ABSTRACT

M.Sc. Audrey Sharon Payne Animal Science

EFFECT OF VITAMIN A ON GROWTH AND REPRODUCTION OF RABBITS

Studies were undertaken to determine the minimum levels of vitamin A required in the diet for growth and reproduction in rabbits. Rations designed to provide vitamin A at levels of 0, 3, 6 and 12 micrograms per kilogram of body weight per day ($\mu g/W_{kg}/day$) were fed to 12 male and 12 female weanling rabbits and to eight mature males. Since feed consumption for all groups of weanling rabbits was greater than anticipated, the levels of vitamin A were slightly higher than expected. Overall growth performance and well-being of the weanling rabbits improved as the dietary vitamin A level increased. Six $\mu g/W_{kg}/day$ was required to sustain growth, in the absence of stress.

Vitamin A appeared to be more critical for the initiation than for the maintenance of spermatogenesis. The minimum level needed to satisfy both requirements appeared to lie close to 6 μg. The presence of pathological abnormalities in the offspring of dams that had received the highest level of vitamin A since weaning suggested that a vitamin A intake slightly in excess of 12 $\mu g/W_{kg}/day$ was inadequate to support satisfactory reproductive performance in breeding females.

EFFECT OF VITAMIN A ON GROWTH AND REPRODUCTION OF RABBITS

by

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I. INTRODUCTION

Pathological manifestations of avitaminosis A were reported long before the factor responsible for the deficiency was isolated and identified as vitamin A per se. Blindness associated with a scarcity of forage was reported in donkeys in early Biblical times--"their eyes did fail, because there was no grass" (Jeremiah 14: 6). Presumably the blindness resulted from lack of the vitamin A precursor, carotene, which would normally have been supplied by the forage. However, the identification of vitamin A as the factor responsible for the deficiency was not demonstrated until the early part of the present century.

Clinical manifestations of avitaminosis A vary but growth suppression, epithelial tissue keratinization and vision impairment are probably the best known. Subclinical lesions are less clearcut and hence more difficult to detect.

No systematic investigation has thus far been specifically devoted to the quantitative determination of the minimum level of vitamin A necessary to prevent deficiency symptoms and to permit normal growth and reproduction in rabbits of both sexes. Failure to establish definite vitamin A requirements for rabbits appears to be a serious omission

when one considers that rabbit rearing for meat production is rapidly developing into an important commercial industry. Under commercial conditions, four to five litters per year can be produced from a single breeding doe, whose efficiency is reflected in the number of pounds of rabbit meat marketed per litter. Large litters are obviously imperative if does are to attain their maximum meat producing potential. In addition to their meat producing capacity, certain breeds of rabbits are reared exclusively for pelt or fur production, while others are employed extensively as laboratory animals. These varying uses demonstrate the economic significance of the rabbit and emphasize the need for supplying all nutrients at levels compatible with health and optimum productive performance. The present study was thus undertaken in an attempt to establish the minimal levels of vitamin A that would satisfy the growth, health and reproductive requirements of both male and female rabbits. The four levels of vitamin A used in this study (0, 3, 6 and 12 $\mu g/W_{kg}/day$) were selected so as to provide a range from zero to a level expected to be adequate for growth and reproduction. The overall study was divided into three main experiments, designed to show the effect of vitamin A level on growth and pathology of weanling rabbits, on semen production and on

female reproductive performance, including normal and pathological development of offspring.

II. REVIEW OF LITERATURE

EFFECT OF VITAMIN A ON GROWTH

It has long been recognized that vitamin A is essential for satisfactory growth. As early as 1916, Hart et al. reported that the feeding of a wheat diet had an adverse effect on the growth of swine. The cause of this depression was clarified by Steenbock and Coward (1927) who demonstrated that the vitamin A activity of wheat was practically nil. Confirmation of this diagnosis is given by the fact that wheat contains essentially no carotene (Crampton, 1956). Beta carotene (β-carotene) is the most important of the carotenoid precursors of vitamin A present in green feedstuffs (West and Todd, 1963). These carotenoids are converted to vitamin A in the intestine of the animal by which they are consumed.

Similar growth deceleration resulting from the consumption of vitamin A depleted diets was observed in rats (Brenner et al., 1942; Lewis et al., 1942) and in Syrian hamsters (Salley et al., 1959). Rams fed a low carotene basal diet gained less than comparable males given daily vitamin A supplements (Lindley et al., 1949). However, Eaton et al. (1951) saw no appreciable depression of gain

in calves fed on a vitamin A deficient regime even when plasma vitamin A fell to less than four micrograms per cent. Nelson et al. (1962) attributed response inconsistencies of this type to differences in severity of vitamin A deficiency as measured by the presence or absence of clinical manifestations. The validity of this theory was challenged by the work of Blakemore et al. (1957) who observed that out of twelve calves studied, six normal and six deficient, the greatest weight increase was for one calf that exhibited distinct clinical deficiency symptoms.

Results reported on avitaminosis A in rabbits agreed unanimously that vitamin A deficiency was detrimental to growth (Phillips and Bohstedt, 1938; Perlman and Williard, 1941; Mann et al., 1946; Pirie and Wood, 1946; and others). The investigations by Mann et al. (1946) and by Pirie and Wood (1946) suggested that weight loss was the initial symptom of deficiency in the rabbit.

