HEAT OUTPUT OF THERMOPHILES OCCURRING ON WOOL

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In an experiment described by Walker and Williamson (1957), the temperature of a bale of slipe wool (wool removed from the pelt by a chemical depilatory applied to the flesh side, a process described in detail by Wright (1921)), which had been packed wet, rose from 17 to 71 C in 48 hr. This corresponds to an average generation of heat over this period of at least 3.5×10^{-4} cal:sec:g (of dry wool), neglecting heat losses. The peak rate of generation of heat would be somewhat in excess of this. Other bales of slipe wool behaved similarly. Walker and Williamson (1957) assumed that the heating was caused by bacteria, but made no attempt to prove this. The present investigation was undertaken to determine whether bacteria are able to generate heat outputs of this magnitude in wet slipe wool.

Several workers have investigated the adiabatic heating of wet biological materials (e.g., Carlyle and Norman, 1941; Prat, 1952). It is usually accepted that 76 C is the maximal temperature that can be reached by the action of thermophilic microorganisms, because their metabolism ceases beyond this limit (Edwards and Rettger, 1937). Carlyle and Norman (1941) estimated that the maximal heat output of wet straw is approximately 26×10^{-4} cal:sec:g (of dry straw) and occurs at about 40 C with bacterial populations up to 1.6×10^{9}/g corresponding to a heat output of 1.6×10^{-12} cal:sec:cell. A second maximum, due to thermophiles, occurs at 60 C giving approximately 15×10^{-4} cal:sec:g (of dry straw) in the presence of only 1.6×10^{6} cells/g, which corresponds to 950×10^{-12} cal:sec:cell. But these authors state that they do not regard their thermophilic counts as entirely satisfactory. Prat (1952) measured the heat output at 24 C of 24 species of moistened seeds and after 24 hr obtained heat outputs ranging from 1.4 to 5.5 $\times 10^{-4}$ cal:sec:g, which he concluded were of biological origin. No bacterial counts were given.

Bayne-Jones and Rhees (1929) studied the heat output of *Escherichia coli* in slightly aerated nutrient solutions at 37.5 C and the highest heat output recorded was only 0.30×10^{-4} cal:sec:ml at a concentration of 0.32×10^{8} bacteria/ml, corresponding to 0.9×10^{-12} cal:sec:cell. Even this heat output was only maintained for about 1 hr, and dropped considerably as the population increased. The greatest heat output per cell was 2.1×10^{-12} cal:sec:cell at the low population of 0.072×10^{8} cells/ml. No more recent measurements of heat output per organism have been found, but an interesting application of bacterial calorimetry is the work of Jeney (1949) who measured the effectiveness of penicillin by its depression of heat given off by bacterial broths. The maximal heat output before the addition of penicillin was approximately 0.9×10^{-4} cal:sec:ml but no bacterial counts were done. Zobell, Sisler, and Oppenheimer (1953) postulated bacterial heating to account for temperature rises of mud at lake bottoms and in their calculations which are based on consumption of organic matter in seawater, assume a heat generation of 0.01×10^{-12} cal:sec:cell, but state that they believe this to be a very conservative estimate. These published results would suggest that the bacterial heat output in nutrient broths is much smaller than in straw and seeds, but at the same time the heat output per cell in straw at 60 C appears unreasonably large. The present paper investigates these discrepancies.

METHODS

The wool used in this work was lamb's slipe wool (Wright, 1921) either directly obtained from the freezing works and with a water content of about 80 per cent regain (g water/100 g dry wool) or soaked in water and centrifuged in the laboratory to the same regain. Fresh wool from the freezing works and soaked wool reacted similarly. A typical first-grade slipe wool has a pH of 8.5 to 9.5 and contains about 3.5 per cent ether solubles and 1.5 per cent subsequent alcohol soluble matter. A few experiments on scoured

wool were also performed. Four lines of approach were used: (i) Investigation of optimal conditions for counting thermophiles; (ii) the measurement of heat output of wet slipe wool and scoured wool aerated with saturated air; (iii) experiments to prove that the entire heat output of slipe wool at 60 C is due to microbiological activity; and (iv) measurement of heat output of wool thermophiles in well aerated nutrient broth.

Bacterial counts. Bacterial counts were performed after vigorously shaking approximately 1 g of wool for 2 min with 100 ml of sterile Ringer's solution, and plating out at least three known dilutions. In experiments on thermophiles, the Ringer's solution was at 60 C, and for mesophiles at 37 C. Subsequently the sample of wool was oven dried to determine its exact weight.

