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THE DISINFECTION OF THE AIR OF COOL ROOMS WITH LACTIC ACID

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INTRODUCTION

The method used in Australia for a number of years for the disinfection of meat chilling rooms involves the liberation of formaldehyde vapour by heating proprietary preparations of paraformaldehyde (Anon. 1938). The disadvantages of this method are that it is sometimes necessary to leave the rooms empty for 24-36 hours, or until all the formaldehyde vapour has dissipated, and care has also to be taken to avoid placing beef in chilling rooms still containing perceptible amounts of formaldehyde.

Bourdillon, Lidwell, and Schuster (1948a) tested a number of compounds as air disinfectants in hospitals and dining-rooms and found lactic acid to be the most effective of all those tested. Consequently, and because of the availability of this acid and its low toxicity, experiments were carried out to measure its performance under cold room conditions. It was also of interest to ascertain whether the paraformaldehyde method, with its attendant disadvantages, could be dispensed with through the use of lactic acid.

MATERIALS AND METHODS

The initial experiments were carried out in a small refrigerated room with a volume of about 10.5 cu m, in which the temperature and relative humidity were varied from 0-10°C and from 75-90% respectively. Micro-organisms were introduced into the test room from an atomizing device and the air was sampled by means of a slit sampler (Bourdillon, Lidwell, and Schuster 1948b). The disinfectant was introduced with the spray described

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by Kaess and Weidemann (1962).

The organisms tested were all isolated from chilled beef and were all psychrophilic. They were: *Penicillium* M107, *Sporotrichium* 74, Yeast 18, *Pseudomonas* 1482, *Lactobacillus* 89 and *Micrococcus* 34, from the culture collection maintained in the laboratory.

The rate of loss of cells from the air was expressed in terms of decrease of $\log_e$ population per hour and represented by the symbol K, as defined and calculated by Bourdillon *et al.* (1948*b*). The death rate, K_D, due to the disinfectant was calculated from the formula: $K_D = K_2 - K_1$, where K_2 and K_1 represent the rate of removal of organisms in the presence and absence of the disinfectant.

Experiments were also carried out in commercial chill rooms of 850 - 1100 cu m total capacity. In these there was no control of relative humidity but the temperature was sometimes adjusted to the required level. The relative humidity during an experiment was measured with an Assman hygrometer. Some of the rooms were cooled by a forced-draft system, and the others by convection only.

No micro-organisms were introduced into the chillers, the natural population being used for evaluating the effectiveness of disinfectants. The air was sampled at five positions in the room, the slit sampler being placed on a trolley with a rubber hose pointing vertically upwards so that each sampling position was about half-way between floor and ceiling. Nutrient agar plates were rotated at two revolutions per minute and exposed for one minute, so that 28 litres of air was sampled each time. All plates were incubated at 20°C for four days before counting the colonies.

Lactic acid was sprayed from a 50% v/v solution by means of a spray-painting unit set to deliver about 40 ml per minute as a fine aerosol. The chillers were treated with formaldehyde by heating paraformaldehyde in four small spirit stoves (Anon. 1938).

Disinfection of the wall population was followed by wiping areas of about 1000 sq. cm with sterile cotton-wool swabs moistened with sterile saline. The swabs were placed in 100 ml sterile saline, vigorously shaken, and the washings diluted and plated.

The effect of the disinfectants on both air and walls was recorded as the percentage reduction of the original population present.

RESULTS AND DISCUSSION

Lactic acid disinfection

In the more closely controlled experiments, carried out in the small experimental test room, lactic acid was more effective at 75-85% R.H. than at 90% R.H. With only one exception, a maximum of activity was reached at a concentration of 300 mg lactic acid/cu m. The exception was Penicillium M107 at 90% R.H. and 0-1°C. Excluding this case, all other combinations of organisms, temperature, and relative humidity gave no increase in death rate with further increase in concentration.

Plots of the death rate K_D against temperature for two species of mould, a yeast, and three types of bacteria, at two levels of spray concentration, revealed that the effect of temperature was most marked under conditions where the maximum kill had not been reached.

Disinfection rate increased with temperature up to 10°C, the highest temperature tested. The graphs also revealed that moulds are more resistant than yeasts and bacteria, and that bacteria are the least resistant. The best conditions for lactic acid disinfection in the test rooms were thus shown to be 75-85% R.H., 5-10°C, and a lactic acid concentration in the air of 300 mg/ cu m.