Considerable variation in the interval of time between the introduction of vitamin A deficient diets and either inhibition of growth or onset of ocular lesions was observed in the literature (61 days, Nelson and Lamb, 1920; 2 - 5 months, Hetler, 1934; 3 - 6 months, Mellanby, 1935; 70 - 100 days, Rao, 1936; 3 - 3½ months, Phillips and Bohstedt, 1938; 4 - 8 months, Perlman and Williard, 1941; about 2 months, Mann et al., 1946; 2 - 4 months, Pirie and

Wood, 1946). These variations were probably influenced by differences in breed of rabbit, age at initiation of treatment, previous vitamin A intake, as well as nature and purity of the deficient diets fed.

Appetite depression has been described by Braman et al. (1935) as the "most prominent early stage effect of vitamin A deficiency" in rats. Inappetence of varying severity has also been observed in other avitaminotic species including cattle (Eaton et al., 1951; Blakemore et al., 1957; Spratling et al., 1965), sheep (Lindley et al., 1949; Sosa, 1965) and rabbits (Nelson and Lamb, 1920; Mann et al., 1946). Since growth rate largely depended upon quantitative feed consumption, Orr and Richards (1934) speculated that growth depression in vitamin A deficiency was the result of pathological lesions that depressed appetite rather than of any direct "growth promotion or inhibition effects." The technique of paired feeding had previously been employed by Sampson and Korenchevsky (1932a) in an attempt to evaluate this proposal. Daily feed intake of the control member of a given rat pair was adjusted to equal the amount consumed on the previous day by its vitamin A deprived counterpart. Differences in growth rate were then directly attributable to vitamin A content, unconfounded by consumption rate variations. They estimated that 40 per cent of the weight loss

incurred in vitamin A deficiency was due to appetite depression while the remaining 60 per cent could be directly linked to the vitamin A content of the diet. Growth per unit of intake was thus lower on the depraved ration, even when total feed consumptions for the two diets were identical. This conclusion was substantiated by Braman et al. (1935) who recorded total gains of 133.49 and 122.60 grams for pair fed rats that consumed 642.0 and 643.3 grams of yellow and white corn, respectively. Morrison (1956) estimated that the vitamin A activity per pound of yellow dent corn was in excess of 2,000 international units while that of white dent corn was essentially nil. Braman et al. (1935) confirmed their initial results when they fed rats equicaloric amounts of two rations, one moderately vitamin A deficient and the other carotene-supplemented. In a later study, Mayer and Krehl (1948) cited decreased feed efficiency as the earliest reliable indication of avitaminosis A in rats. Males generally showed this decrease before females. Muelder and Kelly (1941) were of the opinion that observed weight gains in rats were more influenced by caloric intake than by level of administered vitamin A. They furthermore demonstrated that the proportion of weight increase attributable to caloric intake rose as the vitamin A content increased. Their study was not strictly comparable with that of Sampson and

Korenchevsky (1932a) since rations fed to the members of the controlled rat triads in the former study yielded low levels of vitamin A (1, 3 or 6 USP XI units per day) while rations fed in the latter study were presumably closer to being truly vitamin A deficient. Comparable quantitative studies have not been reported on the relative significance of inappetence in the growth depression of vitamin A deficient rabbits.

Various studies (Brenner et al., 1942; Paul and Paul 1946; Lindley et al., 1949; and others) have demonstrated the enhancement of growth by the addition of vitamin A increments to the deficient basal diet, although there have also been occasional failures to obtain differential growth response at varying levels of vitamin A supplementation, such as that reported by Nelson et al. (1962) in swine fed vitamin A at levels of 2, 4, 6 or 12 micrograms per pound of liveweight daily. Reifman et al. (1943) found that the rate of vitamin A absorption from the gastro-intestinal tract of rats was directly proportional to the concentration of the vitamin administered. Total vitamin A absorbed, as a percentage of total vitamin A fed, declined with increased concentration. Coward et al. (1931) proposed that mean growth response, in terms of weight increase, was proportional to

the level of vitamin A activity in the cod liver oil administered, provided growth was not restricted by other nutrient inadequacies. The validity of this proposal was demonstrated in the rat by Sherman and Batchelder (1931), Lewis et al. (1942) and Paul and Paul (1946). From studies with poultry, Almquist (1953) deduced that a linear relationship existed between the logarithm of the vitamin A intake level and the corresponding growth response, except where the dosage levels were very minute. If this relationship also exists in mammals, it could perhaps explain the failure of Lewis et al. (1942) to demonstrate any further enhancement of weight gain in rats at daily vitamin A intakes above 25 I.U. This would appear to indicate that the maximal physiological limit of response had been attained at the 25 I.U. level. Caution must be exercised in making interpretations of this type since the presence of a particular cause-effect relationship in poultry does not always guarantee its applicability in mammalian species.

