

Concepts of Citrate Production and Secretion by Prostate

1. Metabolic Relationships

Leslie C. Costello and Renty B. Franklin

Department of Physiology, University of Maryland Dental School, Baltimore

Accumulation and secretion of extraordinarily high levels of citrate are principal functions of the prostate gland of humans and other animals. To achieve this, prostate secretory cells must possess unique metabolic relationships which distinguish them from virtually all other cells. Furthermore, citrate metabolism is markedly altered in benign prostatic hyperplasia (BPH) and in prostatic carcinoma (CA). This review assimilates existing information and presents current concepts related to 1) the pathway of metabolism associated with net citrate production, 2) the involvement of transporting mechanisms associated with citrate secretion, 3) energy implications of citrate production, 4) altered metabolic relationships in BPH and CA, and 5) the importance of citrate relationships as biochemical markers for characterizing prostate secretory epithelial cells. It is hoped that this review will bring attention to the importance and urgency of elucidating and understanding the metabolic relationships associated with citrate production by normal and neoplastic prostate epithelial cells. Research in these areas has been severely neglected despite the fact that the combined incidence of BPH and CA constitutes the most prevalent neoplastic disease among men.

Key words: aspartate transport, prostatic fluid, prostate energy metabolism, mitochondrial aspartate aminotransferase, glutamate-aspartate-citrate pathway, zinc metabolism

INTRODUCTION

We concluded our last review in 1978 [1] on the subject of prostate citrate production with the statement, "It is hoped that this discussion has generated interest in the many unresolved aspects of physiological and pathological relationships of prostate citrate metabolism that must be studied". At that time it was fair and accurate to state that the prostate was among the least understood and least studied (regarding intermediary metabolism and regulation) of all the tissues in the body. More than a decade has elapsed and the preceding assessment is, unfortunately, still applicable. This, despite the fact that the combined incidence of benign prostatic hyperplasia and prostatic carcinoma has increased by 50% during this period and now constitutes the most prevalent neoplastic disease among men.

In this current presentation, we will attempt to refocus attention to, and redirect interest in, the unique and major function of the prostate gland, namely the production and secretion of citrate. We will integrate and assimilate recent and past information

Received for publication August 31, 1990; accepted September 20, 1990.

Address reprint requests to Dr. Leslie C. Costello, Department of Physiology, University of Maryland Dental School, 636 West Baltimore Street, Baltimore, MD 21201.

into current concepts regarding metabolic relationships involved in prostate citrate production and the pathological implications associated with prostatic neoplasms. This presentation does not deal with the important issue of hormonal regulation of prostate citrate production, which will be the subject of a sequel now in preparation. We will not attempt to provide an exhaustive review of all published work since our last review. We are taking some license to develop and to offer our concepts to our colleagues for their consideration and scrutiny. It is hoped that this will rekindle interest and will amplify and accelerate research activities to address the many important, yet unresolved, questions associated with the function of prostate citrate production. Consequently, we must acknowledge our appreciation of the contributions of many investigators, some of whom might not be specifically cited. In this regard we humbly offer a special acknowledgment to Dr. Charles Huggins whose pioneering work in prostate citrate metabolism has been inspirational and whose insight and foresight have proven to be incredibly accurate.

CITRATE PRODUCTION—THE UNIQUE CHARACTERISTIC OF PROSTATE

First, the important functional relationship of uniquely high citrate production by prostate must be re-emphasized. In humans and many other animals, semen contains an extremely high concentration of citrate, often in the range of 5–50 mM [2,3]. By contrast, the citrate concentration of blood plasma is generally maintained in the range of 0.07–0.12 mM. Consequently, as represented in humans, the citrate concentration of semen is 40–400-fold greater than the plasma concentration. However, not all animals (notably dogs) produce semen with high citrate levels. The source of seminal citrate varies in different animals. In humans and other primates, the prostate gland is the principal source of citrate. In pigs and bulls the seminal vesicles provide the major source of seminal citrate; however, the pig prostate is also a citrate-producing gland. In rats, the ventral prostate (VP), seminal vesicles and lateral prostate are citrate-producing glands. While we recognize these species relationships, our discussion will deal mainly with the role of prostate as the citrate-producing gland, particularly since this represents the situation in humans.

This function resides within the secretory epithelial cells of the prostate and requires unique metabolic relationships to permit accumulation and secretion of enormous amounts of citrate. Consequently in prostate, citrate is mainly an end product of metabolism. By contrast, “typical” cells use citrate as a major source of energy (Krebs cycle oxidation) or as a source of cytosolic acetyl CoA for fatty acid synthesis. Consequently, prostatic secretory epithelial cells uniquely sacrifice an enormous potential energy source by accumulating and secreting, rather than using, such high levels of citrate. These relationships underscore the importance of citrate production as a major function of the prostate. Therefore, we submit that this is the most distinguishing function which cells must exhibit in order to be considered normal prostatic secretory epithelial cells. The following discussion deals with the metabolic relationships of citrate production and secretion and with the pathological relationships associated with prostatic citrate production.

CITRATE CONCENTRATION AND DISTRIBUTION IN PROSTATE

In humans, the citrate concentration of prostatic fluid ranges from 24 to 130 mM [4], whereas, the blood plasma citrate concentration is maintained at 0.1 mM.

Consequently, prostatic fluid citrate concentration is 240–1,300 times greater than the plasma concentration. Although prostatic fluid citrate values vary considerably, this relationship exists in all animals containing citrate-producing prostate glands. The citrate concentration of human prostatic tissue (nmoles/g wet weight) ranges from about 1,000 in carcinoma (CA) to 12,000 in benign hyperplasia (BPH). To our knowledge and most surprisingly, actual measurements of citrate levels in normal human prostate have not been reported. A representation of the normal citrate relationships in prostate tissue can be obtained from animal studies. In VP, the tissue citrate concentration is about 3,000–4,000, and in pig prostate the value is about 4,300. In bonnet monkeys, the citrate concentration is about 1,800 in cranial prostate and 6,000 in caudal prostate [5]. Based on these relationships and assuming that normal prostate citrate values will be between the high BPH values and low CA values, we would estimate that normal human prostate tissue citrate concentration might be in the range of 4,000–6,000. By contrast, all other soft tissues generally contain citrate in the range of 100–400 nmoles/g. Consequently, citrate-producing tissue normally contains 10–60 times more citrate than other soft tissues in the body.

In typical soft tissues (e.g., muscle, liver), measurements of the tissue citrate levels are generally representative estimates of the intracellular citrate concentration. In such tissues, the intracellular compartment comprises the major component. In addition, the extracellular fluid compartment generally contains low levels of citrate (equivalent to the plasma level of about 0.1 mM). However, these situations do not exist in prostate tissue. First, the luminal content of the acinar structure comprises a major extracellular component that reduces the proportion of tissue weight or volume which is represented by the intracellular compartment. Second, the luminal content of prostatic fluid contains an extremely high citrate concentration. Consequently, the determination of prostate tissue citrate concentration does not provide a reasonable estimate of the intracellular citrate concentration. In human prostate, these problems are compounded by the additional presence of large amounts of stromal tissue which are not associated with the citrate producing function of prostate. Nevertheless, it has become important to determine the distribution of citrate in prostate, particularly the epithelial intracellular concentration. For this, VP provides an appropriate model. Teased VP fragments cleaned of extraneous connective and vascular tissue contain predominantly acinar structures with a minimal amount of stromal tissue (about 10%). Furthermore, secretory epithelium represents about 85% of the cell mass.

