Epidemiologic Observations on the Use of Glycol Vapors for Air Sterilization*

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THE clearly established bactericidal and viricidal effect of glycol vapors on air-suspended microörganisms has presented a new means for attack on the problem of control of air-borne infection. Following intensive laboratory studies on the fundamental aspects of this problem, which have been well summarized in a recent publication,[1] certain practical studies were necessary before full scale clinical application could be attempted. These included investigation of possible fire hazards in the use of these compounds,[2] observations on the properties of glycol vapors in large spaces, and the development of apparatus for generation and distribution of the vapor.[3, 4, 5]

The encouraging outcome of these studies led to a large scale field trial to determine if the incidence of air-borne infections can be reduced by treating living quarters with triethylene glycol (TEG) vapor in effective concentrations.‡ Triethylene glycol was used in this study since it is effective in much lower concentrations than propylene glycol and has been shown to be similarly non-toxic in the recommended concentrations.

MATERIALS

Location of Study—Two two-story barracks, each consisting of two wings, were selected for study. These buildings were divided into eight dormitories, each 120 x 30 x 9 ft; each dormitory housed approximately 80 men in 40 double bunks about 1½ ft. apart. It was decided to use four of the dormitories for test, and four for control purposes. The east wing of one building and the west wing of the other (four dormitories) were used as tests; the remaining four dormitories served as controls. We started with 320 test individuals and 320 controls, which were replaced by a new group of men at 6 week intervals. We were able to observe three such groups, or a total of 1,000 men in the test group and 1,000 in the controls. This number is approximate since some men were transferred and exchanged during our ob-

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† With the technical assistance of Margaret Mellody and Silva Trautman.

‡ The engineering aspects of this test have been described in detail elsewhere.[6]

servation periods. An intermingling of personnel occurred during the day in mess halls, classrooms, drill halls, recreation halls, etc., but since we believed that the greatest incidence of cross-infections and the greatest degree of air contamination occurs in sleeping quarters, it was felt that significant results could be obtained by treating only those quarters.

The problem of maintaining comfortable room temperature was difficult. A single outside thermostat controlled the heat input of each building (two test and two control dormitories) with no available means for the closure of individual steam radiators. Thus, with closed windows and doors in test dormitories, it was necessary to bring in large quantities of fresh cold outside air through the duct system described below, to keep the test room temperatures within comfortable limits. Despite this measure there were occasional uncomfortable rises in temperature, necessitating the opening of windows, with a resultant lowering of glycol concentrations. Temperature adjustment of the control dormitories was left to the discretion of the occupants.

Since it has been shown that dust is of importance in air contamination, and since sleeping quarters are notoriously high in dust content due to bedclothes, etc., it was decided to effect a simple dust control measure by treating the floors in both test and control dormitories with a light floor oil.

Triethylene Glycol (TEG) — The chemical, physical, and pharmacological properties of triethylene glycol have previously been described.[1] In brief, it can be stated that the vapor is effective in low concentrations, it is nontoxic and non-inflammable in these concentrations, the cost is nominal, and no odor is perceptable. A relative humidity of 25 to 60 per cent is desirable for optimum bactericidal action, but killing of air-suspended microörganisms occurs in a humidity range of 20 to 80 per cent. A special "air sterilization grade" of triethylene glycol was supplied by the Carbide and Carbon Chemicals Corporation. This material is highly refined and free from the impurities commonly present in the glycol purchased on the open market.

Vaporizer—A great deal of effort and time was expended in the development of a method of glycol generation which would introduce glycol-vapor at a predetermined controllable rate.

Glycol-vapor was generated by vaporization from an aqueous glycol solution. Since this is a miscible binary mixture, the temperature at which boiling takes place varies with the concentration of the particular mixture. When a glycol-water solution is heated, water, being the more volatile component, vaporizes more readily; however, both water and glycol vapors are delivered from the boiling mixture. The more rapid loss of water results in a solution richer in glycol and raises the boiling point of the mixture. It can be seen that if the relative proportions of the boiling glycol and water are kept constant, the respective proportion of glycol- and water-vapor emitted from the vaporizer will remain constant and the total output of vapor will depend on the heat input or rate of boiling.

This vaporizer aids in maintaining desired humidities as well as obtaining effective glycol concentrations, and is described in detail in a previous publication.[4]

Duct and Fan System—The size and shape of the dormitories, and a double row of lockers extending the length of the room necessitated the construction of a duct system to distribute glycol and water-vapor uniformly throughout the space.

