μg phosphorus/h/mg wet weight retina to 5·7 μg phosphorus/h/mg wet weight retina, that is, a 3·3-fold increase.

Although the Na–K ATPase activity is considerably lower in the immature as compared with adult retina, nevertheless, if the hypothesis put forward earlier were true, the fact that it is present would imply that there should be some calcium effect on the immature respiratory rate.

Results obtained with rabbit retinæ[1] have indicated that the respiration affected is that derived from direct glucose oxidation and that (as in the rat retina[10]) this forms only 30 per cent of the respiratory activity of the immature tissue. Considering the fact that there is a 2–3-fold increase in the rate of respiration during maturation and that glucose oxidation then forms 60–70 per cent of the total[1,10], this actually corresponds to an approximate 6-fold increase in the rate of direct glucose oxidation via the tricarboxylic acid cycle.

Thus it is apparent that both components of the system under consideration are substantially lower in the immature than in the adult retina. Furthermore, although, relative to the amount of glucose which undergoes direct oxidation, the calcium-inhibited ATPase activity is actually greater in the former tissue, this is also true of the portion of ATPase activity which is unaffected by calcium, and it may be that the relative level of this latter enzyme in the immature tissue is sufficient to maintain the constancy of the respiratory rate when calcium is present in the incubating medium.

Mary J. Towlson

Department of Physiological Optics,
Institute of Ophthalmology,
Judd Street, London, W.C.1.

[1] Cohen, L. H., and Noell, W. K., *J. Neurochem.*, **5**, 253 (1960).
[2] Dickens, F., and Greville, G. D., *Biochem. J.*, **29**, 1468 (1935).
[3] Krebs, H. A., *Biochim. Biophys. Acta*, **4**, 249 (1950).
[4] Skou, J. C., *Biochim. Biophys. Acta*, **23**, 394 (1957).
[5] Skou, J. C., *Biochim. Biophys. Acta*, **42**, 6 (1960).
[6] Bonting, S. L., Simon, K. A., and Hawkins, N. M., *Arch. Biochem. Biophys.*, **95**, 416 (1961).
[7] Kornbleuth, W., Yardeni-Yaron, E., and Wertheimer, E., *Arch. Ophthal.*, **50**, 45 (1953).
[8] Reis, J. L., *Biochem. J.*, **48**, 548 (1951).
[9] Potter, V. R., Schneider, W. C., and Leible, G. J., *Cancer Res.*, **5**, 21 (1945).
[10] Reading, H. W., and Sorsby, A., *Vision Res.*, **2**, 315 (1962).

A Sulphuric Acid Reagent for the Colorimetric Determination of Testosterone

This communication describes a new, simple, yet sensitive and very reproducible technique for the spectrophotometric determination of testosterone, surpassing the known specific reactions with sulphuric acid reagents[1–7] with regard to precision, sensitivity and homogeneity of blanks.

Heating the steroid with the reagent and subsequent shaking with diluted acid results in a stable blue solution with a greenish tint and a specific absorbance spectrum with a strong peak at 620 mμ. In the range of 1–20 μg/ml. the molecular extinction coefficient $\varepsilon_m = 14 \times 10^3$ is by low coefficients of variation ($CV < 4$) independent of the testosterone concentration. Various steroids have been tested with respect to colour developed and molecular extinction coefficient, indicating that the reaction is nearly specific for testosterone, *cis*-testosterone and androst-4-ene-3,17-dione.

The sulphuric acid reagent comprised 25 mg $NH_4Fe(SO_4)_2 + 12H_2O$ dissolved in 1 ml. of a solution containing 1 mg per cent $KMnO_4$ in 20 per cent sulphuric acid heated in a seething water bath for 2 min, cooled and brought to a volume of 100 ml. with concentrated sulphuric acid (1·84). Stored in a refrigerator the colourless reagent is stable for at least three weeks.

To a solution of 1–20 μg testosterone in 0·02 ml. ethanol 0·5 ml. reagent is added. The mixture is heated for 5 min in a seething water bath and cooled for 3 min in an ice water bath, 0·5 ml. 3 per cent aqueous acetic acid is added, and mixing is performed by gently shaking. The absorbance is measured at 620 mμ against a blank containing all reactants except steroid. All chemicals used were analytical reagent grade.

The foregoing method represents a basis for the determination of testosterone in biological material.

This work was supported by the Deutsche Forschungsgemeinschaft.

Lothar Sachs

Universitäts-Frauenklinik,
Kiel, Germany.

[1] Koenig, V. L., Melzer, F., Szego, C. M., and Samuels, L. T., *J. Biol. Chem.*, **141**, 487 (1941).
[2] Nielsen, A. T., *Acta endocrinol.*, **1**, 362 (1948).
[3] Hosoi, M., *J. Pharm. Soc. Jap.*, **75**, 1506 (1955).
[4] Martin, E. A., *Rev. Canad. Biol.*, **14**, 399 (1956).
[5] Konii, S., *J. Pharm. Soc. Jap.*, **81**, 1016 (1961).
[6] Oertel, G. W., *Acta endocrinol.* **37**, 237 (1961).
[7] Martin, R. P., *Acta endocrinol.*, **40**, 263 (1962).

Effect of Growth Hormones on the Development of Invertase associated with Cell Walls

The enzyme invertase has been shown to develop in washed aerated slices of storage tissues of higher plants[1,2]. Using Jerusalem artichoke stem tubers[2] we have shown that this development is likely to be an expression of protein synthesis as it is suppressed by known inhibitors of this process.

This communication reports that invertase synthesis by aerated slices of artichoke tuber is markedly inhibited by indolyl-3-acetic acid (IAA) at physiological concentrations (Table 1): this is of particular interest since the enzyme appears to be associated entirely with the cell walls[2,3], and has defied attempts to render it soluble. The hormone has no direct effect on the activity of the developed enzyme.

Various compounds bearing some relationship to the IAA molecule, and of some physiological significance, were tested for comparable activity (Table 2). Apart from indolyl-3-acetonitrile (IAN), none of these approached IAA in inhibitory activity, but both indole and hydroxyproline had significant effect. It has been reported that certain chelating compounds simulate IAA in biological activity[4]: Table 2 shows that two of them were inactive in this system. The comparison between proline and hydroxyproline is of particular interest; hydroxyproline is a known constituent of some cell-wall protein[5], including that of the artichoke[6], and Cleland[7] has recently reported that hydroxyproline is an inhibitor of auxin-induced cell elongation—an effect which he ascribes to interference with protein metabolism. The hydroxyproline of the cell wall

Table 1. Effect of IAA on invertase synthesis. Slices of artichoke tuber shaken for 24 h in IAA at concentrations shown. For enzyme assay disks were transferred to 0·025 M sucrose and reducing sugar estimated after 1 h

Concentration (M) of IAA	Percentage inhibition of invertase synthesis
Water control	0
10^{-7}	15
5×10^{-7}	8
5×10^{-6}	13
5×10^{-5}	35
5×10^{-4}	70
10^{-3}	78

Table 2. Effect of IAA analogues and other compounds on invertase synthesis. Conditions as for Table 1; compounds used at 10^{-3} M

	Percentage inhibition of invertase synthesis
Water control	0
IAA	80
IAN	66
Indole	43
Tryptophan	5
Sodium propionate	0
Pyrollidine	2
Proline	7
Hydroxyproline	34
Ethylenediamine tetraacetic acid*	5
8-Hydroxyquinoline*	7

* Chelating agents.