Farnsworth [6,7] suggested that most of the citrate content of prostate resided in the secretory extracellular space, and that the intracellular concentration of citrate was not uniquely high. We [1] suggested that, although prostatic secretion likely accounted for the major component of the tissue citrate, the intracellular compartment of epithelial cells probably also contained significantly high levels of citrate. This conclusion was based on our observations that pressed and washed VP fragments retained high citrate content, about 1,000 nmoles/g as compared with 3,000 nmoles/g for untreated prostate fragments. More recently we used the method of “washout kinetics” [8] to ascertain the distribution of citrate in VP. The washout kinetics indicate that the citrate concentration of the intracellular compartment is approximately 1.2 mM. This would be 3–10 times higher than the expected intracellular citrate concentration of other soft tissues. Even after prolonged (6 hr) washout, about 50% of the intracellular citrate remains in the cellular compartment. [Littleton and Cooke (personal communication) determined the citrate content of subpopulations of

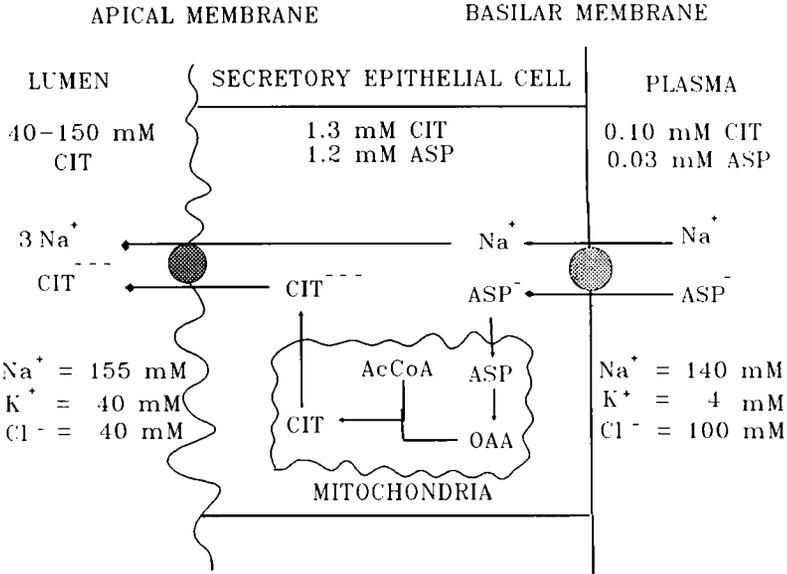


Fig. 1. Proposed distribution and transport of citrate and aspartate in prostate secretory epithelial cells. A high affinity, Na⁺-coupled aspartate transporter at the basilar membrane is responsible for the import of aspartate against a concentration gradient. Intracellular aspartate is converted to citrate. The apical membrane contains an Na⁺-coupled citrate transporter, which is necessary for the secretion of citrate against a large concentration gradient. This Na⁺-coupled citrate transporter results in Cit³⁻, rather than Cl⁻, being the major anion in prostatic fluid. Although not represented in the illustration, the luminal Na⁺ concentration will likely vary in proportion to the Cit³⁻ concentration.

VP cells obtained by density gradient centrifugation. The citrate concentration of the secretory cells was 3–4 times greater than the other cell types.] We would conclude that 1.2 mM is representative of the secretory epithelium which comprises the major cellular component of VP fragments. Unfortunately, no information exists regarding the intracellular distribution of citrate. At this time we propose that the intracellular concentration of 1.2 mM likely approximates the cytosolic concentration of citrate. Based on these assumptions the citrate gradient across the basilar cell membrane (cytosol to plasma ratio) would be of the magnitude of 12:1. If these relationships established with VP were applicable to human prostate which contains extremely high citrate levels in prostatic fluid, the approximate gradient at the apical membrane (cytosol: luminal content) could be of the magnitude of 1:100. This requires that an active citrate transport process is probably associated with the secretion of citrate.

These unique citrate relationships (Fig. 1) necessitate some consideration of the requirements and mechanisms by which these conditions are maintained by the secretory epithelial cells. The existing conditions would tend to result in an efflux of citrate into circulation. This would be counterproductive to the function and process of prostatic fluid citrate secretion. It is likely that the intracellular citrate exists in at least three forms. Some portion is chelated by the divalent cations Mg²⁺, Ca²⁺, and Zn²⁺; some citrate is in the form of citric acid; and the major portion is represented as citrate anions (Cit³⁻). The diffusion of intracellular Cit³⁻ across the plasma membrane should be difficult. Consequently, efflux of Cit³⁻ down the concentra-

TABLE I. Citrate and Electrolyte Composition of Prostatic Fluid*

| | Na ⁺ | K ⁺ | X ⁺ | T ⁺ | Cl ⁻ | Cit ³⁻ | Y ⁻ | T ⁻ |
|----------|-----------------|----------------|----------------|----------------|-----------------|-------------------|----------------|----------------|
| Human PF | 155 | 40 | 30 | 225 | 40 | 170 | 15 | 225 |
| dog PF | 160 | 5 | 5 | 170 | 160 | 2 | 8 | 170 |
| Pig SVF | 30 | 50 | 6 | 86 | 3 | 80 | 3 | 86 |

*Values estimated from the data presented by Huggins (1947) and Mann and Lutwak-Mann (1981). Values are presented as milliequivalents/liter. Pig SVF refers to seminal vesicle fluid. X⁺, other cations; T⁺, total cations; Y⁻, other anions; T⁻, total anions.

tion gradient across the basilar cell membrane is likely minimal. The citric acid form is likely more permeable and could efflux into circulation thereby resulting in some loss of Cit³⁻. Overall, these conditions would tend to result in the retention of citrate within the cell where it is synthesized. Supporting these suggestions is the difficulty observed in “washing out” citrate from prostate epithelial cells. However, some consideration must be given to the possibility that a citrate transport process might exist at the basilar cell membrane. Such a citrate transporter could achieve two purposes: (1) it could provide for a reuptake of effluxed citrate, thereby preventing citrate loss from the epithelial cells; or (2) it could provide a mechanism for the net uptake of citrate from circulation as a source of citrate for secretion by these cells. An important clue to this problem would be provided by citrate extraction studies, i.e., arteriovenous differences across the prostate. Does the prostate gland extract citrate from circulation?; or does the gland add citrate to circulation? Unfortunately, the organizational relationships of venous circulation of prostate make such studies extremely difficult. We are attempting to resolve this issue by utilizing an isolated VP perfusion system. However, other animal models, in the absence of human studies, might be considered.

The situation at the apical membrane is even more imposing than that of the basilar membrane. Here the extracellular (prostatic) fluid citrate content can be a hundred times greater than the intracellular citrate concentration. Citrate secretion would have to require a citrate transport mechanism associated with the apical membrane. Prostatic secretion has generally been viewed to involve apocrine and merocrine processes. One might suggest that citrate is “packaged” into a secretory structure which ultimately becomes a component of acinar content (prostatic fluid) via one of these two processes. However, the important observation of Huggins [9] regarding electrolyte composition of prostatic fluid would not support such a proposal (Table I). Human prostatic fluid is most unique in that Cit³⁻, not Cl⁻, is the major attendant anion for sodium. This relationship also exists in citrate-producing glands of other animals [2]. In pig seminal vesicle fluid, which also contains very little Cl⁻, Cit³⁻ is the major anion that provides electrochemical balance. In contrast, dog prostatic fluid, which contains very little citrate, contains Cl⁻ as the major anion. From these data it appears that the citrate concentration (meq/liter) of these reproductive fluids can be estimated by the formula

$$(\text{Cit}^{3-}) = (\text{Na}^+) + (\text{K}^+) - (\text{Cl}^-).$$

This relationship exists regardless of the osmolarity and cation composition. It is both interesting and significant to note the differences between human prostatic fluid and pig seminal vesicle fluid, both of which contain very high citrate levels. The latter is

hyposmotic and contains more K^+ than Na^+ , whereas the former is hyperosmotic, with Na^+ being the major cation. In both cases, K^+ concentration is high compared to plasma. Yet, in both cases, Cit^{3-} is the major anion, and the formula presented above is applicable.

These relationships suggest that prostate epithelial cells function as transporting cells in which Na^+ and Cit^{3-} transport are important activities. Aspartate transport appears to be another important activity of prostate epithelial cells that might be linked to Na^+ transport. It is extremely important to recognize that the secretory process eliminates Cl^- from the prostatic fluid. These conditions can be likened to the pancreatic acinar cells, which transport sodium from circulation to pancreatic fluid, and which involves a coupled HCO_3^- transport mechanism. As a result HCO_3^- (which is synthesized intracellularly as is citrate in prostate) replaces Cl^- as the major anion of pancreatic fluid. Such relationships cause us to propose a similar mechanism in prostate that we would modify to include aspartate transport (Fig. 1). This hypothesis proposes that coupled transport of Na^+ and Asp^- (described below) occurs at the basilar cell membrane to permit uptake from circulation. The Asp^- is converted intracellularly to Cit^{3-} . Cit^{3-} is then secreted into the acinar lumen via a Na^+ coupled citrate transporter associated with the apical membrane. In this mechanism, electrochemical neutrality is achieved and the requirement for Cl^- is eliminated. Such a mechanism is, in part, supportive of the proposal of Farnsworth [7] that a Na^+/K^+ ATPase transport mechanism might be associated with citrate secretion. For simplification, we have represented a stoichiometry of $3 Na^+/Cit^{3-}$. It is conceivable that Cit^{3-} is transported with H^+ and $2 Na^+$, which could result in dissociation of H^+ in lumen, thereby contributing to the lower pH (about 6.6) of prostatic fluid. Citrate transporters have been described in various cells and systems and the likelihood for its existence in relation to prostate citrate secretion seems extremely plausible. That these fascinating, important, and unique transport relationships have not been rigorously explored since the early reports of Huggins and of Mann is most unfortunate. Certainly methods, techniques, and models are now available to pursue such studies.