Figure 1 shows the general arrangement of the equipment. For clarity the lockers and two nearby bunks are not shown. At the far left is the fresh air

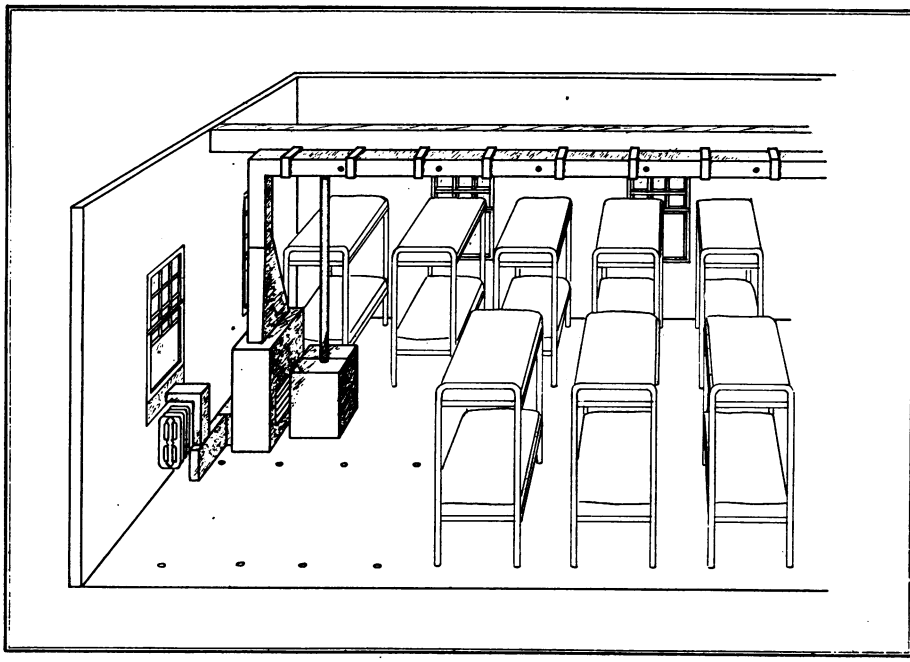

FIGURE 1—Diagrammatic representation of duct, fan unit, and vaporizer as installed in dormitory

intake coming from a special fitting in the window. The fresh air enters the back of the blower fan unit through a set of dampers. Recirculated room air enters the front of the fan unit through the louvres (visible in the drawing) and another set of dampers. Both sets of dampers may be controlled by a lever mounted on the top of the fan cabinet. Thus the air output can be varied uniformly from 100 per cent fresh air to 100 per cent recirculated air. The fan output may be varied in six steps to produce from 750 to 1,700 cu. ft. per minute at free delivery of the fan. The air is blown from the fan into the pyramid shaped adapter piece, and then enters the duct through a right-angled elbow provided with turning vanes. The duct is of laminated asbestos construction, specially treated to resist the absorption of glycol.* The air leaves the duct through circular openings $2^3/_{32}$ in. in diameter, spaced 5 ft. apart, on each side of the duct. The vaporizer is mounted on the floor in front of the fan unit and delivers the glycol-water vapor through a well insulated 1¼ in. pipe to the distributing duct.

Sampling Tables—To facilitate the taking of glycol and bacterial samples in the crowded dormitories, special sampling tables were built (Figure 2). These were sturdily constructed of ¾ in. pipe and pipe fittings. Rubber-tired wheels are fitted to the front legs and pipe caps to the rear legs. A motor vacuum pump unit provided with an extension cord is mounted on the horizontal braces by pipe straps forming, in effect, a vibration-free, three-point suspension. On the front end of the table top is fastened a rack for holding test tubes for glycol samples and "Moulton air samplers" for bacterial samples. The rack proved not

* This material was supplied by the Philip Carey Mfg. Co., Lockland, Ohio.