Disinfection of commercial chillers with lactic acid was at first investigated under conditions normally prevailing in the chillers - i.e. a low temperature (0-2°C) and a high relative humidity (90-99%). The small-scale experiments have shown these conditions to be not the most favourable for disinfection by means of lactic acid.

Effect of Spraying Concentration.- Table 1 shows the effect of the concentration of lactic acid in the air on disinfection in the commercial rooms. It can be seen that a concentration of 300 mg/cu m gave a reduction of air-borne population of only about 60% after 3 hours, with the temperature at 1°C and relative humidity at 99%. These were the normal conditions. At about the same temperature (2°C) a concentration of 500 mg lactic acid/cu m gave about a 90% reduction in 3 hours. A concentration of 1000 mg lactic acid/cu m gave only a slightly better result than this, even when the air was sampled after 14 hr. However, when the lactic acid was heated for 2 hours at 121°C before use, a concentration of 300 mg/cu m was sufficient to give 90% reduction in the air-borne population; increasing the concentration of pre-heated lactic acid to 500 mg/cu gave no greater reduction. The heating of lactic acid under the above conditions is reported to cause depolymerization (Umbreit, Burris, and Stauffer 1959).

Effect of Atmospheric Environment.- Table 1 also shows the effect of temperature and relative humidity on disinfection with lactic acid aerosol in the commercial rooms. It was noted that, with unheated lactic acid at an air concentration of 300 mg/cu m, increasing the room temperature to 6-10°C gave 80-90% reduction of population. This confirms the results in the test room, where it was shown that an increase in temperature favoured lactic acid disinfection.

No greater improvement was obtained by decreasing the relative humidity to about 70%. Thus, a concentration of 300 mg unheated lactic acid/cu m gave nearly 90% reduction in population, but increasing the concentration to 500 mg lactic acid/cu m at this relative humidity had little further effect. A 90% reduction in the air-borne micro-organisms in the chillers seems to be the maximum reduction attainable under all conditions tested.

To summarize, a 90% reduction of air-borne micro-organisms can be obtained by any of four methods:

(1) Increasing the room temperature to 5-6°C and using 300 mg unheated lactic acid/cu m when the relative humidity is high.

(2) Decreasing the relative humidity to 70% and using 300 mg unheated lactic acid/ cu m when the temperature is low.

(3) Using 500 mg unheated lactic acid/cu m when the temperature is low and relative humidity is high (normal conditions).

(4) Heating the lactic acid for 2 hours at 121°C and using 300 mg/cu m of this heated lactic acid, again under normal conditions of temperature and relative humidity.

The Effect of Repeated Applications of Lactic Acid.- During some experiments the chillers were being used for normal commercial production. In one case the chilling room had a low initial population of micro-organisms and in the other the initial population was high. In the first case, i.e., with the low initial population, the population after two sprayings was only 13% of the initial, even though the room was constantly being loaded and unloaded. With the high initial population, the population after a period of 57 days (with four sprayings over a period of 29 days) was only about 26% of the initial. At 36 days the population was down to 13% of the initial. During the last three weeks no further treatment was given and the population rose by only 13% (of the initial) during this time.

Frequent use of lactic acid had the effect of reducing the air-borne population, irrespective of the initial level of contamination. However, no reduction of the wall population was achieved with lactic acid disinfection. In all these experiments in chill rooms, it was found that approximately 90% of the organisms remaining in the air were moulds.

Formaldehyde disinfection

Disinfection with formaldehyde was carried out to ascertain the relative effectiveness of the two disinfectants under conditions prevailing in the meat chilling rooms.

In only one instance out of four a 90% reduction in air-borne population was achieved in 14 hours. In three of these instances there was no reduction at all. In a further four experiments, with samplings at 38 hours, the reduction ranged from 83% to 91%. In two other experiments, when samples were taken at 24 hours, the reduction achieved was 66% in one case, and 88% in the other. In an experiment with samplings at 14, 24, and 38 hours, the air-borne population continued to decrease with time, with a 33% reduction at 14 hours, 64% at 24 hours, and 89% after 38 hours. As with lactic acid, only a 90% final reduction of air-borne micro-organisms was achieved.

