PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 172, 139–152 (1983)

The Function of Vitamin A: Current Concepts[1] (41537)

MAIJA H. ZILE[2] AND MALFORD E. CULLUM

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824

Visual function of vitamin A. Somatic functions of vitamin A: a. differentiation. b. growth. *Reproduction. Membrane phenomena. Conclusions. Addendum.*

The function of vitamin A has remained an enigma even though this vitamin was one of the earliest to be discovered (1). The knowledge that has accumulated from more than half a century of investigations points to a multitude of physiological and biochemical changes that take place when the mammalian body has insufficient vitamin A. At this time it has become evident that at least three different physiological functions are dependent on proper vitamin A nutrition:

(1) Somatic function or systemic function—this encompasses growth and differentiation, and for vitamin A is best understood in terms of development and differentiation of epithelial structures and bone (2–6). Forms of vitamin A capable of supporting somatic functions include retinol, retinyl esters, retinal and retinoic acid.

(2) Reproduction—vitamin A is essential for spermatogenesis, oogenesis, placental development, and fetal and embryonic growth. While retinol, retinyl esters, and retinal are active for these functions, retinoic acid is *not* able to support all aspects of mammalian reproduction (2, 3, 7).

(3) Visual process—it is firmly established that vitamin A is required for vision in the dark, possibly also for color perception. The active form of vitamin A for this function is the aldehyde, retinal (8), derived from retinyl esters and retinol. Retinoic acid is inactive (9).

Some of the most significant developments in the area of vitamin A function and metabolism are outlined in this introductory section.

Utilization of vitamin A in the body is a tightly regulated process; there are several sites of control:

(1) Transport of vitamin A from its storage site in liver to tissues is in the form of retinol bound to its specific carrier protein, retinol-binding protein (10, 11). Retinoic acid is apparently transported by serum albumin (12).

(2) There is control at the level of entry into cells: it has been shown that a specific cell surface binding protein accepts the vitamin from its carrier protein and then transfers it across the membrane into the cell (13, 14).

(3) At the cellular level vitamin A appears to be bound to a cellular receptor protein: cytosolic binding proteins have been found that specifically bind retinol, retinal and retinoic acid (15–18), and there is evidence of nuclear receptors specific for retinol and retinoic acid (15, 18–23).

(4) Computer modeling studies suggest that plasma retinol cycles repeatedly in a "futile cycling" process that interfaces target issues, circulation, and body pools (M. H. Green, personal communication).

The question now arises: what exactly does vitamin A do at the cellular and molecular level? Although a great deal of effort has been expended in many laboratories to answer this question, the function of this important nutrient is still unknown. At the present time there are three working models offered to explain the mode of action of vitamin A.

One is based on the mode of action of vitamin A in vision, where, in the form of 11-*cis*-retinal, it functions as the prosthetic group on the visual protein, opsin, in the rhodopsin cycle ((8, 9), reviewed in (5, 6)). This molec-

[1] Michigan Agricultural Experiment Station Journal Article 10505. Partially supported by USDA Grant 81-CRCR-1-0700 to M.H.Z. Many thanks to Drs. D. R. Romsos, C. C. Sweeley, and S. M. Ferguson-Miller for critically reading the manuscript. We thank Ms. Janeen Hunt for expert typing of the manuscript.

[2] To whom all correspondence should be addressed.

0037-9727/83/020139-14$01.50/0

ular mechanism of action for vitamin A, however, so far has not been found applicable to other target sites of vitamin A function.

To explain the mode of action of vitamin A in reproductive and general somatic functions, such as growth and differentiation, there are currently two models under active investigation:

(1) Vitamin A acts in a coenzyme fashion as a sugar carrier for glycoprotein biosynthesis. Work is in progress in several laboratories to seek substantiating evidence for this hypothesis (5, 6, 24).

(2) Vitamin A functions via a specific receptor protein (16, 17). Since cellular binders for all active forms of vitamin A have been found (16, 17, 25, 26), this working model has received wide acceptance.

Functions of vitamin A must be intimately linked to its metabolism. Simultaneously with research ongoing in the area of vitamin A function, there has been a great deal of stimulating work in the area of vitamin A metabolism. Of interest has been the work of Wolf and De Luca, who have focused on retinyl phosphate as the metabolically active form of retinol. Retinyl phosphate has been detected in several tissues and is biologically (growth response) as active as retinol (27, 28). Retinyl phosphate combines with monosaccharides such as mannose and can transfer this sugar to a protein (24). The physiological significance of retinyl phosphate and the sugar–lipid intermediate formed remains to be established.

Extensive research has developed around the concept that retinoic acid or a metabolite of it might be the "active form" of vitamin A for its somatic function. This concept continues to be viable because reductive metabolism (to retinol, retinal, or retinyl esters) does not appear to occur *in vivo*. Storage forms (retinyl esters) are not generated and the metabolism of retinoic acid is rapid. Metabolism of retinoic acid is still not fully elucidated and thus metabolites remain yet to be identified and tested for vitamin A activity.

Several important developments have significantly contributed to the understanding of metabolism of retinoic acid:

(1) The realization that retinoic acid exists in many tissues and is a physiological metabolite of all known vitamin A-active compounds (29–32). This knowledge confirmed what was previously only speculation concerning the important role of retinoic acid as a vitamin A-active compound.

(2) The discovery that vitamin A compounds have anticarcinogenic potential and that among natural vitamin A compounds retinoic acid is the most promising antitumor agent (33–35). This finding has generated a tremendous interest within the medical field and has expanded our understanding of vitamin A in nutrition and metabolism.

(3) Geometric isomers of retinoic acid occur *in vivo* in certain target organs and may have physiological functions (36, 37).

(4) The development of new chromatographic and isolation technology for the separation of organic compounds. Methods such as liquid-gel partition chromatography (30, 38), improved isolation methods (36, 39), and especially high-pressure liquid chromatography [HPLC] (40–42) have practically revolutionized the field of vitamin A metabolism allowing separation of vitamin A compounds from each other and their isomers. Unfortunately, much of the earlier work on vitamin A compounds must now be viewed with some reservation because earlier methods permitted the destruction of the very labile vitamin A compounds.

It has now been possible to characterize several tissue metabolites of retinoic acid. One such metabolite is 5,6-epoxyretinoic acid (43), previously thought to be a highly active vitamin A compound (44, 45), but now proven to be almost totally inactive (46). Other recently identified metabolites of retinoic acid include 4-oxoretinoic acid and 4-hydroxyretinoic acid (47, 48); they, too, are biologically inactive and represent early catabolic products of vitamin A metabolism.

Another newly characterized metabolite of retinoic acid is retinoyl glucuronide (49–51) originally isolated and studied by Olson and co-workers (52–54). Postulated to be primarily a biliary metabolite of retinoic acid, retinoyl glucuronide was found to be 30–100% as active as all-*trans*-retinoic acid (52) and has been thought to represent the major excretory product of retinoic acid metabolism (53). However, the recent demonstration of only very small amounts of all-*trans*-retinoyl gluc-

uronide in bile (49–51) suggests that glucuronidation is a minor metabolic pathway for retinoic acid elimination. The excretion of other polar metabolites of retinoic acid as glucuronides is, however, very probable.

Of particular interest is the report that retinoyl glucuronide is the *major intestinal metabolite* from administered physiological concentrations of retinoic acid to vitamin A-deficient, bile duct-cannulated rats (36). The authors suggest that glucuronidation of retinoic acid to form the mixed anhydride-ketal, may have a physiological function in tissues.