CITRATE METABOLISM OF PROSTATE SECRETORY EPITHELIAL CELLS

Two possible mechanisms could account for the source of accumulated and secreted citrate. The secretory epithelial cells might transport citrate from circulation across the cell and finally into the acinar lumen. Unfortunately, no information exists regarding this possibility. The second possibility is that citrate is synthesized by the secretory epithelial cells. This has been the area of our major focus over the past decade. Current evidence supports the view that a major function of the secretory epithelial cells is to synthesize and to accumulate as well as to secrete citrate. Based on our studies mainly with VP, prostatic epithelial cells contain the unique metabolic relationships and pathways to synthesize and to accumulate citrate (Fig. 2). We refer to this as *net citrate production*, which involves two metabolic considerations: (1) the pathway and rate of citrate synthesis, and (2) the capability and rate of citrate oxidation. To achieve net citrate production, the rate of citrate synthesis must exceed the rate of citrate oxidation, and the resultant citrate produced must remain unused. This relationship was recognized by Huggins in 1947 [9]. The accumulated citrate is then available for secretion.

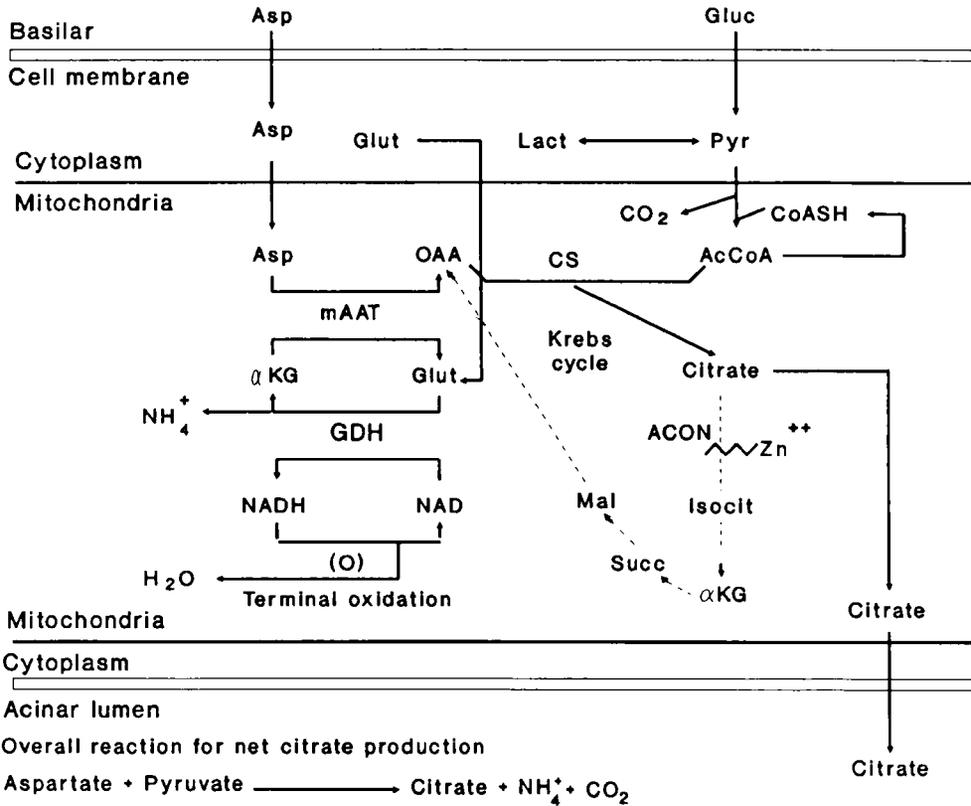


Fig. 2. Proposed pathway of net citrate production and secretion by prostate secretory epithelial cells. Aspartate provides the intramitochondria source of oxalacetate, and glucose provides the source of acetyl CoA for citrate synthesis. Citrate oxidation is limited at the aconitase step, which allows for net citrate production. Accumulated citrate is secreted into the acinar lumen. Asp, aspartate; Gluc, glucose; Glut, glutamate; Lact, lactate; Pyr, pyruvate; OAA, oxalacetate; AcCoA, acetyl CoA; Isocit, isocitrate; α KG, α -ketoglutarate; Succ, succinate; Mal, malate; mAAT, mitochondrial aspartate aminotransferase; GDH, glutamate dehydrogenase; CS, citrate synthase; ACON, aconitase.

Citrate Oxidation

Essential for the capability of prostate secretory cells to accumulate citrate must be their relative inability to oxidize citrate. The entire metabolic pathway of citrate synthesis and oxidation is a function of the Krebs cycle which resides within the mitochondrial compartment. In "typical" cells, mitochondrial citrate is readily subjected to Krebs cycle oxidation from which energy production is derived, or it is shuttled out of mitochondria into cytosol where it is cleaved by citrate lyase to provide AcCoA for fatty acid synthesis. Prostatic secretory cells must present a different situation, which will permit a significant portion of the citrate synthesized to be accumulated.

Barron and Huggins [10] suggested that citrate accumulation in prostate resulted from a lack of citrate oxidation. Humphrey and Mann [11] reported that rat seminal vesicle (a citrate-producing gland) contained little aconitase. This led to speculation that low aconitase might be limiting citrate oxidation. However, other early reports

suggested the presence of aconitase in prostate. The confusion associated with these reports apparently resulted largely from the type of tissue preparations employed. For example, Franklin et al. [12] reported that VP contains both cytosolic (c-) aconitase and mitochondrial (m-) aconitase as is the case in many other tissues. Indeed the c-aconitase isozyme appears to be more prevalent and the m-aconitase somewhat low in VP. Consequently early studies, deprived of the benefit of such subsequent revelations, which employed whole tissue homogenates and extracts, would likely and understandably have misinterpreted the presence of c-aconitase as a mitochondrial enzyme for citrate oxidation. The first direct study of Krebs cycle enzymes in isolated prostate mitochondria was reported by Costello et al. [13]. This report clearly demonstrated that VP mitochondria contained isocitrate dehydrogenase (IDH) and suggested that m-aconitase activity was the likely step which would limit citrate oxidation. Subsequent early studies from our laboratory supported the proposal that VP had a limited capability to utilize citrate and that m-aconitase reaction was possibly a key step in limiting citrate oxidation. These and other early studies were discussed in our previous review [1], which led to our proposal of a unique limited aconitase associated with prostate mitochondria. Costello et al. [14] presented the first report involving the direct assay of m-aconitase activity in VP mitochondria. The studies revealed that m-aconitase activity appeared to be relatively low in prostate and that citrate oxidation was limited at the aconitase step. These conclusions were corroborated by Harkonen et al. [15]. They demonstrated that fluoroacetate had no effect on the formation of citrate in prostate whereas all other tissues exhibited an increase in citrate production. In addition, fluoroacetate had a minimal effect on citrate oxidation (which was already low) by prostate. The results indicated that the aconitase inhibitor was ineffective in prostate due to an already limited aconitase activity. Coupled to the low aconitase activity and high citrate concentration was the observation of a high citrate: isocitrate ratio which characterizes prostate tissue [16] (Table II). In most soft-tissues the citrate:isocitrate ratio generally approximates 10:1, whereas in prostate it approximates 30:1.

Aconitase (aconitate hydratase) catalyzes the interconversion of citrate and isocitrate by two readily reversible reactions:



At equilibrium, m-aconitase establishes a ratio of citrate = 88: cis-aconitate = 4: isocitrate = 8. Typically, m-aconitase belongs to the class of enzymes that governs reversible equilibrium reactions, exhibits high activity, is present in excess, and is not considered a regulating enzyme in the metabolic activity of typical cells. These characteristics ensure that, during typical normal cellular metabolism, the m-aconitase equilibrium is established in association with the Krebs cycle and is reflected by the typical cellular steady-state ratio of 10:1 for citrate to isocitrate regardless of the level of citrate. Whereas tissue measurements generally approximate the cellular composition, this assumption is not applicable to prostate tissue. The cellular citrate to isocitrate ratio of prostate epithelial cells is not known. Most of the prostate tissue citrate might be sequestered in the acinar lumen content in the absence of a proportional amount of isocitrate. If so, the 30:1 ratio observed with prostate tissue would not be representative of the ratio maintained within the epithelial cells. Under these

TABLE II. Citrate and Isocitrate in Prostate Tissue

| | VP | Pig | Human BPH | Rat kidney |
|------------------|--------|--------|-----------|------------|
| Cit (nmol/g) | ≈3,000 | ≈4,000 | ≈11,000 | ≈275 |
| Isocit (nmol/g) | ≈80 | ≈100 | ≈400 | ≈30 |
| Cit/isocit ratio | ≈37 | ≈40 | ≈27 | ≈9 |

conditions, the true citrate to isocitrate ratio of prostate epithelial cells might approach the typical ratio of 10:1. One would expect the secretory content and volume of prostatic fluid to be considerably variable across different preparations. Therefore, given the high citrate level of luminal content, the citrate to isocitrate ratio of prostate tissue should vary correspondingly. However, the high citrate:isocitrate ratio is consistently observed in various prostate tissue preparations (Table II). The absence of this variability suggests that the citrate to isocitrate ratio of prostatic fluid approximates the intracellular ratio. Therefore, we suspect that the 30:1 ratio might reflect the cellular composition as well as the luminal composition.

