that bovine TSH preparations of varying degrees of purity were neutralized by similar amounts of an anti-bovine TSH serum per unit of TSH activity, support this specificity. Qualitative data reported by others have indicated neutralization of the biological activity of both human and bovine TSH preparations by anti-bovine TSH sera(9,10,11). The quantitative data of the present studies, with agreement between two bioassay methods using markedly different indices of response, demonstrated that considerably more anti-bovine TSH serum was required to neutralize the biological effect of human TSH than that of bovine TSH. This fact suggests structural differences in the hormones themselves or their association with other species-specific proteins present in the preparations (*cf.* Dedman, Fawcett, and Morris(12)).

The biological activity of purified bovine TSH and that of an I^{131}-labeled derivative of the same material were neutralized by similar amounts of antiserum. Thus the iodinated preparation maintained its immunologic integrity, as well as its biological activity.

Summary. Two kinds of immunological comparisons between several bovine and human preparations of thyrotropin (TSH), including an I^{131}-labeled bovine preparation, were made with the use of a rabbit antiserum against a purified bovine preparation. Using erythrocytes sensitized with a sample of the immunizing antigen, the inhibition of hemagglutination demonstrated cross-reaction between a crude human TSH preparation and anti-bovine TSH serum, but degree of inhibition was not proportional to biological activity when 3 bovine preparations were compared. Both the *in vivo* mouse I^{131} release and the *in vitro* bovine thyroid slice bioassay showed that the antiserum neutralized TSH. Results from the 2 assays were in agreement. Crude human TSH preparations required considerably more antiserum per unit of biological activity than did either crude or purified bovine TSH preparations. The labeled bovine preparation retained both biological potency and immunological integrity.

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An Antitussive Property of Diphenhydramine in Dogs. (27591)

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The clinical popularity of expectorant mixtures containing diphenhydramine (Benadryl®) hydrochloride has usually been ascribed to this agent's primary antihistaminic action and its secondary sedative, anticholinergic and local anesthetic properties. Examination of the drug for an outright antitussive effect was long delayed, but in 1960 we eventually completed an experiment in dogs to test for this property. Independently and almost simultaneously, H. A. Bickerman (personal communication) has found that 50 mg diphenhydramine hydrochloride orally in man is roughly equivalent to 15 mg codeine

(salt) in suppressing cough induced by citric acid aerosol. Subsequently, we have learned of positive results by 3 further laboratory methods, one unpublished (H. C. Ferguson, personal communication, 1961) and two published(2).

Method. Threshold amounts of anhydrous ammonia required to elicit cough were determined in unanesthetized mongrel dogs after healing from surgical placement of a polyethylene cannula through the ventral wall of the trachea, low in the neck. The cannula and the method of introducing ammonia through it differed in detail from that described earlier(3). During anesthesia, the indwelling end of a polyethylene tube (PE-100; O.D. 0.060 inch) is slipped through a minimal hole drilled diagonally through a tracheal ring, and fastened onto the trachea by means of steel sutures passing between it and a collar of tubing (PE-205; I.D. 0.062 inch) heat-sealed to it.* Optimal protrusion into the tracheal lumen prevents slipping out and avoids erosion of the opposed tracheal wall. The external end is brought through the muscle layer and through a minimal stab incision in the skin adjacent to the main midline incision.

The dog is trained to hang in a sailcloth sling during test, with its feet loosely hobbled, with muzzling for partial restraint of head and neck movements, and with eyes covered to reduce conditioning to the procedure. Air from the house line passes continuously into the cannula through a needle valve and small bubble bottle, at a rate just sufficient to prevent tracheal fluid from creeping into the cannula. A small water manometer on the bubble bottle serves to indicate obstructions. Anhydrous ammonia is released from a small cylinder into a double-walled rubber balloon at essentially atmospheric pressure. From this frequently flushed balloon the ammonia is measured through a 3-way valve into a syringe and then injected at a rate of approximately 0.1 ml/sec. The ammonia enters the air system *via* one branch of a Y of 20-gauge needle stock continuous with the cannula. Connections to the bubble bottle and syringe are made with PE-100 polyethylene tubing. Threshold tussal sensitivities to ammonia were determined to the nearest 0.05 or 0.1 ml every 15 minutes for 2½ hours, by an interval splitting approach. The assigned drug treatment was administered subcutaneously immediately after the third determination.