The plates with wool mesophiles were incubated for 2 days at 37 C using a tryptone glucose extract agar medium (Difco). No attempt was made to isolate any species from the mixed flora occurring in the wool.

The counting of thermophiles has recently been reviewed by Neilson, MacQuillan, and Campbell (1957) who found existing techniques gave variable results and recommended a procedure that gave them counts up to 4.4×10^8 thermophiles/g which they claim to be higher than previously recorded counts. These authors had some difficulty with plates drying out, and although they carefully investigated the effects of the pH of the diluent, apparently did not study the effect of pH of their media. They do not quote the previous work of Imsenecki and Solnzeva (1945) who obtained thermophile counts in broths at 60 C up to 4×10^8/g, and found that, on strongly aerating their broths, counts up to 2.3×10^9/g could be obtained and therefore concluded that thermophilic growth is limited by availability of oxygen.

In the procedure used here, all plates were incubated in loosely closed tins, containing open beakers of water. It was established that the humidity inside the tins was 89 per cent at 60 C (the optimal growth temperature) and under these conditions no appreciable drying out of plates was noticed even after 2 weeks. Initially only tryptone glucose extract agar medium was used, but it was quickly noticed that the counts of the small thermophile colonies varied greatly with pH of the medium. Media in a range of phosphate, glycine, and borate buffers between pH 5.8 and 10.0 were tried, and optimal plating conditions were found to be in a borate buffer at pH 8.4. In a glycine buffer at the same pH equal counts, but smaller colonies, were obtained, whereas phosphate buffered or unbuffered media gave far lower counts. The final medium was prepared from 24 g tryptone glucose extract agar with 7.5 g agar (to improve the rigidity of the medium at elevated temperatures) in 1 liter, containing 50 ml N HCl, 250 ml 0.2 M H_3BO_3, and 60 ml of filtered N NaOH in which 6 g of dry slipe wool had been hydrolyzed. (Hydrolyzed wool was found to increase the colony size considerably.) Incubation for 16 hr was found sufficient at 60 C.

It was shown that at least 95 per cent of all extractable organisms were in the Ringer's solution by counting bacteria on wool extracted a second and third time with fresh Ringer's solution. Thermophile counts performed on a Ringer's extract were substantially unaltered after storing the extract in a closed bottle at 60 C for up to 1 week.

Heat output measurements. Heat outputs of bacteria have often been measured by observing temperature rises in Dewar flasks and correcting for heat losses. It was not considered possible to use this method when the contents of the flask have to be continuously strongly aerated. The method chosen depends on measuring the temperature at the center of a flask filled with wool, when heat losses just balance heat production. The system is calibrated electrically, and calculations involving the thermal conductivity and specific heat of wool show that equilibrium is always established in less than 6 hr, so no readings were taken before this time. The advantage of this method is that no correction for thermal losses is needed as they are allowed for by the electrical calibration. The principal heat loss is along the copper wire of the thermocouple and amounts to approximately 10 per cent of the total heat produced. The gas passing into the flask will cause no appreciable heat loss provided it is saturated with water vapor.

The heat output measurements were performed by filling a 2-liter three-necked glass flask with 400 g of slipe wool at 80 per cent regain. Small samples of wool could be removed periodically with sterile forceps for bacterial counts. The flask was fitted with glass tubes for inlet and outlet of gas at diametrically opposite sides and a glass tube, sealed at the tip, was positioned at the center and was used for inserting one junc-

tion of a copper/constantan thermocouple, whereas the other junction was in a tube filled with oil, which was in a well lagged water tank in which the flask was completely immersed. The temperature of the bath was controlled to ±0.02 C by a mercury contact thermometer, and checked with National Physical Laboratory calibrated thermometers. The differential thermocouple then measured the excess temperature at the center of the flask, and under equilibrium conditions this was shown to be directly proportional to the heat output of any reaction occurring.

The filled flask was calibrated by preparing a long spiral of enameled nichrome wire (diameter 0.013 in.), winding acetone extracted wool around the wire, and working the spiral and thermocouple sheath into the flask, which was then sealed. An X-radiography confirmed that the wire distribution within the flask was uniform and the thermocouple was at the center of the flask. Current from a storage battery was then passed through the wire and figure 1 shows the calibration of wattage against temperature differential (after 12 hr) using wool at different regains (water contents) whose dry weight in all cases was 220 g. For air dried wool of 15 per cent regain and moist wool of 40 per cent regain, the calibration was reproducible at bath temperatures ranging from 20 to 90 C. For wet wools the contribution of liquid water to the thermal conductivity becomes noticeable and the calibration was less accurate and tended to alter with time as some water collected at the bottom of the flask, reducing the thermal conductivity in the middle. Figure 1 shows calibrations at 60 C, each curve representing at least two different packings of the flask. For 15 per cent and 40 per cent regain, the accuracy of calibration is estimated to be ±10 per cent, whereas at 80 per cent regain, the accuracy is at least ±30 per cent.

