THE FAILURE OF BROMAZINE HYDROCHLORIDE TO AFFECT THE OUTPUT AND COMPOSITION OF RESPIRATORY TRACT FLUID

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Respiratory tract fluid is the term applied[1] to a watery fluid which may be collected through a cannula ligated into the trachea of a lightly anæsthetised animal whose inhaled air is warmed to body temperature and saturated with water vapour. This fluid is usually colourless and contains many of the inorganic ions found in blood plasma, protein and non-protein nitrogen, phospholipid, cholesterol, cholesterol esters, neutral fat, some cellular formed elements, mucin and often bits of mucus[2]. Respiratory tract fluid is carried upward to the epiglottis by the action of cilia in the mucosa lining the respiratory tract, and is the natural demulcent of mucous surfaces below the epiglottis, as is saliva above the epiglottis. An established pharmacological action of expectorants, such as ammonium chloride, ipecacuanha and potassium iodide, is their ability to augment the output of demulcent respiratory tract fluid and thus soothe irritation of the respiratory mucosa below the epiglottis, which irritation may otherwise produce cough[3].

Cough mixtures in which an antihistaminic drug is listed as the basis or adjuvant, usually the basis, have been made available commercially to physicians in Canada, Great Britain and the United States of America during the past three years[4]. Various reasons have been advanced for inclusion of such a drug in a mixture designed for the treatment of cough. The local anæsthetic properties of antihistamines have been conceived as, "markedly augmenting the soothing properties of the syrupy vehicle and thereby allaying the cough by abolishing the afferent impulse of the cough reflex," from the pharynx[5]. Mepyramine has been claimed[6] to, "promote decongestion of the respiratory tract—in cases of upper respiratory infection associated with cough." Phenyltoloxamine is claimed[7] to have antitussive properties, "through its local anæsthetic effect." A mixture of prophenpyridamine maleate with ammonium chloride and ephedrine sulphate is claimed[8] to relieve a dry cough by, "lubrication through increased secretions in the tracheobronchial tree."

There are 5 possible mechanisms whereby antihistamines, or any other drugs, can obtund cough[9]. First, they may have a pharyngeal demulcent action and thereby lessen the intensity of tussal stimuli arising from irritation of the respiratory mucosa above the epiglottis. This possibility has been noted in the previous paragraph. Unless the drug is administered in the form of a lozenge or as a frequently used linctus, its possible antitussive activity as a pharyngeal demulcent would be short-lived. Further, the chief purpose of an antitussive pharyngeal

demulcent is to augment the flow of saliva and there is no indication that antihistamines possess this virtue.

Secondly, they might augment the output of demulcent respiratory tract fluid and by this expectorant action lessen or abolish tussal irritation of the respiratory mucosa below the epiglottis. Diphenhydramine hydrochloride[4] and chlorprophenpyridamine maleate[3] have been investigated from this point of view and have been reported to have no effect upon the output and composition of respiratory tract fluid. This does not mean necessarily that all antihistamines are devoid of expectorant action and the study to be reported below was undertaken to investigate the possible expectorant properties of a third antihistamine. There are other possible sites of origin of the cough reflex upon which antihistamines might act but the two noted above are the most common.[9]

Thirdly, they might obtund cough by depressing the medullary cough centre as do opiates and other narcotics. While this possibility cannot be gainsaid, central antitussives are all narcotics and there is no indication as yet that antihistamines have narcotic properties.

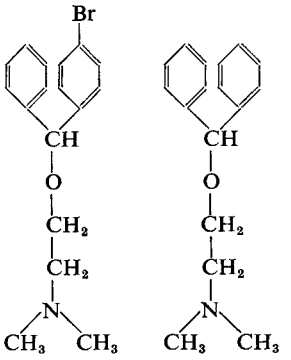
Fourthly, they might depress or suppress cerebral cortical reaction to cough stimuli and to coughing. Undue cortical apprehension makes a person acutely aware of tussal stimuli and accentuates the importance attached to the act of coughing. By their sedative side reaction, antihistamines could very well allay undue apprehension toward cough.

