

Fig. 3. Detail of final chamber in *Globorotalia truncatulinoides* from Fig. 1B, showing calcite crust as a third layer over the bilamellae.

maximum test dimension of about 685 μ and their test walls are rarely thicker than 20 μ (Figs. 1A and 2A).

Third, bathypelagic specimens of *G. truncatulinoides* collected in plankton tows deeper than 500 m and the majority of empty tests from the ocean floor have heavily encrusted outer test walls, which contrast sharply with the thin, transparent walls of epipelagic specimens. The specimen we have selected from several dozen sectioned shells comes from an ocean bottom sample in the North Atlantic and has a maximum test dimension of 690 μ and a maximum wall thickness of 50 μ (Fig. 1B). In addition to the original bilamellar portion, it has a third layer or calcite crust (Fig. 3) which can be seen to increase in thickness over the earlier chambers. The calcite crust, shown as a stippled area in the schematic diagram (cover), has a thickness of 33 μ that is twice that of the earlier bilamellar portion.

Bé and Ericson (6) have discussed in more detail the shell thickening of *Globorotalia truncatulinoides* which takes place at increasing depths, and Bé (7) described a similar phenomenon for *Globigerinoides sacculifer* (Brady) — *Sphaeroidinella dehiscens* (Parker and Jones). Bé and Ericson concluded that those *G. truncatulinoides* inhabiting water depths greater than 500 m possessed test walls up to twice the thickness of those living near the surface. Our present observations corroborate Hofker's view that the unusually thick outer wall, in contrast to the relatively thin septa and inner walls, is indeed due to "secondary thickening." In our opinion, this secondary thickening is a deep-water phenomenon of calcium-carbonate secretion that is superimposed upon the original, bilamellar test.

Our data indicate that two basic calcification processes are at work in most species of planktonic Foraminifera. Bilamellar shell growth occurs in the epipelagic zone and accounts for the development of the overall test. When the individual descends to deeper habitats, chamber additions to the test are retarded or cease in favor of a progressive increase in wall thickness. This calcite crust deposition is a terminal process in response to some yet unknown stimulus in deeper waters.

The existence of a calcite crust over the original, bilamellar test should be considered in taxonomic studies of lamellar Foraminifera. Emiliani (9) showed on the basis of oxygen isotopic composition that various species of planktonic Foraminifera deposited their shell material at different average depths and that *G. truncatulinoides* occupied the deepest habitat of the nine species he examined. Since the calcite of a mature individual's test is secreted over a considerable range of temperature and depth (23°C to less than 6°C from the surface to a depth greater than 1000 m for *G. truncatulinoides*), determinations of oxygen isotopic ratios in such foraminiferal shells would produce only average values. We agree with Emiliani (9) that his measured temperature differences were due to different depth habitats or the season of average shell deposition or both. We amplify his results with our field observations that shell deposition begins in the euphotic zone and probably continues to well below a depth of 1000 m. This depth factor requires more thorough investigation in order that its implications in paleotemperature studies employing oxygen isotopic composition of planktonic foraminiferal tests can be evaluated.

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Protein as the Mitochondrial Site for Action of Uncoupling Phenols

Abstract. *Phenolic reagents which uncouple oxidative phosphorylation are bound to the protein moiety of mitochondria. This interaction may induce configurational changes in the enzymes of oxidative phosphorylation, and these changes could be responsible for the uncoupling phenomenon.*

Most reagents which uncouple oxidative phosphorylation in isolated animal mitochondria are highly lipophilic. The efficacy of these uncoupling reagents, particularly the substituted phenols, has been related to the extent of their lipid solubility (1). The inference from such studies, together with the knowledge that phospholipid is a major constituent of mitochondria, has led to the assumption that these uncoupling phenols are dissolved in the lipid moiety of the mitochondrion and exert their action in this milieu (2). The possession of lipid solubility by uncoupling reagents is not necessarily indicative that lipids are the mitochondrial constituents taking part in the critical interaction.

Studies in our laboratory have shown that uncoupling phenols, when added to suspensions of mitochondria, are bound to the protein portion of these organelles, and we believe that it is this interaction which is responsible for their uncoupling activity. This report summarizes the evidence for such a conclusion, and we present the results of two particularly relevant experiments.

Bovine serum albumin restores the capacity for oxidative phosphorylation to isolated rat liver mitochondria uncoupled by various substituted phenols. Moreover, the addition of this protein together with adenosine triphosphate largely restores morphological integrity to mitochondria swollen by pentachlorophenol (3). Serum albumin exerts these beneficial effects by tightly binding the uncoupling phenol and thereby removing it from mitochondria. These results, in conjunction with numerous reports demonstrating the capacity of

serum albumin to bind other lipophilic compounds (4), suggested to us that mitochondrial protein may possess a similar capacity to bind lipophilic reagents. This suggestion was substantiated by the following experiments (5): (i) Chromatography on Sephadex G-25 of extracts of mitochondria—disrupted by high frequency sound—containing bound pentachlorophenol or 2,4-dinitrophenol revealed that these compounds migrated with the protein-containing moiety and not with the released lipids. (ii) Treatment of intact mitochondria with reagents such as 8M urea or guanidine, which cause conformational changes in protein released pentachlorophenol from its mitochondrial binding site. (iii) The time required for heat coagulation of mitochondria containing pentachlorophenol was increased compared to untreated mitochondria. Such increased time for thermal denaturation also was observed with serum albumin after its reaction with pentachlorophenol. (iv) Mitochondria, depleted to various degrees of their lipid content,

bound pentachlorophenol to the same or to a greater extent than intact mitochondria.

