

In this way it was possible to show that only a part of the easily hydrolysable phosphorus present in the experimental material is adenosinetriphosphate, whereas as much as 60 per cent (in some preparations up to 80 per cent) of the 7-minutes hydrolysable phosphorus was identified as inorganic pyrophosphate. This was found to be localized mainly in the ductus ejaculatorius simplex (2,200 mgm. per cent phosphorus). It was converted into silver pyrophosphate which had a content of 71.4 per cent silver, as against 71.2 per cent required theoretically. The crude manganese precipitate was also found to contain a very small admixture of metaphosphate, and a small quantity of yet another metaphosphate compound was detected in the supernatant solution. The whole of the metaphosphate could be precipitated by the procedure used by Mann⁴ for metaphosphate from moulds (precipitation with sodium hydroxide at pH 5.2 from a trichloroacetic-ethanol extract).

Hitherto the evidence for the occurrence of inorganic pyrophosphate in Nature was confined mainly to moulds⁴ and yeast⁶. Our results, however, provide a proof of the occurrence of inorganic pyrophosphate in an animal tissue. They lend, at the same time, new significance to observations of other authors^{6,7} concerning the role of inorganic pyrophosphate in the intermediary metabolism of cells.

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A Simple Gasometric Method for the Analysis of the Fermentation-inhibiting Properties of Antimicrobial Agents

RECENTLY, Heim and Poe in the United States introduced a very convenient method for evaluating antimicrobial properties of organic compounds¹. They used, for example, *Aerobacter aerogenes* and *Saccharomyces cerevisiae* as test strains, and based the estimation on the volume of gas found on incubation in lactose and glucose broth respectively for 72 hr. or less at 37° C. We have applied the same principle as a convenient method for estimating fermentation-inhibiting properties.

As an inoculum a 10 per cent suspension of fresh baker's yeast (*Saccharomyces cerevisiae*) is used, in a concentration of 1 vol. per cent. This method of inoculation—which can be carried out in any laboratory, not necessarily specializing in microbiology—is prescribed by the Dutch Food Law in a test for establishing qualitatively the presence or absence of preservatives in fruit juices². Counts on 'Difco' malt agar at 25° C. showed that such an inoculation corresponds to about 10⁷ yeast organisms per ml. Variations due to different inoculations or to differences in the age of the baker's yeast used do not impair the consistency of the results obtained, since blanks are always carried out to eliminate these fluctuations.

Commercially bottled depectinized filtered apple juice ($n_D^{20} \sim 1.350$, corresponding to about 12 per cent of sugars; pH about 3.5) was chosen as the medium. This juice is a satisfactory source of nutrients for yeasts³ and possesses a fair buffer capacity. A further merit of this medium is that it is generally commercially available in Holland as a sterile liquid, free from preservatives; the latter are forbidden by the Food Law. Hence from this point of view, too, the method is generally applicable in purely chemical laboratories.

Incubation is carried out in sterilized ($\frac{1}{2}$ hr. at 170° C.) Einhorn tubes for 12–48 hr. at 25° C. The volume of gas formed is not recalculated to standard pressure. The inaccuracy involved by omitting this recalculation is about 1.2 per cent. This is significantly less than the precision of the technique, which in the 'best' replicates did not surpass 4.3 per cent.

The method has been used successfully in our laboratory for more than a year in testing preservatives and in detecting the same. It should be noted, however, that this method of detection and evaluation is only applicable to those preservatives which in the concentrations usually found in practice exert a powerful specific inhibition of yeast fermentation.

There exist some agents, for example, sulphonamides and antibiotics, which selectively inhibit the multiplication of micro-organisms. Such agents as a rule only become active after some divisions have occurred⁴. According to our measurements, this means that in the present test their action begins after a period of the order of 10 hr., that is, when fermentation as a rule is greatly advanced. Hence such agents do not greatly influence the results of the present fermentation test. To detect and evaluate compounds acting in the latter way a low inoculation (of the order of 10⁴/ml.) must be used. The test then takes 48–96 hr. Work along these lines is in progress.

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Penetration of Benzpyrene through the Intact Skin of New-Born Mice

WHEN the skin of the mouse is painted with a benzene or an acetone solution of a carcinogenic hydrocarbon, it is found afterwards that a part of the carcinogen remains in the keratinized layer of the epidermis, while some of the carcinogen (fluorescent material) immediately enters the pilo-sebaceous apparatus and is found dissolved in the lipid droplets within the gland cells. Several authors have suggested that the pilo-sebaceous apparatus plays an important part in the genesis of skin cancer by providing channels through which the carcinogen may penetrate the epidermis¹. It was thought that a study of carcinogenic action on new-born mice^{2,3,4} would throw light on this problem, for it has been observed that new-born mice (2–10 hr. after birth) are refractory to a single application of methylcholanthrene. The