Another recent finding is that in the intestinal mucosa, a target tissue for vitamin A action, retinoic acid exists in an equilibrium mixture, 2:1, of its all-*trans* and 13-*cis* forms, respectively (36). Although both forms appear to be equally active in the somatic function (growth response) (55), it has not been established if each form is active on its own account or because of a conversion to the other isomer. It is possible that *in vivo isomerization* of all-*trans*-retinoic acid is *crucial* to the cellular function of retinoic acid. Thus, 13-*cis* isomerization may be the initial physiological event in the action of retinoic acid at the cellular level, followed by further metabolic reactions. This idea gains support from studies with vitamin A-deficient rats: in the small intestinal mucosa of retinoic acid-dosed, bile duct-cannulated rats a similar equilibrium exists between the 13-*cis* and all-*trans* retinoyl glucuronides (36). Also, the 13-*cis* isomer of 4-keto-retinoic acid, although inactive, has been found to be a metabolite of all-*trans*-retinoic acid in liver (48). Recently, several isomers of retinoic acid have been detected in varying amounts in a number of tissues after administration of all-*trans*-retinoic acid to vitamin A-sufficient rats (37). *Cis-trans* isomers of retinol and retinal are also present in livers of mammals, birds, and fish (56, 57), suggesting that they are naturally occurring forms of vitamin A. The importance of isomerization for a particular expression of vitamin A activity thus may not be limited only to the rhodopsin cycle in retina but may be of a more universal occurrence.

Progress in elucidation of vitamin A metabolism has not yet led to an understanding of the function of vitamin A. Presently the function of this vitamin can not be explained by a single unifying mechanism involving a common metabolic intermediate. Equally active forms of vitamin A, such as retinol, retinal, and retinoic acid, may in fact each act by divergent mechanisms and may possibly even affect different functions in specific target tissues.

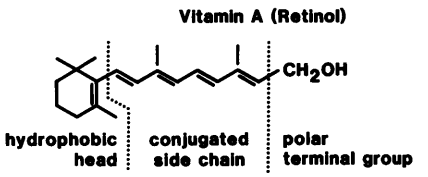

FIG. 1. Structure of vitamin A.

A brief examination of the vitamin A molecule (Fig. 1) reveals three important features: (1) a hydrophobic head: the β-ionone ring; (2) a conjugated isoprenoid side chain: this conjugated double bond system lends itself to isomerization (by light, or enzyme, or heat) and thus allows for a change in shape of the molecule; (3) a polar terminal group that can be enzymatically or chemically modified to become an ester as in retinyl palmitate or an aldehyde (-CHO) as in retinal or be oxidized to a very polar carboxylic (-COOH) group as in retinoic acid.

It is clear that a molecule with such diverse chemical properties of its components may readily enter into several different biochemical reactions, the choice of reaction type governed by endogenous and exogenous influences in the biological environment surrounding it.

Our discussion to follow is primarily directed at an examination of the current understanding of physiological function(s) of vitamin A in light of present and past concepts of possible mechanism(s) of action.

Visual Function of Vitamin A. Vitamin A, because of the diversity of its molecular components, can serve in various different biochemical functions at the molecular level. It is obvious that in the course of evolution advanced organisms have utilized this unique molecule in several biological systems.

The best example is the function of vitamin A aldehyde, retinal, in vision. A change in shape of the molecule, such as that resulting from isomerization of all-*trans*-retinal to 11-*cis* form, allows this molecule to fit the surface of a specific glycoprotein, opsin, located in the

rods of the retina, where it is bound to protein in an imine linkage (Schiff base). Here retinal acts as a specific chromophore that responds to white light by isomerization to all-*trans* form, releasing retinal from the protein and thus causing a change in charge distribution on the protein and initiating events that lead to neurotransmission. This process was discovered by Wald (8) and has been intensively studied and reviewed (5, 6).

The unique properties of vitamin A are also thought to account for color perception. In the *cones* of the retina, 11-*cis*-retinal fits into three specific cone opsins, each of which provides a different environment in terms of distribution of negative charges along the length of the molecule and enables retinal to absorb at visible (longer) wavelengths (bathochrome shift). When a wavelength characteristic of a certain color (e.g., red) enters the eye, the 11-*cis* retinal that is bound to opsin in the "red" cones can absorb this light and is isomerized to its all-*trans* form. This change in shape of the molecule dissociates the chemical bonds between retinal and the protein, causes a redistribution of charges and a change in current, that finally results in a signal to brain, perceived as red color.

This experimental model for color perception, described above, has been recently proposed by Honig *et al.* (58, 59) and is expected to be applicable to all color-perceiving visual systems.

Much of the understanding of the role of retinal as chromophore stems from studies on the purple membrane in *Halobacterium halobium* (60–63). This membrane contains the protein bacteriorhodopsin with all-*trans*- and 13-*cis*-retinal as its chromophore. Since bacteriorhodopsin is a small molecule (in contrast to the large protein molecules in rods and cones of the vertebrate eye), it has been possible to progress much more rapidly in understanding the functioning of the protein–retinal complex, the chemistry of which is similar to that of the visual pigment, rhodopsin. In the bacterial membrane system the energy of light, absorbed by the retinal chromophore on the bacteriorhodopsin, is converted into an electrochemical proton gradient across the membrane, providing a source of energy for this unusual salt-adapted microorganism.

Thus, molecular functions of several retinal pigments have been clearly established. Even as bacteriorhodopsin is a light-driven proton pump (61–63), another retinal pigment, halorhodopsin, has been found to function as a light-driven sodium ion pump (64). Finally, in higher organisms, rhodopsin in the eye enables them to utilize the visible region of the spectrum and transform a light stimulus into an electrical signal that causes neurotransmission and perception of dim light and, possibly, also perception of color.

The specific protein–retinal interactions that enable vitamin A to perform light-absorbing functions are dependent not only on the photosensitivity of retinal because of conjugated double-bond system, but also on hydrophobic properties of the β-ionone ring of the molecule and on the reactivity of the aldehyde group on the side chain terminal; all parts of the molecule are essential for this biochemical event to be accomplished.

The Somatic Functions of Vitamin A. *a. Differentiation.* It is universally accepted that vitamin A is required for growth and differentiation. These functions, however, are inseparable processes in higher organisms, involving complex functioning of many tissues and cells. Indeed, the earliest symptom of vitamin A deficiency—a loss of appetite and an ensuing decrease of growth, is followed by multiple changes in tissue morphology, mostly involving changes in epithelial tissue differentiation. All this inevitably points to an involvement of vitamin A in some very fundamental process.

A general observation of the effect of vitamin A upon differentiation is that the vitamin most certainly exerts its action upon bipotential cells: cells that have retained their differentiating ability in the growing and mature animal and can differentiate in more than one direction. Vitamin A deficiency effects at the cellular level are most readily seen in those differentiating tissues that have a rapid turnover rate, such as epithelia of oral cavity, respiratory tract, gastrointestinal tract, urinary tract, and ducts of various secretory glands (2, 3, 6, 65). Similarly, effects of vitamin A on embryonic development (66–68), development of bone and teeth (69, 70), maintenance of spermatogenesis (7, 71), and limb regeneration (72–75) have also been explained by

an effect upon differentiation. Early (76) as well as recent (reviewed in (25)) studies with cell cultures have substantiated the involvement of vitamin A in cellular differentiation.

Regulation of differentiation of eukaryotic cells is still one of the major unsolved problems in biology. Programming of cell function has multiple control mechanisms that are indirectly influenced by environmental factors. It has become increasingly clear that vitamin A nutriture may be one such factor involved in regulation of differentiation of certain tissues.

It is well established that lowered immunoresponsiveness is a sign of vitamin A deficiency (reviewed in (6, 77, 78)) and that an increased level of dietary vitamin A provides protection against infection (reviewed in (77)). Certain xenobiotics, such as TCDD (tetrachlorodiphenyldioxane) (79, 80), ethanol (81), phenobarbital (82, 83), PBB (polybrominated biphenyls), and PCB (polychlorinated biphenyls) (84–86) increase vitamin A turnover from liver stores. Other stress factors, such as infection, accentuate vitamin A utilization and decrease assimilation ((87), reviewed in (88)). It is clear that optimal vitamin A stores and intake have a protective effect against adverse enviromental factors that could lead to misguided gene expression and altered cell phenotype. A case in point is the accumulating epidemiological evidence that adequate vitamin A nutriture has a significant protective effect against lung cancer in smokers (89, 90).