With respect to variations among individual animals on a particular dietary regime, Coward (1932) reported that standard deviations for weight increases were similar on vitamin A deficient and normal diets. The magnitude of mean weight increases and of corresponding vitamin A doses

did not affect the range of accuracy but more females than males were required to obtain equal accuracy when growth was the parameter considered. Williams and Pelton (1966) formulated a theory of "biochemical individuality" to explain the wide within treatment variations observed in weight gain, lifespan and time of appearance of pathological lesions in rats fed 0, 1, 8 or 64 I.U. of vitamin A daily. They have suggested that differential response of individual animals to the same treatment may be due to individual differences in absorption, storage or retention of the vitamin. Sherman and Batchelder (1931) noticed that weight losses for groups of rats on low vitamin A levels were subject to more individual variation than were weight losses for comparable groups of rats on deficient vitamin A levels.

Frape et al. (1959) observed that thyroxine secretion in young pigs was depressed when rations deficient in vitamin A were consumed. A similar reduction in thyroid secretion rate was observed in pigs fed very high levels of vitamin A (more than 1,600 I.U. per pound of feed). The possibility was entertained that differential growth response to varying levels of vitamin A could be explained on the basis of alterations in thyroid secretion rates. The observed reduction in thyroxine secretion in vitamin A deficient pigs was consistent with the hyperplasia of the

thyroid gland reported by Jungherr et al. (1950) in dairy bulls and with the hypertrophy and atrophy of the thyroids noted by Coplan and Sampson (1935) in female and male rats, respectively. Turner (1955) and Smith and Jones (1957) have discussed the thyroxine-thyrotropin feedback mechanism responsible for the maintenance of normal thyroid function. Their comments verified that hyperplasia, hypertrophy and atrophy could all result from the inability of the thyroid gland to increase its thyroxine output in response to elevated thyrotropic stimulation. Size and number of thyroid secretory cells were increased in an unsuccessful attempt to compensate for the deficient activity of individual cells. However, results of the study by Frape et al. (1959) indicated a poor relationship between the rate of thyroid secretion and growth. It therefore appeared unlikely that the effect of vitamin A on growth was mediated through alterations in thyroidal secretion rates. Johnson and Baumann (1948) concluded that vitamin A utilization in the rat depended less on the thyroid-regulated basal metabolic rate than on growth rate. Growth rate would clearly be reflected in body weight. Confirmation of this observation in other species led to the acceptance of body weight as the basis for computation of mammalian vitamin A requirements (Guilbert

and Loosli, 1951). Guilbert et al. (1940) suggested that 4-6 micrograms of vitamin A per kilogram of body weight per day ($\mu g/W_{kg}/day$) was the minimum requirement for normal growth and well-being in most species. This level permitted little or no storage of vitamin A. A threefold increase in the minimal vitamin A level was required for satisfactory reproduction and significant storage to occur. In their textbook, Crampton and Lloyd (1959) quoted 4 $\mu g/W_{kg}/day$ as the minimal vitamin A requirement for growth with 12 $\mu g/W_{kg}/day$ being required for storage and reproduction in most mammals.

In the rabbit, as in other species, mature body weight was dependent upon growth rate in earlier life. Casady (1961) calculated growth rates for a large population of New Zealand White rabbits at four different stages in the overall growth period - birth to 3 weeks, 3 to 8 weeks, 8 to 14 weeks and 14 weeks to 5 months. Rabbits in this study were weaned onto a stock colony diet at eight weeks of age. Recorded daily rates of gain for the four stages of growth were 15.1, 41.5, 33.2 and 16.7 grams, respectively. An average daily gain of 36.5 grams was observed by Macartney (1966) in a group of four rabbits fed a ration containing 40 per cent dehydrated alfalfa meal. This value was based on rabbit growth in the four week period from weaning to

eight weeks of age. The N.A.S.-N.R.C. (1966) considered 31.8 grams (.07 lb) to be the average daily gain for growing rabbits that weighed 4 to 7 pounds.

Although specific vitamin A and carotene requirements based on body weight have been ascertained for most other mammalian species, no definite recommendations have been made as to the quantitative needs of the rabbit for vitamin A (Aitken and Wilson, 1962; Branion, 1966; N.A.S.-N.R.C., 1966).

Coward (1953) suggested that a vitamin A level of 4 $\mu g/W_{kg}/day$ or a carotene level of 25 $\mu g/W_{kg}/day$ should theoretically satisfy the vitamin A requirements of the rabbit since these levels were adequate for most other classes of livestock. Her theoretical estimate was no doubt based upon the report of Guilbert et al. (1940) that 4-6 $\mu g/W_{kg}/day$ was the minimum vitamin A requirement for growth in most mammalian species. Phillips and Bohstedt (1938) found that a carotene level of 50 $\mu g/W_{kg}/day$ fed in the rabbit ration prevented deficiency lesions and permitted normal growth and reproduction while a level of 30 $\mu g/W_{kg}/day$ was inadequate to even sustain growth. The lactation period was not studied. As in the other mammalian species observed by Guilbert et al. (1940), reproductive requirements for vitamin A in

rabbits were probably higher than the needs for growth.

