functioning to produce a limited number of excreted substances, also demonstrates large regions of inactive, pycnotic chromatin.[9]

Thus, facultative heterochromatization in the coccids may be a visible manifestation of what in other organisms is the normal mechanism of a step-by-step differentiation involving intercession at the level of RNA synthesis. On the other hand, it may be the special expression of a mechanism brought into play only when large parts of the genome are simultaneously turned off. Regardless of the specific significance for development in other organisms, coccids provide a unique system for testing cytochemical hypotheses of differential gene expression.

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COMPARTMENTATION OF THE MITOCHONDRION

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One of the classical problems posed by the mitochondrion can be formulated by the following question. How is ATP generated in the mitochondrion, made available to systems external to the mitochondrion? The simple solution of the problem in terms of a molecule each of ADP and P_i entering the mitochondrion by passive diffusion, the same molecules being then joined to one another in pyrophosphate linkage during oxidative phosphorylation, and finally leaving the mitochondrion as ATP, is excluded by a large body of evidence. There are barriers which

separate the entering molecules of ADP and P_i from the molecules which are joined to form ATP. Thus, two uncertainties exist. How do ADP and P_i reach the intramitochondrial sites where oxidative phosphorylation proceeds, and how is ATP, generated in the interior, transported to the outside of the mitochondrion? Evidence will be presented that a unique set of enzymological principles provides the key to the solution of this dilemma.

Before any progress could be made in clarifying the central question raised above, information was required in respect to: (1) the permeability of the mitochondrial membranes to solutes; (2) the arrangement of the mitochondrial membranes; (3) the localization of the units concerned in oxidative phosphorylation; and (4) the interior lanes of communication between the different parts of the mitochondrion. Sufficient physiological and ultrastructural data are now available to permit a systematic examination of the problem of the delivery of internally generated ATP to systems external to the mitochondrion.

The Ultrastructure of the Mitochondrion.—For present purposes we may consider the mitochondrion as a composite of an outer membrane layer and an inner membrane layer with a space between these two layers[1, 2] (cf. Fig. 4 of ref. 2). The inner membrane layer invaginates into the interior in the form of hollow cristae. The space within the crista is continuous with the space between outer and inner membrane.[2] Without specifying whether the inner membrane that makes up the walls of the cristae has the same composition as the inner membrane that parallels the outer membrane, we can invoke evidence that the entire inner membrane, regardless of location, is lined on its interior side with a matrix material (structural protein network). This matrix lining possibly underlies why the inner membrane is relatively impermeable to solutes while the outer membrane is relatively permeable. The units which carry out oxidative phosphorylation may be provisionally identified with the subunits of the inner membrane that constitute the walls of the cristae.[3] These subunits have been called the elementary particles.[3] The matrix side of the inner membrane may be considered as a structure-filled space in contradistinction to the structureless space that separates outer and inner membranes.

The interpretation of the ultrastructure of the mitochondrion given above has facets that are not essential to the argument (these have been introduced as an aid to visualization) and facets which are essential. The latter are: (1) a marked difference in properties between outer and inner membrane; (2) a fluid-filled space that separates outer and inner membrane and is continuous with the space within the cristae; (3) the localization of the matrix on the interior side of the inner membrane. The first has been clearly established. The fact of a structureless space between membranes is generally accepted but the fluid nature of the space is still an assumption.

Passive Diffusion of Solutes into the Mitochondrion.—Small molecules uniformly can penetrate a limited portion of the mitochondrial water too rapidly to be measured.[4, 5] We have not attempted to determine the upper limit to the size of a molecule that is still compatible with rapid penetration. In our studies[4] evaluation of the degree of penetration has been based on the assumption that polydextran of molecular weight $60\text{–}90 \times 10^3$ does not penetrate the mitochondrion. The extramitochondrial water volume in the pellet of sedimented mitochondria is then

evaluated from the concentration of polydextran per ml. By this criterion at least 25–30 per cent of the mitochondrial water is penetrated by all small solute molecules.[4] As far as the particular molecules of immediate interest are concerned (adenine nucleotide, inorganic phosphate (P_i) divalent metal ions, and substrates), all these can rapidly penetrate by passive diffusion into the mitochondrion but not into all sectors of the mitochondrion. The rapidly penetrable sector accounts for no more than 30–50 per cent of the total water present in the mitochondrion. We shall equate this rapidly penetrable sector with the space that separates outer and inner membranes and equate the outer membrane layer with the readily permeable layer since this model appears to explain best the observed behavior of the mitochondrion.[4] Some incisive studies of Whitaker offer strong support for this interpretation.[2]

When mitochondria which have been passively penetrated by solutes are washed in various media, the bulk of the solute is washed out just as readily as it penetrated. The outer membrane thus imposes a barrier neither to the entry nor to the exit of molecules of low molecular weight.