Differentiation is almost certainly based upon gene expression. Many biochemical studies suggest that vitamin A affects nuclear events ((91–98), reviewed in (6)), but the exact molecular site of its action has not been identified.

The mechanism of action of steroid hormone-induced differentiation has been successfully explored by the receptor model (99, 100); this model proposes that specific proteins facilitate interactions with nucleus. The model is also being applied to study the role of vitamin A in differentiation. This approach to elucidation of vitamin A function at the molecular level, pioneered by Chytil and Ong (16, 17, 101), has gained wide acceptance because cytosolic and nuclear binding proteins for vitamin A-active compounds have been found in many tissues (16–23) and their concentrations appear to be regulated by vitamin A status (101).

Additional support that vitamin A modifies genomic expression comes from the recent demonstration of nuclear binding of retinol in liver (18, 23) and the observation that retinol regulates the concentration of poly(A) RNA (mRNA) in several target tissues (97, 101). In epidermal cells it has been possible to correlate alterations in mRNA concentration with appearance of specific keratins (97, 98). The exact nuclear sites of these events remain to be identified.

Cellular binding proteins for vitamin A-active compounds have also been detected in some transformed cells ((16) reviewed in (25)), suggesting that a vitamin A–receptor complex is involved in expression of the anticarcinogenic properties of this vitamin. Presence of cellular binding proteins, however, does not always correlate with retinoid-induced effects on proliferation or differentiation.

Although the receptor model is an excellent tool for investigating the specific site of action of vitamin A at the molecular level, this mechanism may not account for all vitamin A functions. A correlation between the presence of cellular vitamin A binding proteins and gene regulation is not of universal occurrence. An example is the abundance in the retina of "receptors" for almost every known vitamin A-active compound (26, 103), while retina is a tissue where the function of vitamin A in the visual cycle is known *not* to involve gene expression. Thus the physiological function(s) of these cellular binding proteins is not clear. There is recent evidence that in retina the proteins may function as inter- and intracellular carriers for the various forms of vitamin A (26, 102, 103). It is also possible that the proteins may be enzymes for the interconversion of the vitamin A compounds or they may be needed to stabilize the labile vitamin A compounds or to decrease the toxicity of free retinol and retinoic acid (104).

An argument against the receptor model has been raised in view of the data that there are vitamin A target tissues without detectable vitamin A-binding proteins as well as that there are nontarget tissues containing binding proteins (16, 17). It is likely, however, that these discrepancies exist because of our present incomplete knowledge of vitamin A target

tissues and the inability to detect small amounts of vitamin A binding proteins. The availability and application of sensitive immunochemical (105, 106) and radioimmunoassay (103, 107, 108) techniques may abrogate these differences.

The striking morphological changes in certain epithelial tissues that are the result of alterations in vitamin A nutrition (3, 5, 6, 65), are often accompanied by altered mucus secretion. Thus, a role of vitamin A in glycoprotein metabolism has been suggested to explain its effect on differentiation (5, 6, 24). The ability of vitamin A to alter glycoprotein biosynthesis has been documented in many *in vivo* and *in vitro* systems (5, 6, 24). Wolf and De Luca propose a *coenzyme* role for vitamin A, where retinol, as retinyl–phosphate–sugar complex, functions in membranes in post-translational transfer of monosaccharides to an acceptor protein, this resulting in synthesis of a specific glycoprotein that may affect cellular differentiation (5, 6, 24).

At the present time it has not been possible to demonstrate the existence of a protein that specifically accepts a sugar moiety from vitamin A. Furthermore, the model is not applicable to retinoic acid, even though this vitamin A-active compound stimulates glycoprotein synthesis and fulfills the somatic functions of vitamin A. Also lacking is a working hypothesis as to how a change in the biosynthesis of a vitamin A-dependent glycoprotein might result in modulation of cellular phenotype.

The demonstration that retinoic acid can regulate release of the glycoprotein, fibronectin, in an enucleated cell (109) indicates that *cytoplasmic* function may be another molecular mechanism for vitamin A.

b. Growth. Elucidation of somatic functions of vitamin A becomes even more complicated in the light of some important concepts of vitamin A action that have received very limited attention.

Pathological lesions associated with vitamin A deficiency in animals have been described in great detail (2, 3, 110), but it is not known what initial biochemical events are responsible for the eventual morphological changes. Physiological and biochemical changes have been observed in young animals when their dietary vitamin A is withheld even though they still have adequate amounts of vitamin A stored in their livers, and therefore have an endogenous supply of vitamin A that is supposedly "available" for release to needy tissues. The outward appearance of such animals, their growth rate, and their serum vitamin A give no indication of an ensuing vitamin A deficiency; the animals are in apparent good health. However, the development of tissues that have been examined was found to be restricted (77, 111). One explanation could be that the amount of vitamin A adequate to maintain body weight and general health may *not* be sufficient for optimal growth and development of *all* tissues, as is the case with testes (112). The mechanism(s) regulating the release of retinol–RBP from liver of *normal* animals is not understood. This process might partially depend on newly absorbed (dietary) vitamin A, mostly retinyl esters. In the absence of this exogenous stimulus, the rate of release of the retinol–RBP complex may be sufficient to keep the blood concentration of retinol normal, but *not* sufficient to satisfy the needs of rapidly proliferating tissues.

The principal cause of an impairment in growth in a nutritional insufficiency might be connected with alterations in protein metabolism (113). The initial biochemical effect thus could be either at the nuclear level or epigenetic; for example, some function of vitamin A may be concerned with maintenance of stability or specificity of complex proteins, particularly in membranes.

Recent studies have provided evidence that rapidly proliferating tissues are very sensitive to suboptimal vitamin A nutrition: DNA synthesis phase in the small intestinal crypt cells is prolonged (111), addition of new cells to growing organs is decreased (77), and DNA labeling index (114), cell mitoses (115), and DNA synthesis (116, 117) are decreased in several tissues of animals prior to manifestation of external deficiency symptoms. The lowering in DNA synthesis activity may be a conservation mechanism for vitamin A in the presence of a limited supply of this micronutrient. Under suboptimal nutritional conditions, cells cease to multiply and, to survive, may revert to a differentiated state that is better adapted to existing nutritional conditions.

Temporal effects in target tissues of decreased DNA synthetic activity, followed by hyperplasia, might also be explained by a proliferative dependence for vitamin A in rapidly dividing target cells. Such cells would be trapped in G_0 permitting other juxtaposed cells, not dependent on vitamin A, to continue proliferating. Recent *in vivo* experiments, using vitamin A-depleted and -repleted animals, suggest that both mechanisms may be operating simultaneously in tracheal epithelium (114, 118, 119). Whether these changes in cellular behavior caused by vitamin A insufficiency are phenotypic alterations of target cells or the "overgrowth" of other nondependent cell types *in vivo*, cannot be answered with our present knowledge.

Studies with rats in germ-free environment (120, 121) have been very useful in separating various components associated with vitamin A function: growth, differentiation, reproduction, vision. The germ-free experimental model illustrates clearly the vitamin A requirement for reproduction, vision in the dark, and epithelial tissue differentiation: such animals cannot reproduce or see in the dark; many epithelia undergo keratinization; accumulated keratinized debris obstructs vital passages to organs and is often the primary cause of death of the animal.

