Thorax (1970), **25**, 737.

Clinical and physiological evaluation of mucolytic agents nebulized with isoproterenol: 10% N-acetylcysteine versus 10% 2-mercaptoethane sulphonate

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In a controlled five-week cross-over study in 12 patients with chronic bronchitis, the effects of nebulized 10% N-acetylcysteine plus isoproterenol were compared with a new drug, sodium 2-mercaptoethane sulphonate, in 10% concentration also nebulized with isoproterenol. Both combinations were compared with a control aerosol of saline and a second control aerosol of saline plus isoproterenol. Both 10% 2-mercaptoethane sulphonate and 10% N-acetylcysteine were highly effective in thinning mucoid, mucopurulent or purulent sputum. Subjective improvement in these patients, however, was related to the isoproterenol rather than to the mucolytic agents. When nebulized with isoproterenol, neither mucolytic agent was associated with bronchospasm in any patient. Physical findings, spirometric values, and sputum volume were unaffected by these mucolytic agents.

One of the major problems in the treatment of patients with acute or chronic pulmonary disease is the management of thick, tenacious secretions. In recent years a number of agents designed to liquefy bronchopulmonary secretions have been introduced. In order to evaluate the effectiveness of such agents in an objective manner, we have developed an instrument, now termed the Fluid Consisto-viscosimeter (Hirsch and Kory, 1967; Hirsch, Kory, and Hamilton, 1966), which is capable of measuring the consistency (apparent viscosity) of heterogeneous substances such as sputum.

Using this instrument, we have shown in a short-term clinical study that nebulization of 10% N-acetylsteine (N-ac) was as effective as 20% N-ac in thinning sputum. More recently, in a seven-week controlled clinical study (Kory, Hirsch, and Giraldo, 1968), we demonstrated the sputum-thinning effectiveness of 20% N-ac nebulized with racemic epinephrine. Following these reports we received a number of enquiries as to the effectiveness of 10% N-ac nebulized with isoproterenol (Iso).

In our most recent study, we described a new method for the *in vitro* measurement of the sputum-liquefying efficacy of drugs, and have applied the method to the evaluation of several new agents (Hirsch, Zastrow, and Kory, 1969). One of these agents, the sodium salt of 2-mercaptoethane sulphonic acid (MES), appeared to be at least as potent as N-ac but had a less disagreeable odour.

The present study was designed, therefore, to determine: (*a*) whether 10% N-ac + Iso is effective in liquefying sputum; (*b*) the comparative effectiveness of 10% MES + Iso in thinning sputum; (*c*) the effect of saline + Iso on sputum consistency as compared to the above regimens; and (*d*) the clinical and physiological responses of patients with chronic bronchitis to the above regimens in a controlled study.

MATERIALS AND METHODS

Twelve patients with relatively stable chronic airways obstruction and productive coughs were admitted to the Emphysema Study Unit of Wood Veterans' Administration Hospital. All the patients had airway obstruction of varying severity and reversibility, and one also had a history of chronic bronchial asthma. Before entering the study, each patient was given appropriate therapy with oral bronchodilators, expectorants and, when necessary, antibiotics in order to achieve clinical stability. The therapeutic regimen for each patient, once established, was maintained with virtually no change throughout the study.

TABLE I

PLAN OF STUDY OF NEBULIZED DRUGS

Week	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
1	Sal	Sal	Sal	Sal	Sal	Sal
2	Sal+Iso	N-ac+Iso	MES+Iso	MES+Iso	Sal+Iso	N-ac+Iso
3	N-ac+Iso	MES+Iso	Sal+Iso	N-ac+Iso	MES+Iso	Sal+Iso
4	MES+Iso	Sal+Iso	N-ac+Iso	Sal+Iso	N-ac+Iso	MES+Iso
5	Sal	Sal	Sal	Sal	Sal	Sal

Sal=3·0 ml. of isotonic saline. Sal+Iso=2·5 ml. of isotonic saline plus 0·8 ml. of 1:200 isoproterenol.
N-ac+Iso=2·5 ml. of 10% N-acetylcysteine plus 0·8 ml. of 1:200 isoproterenol.
MES+Iso=2·5 ml. of 10% 2-mercaptoethane sulphonate plus 0·8 ml. of 1:200 isoproterenol.

