TABLE I.—ANALYSIS OF PRODUCTS CONTAINING DEXTROMETHORPHAN HYDROBROMIDE

Dextromethorphan Hydrobromide Product No.	Amt. Claimed	Amt. Found	% Label Claim
1[a]	0.9 Gr./fl. oz.	0.906 Gr./fl. oz.	100.7
2[b]	15 mg./5 ml.	14.93 mg./5 ml.	99.5
3[c]	5 mg./5 ml.	5.18 mg./5 ml.	103.6
4[d]	7.5 mg./5 ml.	7.76 mg./5 ml.	103.5

[a] Marketed as Cheracol D by The Upjohn Co., Kalamazoo Mich. [b] Marketed as Robitussin D M by A. H. Robins, Richmond, Va. [c] Marketed as Thorexin by Isodine Pharmacal Corp., New York, N. Y. [d] Marketed as Actin by Chesebrough-Pond's, Inc., New York, N. Y.

Sample Treatment.—Pipet a sample equivalent to 15 mg. of dextromethorphan hydrobromide into the reservoir, rinse the pipet with distilled water, and add to the reservoir.

Add distilled water to the sample to make the volume approximately 100 ml. and mix well.

Allow the sample solution to flow through the resin bed at the rate of 2–3 ml./min. Wash the column by adding 100 ml. of distilled water and allow it to flow through the resin at the rate of 5 ml./min.

Traces of aromatic amines from flavors or coloring agents are removed by allowing 50 ml. of 0.05 *N* hydrochloric acid in 60% methanol in water to flow through the column at 5 ml./min.

Position a 200-ml. volumetric flask under the column and add 190 ml. of 1.0 *N* hydrochloric acid in 60% methanol in water. Allow this to flow through the column at the rate 3 ml./min. The volume is adjusted with 1.0 *N* hydrochloric acid in 60% methanol in water.

Determination.—The ultraviolet spectrum of the sample effluent and of the working standard is recorded with a suitable spectrophotometer. Absorbance units should be used.

Using the baseline technique determine the absorbance at the maximum at about 278 mμ. Calculate the amount of dextromethorphan hydrobromide present from the standard values obtained at the same time the sample is analyzed

DISCUSSION AND RESULTS

Standard solutions, when subjected to the above procedure, yield an average recovery or accuracy of 99.04% with a standard deviation of ±0.52% based on ten determinations. The data for the analysis of marketed products are shown in Table I.

The analytical grade resin used was found to be satisfactory without pretreatment except for washing with a simple water rinse.

A slightly raised baseline was noted with some products where a small portion of the coloring agent was held and eluted with the sample. This did not cause any problem since the baseline technique was used in the calculations.

Other ingredients present in the various formulations included potassium guaiacolsulfonate, ammonium chloride, tartar emetic, sodium citrate, glyceryl guaiacolate, white pine and wild cherry bark extractives, and various common ingredients in syrups and elixirs.

SUMMARY

A method for the isolation and determination of dextromethorphan has been presented. The procedure has been used successfully on commonly available liquid dosage forms and the results are accurate and reproducible.

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Effect of Red Cedar Chip Bedding on Hexobarbital and Pentobarbital Sleep Time

By HUGH C. FERGUSON*

Male albino mice, in a red cedar chip environment, show a decreased sleep time to sodium hexobarbital and sodium pentobarbital. The effect appears to be reversible.

THROUGHOUT the years various papers have been published citing controllable factors that modify drug action and toxicity in experimental animals. Examples of these are the age of the animal reported by Petty and Karler (1), cage design by Winter and Flataker (2), exercise and limitation of movement by Hardinge and Peterson (3), strain difference by Weaver and Kerley (4), type of anesthetic and pain response by Gutman and Charmovitz (5), grouping and amphetamine toxicity by Chance (6), and volume of fluid administered by Ferguson (7). Thus, by taking advantage of such knowledge, variations in results might be minimized within this laboratory and from laboratory to laboratory.