In the germ-free environment rats can live and maintain their body weight in the absence of vitamin A. The experimental model illustrates a very important concept: if all stresses are removed, animals can survive without vitamin A. However, if demands are imposed upon the body, such as growth or need for rapid tissue regeneration or turnover, the animal *must* have vitamin A to survive. This concept is also supported by the observation that the requirement of vitamin A depends on the rate of growth (122–126, also reviewed in (6)). Similarly, bone lesions due to inadequate vitamin A nutrition are observed only in young, growing animals (2–6) and are probably related to the need for this vitamin in proliferation and differentiation of bone cells, particularly osteoblasts and osteoclasts. Despite extensive studies with hypo- and hypervitaminotic-A animals (2–6), regulation by vitamin A of bone growth and development is still completely obscure.

Reproduction. The requirement of vitamin A for reproductive functions in higher animals has been known since 1922 (127). In studies with animals restricted to vitamin A-deficient diets and repleted with various active forms of vitamin A, Thompson *et al.* (7) established a dichotomy of vitamin A function: while retinoic acid maintained somatic epithelial function in testes, it did not maintain germinal epithelium or spermatocytogenesis. The reduced forms of vitamin A, retinol, or retinal, are required for these functions. In female rats, hormone production, oogenesis, fertilization, and implantation are supported equally well by retinoic acid, retinol, and retinal, but the reduced forms are required for placental and fetal development.

Recent studies, however, suggest that in addition to its general, somatic role in epithelial maintenance, retinoic acid *does* have a specific function in testes: it supports testosterone biosynthesis in Leydig (interstitial) cells (128). Both retinol and retinoic acid appear to be required for complete testicular function. It remains to be determined why retinoic acid alone *can* support spermatogenesis and oogenesis in birds (129).

The partial vitamin A activity of retinoic acid has been very useful in sorting out the various functions of vitamin A, particularly in separating the multiple somatic lesions (that often lead to complicating consequences) from the more specific effect of vitamin A on reproduction (and vision). At this time it is still not clear what biochemical systems are involved.

Wald and Dowling suggested (9) that retinol serves merely as a precursor to two active forms of vitamin A: retinal and retinoic acid. This idea is still a valid model for proposing a function for *retinal* in reproduction: (a.) retinal supports *all aspects* of reproduction (7); (b.) retinol–retinal interconversions occur in tissues ((30, 130) discussed in (6)). Cellular binding proteins for retinal have been detected in eye tissues (26, 102, 158); no data on other tissues are available. At this time one can not assume that retinal is functional only in vision. Present evidence supports the concept that reproduction in higher animals is an example of a specialized system that utilizes several available active forms of vitamin A, each for its unique potential.

The *mechanisms of action* of vitamin A

proposed for maintenance of growth and differentiation have also been suggested for the function of vitamin A in reproduction. Cellular binding proteins for both retinol and retinoic acid have been isolated from testes, ovary and uterus (16), oviduct (131) and mammary gland (132). Extensive studies with testicular retinol- and retinoic acid-specific binding proteins have strengthened the view that the action of vitamin A compounds in reproduction is mediated by specific intracellular carrier proteins and that vitamin A exerts its effect on reproduction at the gene level via a nuclear receptor.

Membrane Phenomena. Vitamin A, when added in excess to biological systems, acts on cellular membranes and glycocalyx. Membrane effects have shown a high degree of correlation with biological activity. As a result of these observations as well as the surface-active effect of vitamin A (see Fig. 1), a working hypothesis has been proposed by Lucy (133) that one function of this vitamin may be to physically alter lipoprotein membranes and thus to affect differentiation.

Effects of vitamin A on membrane structure and function have been studied extensively in biological and physical experimental systems. When high nonphysiological concentrations of vitamin A are employed, biological studies demonstrate that free retinol (not bound to its physiological carrier, RBP), labilizes membranes causing leakage of enzymes (134–136). Lucy (133) has suggested that physiological concentration of vitamin A might similarly regulate release of membrane-enclosed enzymes essential for normal metabolic processes and thus account for the varied functions influenced by vitamin A.

For explanation of the physiological function of vitamin A the membrane model has been discounted at this time, since there are several lines of evidence that indicate that the hypervitaminotic effects of vitamin A are *not related* to the physiological function of this vitamin. For example, α-retinol, an analog of retinol capable of producing the membrane effects, has very low growth-promoting activity (135). Also, an excessive amount of retinol, when added to cell culture in its physiologically circulating form (bound to RBP), did not cause the deleterious alterations in membrane structure and function that are associated with the action of free retinol (137). Furthermore, considering the very low concentration of vitamin A in membranes (except in the membrane discs of retinal rods), it is difficult to envision this compound in the role of a structural component.

Much of the available evidence, however, *does* point to *membranes* as the most likely biological structures for the site of action of vitamin A (138–144). Also, length of the vitamin A molecule is appropriate to span the lipid bilayer of some cellular membranes (145).

Although vitamin A is distributed among various soluble intracellular compartments, some of it is *always* associated with membranous structures (138, 139, 146, 147). Furthermore, in recent studies demonstrating the delivery of retinol via cellular receptor to nucleus, it was noted that free retinol was associated with the nuclear membrane (23).

Evidence is increasing that membranous structures play an essential part in many cellular events. Since vitamin A influences the *biosynthesis of glycoproteins*, characteristic components of membranes, it may affect cellular differentiation by a nonnuclear mechanism. Glycoproteins on the surface of mammalian cells are important in a variety of membrane-mediated functions, such as cell adhesion and communication. *In vitro* studies have demonstrated that these cellular interactions are affected by vitamin A ((140–143), reviewed in (25)). Evidence also suggests that changes in membrane glycosylation affect cell surface recognition mechanisms (148–150) and that glycosylation of membrane-associated proteins may be an integral part of cellular differentiation (151).

Recent morphogenesis studies indicate that vitamin A compounds exert striking systemic effects on the anatomical patterns of regenerating limbs in amphibians (72–75) and developing limb buds in chick and mouse (152–155). Maden (74) suggests that the effect of retinoids on cell surface glycoprotein glycosylation, on gap junction formation, and on secretion of matrix proteins may be responsible for alterations in cellular communication during limb pattern formation and thus may influence the determination and maintenance of tissue positional information.

The highly reactive nature of the molecular

species of vitamin A indicates that this compound could be involved in electron transfer. Lucy ((133), see also (6)) has suggested that vitamin A may be involved in the *electron transfer* chain of the membrane-bound enzymes [e.g., Cyt P_{450}-mixed function oxidases] in microsomes. This possibility is strengthened by the observation in model systems that flavins catalyze the isomerization of retinal (156).

Elucidation of the molecular function of retinal as a photosensitive chromophore, powerfully demonstrates how the various parts of the vitamin A molecule can be utilized by the cell to accomplish a biological task. The photoprocess (photoisomerization) induces a conformational change in membrane protein, this leading to an increased permeability of the membrane to ions and to the consequent generation of a charge. While not directly applicable to the elucidation of other physiological functions of vitamin A, the retinal chromophore *does* provide us with an additional working model: in the somatic cells vitamin A-active compounds could be *enzymatically isomerized* and then utilized, in a similar fashion, to perform some fundamental biochemical function.

It is definitely premature to speculate whether some action of vitamin A at the gene level affects membrane function or whether some function of vitamin A at the membrane site alters gene expression.

Conclusions. All available evidence indicates that vitamin A has a very complex function in the body, involving cellular proliferation, differentiation, as well as specialized functions, such as vision and reproduction.

The search for a link between a biochemical reaction, initiated by vitamin A, and a physiological or morphological change, has progressed in several directions. The following models are being explored to study the regulation of molecular events by vitamin A:

(1) Isomerization of vitamin A to affect conformation and charge of complex proteins. Although this model has been useful to elucidate the function of vitamin A in vision, so far it has not been found applicable to somatic functions of this vitamin. It is likely that in higher organisms an enzymatic isomerization will link a critical biochemical event to the physiological change elicited by vitamin A.

(2) Regulation of nuclear events via specific vitamin A–cellular binding protein complexes. This model appears to explain the effect of vitamin A in certain target epithelia. However, specific alterations in proliferative or differentiative response do not always correlate with presence of vitamin A binding proteins.