Thermodynamically, the equilibrium of the aconitase reaction cannot be altered. However, the intracellular steady-state ratio of citrate to isocitrate will depend on the relative activities involved in citrate synthesis, the aconitase reaction, and isocitrate oxidation. In prostate, the rate of citrate synthesis possibly exceeds the rate at which m-aconitase can establish its equilibrium. Citrate which is synthesized would accumulate at a faster rate than its conversion by aconitase to isocitrate. The isocitrate generated should be readily oxidized via IDH activity. Under these conditions, both the citrate level and citrate to isocitrate ratio would be markedly increased in the prostate epithelial cells. In other words, the steady-state citrate to isocitrate ratio in these cells would be markedly higher than the ratio which m-aconitase attempts to establish, whereas in "typical" cells the steady-state level of citrate to isocitrate approximates the equilibrium (10:1) established by m-aconitase. Conversely, citrate levels might be increased under circumstances in which the cellular steady-state citrate to isocitrate ratio is retained at about 10:1. This would be achieved as long as the m-aconitase activity was in excess (not rate-limiting) of the rate of citrate synthesis and isocitrate oxidation. If the rate of citrate synthesis is increased, but m-aconitase activity was still in excess, we would expect that the concentration of citrate would be increased, m-aconitase would still establish its equilibrium, isocitrate would be proportionately increased, and the cellular steady-state 10:1 ratio would be achieved. Based on these possibilities, the steady-state citrate: isocitrate ratio of prostate epithelial cells during net citrate production needs to be determined. Direct studies with isolated epithelial cells should resolve this important issue.

The existence of a rate-limiting m-aconitase in prostate epithelial cells would be in contrast to the "typical" relationship of this enzyme in other cells. However, some examples of a similar situation have been reported. Most significantly, Hernanz and de la Fuente [17] reported that m-aconitase activity was decreased, and kinetic properties were markedly altered in ascites tumor cells. These workers emphasized the potential importance of this unexpected relationship to the study of cancer biochemistry. Furthermore, Boquist et al. [18,19] reported that m-aconitase is specifically inhibited in alloxan-induced diabetes and in genetic diabetes which results in high citrate levels in kidney, liver, and pancreatic islets. Consequently, conditions appear to exist that result in alterations of the typical m-aconitase characteristics and rela-

tionship in cell metabolism. Since prostate epithelial cells represent a specialized situation requiring maximal citrate production, a unique aconitase relationship would provide the most effective and most plausible mechanism to achieve this purpose.

Three possible mechanisms could be involved in the unique rate-limiting characteristic of prostate m-aconitase activity.

1. Prostate epithelial cells might inherently contain a low level of m-aconitase enzyme. This would be reflected in a low total activity of m-aconitase in relation to the capability to synthesize citrate. Under such conditions, no unique or special characteristics or properties of m-aconitase would be required to effect citrate accumulation.

2. The m-aconitase activity might contain some unique kinetic properties. For example, the K_m for citrate might be uniquely high, which would require the accumulation of high levels of citrate to obtain maximal activity.

3. The m-aconitase activity of prostate epithelial cells might be uniquely inhibited. This possibility is of special consideration in view of our report that zinc might be an inhibitor of prostate m-aconitase [14]. Consequently, we focus on this relationship, although all three possibilities need to be investigated.

Costello and Franklin [14] provided initial evidence that zinc might be an inhibitor of prostate m-aconitase activity. We observed that millimolar concentrations of zinc could markedly inhibit the m-aconitase activity of isolated prostate mitochondria. However, no information exists regarding the *in situ* levels and possible association of zinc and prostate m-aconitase. Zinc, like citrate, is uniquely accumulated and secreted by prostate. The accumulation of zinc in prostate varies widely in different species (numerous reports; see Byar, [20], for an excellent review). Generally, the intracellular distribution of zinc in prostate epithelium is about 70% in nucleus, 24% in mitochondria, and 6% in cytosol [21–23]. Some correlations exist between zinc accumulation and citrate production in prostate: (1) testosterone stimulates zinc uptake and concentration in prostate, which parallels its stimulation of citrate accumulation and secretion; (2) prolactin stimulates both zinc uptake and citrate production in lateral prostate; (3) both zinc and citrate levels are increased in BPH and are markedly decreased in CA; and (4) in bonnet monkeys, the caudal prostate contains the most citrate and accumulates the most zinc, whereas the cranial prostate contains lower levels of both citrate and zinc. These correlations by no means establish that zinc increases citrate levels by an effect on aconitase. However, this possibility becomes more compelling in view of our initial direct studies indicating that prostate m-aconitase might be inhibited by zinc. On balance, some information exists that might argue against this zinc–aconitase–citrate relationship. For example, dog prostate contains very high zinc levels but relatively low levels of citrate. Rat VP is high in citrate and relatively low in zinc content. However, the specific protein binding of zinc must be considered, rather than total zinc content of tissue. This is supported by our preliminary data in which VP mitochondria appear to contain 10 times more zinc than kidney or liver although the tissue levels of zinc are similar (Table III). Unfortunately, little information exists concerning the accumulation and binding of zinc by specific prostate mitochondrial proteins. Consequently, the issue of zinc–aconitase–citrate relationship in prostate needs to be addressed.

Although the chemistry and structure of aconitase have been extensively studied [24–26] in other tissues, zinc has never been reported to be an inhibitor of m-

TABLE III. Zinc Content of Prostate, Kidney, and Liver*

| | Tissue zinc | Mitochondrial zinc |
|--------|--------------------|-------------------------------|
| Kidney | 22 $\mu\text{g/g}$ | 0.06 $\mu\text{g/ml}$ protein |
| Liver | 25 | 0.06 |
| VP | 19 | 0.65 |
| DLP | 116 | 1.60 |

*Values are unpublished data from our laboratory. DLP, dorsolateral prostate.

aconitase. Presumably, Zn^{2+} does not readily gain access to the active site of the m-aconitase enzyme studied in other tissues. Of considerable interest is the observation that ferredoxin protein (an iron-cluster protein like aconitase but a much smaller protein) can incorporate Zn^{2+} into the active site region [27]. Plank et al. [28] have suggested that alterations in the amino acid residues will likely alter the functional characteristics of aconitase-type enzymes. This principal might be applicable to prostate m-aconitase as an adaptation associated with the unique function of limiting citrate oxidation. Conceivably, the structure of prostate m-aconitase might be different from other tissues, thereby providing for binding of Zn^{2+} , resulting in inhibited activity. Should zinc prove to be an inhibitor of prostate m-aconitase, an exciting investigation of the structural and genetic relationships of this enzyme would be plausible.

Citrate Synthesis

The accumulation and secretion of high levels of citrate require that citrate is a major end product of metabolism of the prostate secretory epithelial cells. A result of this is the loss of six carbons from the metabolic pool of these cells. Consequently, continual 6-carbon sources are required to maintain net citrate production. The 6-carbon sources must include a 4-carbon source of oxalacetate (OAA) and a 2-carbon source of acetyl CoA (AcCoA). Over the past decade we have been engaged in the identification of the carbon sources and the metabolic pathway associated with the synthesis and net production of citrate.