The vitamin A requirements of rabbits for growth, reproduction and lactation were apparently satisfied by feeding a purified diet that provided 12,000 I.U. of vitamin A per kilogram of ration (Hogan and Hamilton, 1942). The relatively high vitamin A content of this ration suggests that the level used was probably in excess of the minimum required for satisfactory growth and reproductive performance. Wooley and Sebrell (1945) observed nearly normal growth in rabbits fed for a limited period of time on a purified diet that contained 6,525 U.S.P. units of vitamin A (19,575 U.S.P. units of carotene) per kilogram of ration. Mellanby (1935) reported that 1-3 mg of carotene fed daily prevented deficiency lesions in rabbits. Once pathological deficiency symptoms appeared, immense therapeutic doses of vitamin A were required to restore growth and correct the anomalies (Phillips and Bohstedt, 1938; Mann et al., 1946; Pirie and Wood, 1946). No systematic experiment, designed specifically to determine the quantitative requirements of the rabbit for vitamin A during growth, maintenance or reproduction, has been reported in the literature surveyed.

The satisfaction of these unspecified requirements for vitamin A has warranted some consideration, however. Sandford (1957) expressed doubt that vitamin A deficiency would occur

in rabbits fed normal rations that contained green feedstuffs. Sandford's concept is probably valid, provided the green feedstuffs used are as high in carotene content as the good quality alfalfa described by Aitken and Wilson (1962). The latter two workers estimated that a ration consisting of alfalfa hay, with or without alfalfa meal, would provide a 3 kilogram rabbit with 7,700 to 9,600 µg carotene. A winter ration containing clover hay, with or without timothy hay, and a little maize meal, with or without a little alfalfa meal, was calculated to supply about 2,900 to 3,900 µg carotene to a 2.5-3 kilogram rabbit. It is obvious that the alfalfa ration provides more than twice as much carotene as the other ration. Sun curing of forages resulted in severe losses of carotene through oxidation (Seshan and Sen, 1942). Heat, light and moisture accelerated the destruction. Rabbits ingested less forage when its quality was poor (Aitken and Wilson, 1962). With commercial rations, laboratory-determined vitamin A contents were sometimes less than those claimed by the manufacturer (Slanetz, 1943). In addition, vitamin A losses of up to 32 per cent were reportedly incurred during the pelleting of commercial rations (Bierer and Vickers, 1958).

PATHOLOGY OF VITAMIN A DEFICIENCY

In addition to growth depression, a host of other pathological symptoms have been attributed to or linked with vitamin A deficiency. From their survey of the literature, Jubb and Kennedy (1963) inferred that vitamin A was intimately involved in five main bodily processes - vision, embryogenesis, osteogenesis, neurological well-being, and maintenance of epithelial integrity. They used this arbitrary classification as a basis for their discussion of pathological lesions associated with avitaminosis A.

A massive volume of literature has accumulated on the pathological symptomatology of vitamin A deficiency in individual species. O'Donoghue (1954) has summarized the established signs of avitaminosis A in cattle, as reported by various workers from both clinical and experimental studies. Gross symptoms of deficiency included night blindness (nyctalopia), papilledema, lacrymation, corneal opacity, keratitis, syncope, incoordination, nervous derangement, increased cerebrospinal fluid pressure (CSFP) and increased susceptibility to infection, particularly of digestive and respiratory tracts. In addition to these, inappetence, apathy, pityriasis, dull coat, poor heat tolerance (Spratling et al., 1965), convulsions (Moore et al., 1935;

Jungherr et al., 1950; Spratling et al., 1965), anasarca (Creech and Seibold, 1943; Madsen et al., 1947), skin eruptions (Alvarez, 1947) and increased urinary output (Woelfel et al., 1965) were observed. Bleaching or mottling of the tapetum lucidum and tapetum nigrum (Moore, 1939, 1941), swelling and hemorrhaging of the optic disc (Spratling and Bridge, 1966), exophthalamus (Moore, 1939; Jungherr et al., 1950; Eaton et al., 1951), corneal rupture and iris prolapse (Alvarez, 1947) and blindness due to optic nerve constriction (Moore et al., 1935; Blakemore et al., 1957) were also reported. In most instances these observations were confirmed by other workers. Gross and histological post-mortem examination of organs, other than eyes, showed squamous metaplasia of parotid glands (Jungherr et al., 1950; Eaton et al., 1951; Helmboldt et al., 1953; Nielsen et al., 1966a), cystic pituitaries (Madsen et al., 1942; Spratling et al., 1965), vascular alterations (Creech and Seibold, 1943), focal necrotic hepatitis (Jungherr et al., 1950; Helmboldt et al., 1953; Spratling et al., 1965), nephritis (Langham et al., 1941; Spratling et al., 1965), keratinization of prepuce, reticulum and rumen (Nielsen et al., 1966b) and pulmonary infections (Spratling et al., 1965) to be the pathological lesions most frequently encountered

in cases of hypovitaminosis A. A subject of great controversy has been the possibility of an existing relationship between vitamin A deficiency and urinary calculus formation. According to studies by Swingle and Marsh (1956), vitamin A deficiency could be a predisposing factor in the development of urolithiasis, but not the direct cause.