(3) Modulation of glycoprotein biosynthesis by participation in sugar transfer reactions. The importance of glycoprotein processing in cellular control mechanisms makes this hypothesis attractive. Demonstration of a specific protein acceptor and identification of a retinoic acid intermediate would further substantiate this hypothesis.

(4) Membrane phenomena. It is possible that the ability to penetrate and modify biological membranes is the basis for some physiological activity of vitamin A such as stabilizing complex protein, i.e., membrane-bound enzymes.

(5) Electron transfer. The conjugated double-bond system of vitamin A molecule can provide an electron mobility that might be utilized for some systemic actions.

It is certain that a single unifying mechanism cannot explain the molecular action of vitamin A. Several active forms of this vitamin exist in the body: retinol, retinal, and retinoic acid. They may function each in a specific tissue or simultaneously in the same tissue, possibly each modulating cellular behavior by a different mode of action.

The significance of retinoic acid is still not clear. Is retinoic acid a partially active metabolite of vitamin A or *must* retinol and retinal be converted to retinoic acid for the vitamin A function in growth and maintenance of somatic epithelial tissues? So far, it is not known whether the biochemical event caused by retinoic acid is specific only for retinoic acid, because the reduced forms of vitamin A are as effective as retinoic acid and can be physiologically metabolized to retinoic acid. A specific function could be ascribed to retinoic acid with the demonstration of a differentiation product specific only for retinoic acid in a target tissue that responds to all forms of vitamin A.

Addendum. Vitamin A research has been characterized by a considerable progress in recent years. This is reflected not only in the vast number of publications in the area, but also in the availability of several excellent *recent* reviews. Particularly informative is the *comprehensive survey* and evaluation of the vitamin A field by Wolf (6). Other recent reviews dealing with *specific* areas include: an update of RBP research (11), an examination of natural and synthetic vitamin A compounds (collectively designated as retinoids) and their *in vivo* and *in vitro* effect on neoplasia (25); a review on the involvement of vitamin A in sugar transfer reactions in mammalian membranes (24); a discussion of cellular binding proteins for vitamin A compounds (16, 18); a general review of vitamin A, including nutritional information, and chemical and biological assay procedures (88); reviews on vitamin A and cancer (33–35); a review on vitamin A deficiency as a public health problem in many parts of the world (157) and a review of retinoids in ocular tissues (158).

1. McCollum EV, Davis M. The nature of dietary deficiencies of rice. J Biol Chem **23**:181, 1915.
2. Moore T. Vitamin A. New York, Elsevier, 1957.
3. Wolbach SB. Effects of vitamin A deficiency and hypervitaminosis A in animals. In: Sebrell WH, Harris RS, eds. The Vitamins. New York, Academic Press, Vol I:pp106–137, 1954.
4. Barnicot NA, Datta SP. Vitamin A and bone. In: Bourne GH, ed. The Biochemistry and Physiology of Bone. New York, Academic Press, Vol II:pp197–229, 1972.
5. De Luca LM. Vitamin A. In: DeLuca HF, ed. Handbook of Lipid Research. The Fat Soluble Vitamins. New York, Plenum, Chap 1, pp1–67, 1978.
6. Wolf G. Vitamin A. In: Alfin-Slater RB, Kritchevsky D, eds. Human Nutrition. New York, Plenum, Vol 3B:pp97–203, 1980.
7. Thompson JN, Howell JMcC, Pitt GAJ. Vitamin A and reproduction in rats. Proc Roy Soc London **159**:510–535, 1964.
8. Wald G. The visual function of vitamin A. In: Vitamins and Hormones. New York, Academic Press, Vol 18:pp417–430, 1960.
9. Dowling JE, Wald G. The biological function of vitamin A acid. Proc Nat Acad Sci USA **46**:587–608, 1960.
10. Kanai M, Raz A, Goodman DeW. Retinol-binding protein: Transport protein for vitamin A in human plasma. J Clin Invest **47**:2025–2944, 1968.
11. Rask L, Anundi H, Böhme J, Eriksson U, Frederiksson A, Nilsson SF, Ronne H, Vahlquist A, Peterson PA. The retinol-binding protein. Scand J Clin Lab Invest **40**, Suppl 154, pp45–61, 1980.
12. Smith JE, Milch PO, Muto Y, Goodman DS. The plasma transport and metabolism of retinoic acid in the rat. Biochem J **132**:821–827, 1973.
13. Heller J. Interactions of plasma retinol-binding protein with its receptor. Specific binding of bovine and human retinol-binding protein to pigment epithelium from bovine eyes. J Biol Chem **250**:3613–3619, 1975.
14. Rask L, Peterson PA. In vitro uptake of vitamin A from retinol-binding plasma protein to mucosal epithelial cells from the monkey's small intestine. J Biol Chem **251**:6360–6366, 1976.
15. Ong DE, Chytil F. Changes in levels of cellular retinol- and retinoic acid-binding proteins of liver and lung during perinatal development of rat. Proc Nat Acad Sci USA **73**:3976–3978, 1976.
16. Chytil F, Ong DE. Cellular-binding proteins for compounds with vitamin A activity. In: Receptors in Hormone Action. New York, Academic Press, Vol II:pp573–591, 1978.
17. Chytil F, Ong DE. Cellular retinol- and retinoic acid-binding proteins in vitamin A action. Fed Proc **38**:2510–2514, 1979.
18. Chytil F. Liver and cellular vitamin A binding proteins. Hepatology **2**:282–287, 1982.
19. Sani BP, Donovan K. Localization of retinoic acid-binding protein in nuclei and the nuclear uptake of retinoic acid. Cancer Res **39**:2492–2496, 1979.
20. Rao MRS, Prasad VR, Padmanaban G, Ganguly J. Isolation and characterization of binding proteins for retinol from the cytosol, nucleosol and chromatin of the oviduct magnum of laying hens. Biochem J **183**:501–506, 1979.
21. Russell P, Wiggert B, Derr J, Albert D, Craft J, Chader G. Nuclear uptake of retinoids: Autoradiographic evidence in retinoblastoma cells in vitro. J Neurochem **34**:1557–1560, 1980.
22. Takase S, Ong DE, Chytil F. Cellular retinol-binding protein allows specific interaction of retinol with nucleus in vitro. Proc Nat Acad Sci USA **76**:2204–2208, 1979.
23. Liau G, Ong DE, Chytil F. Interaction of the retinol/cellular retinol-binding protein complex with isolated nuclei and nuclear components. J Cell Biol **91**:63–68, 1981.
24. De Luca LM. The direct involvement of vitamin A in glycosyl transfer reactions in mammalian membranes. In: Vitamins and Hormones. New York, Academic Press, Vol 35:pp1–57, 1977.
25. Lotan R. Effects of vitamin A and its analogs (retinoids) on normal and neoplastic cells. Biochim Biophys Acta **605**:33–91, 1980.
26. Saari JC, Bredberg L, Garwin G. Endogenous retinoids associated with three cellular retinoid-binding proteins from bovine retina. Fed Proc **41**:861 (Abstract), 1982.

