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The Uptake of Biologically Active Substances by Lysosomes

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Living cells exposed to acridine orange show metachromatic fluorescent staining of subcellular structures (Allison & Young, 1964). Recent observations have shown that uptake of the dye also occurs readily in isolated lysosomes and other organelles (Dingle & Barrett, 1967). Further investigations into the mechanism of this phenomenon have led incidentally to a rapid method for the examination of fractions obtained during the separation of subcellular particles. In this technique 50 μ l. of each fraction is mixed with an acridine orange solution in suspension medium to give a concentration of 50 μ g. of dye/ml. After allowing 5 min. for uptake of the acridine orange, slides are examined by fluorescence microscopy with blue-violet dark-ground illumination and a yellow secondary filter with a magnification of 220 $\times$. Under these conditions nuclei (pale green), mitochondria (green), lysosomes (bright orange) and

membrane fragments (green) can be readily identified and counted. Among the advantages of this method are its speed and convenience, so that the purity of large numbers of fractions can be estimated and the organelle morphology can be examined immediately. Thus it has been found that counts of lysosomes in gradient-centrifugation fractions agree well with measurements of specific enzyme activity, and damage to organelles resulting in mitochondrial swelling or lysosomal abnormalities can be easily recognized. Further, it has been found that fractions apparently pure by conventional enzymological methods may, in fact, be seriously contaminated.

Measurement of the uptake of tritium-labelled acridine orange by isolated organelles has shown that, at the concentration used for staining, lysosomes take up several times as much of the dye as mitochondria. This factor may be sufficient to explain the orange fluorescence of the lysosomes, since solutions of the dye show a shift from green to orange emission with increasing concentration, but a specific molecular 'stacking' effect at lower concentration cannot yet be precluded. The possibility exists that the acridine orange-binding substance extracted from lysosomes and shown to bind dye after gel electrophoresis (Barrett & Dingle, 1967) may be present as a component of the lysosomal membrane. The bright orange fluorescence observed in the fine filamentous processes of lysosomes subjected to mechanical stress is compatible with a high concentration of dye within the membrane, as is the peripheral orange fluorescence of small vesicles sometimes observed within large lysosomes of rat kidney.

Experiments *in vivo* still in progress demonstrate that acridine orange injected intraperitoneally into rats persists in the kidney lysosomes, but not the mitochondria, for at least 1 week. These findings are similar to those obtained with labelled dimethyl-benzanthracene (Dingle & Barrett, 1967), and with [14 C]cetyltrimethylammonium bromide, which remains in the lysosomes for more than 4 weeks. The binding of acridine orange is thought to be a useful model system in which to study the processes by which lysosomes bind biologically active substances.

Results so far obtained support the hypothesis (Barrett & Dingle, 1967) that kidney lysosomes contain a strongly acidic lipid capable of binding lipophilic and cationic molecules. This substance may confer upon lysosomes a role in the scavenging and metabolism of drugs, carcinogens and other toxic materials from the cytoplasm.

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