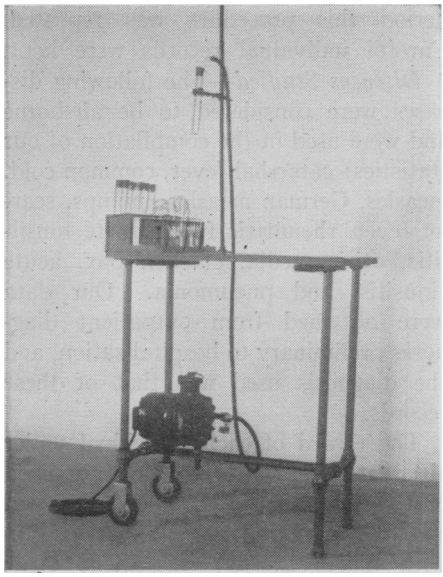

FIGURE 2—Table used for obtaining glycol and bacterial samples

only convenient but it also reduced breakage. A ¼ in. pipe in a floor flange bolted to the table top serves as a ring-stand for holding test tubes and air samplers while samples are being taken. There is ample space on the table for writing purposes. These tables made it possible to take air samples in all parts of the dormitories and at any desired height.

Operation of Equipment—Following the installation described above measurements were made to obtain the relative air velocities through the various outlets of the duct, and air samples were also taken at the openings for glycol determinations. The results showed remarkably uniform distribution of air and glycol, and demonstrated that complete mixture of glycol and air had occurred before the first opening was reached, and that there was no perceptible loss of glycol as the air flowed through the duct.

For adequate ventilation it was decided to allow not less than 25 cu. ft. per minute of outside air per individual. Since it was estimated that the uncontrolled normal infiltration of air was equivalent to two air changes per hour, the fan speed was set to deliver from 1,000 to 1,200 cu. ft. per minute of outside air. Even in well constructed buildings there is a considerable amount of infiltration and exfiltration which cannot be determined accurately; it depends not only on the general tightness of construction, particularly around windows and doors, but also on wind velocity and direction. To reduce this inaccuracy to a minimum it is essential to keep all doors and windows closed and to depend on bringing in fresh air by means of the fan alone. Therefore, every effort was made to keep the doors and windows of the treated rooms closed. All air entering the duct was "glycolized." Ventilation in the control room was at the discretion of the occupants; it is believed that the number of air exchanges was greater than in the test quarters. Since the volume of the room was 33,000 cu. ft., with the equivalent of four air changes per hour, it was calculated that a glycol delivery of 0.04 lb. per hour would be needed to maintain a concentration of approximately 0.004 mg. TEG per liter of air. To obtain this output the operating vaporizer temperature was set at 280° F., using a 1,000 watt heater. This setting gave a concomitant water delivery of 2.5 lb. per hour.

METHODS

Glycol Determinations—Since preliminary observations showed uniform distribution of glycol throughout the treated space it was decided to take all air samples for glycol analysis at one fixed location in each test room. Air samples were taken at 2:00 A.M., 5:00 A.M., and 8:00 A.M. daily. These hours were chosen to coincide with the times at which air samples for determination of bacterial content were taken. Four cu. ft. of air were collected and analyzed for glycol content by a modification of

the technique described by Wise, *et al.*[7] The results were recorded as mg. TEG per liter of air.

Bacterial Determinations — Samples were taken in all the dormitories at the hours noted above. Since it is well known that movement of individuals within a space produces a sharp rise in the number of air-borne bacteria, we attempted to determine the air contamination at the highest and lowest point of room activity. At 2:00 A.M. the men were asleep, at 5:00 A.M. (Reveille) activity was greatest, and 8:00 A.M. the men had left their quarters.

Using a Moulton bacterial sampler, 10 cu. ft. of air were collected at the same position as used for glycol samples. Pour plates were made with 1 ml. of the sample, using blood agar media, and colony counts were recorded as colonies per cu. ft. of air, noting the number of colonies of hemolytic streptococci. Aliquots were also plated in media to which gentian violet (1–500,000) had been added.[8] The latter procedure was used to enable us to determine the presence of hemolytic streptococci more readily, but was not entirely satisfactory and was discontinued.

Temperature and Humidity—These observations were also made at 2:00 A.M., 5:00 A.M., and 8:00 A.M. Individual records were kept for each dormitory, including both test and controls. A sling psychrometer was used and wet and dry bulb readings were recorded. Records on outside weather conditions were also kept.

Throat Cultures—At the beginning of each observation period throat cultures were taken of each individual, both in the test and control groups. Swabs of the throat were placed in tubes containing 1 ml. of tryptose-phosphate broth (for convenience in carrying) and subsequently plated on blood agar. At the end of the 6 week period this procedure was repeated. Careful individual records were kept.