The issue of the 4-carbon source of OAA was recognized by Barron and Huggins [29], who suggested that amino acid transamination might be important. Awapara [30,31] provided additional information in support of this proposal. Thirty years elapsed when Toghrol et al. [32] and Franklin et al. [33] demonstrated that VP mitochondria could stoichiometrically convert aspartate + AcCoA to citrate in the presence of αKG thereby demonstrating the presence of mitochondrial aspartate aminotransferase (mAAT). That aspartate might be a 4-carbon source of OAA was enhanced by the observation of extremely high aspartate levels in VP (Table IV). However, the endogenous concentration of αKG is very low in VP as might be expected, since citrate oxidation is minimal. By contrast glutamate levels are extremely high. Therefore, it was necessary to incorporate a mechanism for the generation of αKG for aspartate transamination. This was accomplished by the demonstration of glutamic dehydrogenase (GDH) in VP mitochondria and that aspartate in the presence of glutamate + AcCoA was stoichiometrically synthesized to citrate [34]. We refer to the coupling of mAAT, GDH, and citrate synthase (CS) reactions as the glutamate–aspartate–citrate pathway of prostate mitochondria (Fig. 2).

Since the presence of these enzymes is not unique to prostate mitochondria, the

TABLE IV. Rat Tissue Levels of Citrate, Aspartate, Glutamate and α -Ketoglutarate*

| | Citrate | Aspartate | Glutamate | α -Ketoglutarate |
|--------|-----------------|-----------------|-----------------|-------------------------|
| VP | $\approx 4,000$ | $\approx 3,000$ | $\approx 4,000$ | ≈ 26 |
| Kidney | ≈ 420 | ≈ 420 | $\approx 2,300$ | ≈ 230 |
| Liver | ≈ 160 | ≈ 275 | $\approx 1,400$ | — |
| Plasma | ≈ 180 | ≈ 30 | ≈ 60 | — |

*Values taken from Franklin et al. (1982). Values are expressed as nmols/g tissue.

reason for the operation of this unique pathway must be emphasized. Limited Krebs cycle regeneration of mitochondrial OAA is essential for the mAAT reaction to proceed in the direction of aspartate transamination to OAA [34,35]. This coupled to the unique composition of substrates and kinetic properties of mAAT and GDH in prostate favor OAA production from aspartate. It is fascinating to recall that aspartate is typically characterized as a nonessential amino acid. In typical tissues, aspartate is synthesized from OAA generated by Krebs cycle activity which is the reverse direction of the mAAT reaction. However, in prostate, aspartate is an essential amino acid required for the function of citrate production and secretion.

The demonstration of the glutamate-aspartate-citrate pathway in isolated mitochondria does not establish that the pathway is operational in intact secretory epithelial cells. Consequently, a series of studies was conducted which ultimately demonstrated that isolated epithelial cells could generate net citrate production from utilization of exogenous aspartate [36–38]. The combination of these studies support the proposal that aspartate, via the mitochondrial glutamate-aspartate-citrate pathway, can serve as a major source of mitochondrial OAA for citrate synthesis by prostate epithelial cells. We must emphasize that this pathway has thus far been identified only in VP. It remains to determine whether this is applicable to other citrate-producing glands, and most importantly to human prostate. Conceivably other carbon sources and pathways, in addition to or in place of aspartate, might be involved in the production of OAA for citrate synthesis. Glucose has been proposed as the 6-carbon source by some investigators [15,39]. However, the same studies demonstrated the production of CO_2 from glucose. Under such conditions, glucose cannot provide the total 6-carbon for net citrate production. Since those studies, like many others, employed ^{14}C incorporation into citrate, they do not represent net citrate production. Such studies could represent citrate flux through the Krebs cycle. We must emphasize that some limited citrate oxidation is evident in prostate [12], but this represents a small proportion of the citrate synthesized and does not constitute net citrate production. Net citrate production is determined as accumulated citrate levels, not by incorporation of ^{14}C label into citrate. Under the conditions employed in our studies with either prostate fragments or isolated epithelial cells, we have not successfully demonstrated significant citrate production when glucose was the only carbon source [38]. In addition, we have not observed that pyruvate could provide the 4-carbon source, and we have no evidence for pyruvate or phosphoenolpyruvate carboxylation as sources of mitochondrial OAA and citrate synthesis. However, these and other possibilities still require intense investigation.

Attention is now directed at the 2-carbon source of AcCoA for citrate synthesis. Several investigators have demonstrated that prostatic tissue can use glucose via aerobic glycolysis [39,40]. Studies in our laboratory (unpublished information) also

demonstrated that VP fragments readily used glucose. Furthermore, Harkonen et al. [41] had reported that VP could readily oxidize pyruvate. Consequently, it was reasonable to expect that glucose, via pyruvate oxidation, would provide AcCoA for net citrate production. That VP tissue fragments incorporated [^{14}C]glucose into citrate [15] added to this likelihood. Despite such expectations, we were repeatedly unable to demonstrate that physiological levels of glucose or pyruvate (in the presence of aspartate to generate mitochondrial OAA) would result in net citrate production by VP fragments. Therefore, we attempted to stimulate net citrate production with other potential physiological 2-carbon sources including fatty acids, ketone bodies, acetate and others. The results (unpublished) were discouragingly negative. These studies, like those of other investigators, employed small pieces of VP tissue, which we called prostate fragments. We concluded that such preparations might not be advantageous for metabolic studies of net citrate production. First, these preparations retain a high concentration of citrate and other metabolites. Such conditions could make it difficult to identify citrate changes in the presence of a high endogenous citrate concentration; or effects of added substrates could be masked by endogenous substrates. Second, the exposure of the epithelial cells to the external environment (media) might be limiting in regard to availability of oxygen and exogenous substrates. Consequently, we turned our attention to the preparation and use of isolated epithelial cells derived from collagenase treatment of the VP prostate fragments for these and subsequent studies [37,38]. This resulted in the successful demonstration that glucose, via pyruvate oxidation, can serve as the source of AcCoA for citrate synthesis by VP epithelial cells (Fig. 2). Since we have not obtained comparable results with other potential 2-carbon sources with this system, we believe that glucose oxidation is the physiological 2-carbon source for net citrate production. Studies with other citrate-producing glands and human prostate are necessary to establish this as a general pathway common to all such glands. Based on our experience, we would suggest that such studies attempt to employ cell preparations rather than pieces or homogenates of intact prostate tissue.

ASPARTATE TRANSPORT

Aspartate occupies a key role as a 4-carbon substrate for citrate production. VP tissue contains uniquely high levels of aspartate ($\approx 3,000$ nmoles/g). The possibility that prostate epithelial cells might contain a high intracellular aspartate pool which could be readily available for citrate synthesis had to be considered. Washout kinetics studies [8] demonstrated that aspartate was distributed in the approximate ratio of 52% extracellular (mainly luminal content) and 48% intracellular. From this, the intracellular concentration (based on cell water volume) was calculated at 1.3 mM. By contrast, the plasma concentration of aspartate is 0.03 mM, thereby creating an intracellular: extracellular gradient of ≈ 43 across the basilar cell membrane.

What is the source of intracellular aspartate? Aspartate is possibly extracted (transported) from circulation and/or derived from protein catabolism. Our studies [8] demonstrate that little or no total change in endogenous aspartate levels occurs in "resting" prostate preparations. In addition, the endogenous level of asparagine remains constant and prostate tissue contains no demonstrable asparaginase activity (personal observations). The combination of these circumstances seem to argue against the likelihood that catabolic processes or endogenous sources provide the

major source of intracellular aspartate. We propose that prostate derives its aspartate mainly from circulation. For this to occur and to prevent the net efflux of aspartate from the cell requires that prostate epithelial cells contain an active transport mechanism at the basilar cell membrane. Consistent with this is the evidence for the existence of a Na^+ dependent, high affinity aspartate transporter in VP isolated epithelial cells [8]. The K_m was calculated as 0.01 mM. Since the plasma concentration of aspartate is 0.03 mM, this transporter would be extremely operational at the basilar cell membrane. Quite surprisingly was the demonstration of a low affinity transporter, also Na^+ dependent, but with a K_m of 0.8 mM. At this K_m , such a transporter would not be effective for the import of aspartate from circulation. However, it could be functioning at the apical cell membrane in determining the aspartate concentration of luminal content and maintaining an appropriate high intracellular content. In support of this concept is our recent observation (unpublished information) that the pH optimum for the low affinity transporter is 6.0–6.5, and at pH 7.2 transport is negligible. By contrast, the high affinity transporter is operational at pH 7.4. These pH effects correspond to the low pH at the luminal region and the higher pH at the circulatory region. Therefore, we propose the existence of a high affinity transporter associated with the import of aspartate from circulation at the basilar cell membrane, and a low affinity aspartate transporter at the apical membrane required for re-uptake of aspartate from luminal content. Na^+ -coupled aspartate transporters have been identified in many cells. Notably, Wingrove and Kimmich [42,43] reported the presence of Na^+ -coupled high and low affinity transporters in intestinal epithelial cells. In kidney proximal tubules, Na^+ -aspartate inwardly directed cotransport occurs at both the luminal and basilar membranes to maintain the high aspartate concentration in tubular cells [44]. When one also considers the Na^+ and citrate relationships described above, the role and function of prostate epithelial cells in electrolyte (including organic anions) transport seem of paramount importance. We are continuing studies in characterizing these transport mechanisms and invite the interest of others in this exciting research.

