kg. for *d*-tubocurarine chloride. Hayatin methiodide thus appeared to be 2.13 times as potent as *d*-tubocurarine chloride (Table VI). The HD50 for *d*-tubocurarine chloride obtained by this procedure agrees with the value of between 0.110 to 0.115 mg./kg. obtained by Collier, Paris, and Woolf (1948). Everett (1948) and Hoppe (1950), however, gave higher values for the HD50—of 0.15 mg./kg. and 0.146 mg./kg. respectively.

The ratio of HD50 to LD50 was 1.34 for hayatin methiodide, as compared with 1.28 for *d*-tubocurarine chloride. After rapid intravenous injection of hayatin methiodide, symptoms similar to those produced by *d*-tubocurarine chloride appeared. At first there was paralysis of the hind legs followed by respiratory depression and marked skeletal muscle incoordination, then slight restlessness; head-drop finally occurred within 1 to 2 minutes. Data showing the duration of head-drop and limb muscle paralysis with different doses of hayatin methiodide are given in Table II. It is evident that the duration of paralysis of neck and limb muscles was prolonged with increase of dose.

TABLE II

DURATION OF HEAD-DROP, AND TIME FOR COMPLETE RECOVERY FROM LIMB PARALYSIS, AFTER INTRAVENOUS ADMINISTRATION OF VARIOUS DOSES OF HAYATIN METHIODIDE TO RABBITS

| Dose (mg./kg.) | No. of Animals Used | Duration of Head-drop (min.) | Duration of Limb Paralysis (min.) |
|---|---|---|---|
| 0·052 | 10 | 1·8±1·88 | 2·3±2·12 |
| 0·054 | 10 | 2·0±1·82 | 4·5±2·31 |
| 0·057 | 10 | 2·1±1·39 | 5·0±2·26 |
| 0·065 | 10 (a) | 4·0±3·34 | 6·34±3·22 |
| 0·070 | 10 (b) | 6·96±2·85 | 8·67±3·39 |
| 0·075 | 10 (c) | 9·04±4·16 | 11·19±4·16 |

(a) 3 died after 8·25±1·95 min.
(b) 6 ,, ,, 7·18±2·06 ,,
(c) 6 ,, ,, 10·15±1·61 ,,

The potencies of these two drugs were also compared in rabbits by a cross-over test. By this method the mean head-drop dose was 0.0840± 0.0176 mg./kg. for hayatin methiodide and 0.1713±0.0364 mg./kg. for *d*-tubocurarine chloride. The standard error of the difference was 0.0283, with "t"=0.79; the difference is thus significant. By this method hayatin methiodide was found to be 2.04 times as active as *d*-tubocurarine chloride. The mean head-drop dose for

hayatin methiodide was found to be much lower than the figure obtained previously (Pradhan *et al.*, 1952), probably because a purer sample of the methiodide was available. Our figure for *d*-tubocurarine chloride appears to be a little higher than that reported by other workers.

The " cumulative " effect of hayatin methiodide, as manifested by the variation in response of the head-drop when the drug was given on successive days, was also studied. Three groups of five rabbits were each given a different dose of the drug (0.050, 0.052, and 0.055 mg./kg.), by rapid intravenous injection, daily for five successive days. The results are given in Table III. It appears from the data that there was no consistent alteration in sensitivity with repeated dosing.

TABLE III

DAY-TO-DAY VARIATION IN HEAD-DROP RESPONSE OF RABBITS TO HAYATIN METHIODIDE GIVEN INTRAVENOUSLY ON 5 SUCCESSIVE DAYS

| Dose (mg./kg.) | Rabbit No. | Head-drop Responses. Day: 1 | 2 | 3 | 4 | 5 | Total No. Head-drops/ Total No. Injections |
|---|---|---|---|---|---|---|---|
| 0·050 | 37 | − | − | − | − | − | 10/25 |
| | 38 | − | + | − | + | + | |
| | 39 | − | + | + | + | − | |
| | 41 | + | − | − | + | + | |
| | 51 | − | + | − | − | − | |
| 0·052 | 42 | − | − | + | + | + | 12/25 |
| | 43 | − | + | − | + | + | |
| | 44 | + | +? | − | + | + | |
| | 45 | + | + | − | − | − | |
| | 47 | − | − | − | − | − | |
| 0·055 | 16 | + | + | + | + | + | 23/25 |
| | 22 | + | + | + | + | + | |
| | 53 | − | +? | + | + | +* | |
| | 55 | + | − | + | + | + | |
| | 56 | + | + | + | + | + | |

