

including the  $\text{BaSO}_4$  treatment, was the same in all species, it seems unlikely that the mitochondria were altered by the addition of  $\text{BaSO}_4$  to the homogenate. Furthermore, rat-liver-mitochondrial preparations so treated had an unimpaired capacity for oxidative phosphorylation and for swelling. Other factors, apart from the presence or absence of long-chain fatty acids, may be important. For example, autolytic destruction and in particular lipolytic action, though minimised by rapid preparation and low temperatures prior to incubation, may be a decisive factor and may be higher in some species than in others. Also the relative amount and type of bile acids present in the preparation may be an important factor.

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### Phosphorus levels of normal and conditioned tobacco callus tissue

When higher-plant tissues are first isolated it is found that they possess an absolute requirement for auxin and a cell-division factor for continued growth in culture. Some of these tissues when grown for sometime in a culture medium containing an auxin such as indoleacetic or naphthaleneacetic acid develop the capacity to grow indefinitely in an auxin-free medium<sup>1-3</sup>. These tissues have been termed habituated or conditioned. In an effort to characterize this type of auxin-independent growth phosphorus compounds in normal and conditioned tissues were investigated.

Conditioned callus tissue of *Nicotiana tabacum* var. White Burley was subcultured on a modified MOREL's medium<sup>4</sup> containing 0.5 g  $\text{Ca}(\text{NO}_3)_2$ , 0.5 g  $\text{KH}_2\text{PO}_4$ , 0.125 g  $\text{KNO}_3$ , 0.125 g  $\text{MgSO}_4$ , 20 g glucose, 0.5 ml Bertholet's solution, 10 mg cysteine hydrochloride, 1 mg aneurine and 5 g of agar per l. Normal tissue was subcultured on this medium supplemented with 100 ml/l coconut milk and a final concentration of  $10^{-8}$  M naphthaleneacetic acid. Subcultures were made with pieces of tissue approx. 20 mg fresh wt. and were grown at 25° with a light intensity of 200 ft-candles. Phosphorus compounds of 4-week-old cultures were fractionated by the method of HOLDEN<sup>5</sup>. Orthophosphate and acid-soluble phosphorus were removed from 1-g samples of tissue with 10-ml aliquots of 0.2 N  $\text{HClO}_4$  in the cold. Phosphorus soluble in ethanol-ether was extracted with three successive 5-ml aliquots of neutral

ethanol-ether (3:1). Extraction with 1 N  $\text{HClO}_4$  at 28° for 15 h was found to give incomplete extraction of RNA phosphorus, as RNA phosphorus was shown to be present in the DNA fraction by the orcinol procedure of BROWN<sup>6</sup>. A better separation was obtained by extracting for 24 h at 20° with 10 ml 1 N  $\text{HClO}_4$  and washing the tissue with another 10-ml aliquot of 1 N  $\text{HClO}_4$ . DNA phosphorus was extracted by 10 ml of 1 N  $\text{HClO}_4$  overnight at 37° and the tissue washed with another 5-ml aliquot of 1 N  $\text{HClO}_4$ . Separation of DNA phosphorus and RNA phosphorus by this procedure was in good agreement with separations carried out by the SCHMIDT-THANNHAUSER<sup>7</sup> procedure. After  $\text{HClO}_4$  digestion phosphorus was determined colorimetrically in the extracts by a modification of the method of KING<sup>8</sup>. Orthophosphate was removed from the first fraction by the method of LIPMANN AND TUTTLE<sup>9</sup>, and the acid-soluble bound phosphorus determined after  $\text{HClO}_4$  combustion. Dry weights were determined by heating the tissue in an oven at 100° for 24 h. For both normal and conditioned tobacco callus tissue the dry wt. was  $10 \pm 1\%$  of fresh wt.; thus the water content of both tissues is the same.

TABLE I  
COMPARISON OF PHOSPHORUS COMPOUNDS OF NORMAL AND CONDITIONED  
TOBACCO CALLUS TISSUE

	<i>µmole-P/mg dry wt.</i>	
	<i>Normal tissue</i>	<i>Conditioned tissue</i>
Orthophosphate	8.1	21.6
Acid-soluble organic P	2.0	2.0
Lipid P	0.3	1.8
RNA P	0.8	2.8
DNA P	0.5	0.6
Total P	11.7	28.8

The conditioned tissues were found to possess a higher level of phosphorus compounds compared with normal tissue (Table I). The conditioned tobacco callus tissue resembles a typical plant neoplasm<sup>10</sup> such as crown gall in its very high phosphorus level, although unlike crown-gall tumours the acid-soluble organic phosphorus does not show an increase.

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