Diseases Studied—The following diseases were considered to be air-borne and were used in the compilation of our statistics: catarrhal fever, common cold, measles, German measles, mumps, scarlet fever, rheumatic fever, acute tonsillitis, otitis media, chicken pox, acute sinusitis, and pneumonia. Our data were obtained from outpatient diagnoses preliminary to hospitalization, and the diagnosis used was that of these records.

The record of each individual under observation in this study thus consisted of (1) throat culture at beginning and end of test period; (2) hospitalization with diagnosis, (3) outpatient visits with diagnosis; and (4) dormitory and bunk location.

DATA

Glycol Concentrations — Although it has been shown that for immediate killing of air-suspended organisms a concentration of 0.005 mg. TEG per liter of air is desirable, it was our belief that sufficient bactericidal and viricidal effect would be produced by the use of a somewhat lower concentration. We further realized the difficulties inherent in the enforcement of the rule for closed windows and doors at all times. Thus fluctuations in concentration may have occurred at times other than those at which analyses were taken.

The results of our analyses were quite constant. Although we occasionally obtained values as high as 0.010 and as low as 0.001 mg. TEG per liter, for the most part the concentration was between 0.0025 and 0.003. At no time was there any fog formation. It is interesting to note that we obtained a small but significant correlation between wind velocity and glycol concentration.

Frequent interrogation of the men concerning possible effects of the vapor elicited no evidence of irritation of the respiratory tract.

Temperature and Humidity—The room temperatures both in test and control dormitories were higher than desirable, averaging 72° F. in the control spaces and 75° F. in the test. The relative humidities recorded in the test and control dormitories showed an average approximately 5 per cent higher in the former. We were able to maintain the relative humidity within the optimum range for bactericidal glycol activity.

Bacterial Counts — Many workers have noted the extreme fluctuations of air bacterial content occurring in occupied spaces. We also observed this phenomenon. The most important factor influencing the variation in number of air-suspended organisms is the amount of movement in the room. We found that in the samples taken at 2:00 A.M. bacterial counts were relatively low as compared with the number of recoverable bacteria at the time of Reveille.

The overall reduction due to the action of glycol is shown in Figure 3. Each point of the curves represents a logarithmic average of the twelve readings obtained from four dormitories at three different times during the day. Large fluctuations have been reduced by 3-point moving averages.[9]

The occurrence of hemolytic streptococci in the controls was extremely sporadic. Many days often passed without their appearance, but isolated samples frequently contained hundreds of colonies. A great reduction of hemolytic streptococci in the test spaces was observed.

Incidence of Infection—For approximately the first half of each observation period, there was little difference in hospital admission rate between control and test. Following this inconclusive phase, however, a definite effect was noted. For the first entire 6 week period there were 63 admissions from the control group and 56 from the test,

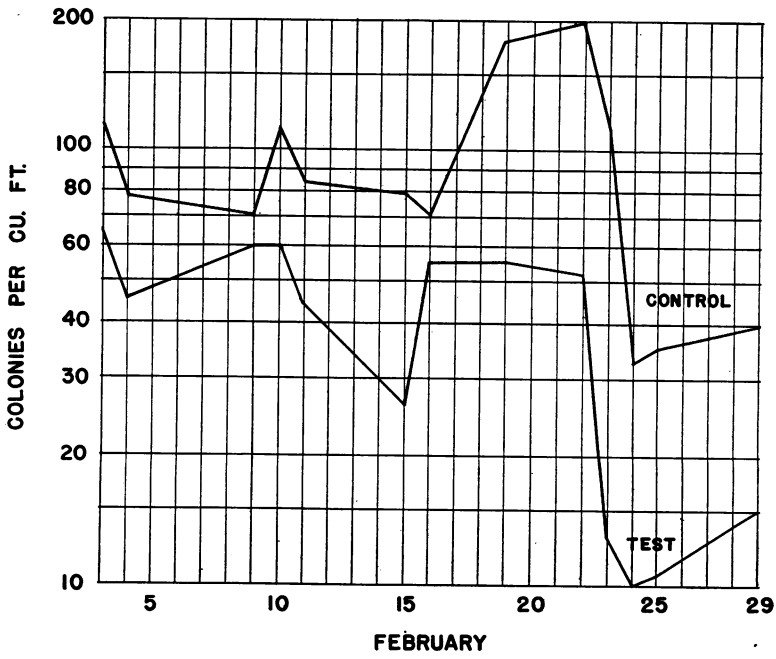

FIGURE 3—Daily bacterial counts in test and control dormitories, showing reduction obtained in glycol-treated spaces

representing a reduction of 11 per cent. During the last 17 days, there were 19 admissions from the control and 7 from the test, or a reduction of 63 per cent.