Energized Translocation of Solutes into the Mitochondrion.—When mitochondria carry out citric cycle oxidations in presence of divalent metal and phosphate ions, the ions in question are actively translocated[6–11] and deposited into the interior as $Mg_3(PO_4)_2$, $Ca_3(PO_4)_2$, etc. These translocated ion deposits are not readily removed from the mitochondrion by washing in various media. The available evidence suggests that translocation involves the energized movement of ions from the space between membranes across the inner membrane into the matrix space.[12, 13] Thus, in our proposed model an energy-requiring process is necessary to move ions through the inner membrane to the matrix space.

Osmometer Characteristics of the Inner Membrane.—When mitochondria are exposed to solutions of sucrose of increasing molarity, the water content of the mitochondrion varies inversely with the osmolarity of the suspending medium in a straight line relation.[14, 15] The mitochondrion thus conforms to a perfect osmometer. Since the outer membrane appears to be freely permeable to solutes, it follows that the osmometer properties have to be ascribed to the inner membrane. The perfect osmometer is by definition a membrane system which is completely impermeable to solutes but permeable to water, and this description can now be applied to the inner-mitochondrial membrane.

Bound Coenzymes and Small Ions in the Mitochondrion.—There is a set of well-established mitochondrial phenomena (latency of enzymes, osmometer properties, bound coenzymes, and energized translocation) that have been satisfactorily interpreted in terms of an impenetrable outer membrane. How can these phenomena be rationalized in terms of a readily permeable outer membrane? Clearly, some new considerations will have to be invoked if the notion of impenetrability is to be retained.

An intact mitochondrion has a complement of essential coenzymes, adenine nucleotides, divalent metal ions, and inorganic phosphate. The level of each of the coenzymes and of the various ions is fairly uniform from one batch to another of mitochondria of a given source. These essential molecules cannot be removed from intact mitochondria by a washing procedure nor can the levels be augmented by exposing mitochondria to high concentrations of any of these species and then

removing by washing whatever is not bound. Clearly, a set of small molecules is sequestered within the mitochondrion in such a manner that these are retained at a level sufficient for maximal activity of the dependent systems. There are devices (attachment to tight binding sites, clathrate type of sequestering, etc.) which insulate from the rest of the mitochondrion a set of small molecules including ADP and P_i and prevent these from leaving the mitochondrion. This and the observed incomplete exchange with external component lead to the notion that a set of coenzymes and selected ions is locked up into impenetrable mitochondrial structures, and by virtue of this isolation these molecules or ions which are present in fixed concentrations are not in equilibrium with the same molecules presented externally to the mitochondrion.

Binding of Adenine Nucleotide and P_i by Mitochondria.—When intact mitochondria are incubated with C^{14}-labeled adenine nucleotide or P^{32}-labeled inorganic phosphate, label is incorporated into the mitochondrial nucleotide in a form that cannot be removed merely by washing mitochondria in 0.25 *M* sucrose.[16-18] This labeled nucleotide, in a form which is resistant to washing, has to be distinguished from the form of the nucleotide of P_i that is readily washed out.[18]

An easier way to visualize the dual character of the bound phosphate and nucleotide would be in terms of the following description. About half of the total bound nucleotide or phosphate is exchangeable with externally added labeled molecules.[18] The rest of the bound nucleotide or phosphate is not exchangeable, and never becomes labeled by exposure of the mitochondrion to labeled molecules. The extent of incorporation of labeled nucleotide or P_i into the mitochondrion in a form resistant to washing provides a measure of the exchangeable sector of the bound nucleotide or phosphate. It has to be noted that the constancy of the level of bound adenine nucleotide and phosphate in the mitochondrion is not incompatible with an exchangeable moiety.