In each of 10 dogs in independently randomized order, we studied all treatments, at intervals no shorter than 5 days in any one animal. Treatments, contained in 0.1 ml of 0.9% NaCl per kg body weight, were 4 mg codeine phosphate (USP) and 4 and 8 mg diphenhydramine hydrochloride per kg, and vehicle.

In view of evidence that such threshold volumes of ammonia are approximately lognormally distributed(3), we transformed them to their logarithms for analysis.

Results. Analysis of covariance (Table I) of log thresholds after treatment on the average of second and third log thresholds before treatment revealed an expected correlation (*cf.* 3), evident in terms of the *total* 2-hour effects. Uniform adjustment of the mean thresholds at various times after treatment, according to the estimated average linear relationship to thresholds before treatment, enabled plotting the time course of treatment effects from a common origin in Fig. 1.

These temporal courses of treatment effects indicate a decrease in tussal sensitivity with both drugs within 15 minutes after injection. Peak rises in threshold occurred at 30 minutes with codeine phosphate and at 45 and 75 minutes with diphenhydramine hydrochloride. Drug effects were significant and were significantly dose-graded (Table 1).

The relationships of dosage to *peak*,† and to *total* log thresholds (sum of 8 log thresholds after treatment) are shown in Fig. 2. The experimental design provides no test of linearity in dose effect relationship or of parallelism between the two

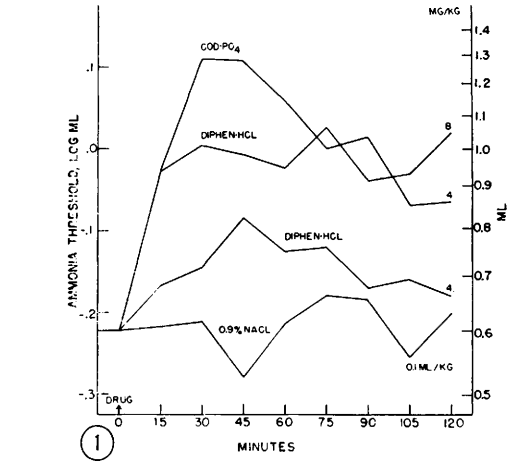
* We are indebted to C. E. Rosiere for preliminary work with this cannula design.

† *Peak* threshold following the particular treatment is the threshold occurring at that period of observation when the log threshold was highest as an average for all 10 dogs.

TABLE I. Analysis of Variance of Log Thresholds.

Source of variation	Peak		2-hr total	
	Degrees of freedom	Variance ratio (F)	Degrees of freedom	Variance ratio (F)*
Dogs	9	7.91†	9	.18
Drugs *vs* NaCl	1	17.21†	1	12.11†
Diphenh. 4 & 8 *vs* cod.	1	5.82‡	1	3.48
Diphenh. 4 *vs* diphenh. 8	1	5.96†	1	4.65‡
Covariance§	—	—	1	4.84‡
Residual	27	1.00 (.0229)‖	26	1.00 (1.0609)‖

* Reduced to accommodate uncertainties in covariance adjustment(4).
† $P \leq .01$.
‡ $.05 > P > .01$.
§ Thresholds after treatment on thresholds before treatment, independently of treatments or dogs ($b = 0.515$).
‖ Actual residual variance.



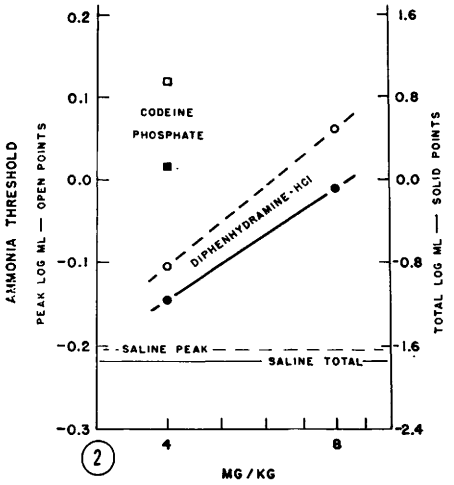


FIG. 1. Mean time-effect curves. All values are adjusted to those expected had all thresholds before treatment been the same (covariance, Table I).

drugs. Tentatively assuming these requirements of comparative bioassay to prevail, one could provisionally compute diphenhydramine hydrochloride to be 0.4 as potent as codeine phosphate (USP) in terms of both peak $(0.02 \text{ to } 0.6)_{95 \text{ per cent}}$ and 2-hour $(0.02 \text{ to } 0.7)_{95 \text{ per cent}}$ effects.