* Died afterwards.

*Cat and Dog Nerve-muscle Preparations.*— Though there was considerable individual variation in the relation between the dose of hayatin methiodide and the percentage paralysis of the gastrocnemius, the dose-response curve appeared to be linear ($Y = 37.22 + 136.76\ x$) over the range of doses used. The average time taken for complete recovery of the nerve-muscle preparation was approximately an hour in each instance. There was no evidence to indicate an antagonism between *d*-tubocurarine chloride and hayatin methiodide when both were injected in the same animal at suitable intervals; on the other hand a slight synergistic effect was sometimes manifested.

A comparison of hayatin methiodide and *d*-tubocurarine chloride was carried out, both the drugs being administered to the same animal at suitable intervals. Table IV shows the results.

TABLE IV

COMPARISON OF PARALYSING POTENCY OF HAYATIN METHIODIDE AND *d*-TUBOCURARINE CHLORIDE ADMINISTERED INTRAVENOUSLY TO DOGS

| Dog No. | *d*-Tubocurarine Chloride (mg./kg.) | Hayatin Meth. (mg./kg.) | Max. Twitch Depression (% of Control) | Ratio— Hayatin Methiodide/ *d*-Tubocurarine Chloride |
|---|---|---|---|---|
| 56 | 0·29 | — | 75 | 1·01 |
| | — | 0·29 | 76 | |
| 57 | 0·26 | — | 49 | 1·35 |
| | — | 0·26 | 66 | |
| 58 | 0·33 | — | 93 | 0·97 |
| | — | 0·33 | 90 | |
| 146 | — | 0·10 | 41 | 1·22 |
| | 0·10 | — | 50 | |
| 149 | — | 0·20 | 67 | 1·15 (Average 1·14) |
| | 0·20 | — | 77 | |

Excellent agreement in the potency ratio between hayatin methiodide and *d*-tubocurarine chloride was observed in different animals. The Table shows that by this method hayatin methiodide was approximately 1.14 times as potent as *d*-tubocurarine chloride in dogs.

When hayatin methiodide depressed or even abolished the contraction of the gastrocnemius muscle indirectly stimulated through the nerve, the response to direct electrical stimulation of the same muscle still persisted (Fig. 1a). This was also so with *d*-tubocurarine chloride (Fig. 1b). The amplitude of the muscle contraction to direct stimulation was, however, depressed by about 36% of the control value during complete neuromuscular blockade produced by either drug. This observation tends to show that both hayatin methiodide and *d*-tubocurarine act on the muscle directly as well as on the neuromuscular junction.

The effect of frequency of stimulation on the degree of paralysis in neuromuscular preparations has been stressed. Thus Briscoe (1936) found, with the cat femoral-quadriceps preparation, that though after tubocurarine the contraction increased in magnitude in direct proportion to the frequency of stimulation up to 240 per second, it became merely a brief twitch at the highest frequencies. With cat's sciatic-gastrocnemius preparations hayatin methiodide was found to depress the muscular contraction more with increase of frequency (0.25, 0.5, 1, 2 pulses/sec.) (Fig. 2).

