

produced in the mycelia, but in mutant gamma, which lacks ATP-sulphurylase, the only radioactive compound produced was $^{35}\text{SO}_4^{2-}$. A further point requiring clarification was whether the *in vivo* release of sulphate from choline sulphate was the result of the direct action of a sulphatase or only followed after the catabolism of the carbon skeleton. That a choline sulphatase was operative *in vivo* was implied by the demonstration that choline sulphate could act as a source of sulphur and of choline for two cholineless mutants (ATCC 14078 and 14714) of *Neurospora crassa*^{16,17}.

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Acid Nucleases of the Bovine Adrenal Medulla

THE chromaffin granules of the adrenal medulla are characteristic cell organelles which serve the function of amine storage. Earlier work has shown that these granules are distinct from mitochondria¹.

A relationship between lysosomes and secretory granules has been discussed², particularly where the secretion product of the granules is an enzyme. However, a relation between the chromaffin granules and lysosomes may also have to be considered³. It is known that the chromaffin granules, like the lysosomes, release their contents into hypotonic media.

A possible connexion between lysosomes and chromaffin granules is of particular interest in view of a recent report on the occurrence of a ribonuclease (RNase) in the chromaffin granule fraction⁴.

During a recent investigation of the soluble protein obtained from a lysate of chromaffin granules it was noted that the RNase was present in the soluble lysate and that the latter also contained a deoxyribonuclease (DNase), and we have examined some of the properties of these enzymes.

Yeast RNA and calf thymus DNA were used as substrates. After an incubation for 1 h at 37°C the RNA or DNA was precipitated and the acid-soluble nucleotides determined by measuring the absorption at 260 m μ ; this was supplemented, for confirmation, by total phosphate analysis.

Purified fractions of chromaffin granules were lysed in *tris*-sodium succinate buffer of pH 5.9 and of low ionic strength ($I \approx 0.015$), as recently described⁵. After lysis, more than 80 per cent of both RNase and DNase activities were recovered in the supernatant fluid on centrifugation. The soluble lysate was fractionated in the same buffer on a column of 'Sephadex G-200'. The enzymes could be

separated from 50 per cent of the total soluble protein. The enzymic activities emerged in two overlapping peaks; the distribution coefficient, K_d (ref. 6), was 0.201 for RNase and 0.178 for DNase. The enzymatic activities for the peak fractions were determined and expressed in terms of μM of acid-soluble P/mg protein/h; these values were 2.74 for RNase and 2.72 for DNase. In the experiments to be described below, pooled fractions from each peak were used.

The RNase of the soluble lysate from bovine adrenal medulla differed from the RNase of bovine pancreas in that it acted on RNA 'core', and in its thermostability and in its pH optimum; the latter was 5.5 for the adrenal enzyme, as compared with 7.3 for the pancreas enzyme⁷. The pancreas enzyme is known to be very stable to heat. On the other hand, the rat liver lysosomal enzyme has a pH optimum similar to that of the adrenal enzyme and is thermostable⁸; it also acts on RNA 'core'⁹.

The optimal pH for the adrenal medullary DNase, in sodium acetate buffer of $I = 0.136$, was 4.6. This enzyme was not inhibited by Cu^{2+} concentrations up to $2 \times 10^{-4} \text{ M}$, whereas the RNase of the adrenal lysate showed a 50 per cent inhibition with $2.5 \times 10^{-5} \text{ M Cu}^{2+}$.

The observations show that the RNase described by Philippu and Schümann⁴ is present in the soluble protein fraction and also that an acid DNase is present in the same lysate. The material used in this study was obtained from the bovine adrenal medullary 'large granule' fraction by ultracentrifugation over a sucrose density gradient. Although a possible contamination of the chromaffin granules by either mitochondria or microsomes can be excluded as a source of the enzymatic activities studied, the possibility remains that the two enzymes are lysosomal in origin. Two alternative interpretations offer themselves: either the presence of the two acid hydrolases is an expression of a close relationship between chromaffin granules and lysosomes, or the two enzymes are located not in the chromaffin granules but in lysosomes which might be present in the particulate fraction isolated from the chromaffin tissue.

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Enzymatic Synthesis of the Sugar Esters of Hydroxy-aromatic Acids

GLUCOSE esters (1-O-acyl derivatives) of hydroxycinnamic acids have recently been shown to be widely distributed among higher plants¹. They are also the most common derivatives produced when the free acids are fed to a wide variety of plants even in cases where the species examined contained either a different derivative of the acid in question or no trace of it at all². In spite of this, however, comparatively little is known of the way in