Using this method, cells of yeast and *Bacillus* showed a faintly uniformly stained blue-violet cytoplasm. With a Gram-negative organism (*Azotobacter*) the cytoplasm was practically invisible under light microscope though observable under the phase microscope.

These results show that it is possible at present using ultra-thin sections to observe directly that the Gram stain is located in the cytoplasm of the microbial cell.

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VETERINARY SCIENCE

Specific Gravity of Live Sheep

DURING the past two years three experiments were carried out to determine the feasibility of estimating the specific gravity of live sheep by water displacement. This work was stimulated by the reports of high correlations between carcass specific gravity and the chemical composition of the carcass published by numerous authors and summarized in ref. 1. Moreover, recent work² emphasizes that subjective methods of estimating the proportion of fat in the carcasses of lambs are unsatisfactory.

In the first experiment 30 Merino wethers underwent a starvation period of 40 h and were afterwards weighed in air and then weighed in water twice. The animals were killed and their carcasses weighed in air and water. From these readings the live and dead specific gravities for each animal were calculated, and the sample correlation coefficient for these two measurements was 0.52. However, it was found from the repeated weighings in water that the live specific gravity of 6 sheep had probably been inaccurately estimated, and when these animals were omitted from the analysis, the correlation between live and dead specific gravities increased to 0.59.

The purpose of the second experiment was to improve the general technique, and, in the process, to determine the correlation between repeated specific gravity determinations made on the same sheep during different starvation periods. Fifteen Merino wethers were anaesthetized and weighed in water to a greater accuracy than was possible on the previous occasion, and then returned to pasture. This procedure was repeated six days later and a correlation of 0.46 was obtained between repeated measurements of specific gravity.

The aim of the final experiment was to determine whether or not the technique which had been developed was in fact sufficiently sensitive to pick up between sheep differences in specific gravity. Twenty-two wethers were starved for 40 h, weighed in air, anaesthetized and then weighed in water. The sheep were returned to their pens overnight, and were not allowed access to food or water and a further determination of specific gravity was made on the following day. The correlation between repeated determinations of specific gravity within the same starvation period was 0.66 and it was found that the specific gravity means of the 22 sheep for both days were exactly the same.

The conclusion drawn from this work is that specific gravity can be determined for the live sheep by fluid displacement techniques. The correlations, which were all statistically significant with a one tail test, indicate reasonable repeatability between successive determinations and, also, that live and dead specific gravity values are related. Since the technique and equipment used during this series of experiments were fairly rough, it does not seem unreasonable to postulate better results with improved methods. Certainly, air-displacement investigations, al-

though costly, should give greater accuracy and repeatability.

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CYTOLOGY

Determination by Ammonium of the Manner of Yeast Nuclear Division

ALTHOUGH it is questionable whether mitosis and meiosis in the classical sense occur in *Saccharomyces cerevisiae*¹, there is no doubt that the nuclear divisions observed are genetically equivalent to those of higher forms of life^{2,3}. In sporogenesis, spore walls enclose the haploid nuclei resulting from meiosis, and thus presence of spores in a cell demonstrates that meiosis has occurred. But absence of spores is inadequate evidence that meiosis has not occurred: following formation of the tetrad of haploid nuclei spore walls may conceivably fail to form. Thus although the proportion of sporulated cells in a population is easily determined without staining, a nuclear staining schedule must be utilized for reliable assessment of meiotic frequency. Environmental conditions favouring sporulation are known to differ in many respects from those that promote growth⁴. Comparison of the response of yeast sporulation and growth to nutritional and other factors may therefore help explain how the manner of division (mitosis or meiosis) is determined, provided that the treated cells are examined utilizing a nuclear staining technique.

Cells of a bakers' yeast isolate (*F493*) precultured two days in Wickerham's⁵ yeast nitrogen base (Difco) with 1 per cent glucose as carbon source were added at a cell density of 4 million per ml. to 180-ml. pharmaceutical bottles containing 8 ml. volumes of liquid and incubated for three days at $27 \pm 1^\circ \text{C}$ on their flat sides (to aid aeration). Fuller details of the culture procedure are given elsewhere⁶. The liquid in the bottles was 0.05 M pH 5 phthalate buffer or buffer + 0.01 M ammonium sulphate or buffer + 0.02 M sodium acetate or buffer + salts-vitamins or various combinations of these. The salts-vitamins were as in yeast nitrogen base except that ammonium sulphate, amino-acids, folic acid, *p*-aminobenzoic acid, and riboflavin were omitted. The final concentration of salts-vitamins in the liquid was one-third of that recommended for yeast nitrogen base. Following incubation a sample of cells from each bottle was seeded on a piece of ultra-thin 'Millipore' filter and Giemsa-stained by the method described elsewhere⁷. From each sample, 200 randomly selected cells were examined and classed in one of the five stages of sporogenesis illustrated by Pontefract and Miller¹ (see footnote to Table 1).

The figures in Table 1 are the averages of the results of two experiments, each of which was done in duplicate. It is evident that few nuclei advanced even so far as the first stage of sporogenesis in the absence of the acetate carbon source; but in its presence more than half the cells sporulated and many of the remainder were in various stages of sporulation. Addition of salts-vitamins caused a small reduction of acetate-induced sporulation which may be unimportant, as in other experiments¹ it had no effect. Ammonium sulphate reduced acetate sporulation to 8.5 per cent, and whereas 60 per cent remained in the vegetative state, approximately one quarter entered stage 1. Apparently the later stages of meiosis are more sensitive to this substance. When all ingredients were present, 90 per cent of the nuclei remained in the vegetative state and, as expected, the greatest cell increase occurred.