The onset, development, and degree of paralysis in different muscles, after intravenous administration of hayatin methiodide to cats and dogs anaesthetized with a barbiturate, followed the same pattern as is seen with similar doses of *d*-tubocurarine chloride. As with mice and rabbits, the limb muscles were affected first and the respiratory muscles, especially the diaphragm,

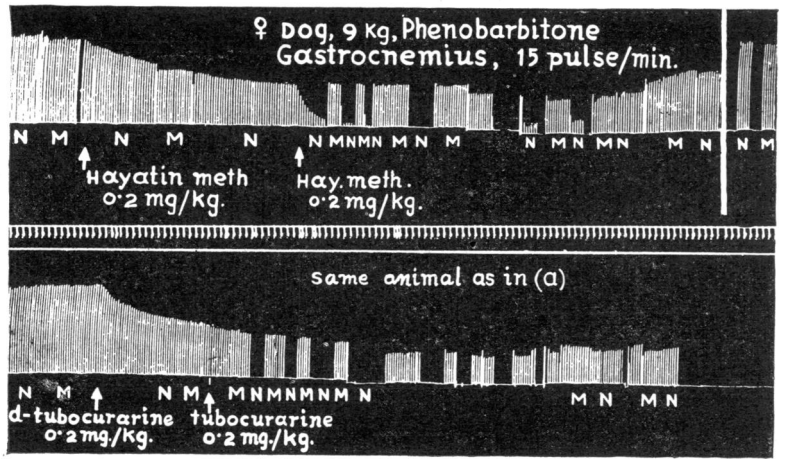



FIG. 1.—Showing depression of gastrocnemius contraction by (a) hayatin methiodide and (b) *d*-tubocurarine chloride, when the muscle is stimulated directly (M) and via the sciatic nerve (N).

last of all. The individual limb muscles also showed different degrees of susceptibility to both drugs. Simultaneous records of gastrocnemius and tibialis contractions, along with the movements of the intercostal muscles and the diaphragm, showed that *d*-tubocurarine chloride, in a dose of 0.2 mg./kg., caused 67% paralysis in the gastrocnemius, 45% in tibialis, with some stimulation of diaphragmatic movement; hayatin methiodide, in the same dose, caused 77% paralysis in both gastrocnemius and tibialis together with very slight depression of intercostal but stimulation of diaphragmatic movement (Fig. 3). Diaphragmatic movements were stimulated to a greater extent at doses which completely paralysed the skeletal muscles—probably as a compensatory measure for the depression of the intercostals. Complete diaphragmatic paralysis could, of course, be obtained with sufficiently high doses.

To find out the margin of safety for this drug, the doses causing complete paralysis of limb muscles and doses causing complete respiratory paralysis were determined. A few cats and dogs were given 0.1 mg./kg. doses every minute, until paralysis of limb muscles and of respiration was obtained. Table V gives the results of such experiments. The ratio between the two doses,

TABLE V

RATIO OF GASTROCNEMIUS AND RESPIRATION PARALYSING DOSES OF HAYATIN METHIODIDE IN CATS AND DOGS

| Experiment No and Animal Used | (1) Dose to Cause Complete Paralysis of Gastrocnemius (mg./kg) | (2) Dose to Cause Complete Paralysis of Respiration (mg /kg.) | Ratio (2)/(1) |
|---|---|---|---|
| 158-dog | 0·5 | 0·8 | 1·6 |
| 159-cat | 0·1 | 0·2 | 2 |
| 162-cat | 0·4 | 0·9 | 2·25 |