ENERGY IMPLICATIONS OF CITRATE METABOLISM

Most cells rely on the operation of the Krebs cycle to obtain sufficient ATP to support their functions and activities. Citrate oxidation represents the first step in the Krebs cycle by which glucose and fatty acid oxidation can be completed. Glucose oxidation through pyruvate oxidation to AcCoA will provide 8 moles ATP/mole glucose. If no other significant alternate pathways are available for entry of glucose carbon for oxidation coupled to ATP production, the accumulation of unoxidized citrate will result in the potential loss of 24 moles ATP/mole glucose used. In other words, the accumulation and secretion of citrate involves the sacrifice of 67% of the potential energy available to the cells from glucose oxidation. (Note: An additional 6 moles ATP could result from oxidative phosphorylation of glycolytic generated NADH.) Likewise, the utilization of fatty acids for energy production will also be markedly impaired by limited citrate oxidation. However, other entry sites for intermediates to be oxidized with coupled ATP production can exist in mitochondria. Unfortunately, virtually no information exists regarding such pathways in prostate. We would doubt that any mitochondrial pathways that would ultimately result in appreciable OAA production would exist for reasons already discussed in relationship

to the operation of the glutamate-aspartate-citrate pathway. VP can oxidize isocitrate and possesses mitochondrial IDH; but with limited aconitase, a significant physiological source of isocitrate is unknown [13,16]. Prostate mitochondria appear to contain other Krebs cycle oxidative enzymes, e.g., succinic dehydrogenase, malate dehydrogenase. Whether substrates for these and other oxidative reactions are physiologically available to bypass citrate entry is questionable. Astoundingly, no study or combination of studies has yet been reported regarding the complete operation of the Krebs cycle in prostate mitochondria. Furthermore, no information exists regarding the steps and efficiency of ATP production coupled to mitochondrial oxidation. Today, such statements cannot be made in regard to most other tissues in the body. When one recognizes the unique citrate relationship of prostate, the danger of extrapolating prostate metabolic relationships from studies conducted with other tissues cannot be overemphasized.

Prostate tissue has been characterized in relation to other tissues as being of "low respiration", i.e., low QO_2 [9]. In addition, prostate tissue has a high capability to utilize glucose via aerobic glycolysis. These characterizations are consistent with the existence of low citrate oxidation. Our studies with VP fragments (unpublished) have consistently demonstrated that over 85% of glucose utilized can be recovered as mainly lactate plus some pyruvate. Clearly, as reported by others, complete oxidation of glucose for energy production is not a characteristic of prostate. However, because of the complexities and difficulties imposed by the cellular organization of intact prostatic tissue, studies with isolated epithelial cells are required. It is essential to relate the oxygen requirements, energy production, and intermediary metabolism of these cells which cannot be accurately achieved with prostate tissue slices or fragment preparations.

The emphasis of a limited citrate oxidation might be misinterpreted as a complete absence of citrate oxidation in prostate epithelial cells. Convincing evidence exists in support of the conclusion that some citrate is oxidized via the Krebs cycle [12,14,39]. However, this represents a small portion of the total citrate synthesized most of which is accumulated [12]. We have estimated that VP mitochondria are capable of synthesizing citrate via the glutamate-aspartate citrate pathway at a rate 4–8 times greater than the rate of citrate oxidation. One could expect that limited citrate oxidation via the Krebs cycle, most likely coupled to ATP production, will provide some source of energy. The presence of Krebs cycle oxidative enzymes, and the presence of the terminal electron transport system in prostate mitochondria [13] support this likelihood. The observations that testosterone stimulates respiration of prostate and oxidation of glucose and pyruvate caused Harkonen [38] to propose that increased energy requirements of the prostate might be achieved through increased mitochondrial oxidation. We had expected the opposite effect in rationalizing that testosterone could increase citrate production by increasing the rate of citrate synthesis and/or decreasing the rate of citrate oxidation. Instead, Franklin et al. [12] demonstrated that testosterone increased both rates, which resulted simultaneously in increased net citrate production and increased citrate oxidation. In retrospect, this is an ideal mechanism for testosterone to meet the increased energy requirements of the cell demanded by its effect on increasing citrate production and other cellular activities. Furthermore, the glutamate-aspartate-citrate pathway might also provide a pathway for energy production. The oxidative deamination of glutamate (GDH reaction) and recycling of glutamate require oxidation of NADH. We propose (Fig. 2)

that NAD is regenerated by oxidation of NADH via the electron transport system. This mitochondrial oxidative process would likely be coupled to phosphorylation for generation of ATP. Studies regarding oxidative phosphorylation by prostate mitochondria are essential and urgently required to understand the energy relationships of the prostate.

CITRATE RELATIONSHIPS IN NORMAL AND NEOPLASTIC HUMAN PROSTATE

That prostate citrate levels are extremely high in BPH and extremely low in CA is well established [1,9,45,46]. The tissue citrate levels range from 1,000 nmoles/g for CA to 12,000 for BPH. As discussed earlier [1], human tissue samples vary greatly, especially in the relative amounts of stroma versus glandular components. Since the stroma contains little citrate (about 100 nmoles/g), its presence lowers the tissue citrate concentration. When based on glandular epithelial content, the citrate concentration of BPH can range from 23,000 to 48,000 nmoles/g as compared with approximately 2,000 in CA. The true value for CA is probably even lower if one corrects for the presence of some normal glandular components, which likely coexists with the neoplastic tissue. The consistency of this citrate relationship in prostate neoplasms was emphasized by Cooper and Farid [45]. These workers suggested that a decrease in citrate (specifically a decrease in the citrate to lactate ratio) could serve as a biochemical marker for the progression of malignancy. Since the 1978 review [1] of these early studies, no significant contributions or advancements in this area have been forthcoming.

Elucidation of the metabolic alterations which account for the dramatic change in citrate associated with these transformed cells is essential. One possible mechanism might be that in CA the transformed epithelial cells have developed the machinery to oxidize citrate. Perhaps the unique aconitase properties which characterize normal prostate are lost in malignant cells. Furthermore, if these transformed cells do oxidize citrate via Krebs cycle, the conditions required for the operation of the glutamate-aspartate-citrate pathway for net citrate synthesis would no longer be established. The consequences would be increased citrate oxidation, decreased citrate synthesis, and decreased net citrate production. By contrast, BPH cells might be characterized by an increase in the rate of citrate synthesis and a decrease in the rate of citrate oxidation. Unfortunately, no information currently exists regarding the biochemical transformations associated with citrate production by neoplastic prostate cells. Until these questions are resolved, issues concerning the induction of these transformations cannot be addressed. Therefore, the pathogenetic relationships of prostate neoplasms remain a mystery.

All these discussions, while important, are speculative when applied to human prostate. Virtually no information exists concerning citrate-related metabolism of normal human prostate. The lack of availability of particularly normal human prostate makes such research extremely difficult. The complexity of the human prostate adds to the difficulty. The morphology and ontogeny impose a heterogeneous organization of various cell types, diverse tissues and different anatomic regions within the prostate structure [47]. There is no reason to expect that the functional and metabolic activities of citrate production reside uniformly throughout the different regions of the human prostate or uniformly throughout the glandular structure of the prostate. In

animals containing discretely different prostate regions (e.g., monkey, pig, rat), the citrate relationships are also discretely different. Indeed it would be most useful to identify regional differences of citrate production in normal human prostate and in prostate neoplasms. Because of its availability, BPH tissue has been employed for metabolic studies including some aspects of citrate-related metabolism. One must cautiously consider that such studies represent the combined metabolic activities of epithelial and stromal tissue, secretory and nonsecretory cells, neoplastic and normal tissue. Interpretation of resulting data in relationship to the metabolism of the secretory epithelial cells is most difficult and possibly misleading.

Because of these difficulties, animal models and cell cultures have become important in attempting to understand the functional, metabolic, and pathological relationships of the human prostate. In contrast to most other tissues, the appropriateness of experimental models employed to represent the activities of human prostate must be seriously addressed. For example, rat liver provides an excellent model to elucidate functional and metabolic relationships which can serve as a foundation representative of human liver. Furthermore, direct studies with human liver and/or comparative studies with other animals can be accomplished to verify such relationships. For the most part, the morphological, histological, embryological, metabolic, and physiological relationships are common to the liver independent of species. Therefore, for many tissues (e.g., muscle, heart, liver, kidney, brain), appropriate animal models have been well established. Unfortunately this situation does not exist for prostate, especially regarding the citrate relationships of prostate.

