Growth of staphylococci on nutrient agar at low temperature (12°C).

It is well recognised that staphylococci will grow over a wide range of temperatures and that the minimum of the range is relatively low. There appears to be no documented evidence on the subject, however, particularly with reference to any species differences that might exist within the genus. Routine bench experience in hospital laboratories commonly gives the impression that *Staphylococcus aureus* grows much more readily at bench temperatures than coagulase negative species. Some preliminary experiments designed to provide objective data on this matter showed this to be correct. At a temperature of 15°C all of a small batch of *S aureus* isolates grew abundantly on nutrient agar within five days, whereas non-pigmented coagulase negative staphylococci grew either slowly or not at all within that time. Several pigmented isolates of this category did, however, grow as well as the coagulase positive organisms. It was then established, again with small batches, that all coagulase positive organisms also grew well at 12°C, whereas none of the non-pigmented coagulase negative isolates showed visible growth within 20 days. Again, several of the pigmented isolates grew well.

More extensive trials were then set up at this temperature (12°C), with all pigmented coagulase negative organisms excluded. Testing for coagulase was done by a tube method using human plasma. Colony appearance, coccoid morphology, Gram stain positivity, and catalase positivity were the only other characteristics observed in the selection. Single colony isolates were selected randomly from routine cultures mainly of wounds, skin, and nares and cultured in nutrient broth. Early stationary phase growth was used for inoculations and the broth cultures were diluted 1/200. About 0.2 ml of each suspension was inoculated on to a nutrient agar slope for low temperature incubation and on to a nutrient agar plate to check for growth at 25°C. The agar medium was blood agar base no 2 (Oxoid). Slopes were used in preference to plates for the low temperature studies to reduce contamination, which was inevitable on plates incubated for such long periods. The slopes were placed in sealed containers to reduce evaporation and then incubated in a cabinet maintained at 12°C. They were examined initially at weekly intervals and later more irregularly over at least 60 days. The container of inoculated tubes was

Growth of staphylococci on nutrient agar slopes at 12°C

Staphylococci	Growth at 12°C	
	No of isolates	% Showing growth
Coagulase positive	150	100
Coagulase negative	300	7

given a preliminary cooling at 4°C for about 30 minutes before placing it in the cabinet in order to reduce the period of exposure to bench temperature and to effect a more rapid equilibration with the experimental temperature.

The table summarises the results of the study. All of the coagulase positive organisms tested yielded heavy growth within seven days, whereas only 7% of the coagulase negative organisms yielded visible growth during incubation periods ranging from 60 to 150 days. Most of the organisms in the latter category which grew did so in about the same time as that taken by the isolates of *S aureus*. A few, however, grew slowly or poorly. A large proportion of the coagulase negative isolates which failed to grow at 12°C also remained negative when transferred to a temperature of 37°C, indicating that the low temperature incubation had been lethal.

Using API methods some of the coagulase negative low temperature positive isolates were speciated and included: *S warneri*, *S hyicus*, *S cohnii*, and *S xylosus*. Coagulase negative, low temperature negative isolates included: *S epidermidis*, *S simulans*, and *S haemolyticus*.

This work, although essentially exploratory, nevertheless showed unequivocally that sharp distinctions existed among the staphylococci with respect to their minimum growth temperatures on nutrient agar slopes. Obviously, the culture medium and other growth conditions such as aeration would be expected to modify the results. While no special measures were employed for critical temperature control, random mixing of the slopes in the containers ensured that no serious bias was introduced in this aspect of the experimental design.

The finding that the coagulase positive staphylococci all grew well at 12°C was not unexpected, but we were surprised that such a large fraction of the coagulase negative organisms failed to grow and, in fact, died at that temperature. With this latter group minimum temperature for growth obviously covers a fairly wide range, with a probable upper limit of about 15°C.

Pigmented coagulase negative staphy-

lococci appear to resemble *S aureus* in their growth temperature patterns but both groups need further investigation at temperatures lower than those used here to provide this data.

The taxonomy of the coagulase negative staphylococci is a highly controversial subject. Low temperature growth studies may provide some useful data for this purpose. Obviously, no useful conclusions can be drawn from the few examples of speciation recorded here.

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DI ANNEAR

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Is the ratio of total cholesterol to albumin useful as a predictor for the risk of coronary heart disease?

In an attempt to diminish the costs of public health care, Nanji and Reddy¹ suggest the use of the total cholesterol to albumin ratio (TC:Alb) for predicting whether an individual has a total cholesterol to high density lipoprotein cholesterol ratio (TC:HDL-C) below or above the discrimination value of 5—that is, normal or diseased, respectively. TC:Alb might then be used to predict the risk of coronary heart disease. We have several criticisms of their paper.