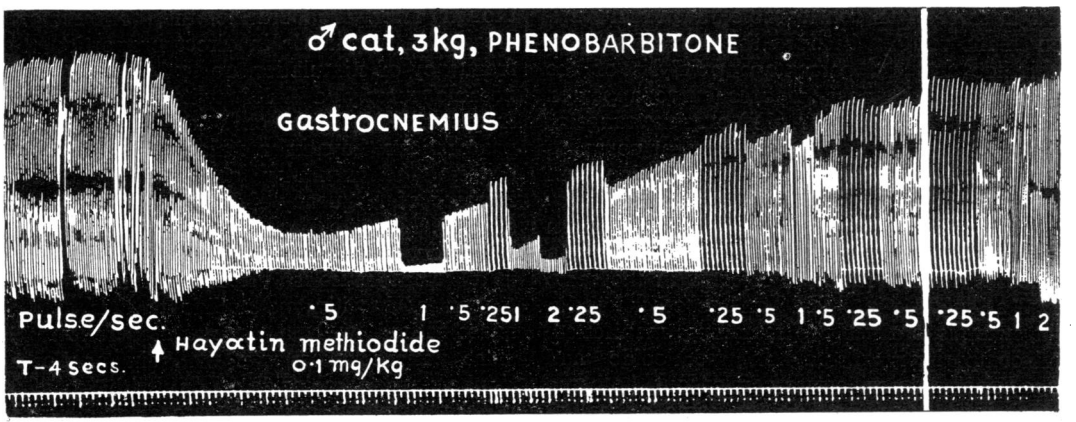
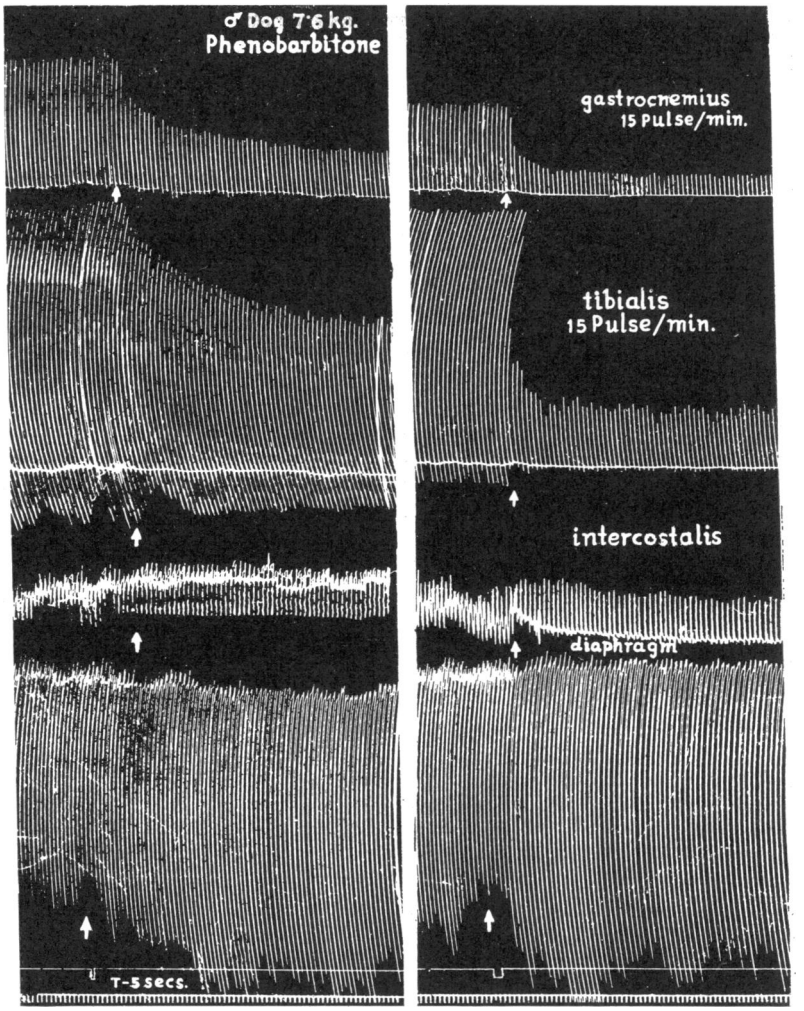



FIG. 2.—Effect of frequency of electrical stimulation on the degree of paralysis of the gastrocnemius muscle produced by hayatin methiodide. The pulse frequencies per second (0.25, 0.5, 1.0, and 2.0) are indicated on the record.

indicating the margin of safety, was about 2. This compares with a corresponding ratio of 1.25 for *d*-tubocurarine chloride and 2.2 for decamethonium iodide (Robson and Keele, 1950).