Data from the second 6 week observation period showed comparable values, i.e., 63 from the control and 55 from the test (13 per cent reduction). The last 17 days showed 34 cases as compared with 12 (65 per cent reduction).

The third observation period produced inconclusive data. This was due to the fact that conditions produced by high outside temperatures and unsatisfactory overheating of the sleeping quarters during the last 3 weeks of the test resulted in opening of windows, effecting a lowering of the glycol concentration. Combining the first two periods, we find a total of 126 hospital admissions from the control dormitories and 111 from the test quarters, or a reduction of 12 per cent. For the final 17 days of both periods, there are 53 from the control as compared to 19 from the test, representing a reduction of 64 per cent.

Table 1 records our experience in a study of a small epidemic of mumps. For the first 3 weeks (the recognized incubation period of this disease) there was a fairly comparable rate of admissions from both the test and controls. Thereafter there were only 4 additional cases from the test barracks while 14 cases occurred in the control dormitories.

TABLE 1

Record of Hospitalizations for Mumps, Showing Effect of Glycol Following Mumps Incubation Period

	Test	*Control*
Total Number of Hospitalizations in 24 day period	9	9
Total Number of Hospitalizations for succeeding 17 days	4	14

There was no apparent correlation between total incidence of infection and outside weather conditions.

Throat Cultures—The results of these observations were most striking. A surprising number of individuals were found to harbor hemolytic streptococci in their throats. The average incidence in incoming men was 24 per cent. This figure represents the average percentage found in a total of approximately 2,000 men (three test periods).

A sharp reduction occurred in individuals exposed to the action of glycol vapors in their sleeping quarters. This is shown in Table 2 which represents the percentage of positive throat cultures in test and control men. The original incidence of 34.2 per cent in this test group fell to 8 per cent at the end of the 6 week period. This may be compared to the reduction from 27.3 per cent to 24.1 per cent in the controls. A similar effect was produced in the first 6 week period during which the incidence of positive findings in the test fell from 24 to 14 per cent, while in the control there was a rise from 22 to 25 per cent. In the third period little significant change was noted. These data are reported on a percentage basis rather than in numbers of individuals, since a transfer of men occurred during the observation period and there were some individuals from whom we could not obtain cultures at both the beginning and end of the test period. We cannot state at what time during the test period the change noted above occurred since only two cultures were

TABLE 2

Incidence of Positive Throat Cultures (hemolytic streptococci) in Test and Control Dormitories Before and After 6 Week Observation Period

	First Period		*Second Period*	
	Test	*Control*	*Test*	*Control*
Average incidence in incoming men, per cent	24	22	34	27
Average incidence in outgoing men, per cent	14	25	8	24

taken from each individual. It would be of interest to have more frequent cultures taken.

An attempt was made to analyze further the above data. This was done in the manner illustrated in Table 3 which represents the 6 week period from January 29 to March 10. Records were kept to show losers (positive initial throat culture, negative final throat culture); gainers (negative initial throat culture, positive final throat culture); and keepers (positive initial throat culture, positive final throat culture). Statistics on the first period were similar to those shown in the table, while little effect occurred in the third period.

The most significant finding was the greater number of losers, and the smaller number of gainers and keepers in the test as compared to the controls.