Fifthly and finally, they could depress the motor side of the cough reflex. Hillis and Kelly[10] induced cough by rapid intravenous injection of 5 mg. of α-lobeline hydrochloride, which acts upon the carotid chemoreceptors, and found in one experiment per drug upon one human subject that diphenhydramine hydrochloride, mepyramine maleate and promethazine hydrochloride did not suppress cough. In these experiments, interruption of nerve impulses by use of hexamethonium iodide completely suppressed cough in 10 experiments upon 5 subjects.

The possibility that antihistamines might act as expectorants and augment the output of respiratory tract fluid has been under investigation in this department at Queen's University for 3 years. Effective antitussive agents are needed in Canada where cough is one of the most common complaints in a long winter season. Two antihistamines have been reported previously from these laboratories to have no effect upon the volume output and the physical and chemical properties of respiratory tract fluid, namely diphenhydramine hydrochloride[4] and chlorprophenpyridamine maleate[3]. A third recently made available in Canada, bromazine (ambodryl) hydrochloride, has been found also to be without influence upon the output and properties of respiratory tract fluid.

The chemical structure of this compound, compared with that of diphenhydramine, is shown opposite.

In 1950, Chen, Ensor and Clarke[11] found that substituting methyl, ethyl and halogen atoms in the *para*-position of one of the benzene rings of diphenhydramine, produced compounds with increased antihistaminic potency, as measured by ability to protect against histamine-induced bronchospasm in guinea-pigs or suppression of histamine-induced spasm



Bromazine Diphenhydramine

The chemical structure of bromazine (ambodryl) compared with that of diphenhydramine (benadryl), showing the chemical similarity of the two antihistaminic drugs.

of isolated intestinal strips, or with decreased acute toxicity or with both. The new *p*- substituted compounds bore the same chemical and pharmacological relation to diphenhydramine as does mepyramine maleate, B.P., to tripelennamine hydrochloride, U.S.P., mepyramine (pyrilamine, anthisan, neo-antergan) being *p*-methoxytripelennamine. Of the various *p*-substituted diphenhydramines which Chen, Ensor and Clarke studied[11], bromazine was found, in preliminary trials, to have an insignificant incidence of drowsiness and atropine-like effects in man. A clinical trial of bromazine hydrochloride by McGavack, Shearman, Weissberg, Fuchs, Schulman and Drekter[12] at the Metropolitan Hospital, Welfare Island, New York City, disclosed no untoward reactions from daily doses below 100 mg. and a mean of 6·2 per cent. incidence of drowsiness, dry mouth, diarrhœa and anorexia from daily doses of 75 to 300 mg. Thomas and Kelly[13] reported side reactions in 16 per cent. of patients at Richmond, Virginia, given 10 to 200 mg. daily of bromazine hydrochloride. Later reports in 1952 from these same two clinics[14,15] emphasised that bromazine has antihistaminic properties like those of diphenhydramine but with a much lower incidence of drowsiness and other side effects.

Method

The effect of bromazine hydrochloride was measured upon the output and properties of respiratory tract fluid in cats, rabbits, guinea-pigs and albino rats. The animals were anæsthetised to the lower level of Guedel's Plane I by the intraperitoneal injection of urethane, B.P., in a dose of 1 g./kg. of body weight administered as 4 ml. of a 25 per cent. solution in distilled water. Local and supplementary general anæsthesia were administered as indicated. A T-cannula was ligated into the trachea, the inhaled air warmed to body temperature and saturated with water vapour in an air-conditioning chamber and respiratory tract fluid collected in a graduated, 15 ml. centrifuge tube. The technique was similar to that originally described by Perry and Boyd[1] and as modified by Boyd and

Ronan[16] and by Boyd, Perry and Stevens[17]. The output of respiratory tract fluid was expressed as ml./kg. of body weight per 24 hours.

The usual procedure was to arrange the animal for collection of respiratory tract fluid in the morning and to make half-hourly readings of volume output over a period of 3 hours. Previous experience indicated that this was sufficient time to establish a normal rate of flow of fluid. The bromazine hydrochloride was then administered in the selected dose in terms of mg./kg. of body weight and further half-hourly readings of volume output were recorded for a further period of 4 hours. Some animals survived until the second day and a few lasted to the third day. These were given an hypodermocylsis of saline solution and additional anæsthesia.