27. Rosso GC, De Luca L, Warren CD, Wolf G. Enzymatic synthesis of mannosyl retinyl phosphate from retinyl phosphate and GDP-mannose. J Lipid Res **16**:235–243, 1975.
28. Frot-Coutaz JP, Silverman-Jones CS, De Luca LM. Isolation, characterization and biological activity of retinyl phosphate from hamster intestinal epithelium. J Lipid Res **17**:220–230, 1976.
29. Emerick RJ, Zile M, DeLuca HF. Formation of retinoic acid from retinol in the rat. Biochem J **102**:606–611, 1967.
30. Ito YL, Zile M, Ahrens H, DeLuca HF. Liquid-gel partition chromatography of vitamin A compounds; formation of retinoic acid from retinyl acetate in vivo. Lipid Res **15**:517–524, 1974.
31. DeRuyter MG, Lambert WE, DeLeenheer AP. Retinoic acid: An endogenous compound of human blood. Unequivocal demonstration of endogenous retinoic acid in normal physiological conditions. Anal Biochem **98**:402–409, 1979.
32. Shidoji Y, Hosoya N. Competitive protein-binding radioassay for retinoic acid. Anal Biochem. **104**:457–463, 1980.
33. Bollag W. Retinoids and cancer. Cancer Chemother Pharmacol **3**:207–215, 1979.
34. Newberne PM, Rogers AE. Vitamin A, Retinoids and Cancer. In: Newell GR, Ellison NM, eds. Nutrition and Cancer: Etiology and Treatment. New York, Raven Press, pp217–231, 1981.
35. Sporn MB, Newton DL. Retinoids and chemoprevention of cancer. In: Zedeck MS, Lipkin M, eds. Inhibition of Tumor Induction and Development. New York, Plenum, Vol 3:pp71–100, 1981.
36. Zile MH, Inhorn RC, DeLuca HF. Metabolism in vivo of all-trans retinoic acid. Biosynthesis of 13-cis-retinoic acid and all-trans- and 13-cis-retinoyl glucuronides in the intestinal mucosa of the rat. J Biol Chem **257**:3544–3550, 1982.
37. Sundaresan PR, Bhat PV. Ion-pair high pressure liquid chromatography of cis-trans isomers of retinoic acid in tissues of vitamin A-sufficient rats. J Lipid Res **23**:448–455, 1982.
38. Nystrom E, Sjovall J. Separation of lipids on methylated Sephadex. Anal Biochem **12**:235–248, 1965.
39. Ito Y, Zile M, DeLuca HF, Ahrens HM. Metabolism of retinoic acid in vitamin A-deficient rats. Biochim Biophys Acta **369**:338–350, 1974.
40. Frolik CA, Tavela TE, Sporn MB. Separation of the natural retinoids by high-pressure liquid chromatography. J Lipid Res **19**:32–37, 1978.
41. McCormick AM, Napoli JL, DeLuca HF. High-pressure liquid chromatographic resolution of vitamin A compounds. Anal Biochem **86**:25–33, 1978.
42. McKenzie RM, Hellwege DM, McGregor ML, Rockley NL, Riquetti PJ, Nelson EC. Separation and identification of geometric isomers of retinoic acid and methyl retinoate. J Chromatogr **155**:379–387, 1978.
43. McCormick AM, Napoli JL, Yoshizawa S, DeLuca HF. 5,6-Epoxyretinoic acid as a physiological metabolite of retinoic acid in the rat. Biochem J **186**:475–481, 1980.
44. John KV, Lakshmanan MR, Cama HR. Preparation, properties, and metabolism of 5,6-monoepoxyretinoic acid. Biochem J **103**:539–543, 1967.
45. Krishna Mallia A, John J, John KV, Lakshmanan MR, Jungalwala FB, Cama HR. Biological activity of epoxides of vitamin A. Indian J Biochem **7**:102–103, 1970.
46. Zile MH, Inhorn RC, DeLuca HF. The biological activity of 5,6-epoxyretinoic acid. J Nutr **110**:2225–2230, 1980.
47. Frolik CA, Roberts AB, Tavela TE, Roller PP, Newton DL. Isolation and identification of 4-hydroxy- and 4-oxoretinoic acid. In vitro metabolites of all-trans-retinoic acid in hamster trachea and liver. Biochemistry **18**:2092–2097, 1979.
48. Frolik CA, Roller PO, Roberts AB, Sporn MB. In vitro and in vivo metabolism of all-trans- and 13-cis-retinoic acid in hamsters. J Biol Chem **225**:8057–8062, 1980.
49. Zile MH, Schnoes HK, DeLuca HF. Characterization of retinoyl-β-glucuronide as a minor metabolite of retinoic acid in bile. Proc Nat Acad Sci USA **77**:3230–3233, 1980.
50. Swanson BN, Frolik CA, Zaharevitz DW, Roller PP, Sporn MB. Dose-dependent kinetics of all-trans retinoic acid in rats. Biochem Pharmacol **30**:107–113, 1981.
51. Zile MH, Inhorn RC, DeLuca HF. Metabolites of all-trans-retinoic acid in bile: Identification of all-trans- and 13-cis-retinoyl glucuronides. J Biol Chem **257**:3537–3543, 1982.
52. Nath K, Olson JA. Natural occurrence and biological activity of vitamin A derivatives in rat bile. J Nutr **93**:461–469, 1967.
53. Lippel K, Olson JA. Origin of some derivatives of retinoic acid found in bile. J Lipid Res **9**:580–586, 1968.
54. Olson JA. Some aspects of vitamin A metabolism. In: Harris RS, Wool IG, Loraine JA, eds. Vitamins and Hormones. New York, Academic Press, Vol 26:pp1–63, 1968.
55. Zile M, Emerick RJ, DeLuca HF. Identification of 13-cis retinoic acid in tissue extracts and its biological activity in rats. Biochim Biophys Acta **141**:639–641, 1967.
56. Braekkan OR, Myklestad H, Njaa LR, Utne F. Vitamin A isomers in the livers of rats and chicks. Nature (London) **186**:312, 1960.
57. Stainer DW, Murray TK, Campbell JA. Isomerization of 11-cis vitamin A in vivo. Canad J Biochem Physiol **38**:1219–1222, 1960.
58. Arnaboldi M, Motto MG, Tsujimoto K, Balogh-Nair V, Nakanishi K. Hydroretinals and hydrorhodopsins. J Amer Chem Soc **101**:7082–7084, 1979.
59. Honig B, Dinur U, Nakanishi K, Balogh-Nair V, Gawinowicz MA, Arnaboldi M, Motto MG. An ex-

ternal point-charge model for wavelength regulation in visual pigments. J Amer Chem Soc **101**:7084–7086, 1979.

