showed numerous well defined inclusion bodies of distemper.

Immunity. In tests for immunity, each of 5 animals that survived initial inoculation of the viral mixture was given intravenously, after 30 days, 1 ml of a suspension of single virus, followed in 21 days by a similar inoculation of the other virus. After inoculation, each animal was carefully observed, temperatures taken and leukocyte counts made. None of these animals showed signs of illness from inoculation with either infectious hepatitis virus or distemper virus. This indicated that immunity had developed against both of these diseases from the viral mixture.

Discussion. Following inoculation of infectious canine hepatitis virus and distemper virus simultaneously into dogs, both viruses were present in the blood during the acute phase of illness, inclusion bodies typical of distemper and infectious hepatitis virus were found in dogs that died, and immunity developed against both viruses. The conclusion was reached that dual infection had been produced in dogs.

A comparison of clinical signs shown by dogs that had received the mixture of viruses with those produced by each virus singly showed that signs of illness were more severe and a higher mortality rate was produced by the mixture. This finding may explain certain fatalities heretofore attributed to either distemper or infectious hepatitis, especially in instances where distemper virus is the precedent infection and thereby enhances the effects produced by infectious hepatitis.

The whole subject of dual infections or its converse, interference, presents many interesting aspects(6,7) particularly when related to the subject of immunization. Obviously, combinations of 2 or more viruses that produce dual or multiple infections simultaneously can permit combined vaccination procedures. In the case of infectious hepatitis and distemper, Poppensiek(8) has shown that dogs can be immunized consistently by a mixture of both viruses.

Summary. Following inoculation of distemper virus and infectious canine hepatitis virus singly and mixed, it was found that the mixture of viruses produced a more severe type of illness than either virus alone. In the same dog, both viruses were present in the blood during the acute phase of illness, inclusion bodies of both viruses were found and immunity against both viruses developed.

1. Baker, J. A., Richards, M. G., Brown, A. J., and Rickard, C. G., *Proc. Book, A.V.M.A.,* 1950, 87th Ann. Meet., 242.

2. Carre, H., *Comp. rend. Acad. Sci.,* 1905, v140, 689, 1849.

3. Rubarth, S., *Acta. Path. et Microbiol. Skand,* 1947, v69, 1.

4. Lewis, G., *N. Am. Vet.,* 1950, v31, 743.

5. Brunner, K. T., Scheitlin, M., und Stunzi, H., *Schweiz. Arch. f. Tierheilkde,* 1951, v93, 443.

6. Rivers, T. M., *Viral and Rickettsial Infections of Man,* 1952, Lippincott, Philadelphia.

7. Henle, W., *J. Immunol.,* 1950, v64, 203.

8. Poppensiek, G. C., *Proc. Book, A.V.M.A.,* 1952, 89th Ann. Meet., in press.

Received October 20, 1952. P.S.E.B.M., 1952, v81.

Antitussive Action of *d*-Isomethadone and *d*-Methadone in Dogs. (19912)

CHARLES A. WINTER AND LARS FLATAKER.

From the Merck Institute for Therapeutic Research, Rahway, N. J.

In the management of unproductive or useless cough (tussal insufficiency), potent analgesic drugs are generally employed. Among the active cough suppressants which have been used for this purpose are morphine, ethyl-morphine, codeine, dihydrocodeinone, and methadone. In addition to their antitussive action, these drugs have a general hypnotic effect, they are respiratory depressants, they are often nauseant, they produce constipation, tolerance develops to their use, and above all they possess addiction liabilities.

With the exception of the recent report of Toner and Macko(1), on experiments per-

TABLE I. Suppression of Cough in 11 Dogs by Orally Administered Codeine.

Dog No.	Drug	Dose, mg/kg	No. of coughs/5 min. Before drug Hr 2	Hr 1	Hr 0	After drug Hr .5	Hr 1	Hr 2	Hr 3
1	None (controls)	—	—	—	40	—	47	38	—
2		—	—	—	14	—	10	12	—
3		—	—	—	34	—	55	46	—
4		—	—	—	42	—	47	56	—
5		—	—	—	18	—	29	21	—
6	Codeine phosphate	.25	—	30	28	25	32	28	—
7		.25	—	19	19	18	2	3	14
8		.5	12	16	15	—	3	12	—
9		.5	—	21	19	—	10	13	—
10		.5	28	26	28	9	6	18	—
11		1	17	21	23	0	1	9	24

formed on anesthetized cats, objective evidence is lacking that potent antitussive activity is possessed by compounds having little or no narcotic or analgesic effect. This communication presents such evidence in unanesthetized dogs. It is generally known that the analgesic and narcotic activities of *dl*-isomethadone and *dl*-methadone reside mainly in the *levo* isomer, the *dextro* isomers being relatively free of these effects. We have obtained evidence, however, that *d*-isomethadone and *d*-methadone possess an appreciable degree of antitussive activity.