As a consequence of these findings, an insoluble protein residue, completely devoid of lipids and water soluble components, was prepared from isolated rat-liver mitochondria (6). This protein was tested for its capacity to interact with uncoupling phenols and was comparable in this respect to intact mitochondria. The data in Table 1 reveal clearly that the mitochondrial protein had an affinity for representative halo- and nitrophenols equal to or greater than that of intact mitochondria. Additional experiments showed that the parameters of binding of these uncoupling phenols to both intact mitochondria and to the isolated mitochondrial protein were similar with respect to pH, concentration of phenols, and concentration of mitochondria or mitochondrial protein (5). In a homologous series of halophenols, the absolute amount bound to isolated mitochondrial protein was related to the efficacy of these compounds as uncouplers of oxidative phosphorylation with intact mitochondria, and also related to the ease with which such phenols were removed from intact mitochondria by simple washing procedures.

Further evidence that uncoupling phenols interact with the protein moiety of intact mitochondria was provided by difference spectrophotometry (Fig. 1). Graph A is the absorption spectrum of 0.01M lipid-free serum albumin (11), in 10mM tris buffer at pH 7.5. The solid line in graph B is the difference spectrum of albumin which had been reacted with pentachlorophenol in a molar ratio of 1:5, with the reference cell containing the same concentration of the halophenol. Pentachlorophenol alone in aqueous solution at pH 7.5, as the phenolate ion, has two absorption maxima, at 320 and at 248 m μ (dashed line, graph B). Pentachlorophenol, when dissolved in lipophilic solvents, or when interacted with a phospholipid such as lecithin, exhibits an absorption maximum at 305 m μ . In contrast, the two peaks attributable to pentachlorophenol in the spectrum of albumin reacted with the halophenol are at 330 and 263 m μ , indicating a shift in the absorption maxima of pentachlorophenol when it interacts with this protein (graph B). Such modifications in absorption maxima are indicative of small-molecule binding to protein (7). Proteins which do not bind pentachlorophenol, such as ovalbumin,

do not exhibit a modified absorption spectrum. Graphs C and D represent analogous experiments performed with suspensions of intact mitochondria. It is evident from the peaks at 330 and 263 m μ (graph D) that a modification of the spectrum occurred with mitochondria in the presence of pentachlorophenol similar to that which occurred with serum albumin. Treatment of mitochondria containing pentachlorophenol with 1.5 percent deoxycholate and 0.1N NaOH abolished the absorption maxima at 330 and 263 m μ observed in graph D. This treatment released pentachlorophenol bound to intact mitochondria or to the isolated mitochondrial protein.

Phenol	Phenol bound (%)	
	Intact mitochondria	Mitochondrial protein
Pentachlorophenol	53	70
2,3,5-Trichlorophenol	44	48
2,4-Dichlorophenol	25	38
2,4-Dinitrophenol	7	7

do not exhibit a modified absorption spectrum.

The results of these experiments, together with the foregoing observations, provide cogent evidence that pentachlorophenol interacts with the protein moiety of the mitochondrion. Accordingly, it seems quite possible that the biochemical effect of uncoupling phenols may be a consequence of such protein-phenol interaction. This view is compatible with many diverse observations regarding the action of uncoupling reagents. For example, the different biochemical sites of action presumed for various uncoupling reagents and inhibitors of electron transport, having in common marked lipid solubility, as well as the differing concentrations necessary for their maximum activity, would be a reflection of their interaction with

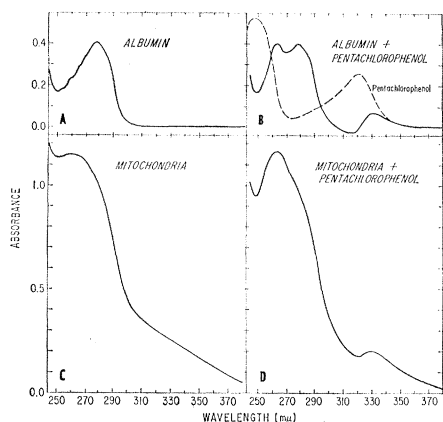


Fig. 1. Spectrophotometric evidence for the binding of pentachlorophenol to serum albumin and to protein of intact mitochondria. Graph A is the absorbance spectrum of 0.01M lipid-free serum albumin (11), in 10mM tris buffer at pH 7.5. The solid line in graph B is the difference spectrum of the same amount of albumin after its interaction with 0.05mM pentachlorophenol at pH 7.5 (compare 12). The absorption spectrum of this concentration of pentachlorophenol is shown in the dashed line of graph B. Graph C represents the absorbance spectrum of rat-liver mitochondria (2 mg protein) suspended in tris buffer at pH 7.5. Graph D is the difference spectrum of this amount of mitochondria after its interaction with 0.25mM pentachlorophenol at pH 7.5. For the difference spectra (graphs B and D) the reference cell contained a concentration of pentachlorophenol equal to that in the experimental cell. A Cary model 14 spectrophotometer with 1-cm silica cells was used.