This investigation was designed to reduce the effect of the natural fluctuations of the disease upon results of the study and the effects of one regimen upon another. The plan of study is outlined in Table I. The patients were divided into six groups of two patients each. The first and last (fifth) week of the study were control periods for all of the six groups during which the patients received nebulized isotonic saline. During the second, third, and fourth weeks each group rotated through the three treatment regimens in a different order. All agents were administered by nebulization with a Mark VII Bird IPPB respirator at 8 a.m., 12 noon, and 6 p.m. All sputum was collected during the control and treatment periods from the beginning of one IPPB nebulization to the beginning of the next. These specimens were designated the 'morning', 'afternoon', and 'overnight' collections.

Each specimen was visually assessed as to its type (mucoid, mucopurulent, or purulent) and placed in a freezer at −20° C. immediately thereafter. At a convenient time the sputum was thawed, and its volume and consistency were measured in the Fluid Consisto-viscosimeter. This instrument was developed in our laboratories and has been described in detail elsewhere (Hirsch and Kory, 1967; Hirsch *et al.*, 1966; Kory *et al.*, 1968). The principal part of the unit is a 10-ml. stainless steel syringe with a hollow plunger carefully machined to the syringe barrel (Fig. 1). At the lower end of the plunger is a stainless steel disc, 1/32 in. (0·8 mm.) thick, which is held in place against the bottom of the plunger by a thin rod. This rod extends up through the hollow plunger and is secured by a knurled nut. Symmetrically arranged on the disc are eight holes, each 1·6 mm. in diameter. Threaded into the bottom end of the syringe barrel is a Statham P-23D strain gauge pressure transducer secured tightly in place with the aid of O-ring seals. This assembly is mounted on a modified Harvard infusion pump which is placed upright on a box containing the controls for the instrument. The tracing is recorded on a

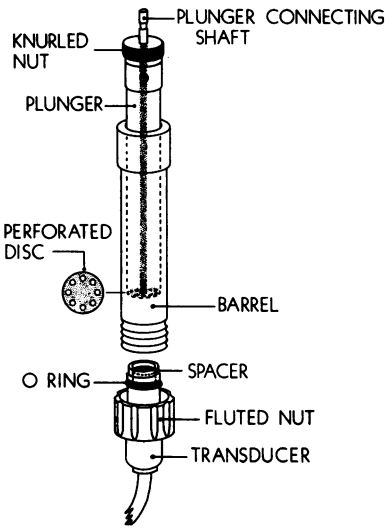

FIG. 1. *Diagram of the syringe assembly showing the hollow plunger with the perforated disc at the bottom, the barrel and the pressure transducer.*

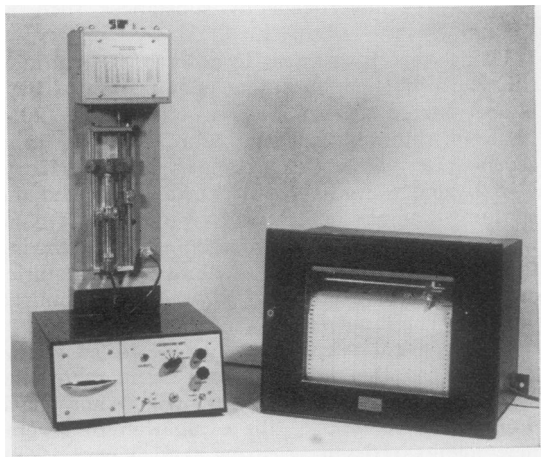

FIG. 2. *The Fluid Consisto-viscosimeter and the recorder.*

1-mV potentiometric strip chart recorder (Fig. 2).