The slipe wool was aerated, by passing air at uniform controlled rates through three humidifiers in series immersed in the thermostat tank, before it entered the reaction flask. This ensured both that the air was at tank temperature and fully saturated, thus causing no heat loss by evaporating water from the wool, provided its temperature was not much above bath temperature. After leaving the wool, the air passed through a recording thermal conductivity type carbon dioxide analyzer. This apparatus was calibrated and frequently checked by absorbing the carbon dioxide for known periods in sodium hydroxide solution and titrating with standard hydrochloric acid. Carbon dioxide and oxygen were also determined as a percentage of out-

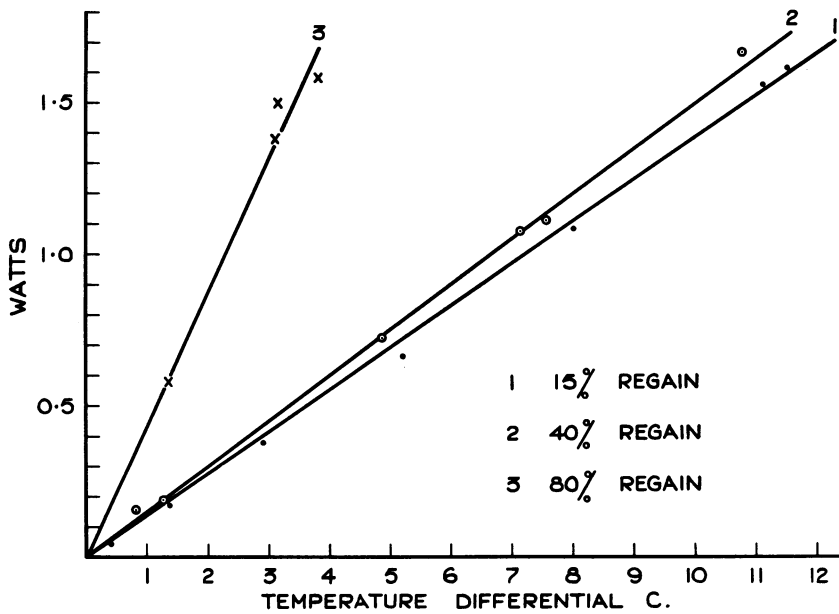

Figure 1. Electrical calibration of a 2-liter reaction flask containing acetone extracted wool (220 g dry weight) at different regains.

flowing air, by absorption in alkali and alkaline pyrogallol. Making a correction for diminution in volume allowed the oxygen absorbed from the incoming air to be calculated. The air flow rate was chosen so that the carbon dioxide concentration was between 1 and 4 per cent, as small amounts of carbon dioxide are beneficial to bacterial growth (Harris, 1954, showed that 0.8 per cent carbon dioxide stimulated the metabolism of 38 bacterial species), whereas 4 per cent was considered to be small enough not to impair their metabolism.

The heat output of aerated broths was determined by inoculating 200 ml of sterile broth with 5 ml of an extract of slipe wool, made with Ringer's solution at 60 C. The broth, containing approximately 2×10^6 thermophiles per ml was uniformly spread on the surface of 200 g asbestos fiber, which had been washed in running water and dried in an oven at 110 C for 2 days. The resulting mixture showed very few heterogeneities, was packed into the 2-liter flask, and measurements were taken in the same way as for slipe wool.

RESULTS

Although no detailed investigation of the types of thermophilic bacteria in wool was made, a typical plate, which contained about 200 colonies, and had been incubated at 60 C and used for counting wool thermophiles, was examined more closely. Six colonies were picked off, subcultured, and characterized as far as possible according to the methods of Smith, Gordon, and Clark (1952). Three of the organisms were identified as *Bacillus coagulans* Hammer and two as *Bacillus stearothermophilus* Donk. The remaining organism, which showed the characteristics of *Bacillus sphaericus* Neide, appeared to be a contaminant, as its maximal growth temperature was 47.5 C.

