induced a 15% increase and 10 μM-GA_3 + 1 μM-IAA caused a 50% decrease in activity for the 4-day period. Hydroxylamine (3mM) caused a twofold increase and 4mM-ammonium chloride caused a 50% increase over the same period.

A methanol extract of embryos from 1-day-old seedlings induced a 15% increase in activity, whereas an aqueous diffusate of such embryos induced a twofold increase, which increased to 2·7-fold after ethyl acetate extraction of the diffusate.

These results strongly suggest that the increase in phytase activity in the bran of germinating wheat is induced by a factor from the embryo. The nature of the factor is unknown.

When bran from ungerminated wheat was incubated at pH 5·0, release of reserves of macronutrient elements into the medium was complete within 24hr., the rate of release being unaffected by GA_3, IAA, kinetin or any combination of these hormones at physiological concentration. However, K^+ release was stimulated by Na^+ and by oligomycin and inhibited by 2mM-Ca^{2+}, in the presence of Na^+, by F^- and by 2,4-dinitrophenol. Oligomycin also stimulated the release of Mg^{2+} and $H_2PO_4^-$. Poly-L-lysine inhibited Mg^{2+} release, whereas 1mM-$H_2PO_4^-$ completely inhibited the release of $H_2PO_4^-$ but was without effect on K^+ release.

Different mechanisms thus appear to exist for the release of each inorganic ion and these mechanisms might be linked in some way with energy metabolism.

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Factors Governing the Contents of Ferrous and Ferric Ions in Forest Soils

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The E_h of most soils ranges from +200 to +800mv and reflects the Fe^{3+}/Fe^{2+} balance. In the normal range of acidity found in soils the Fe^{3+} concentration in solution is governed by the solubility product of ferric hydroxide. Thus soil E_h is determined primarily by the pH and the concentration of Fe^{2+} in the soil solution. From thermodynamic considerations Ponnamperuma, Tianca & Loy (1967) derived the following equation for the E_h of a Fe^{3+}/Fe^{2+} system in soil:

$$E_h \text{ (volts)} = A - 0{\cdot}06 \log Fe^{2+} - 0{\cdot}177 pH$$

where A is a constant based on the Fe^{3+}/Fe^{2+} standard electrode potential and having the theoretical value +1·06v. E_h will thus change in response to any environmental factor that alters the Fe^{2+} concentration or the pH.

Measurements of E_h *in situ* were made over a period of 2 months on a brown forest soil and a well-developed podsol (both developed from Silurian shale) by using an instrument developed from those described by Poel (1960) and Armstrong (1967). Data for E_h, pH and Fe^{2+} concentration in the soil solution were fitted into the theoretical equation. For all horizons except the eluvial horizons of the podsol (A_0 and A_2) the constant fell within the range 1·01–1·08v (mean 1·03v), in good agreement with the theoretical value. The A_0 and A_2 horizons of the podsol, however, gave a mean value of 0·87v. These horizons were peculiar in that the iron in solution was largely in the form of complexes; in the A_0 96% and in the A_2 67% of the iron in solution was in this form, and the equation is not directly applicable in such circumstances.

Restriction of oxygen diffusion by water is frequently used to explain low E_h values. We found that for the horizons conforming to the theoretical equation there was, within the range cited above, a highly significant positive linear correlation between E_h and the volumetric proportion of the air space in the soil.

For the soils examined we have found that the E_h measurements can be made quickly and satisfactorily in the field and are generally related to moisture state. E_h measurements agreed with theoretical prediction except on horizons indicating marked iron mobilization. It is likely that in most soils E_h and pH are biologically significant parameters of the physical environment.

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A Precursor of Benzyl Methyl Ketone in Amphetamine Urine

By L. G. Dring, R. L. Smith and R. T. Williams (*Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*)

Benzyl methyl ketone (phenylacetone) was detected in the acid-hydrolysed urine of man, dog or rabbit dosed with amphetamine (Dring, Smith & Williams, 1966). The ketone was not found free in

the urine but as a precursor (X). The rat, mouse, guinea pig and rhesus monkey did not excrete X or the ketone after being dosed with the drug. In the rabbit, as much as 22% of the dose of amphetamine (10mg./kg.) was excreted as precursor or precursors of the ketone, whereas man excreted only 2–3% and the dog 1%.