A cumulative effect of hayatin methiodide was manifested in nerve-muscle preparations of cats and dogs. Some doses of the drug were repeated in the same animal after the amplitude of muscle contraction had returned to its original level. It was found that the second dose produced paralysis of a greater degree and of a longer duration than did the first dose. The initial injection of 0.1 mg./kg. of the drug caused 71% paralysis in the gastrocnemius of a cat, with recovery after nine minutes; repetition of the same dose, however, caused 100% paralysis, with complete recovery after 13 minutes (Fig. 4).




(a) Tubocurarine chloride 0·2 mg./kg. (b) Hayatin methiodide 0·2 mg./kg.

FIG. 3.—To show variation in the response of different muscles to the action of (a) *d*-tubocurarine chloride, and (b) hayatin methiodide. Dose of each drug, 0.2 mg./kg. The two upper rows of tracing show induced contractions in gastrocnemius and tibialis muscles respectively: the two lower the spontaneous movements of intercostal and diaphragmatic muscles.

*Effect of Neostigmine.*—As with *d*-tubocurarine chloride, neostigmine methyl sulphate antagonized the curariform effect of hayatin methiodide. In rabbits, intravenous injection of 0.05 mg./kg. of neostigmine, one minute before the injection of hayatin methiodide, increased the HD50 from 0.0523 mg./kg. to 0.1182 mg./kg. (i.e. 2.25 times), and the LD50 from 0.0703 mg./kg. to 0.1972 mg./kg. (i.e. 2.8 times). With *d*-tubocurarine chloride the corresponding increases in the HD50 and LD50 were 1.7 and 2.1 respectively (Hoppe, 1950).

In dog-nerve muscle preparations also, neostigmine exerted its antagonistic effect on the action of hayatin methiodide. As with *d*-tubocurarine chloride, neostigmine, given in doses of 0.05–0.1 mg./kg. during curarization with hayatin methiodide, caused mainly a shortened duration of, and a more rapid recovery from, the muscular paralysis (Fig. 5). On simultaneous administration of hayatin methiodide and neostigmine, though paralysis was not completely prevented, its degree and duration were markedly diminished. Thus a dose of 0.2 mg./kg. hayatin methiodide produced 67% paralysis of the gastrocnemius muscle of a dog lasting for more than 20 minutes, whereas a combination of 0.2 mg./kg. of the drug with 0.005 mg./kg. of neostigmine produced in the same animal only 28.5% paralysis, complete recovery occurring within six minutes. Another combination, of 0.3 mg./kg. of the drug with 0.08 mg./kg. of neostigmine, caused only 30% paralysis lasting

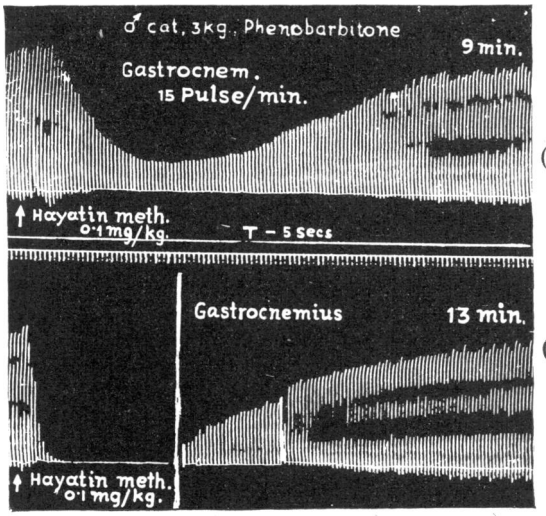



FIG. 4.—To show cumulative action of successive equal doses (0.1 mg./kg.) of hayatin methiodide on the degree and duration of paralysis of the gastrocnemius muscle. Upper record (a) shows effect of first dose; lower record (b) that of second dose. Partial paralysis in (a), with complete recovery in 9 min. Complete paralysis in (b), with recovery in 13 min.