Atractyloside as a Reagent for Probing Mitochondrial Compartmentation.—Recent studies with atractyloside,[19, 20] a potent inhibitor of oxidative phosphorylation and other reactions involving adenine nucleotides in intact mitochondria, have led us to the concept that this compound interferes with the access of extramitochondrial ADP to the sites of phosphorylation within the mitochondrion.[18] A similar conclusion has been reached by Klingenberg[5] and by Chappell and Crofts[21] and is based on the following observations. (1) Atractyloside does not affect phosphorylation or its partial reactions in submitochondrial particles.[18, 20, 21] There is, however, some evidence that in the preparation of ETP_H, atractyloside added to mitochondria prior to sonic irradiation retains some activity in the resulting particles. (Further investigation of this point is projected.) (2) The incorporation of externally added labeled ADP and ATP into the mitochondrion is blocked by atractyloside.[23] Since this reaction represents an exchange between bound and external nucleotide,[5, 18] it appears that the accessibility of external ADP to intramitochondrial binding sites is blocked by this reagent. (3) In addition, Chappell and Crofts[21] have shown that while atractyloside inhibits conversion of external ADP to ATP during both oxidative phosphorylation and substrate level phosphorylation, it has no effect on the conversion of internal ADP to ATP implemented by the same phosphorylation mechanisms. There is also no effect of atractyloside on internal ATPase while the same activity operative on externally added ATP is inhibited.[21]

These authors list several other reactions in this category.[21] (4) While mitochondrial myokinase is not inhibited by atractyloside,[18, 21] the conversion of added AMP to ADP via interaction with bound ATP is inhibited by atractyloside.[18] If myokinase is indeed localized primarily on the exterior of the mitochondrion,[21] then it follows that bound ATP cannot interact with external ADP or AMP in the presence of this inhibitor. If myokinase is also present within the mitochondrion,[16] then the same result would obtain if atractyloside blocked the entrance of AMP into the area of myokinase localization. (5) About 30 per cent of mitochondrial water is rapidly penetrated by added adenine nucleotide,[4] and this penetration is not inhibited by atractyloside.[4, 5] (6) An additional piece of evidence of as yet undetermined significance is that about 10 per cent of the exchangeable bound adenine nucleotide is displaced by atractyloside.[18] It has not yet been established that this displacement is directly related to the action of atractyloside.

Several possibilities appear open to explain the experimental results cited above. The first is that atractyloside blocks a "permease" type activity[21] in the inner membrane of the mitochondrion. In this model, external ADP would pass into the interior of the mitochondrion where it would exchange with the bound internal components and be phosphorylated to ATP by direct interaction with the energy transport system. The entrance and exit of adenine nucleotide would be mediated by a "permease" system[25] located in the inner membrane. Such a model would require that a portion of the bound adenine nucleotide be held in a form or location which does not permit exchange with external nucleotide. If a small amount of adenine nucleotide were bound by the "permease," then the observed displacement of a portion of the bound nucleotide might be explained by competitive interaction of atractyloside with the enzyme. Such an arrangement would also be compatible with the fact that atractyloside inhibition can be overcome by additional ADP.[19, 24]

Another possible explanation cited by Chappell and Crofts[21] is that atractyloside interferes with the transfer of a phosphoryl group across a membrane which is impermeable to nucleotide. We feel that the thesis which will be developed below also provides a satisfactory explanation for the experimental facts.

The Concept of the Impenetrable Unit.—On the basis of the experimental evidence cited above, it is permissible to assume that the repeating unit of oxidative phosphorylation contains fixed amounts of both adenine nucleotide and inorganic phosphate which are closely associated with the sites where ATP is synthesized from ADP and P_i. The unit of oxidative phosphorylation is visualized as a sealed chamber into which solutes neither enter nor leave. The nonexchangeable moiety of the bound adenine nucleotide and inorganic phosphate may be identified with the ADP and P_i in the sealed chamber of the repeating unit.

We further postulate that the membrane which encloses the repeating units of oxidative phosphorylation is the site of an enzyme which is linked to ADP and P_i on both the interior and exterior face of the membrane. This enzyme which we have elected to call a mesomerase catalyzes the reversible transfer of the pyrophosphate bond from the pair of ligands on the one surface to the pair on the other surface (Fig. 1). In this way ATP generated within the repeating units is transported to the outside not in the form of the whole nucleotide molecule but simply in the form of the high-energy bond. Atractyloside specifically inhibits the activity of

the membrane-bound mesomerase, and this inhibition could be due to a displacement on the exterior surface of ADP by atractyloside. Mesomerase in the mitochondrion controls oxidative phosphorylation by regulating the regeneration process by which ADP and P_i are made available for another cycle of synthesis.