Salivation following ammonia administration was observed in some dogs both before and after drug treatment. We assigned a score of unity to each observation period in which salivation occurred, and analyzed the variance of such scores as well as of their square roots. Use was made of covariance of total salivation after treatment on the average salivation in the second and third periods before treatment, as summarized in Table II. Salivation occurred much more with codeine treatment than with any other treatment. There was no evident effect on salivation by either dose of diphenhydramine hydrochloride.

Discussion. During the past decade several agents possessing local anesthetic and antispasmodic and/or anticholinergic properties were found to be antitussive, and some of these are in use. Diphenhydramine would now seem to be such an agent, possessing, in addition, a primary antihistaminic property.

The mechanism of antitussive effectiveness of such agents is not clear. It seems most dif-

FIG. 2. Dose-*peak* log threshold and dose-*total* log threshold relationships. *Total* log thresholds are adjusted in terms of their significant covariance on log thresholds before treatment (Table I).

TABLE II. Analysis of Variance of Salivation Scores.

Source of variation	Degrees of freedom	Variance ratio (F)* Salivation score	$\sqrt{\text{Salivation score}}$
Dogs	9	2.68†	3.43‡
Codeine *vs* diphenh. & NaCl	1	15.98‡	13.45‡
Diphenh. 4 & 8 *vs* NaCl	1	.34	.79
Diphenh. 4 *vs* diphenh. 8	1	.00	.24
Covariance§	1	17.56‡	8.57‡
Residual	26	1.00 (2.34)‖	1.00 (.4060)‖

* Reduced to accommodate uncertainties in covariance adjustments.
† $.05 > P > .01$.
‡ $P \leq .01$.
§ Salivation after treatment on salivation before treatment, independently of treatments or dogs.
‖ Actual residual variance.

ficult to assess any part played by local anesthetic concentrations of drug in pulmonary tissue, as proposed by Bucher(5) for some such agents. Any substantial role of a peripheral anticholinergic property in the test employed would seem denied by negative antitussive results‡ with the more strongly anticholinergic(7) methyl quaternary derivative. This agrees with the observation that diphenhydramine, itself, failed to influence salivation significantly in the doses used. The antitussive action of most of these agents has been predicted from effects on reflexes arising from stimulation of the superior laryngeal nerve in anesthetized animals, a situation bypassing any peripheral afferent factor. We suppose that the antitussive effect of diphenhydramine may be central in major part.

Summary. Diphenhydramine hydrochloride was estimated to be about 0.4 as potent as codeine phosphate in terms both of peak and of 2-hour total effect of subcutaneous administrations, in raising tussal thresholds to ammonia in unanesthetized dogs.

The authors are grateful to Dr. R. W. Fleming, with his long interest in benzhydryl alkamine ethers, for persistent encouragement to schedule this study.

‡ An antitussive property once inferred for the quaternary compound(6) could not be confirmed even with strongly mydriatic doses, either in terms of the ammonia tussal threshold in dogs (Rosiere and Winder, unpublished work done in 1953) or in terms of amount of coughing in dogs subjected to sulfuric acid mist (C. A. Winter, personal communication in 1953).

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Studies with Tularemia Vaccines in Volunteers.* VI. Assessment of Role of Properdin in Resistance. (27592)

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Recently there has been resurgence of interest in nonspecific resistance to infection (1). The role of properdin in resistance to infectious disease is not clear(2). Previous studies from this laboratory(3-7) have been concerned with clinical and serologic aspects of experimental tularemia in vaccinated and non-vaccinated volunteers after intracutaneous or respiratory challenge. Both Foshay killed (phenolized) and viable attenuated vaccine stimulated production of antibodies, but there was no correlation between inci-

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