Staggery gait, incoordination, abnormal head attitude (O'Donoghue, 1954), bilateral corneal opacity and related ocular defects (Lucas et al., 1955) observed in sheep were attributed to a lack of vitamin A. Eveleth et al. (1949) reported that excitability, pica and lordosis were also characteristic symptoms of vitamin A deficiency in sheep. In swine, vitamin A inadequacy was defined by a similar variety of symptoms - nyctalopia, incoordination, posterior paresis with subsequent partial paralysis, lordosis, spasms, seborrhea, increased cerebrospinal fluid pressure, reduced plasma vitamin A and a tendency to carry the head tilted to one side as a result of inner ear infection (Hentges et al., 1952). Constriction and degeneration of optic nerves were noted. Deaths due to pneumonia were also discernible. Additional symptoms reported by Elder (1935) in an earlier pig study included nervousness, blindness, dermititis, intense itching, diarrhoea, emasciation,

lowered disease resistance and myelin degeneration of spinal chord and sciatic nerves.

Heaton et al. (1955) listed defective scotopic vision, retarded growth, hyperkeratosis (including xerophthalmia), skeletal abnormalities and disturbed sexual processes as the accepted indicators of avitaminosis A in rats. Paul and Paul (1946) demonstrated reduced incidence of ocular lesions and increased lifespan when daily dose of vitamin A was increased from 1 to 20 U.S.P. units per 100 grams of body weight. Aberle (1934) described the development of paralysis in vitamin A deficient rats, commencing with clumsiness and incoordination and climaxing in spasms, urinary incontinence and complete loss of neurological control. Vaginal cornification preceded nervous disturbances. Frequently xerophthalmia and weight loss occurred prior to neural malfunction as well. Coward (1935) noted that weight reduction generally occurred before ocular symptoms appeared. Ataxia and incoordination were attributed to the nervous tissue degeneration observed by Aberle (1934) and Wolbach and Bessey (1940). The latter workers suggested that this degeneration was due to pressure resulting from unequal rates of growth in bone and nerve. Vitamin A deficiency theoretically caused cessation of bone growth but did not inhibit nerve development proportionately. The enlarging nerve was

thus constricted with the resultant pressure being detrimental to normal function and integrity of the nerve. However, Mellanby (1941, 1947), from work with dogs, proposed that bone overgrowth (dysplasia) rather than undergrowth was responsible for the detrimental pressure on the nerve. Vitamin A was believed to regulate bone development through its control of osteoclastic and osteoblastic activities. The possibility that vitamin A acted directly on the maintenance of nervous tissue integrity was considered by O'Donoghue (1955). Jubb and Kennedy (1963) have suggested that the neurological lesions resulted from increased production of cerebrospinal fluid rather than from osteogenic alterations. They based this hypothesis on the reported incidence of increased cerebrospinal fluid pressure (CSFP), accompanied by incoordination and papilledema, in mature vitamin A deficient cattle in which skeletal growth had ceased prior to instigation of deficiency treatment. This idea was supported by the work of Woollam and Millen (1956), who observed increased cerebrospinal fluid pressure in chicks fed a low carotene diet, even though neurological deficiency lesions were absent.

Fatty livers, distended bladders and stomachs, as well as infected submaxillary glands were observed in the vitamin

A deficient rats studied by Heaton et al. (1955). These findings were in partial contrast to those obtained earlier by Aberle (1934), who observed no significant lesions in intestine, stomach, liver or salivary glands. He did, however, notice infection of kidneys, bladder, lungs, trachea and basal glands of tongue.

Distinctive vitamin A deficiency symptoms have been established for most of the other common mammalian species including the guinea pig and monkey (Hetler, 1934), the dog (Emmel, 1940) and the fox (Bassett et al., 1946). Ocular and nervous lesions were the predominant characteristics in most instances.

It has already been indicated in the discussion on growth that ataxia, growth depression and ocular lesions were the initial symptoms of avitaminosis A in rabbits. Phillips and Bohstedt (1938) estimated that in about 50 per cent of the observed cases, ataxia and equilibrium loss occurred prior to or concurrently with the ocular lesions. The earliest ocular alteration consisted of corneal epithelium metaplasia with dense plaques of whitish, squamous, keratinized cells forming on the cornea (Mann et al., 1946). The full chronological sequence of eye changes has been outlined by Phillips and Bohstedt (1938). The condition

commenced as a fleeting or persistent ophthalmia accompanied by hyperemia and congestion. An erosive dry keratitis followed; it gradually progressed from Bitot's spots to corneal opacity and terminated in blindness, without stenosis of the optic foramen. The fact that optic foramen constriction occurred in vitamin A deficient cattle (Moore et al., 1935; Blakemore et al., 1957) but not in vitamin A deficient rabbits was attributed by Phillips and Bohstedt (1938) to differences in the skull shapes of the two species. In the interstitial type of keratitis the cornea assumed a ground glass-like appearance. The final ocular condition was described by Hetler (1934) as an advanced xerosis of corneal epithelium, bulbar conjuctiva and palpebral conjunctiva and by Mann et al. (1946) as a keratinization of the conjuctiva, especially in the lower fornix. Similar observations of climactic conjunctivitis and blindness in rabbits were made by Safarov and Dzul'faev (1961). Mann et al. (1946) suggested that this relative delay in development of conjunctivitis in the rabbit was explainable on the basis that the conjunctiva was completely covered, and thus protected from the air, by the eyelid. Slight vascularization accompanied conjuctivitis but no ulceration or affection of the substantia propria, nerve fibers or retina was

noted. The smoky appearance of the conjunctiva was due to outward chromatophore migration. A purulent eye discharge (Hetler, 1934) and sluggish light reflexes (Phillips and Bohstedt, 1938) accompanied the structural alterations. Further reports of xerophthalmic development in vitamin A deficient rabbits were presented in the work of Mellanby (1934, 1935) and Pirie and Wood (1946).