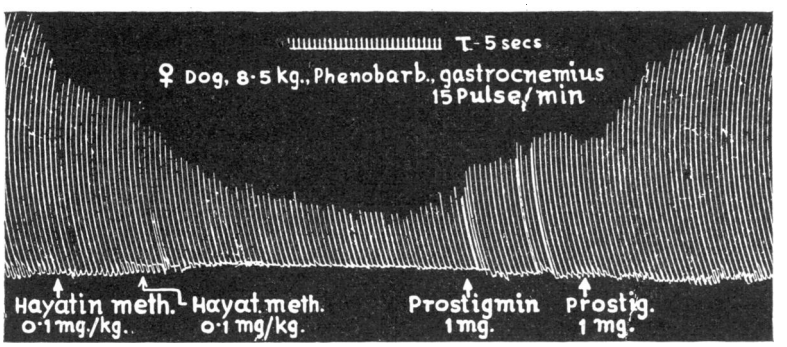



FIG. 5.—To show the antagonism of neostigmine to the curariform action of hayatin methiodide on the gastrocnemius.

for nearly 18 minutes. In another animal hayatin methiodide in a dose of 0.1 mg./kg. caused 60% paralysis of the gastrocnemius muscle, lasting for one hour. When, however, neostigmine, 0.1 mg./kg., was administered two minutes previously the paralysing effect of hayatin methiodide was completely prevented. When the hayatin methiodide was given ten minutes after the neostigmine, only 40% paralysis was obtained and recovery was quick. Neostigmine exerted a marked beneficial effect on respiration when this was seriously affected by hayatin methiodide (Fig. 6).

Previous tetanization antagonized the paralytic effect of hayatin methiodide as it does that of *d*-tubocurarine chloride.

*Frog Rectus Abdominis Muscle and Antagonism to Acetylcholine.*—Hayatin methiodide did not show any effect on the frog rectus abdominis even at a concentration of 1 in 10,000 (1 mg. in 10 ml.). On the other hand 0.2 mg. of hayatin methiodide could prevent the contraction produced by 0.01 mg. of acetylcholine and not more, whereas 0.1 mg. of hayatin methiodide failed to antagonize 0.01 mg. acetylcholine. Thus hayatin methiodide appeared to antagonize the action of acetylcholine on the frog's rectus though it did not itself produce any muscular contraction. In this way its action resembles that of *d*-tubocurarine chloride and not that of decamethonium.

### *Toxicity*

*Acute Toxicity.*—The symptoms were more or less the same in all the species studied. Ataxia of hind limbs, incoordination of skeletal muscles, and respiratory embarrassment were the usual manifestations. When respiration became more affected, twitching or even convulsions occurred and urination followed; the animal finally died of respiratory paralysis usually within 30 to 50 minutes, rarely after an hour. At sublethal dosages, convulsive movements disappeared when respiratory movements were re-established. Salivation was noticed in some of the dogs and rabbits.

Lethal doses of hayatin methiodide in various species of animals are given in Table VI and compared with those of *d*-tubocurarine chloride.

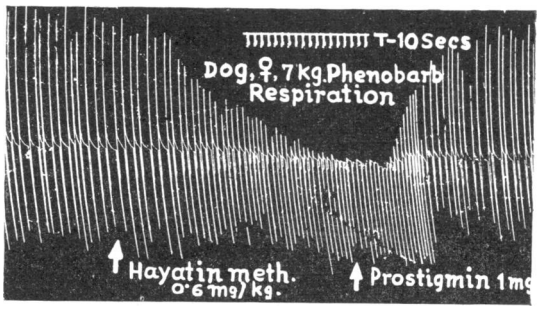



FIG. 6.—To show beneficial effect of neostigmine on respiratory movements depressed by hayatin methiodide.