We, like most other investigators, have employed rat ventral prostate as a model to elucidate functional and metabolic relationships which can be extrapolated to represent human prostate. We believe that the citrate-related pathways which have been identified in VP might prove to be applicable to all citrate-producing prostate glands, including human. However we must recognize that, while being analogous in regard to the function of producing and secreting citrate, VP and human prostate are not homologous. By contrast, rat lateral prostate (LP) and dorsal prostate (DP) are homologous to human prostate [48], but DP is not a citrate producing gland whereas LP is. Can we infer from this that human prostate consists of secretory epithelial cells that function, like LP, as citrate-producing cells and also consists of secretory epithelial cells which, like DP, are not citrate-producing cells? Do differences exist between VP and LP (both citrate producing but ontogenically different) in regard to citrate production and secretion? If so, which is representative of human prostate? In other words, what does the experimental model represent and what limitations need to be considered when employing a particular model. Indeed, distinct differences exist regarding the regulation of citrate production by VP as compared with LP [49,50]. (Note: Hormonal regulation of prostate citrate production will be described in a subsequent review article currently in preparation.) Consequently studies, such as ours, which employed mainly VP must be expanded to obtain sufficient corroborating information to strengthen the plausibility that the established relationships are applicable to human prostate. Ultimately, the final evidence should come from direct studies of human prostate. Until that final achievement, other comparative studies can be expanded to other animal models (e.g., pig prostate, pig seminal vesicle, monkey prostate). If the citrate pathways and function are consistently common to such a variety of species, the application of the relationship to human prostate becomes extremely plausible.

Based on these considerations, we have directed some efforts toward the use of pig prostate (minipigs and boars) as a model for human prostate. The pig prostate is homologous as well as analogous (with respect to citrate production) to human prostate and, therefore, resembles man more closely than rat [51]. Lauwers et al. [51] suggested that the pig can be a suitable experimental model for human prostate relationships. The pig prostate is composed of (1) a disseminate prostate contained within and along the length of the caudal part of the pelvic urethra, and (2) a compact prostate associated with the cranial part of the pelvic urethra. The dorsal portion of the cranial prostate is lobular and lies externally to the urethra. We recently examined the citrate content and histological appearance of these various prostatic regions (unpublished information). The dorsal portion (compact prostate) contained about 5,000 nmoles citrate/g, whereas the disseminate prostate contained about 1,000 nmoles/g. Prostatic fluid obtained from the dorsal portion had a citrate content of approximately 15,000 nmoles/ml. In this regard, the compact prostate of pig possesses citrate-producing characteristics. In fact, these citrate levels were comparable to those observed for the pig seminal vesicle tissue and fluid concentrations although the seminal vesicles (due to size and volume of secretion) are the major citrate-producing structures. Histological examination of the compact region demonstrated the dominance of acinar structures lined with columnar epithelium (secretory cells) with occasional basal cells. Thus far our studies with pig prostate have demonstrated that it provides an extremely valuable model for citrate relationships [49,52], especially when used in conjunction with rat prostate studies. One might also consider the use of pig seminal vesicles as a model for citrate relationships. The seminal vesicles provide a large amount of tissue, particularly glandular epithelium, and a large volume of secretory fluid. From one animal, the seminal vesicles will yield 300–500 ml of seminal vesicular fluid containing about 10,000–20,000 nmoles/ml, which provides a total of about 6 mmoles (≈ 1.2 g) of citrate. The propensity of seminal vesicle glandular epithelium for citrate production is obvious. The drawback to pig seminal vesicle is that it is not homologous to human prostate. Nevertheless, these circumstances seem to dictate that the conventional acceptance of rat prostate as the dominant and best model for prostate studies should be evaluated, and other animal models along with rat should be given careful consideration dependent on the nature of the study.

Prostate cultures have provided, and continue to provide, experimental models for research. Various types of cultures (organ culture, cell culture) derived from various animal and human sources including BPH and CA have been described. The epithelial cells obtained are taken as representative of the progenitor parent cells from which the culture was derived. However, the criteria for such a representation must be given serious consideration. Various biochemical markers have been suggested and employed to characterize cells in culture as prostate epithelial cells. We have attempted to provide a rational basis for the proposition that the most unique and distinguishing characteristic of prostate secretory epithelial cells is their specialized ability to perform the major function of producing and secreting citrate. Consequently, cells in culture must continue to exhibit this characteristic if they are to be represented as normal prostate secretory epithelial cells. Without this ability the cells might be genotypically, but not phenotypically, characterized as prostate secretory epithelial cells; or the culture conditions might be unsuitable for the expression of this function. Under appropriate conditions, VP and pig prostate epithelial cell cultures are capable of producing citrate [49,51–54]. We propose that the following criteria

should represent the existence of normal functional prostate secretory epithelial cells in culture: (1) net citrate production and accumulation are evident, (2) citrate oxidation is low, and (3) net citrate production is stimulated by appropriate hormones (e.g., testosterone). This relationship needs to be extended to cultures representing BPH and CA. Since citrate levels are extremely high in BPH and extremely low in CA, secretory epithelial cells derived from these tissues should retain these respective characteristics. BPH cultures should exhibit the ability to produce citrate; whereas CA cultures should exhibit little or no net citrate production and perhaps should exhibit citrate utilization. Studies and information regarding the metabolism of citrate by cultured prostate epithelial cells are extremely necessary and important.

CONCLUSIONS

1. Citrate production and secretion are major functions of prostate.
2. These functions require unique metabolic activities associated with prostate secretory epithelial cells.
 - a. Citrate is a major end product of metabolism.
 - b. The secretory cells contain a unique m-aconitase activity which limits their capability to oxidize citrate.
 - c. The secretory cells use glucose, via pyruvate oxidation, as the source of acetyl CoA for net citrate synthesis.
 - d. The secretory cells use aspartate via a unique mitochondrial glutamate-aspartate-citrate pathway as the source of OAA for net citrate synthesis.
3. The secretory cells function as transporting cells.
 - a. The secretion of citrate requires a unique citrate transport mechanism coupled to Na^+ transport at the apical membrane.
 - b. The uptake of aspartate from circulation involves an aspartate transporter coupled to Na^+ transport at the basilar membrane.
4. The accumulation and secretion, rather than the utilization, of citrate results in the sacrifice of a major potential energy source. The energy requirements and relationships of prostate secretory epithelial cells are virtually unknown.
5. Metabolic alterations in citrate production are characteristically associated with neoplastic transformation of human prostate epithelial cells.
6. Citrate relationships should be employed as major biochemical markers for characterizing prostate secretory epithelial cells maintained in vitro and in culture.
7. Information concerning all the above relationships is minimal, and intense research is urgently required in all these areas.
8. Extrapolation of these metabolic relationships obtained from studies with "typical" tissues is dangerous and misleading.

ACKNOWLEDGMENTS

We wish to acknowledge the contributions of several investigators who had participated in our studies including Dr. R. Stacey, Dr. G. Littleton, Dr. M.W. Khang, Dr. R. Brandly, Dr. F. Toghrol, Dr. M. Khan, and Ms. V. Akuffo. We thank Dr. George Huggins for reviewing this manuscript and for the helpful suggestions

offered to us. Much of our research included in this paper was supported by NIH research grants DK 28015 and HD 16193.