Other symptoms of avitaminosis A in rabbits included sneezing, nasal discharge (Hetler, 1934), convulsions and slight temperature elevation (Safarov and Džul'faev, 1961). Stiffness of the hind quarters was noted by Mellanby (1935), while partial paralysis, particularly of front quarters, was observed by Phillips and Bohstedt (1938). Unequal wearing of the molar teeth, salivation and slight scouring were also noted in the deficient rabbits by the latter two workers. Probable deafness was reported by Mellanby (1935) but this observation was not substantiated by the work of Perlman and Williard (1941) who found no deterioration in cochlear function, even though nerve fibers were compressed and stretched by new temporal bone formation. Chemical analyses showed reduced vitamin A levels in blood plasma (Mann et al., 1946; Pirie and Wood, 1946) as well as lowered levels of ascorbic acid in aqueous humor of the eyes (Pirie and Wood, 1946) of vitamin A deficient rabbits.

Post-mortem findings were generally consistent with the symptoms observed prior to death. Mellanby (1934, 1935) detected nerve myelin sheath degeneration, particularly in optic and trigeminal nerves, but was unable to ascertain whether the cell nerve or fiber received the initial damage. Myelin degeneration in the sciatic nerve and brachial plexus was also noted (Rao, 1936; Phillips and Bohstedt, 1938). Rao (1936, 1940) found no evidence, however, that loss of neurotrophic control of the ophthalmic division of the trigeminal nerve was responsible for xerophthalmia as had been suggested by Mellanby in 1934. Although carotene administration proved successful in the therapeutic treatment of xerophthalmia, it was ineffective in rectifying the damage incurred by the peripheral nervous system. Vacuolation and slight hypertrophy of the ependymal cells of the choroid plexus was noted by Phillips and Bohstedt (1938). Compact bone decalcification associated with an absence of osteoclasts was the chief osteological defect noted (Hetler, 1934). This observation was supported by Safarov and Dzul'faev (1961) who also described bone softness as being part of the vitamin A deficiency syndrome in rabbits.

Intestinal flaccidity, pulmonary and hepatic hyperemia, nephritic petechial hemorrhages and small urinary bladder stones were detected in young rabbits that died of

suspected hypovitaminosis A (Safarov and Dzul'faev, 1961). Hepatic congestion and hypertrophy were reported by Phillips and Bohstedt (1938), along with nephritic degenerative changes and spleen involvement. Metaplasia and keratinized metaplasia were noted in the epithelia of the renal pelvis (Phillips and Bohstedt, 1938) and in the epithelia of the nasal sinus and turbinate mucous membranes (Hetler, 1934). These observations, coupled with the earlier reports of respiratory disturbances, gastro-intestinal disorders and ocular epithelial alterations, lent support to the theory advanced by De Ruyter and Rosenthal (1936). Their hypothesis, based on rat studies, proposed that the primary manifestations of vitamin A deficiency arose from atrophy of the epithelia of the eye and mucus membranes. The epithelial linings most frequently affected were those of the respiratory, digestive and urogenital tracts but lymphoid tissues also underwent atrophic changes.

A satisfactory explanation for the mode of action of vitamin A has long been sought. Moore (1957) suggested that on the widest possible basis, vitamin A was necessary for the formation of large glucosamine-containing molecules known as mucopolysaccharides. Since these mucopolysaccharides were important quantitative constituents of mucoids, it was considered possible that vitamin A might exert its

effect on mucus-secreting membranes through alterations in biosynthesis of these molecules. According to the *in vitro* studies of Wolf and Johnson (1960), mucopolysaccharide formation in mucus-secreting epithelium was reduced in vitamin A deficiency but increased with the addition of retinol (the alcohol form of vitamin A). In their paper, Wolf and Johnson included an excellent schematic outline of the probable sequence of steps involved in mucopolysaccharide biosynthesis. This outline is reproduced with slight modifications in Figure 1. The work of Wolf and Johnson suggested that vitamin A could be involved in mucopolysaccharide biosynthesis at the point where sulfate was transferred to the polymer to give the sulfated mucopolysaccharide that represents an important constituent of mucus-secreting epithelium. This hypothesis was confirmed by Sundaresan and Wolf (1963) who reported that the initial step in the formation of 3^1 phosphoadenosine 5^1 phosphosulfate (i.e. the formation of adenosine 5^1-phosphosulfate from adenosine triphosphate and sulfate) was severely depressed under conditions of vitamin A deficiency. Thompson (1965), in reviewing this topic, speculated that metabolites of vitamin A could conceivably serve as co-factors for the sulphurylase enzyme that was responsible for the catalysis of this sulfate activation reaction. Reduction in the rate of sulfate activation