The sputum is placed in the barrel. The plunger, with the perforated disc at the bottom, is driven down through the sputum by the pump at a constant velocity (4·12 ml./min.), thereby developing a pressure directly proportional to the thickness (consistency) of the specimen. Figure 3

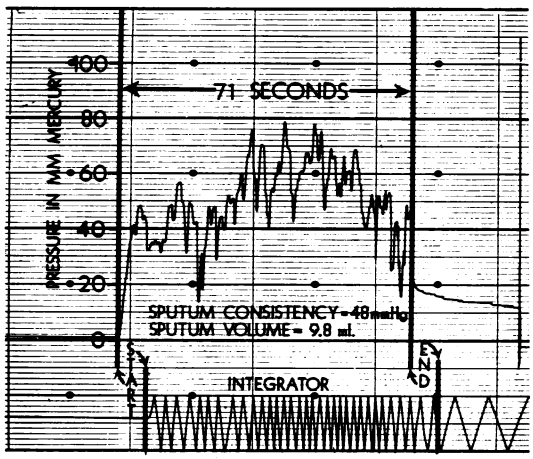

FIG. 3. *A typical record from a sputum consistency measurement. The heavy vertical lines mark the beginning and the end of the traversal of the plunger through the sputum. The upper tracing is a continuous record of the pressure while the lower tracing integrates the area under the upper curve, thereby permitting calculation of the mean pressure in millimetres of mercury. The time interval between the 'start' and the 'end' of the tracing is used with volume factor for the barrel and the velocity factor for the plunger to provide an accurate measurement of the sputum volume.*

is a typical record from a sputum consistency measurement. In order to relate consistency values to viscosity units in general use we have calibrated the instrument with a series of five Dow-Corning 210 silicones. When the pressure in millimetres of mercury is plotted against viscosity in centistokes of the five silicones, the striking linearity permits the establishment of the relationship of 1 mm. Hg = 1,500 centistokes. Pressure measurements alone are meaningful only for instruments with barrel and plunger dimensions and plunger velocity identical to those of our own instrument. For this reason, we have established 'consistency units' to relate our measurements to conventional viscosity units. One consistency unit is equivalent to 1,500 centistokes. The thicker the sputum, the larger the number of consistency units recorded.

The forced vital capacity test was performed on the third and seventh days of each week of the study just before the mid-day IPPB treatment, and again 30 minutes thereafter. Measurements from the forced expiratory spirogram (Kory, Rankin and Snider, 1963) included the forced vital capacity (FVC), the one-second forced expiratory volume ($FEV_{1\cdot0}$), the 200–1,200 ml. forced expiratory flow rate ($FEF_{200\text{-}1,200}$), and the forced mid-flow (FMF).

Observations of the patient's clinical course and side-effects were recorded daily.

RESULTS

Comparison of the treatment and the control periods involve four categories: sputum consistency; sputum volume; spirometric measurements; and the patient's clinical course.

SPUTUM CONSISTENCY The method of evaluation of sputum consistency is illustrated by Table II which shows all the sputum-consistency values for one patient (W.S.) for the entire 35 days of

TABLE II

SPUTUM CONSISTENCY IN CONSISTENCY UNITS—PATIENT W.S.

Day	Week 1	2	3	4	5
	Saline Control	MES + Iso	Sal + Iso	N-ac + Iso	Saline Control
	Morning specimen				
1	104	42	102	30	90
2	120	26	83	38	144
3	132	15	105	50	88
4	120	26	82	22	111
5	62	8	80	4	136
6	132	8	102	10	94
7	104	9	74	8	112
Weekly mean	110	19	90	23	110
	Afternoon specimen				
1	121	22	86	26	96
2	82	3	95	18	132
3	90	12	83	19	91
4	118	14	67	24	52
5	89	2	70	7	107
6	88	0	94	4	108
7	87	6	68	4	137
Weekly mean	96	8	80	14	103
	Overnight specimen				
1	102	48	112	94	96
2	132	44	132	78	93
3	139	45	121	66	102
4	119	86	142	78	84
5	104	75	100	64	126
6	126	104	107	98	125
7	72	70	96	83	130
Weekly mean	113	67	116	80	108

For abbreviations see footnotes to Table I.