Materials and methods. For the production of cough, dogs were placed in a wood and glass box about 56 cm square and 42 cm high. Through 2 holes, a fine aerosol mist of N/2 H_2SO_4 was sprayed into the box by means of 2 Vaponephrin nebulizers operated by compressed air at a pressure of about 400 mm Hg. Many animals have been exposed repeatedly without any signs of ill effects. A count of coughs during a 5-minute exposure was obtained by attaching a throat microphone to the animal, the observer listening by means of headphones. Drugs were administered either in dry form in gelatin capsules, or (more usually) in aqueous solution by stomach tube following an 18-hour food fast. In some experiments, drugs were injected subcutaneously. At least 2 control readings an hour apart were taken before administration of drugs, and subsequent counts were made at appropriate intervals after the drug. About one week rest was allowed for each animal between experiments. In no instance has either tolerance or increased sensitivity either to the procedure or to the drugs been observed. This method is a modification of that used in guinea pigs by Eichler and Smiatek(2).

Results. The consistency of the number of counts obtained from an animal not receiving an antitussive agent, and repeatedly exposed in the chamber, is shown in Table I. The data also demonstrate the responsiveness of the preparation to a known antitussive agent. The number of coughs was markedly reduced in one of 2 animals receiving 0.25 mg per kg of codeine phosphate, and in all those receiving higher doses.

The data in Table II show that both *d*-isomethadone and *d*-methadone produced a degree of cough suppression comparable to that produced by codeine, and the effective doses of the former drugs were only slightly higher than those of codeine. We have been unable to demonstrate a significant degree of analgesia or hypnosis when *d*-isomethadone was tested in rats in doses up to 64 mg per kg subcutaneously or 128 mg per kg orally by a method previously described(3). Others have also found that such effects are minimal in *d*-isomethadone and *d*-methadone, although the *levo* isomers are potent analgesics(4-7). Four experiments with l-isomethadone are included in Table II, for purposes of comparison. According to Eddy *et al.*(7), *l*-isomethadone is about 40 times as potent an analgesic as is the *d* isomer. The data in Table II indicate that it is no more than 4 times as potent an antitussive. It is thus clear that the

TABLE II. Suppression of Cough in Dogs by Orally Administered *d*-isomethadone and *d*-methadone.

Dog No.	Drug	Dose, mg/kg	No. of coughs/5 min. Before drug Hr 1	Before drug Hr 0	After drug Hr .5	After drug Hr 1	After drug Hr 2	After drug Hr 3
12	*d*-isomethadone HCl	.5	28	25	16	18	28	—
13		.5	19	24	13	19	26	—
14		1	56	52	13	14	57	—
15		1	18	24	5	16	18	—
16		1	22	34	9	0	18	24
17		1	19	19	5	2	23	17
16		2	31	29	27	11	7	9
18		2	17	17	0	2	0	19
9		2	15	26	23	2	4	3
6		2	14	19	0	0	17	26
19	*d*-methadone HCl	.5	23	15	6	6	12	14
12		.5	28	30	21	25	20	27
16		.5	18	23	19	25	28	—
7		1	14	21	7	5	17	14
18		1	15	19	5	3	11	5
18	*l*-isomethadone HCl	.25	19	17	0	14	21	20
7		.25	26	29	15	19	24	23
17		.5	23	26	0	0	26	20
16		.5	28	31	0	10	13	24

antitussive activity is not dependent upon analgesic or narcotic effects.

An unexpected finding was that *d*-isomethadone is much less effective as an antitussive agent in dogs when administered subcutaneously than when given by mouth. The following experiment was performed: 6 dogs were selected; 2 received each of the following doses subcutaneously: 1, 2, and 10 mg/kg. The 2 dogs on the highest dose showed nearly complete suppression of coughing, but the other 4 showed no effect within 2 hours. The first 2 were then given 2 mg/kg, and the second 2, 1 mg/kg by stomach tube. Two of these 4 responded by complete or nearly complete suppression of coughing, while the other 2 had about 50% suppression within an hour. Two dogs which received 1 mg/kg of codeine phosphate subcutaneously gave only a moderate response. Although we have not made a detailed comparison of the effectiveness of oral and subcutaneous codeine in dogs, the preliminary indication is that codeine also may be more effective by the former route.

Summary. A method is described for testing antitussive activity in dogs. It is shown that 2 compounds nearly devoid of analgesic or narcotic properties, *d*-methadone and *d*-isomethadone, are active in this test.

1. Toner, J. J., and Macko, E., *J. Pharm. Exp. Therap.*, 1952, v106, 246.

2. Eichler, O., and Smiatek, A., *Arch. exp. Path. u. Pharm.*, 1940, v194, 621.

3. Winter, C. A., and Flataker, L., *J. Pharm. Exp. Therap.*, 1950, v98, 305.

4. Scott, C. C., Robins, E. B., and Chen, K. K., *J. Pharm. Exp. Therap.*, 1948, v93, 282.

5. Cahen, R. L., Epstein, H. J., and Krementz, C. S., *J. Pharm. Exp. Therap.*, 1948, v94, 328.

6. Thorp, R. H., *Brit. J. Pharm. and Chemother.*, 1949, v4, 98.

7. Eddy, N. B., Touchberry, C. F., and Lieberman, J. E., *J. Pharm. Exp. Therap.*, 1950, v98, 121.

Received October 20, 1952. P.S.E.B.M., 1952, v81.