TABLE 3

Per cent Loser, Gainer, and Keeper in Individual Dormitories and Average Total Test and Control

Test	*Per cent Loser*	*Per cent Gainer*	*Per cent Keeper*
Dormitary 1	32	0	4
Dormitory 2	29	6	6
Dormitory 3	28	6	3
Dormitory 4	30	3	3
Control			
Dormitory 1	26	12	0
Dormitory 2	26	13	14
Dormitory 3	12	33	7
Dormitory 4	11	9	11
Average Test	30	4	5
Average Control	19	16	8

A similar system of recording losers, gainers, and keepers was used for the separate dormitories studying their oc-

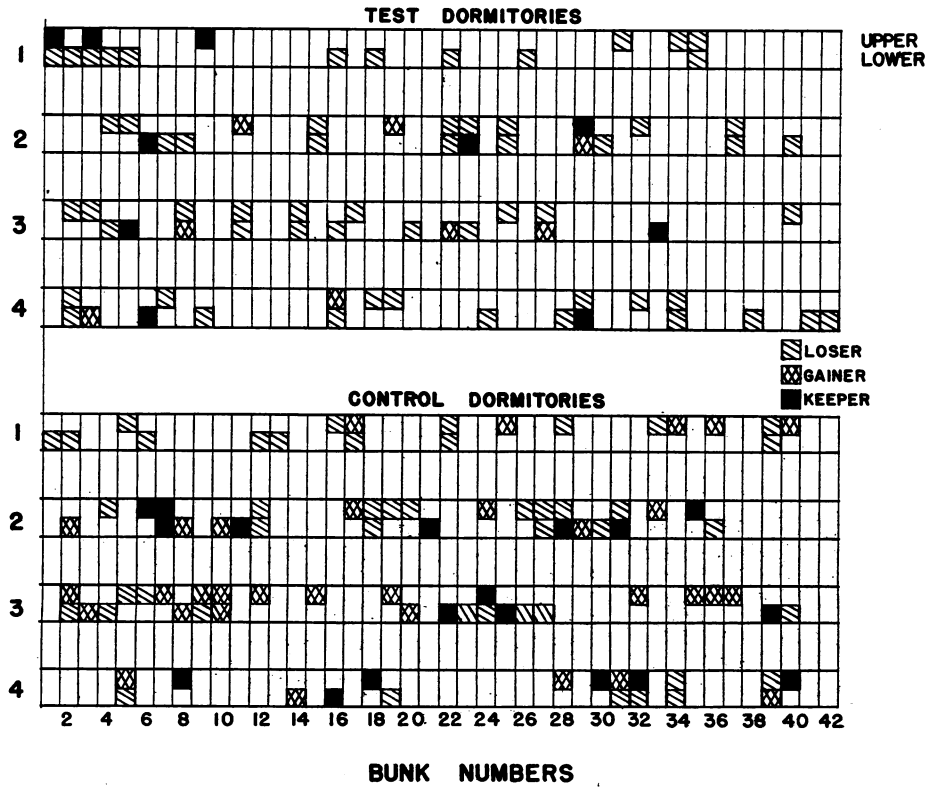

FIGURE 4—Record of individuals according to bunk location, showing losers, gainers and keepers

currence in relation to location of bunks. This is diagrammatically represented in Figure 4.

DISCUSSION

Incidence of Diseases—As stated above, we realized at the outset of our studies that certain limitations as to the conclusiveness of our observations were unavoidable. It is obvious that sources of cross-infections other than sleeping quarters would occur. We were unable to control the men during periods of classroom instruction, drill, eating, and leave. However, we felt that since the greatest reservoir of infection was present in the dormitories (other workers [10, 11] have presented evidence to substantiate this hypothesis), a demonstration of a statistically significant reduction in air-borne disease would be possible.

It was particularly unfortunate that an observation period longer than 6 weeks was not feasible since we appeared to be getting a most encouraging effect after an initial 3 week period of time had elapsed. The interpretation of this observation is not entirely clear but reasonable explanations may be presented.

From a study of Table 1, one can state that the men hospitalized for mumps during the first three weeks received their infection previous to their coming under our observation. This resulted in the fairly equal distribution in cases between test and control groups. It may further be seen that spread of the infection was reduced in the group sleeping in glycol-treated atmosphere. Considering the shorter incubation period of the more acute respiratory diseases, it does not seem possible to explain all our findings on this basis. However, it does suggest that under conditions of crowding and the bringing together of individuals from various walks of life and localities, the incubation period of these infections may be altered.

Another explanation may be considered. During the first few weeks when a number of individuals are placed in close contact, those most susceptible contract disease, and those less susceptible escape. During the entire 6 week period one can postulate the progressive building up of air and bed-clothing contamination in the dormitory. The use of glycol vapors prevents the contamination from reaching the concentration necessary to affect those individuals of lesser susceptibility, while in the control dormitories such action does not occur.

