pathway now available, and hence galactose transfer is inhibited.

Other explanations are also possible. There may be one mechanism for entry of hexoses into the cell, and the separation into two pathways—one uranyl sensitive leading into metabolism—may occur inside the cell. In this case it must be postulated that the uranyl sensitive pathway is not available to glucose initially present in the serosal fluid. Another possibility is that uranyl ions may penetrate only into a limited part of the cell and may affect metabolism of part of the cell into which glucose from the serosal side of the cell does not penetrate.

Of considerable interest is the stimulation of galactose transfer by small concentrations of glucose. Fisher and Parsons[4] showed that glucose and galactose compete for entry, and while the present results support this they also show that provided glucose is present in small concentrations in the mucosal fluid or in higher concentrations in the serosal fluid, it may be able to enter the cell without competing with galactose and by its metabolism stimulate galactose transfer. This would be in keeping with the results of Newey and Smyth[5], who have shown that glucose metabolism can stimulate glycine transfer. It thus appears that glucose metabolism can supply energy for three different transfer systems, that is, galactose, glycine and fluid.

We thank the Medical Research Council and John Wyeth and Brother for grants towards expenses.

H. Newey
P. A. Sanford
D. H. Smyth

Department of Physiology,
University of Sheffield.

[1] Rothstein, A., *Circulation*, **26**, 1189 (1962).
[2] Ponz, F., *Rev. esp. Fisiol.*, **8**, 217 (1952).
[3] Ponz, F., and Lluch, M., *Rev. esp. Fisiol.*, **14**, 217 (1958).
[4] Fisher, R. B., and Parsons, D. S., *J. Physiol.*, **119**, 224 (1953).
[5] Newey, H., and Smyth, D. H., *J. Physiol.*, **164**, 527 (1962).

Identification of Oestradiol and Oestrone in Avian Plasma

Although it has long been held that the plasma of the laying domestic fowl contains oestrogenic substances no direct identification has been reported. Although oestrone, oestradiol and oestriol have been identified in extracts of ovarian tissue from the laying hen[1] and also in urine[2,3], attempts to detect oestrogens in blood have been unsuccessful.

As part of an investigation of factors involved in yolk production in the domestic fowl this problem has been investigated using a technique of double isotope derivative analysis[4] using carbon-14-labelled oestrogens and tritiated dimethyl sulphate as identifying agents.

Plasma from laying hens was treated with hydrochloric acid[5] to hydrolyse conjugated oestrogens, and after extraction with chloroform was separated into fractions presumptively containing oestradiol + oestrone and oestriol[6]. The fractions were methylated with dimethyl sulphate-*T* and, after removal of solvents and decomposition of excess dimethyl sulphate with alkali, were extracted into hexane. Authentic methylated ^{14}C-oestradiol and oestrone were then added and the methyl oestrogens were separated and purified, first by thin-layer chromatography using solvent systems *A*, *B* and *C* of Lisboa and Diczfaluzy[6], followed by chromatography on columns of prepared alumina[6]. Samples of each fraction were counted in a liquid scintillation counter.

It was found that the samples of both methyl oestradiol and methyl oestrone contained tritium, the ratios of tritium/^{14}C becoming constant after several fractionations (Table 1). As a further check on the chromatographic homogeneity, fractions eluted from the second alumina column (Table 1) were re-chromatographed on alumina and the three fractions comprising the beginning, the middle and the end of each elution peak were separately collected. The tritium/^{14}C ratios in each were found to be the same within the limits of experimental error (Table 2). At no time was tritium detectable in chromatographic areas corresponding to authentic methyl oestriol.