Seventeen commercial slipe wools all taken from one freezing works were examined and all gave similar reactions. Bacterial counts, heat outputs, and carbon dioxide production figures of a typical wool after 24 hr at fixed temperatures are given in table 1. After 1 week, heat outputs dropped to about 45 per cent of the original value in the case of mesophiles at 37 C, but only to 85 per cent in the case of thermophiles at 60 C. The metabolism of thermophiles can therefore be considered uniform over several days at their optimal temperature.

From the amount of carbon dioxide evolved, heat outputs were calculated by assuming a heat of combustion of 130 kcal/mole CO_2 produced. This is a reasonable mean value as the literature gives figures varying from about 115 for carbohydrates, 120 to 140 for amino acids, to 160 for long chain fatty acids and alcohols.

At 37 C mesophiles produced 50 ml carbon dioxide per 100 ml oxygen absorbed, whereas at 60 C thermophiles produced 83 ml carbon dioxide per 100 ml oxygen.

A few experiments were performed on scoured wool. Laboratory acetone extracted wool (containing 0.04 per cent ether soluble matter and 0.02 per cent subsequent alcohol solubles) was soaked in distilled water, seeded with wool thermophiles, centrifuged to 80 per cent regain, and examined in the same way as slipe wool. After 24 hr at 60 C it gave off 1.9×10^{-8} g:sec:g (of dry wool) of carbon dioxide and 0.2×10^{-4}

TABLE 1

Carbon dioxide production and heat output (experimental and calculated) of wet slipe wool (first cross bred pelts) after 24 hr at various temperatures; also bacterial population and heat output per cell

Temp (C)	CO_2 Production (g:sec:g dry wool $\times 10^8$)	Mesophiles/g Dry Wool*	Thermophiles/g Dry Wool†	Measured Heat Output (cal:sec:g $\times 10^4$)	Calculated Heat Output (cal:sec:g $\times 10^4$)	Heat Output (cal:sec:cell $\times 10^{12}$)
26	18.5	8.0×10^8	$< 10^3$	11.0	5.5	1.4
37	33.0	24.0×10^8	4.0×10^3	15.0	9.7	0.6
50	14.7	1.5×10^8	6.5×10^8	4.2	4.3	0.5
60	17.5	5.0×10^6	4.0×10^8	6.0	5.2	1.5
70	16.4	5.0×10^5	3.9×10^8	3.4	4.8	0.9
74	9.2	$< 10^3$	5.0×10^8	1.7	2.7	0.3
78	0.8	$< 10^3$	5.0×10^6	0.0	0.2	0.0

* Counted after incubating plates 2 days at 37 C.

† Counted after incubating plates 16 hr at 60 C.

cal:sec:g at a bacterial population of 0.2 × 10^8/g, corresponding to a heat output of 1.0 × 10^{-12} cal:sec:cell. The pH was 7.8. Acetone extracted wool that had been soaked in borate buffer at a pH of 8.0 and commercial scoured wool (containing 0.42 per cent ether soluble matter) gave substantially the same results for carbon dioxide production and thermophile heat output.

If the values in table 1 are to be accepted as bacterial heat outputs, it must be proved, by sterilization and reseeding, that wet wool at 60 C has a negligible heat output due to any possible chemical oxidation.

Experiments with sterilized wool suffer from the disadvantage that most sterilizing procedures also modify the structure of the wool itself. Recently Noval and Nickerson (1959) described a sterilization method using ethylene oxide, which, it is claimed, produces no chemical change in the wool. However, if ethylene oxide is introduced into wool in the apparatus described here, a definite temperature rise is noted, which means that some reaction must be taking place. A simple approach was therefore used which consisted of leaving wool in the normal apparatus with continuous aeration at elevated temperatures for sufficiently long periods to ensure that on returning to 60 C there was a negligible heat output and bacterial population. It was found empirically that 3 days at 90 C was sufficient for this purpose, and figure 2 shows a typical experiment in which slipe wool with normal heat output at 60 C was left at 90 C and showed no detectable heat output or bacterial population for 3 days after returning to 60 C. Reseeding with 10 ml of an extract of slipe wool that had been incubated at 60 C immediately brought about normal bacterial heating. The cycle was repeated and this time the wool on returning to 60 C showed no heating for 7 days until reseeded with bacteria. A drop in temperature to 30 C halted the heat output and carbon dioxide production, which recommenced as soon as the temperature was returned to 60 C. A final temperature rise to 80 C once again stopped the reaction.

