in the excreta of *Galleria mellonella* and *Achræa grisella* suggests a connexion with the unique ability of these insects to digest and assimilate wax.

A detailed report of this work will be published elsewhere.

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Identification of *p*-Cresol as a Toxin in Œstrogen Concentrates from Sheep Urine

DURING a study in this laboratory of the urinary excretion of œstrogens by sheep, the final œstrogen concentrates injected into mice often proved so toxic that bio-assay by the spayed mouse vaginal smear method was impossible. This toxicity was observed in concentrates from pregnant, non-pregnant and spayed ewes. Typical symptoms of lethargy and panting appeared within ten minutes in extreme cases, followed by death in less than two hours. Post-mortem examination usually showed gaseous distension of the entire alimentary tract. When symptoms were less severe, the animals usually recovered slowly over a period of several days.

To detect œstrogenic activity it was necessary to inject the extract from a large volume of sheep urine, an injection of 0·1 ml. of the final oily extract corresponding to as much as 70 ml. of urine. Since the usual extraction methods for œstrogen, including that used[1], would be expected to concentrate other phenolic material also, it seemed likely that toxicity was due to accumulation of simple phenols known to occur in urines. Portions of the toxic concentrates were therefore subjected to steam distillation. The distillates produced the typical toxic symptoms, whereas the residue could be used safely for bio-assay.

The steam distillates from 17·3 litres of ewe pregnancy urine gave on ether extraction 5·1 gm. of phenolic material. Fractionation at atmospheric pressure yielded only one fraction (4·4 gm., b.p. 196–99°), which soon formed crystals (m.p. about 23°, deliquescent). Physical properties were consistent with those of *p*-cresol, and this was further characterized by conversion to the *p*-toluenesulphonate (m.p. 67°, reported m.p. 70° [2]), and to *p*-methyl phenoxyacetic acid (m.p. 135–36°, reported m.p. 136° [3]). The corresponding derivatives prepared from authentic *p*-cresol had the same melting points, and gave no depression of melting point on admixture with the derivatives from the urinary phenol. A small amount of residue in the distillation flask was recovered as a *p*-cresol derivative. Twenty-three litres of urine from another pregnant ewe gave 9·9 gm. of crude phenols, and 16·4 litres from a non-pregnant ewe gave 5·9 gm. of phenols. These on distillation gave only a *p*-cresol fraction, characterized as previously.

Further evidence identifying *p*-cresol as the toxic substance is that injection of pure *p*-cresol into mice produced the typical toxic symptoms.

Campbell and Hey[4] have reviewed the literature on the occurrence of *p*-cresol and other phenols in the urines of animals and humans. *p*-Cresol occurs in most urines, usually with several other phenols; but the concentration is usually low (see also Porteous and Williams[5]). There appears to be no record of the examination of sheep urine for phenols. The urine of stallions gave 0·055 per cent of *p*-cresol[4], while in urine of the pregnant and non-pregnant ewe we find a comparable value of 0·03 per cent. The absence of appreciable amounts of other phenols in sheep urine is of interest.

We have found it most convenient to remove the toxin from the concentrates by carrying out the steam distillation at the stage when the alkaline extract containing the œstrogens had been acidified. To obtain rapid and consistent removal of toxin, it was necessary to saturate the acid solution with sodium chloride before distillation. In experiments in which œstrogens in the form of human pregnancy urine were added to sheep urine, no loss of œstrogenic activity was observed when the steam distillation stage was included. Friedgood *et al.*[6] showed that pure œstrone, œstradiol and œstriol are not steam-volatile, and can be recovered quantitatively. After introducing this modification, it has been possible to complete assays with extracts which previously would have killed the test animals.

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Narcotics and the Phosphates of Brain

IN brain, as in other organs, phosphates form an important link between function and the metabolism which supports function[1]. The levels of inorganic and esterified phosphates in brain *in vivo* have been examined previously; but there is little information on the levels of phosphates during *in vitro* metabolism[2], possibly because of the reports of rapid post-mortem changes in these constituents[3]. We have, however, found that during metabolism *in vitro* the concentration of inorganic phosphate and of phosphocreatine can be restored to stable levels, which depend on metabolic conditions and which can approximate closely to those normal to the brain *in vivo*. Determinations of both phosphates and creatine have been made, following separations by calcium salts and alcohol[4]. Inorganic phosphate has been determined according to Lowry and Lopez[5]. The sum of inorganic phosphate, and phosphates with the lability of creatine phosphate, has been determined by the method of Fiske and SubbaRow[6].

It has therefore been possible to compare the actions of drugs on phosphates during their actions *in vivo* and *in vitro*, and we report now some conclusions with respect to the action of narcotics. These substances inhibit respiration *in vivo*[7] and also *in vitro*[8], and suggestions have been made that depression of cerebral respiration is the primary action of