TABLE III

WEEKLY MEAN SPUTUM CONSISTENCY IN CONSISTENCY UNITS—ALL PATIENTS

Patient	Morning					Afternoon					Overnight				
	Sal	Sal + Iso	MES + Iso	N-ac + Iso	Sal	Sal	Sal + Iso	MES + Iso	N-ac + Iso	Sal	Sal	Sal + Iso	MES + Iso	N-ac + Iso	Sal
Group 1															
B.B.	61	86	24	21	72	75	76	8	17	74	111	140	65	56	112
D.V.	38	36	10	10	40	38	20	8	6	23	28	45	23	26	38
Group 2															
G.B.	56	40	34	34	44	46	34	30	32	39	75	44	44	48	52
W.M.	42	74	10	19	102	41	53	12	11	82	92	72	48	41	92
Group 3															
U.G.	28	46	4	22	58	20	39	1	28	46	28	36	15	24	46
W.S.	110	90	19	23	110	96	80	8	14	103	113	116	67	80	108
Group 4															
W.E.	30	20	5	2	4	38	20	7	3	38	48	26	24	12	48
W.H.	22	16	8	8	14	13	14	3	6	12	39	20	12	14	25
Group 5															
M.S.	10	13	16	16	17	22	41	13	10	16	21	44	27	29	12
D.R.	66	89	29	40	150	74	71	30	11	108	111	127	106	164	140
Group 6															
J.K.	24	24	6	4	24	23	16	5	3	17	35	19	4	8	14
E.K.	53	72	8	10	80	32	65	7	10	61	50	73	26	32	62
Mean	45	51	14	16	63	43	44	11	13	52	63	64	39	45	62

the study. The consistency values of the seven sputum specimens collected from each patient during one week of the study in the morning, afternoon and overnight periods are averaged to provide the *weekly mean sputum consistency* value for that period. It is apparent from this table that the weekly mean sputum consistency values for week 2 (MES + Iso) and week 4 (N-ac + Iso) are consistently lower than either of the saline control periods (weeks 1 and 5) or the saline + Iso period (week 3).

The weekly mean consistency values for the five treatment regimens for all 12 patients are shown in Table III. It may be seen from this table that those patients with relatively thin sputum during the control periods could not clearly demonstrate decreases in consistency to the degree seen in those patients with thick sputum. Eleven of the 12 patients showed distinct lowering of sputum consistency during the MES + Iso and the N-ac + Iso periods, but one patient (M. S.), whose sputum was rather thin, did not respond to either regimen. The mean values for the five regimens for all 12 patients are graphically presented in Figure 4. It is evident from this figure that the mean sputum consistency values for the MES + Iso and the N-ac + Iso periods are lower than both the saline control periods and the saline + Iso periods. For both the MES + Iso and the N-ac + Iso, these differences from the controls are highly significant for the morning and afternoon specimens ($P<0.01$). The differences are less striking for the overnight specimens, $P<0.05$ for the MES + Iso and $P<0.3$ for the N-ac + Iso. The MES + Iso consistency values are slightly lower than for N-ac + Iso, but the difference between these two regimens is not statistically significant. There was no significant

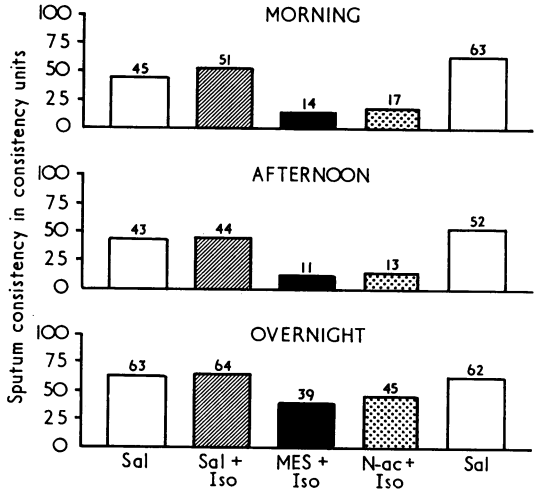

FIG. 4. *Comparison of the effect of* 10% *N-acetylcysteine (N-ac) plus isoproterenol (Iso) with that of* 10% *mercaptoethane sulphonate (MES) plus isoproterenol. For the morning and afternoon specimens the mean sputum consistency values for the N-ac + Iso and MES + Iso periods are significantly lower than both the saline control (Sal) and the saline plus isoproterenol (Sal + Iso) periods.*

difference in sputum consistency between the saline control and the saline + Iso periods.