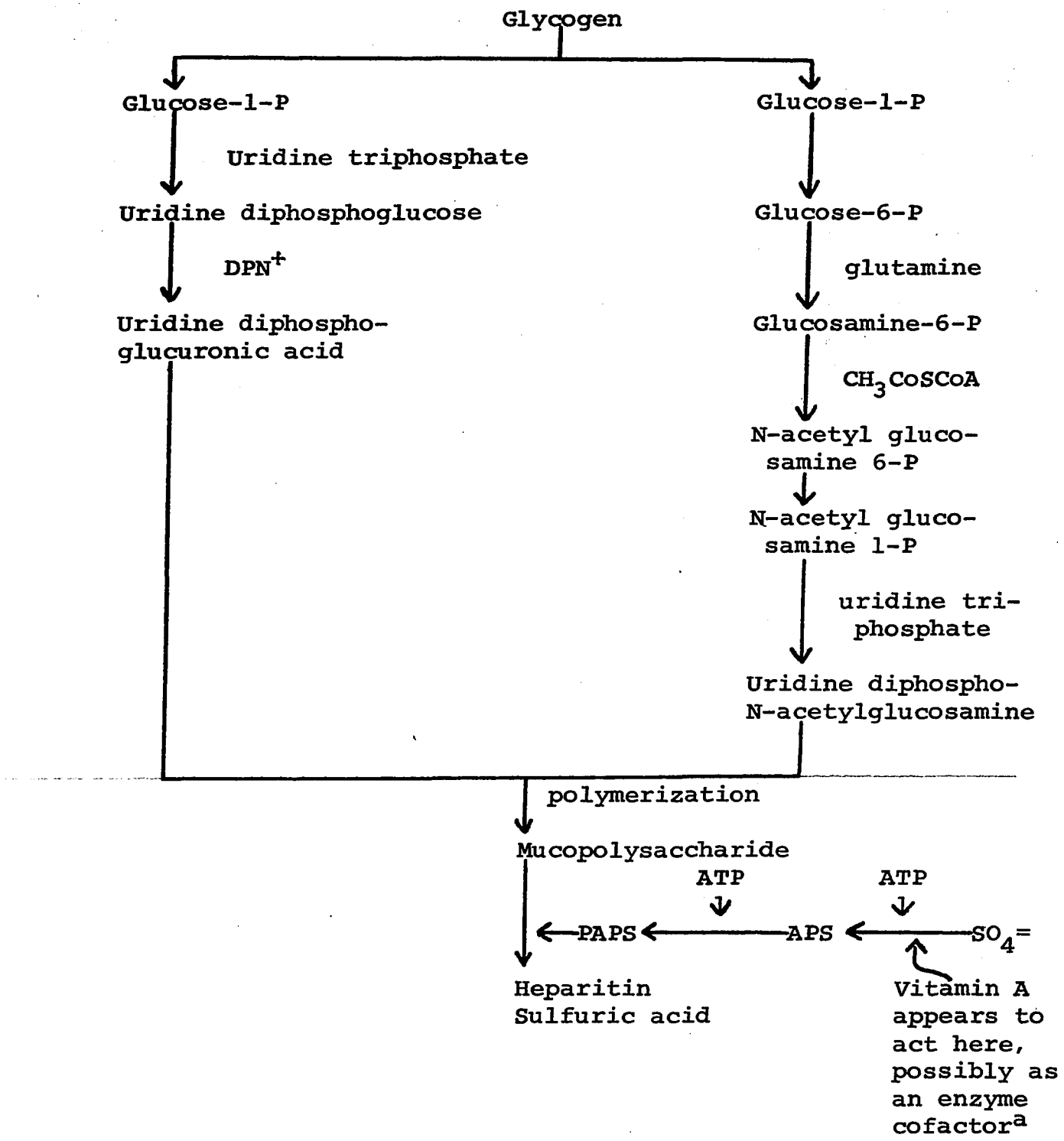

Figure 1. Schematic outline of the possible pathway of mucopolysaccharide biosynthesis, modified from Wolf and Johnson (1960) to show the probable site of action of vitamin A in the synthesis of the sulfated mucopolysaccharide constituents of mucus secreting epithelium.
(See page 28 for Key to Abbreviations).

Key to Abbreviations in Figure 1

P = phosphate
$CH_3CoSCoA$ = acetyl coenzyme A
DPN = diphosphopyridine nucleotide
PAPS = 3^1 phosphoadenosine 5^1-phosphosulfate
APS = adenosine 5^1-phosphosulfate
Heparitin sulfuric acid is the sulfated ester of the mucopolysaccharide.

[a]Based on work of Sundaresan and Wolf (1963) and discussion by Thompson (1965).

could have serious physiological consequences since this reaction plays an important role in the biosynthesis of many sulfur-containing substances, including the sulfated mucopolysaccharides of the epithelium. Degeneration of epithelial tissues could, therefore, have resulted from a decrease in synthesis of sulfated mucopolysaccharides, with this decrease being attributable to absence or inadequacy of the vitamin A cofactor required by the reaction catalysing enzyme.