In this regard we wish to point out that the data on recovery of air-borne bacteria (Figure 3) does not give a true representation of the air bacterial population at all times. The counts include many non-pathogens and, of course, give no index of air contamination by viruses. The most significant observations gathered from the air sampling are the overall reduction in colony count and the practical abolition of hemolytic streptococci in the test dormitories.

The data recorded on the basis of observations in individual dormitories showed a consistency in the number of hospitalizations and infections from each. A study of infections according to the location of bunks in the dormitories suggested a greater degree of isolated cases in the test quarters, as compared to the controls where there were numerous clusters of infections. This was particularly well shown in the study of mumps. Another interesting observation in regard to the mumps epidemic was that in one control dormitory housing a group of men coming from a different station from those in the other seven dormitories, not a single case of mumps occurred. This lends further strength to the belief that most cross-infections occur in sleeping quarters, since there was complete intermingling of men during their work day.

An attempt to evaluate the influence of triethylene glycol vapors on outpatient visits was inconclusive. There were many difficulties encountered, such as incorrect diagnosis, multiple visits by single individuals, frequent visits by men who had no demonstrable infections, etc.

Throat Cultures—It is shown that in groups living in atmospheres containing bactericidal concentrations of triethylene glycol, the incidence of hemolytic streptococci found in the throat is reduced. This is demonstrated clearly in the first two periods where approximately 650 test and 650 control individuals were observed. A breakdown of the data suggests that this reduction is not due to the direct action of the glycol in the respiratory tract but is produced by its effect on the air-borne bacteria. It has been shown that in the test dormitories the difference between the initial and final cultures was caused by a greatly increased number of men developing negative throat cultures with only a small percentage developing positive cultures during the exposure period (Table 3). It would appear that in the test spaces, death of hemolytic streptococci occurs promptly when they are introduced into the air either by expression of the organisms directly from the respiratory tract, or secondarily introduced from bed-clothing, etc. This action prevents the dissemination of organisms to the throats of susceptibles. The increased number of "gainers" (Table 3) in the control dormitories further substantiates this hypothesis.

From a study of the data shown in Figure 4 it would appear that the presence of glycol in the test dormitories prevented the spread of hemolytic streptococci between adjacent bunks. There were very few gainers as compared to the control dormitories where new individuals developed positive throat cultures appearing in clusters radiating from previously demonstrated carriers. Control of the high local bacterial concentration about a carrier's bed produced by direct expression from the respiratory tract and secondary introduction from the bed-clothing is probably effected by glycol vapor.

It is significant to point out the observation made in the third period studied. This group of men served as an interesting control on the first two periods. With an inadequate glycol concentration there was no effect on either the incidence of infections or throat cultures. It would appear, therefore, that in large groups there is some correlation between frequency of occurrence of hemolytic streptococci in the throats of individuals under observation and incidence of respiratory disease in the group as a whole; and that an index of glycol efficiency may be determined by this effect. Although there was no apparent relationship between the incidence of hemolytic streptococci and infection in individuals, our data suggests that if glycolized air prevents the spread of hemolytic streptococci from one person to another, it also prevents the spread of other air-borne microörganisms including viruses.

CONCLUSIONS

1. Bactericidal concentrations of triethylene glycol and optimum humidity conditions were maintained in large living quarters.
2. A reduction in total bacterial air contamination was produced.
3. Hemolytic streptococci were practically eliminated from the air of glycol-treated dormitories.
4. A definite reduction in air-borne infections was effected.
5. Control of a small epidemic of mumps was attained.
6. Prevention of spread of hemolytic streptococci from the throat of one individual to another was demonstrated.

SUMMARY

A practical installation for triethylene glycol generation and distribution

was made in a military camp. Glycol concentrations of 0.0025 to 0.004 mg. per liter of air and optimum relative humidities were maintained. Studies were made on three groups of 640 men, observed for 6 week intervals and equally divided into test and controls; the former sleeping in glycol-treated quarters, the latter in untreated dormitories. An overall reduction in airborne disease of 12 per cent was produced for the entire period, but the statistics on the final 17 days showed a reduction of 64 per cent. Explanations for this phenomenon are presented. The incidence of hemolytic streptococci recovered from throat cultures of men exposed to the effect of the glycol vapors fell dramatically in contrast to the control individuals. There was a definite prevention of spread of these organisms in the dormitories.

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