It is not claimed that the heating procedure caused no change in the structure of the wool, but the fact that artificially heated wool will resume heat production and carbon dioxide evolution repeatedly only after reinoculation proves conclusively that at least the major heat production is due to bacteria. After 1 day at 60 C and then raising to 90 C the carbon dioxide production rate dropped to 4 per cent of its initial value. As many chemical reactions double their rate approximately every 10 C, it can be assumed that a reaction at 60 C will give off one eighth of the carbon dioxide given off at 90 C. On this assumption less than 1 per cent of the

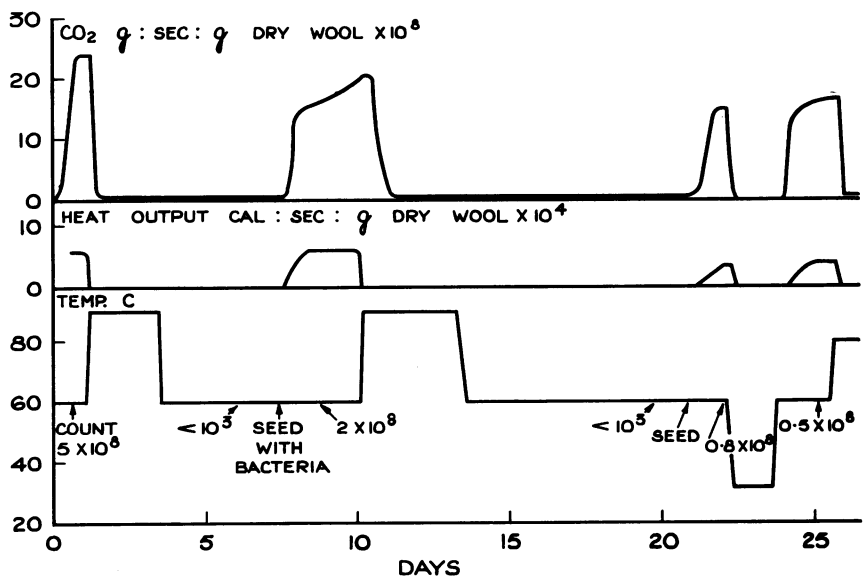

Figure 2. Carbon dioxide production, heat output, and plate counts of heated, reseeded, and cooled slipe wool.

TABLE 2

Carbon dioxide production and heat output (experimental and calculated) of aerated broth at 60 C, seeded with thermophiles; also bacterial populations and heat output per cell

Hr	CO_2 Production (g:sec:ml broth $\times 10^8$)	Measured Heat Output (cal:sec:ml $\times 10^4$)	Calculated Heat Output (cal:sec:ml $\times 10^4$)	Thermophiles/ml	Heat Output (cal:sec:cell $\times 10^{12}$)
2½	25	7.5	7.4		
3	50	13.2	14.8		
4	95	23.0	28.2		
4¾	120	26.0	35.6	1.9×10^9	1.25
5	115	24.5	34.0		
6	95	19.0	28.2		
7	70	15.1	20.8		
8	65	13.0	19.3		
9	37	10.0	11.1		
13	20	2.5	5.9		
19	10	1.4	3.0	2.2×10^8	0.65

heat output at 60 C can be due to nonbacterial oxidation.

Confirmatory evidence that the heat output at 60 C is due to bacterial action is given by the negative temperature coefficient of the heating reaction between 60 and 78 C (table 1), and by an experiment performed at 60 C, in which the changing of air to oxygen reduced the heat output from 6.0×10^{-4} cal:sec:g to 2.2×10^{-4} cal:sec:g. Changing back to air restored the high value, giving 5.2×10^{-4} cal:sec:g. These features are quite imcompatible with a chemical reaction, but are easily reconciled with a biological action whose optimal conditions are in air at 60 C.

Some experiments were performed to give the heat output of thermophiles in broth absorbed on asbestos fiber where it can be easily aerated. An electrical calibration using 200 g asbestos moistened with 200 g water gave a value of 7.4×10^{-4} cal:sec:g asbestos for a central temperature rise of 1 C at 60 C. Although complete thermal equilibrium in the flask is only reached in 3 hr, the central temperature differential is 70 per cent of its final value in ½ hr, 90 per cent after 1 hr, and 96 per cent after 1½ hr. Reactions in broths, involving appreciable heat production and reaching maxima in 4 to 6 hr have, therefore, had a small correction applied to them, to allow for incomplete thermal equilibrium.