60. Oesterhelt D, Stoeckenius W. Rhodopsin-like protein from the purple membrane of *Halobacterium halobium*. Nature New Biol **233**:149–152, 1971.
61. Oesterhelt D. Bacteriorhodopsin as a light-driven proton pump. In: Azzone GS, Klingenberg M, Siliprandi N, eds. Membrane Proteins in Transport and Phosphorylation. Amsterdam, Elsevier, pp78–84, 1974.
62. Schreckenbach T, Walckhoff B, Oesterhelt D. Studies on the retinal-protein interaction in Bacteriorhodopsin. Eur J Biochem **76**:499–511, 1977.
63. Spudich JL, Stoeckenius W. Light-regulated retinal-dependent reversible phosphorylation of *Halobacterium* proteins. J Biol Chem **255**:5501–5503, 1980.
64. Lanyi J. Halorhodopsin—A second retinal pigment in *Halobacterium halobium*. Trends Biochem Sci **6**:60–62, Feb 1981.
65. Parnell JP, Sherman BS. Effect of vitamin A on keratinization in the vitamin A-deficient rat. In: Butcher EO, Sognnaes RF, eds. Fundamentals of Keratinization. Washington, DC, American Association for the Advancement of Science, pp114–131, 1962.
66. Langman J, Welsch GW. Excess vitamin A and development of the cerebral cortex. J Comp Neurol **131**:15, 1967.
67. Kochar DM. Studies on vitamin A-induced teratogenesis: Effects on embryonic mesenchyme and epithelium, and on incorporation of H^3-thymidine. Teratology **1**:299, 1968.
68. Thompson JN. Vitamin A in development of the embryo. Amer J Clin Nutr **22**:1063, 1969.
69. Mellanby EA. A Story of Nutritional Research. Baltimore, Williams & Wilkins, p74, 1950.
70. Hayes KC, Cousins RJ. Vitamin A deficiency and bone growth. I. Altered drift patterns. Calcif Tissue Res **6**:120–132, 1970.
71. Howell JMcC, Thompson JN, Pitt GAJ. Histology of the lesions produced in the reproductive tract of animals fed a diet deficient in vitamin A alcohol but containing vitamin A acid. I. The male rat. J Reprod Fertil **5**:159, 1963.
72. Niazi IA, Saxena S. Relationship between inhibiting influence of vitamin A and developmental stage of regenerating tail in toad tadpoles (*Bufo andersonii*). Indian J Exp Biol **17**:866–868, 1979.
73. Niazi IA, Jangir OP, Sharma KK. Forelimb regeneration at wrist level in adults of Skipper frog *Rana cyanoplyctis* (Schneider) and its improvement by vitamin A treatment. Indian J Exp Biol **17**:435–437, 1979.
74. Maden M. Vitamin A and pattern formation in the regenerating limb. Nature (London) **295**:672–675, 1982.
75. Cooke J. Vitamin A, limb patterns and the search for the positional code. Nature (London) **296**:603–605, 1982.
76. Fell HB, Rinaldini LM. The effects of vitamins A and C on cells and tissues in culture. In: Willman EN, ed. Cells and Tissues in Culture; Methods, Biology and Physiology. New York, Academic Press, Vol I:pp659–699, 1965.
77. Zile MH, Bunge EC, DeLuca HF. On the physiological basis of vitamin A-stimulated growth. J Nutr **109**:1787–1796, 1979.
78. Hof H, Wirsing CH. Anti-infective properties of vitamin A. Z Ernahrungswiss **18**:221–232, 1979.
79. Thunberg T, Ahlborg UG, Johnsson H. Vitamin A (retinol) status in the rat after a single oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch Toxicol **42**:265–274, 1979.
80. Thunberg T, Ahlborg UG, Hakansson H, Krantz C, Monier M. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the hepatic storage of retinol in rats with different dietary supplies of vitamin A (retinol). Arch Toxicol **45**:273–285, 1980.
81. Sato M, Lieber CS. Increased metabolism of retinoic acid after chronic ethanol consumption in rat liver microsomes. Arch Biochem Biophys **213**:557–564, 1982.
82. Tuchweber B, Bhagwan DG, Salas M. Microsomal enzyme inducers and hypervitaminosis A in rats. Arch Pathol Lab Med **100**:100–105, 1976.
83. Backes WL, Krause RF, Canaday WJ. Observations relating phenobarbital induction of cytochrome P-450 to the obligatory destruction or release of vitamin A. Nutr Rep Int **19**:9–14, 1979.
84. Innami S, Nakamura A, Miyazaki M, Nagayama S, Nishide E. Further studies on the reduction of vitamin A content in the livers of rats given polychlorinated biphenyls. J Nutr Sci Vitaminol **22**:409–418, 1976.
85. Kato N, Kato M, Kimura T, Yoshida A. Effect of dietary addition of PCB, DDT or BHT and dietary protein on vitamin A and cholesterol metabolism. Nutr Rep Int **4**:437–445, 1978.
86. Darjono DVM. Vitamin A Status, Polybrominated Biphenyl Toxicosis and Common Bile Duct Hyperplasia. Ph.D. thesis, Michigan State University, 1982.
87. LeFrancois P, Lamblin G, Carles C, Maire G. Variations of some plasma parameters (albumin, prealbumin, retinol binding protein) and urinary parameters during measles in Senegalese children. Ann Nutr Aliment **33**:417–427, 1979.
88. Olson J. Vitamin A. In: Machlin L, ed. Handbook on Vitamins. New York, Marcel Dekker, Chap 1, in press, 1982.
89. Bjelke E. Dietary vitamin A and human lung cancer. Int J Cancer **15**:561–565, 1975.
90. Wald N, Idle M, Boreham J, Bailey A. Low serum-

vitamin-A and subsequent risk of cancer. Preliminary results of a prospective study. Lancet, 813–815, 1980.
91. Zachman RD. The stimulation of RNA synthesis in vivo and in vitro by retinol (vitamin A) in the intestine of vitamin A-deficient rats. Life Sci **6**:2207–2213, 1967.
92. De Luca L, Little EP, Wolf G. Vitamin A and protein synthesis by rat intestinal mucosa. J Biol Chem **244**:701–708, 1969.
93. Johnson BC, Kennedy M, Chiba N. Vitamin A and nuclear RNA synthesis. Amer J Clin Nutr **22**:1048–1058, 1969.
94. Zile M, DeLuca HF. Vitamin A and ribonucleic acid synthesis in rat intestine. Arch Biochem Biophys **140**:210–214, 1970.
95. De Luca L, Wolf G. Mechanism of action of vitamin A in differentiation of mucus-secreting epithelia. J Agr Food Chem **20**:474–476, 1972.
96. Blalock JE, Gifford GE. Retinoic acid (vitamin A acid) induced transcriptional control of interferon production. Proc Nat Acad Sci USA **74**:5382–5386, 1977.
97. Fuchs E, Green H. Multiple keratins of cultured human epidermal cells are translated from different mRNA molecules. Cell **17**:573–582, 1979.
98. Fuchs E, Green H. Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. Cell **25**:617–625, 1981.
99. Jensen EV, DeSombre ER. Estrogen–receptor interaction. Science **182**:126–134, 1973.
100. O'Malley BW, Means AR. Female steroid hormones and target cell nuclei. Science **183**:610–620, 1974.
101. Chytil F, Omori M, Laiu G, Ong DE. Functions of vitamin A in differentiation. Fed Proc **41**:861, 1982 (Abstract).
102. Bridges CDB. Function of vitamin A in vision. Fed Proc **41**:860, 1982 (Abstract).
103. Adachi N, Smith JE, Sklan D, Goodman DS. Radioimmunoassay studies of the tissue distribution and subcellular localization of cellular retinol-binding protein in rat. J Biol Chem **256**:9471–9476, 1981.
104. Ross AC, Takahashi YI, Goodman DS. The binding protein for retinol from rat testes cytosol. Isolation and partial characterization. J Biol Chem **253**:6591–6598, 1978.
105. Ong DE, Chytil F. Immunochemical comparisons of vitamin A binding proteins of rat. J Biol Chem **254**:8733–8735, 1979.
106. Ong DE, Chytil F. Immunochemical studies on cellular vitamin A binding proteins. Ann NY Acad Sci **359**:415–417, 1981.
107. Conrad DH, Wirtz GH. Radioimmunoassay for vitamin A. Fed Proc **31**:685, 1972 (Abstract).
108. Westfall SS, Wirtz GH. Vitamin A antibodies: Application to radioimmunoassay. Experientia **36**:1351–1352, 1980.
109. Bolmer SD, Wolf G. An extra-nuclear function of retinoic acid. Fed Proc **41**:740, 1982 (Abstract).
110. Hayes KC. On the pathophysiology of vitamin A deficiency. Nutr Rev **29**:3–6, 1971.
111. Zile MH, Bunge EC, DeLuca HF. Effect of vitamin A deficiency on intestinal cell proliferation in the rat. J Nutr **107**:552–560, 1977.
112. Bieri JG, Mason KE, Prival EL. Testis requirement for vitamin A in the rat and the effect of essential fatty acids. Int J Vitam Res **38**:312–319, 1968.
113. Miller SA. Nutrition in the neonatal development of protein metabolism. Fed Proc **29**:1497–1502, 1970.
114. Zile MH, Bunge EC, DeLuca HF. DNA labeling of rat epithelial tissues in vitamin A deficiency. J Nutr **111**:777–788, 1981.
115. Sherman BS. The effect of vitamin A on epithelial mitosis in vitro and in vivo. J Invest Dermatol **37**:469–480, 1961.
116. Takahashi YI, Smith JE, Winick M, Goodman DS. Vitamin A deficiency and fetal growth and development in the rat. J Nutr **105**:1299–1310, 1975.
117. Jayaram M, Sarada K, Ganguly J. Effect of depletion of vitamin A, followed by supplementation with retinyl acetate or retinoic acid, on regeneration of rat liver. Biochem J **146**:501–504, 1975.
118. Chopra DP. Induction and retinoid reversal of squamous metaplasia in organ cultures of vitamin A-deficient hamster trachea: Cytokinetic and ultrastructural changes. AACR Abstract 343, 1982.
119. McDowell EM, Keenan KP. Restoration of mucociliary epithelium in tracheas of vitamin A-deficient hamsters. Fed Proc **41**:622, 1982 (Abstract).
120. Bieri JG, McDaniel EG, Rogers WE Jr. Survival of germ free rats without vitamin A. Science **163**:574–575, 1969.
121. Rogers WE Jr, Bieri JG, McDaniel EG. Vitamin A deficiency in the germ-free state. Fed Proc **30**:1773–1778, 1971.
122. Gilbert HR, Hart GH. Minimum vitamin A requirements with particular reference to cattle. J Nutr **10**:409–427, 1935.
123. Paul HE, Paul MF. The relation of vitamin A intake to length of life, growth, tooth structure and eye condition. J Nutr **31**:67–78, 1946.
124. Brown EF, Morgan AF. The effect of vitamin A deficiency upon the nitrogen metabolism of the rat. J Nutr **35**:425–438, 1948.
125. Rechcigl M Jr, Berger S, Loosli JK, Williams HH. Dietary protein and utilization of vitamin A. J Nutr **76**:435–440, 1962.
126. Corey JE, Hayes KC. Cerebrospinal fluid pressure, growth and hematology in relation to retinol status of the rat in acute vitamin A deficiency. J Nutr **102**:1585–1594, 1972.

