tions. Because this enzyme and the folic acid antagonists have an important bearing on the clinical and research aspects of acute leukaemia, the development of these applications may prove very valuable.

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Reduction of Colour Fading in the PNPH Test for DNA in Bovine Spermatozoa

COLORIMETRIC tests are employed routinely for estimating the deoxyribonucleic acid (DNA) content of tissues. The diphenylamine test, originally described by Dische[1], or subsequent modifications of it, are the most frequently used. Other colorimetric tests include Ceriotti's indole[2] and the *p*-nitrophenylhydrazine (PNPH) proposed by Webb and Levy[3]. The latter is the most sensitive, but possesses the disadvantage of rapid colour fading. In 1963 Brown *et al.*[4] proposed a modification of the PNPH test for plant leaf tissue that is more sensitive and stabilizes the colour. This modification, applied to animal tissue, does not increase the sensitivity of the original method, but it does reduce the colour fading. Essentially this test differs from the Webb–Levy technique in the extraction procedure since iso-amyl acetate replaces the butyl acetate. This is then followed by an extraction of ethyl ether to remove residual amyl acetate. Colour is developed in *n*-butyl alcohol made strongly alkaline by the addition of an equal volume of 50 per cent sodium hydroxide.

In this procedure nucleoprotein is extracted according to Borenfreund *et al.*[5]. For our purposes we suspend a 100-mg sample of bovine spermatozoa in a beaker containing 20 ml. of 0·5 M NaCl–0·005 M Na citrate. 2-Mercaptoethanol is then added to a final concentration of 2 per cent and the mixture incubated at 4° C for 2 h with gentle stirring. Trypsin is then added (10 mg) and the incubation continued for 1 h at room temperature[4]. The sample is then centrifuged and the supernatant saved for analysis. Incubation with trypsin is repeated twice, the supernatants combined, and the nucleoprotein precipitated by the addition of two volumes of ethanol. The precipitated nucleoprotein is collected with a glass rod and dried over calcium chloride. DNA is hydrolysed with 5 per cent TCA (trichloroacetic acid) and brought to volume with 5 per cent TCA. Colour is developed by the method of Brown *et al.* and absorbance at 580 mμ used as an indication of the DNA content. Representative comparisons made for reduction of colour between the original method and the present modification are shown in Table 1.

Table 1. COMPARISON OF COLOUR STABILITY BETWEEN THE WEBB–LEVY AND BROWN PNPH COLORIMETRIC TESTS FOR DNA

	Webb–Levy		Brown	
Time (min)	*O.D.*	Loss of colour (%)	*O.D.*	Loss of colour (%)
0	0·1871	0	0·1871	0
5	0·1739	7·06	0·1871	0
10	0·1612	13·84	0·1871	0
20	0·1518	18·85	0·1871	0
40	0·1397	25·33	0·1871	0
60	0·1308	30·09	0·1871	0

As indicated in Table 1, the present modification reduces the loss of colour, amounting to approximately 30 per cent in the Webb–Levy method, to virtually nil in the present modification at the end of 1 h. This reduction in colour fading adds greater precision to the PNPH method for estimating DNA.

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Technicon Autoanalysis of the Ninhydrin-positive Phospholipids of *Lactobacillus casei*

ANALYSIS of the phospholipids of *Lactobacillus casei* by thin-layer chromatography has revealed the presence of an unidentified ninhydrin-positive compound, accounting for 30 per cent of the total phospholipid phosphorus[1]. The identity of this substance and the presence of other ninhydrin-positive compounds have now been determined using a Technicon autoanalyser by the method of Gaby and McCurley[2].

A 47·5-mg sample of bacterial lipid was prepared as described previously[1] and hydrolysed with 2 ml. 6 N hydrochloric acid at 110° C for 24 h in a sealed tube. The hydrochloric acid was removed at 100° C with a rotatory evaporator; 1 ml. water was added and the sample re-dried three times. It was then suspended in 3 ml. 0·1 N hydrochloric acid and 0·5-ml. aliquots were taken for Technicon autoanalysis on a column of Technicon 'Chromobeads *A*' (17μ), using a buffer system with a continuous gradient from *p*H 2·87 to *p*H 5·0 (ref. 3). The results are given in the first column of Table 1. The figures quoted represent the molar quantity of each amino-acid as a percentage of the total ninhydrin-positive material, neglecting ammonia. The bulk of the material, 71·3 per cent, was identified as lysine. In addition, 6·9 per cent of the amino-acids were alanine and 5·5 per cent ornithine. Small quantities of aspartic acid, threonine, serine, glutamic acid, glycine, valine, leucine, isoleucine and phenylalanine were present.

The occurrence of lysine in the phospholipids of lactic acid bacteria has been reported previously by Ikawa[4], and it has also been found in *Clostridium welchii*[5] and *Staphylococcus aureus*[6–8]. It is suggested by Houtsmuller and van Deenen[7] that the formation of membraneous phospholipids containing basic amino-acids may represent a response of the organism to the acidity of the surrounding growth medium.

Table 1. AMINO-ACID CONTENT OF THE PHOSPHOLIPIDS OF *L. casei*

	Extracted phospholipid (molar percentage)	Phospholipid washed with 0·1 N HCl* (molar percentage)
Aspartic acid	1·3	0·6
Threonine	1·0	0·7
Serine	2·4	1·7
Glutamic acid	1·3	0·8
Glycine	3·1	1·6
Alanine	6·9	9·9
Valine	1·7	2·0
Isoleucine	0·9	1·7
Leucine	3·3	3·3
Phenylalanine	1·3	1·4
Ornithine	5·5	0·0
Lysine	71·3	76·3

* Extracted phospholipid washed by the method of de Koning[9].