The Effect of Repeated Applications of Formaldehyde.- In an experiment with a low initial population, formaldehyde prevented any population increase without causing any further reduction. However, it was noticed that on another occasion rooms treated by the abattoir staff with four treatments over a period of six weeks were reduced from a high population to a population approximating to the level obtained in this experiment.

Disinfection by formaldehyde achieved a 60-80% reduction in wall population. This is one advantage of disinfection with formaldehyde, since no reduction in wall population occurred with

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lactic acid.

CONCLUSIONS

A comparison of lactic acid and formaldehyde as chiller disinfectants shows that lactic acid has some distinct advantages over formaldehyde. They are:

(1) Lactic acid is non-toxic whereas formaldehyde is toxic.
(2) Lactic acid achieves a 90% reduction in 3 hr but formaldehyde needs 24-36 hr. This gives a considerable saving in time and chiller space.
(3) Lactic acid is a normal constituent of muscle and its presence, in these concentrations, will not be a problem.

Formaldehyde has the advantage of reducing the wall population, but preliminary tests have shown that an 0.2% v/v active solution of quaternary ammonium compounds gave a reduction of about 95% on the number of bacteria, yeasts, and moulds on the chiller walls. This washing requires very little in the way of time and labour and can be done just prior to loading the rooms with meat. It is likely, therefore, that, in conjunction with this wall washing, lactic acid spraying can provide a safe and useful means of keeping chillers in good prophylactic condition.

SUMMARY

Lactic acid spraying of the room atmosphere was compared with existing methods of disinfecting chilling rooms by means of formaldehyde fumigation.

In a small experimental room it was shown that lactic acid was a very good disinfectant, and a reduction approaching 100% of air-borne microbial flora was achieved. It was most effective against bacteria, with yeasts more sensitive than moulds. The rate of disinfection increased on raising the air

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temperature from 0°C to 10°C. Disinfection was more effective between 75 and 85% R.H. than at 90% R.H.

Lactic acid when sprayed into meat chilling rooms from a 50% v/v solution has been shown to have some disinfecting power. With a concentration in the air of 300 mg pre-heated lactic acid/cu m a decrease in the air-borne population of 90% was obtained in three hours. The effect of repeated sprayings was to keep the population down to a low level, but no effect on the wall population was observed. On the other hand, the conventional paraformaldehyde method required 24-36 hr for a 90% reduction of microbial population. Paraformaldehyde is toxic and must be completely dissipated before loading the rooms with meat, but does give a 60-80% reduction in wall population.

Lactic acid is proposed as being more suitable for chilling room disinfection because of its speed of action and lack of toxicity. Wall growth can be easily retarded by washing the walls with quaternary ammonium compounds.

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TABLE 1. Effect of temperature, relative humidity, and concentration on lactic acid disinfection in meat chilling rooms

Temp. (°C)	Relative Humidity (%)	Lactic Acid (mg/cu m)	Reduction in air population (% of orig.) ½ hr	3 hr	14 hr
1	99	300	46	59	--
2	96	500	54	89	--
2	96	1000	57	91	92
2	96	300*	80	91	--
2	94	500*	79	90	--
6	95	300	65	88	91
10	95	300	50	80+	--
2	68	300	64	89	--
3	69	500	61	90	--

* Lactic acid pre-heated 2 hr at 121°C.

\+ Sampled after 2 hr.

Figure 1. Effect of concentration of lactic acid on K_D values using Penicillium M107 at 0.1°C at different relative humidities.

(1) 90% R.H. (2) 85% R.H. (3) 80% R.H.

(4) 75% R.H.

Figure 2a. Effect of temperature and type of micro-organism on K_D values with lactic acid

(1) Penicillium M107; (2) Sporotrichium 74; (3) Yeast 18; (4) Lactobacillus 89; (5) Micrococcus 34; (6) Pseudomonas 1482. Lactic acid concentration 120 mg/ cu m; 90% R.H.

Figure 2b. Lactic acid at a concentration 300 mg/cu m; 90% R.H.

Figure 3. Effect of repeated disinfections with lactic acid and formaldehyde on the air population of meat chilling rooms.

(1) Spraying with 300 mg heated lactic acid/cu m starting with a low initial population.

(2) Repeated sprayings with 300 mg heated lactic acid/ cu m starting with a high initial population.