In the course of a screening program, control results changed abruptly in one of the author's standard tests. Investigation of all possible varia-

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TABLE I.—EFFECT OF BEDDING ON SODIUM HEXOBARBITAL AND SODIUM PENTOBARBITAL SLEEP TIME

Time, hr.	Sodium Hexobarbital Sleep Time ± S.E., min.		Sodium Pentobarbital Sleep Time ± S.E., min.	
	Corncob	Red Cedar Chips	Corncob	Red Cedar Chips
24	32.4 ± 2.7	31.2 ± 2.1	107 ± 8.1	110 ± 9.2
48	34.6 ± 3.1	16.8 ± 1.5	117 ± 9.8	58.3 ± 2.8
72	31.7 ± 2.1	15.9 ± 1.3	105 ± 10.2	62.2 ± 5.8
96	31.1 ± 2.4	14.1 ± 0.9	121 ± 8.7	55.0 ± 2.4
120	34.5 ± 2.3	15.3 ± 1.1	111 ± 11.0	60.0 ± 3.8
144	34.3 ± 1.9	15.0 ± 1.4	109 ± 9.2	57.1 ± 2.8
168	32.8 ± 2.2	16.1 ± 1.5	125 ± 8.7	61.4 ± 4.5

bles showed that the only alteration was in the bedding material employed. Prior to the erratic results, mice were housed in plastic cages with a ground corncob bedding, and this had been changed to red cedar chips. Since further investigation verified the fact that changing the bedding material altered the pharmacologic response, the following study was undertaken.

Similar results were obtained in two different laboratories: Distillation Product Industries, a Division of Eastman Kodak, and the School of Pharmacy, University of Georgia. Two different sources of male albino mice and red cedar chips were employed in these experiments.

MATERIALS AND METHODS

Male albino mice, obtained from Blue Spruce Farms and the National Laboratory Animal Co., weighing 20–25 Gm. were used in all tests. Each animal was used only once and then sacrificed. Upon receipt of a shipment of animals, half were housed in ground corncob bedding and half housed in red cedar chip bedding in a separate room. Animals were allowed a 24-hr. adjustment period before tests were started. The red cedar chips were obtained from a local pet supply store.

Sleep time, for a group of 10 animals, was measured from the time of administration of sodium hexobarbital (100 mg./Kg. i.p.) or sodium pentobarbital (80 mg./Kg. i.p.) until the return of the righting reflex, indicated by two spontaneous righting responses within a 30-sec. period. The average sleep time and the standard error were determined daily for each group of mice, after the 24-hr. adjustment period.

To determine if the effects were reversible, animals that had been housed in red cedar chips for 120 hr. were then changed to the corncob bedding and sleep time again determined daily employing both sodium hexobarbital and sodium pentobarbital.

RESULTS

Table I shows typical results obtained with sodium hexobarbital and sodium pentobarbital in both control group and those housed in red cedar chips. In both cases there is a significant difference between animals housed in the two bedding materials. The sleep time for mice housed in red cedar chips is approximately 50% less than control groups. This difference is evident at 48 hr. and shows no significant change up to 168 hr. Tests run at 24 hr. were not significantly different from control results.

The results in Table II indicate that the effect produced by red cedar chips on sleep time is reversible. An approach to normal is observed sometime between the 24th and 48th hr. after the mice are removed from the red cedar chip environment; thus the change develops with about the same speed as it is lost.

TABLE II.—EFFECT OF SODIUM HEXOBARBITAL AND SODIUM PENTOBARBITAL ON SLEEP TIME AFTER REMOVAL FROM RED CEDAR CHIPS

Time After Removal, hr.	Sleep Time ± S.E., min.	
	Sodium Hexobarbital	Sodium Pentobarbital
0	16.3 ± 1.3	58.8 ± 2.9
24	21.6 ± 1.9	60.8 ± 4.1
48	32.6 ± 3.1	102.6 ± 7.6
72	33.8 ± 3.2	119.0 ± 6.9
96	33.6 ± 3.2	121.0 ± 7.1

DISCUSSION

These results point to another controllable variable that might possibly exist in pharmacologic evaluations. It is conceivable that one might extrapolate to all phases of pharmacologic screening and wonder whether the manner in which these test animals were housed alters other responses and determinations. Further investigation along these lines is in progress, *e.g.*, effect on other classes of drugs and alterations in metabolism and detoxification. Additional experiments are also being carried out to determine if the volatile material in red cedar chips might be responsible for the change reported.

Thus, investigators should be cautious about the use of red cedar chips in animal rooms for the sole purpose of minimizing odors.

SUMMARY

Male albino mice, in a red cedar chip environment, show a decreased sleep time to sodium hexobarbital and sodium pentobarbital. The effect appears to be reversible.

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