We tried to produce the results of Nanji and Reddy. Our population consisted of arbitrarily chosen outpatients, without liver or kidney disease, attending a general hospital. The Table compares the correlation coefficients obtained in our study with those of Nanji and Reddy. We found no significant correlation (with a level of significance of 5%) between albumin and high density lipoprotein cholesterol. TC:Alb v TC:HDL-C gave a correlation coefficient much lower than that of Nanji and Reddy and of the same magnitude as the correlation coefficient between TC:HDL-C and total cholesterol. So

	Correlation coefficient (r)	
	Nanji and Reddy (n = 122)	Authors' results (n = 46)
Alb v HDL-C	0.32	0.19
TC:Alb v TC:HDL-C	0.89	0.57
TC:HDL-C v TC	0.52	0.55
TC:Alb v TC		0.93

Alb = albumin.

TC = total cholesterol.

HDL-C = high density lipoprotein cholesterol.

the statement of Nanji and Reddy that TC:Alb predicts TC:HDL-C better than total cholesterol alone is questionable. For the sake of clarity, we showed that the correlation coefficient between TC:Alb and total cholesterol was 0.93; as could be expected, dividing total cholesterol by albumin had little effect, because of the relatively small range of reference values of albumin.

2 The high correlation between TC:Alb and TC:HDL-C found by Nanji and Reddy could be the result of a statistical artefact. When screening several correlations, or the same correlation in several groups and taking the highest, it is probable that this high correlation is too high by chance. The difference between the correlation found by Nanji and Reddy and that found by us indicates that such an artefact might be present.

3 Nanji and Reddy also express the relation between TC:Alb and TC:HDL-C in terms of sensitivity and specificity, using TC:HDL-C = 5 as the discrimination value between "not diseased" and "diseased" and TC:Alb = 50 as the discrimination value between a negative and a positive test. For our material we find, using the same discrimination values, a sensitivity of $23/31 = 0.74$ and a specificity of $9/15 = 0.60$, as compared with 0.91 and 0.87, respectively, found by Nanji and Reddy. This reflects the difference in correlations (see 1) and again points to possibly too favourable results of Nanji and Reddy (see 2).

4 Because of the importance of the serum cholesterol concentration in health screening there has been a tremendous effort to standardise the cholesterol assay. This has led to a determination with reduced bias and improved precision. That is why the TC:HDL-C ratio is precise and accurate. Albumin concentration is still dependent on the chosen method and the coefficient of variation (CV) is larger than the CV of the cholesterol assay. Thus merely from the view point of determina-

tion, the TC:HDL-C ratio is preferable to the TC:Alb ratio.

5 The ratio TC:HDL-C is an important predictor for the risk of coronary heart disease. Replacing TC:HDL-C by TC:Alb is likely to weaken the relation with coronary heart disease. The strength of the relation between TC:Alb and coronary heart disease should, however, be studied directly, without the "intermediate" variable TC:HDL-C. Nanji and Reddy themselves suggest such a study in the last sentence of their paper. The search for an indicator for the development of coronary heart disease by relating possible indicators with another indicator should be avoided.

Of course, it is attractive to use the results that are routinely available on multichannel analysers as a less expensive substitute for high density lipoprotein cholesterol measurements. In our opinion, however, albumin is not an alternative for measurement of high density lipoprotein cholesterol.

The economic and social impact of cardiovascular diseases is obvious, but the way Nanji and Reddy¹ suggest savings (see also Nanji² and Philippi and Barrett-Connor³) may diminish the usefulness of screening subjects at risk for coronary heart disease.

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Book Reviews

Orthopaedic Diagnosis. Clinical, Radiological and Pathological Coordinates. HA Sissons, RO Murray, and HBS Kemp. (Pp. 403; 534 figs; £56.) Springer. 1984.

This fascinating and instructive book covers a wide field and can be used both to learn and to test the reader's diagnostic acumen. It consists of a series of 94 individual cases, each with a brief clinical history, prints and descriptions of the radiographs, and a couple of coloured photomicrographs. With this information the reader can make a diagnosis before turning to the answer on the following page. An authoritative short account of each condition is then given along with a radiological mini-atlas illustrating the range of changes and a list of key references. The illustrations are well chosen and most are of excellent quality though a few radiographs are too small to be helpful. The book, as might be expected from the authors, each distinguished in their own field of orthopaedic practice, emphasises the coordination of clinical, radiological, and pathological information in reaching a diagnosis and is aimed at practitioners in all three fields. The pathologist will find this book not just a slightly ego bruising exercise but a source of much helpful, clearly set out, and up to date information which is readily accessible through the excellent index. I warmly recommend this to pathology departments as a useful addition to the standard orthopaedic pathology texts.

MARY E CATT

Microbial Toxins and Diarrhoeal Diseases. Ciba Foundation Symposium 112. (Pp. 286; £27.95.) Pitman. 1985.

One may say "yet another book on microbial toxins and diarrhoea", but having read it, I must admit it has a great deal to offer.