(3) Repeated treatment with formaldehyde vapour.

Note. The lactic acid was sprayed in, or the paraformaldehyde burners lit, at times marked thus ↑ .

ZUSAMMENFASSUNG DER ABHANDLUNG
"DESINFEKTION DER LUFT IN KÜHLRÄUMEN MIT MILCHSÄURE"
von MAXWELL K. SHAW.

Die bekannte Methode, nach der Kühlräume durch Formaldehyde-Ausräucherung desinfiziert werden, wurde verglichen mit der Bestäubung des Raumes durch Milchsäure.

In einem kleinen Versuchsraum wurde gezeigt, dass Milchsäure ein sehr gutes Desinfektionsmittel ist; die Mikroben-Flora in der Luft wurde um beinahe 100 % reduziert. Sie war äusserst wirksam gegen Bakterien, und zwar war Hefe empfindlicher als Schimmel. Die Desinfektionswirkung steigerte sich mit dem Temperaturanstieg von 0° C auf 10° C. Auch war die Wirkung bei einer relativen Luftfeuchtigkeit zwischen 75 und 86 % grösser als bei 90 %.

Milchsäure, die in einer 50-%-igen v/v Lösung in Fleisch-Kühlräumen versprüht wurde, hatte desinfizierende Wirkung. Bei einer Konzentration in der Luft von 300 mg vorgeheizter Milchsäure per m^3 wurde ein 90-%iger Rückgang der Luft-Kleinlebewesen in drei Stunden erzielt. Durch wiederholtes Stäuben konnte die Zahl der Kleinlebewesen auf einem Minimum gehalten werden, es wurde jedoch keine Wirkung auf die Kleinlebewesen an der Wand erzielt. Andrerseits erforderte die übliche Methode mit Paraformaldehyde 24 - 36 Stunden, um die Mikroben um 90 % zu verringern. Paraformaldehyde ist giftig und muss vor dem Füllen der Räume mit Fleisch völlig verschwunden sein. Es reduziert die Wand-Mikroben allerdings um 60 - 80 %.

Infolge seiner schnellen Wirksamkeit und Harmlosigkeit bezüglich Gift wird Milchsäure als geeigneter zur Desinfektion von Kühlräumen betrachtet. Ausbreitung der Wand-Mikroben kann leicht verhindert werden, indem man die Wände mit quarternärer Ammoniak-Verbindung wäscht.

PRECIS D'EPREUVE

"LA DESINFECTION DE L'AIR DES CHAMBRES FROIDES PAR L'ACIDE LACTIQUE"

par MAXWELL K. SHAW

La pulvérisation de l'atmosphère de la chambre par acide lactique fut comparée aux méthodes existantes de désinfection des chambres frigorifiques par fumigation formaldéhyde.

Dans une petite chambre d'essai, il fut démontré que l'acide lactique était un très bon désinfectant et une réduction de près de 100 % des microbes végétaux de l'air fut achevée. Il fut tres efficace contre les bactéries, plus sensible avec les levures qu'avec les moisissures. Le taux de désinfection s'accrut lors de l'élévation de la température de l'air de 0°C à 10°C. La désinfection fut plus efficace entre 75 et 85 % R.H. qu'à 90 % R.H.

Un acide lactique d'une solution de v/v 50% vaporisé dans une chambre frigorifique pour viandes a démontré avoir un pouvoir désinfectant. En concentrant dans l'air 300 mg d'acide lactique préalablement chauffé l'on a pu obtenir en trois heures une diminution de 90 % des microbes de l'air. Des pulvérisations répétées avaient pour effet de garder la quantité des microbes de l'air à un niveau très bas, mais aucun effet sur les microbes des parois ne fut constaté. D'un autre côté, la méthode conventionnelle paraformaldéhyde demande 24-36 heures pour une réduction de 90 % des microbes. Paraformaldéhyde est toxique et doit être complètement dissipé avant l'entrepôt de viandes dans les chambres, mais, il donne une réduction de 60-80 % des microbes muraux.

L'acide lactique est proposé comme étant le plus propre à la désinfection des chambres froides en raison de la rapidité de son action et de son manque de toxiquité. Le progrès des microbes muraux peut être aisément retardé par le lavage des parois avec des compsotions d'ammonium quaternaire.