

Therefore one would expect that significant variations in the labelling index of bone marrow blast cells would be reflected by the labelling index in the blood. It is likely, then, that the lack of diurnal variation in the labelling index applies to all leukaemic blast cells irrespective of where they are located.

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#### Pathological Excretion of 4-Hydroxy-3-methoxyphenyllactic Acid

ONE of the 'spots' frequently appearing on paper chromatograms of the phenolic acids in human urine has been examined in these laboratories for some six years, since it was recognized as being of complex nature. Normally it appears to consist of a mixture of 4-hydroxy-3-methoxy- and 3-hydroxy-4-methoxyphenylhydraacrylic acids, both probably largely derived from dietary chlorogenic acid, the latter isomer sometimes being observed in relatively large amounts following the consumption of citrus fruits containing the flavonoid hesperidin<sup>1</sup>. However, at least two other strongly acidic substances having rather similar  $R_F$  values have occasionally been detected, particularly in extracts prepared from urines which have been refluxed with acid in order to destroy the hydraacrylic acids. The chromatographic properties of these compounds are recorded in Table 1.

Table 1. CHROMATOGRAPHIC PROPERTIES OF 3 PHENOLIC ACIDS

	3-Hydroxy-4-methoxyphenylhydraacrylic acid	Compound A	Compound B (4-hydroxy-3-methoxyphenyllactic acid)
$R_F$ in anisole-acetic acid-water (70 : 29 : 1)	0.34	0.33	0.34
$R_F$ in benzene-propionic acid-water (125 : 72 : 3)	0.15	0.12	0.14
$R_F$ in dioxan-methyl isobutyl ketone-pyridine-water (40 : 40 : 12 : 20)	0.44	0.30	0.32
$R_F$ in isopropanol-ammonia-water (8 : 1 : 1)	0.31	0.35	0.35
Colour with diazotized <i>p</i> -nitroaniline	Violet-blue	Blue	Grey
Colour with diazotized sulphanic acid	Red-orange	Violet-red	Violet-red
Colour with 2 : 6-dichloroquinone chloroimide-borax	Blue	Blue	Negligible

Compound A was investigated in a case of neuroblastoma in which it was excreted in relatively large quantity. A similar spot has been detected in only one other subject, a case of post-operative stress maintained on a noradrenaline drip. The possibility that the substance is a drug metabolite cannot be excluded.

Compound B was particularly prominent in the case of malignant phaeochromocytoma previously reported<sup>2</sup>. Its chromatographic properties are identical with those of 4-hydroxy-3-methoxyphenyllactic acid, itself conveniently isolated as its sodium salt after reduction of

4-hydroxy-3-methoxyphenylpyruvic acid in aqueous ethanol with sodium borohydride. The acid was detected, in small amount, in only one other of 30 phaeochromocytoma urines examined. However, this metabolite of 3 : 4-dihydroxyphenylalanine was also detected readily in one case of neuroblastoma and small amounts appeared to be excreted in 5 of 10 further cases of this condition (cf. ref. 3).

A variety of pathological urines have been examined for the presence of 4-hydroxy-3-methoxyphenyllactic acid, using diazotized *p*-nitroaniline for its detection after paper chromatography<sup>4</sup> of extracts of acid hydrolysed urines equivalent to 1 min excretion or to 1 mg of creatinine excreted. The acid was thus detected in 2 cases of cancer, one of gross kidney deficiency and one of rheumatoid arthritis in total of 72 subjects. The foregoing urines were selected, usually because abnormalities in phenolic acid excretion were revealed, from a much larger series which were examined without recourse to acid treatment; many such urines did not contain interfering hydraacrylic acids, but in none of these was the lactic acid detected. It has not yet been encountered in any normal urine or in urines from subjects under stress. Nevertheless its presence in traces in all urines may be inferred since the closely related 4-hydroxy-3-methoxyphenylpyruvic acid appears to be invariably present in small quantity, detectable as the derived hydantoin formed by condensation with urea under acid conditions. However, excretion of the lactic acid in readily detectable amount seems to be a very uncommon phenomenon which is particularly, if not invariably, associated with the abnormal production of 3 : 4-dihydroxyphenylalanine; it also appears to be usually associated with high excretion of *p*-hydroxyphenyllactic acid.

Allowance for possible confusion with compound A should be made if 4-hydroxy-3-methoxyphenyllactic acid is to be detected on paper chromatograms with diazonium reagents only. In particular, the grey colour obtained with diazotized *p*-nitroaniline (Table 1) may be distinctly blue if the coupling is not carried out under alkaline conditions. Compounds identified as the lactic acid have been reported to yield 'blue-violet'<sup>5</sup> or 'light blue'<sup>3</sup> colours by other workers.

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#### Enhancing Effect of Thymectomy on Hepatotumorigenesis in Swiss Mice following Neonatal Injection of 20-Methylcholanthrene

UNTIL recently, liver has proved refractory to tumour induction by carcinogenic hydrocarbons. Klein<sup>1</sup> reported in 1959 that the increased incidence of liver tumour was attained by initiating repeated oral administration of 3-methylcholanthrene in suckling mice 7-9 days of age. Kelly and O'Gara<sup>2</sup> also noted that liver tumours were found in 6 of 11 male C3H mice after a single subcutaneous injection of 3-methylcholanthrene at birth.

Many tumours induced by chemical carcinogens are antigenic in the syngeneic and even autochthonous hosts<sup>3,4</sup>. This led to the concept, as noted by Miller *et al.*<sup>5</sup>, that "tumour progression might be possible only when the host's mechanism for homograft immunity is either poorly developed or depressed as a result of irradiation or of the chemical carcinogen itself". Depression of immune