Because of the possibility that different therapeutic regimens may be more effective in lowering sputum consistency of a particular type of sputum, all the sputum specimens were classified by visual inspection as to whether they were mucoid, mucopurulent, or purulent. Purulent sputum regularly had a significantly lower consistency than either mucoid or mucopurulent sputum regardless of which treatment was used during the collection period. Furthermore, both the MES + Iso and the N-ac + Iso combinations were of equal effectiveness in lowering the sputum consistency of all three types of sputum. As might be expected because of the 14-hour collection period, the overnight specimen showed a higher consistency than the morning and afternoon specimens whether the specimen was mucoid, mucopurulent, or purulent.

SPUTUM VOLUME As illustrated in Fig. 5, the weekly mean sputum volume values did not change significantly with any of the three drug regimens.

SPIROMETRIC TESTS None of the measurements from the forced expiratory spirogram changed significantly with any of the three treatment regimens.

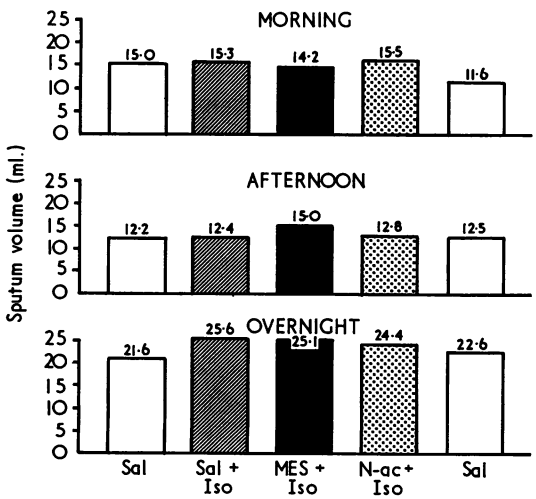

FIG. 5. *Comparison of the effect of* 10% *N-ac + Iso with that of* 10% *MES + Iso on sputum volume. There is no significant change in sputum volume resulting from any of the drug regimens compared to the volume during the control periods.*

CLINICAL COURSE Each patient was questioned daily as to whether the symptoms of cough, wheezing, and dyspnoea were improved, unimproved, or worse. The most striking subjective responses were related to isoproterenol since all three regimens containing isoproterenol provided much greater symptomatic improvement than either of the saline control regimens. However, we were unable to detect any symptomatic difference between saline + Iso, N-ac + Iso, and MES + Iso regimens. Eight of the 12 patients felt that N-ac + Iso allowed them to expectorate with the least effort.

The side-effects included: (1) an episode of blood-streaked sputum in one patient during the initial saline week, and a similar episode in a second patient during the week he received MES + Iso; (2) nausea in one patient associated with N-ac + Iso; and (3) tightness in the chest associated with N-ac + Iso at times in three patients, and with MES + Iso in three other patients. Eight patients considered the taste of N-ac + Iso to be the most disagreeable; three patients considered the taste of MES + Iso to be more disagreeable than N-ac + Iso; and one patient considered the two preparations equally distasteful. No patient in the series considered the taste of either mucolytic agent intolerable or requiring discontinuance of the treatment.

Daily physical examination of the chest showed no significant change in the breath sounds, wheezing, or crepitations attributable to any of the treatment regimens.

DISCUSSION

Our previous clinical study (Kory *et al.*, 1968) showed that the nebulization of 20% N-acetylcysteine (N-ac) plus racemic epinephrine (RE) was an effective combination for lowering sputum consistency. With this combination, bronchospasm, which has occasionally been observed with N-ac alone (Bernstein and Ausdenmoore, 1964), did not occur. In the present study nebulization of 10% N-ac combined with isoproterenol lowered sputum consistency to approximately the same degree as 20% N-ac + RE. Figure 6 compares graphically the mean sputum consistency values resulting from the two N-ac regimens. It is apparent that the sputum consistency values resulting from 10% N-ac + Iso are almost identical to those from 20% N-ac + RE. It is also evident that the control values are lower in the present study (Fig. 6, Study B) because saline was nebulized three times daily dur-

ing these periods whereas, in the previous study (Fig. 6, Study A), there was no nebulization during the control periods. Our recent *in vitro* studies (Hirsch *et al.*, 1969) have shown that the addition of 1·0 ml. of saline to 5·0 ml. of sputum will result in a 34% lowering of consistency.