Table 1. Ratios of Tritium/^{14}C in Extracts of Avian Plasma treated as described in the Text and chromatographed together with Carrier Methyl Oestrogens

Solvent systems	Methyl oestrone T (c.p.m.)	Methyl oestrone ^{14}C (c.p.m.)	Methyl oestrone Ratio (T/^{14}C)	Methyl oestradiol T (c.p.m.)	Methyl oestradiol ^{14}C (c.p.m.)	Methyl oestradiol Ratio (T/^{14}C)
A	$2{\cdot}86\times10^5$	460	621·0	$1{\cdot}67\times10^5$	324	513·0
B	$6{\cdot}9\times10^5$	319	217·0	$20{\cdot}2\times10^3$	293	69·0
C	$4{\cdot}71\times10^5$	126	37·6	$3{\cdot}28\times10^3$	166	19·8
Alumina 1	$1{\cdot}45\times10^5$	73	19·8	$1{\cdot}72\times10^3$	102	18·1
Alumina 2	$1{\cdot}07\times10^5$	57	18·7	$1{\cdot}53\times10^3$	75	20·2

Table 2. Ratios of Tritium/^{14}C in Fractions from the Columns Alumina 2, of Table 1, refractionated on a Fresh Alumina Column[6]

Fraction number	Methyl oestrone T (c.p.m.)	Methyl oestrone ^{14}C (c.p.m.)	Methyl oestrone Ratio (T/^{14}C)	Methyl oestradiol T (c.p.m.)	Methyl oestradiol ^{14}C (c.p.m.)	Methyl oestradiol Ratio (T/^{14}C)
2	97·1	4·5	21·4	415·0	19	21·9
3	$1{\cdot}07\times10^3$	57	18·7	$1{\cdot}53\times10^3$	75	20·2
4	336	17·0	19·8	124·2	16	20·7

The results show that materials are present in hydrolysates of avian plasma which after methylation behave in a manner identical with methyl oestrone and methyl oestradiol in several solvent systems. Such behaviour is considered to be proof of identity and to show the existence of oestradiol and oestrone, or their conjugates, in avian plasma.

We thank Cyril Thornber, Ltd., Mytholmroyd, Halifax, for a generous supply of birds.

J. E. O'Grady
P. J. Heald

Twyford Laboratories, Ltd.,
Twyford Abbey Road,
London, N.W.10.

[1] Layne, D. S., Common, R. H., Maw, M. A., and Fraps, R. M., *Nature*, **181**, 351 (1958).
[2] Ainsworth, L., and Common, R. H., *Nature*, **195**, 77 (1962).
[3] Macrae, H. F., Saharia, W., and Common, R. H., *Poultry Sci.*, **38**, 318 (1959).
[4] Klihan, B., and Peterson, R. E., *J. Biol. Chem.*, **235** 1639 (1960).
[5] Roy, E. J., and Brown, J. B., *J. Endocrinol.*, **21**, 9 (1960).
[6] Svendsen, R., *Acta Endocrinologica*, **34**, 161 (1960).
[7] Lisboa, B. P., and Diczfaluszy, E., *Acta Endocrinologica*, **40**, 60 (1962).
[8] Brown, J. B., *Biochem. J.*, **60**, 185 (1958).

An Extracellular α-L-Arabinofuranosidase secreted by *Sclerotinia fructigena*

An earlier communication[1] reported the resolution of a maceration factor from endopolygalacturonase (PG) in culture filtrates of *Sclerotinia fructigena* Aderh. and Ruhl., by means of gel filtration on dextran Sephadex '*G* 75' and chromatography on 'Ecteola'-cellulose. The biochemical basis of maceration of potato slices by these preparations was not elucidated, but McClendon[2] has demonstrated that, in chromatography on cellulose phosphate of an ultra-filtered and freeze-dried sample of our culture filtrate, maceration of potato disks occurred in two peaks, one associated with a major PG peak and the other with a minor PG peak, with indications that arabanase or galactanase may macerate. We have shown independently that arabinose is liberated from potato fibre by a purified 'maceration factor' preparation free of PG and, subsequent to McClendon's findings, from lupin seed pectate. Incubation of the purified preparation with potato fibre was also accompanied by a release of soluble uronide, as determined by the carbazole method[3] (Fig. 1); the uronide was shown to be of high molecular weight by its failure to pass through a 'Visking' dialysis membrane, suggesting that a partial breakdown of insoluble 'protopectin' had occurred.

'Protopectin' may owe its insolubility to the presence of linkages of uronides with galactans and arabans[4], which are known to consist of chains of α-L-arabino-