Table 2 gives the carbon dioxide production and measured and calculated (assuming 130 kcal/mole CO_2) heat output in a typical experiment at 60 C with 200 ml seeded broth containing beef extract, 3 per cent; tryptone, 5 per cent; and glucose, 1 per cent. Bacterial counts and heat output per cell are also shown.

A similar experiment using a broth containing 2 per cent peptone (the same concentration as that used by Bayne-Jones and Rhees, 1929) gave a maximal carbon dioxide production of 11.2×10^{-8} g:sec:ml broth, a heat output of 4.7×10^{-4} cal:sec:ml at a population of 4.0×10^8 thermophiles/ml. The heat output is therefore 1.15×10^{-12} cal:sec:cell.

An experiment performed with 0.2 per cent mercuric chloride in the seeded broth gave no measurable heat output or carbon dioxide production. It can be concluded that the heat output recorded in table 2 is due to bacterial oxidation of the broth.

DISCUSSION

The greatest heat output per cell of wool thermophiles obtained in this work (1.5×10^{-12} cal:sec:cell on wool and 1.25×10^{-12} in broth) may not necessarily be the greatest heat output per cell possible at a smaller population, but is of the same order as the maximal heat output of *E. coli*, 2.1×10^{-12} cal:sec:cell, obtained by Bayne-Jones and Rhees (1929) in slightly stirred nutrient solutions. However, the maximal total bacterial heat output in nutrient broth obtained here (26.0×10^{-4} cal:sec:ml) is 90 times the value of Bayne-Jones and Rhees (1929). This is undoubtedly due to far more satisfactory aeration and the use of a stronger broth, permitting a maximal population of 2×10^9 thermophiles/ml which is approximately the same as that obtained by Imsenecki and Solnzeva (1945).

It is not surprising that bacterial heat outputs in a broth decrease in the course of a few hours, because all nutrients would be completely oxidized in the course of about 2 days at the maximal rate of carbon dioxide production. Wet wool, however, presents a very rich medium which can be maintained at nearly 100 per cent humidity, and at the same time be well aerated. The heat output on wool can, therefore, be maintained for considerably longer periods.

Carlyle and Norman (1941) estimate that heating straw contains 1.6×10^6 thermophiles/g straw at 60 C. This would correspond to 950×10^{-12} cal:sec:cell but the authors themselves

questioned their thermophile plating technique. Using the counts and heat outputs obtained here, there is no difficulty in explaining the heating of wet slipe wool bales. Walker and Williamson (1957) showed that heat production in a wet wool bale (86.2 per cent regain) corresponds to at least 3.5×10^{-4} cal:sec:g (of dry wool). It can be seen from table 1 that mesophiles generate 15.0×10^{-4} cal:sec:g at 37 C and thermophiles produce 6.0×10^{-4} cal:sec:g at 60 C, and 3.4×10^{-4} cal:sec:g at 70 C, and can therefore easily raise the temperature of a wet bale to 70 C in the observed time. As the heat output at 74 C is 1.7×10^{-4} cal:sec:g and at 78 C 0.0×10^{-4} cal:sec:g, the maximal temperature that the center of the bale could reach is approximately 76 C, when thermophiles cease producing heat. Oxygen starvation may be responsible for a central temperature a few degrees below 76 C. The heat output per cell is of the same order on both slipe and scoured wool, but scoured wool, at the same pH as slipe wool, can only support about $\frac{1}{10}$ of the population of either mesophilic or thermophilic microorganisms. The total bacterial heat output of wet slipe wool is therefore approximately 10 times that of wet scoured wool. Slipe wool apparently contains some substances that promote bacterial growth.

In tables 1 and 2 the heat output of bacteria is compared with a value calculated on the assumption that all the heat results from organic molecules being completely oxidized to carbon dioxide. The satisfactory agreement between experimental and calculated values for thermophiles and the ratio of 1.20 of oxygen absorbed to carbon dioxide produced support this assumption.

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SUMMARY

Up to 10^9 thermophiles/g dry wool can be present on well aerated wet slipe wool. Optimal conditions for counting these have been determined empirically. The heat output per organism has been measured at a number of temperatures and shown to be at a maximum at 60 C. The heat production can be maintained for several days and was shown to be sufficient to heat wet slipe wool bales to over 70 C. The population of thermophiles on scoured wool is lower than on slipe wool, and total heat production is correspondingly less.

In well aerated broths, populations up to 1.9×10^9 thermophiles/ml have been obtained, and the heat output per organism was found to be of the same order as on wool.

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