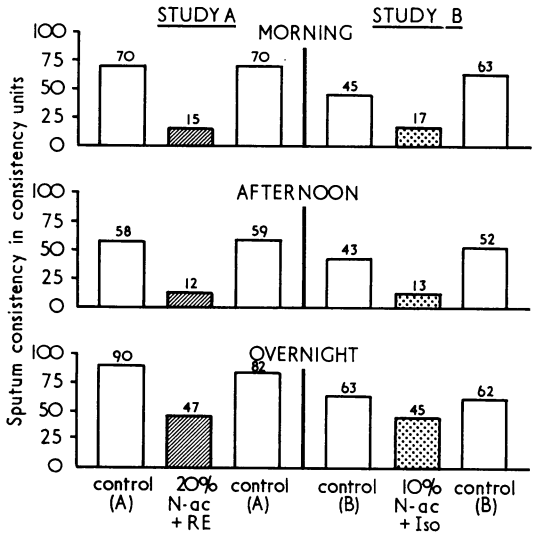

FIG. 6. *Comparison of the mean sputum consistency during nebulization of* 20% *N-acetylcysteine plus racemic epinephrine* (20% *N-ac* + *RE) and* 10% *N-acetylcysteine plus isoproterenol* (10% *N-ac* + *Iso). Nebulization of* 10% *N-ac* + *Iso lower sputum consistency to the same degree as does* 20% *N-ac* + *RE. In study A there was no nebulization during the control periods. In study B normal saline was nebulized three times daily during the control periods.*

In the same *in vitro* study, 10% MES was more effective than 10% N-ac in thinning sputum. In the present clinical study nebulization of 10% MES+Iso resulted in lower mean sputum consistency values than 10% N-ac+Iso in all three daily specimens. This suggests that 10% MES may be more effective not only *in vitro* but also in the clinical situation. These differences, however (between 10% MES+Iso and 10% N-ac+Iso), are not statistically significant.

The lack of effect of either the N-ac+Iso or the MES+Iso on the physical findings and the spirometric values in patients with chronic bronchial disease again demonstrates that the tissue changes responsible for the ventilatory impairment are not easily altered even by effective mucolytic agents. Furthermore, the spirometric tests were performed at those times which would specifically exclude the short-term bronchodilating effects of isoproterenol. The effects of short-term bronchodilatation due to isoproterenol cannot, however, be eliminated from the patient's subjective response. Since even a small amount of bronchodilatation results in considerable improvement in breathing comfort, it is not surprising that subjective improvement was related to the isoproterenol rather than to the mucolytic agents. Furthermore, we have been unable to establish in any of our studies a close correlation between sputum consistency measurements and the ease or difficulty which the patient experiences in coughing up sputum.

The patients were chosen for this study on the basis of their relatively stabile clinical course and, therefore, were able to provide reliable control periods with which drug regimens could be compared. They were *not* chosen because of their need for mucolytic therapy although they did have thick sputum. Patients who have a more acute need for mucolytic agents (in such diseases as laryngotracheobronchitis and post-operative plugging of the bronchi with mucus) often are so seriously ill, have such wide fluctuations in their course, and require so many other simultaneous therapeutic measures that it becomes almost impossible to evaluate mucolytic agents objectively. Nevertheless, we think that the sputum-thinning efficacy of both N-ac and MES demonstrated in the present study should be applicable to acutely ill patients with thick tenacious sputum.

Analysis of the three major types of sputum, i.e., mucoid, mucopurulent and purulent, showed that mucus is the major cause of thick, viscous sputum. This conclusion is in agreement with the findings of Elmes and White (1953) that mucoid and mucopurulent sputum are significantly thicker than purulent sputum.

While the data presented here and in our previous studies substantiate the sputum-liquefying ability of these mucolytic agents, the precise role of these drugs in clinical therapy is not yet clear. It is indeed possible that in some instances the bronchial secretions which had already been propelled to the larger airways, mixed there with the mucolytic agent, and so produced some of our changes. In such instances the secretions may well have been expectorated whether or not mucolysis had been achieved. We think, however, that in at least some of these cases thinning of these secretions may have facilitated expectoration.

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