TABLE VI

COMPARATIVE PHARMACOLOGICAL DATA FOR HAYATIN METHIODIDE AND *d*-TUBOCURARINE CHLORIDE

| Type of Test | Hayatin Methiodide | | *d*-Tubocurarine Chloride | | Relative Activity of Hayatin Methiodide (*d*-Tubocurarine Chloride = 1) |
|---|---|---|---|---|---|
| | No. of Animals | Dose (mg./kg.) | No. of Animals | Dose (mg./kg.) | |
| Subcutaneous ED50 mice | 30 | 0·195 ±0·0187 | 50 | 0·4 ±0·02* | 2·05 |
| ,, LD50 ,, | 30 | 0·355 ±0·0218 | 60 | 0·6 ±0·02* | 1·7 |
| Intravenous LD50 rabbits | 50 | 0·0696±0·0022 | 30 | 0·150 ±0·0122 | 2·15 |
| ,, HD50 ,, | 70 | 0·0526±0·0009 | 30 | 0·1124±0·0113 | 2·13 |
| Intravenous mean head-drop dose in rabbits (by cross-over test) | 10 | 0·0840±0·0176 | 10 | 0·1713±0·0364 | 2·04 |
| Depression of nerve impulse transmission in dogs (Table IV) | | — — | | — — | 1·14 |

* Figures given by Hoppe (1950).

*Chronic Toxicity.*—In rabbits injections of various doses—0.050, 0.052, and 0.055 mg./kg. given for five consecutive days—did not produce any untoward effect or cause significant loss of body weight.

## DISCUSSION

It was reported previously (Pradhan *et al.*, 1952) that hayatin hydrochloride did not show any curariform activity even at 5 mg./kg. doses, whereas its methiodide derivative was active even at a dose of 0.1 mg./kg. The structural formula of hayatin is not known, but the methiodide probably contains a quaternary nitrogen atom and this may be responsible for the curariform activity of the compound. Though this substance works mainly by blocking transmission of nerve impulses at the neuromuscular junction, it has also some direct depressant action on the muscle. A similar property of *d*-tubocurarine chloride has been reported by certain workers (Hoppe, 1950; Acheson, 1948).

The curariform activity of hayatin methiodide seems to resemble that of *d*-tubocurarine chloride more than that of decamethonium. The characteristics are: (i) mice and rabbits show more sensitivity than cats and dogs, (ii) tibialis muscle is not more sensitive than gastrocnemius, being either equally or less sensitive, (iii) *d*-tubocurarine chloride does not antagonize it, but rather potentiates its action, (iv) neostigmine shows definite antagonism, (v) previous tetanization of the sciatic nerve antagonizes the paralysis of the gastrocnemius, (vi) it does not produce contracture on frog's rectus abdominis, but shows antagonism to acetylcholine on this muscle. Hence hayatin may be classified as a pachycurare along with *d*-tubocurarine chloride (Paton, 1951; Bovet, 1951); this might have been assumed from its occurrence in a plant of this genus of Menispermaceae.

## SUMMARY

1. Hayatin methiodide causes paralysis of skeletal muscles in cats and dogs in the same manner as *d*-tubocurarine chloride.

2. In cats and dogs the curariform activity of hayatin methiodide is 1.14 times greater than that of *d*-tubocurarine chloride.

3. On the frog's rectus muscle it antagonizes acetylcholine.

4. In mice hayatin methiodide is 2.05 times as active and 1.7 times as toxic as *d*-tubocurarine chloride when given by subcutaneous injection.

5. In rabbits hayatin methiodide is 2.13 times as active as *d*-tubocurarine chloride by a quantal assay method and 2.04 times as active as the latter by a cross-over test. It is 2.15 times as toxic as *d*-tubocurarine chloride.

6. The margin of safety for both the drugs is very low.

7. The duration of curariform activity for both the compounds seems to be approximately the same.

8. Neostigmine antagonizes the curariform action of both hayatin methiodide and *d*-tubocurarine chloride.

The authors are indebted to Dr. B. Mukerji, Director, Central Drug Research Institute, Lucknow, for his interest in this work, and to Messrs. K. S. Varadan and C. Ray for their technical assistance.

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