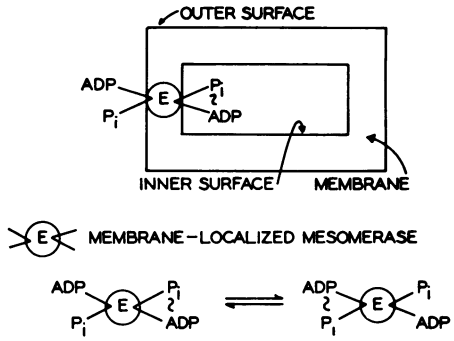

FIG. 1.—Diagrammatic representation of membrane-localized mesomerase in the impenetrable unit of the inner membrane.

The hypothesis of the impenetrable unit with a membrane-bound enzyme catalyzing the transfer of a bond has to account for three phenomena: (1) the inhibition by atractyloside of the incorporation of labeled nucleotide into the bound nucleotide of the mitochondrion; (2) the inhibition by atractyloside of the conversion of externally added AMP to ADP and ATP mediated by internally generated ATP; and (3) the insensitivity of oxidative phosphorylation to atractyloside in submitochondrial particles. The second phenomenon is readily accounted for on the basis that mesomerase-bound ATP is the source of the phosphoryl group for the conversion of AMP to ADP by myokinase action, and this process is catalytic when oxidative phosphorylation is proceeding. The insensitivity of oxidative phosphorylation in ETP_H to atractyloside may be explained simply on the basis that the units of oxidative phosphorylation are no longer impenetrable, and both inorganic phosphate and ADP can now readily penetrate the unit (see also ref. 22). Mesomerase thus no longer controls the interaction of the unit with external ADP and P_i. In line with this interpretation is the fact that ETP_H does not contain bound inorganic phosphate and lacks osmometer properties. It does however contain bound nucleotide, only a small part of which is exchangeable (about 20%)[18] and which in the main is inert (in the sense of not cycling during oxidative phosphorylation). To explain the inhibition of the incorporation of adenine nucleotide into the bound nucleotide of the mitochondrion by atractyloside requires an additional assumption, namely, that the binding sites for adenine nucleotide and inorganic phosphate are accessible or available only when bound ATP is formed on the exterior side by mesomerase action. In other words, the state of the repeating unit controls the capacity to bind adenine nucleotide and phosphate. This binding capacity is lost when mesomerase action is suppressed by atractyloside.

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*SYNTHETIC DEOXYRIBO-OLIGONUCLEOTIDES AS TEMPLATES FOR THE DNA POLYMERASE OF ESCHERICHIA COLI: NEW DNA-LIKE POLYMERS CONTAINING REPEATING NUCLEOTIDE SEQUENCES**

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Chemically synthesized deoxyribo-oligonucleotides containing alternating deoxyadenylate and deoxythymidylate residues [$d(AT)_3$ to $d(AT)_7$][1] have been shown recently to prime the synthesis by *E. coli* DNA polymerase of the high-molecular-weight deoxyadenylate-deoxythymidylate copolymer.[2] The present report describes further work on the utilization of synthetic deoxyribo-oligonucleotides as templates in the DNA-polymerase-catalyzed reactions. Three new reactions have been discovered and are as follows: (1) A mixture of dT_{11},[1] dA_7,[1] dATP, and dTTP gives a high-molecular-weight polymer (dA:dT) consisting of long poly dT and of long poly dA. (2) The omission of dTTP in the above reaction mixture leads to the formation of deoxypolyadenylate which is much longer than the dT_{11} template. (3) A mixture of the decanucleotide, $d(TC)_5$,[1] containing alternating thymidylate and deoxycytidylate units, the decanucleotide, $d(AG)_5$,[1] containing alternating deoxyadenylate and deoxyguanylate units, and the deoxynucleoside triphosphates containing the four bases, T, C, A, and G leads again to a high-molecular-weight product, poly dTC:dAG, in which one strand contains alternat-