The nature of precursor X has been further elucidated. (±)Amphetamine (225mg.) was given to three rabbits over 3 days. The urine was mixed with urine from a rabbit receiving the ^{14}C-labelled drug. Precursor X was separated from the freeze-dried urine by thin-layer chromatography as a pale-yellow solid (20mg.) containing sulphur but practically no nitrogen. On acid hydrolysis it gave benzyl methyl ketone (DNP-hydrazone, m.p. and mixed m.p. 152–153°) and inorganic sulphate. Precursor X was also detected chromatographically in the urine of rabbits receiving [^{14}C]benzyl methyl ketone (1 - phenyl[1 - ^{14}C]propan - 2 - one). When $^{35}SO_4^{2-}$ was given with non-radioactive benzyl methyl ketone, the precursor X located on chromatograms contained ^{35}S. Hydrolysis of precursor X from the urine yielded benzyl methyl ketone (as DNP-hydrazone) and [^{35}S]sulphate (as $Ba^{35}SO_4$).

Gero (1954) reported that benzyl methyl ketone occurs normally in the enol form to the extent of 2·9%. We suggest that precursor X is probably a salt of the sulphate ester of 1-phenylprop-1-en-2-ol, the enol of the ketone, i.e. $C_6H_5 \cdot CH : C(O \cdot SO_3^-) \cdot CH_3$. This structure was supported by nuclear-magnetic-resonance and u.v. spectra.

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The Metabolism of Thiophen and Benzo[*b*]-thiophen

By H. G. Bray and F. M. B. Carpanini (*Department of Physiological Chemistry, University of Birmingham*)

In studies of the metabolic fate of thiophen Heffter (1886), Christomanos (1930) and Chilcote (1945) did not report the identification of any metabolite, although their results suggested that no ethereal sulphate was formed and that the chief metabolite might be a mercapturic acid.

We found that the rabbit excreted about 35% of a dose of thiophen (150mg./kg.) unchanged in expired air and a small amount (about 0·5% of the dose) in faeces. About 40% of the dose was detected in urine as two mercapturic acids, believed to be thienyl 3- and 2-mercapturic acids. No increase in the excretion of glucuronic acid or ethereal sulphate was observed and no evidence of the opening of the ring to form diethyl sulphide, as Christomanos (1930) suggested might occur in the dog, was obtained.

Chilcote (1945) suggested that 2-bromothiophen is metabolized by the rabbit in the same way as is thiophen. In the present investigation, no increase in ethereal sulphate or glucuronic acid was detected and only about 20% of a dose of 2-bromothiophen (120mg./kg.) could be accounted for as mercapturic acid-like compounds in urine. One of these acids is believed to be thienyl 2-mercapturic acid.

Böhm (1941) reported the isolation of a metabolite, believed to be benzothienyl 2-glucuronide, from the urine of rabbits to which benzo[*b*]thiophen had been administered, but we were unable to detect an increase in the excretion of either ethereal sulphates or glucuronic acid by the rabbit dosed with benzo[*b*]thiophen (175mg./kg.). About 80% of the dose appeared in the urine as mercapturic acid-like compounds, four of which could be separated by paper and thin-layer chromatography. One of these has been identified tentatively as benzothienyl 3-mercapturic acid.

A method for the synthesis of thienyl and benzothienyl mercapturic acids from 2-amino-3-chloropropionic acid hydrochloride and the appropriate mercaptothiophen will be described.

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The Metabolism of Cyclohexylamine in Rabbits

By T. H. Elliott, N. Y. Lee-Yoong and Rosaline C. C. Tao (*School of Pharmacy, University of Singapore*)

Bernhard (1937) reported that cyclohexylamine fed to dogs disappears completely. However, in view of the widespread use of sodium cyclamate as a sweetener, and the report by Ohashi (1964) that in the presence of acid and hydrogen peroxide it is hydrolysed to cyclohexylamine, it is of some importance to know the precise metabolic fate of cyclohexylamine.