Bromazine hydrochloride was administered orally by stomach tube in all experiments except as noted below. The drug is a white, crystalline powder which is readily soluble in water forming a colourless solution with a very bitter taste. Each dose selected for study was dissolved in distilled water to a volume of 1 ml./kg. of body weight and washed out of the stomach tube with a further 1 ml./kg. of distilled water. A group of control animals was given distilled water only and in the same volumes noted. Administration of distilled water had no effect upon the volume output of respiratory tract fluid. The doses of bromazine hydrochloride selected for study were 0·5, 5·0, 25·0 and 100 mg./kg. of body weight. This range covered doses from those corresponding to the human therapeutic dose up to doses which produce toxic effects. The toxic effects of this drug are similar to those of diphenhydramine hydrochloride, toxic doses producing clonic convulsions[21]. In animals anæsthetised with urethane, convulsions did not appear.

The experimental work was distributed over one calendar year which should eliminate the seasonal variation in the output of respiratory tract fluid which is encountered in Canada. All animals used were in healthy condition and were not subjected to experimentation until after they had been housed, fed and carefully inspected over a period of a week or longer by the Curator of the animal quarters of this department at Queen's University. The experiments to be reported were performed upon 50 rabbits, 18 cats, 8 guinea-pigs and 8 albino rats.

One chemical and two physical measurements were made upon the respiratory tract fluid. For these measurements, the output of fluid was pooled over two time intervals. The first sample contained respiratory tract fluid which was collected during the first 3 hours after insertion of the tracheal cannula and before administration of any drug. In Figure 1, readings upon this specimen have been labelled "Before." The second pooled sample is recorded as the "After" specimen in Figure 1 and consisted of the output of fluid over the 4 hours immediately following the pooling of the "Before" sample and after administration of bromazine hydrochloride. Upon each pooled sample of fluid, 3 measurements were made. The specific gravity was determined by the method of Barbour and Hamilton[18] using a falling drop densiometer. The relative viscosity, equating to distilled water equals 1·000, was determined by a modification

of the technique of Poiseuille, as described by Findlay[19], using an Ostwald viscosity pipette. The chloride content was measured by the method of King and Bain[20], by reaction with ammoniacal silver iodate, acidifying, filtering and titrating the soluble iodate iodimetrically.