specific mitochondrial proteins. In this regard, it may be pointed out that of a substantial number of purified proteins examined thus far, only serum albumin, myosin, cytochrome *c*, and mitochondrial protein interacted with pentachlorophenol.

The demonstration that representative uncoupling phenols bind to mitochondrial protein suggests the following mechanism for their action: These reagents interact with the mitochondrial proteins participating in oxidative phosphorylation at sites other than the active centers and induce configurational changes, analogous to the allosteric effects proposed by Monod, *et al.* (8). These altered configurations result in modified enzymes whereby coupling activity not only is lost, but other latent enzymatic activities may appear. Such changes may or may not be reversible, depending on the affinity of the proteins for the uncoupling reagent.

The effect of lipid solubility is not discounted by this proposed mechanism. Rather, we envisage that uncoupling reagents must traverse a lipid barrier or a lipid-protein interface to reach the protein sites where they are bound. Consequently, at least two processes take part in their uncoupling action: the permeation of a nonspecific lipid barrier, and the binding by and affinity for specific proteins of the mitochondrion.

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Uric Acid Transport System: Apparent Absence in Erythrocytes of the Dalmatian Coach Hound

Abstract. *The Dalmatian coach hound appears to lack a mediated transport system for uric acid, which can be demonstrated in the erythrocytes of other dogs by hypoxanthine inhibition of uric acid uptake. This transport deficiency may be a manifestation of a genetic defect which has different metabolic consequences in the liver, in the kidney, and in other tissues, depending on the direction of their differentiation.*

Congenital defects in the renal tubular resorption of two groups of amino acids are accompanied by related defects in their intestinal absorption (1). Absorption in such tissues is probably produced by special arrangements of the ordinary uptake or extrusion processes apparently present in all cells (2); as a consequence, genetic defects in renal and intestinal transport may be represented to some degree in the transport activity of other cells in the organism, whatever the course of their differentiation (3). It is in this connection that we have found that a specific transport system for uric acid, present in the erythrocytes of dogs other than the Dalmatian, appears to be largely if not entirely absent in the Dalmatian coach hound.

Uricase has long been recognized to be present in the liver of the Dalmatian (4). In 1938 Klemperer, Trimble, and Hastings found that, although much less uricolytic activity could be observed in surviving liver slices, the amount of uricase activity in suspensions of finely ground liver was not characteristically lower for the Dalmatian coach hound (5). Subsequent investigation has established two pecu-

liarities of purine metabolism in the Dalmatian coach hound:

1) There is a defect in the renal transport of uric acid. This defect was at first supposed to be the simple absence of the characteristic tubular resorption of this substance (6); but subsequent investigation (7), including stop-flow analysis (8, 9), showed that the usual net resorption of uric acid under some conditions can be replaced by a net secretion, also occurring in the proximal tubule (8).

2) There is an additional obstacle to the oxidation of uric acid (5, 6, 10). The plasma uric acid rose much more steeply in the Dalmatian than in other dogs after the ureter was occluded (6); furthermore, the infusion of uric acid caused a larger and more persistent rise in the plasma uric acid of the Dalmatian coach hound, despite faster urinary excretion (10). The most plausible explanation of the metabolic abnormality lies, we believe, in the presence of a generalized defect in a specific transport process for uric acid. This defect may account not only for the decreased renal retention of uric acid, but also for a difficulty of uric acid access to the interior of various cells and to the hepatic uricase.

Blood samples of two unrelated purebred Dalmatian coach hounds were compared with samples from a number of dogs of other breeds. One of these dogs had the form and markings of a Dalmatian coach hound, but was obviously not full blooded, and lacked the peculiarity in uric acid metabolism. Because of the difficulty we have had so far in obtaining Dalmatians, the observations were repeated often on the same animals. The excretion of characteristic proportions of uric acid was established by urine analysis, and the elevated plasma levels were confirmed. Blood cells were separated by centrifugation, and washed three or four times with isotonic 0.11M phosphate solution, pH 7.0, containing 0.1 percent glucose, under conditions tending to eliminate leukocytes. The red blood cells were then incubated for periods up to 80 minutes in 20 volumes of the phosphate solution containing either 0.1 or 0.25mM uric acid-8-¹⁴C. In parallel experiments 1mM hypoxanthine (11) was included in order to inhibit the mediated portion of uric acid entry. Samples of the suspension were then taken and centrifuged for 2 minutes in a refrigerated centrifuge to separate the cells. The sedimented mass