REFERENCES

1. Costello LC, Littleton GK, Franklin RB: Regulation of citrate-related metabolism in normal and neoplastic prostate. In Sharma RK, Criss WE (eds): "Endocrine Control in Neoplasia." New York: Raven Press, 1978, pp 303-314.
2. Mann T: "The Biochemistry of Semen and of the Male Reproductive Tract." New York: John Wiley and Sons, Inc, 1964.
3. Mann T, Lutwak-Mann C: "Male Reproductive Function and Semen." New York: Springer-Verlag, 1981.
4. Huggins C: Physiology of the prostate gland. *Physiol Rev* 25:281-294, 1945.
5. Arunakaran J, Aruldas MM, Covindarajulu P: Effect of prolactin and androgens on the prostate of bonnet monkeys, *Macaca radiata*. 1. Nucleic acids, phosphatases and citric acid. *Prostate* 3:265-273, 1987.
6. Farnsworth WE: Testosterone stimulation of citric acid synthesis in the rat prostate. *Biochim Biophys Acta* 117:247-254, 1966.
7. Farnsworth WE: The normal prostate and its endocrine control. In Griffiths K, Pierpoint CG (eds): "Some Aspects of the Aetiology and Biochemistry of Prostatic Cancer." Cardiff: Alpha Omega Alpha Publishing, 1970, pp 3-15.
8. Franklin RB, Lao L, Costello LC: Evidence for two aspartate transport systems in prostate epithelial cells. *Prostate* 16:137-145, 1990.
9. Huggins C: The prostatic secretion. *Harvey Lect* 42:148-193, 1947.
10. Barron ESG, Huggins C: The citric acid and aconitase content of the prostate. *Proc Soc Exp Biol Med* 62:195-196, 1946.
11. Humphrey GF, Mann T: Studies on the metabolism of semen. 5. Citric acid in semen. *Biochem J* 44:97-105, 1949.
12. Franklin RB, Kahng MW, Akuffo V, Costello LC: The effect of testosterone on citrate synthesis and citrate oxidation and a proposed mechanism for regulation of net citrate production in prostate. *Horm Metab Res* 18:177-181, 1986.
13. Costello LC, Franklin RB, Stacey R: Mitochondrial isocitrate dehydrogenase and isocitrate oxidation of rat ventral prostate. *Enzyme* 21:495-506, 1976.
14. Costello LC, Franklin RB: Aconitase activity, citrate oxidation, and zinc inhibition in rat ventral prostate. *Enzyme* 26:281-287, 1981.
15. Harkonen PL, Kostian ML, Santti RS: Indirect androgenic control of citrate accumulation in rat ventral prostate. *Arch Androl* 8:107-116, 1982.
16. Franklin RB, Costello LC: Isocitrate uptake and citrate production by rat ventral prostate fragments. *Invest Urol* 16:44-47, 1978.
17. Hernanz A, de la Fuente M: Characterization of aconitase hydratase from mitochondria and cytoplasm of ascites tumor cells. *Biochem Cell Biol* 66:792-795, 1988.
18. Boquist K, Ericsson I: Inhibition by alloxan of mitochondrial aconitase and other enzymes associated with the citric acid cycle. *FEBS* 178:245-248, 1984.
19. Boquist L, Ericsson I, Lorentzon R, Nelson L: Alterations in mitochondrial aconitase activity and respiration, and in concentration of citrate in some organs of mice with experimental or genetic diabetes. *FEBS* 183:173-176, 1985.
20. Byar DP: Zinc in male sex accessory organs: Distribution and hormonal response. In Brandes D (ed): "Male Accessory Sex Organs." New York: Academic Press, Inc., 1974, pp 161-171.
21. Feustel A, Wennrich R: Zinc and cadmium in cell fractions of prostate cancer tissues of different histological grading in comparison to BPH and normal prostate. *Urol Res* 12:147-150, 1984.
22. Kar AB, Chowdhury AR: The distribution of zinc in the subcellular fractions of Rhesus monkey and rat prostate. *J Urol* 96:370-371, 1966.
23. Kar AB, Chowdhury AR: The distribution of zinc in the subcellular fractions of human prostate. *Curr Sci* 37:375, 1968.
24. Robbins AH, Stout CD: The structure of aconitase. *Proteins* 5:289-312, 1989.

25. Beinert H, Kennedy MC: Engineering of protein bound iron-sulfur clusters. *FEBS* 186:5–15, 1989.
26. Emptage MH: Aconitase: Evolution of the active-site picture. *ACS Symp Ser* 372:343–371, 1988.
27. Surerus KK, Munck E, Moura I, Moura JJ, Le Gall J: Evidence for the formation of a $ZnFe_3S_4$ cluster in *Desulfovibrio gigas* ferredoxin II. *J Am Chem Soc* 109:3805–3807, 1987.
28. Plank DW, Kennedy MC, Beinert H, Howard JB: Cysteine labeling studies of beef heart aconitase containing a 4Fe, a cubane 3Fe, or a linear 3Fe cluster. *J Biol Chem* 264:20385–20393, 1989.
29. Barron ESG, Huggins C: The metabolism of the prostate: Transamination and citric acid. *J Urol* 55:385–390, 1946.
30. Awapara J: The action of sex hormones on metabolism of amino acids in the prostate. *Tex Rep Biol Med* 10:22, 1952.
31. Awapara J: The influence of sex hormones on the transaminase of the accessory sex organs of the male rat. *Endocrinology* 51:75–79, 1952.
32. Toghrol F, Franklin RB, Costello LC: Citrate synthesis from fatty acids and amino acids in rat ventral prostate. *Enzyme* 25:371–376, 1980.
33. Franklin RB, Brandly RL, Costello LC: Mitochondrial aspartate aminotransferase and the effect of testosterone on citrate production in rat ventral prostate. *J Urol* 127:798–802, 1982.
34. Franklin RB, Costello LC: Glutamate dehydrogenase and proposed glutamate–aspartate pathway for citrate synthesis in rat ventral prostate. *Urology* 132:1239–1243, 1984.
35. Lowenstein JM: Ammonia production in muscle and other tissue: The purine nucleotide cycle. *Physiol* 52:382, 1972.
36. Costello LC, Akuffo V, Franklin RB: Testosterone stimulates net citrate production from aspartate by prostate epithelial cells. *Horm Metab Res* 20:252–253, 1988.
37. Costello LC, Akuffo V, Franklin RB: Net citrate production by isolated prostate epithelial cells. *Enzyme* 39:125–133, 1988.
38. Costello LC, Franklin RB: Prostate epithelial cells utilize glucose and aspartate as the carbon sources for net citrate production. *Prostate* 15:335–342, 1989.
39. Harkonen PL: Adrogenic control of glycolysis, the pentose cycle and pyruvate dehydrogenase in the rat ventral prostate. *J Steroid Biochem* 14:1075–1084, 1981.
40. Barron ESG, Huggins C: The metabolism of isolated prostate tissue. *J Urol* 51:630–634, 1944.
41. Harkonen P, Isotala A, Santti R: Studies on the mechanism of testosterone action on glucose metabolism in the rat ventral prostate. *J Steroid Biochem* 6:1405–1413, 1975.
42. Wingrove TG, Kimmich GA: High affinity L-aspartate transport in chick small intestine. *Am J Physiol* 252:C105–C114, 1987.
43. Wingrove TG, Kimmich GA: Low-affinity intestinal L-aspartate transport with 2:1 coupling stoichiometry. *Am J Physiol* 255:C737–C744, 1988.
44. Ullrich KJ: Sugar, amino acid, and Na^+ cotransport in the proximal tubule. *Ann Rev Physiol* 41:181–196, 1979.
45. Cooper JE, Farid I: The role of citric acid in the physiology of the prostate. A chromatographic study of citric acid cycle intermediates in benign and malignant prostatic tissue. *J Surg Res* 3:112–121, 1963.
46. Cooper JE, Farid I: The role of citric acid in the physiology of the prostate. Lactic/citrate ratios in benign and malignant prostatic homogenates as an index of prostatic malignancy. *J Urol* 92:533–536, 1964.
47. McNeal JE: The anatomic heterogeneity of the prostate. In Murphy GD (ed): “Models of Prostate Cancer.” New York: Alan R. Liss, Inc., 1980, pp 149–160.
48. Price D: Comparative aspects of development and structure in the prostate. *Natl Cancer Inst Monogr* 12:1–27, 1963.
49. Grayhack JT, Lebowitz A: Effect of prolactin on citric acid of lateral lobe of prostate of Sprague-Dawley rats. *Invest Urol* 5:87–94, 1967.
50. Franklin RB, Costello LC: Prolactin directly stimulates citrate production and mitochondrial aspartate aminotransferase of prostate epithelial cells. *Prostate* 17:13–18, 1990.
51. Lauwers H, Simoens P, Nicaise M, de Vos NR: Structure of the prostate and the pelvic urethra in barrows and the changes induced by diethylstilbestrol. *Acta Anat* 110:327–335, 1981.
52. Khan MA, Seibel W, Franklin RB, Provenza DV, Costello LC: Growth pattern and citrate production in organ cultures of rat ventral prostate. *Prostate* 3:391–403, 1982.
53. Costello LC, Khan MA, Franklin RB: Preliminary studies on the cultivation and characterization of mini-pig prostate epithelial cells. *Cell Biol* 12:637–649, 1988.

46 Costello and Franklin

54. Lostroh AJ: Regulation by testosterone and insulin of citrate secretion and protein synthesis in explanted mouse prostate. *Proc Natl Acad Sci USA* 60:1312–1318, 1968.
55. Franklin RB, Khan MA, Costello LC: Testosterone stimulation of mitochondrial aspartate aminotransferase in organ cultures of rat ventral prostate. *J Steroid Biochem* 20:709–713, 1984.