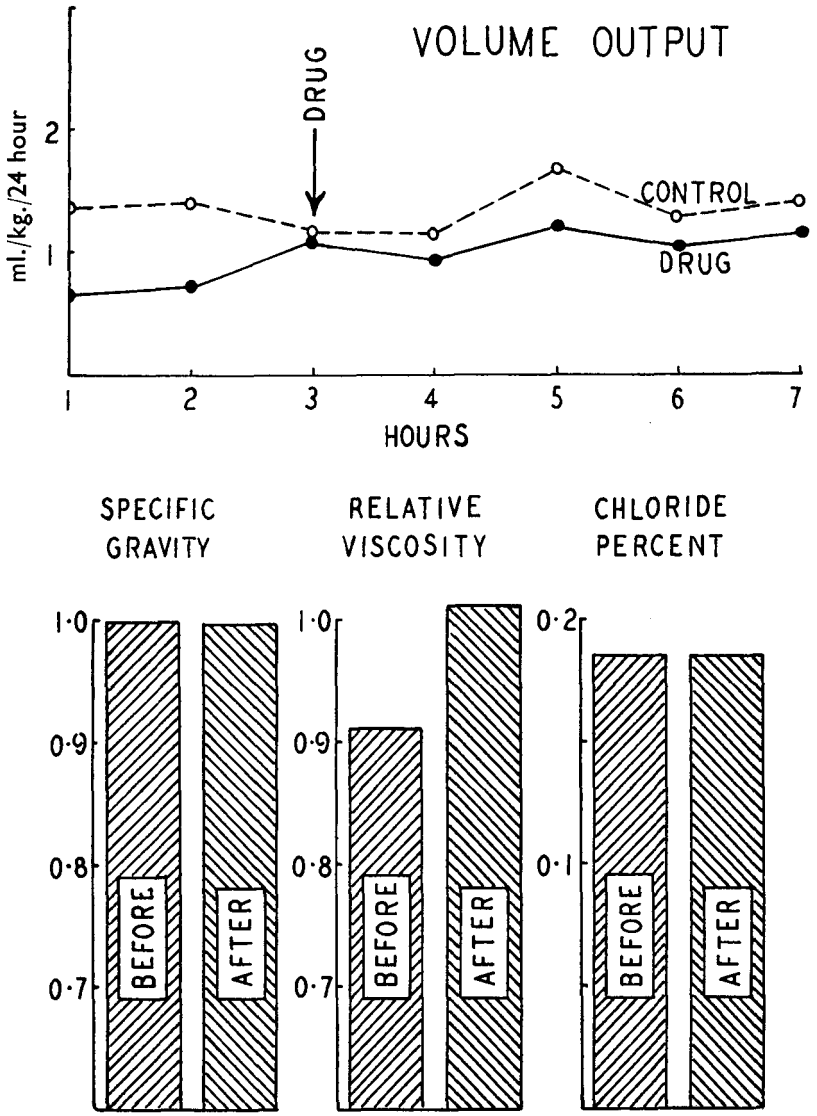


FIG. 1. A chart illustrating the lack of action of bromazine hydrochloride, administered orally to rabbits in doses of from 0·5 to 100 mg./kg. of body weight, upon the volume output, specific gravity, relative viscosity and chloride content of respiratory tract fluid.

RESULTS

The oral administration of bromazine hydrochloride in doses indicated above had no effect upon the volume output, specific gravity, relative viscosity and chloride content of respiratory tract fluid in rabbits, cats, guinea-pigs and albino rats. To emphasise the negative nature of the results obtained and to exemplify the various measurements, the data upon rabbits were averaged and the resultant means have been chartered in Figure 1. The apparent increase in relative viscosity of respiratory tract fluid after administration of bromazine hydrochloride, shown in Figure 1, was insignificant and values of the same order were obtained in control rabbits given no drug.

The bromazine hydrochloride was administered by mouth because this is the usual route of administration in man when antihistaminic therapy is used for cough. Also, if there were any possibility of a reflex expectorant action from the stomach, it would appear following the oral route of administration. Because of the local anæsthetic action of many antihistaminic drugs, it did not appear likely that there would be any gastric reflex stimulation of the output of respiratory tract fluid—and actually none was obtained. Further, parenteral administration of bromazine hydrochloride slowly produces irritation and ulcer formation at the site of injection if sufficient of the drug be given[21]. While antihistamines are readily absorbed from the gastro-intestinal tract, it was deemed advisable to try the parenteral route of administration. Hence, bromazine hydrochloride was administered subcutaneously to 8 rabbits in doses of 5 and 25 mg./kg. of body weight. There followed no change in the volume output nor in the measured physical and chemical properties of respiratory tract fluid in these eight rabbits.

It might be argued that since bromazine is an histamine-antagonising agent, its effect should be tried against an action of histamine. Hence, histamine acid phosphate was administered subcutaneously in doses of 0·01, 0·1 and 1·0 mg./kg. of body weight to cats and rabbits arranged for the collection of respiratory tract fluid. Histamine was found to have no effect upon the volume output of respiratory tract fluid, nor upon its specific gravity, relative viscosity and chloride content.

As noted above, bromazine hydrochloride has some atropine-like action, though to a lesser extent than has diphenhydramine. The possibility was considered that bromazine hydrochloride might affect the output of respiratory tract fluid previously increased by administration of cholinergic drugs. From the studies of Boyd and Lapp[22], methacholine chloride was selected as a suitable parasympathomimetic agent. It was administered by stomach tube in a dose of 10 mg./kg. of body weight to 3 rabbits arranged for the collection of respiratory tract fluid. An hour and a half later, when the volume output of fluid was increased two- to threefold, bromazine hydrochloride was given by stomach tube in doses of 25 and 50mg./kg. of body weight. The 25 mg./kg. dose had no effect and the 50 mg./kg. dose had but a slight atropine-like action in lessening the output of fluid augmented by methacholine chloride. The specific

gravity, relative viscosity and chloride content of respiratory tract fluid were again unaffected by administration of bromazine hydrochloride.

Summary

1. Bromazine (ambodryl) hydrochloride administered in doses of from 0·5 to 100 mg./kg. of body weight to rabbits, cats, guinea pigs and albino rats, had no effect upon the volume output, specific gravity, relative viscosity and chloride content of respiratory tract fluid.

2. The relation of this finding to the pharmacological actions and therapeutic uses of antihistaminic drugs in the treatment of cough has been discussed.

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