127. Evans HM, Bishop KS. On an invariable and characteristic disturbance of reproductive function in animals reared on a diet poor in fat soluble vitamin A. Anat Rec **23**:17, 1922.
128. Appling DR, Chytil F. Evidence of a role for retinoic acid (vitamin A-acid) in the maintenance of testosterone production in male rats. Endocrinology **108**:2120–2123, 1981.
129. Thompson JN, Howell JMcC, Pitt GAJ, McLaughlin EI. The biological activity of retinoic acid in the domestic fowl and the effects of vitamin A deficiency on the chick embryo. Brit J Nutr **23**:471–490, 1969.
130. Fidge NH, Shiratori T, Ganguly J, Goodman, DS. Pathways of absorption of retinal and retinoic acid in the rat. J Lipid Res **9**:103–109, 1968.
131. Das RC, Sarada K, Murthy SK, Ganguly J. Isolation of a receptor for retinol from the oviduct magnum of laying hens. Indian J Biochem Biophys **15**:251–254, 1978.
132. Mehta G, Moon RC. Hormonal regulation of retinoic acid binding proteins in the mammary gland. Biochem J **200**:591–595, 1981.
133. Lucy JA. Some possible roles for vitamin A in membranes: Micelle formation and electron transfer. Amer J Clin Nutr **22**:1033–1044, 1969.
134. Fell HB, Dingle JT. Studies on the mode of action of excess vitamin A. 6. Lysosomal protease and the degradation of cartilage matrix. Biochem J **87**:403, 1967.
135. Pitt GAJ. Vitamin A. In: Isler O, ed. Carotenoids. Basel and Stuttgart, Birkhauser Verlag, pp718–741, 1971.
136. Goodall AH, Fisher D, Lucy JA. Cell fusion, haemolysis and mitochondrial swelling induced by retinol and derivatives. Biochim Biophys Acta **595**:9–14, 1980.
137. Dingle JT, Fell HB, Goodman DS. The effect of retinol and of retinol binding protein on embryonic skeletal tissue in organ culture. J Cell Sci **11**:393, 1972.
138. Roels OA, Anderson OR, Lui NST, Shah DO, Trout ME. Vitamin A and membranes. Amer J Clin Nutr **22**:1020–1032, 1969.
139. Adhikari HR, Mullick RS, Vakil UK, Screenivasan A. Alterations in structure and function relationship in cellular and subcellular membranes in vitamin A-deficient rat. World Rev Nutr Diet **31**:107–113, 1978.
140. Elias PM, Friend DS. Vitamin A-induced mucous metaplasia. An in vitro system for modulating tight and gap junction differentiation. J Cell Biol **68**:173–188, 1976.
141. Dion LD, Blalock JE, Gifford GE. Retinoic acid and the restoration of anchorage dependent growth to transformed mammalian cells. Exp Cell Res **17**:15–22, 1978.
142. Adamo S, De Luca LM, Akalovsky I, Bhat PV. Retinoid-induced adhesion in cultured transformed mouse fibroblasts. J Nat Cancer Inst **62**:1473–1478, 1979.
143. Yaar M, Stanley JR, Katz SI. Retinoic acid delays the terminal differentiation of keratinocytes in suspension culture. J Invest Dermatol **76**:363–366, 1981.
144. Meeks RG, Zaharevitz D, Chen RF. Membrane effects of retinoids: Possible correlation with toxicity. Arch Biochem Biophys **207**:141–147, 1981.
145. De Luca LM, Bhat PV, Sasak W, Adamo S. Biosynthesis of phosphoryl and glycosyl phosphoryl derivatives of vitamin A in biological membranes. Fed Proc **38**:2535–2539, 1979.
146. Jones G. The Intracellular Distribution of Vitamin A in Rat Liver. Ph.D. thesis. Univ of Liverpool, 1971.
147. Berman ER, Segal N, Feeney L. Subcellular distribution of free and esterified forms of vitamin A in the pigment epithelium of the retina and in liver. Biochim Biophys Acta **572**:167–177, 1979.
148. Roseman S. The synthesis of complex carbohydrates by multiglycosyl transferase systems and their potential function in intracellular adhesion. Chem Phys Lipids **5**:270–297, 1970.
149. Roth S. A molecular model for cell interactions. Annu Rev Biol **48**:541, 1973.
150. Ashwell G, Morell AG. Membrane glycoproteins and recognition phenomena. Trends Biochem Sci **2**:76–78, 1977.
151. Weiser MM. Intestinal epithelial cell surface membrane glycoprotein synthesis. II. Glycosyltransferases and endogenous acceptors of the undifferentiated cell surface membrane. J Biol Chem **218**:2542–2548, 1973.
152. Hassell JR, Pennypecker JP, Yamada KM, Pratt RM. Changes in cell surface proteins during normal and vitamin A-inhibited chondrogenesis *in vitro*. Ann NY Acad Sci **312**:406–409, 1978.
153. Pennypacker JP, Lewis CA, Hassell JR. Altered proteoglycan metabolism in mouse limb mesenchyme cell cultures treated with vitamin A. Arch Biochem Biophys **186**:351–358, 1978.
154. Gallandre F, Kistler A. Inhibition and reversion of chondrogenesis by retinoic acid in rat limb bud cell cultures. Wilhelm Roux's Arch fur Entwicklungsmech Org **189**:25–33, 1980.
155. Kwasigroch TE, Kochhar DM. Vitamin A alters the internal viscosity of fragments of limb-bud mesenchyme. J Embryol Exp Morphol **59**:325–339, 1980.
156. Futterman S, Rollins M. The catalytic isomerization of all-*trans*-retinal to 9-*cis*-retinal and 13-*cis*-retinal. J Biol Chem **248**:7773–7779, 1973.
157. Sommer A. Nutritional blindness: Xerophthalmia and keratomalacia. Oxford, Oxford Univ Press, 1982.
158. Chader GJ. Retinoids in ocular tissues: Binding proteins, transport, and mechanism of action. In: McDivitt D, ed. Cell Biology of the Eye. New York, Academic Press, pp377–433, 1982.

Received July 23, 1982. P.S.E.B.M. 1983, Vol. 172.