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Changes of the intracellular water space produced by antibacterial drugs in relation to membrane permeability and membrane lesions

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Some conflicting results on membrane response to antibacterial action have led to a systematic investigation of methods commonly used to assess membrane damage. Measurement of protein leakage is of no value, at least in the case of *E. coli* K 12 and B (Rausa, Cannizzaro, Arena & Gebbia (1966); Rausa, Arena, Gebbia & Guardo (1967); Rausa, Gebbia, Guardo & Persico (1967); Rausa, Arena, Gebbia & Guardo (1967)). The CTR–strain of *E. coli* B is classified as cryptic to citrate: that is, it is classified as a strain impermeable to citrate, although possessing the enzymes required to oxidize it. We have, however, found that CTR + and CTR – strains take up equal quantities of ^{14}C-citrate, although the CTR – mutant has been said to utilize citrate only after membrane damage (Krampitz, 1961). Part of the label which is retained by the cryptic cells is recovered as $^{14}CO_2$ or in the various fractions of the extract, especially in protein suggesting that accumulation within the micro-organisms is partly due to incorporation.

Measurement of water space has also given conflicting results. Thus measurement of water space in *E. coli* K 12 gives different results according to the technique and type of tracer used: for instance, after centrifugation at 12,000 *g* the water space is 25–30% by $Na_2\,^{35}SO_4$, 60–70% by ^{14}C-urea, and 80–90% by ^3H_2O. The water space of a *Shigella sonnei* strain, of which the cells are normally unable to oxidize lactose, increases substantially after centrifugation at 200,000 *g* and the cells then oxidize lactose. In several mutants, labelled lactose crosses the membrane of "cryptic" cells to the same extent as that of ordinary ones, apparently by diffusion. This

suggests that the widely accepted opinion that lactose is transported into the cells by the activity of a specific permease is not valid in all cases. Further drugs such as streptomycin, which inhibit permease synthesis, do not affect penetration of lactose. Lactose is, however, freely released by the cells, showing the membranes are permeable in both directions.

While experimental work is still in progress, it appears that current theories on membrane permeability and the action of drugs on the membrane need be carefully reconsidered. We believe that measurement of the water space available to single drugs provides a direct and reliable index of membrane permeability.

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Inhibition of oxidative phosphorylation by atractyloside in digitonin particles of mitochondria

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The effect of atractyloside on digitonin fragments of rat liver mitochondria was first studied by Vignais & Vignais (1961). They found that atractyloside inhibits the phosphate-adenosine triphosphate exchange reaction and 2, 4-dinitrophenol-stimulated adenosine triphosphatase but was inactive on phosphate uptake coupled with beta-hydroxybutyrate oxidation and on magnesium-stimulated adenosine triphosphatase. The ineffectiveness of atractyloside on the phosphate uptake could be explained by the high amount of adenosine diphosphate present in the incubation medium. In fact, an increase in the concentration of adenosine diphosphate competitively removed the effect of atractyloside on oxidative phosphorylation (Bruni, Contessa & Luciani, 1962).

To clarify this point, the effect of atractyloside on the digitonin particles of rat liver mitochondria was reinvestigated. It was found: (*a*) in agreement with Vignais & Vignais (1961) atractyloside inhibits the 2, 4-dinitrophenol-stimulated adenosine triphosphatase, the phosphate-adenosine triphosphate exchange reaction but it is ineffective on magnesium-stimulated adenosine triphosphatase; (*b*) using low concentrations of adenosine diphosphate, atractyloside fully inhibits the phosphorylation coupled with beta-hydroxybutyrate oxidation; (*c*) the binding of adenosine-diphosphate to digitonin submitochondrial particles is also sensitive to atractyloside.

These results show that submitochondrial particles prepared with digitonin react to atractyloside differently from phosphorylating particles prepared by sonic disinte-