As early as 1933, Turner and Loew had noted that carotene appeared to protect the upper respiratory tract from bacterial invasion. The recent findings on the mode of action of vitamin A would suggest that carotene acted to maintain the integrity and health of the respiratory epithelial tissues and thus rendered them more resistant to

bacterial invasion. Diehl (1960) reported that coccidia-infected rabbits, with extensive liver damage, had lower hepatic vitamin A reserves than did non-infected rabbits. He cautioned, however, that the poorer vitamin A storage might be an indirect result of lowered hepatic vitamin E. The occurrence of pulmonary and intestinal infections in other vitamin A deficient animals (Elder, 1935; Spratling et al., 1965) can also be explained on the basis of epithelial degeneration, accompanied by increased susceptibility to bacterial and parasitic invasions.

EFFECT OF VITAMIN A ON MALE REPRODUCTION

Only one report was found that dealt with the effect of avitaminosis A on reproduction in male rabbits (Chevrel and Cormier, 1948). They described extensive testicular degeneration in bucks fed a vitamin A deficient diet. The observed lesions were similar to those that accompanied avitaminosis E but the inclusion of 10 to 20 milligrams of tocopherol daily in the diet eliminated any possibility that vitamin E inadequacy could be responsible for the testicular deterioration.

More extensive investigations have been conducted on the effects of avitaminosis A in males of other species.

Guilbert and Hart (1935) reported histopathological lesions in the seminiferous tubules of a bovine that died while vitamin A deficient. Seminal deterioration was noted in a second vitamin A deficient bull but improvement in the semen quality of this male was observed after vitamin A administration, suggesting that the testicular damage incurred during the deficiency was repairable. Later workers (Erb et al., 1944; Hodgson et al., 1946; Madsen et al., 1948) verified the occurrence of histopathological lesions in the testes of vitamin A deficient bulls. In vitamin A deficient dairy calves, spermatogenesis was inhibited by degeneration and necrosis of the germinal epithelium lining the seminiferous tubules (Thorp et al., 1942). Pyknotic and degenerate nuclei were characteristic before epithelial destruction was complete but the interstitial cells remained relatively unaffected. A testicular sample obtained by biopsy from a 17-month old bull that had been fed a deficient ration for four months showed almost complete absence of lumenal spermatozoa (Erb et al., 1944). In a later study with mature A-avitaminotic bulls, Bratton et al. (1948) detected few spermatogonia and only small numbers of spermatocytes and immature spermatids in the seminiferous tubules. Spermatogenesis had not passed the spermatogonial

stage in the majority of young dairy bulls fed a carotene deficient diet for 205 days by Jungherr et al. (1950). While isolated primary and secondary spermatocytes were present, there was no indication of systematic spermatozoan maturation. It was not uncommon, however, to find abnormal and normal tubules almost adjacent to each other in some of the testes studied. Incomplete spermatogenesis was similarly observed in Baladi bulls that had been fed from the age of eight to twelve months on a diet deficient in both carotene and vitamin A (Ghannam et al., 1966). Spermatogonia were not appreciably affected but Leydig cells were detrimentally influenced. A vitamin A level of 30 I.U./W_{kg}/day appeared adequate to permit normal spermatogenetic function, however.

Avitaminosis A incurred at, or prior to, puberty appeared more detrimental to bovine male reproductive performance than did that sustained after the attainment of sexual maturity (Hodgson et al., 1946; Erb et al., 1947). Not only was libido inhibited and spermatogenesis inhibited or reduced as a consequence of the prepubertal vitamin A deficiency, but there was also strong indication that testicular damage might be permanent, particularly when accompanied by pituitary cysts (Erb et al., 1947). In mature bulls rendered A-avitaminotic after the onset of sexual maturity,

however, libido was retained even after physical incoordination hampered or prevented mounting (Bratton et al., 1948). The presence of pituitary cysts, together with histological alterations in the anatomy of the anterior pituitary, were suspected as possible etiological factors that influenced the delay of sexual maturity in prepubertal bulls. Madsen et al. (1948) postulated that the cysts could exert a "partial hypophysectomy" effect by replacing or constricting enough of the glandular parenchyma to cause interference with hypophyseal hormonal function, probably involving interstitial-cell-stimulating and follicle stimulating hormones (ICTH and FSH) described by Wright (1965). A moderate degree of spermatogenesis was noted, however, in calves that developed pituitary cysts following the consumption of rations containing marginal carotene levels (Nielsen et al., 1966b). The pars distalis of the hypophysia thus appeared to be little affected by the cysts at the time of puberty.

Semen abnormalities associated with bovine avitaminosis A have been demonstrated by several investigators. In 1942, Anderson observed that the pH of normal semen was negatively correlated with ejaculate volume, spermatozoan concentration and spermatozoan motility. Increased pH, low spermatozoan concentration, low spermatozoan motility, high per cent abnormal spermatozoa and poor storage quality were the prominent characteristics of the semen

of A-avitaminotic bulls observed by Hodgson et al. (1946). However, poor quality did not completely eliminate the fertility of the semen since a number of cows were successfully impregnated with semen from deficient bulls. Semen improved when carotene or pregnant mare serum was administered to the bulls. The percentage of motile spermatozoa decreased and the percentage of abnormal spermatozoa increased with progressive deficiency in the mature bulls studied by Bratton et al. (1948). Semen volume varied but a decline in spermatozoan concentration was noted in one of the experimental subjects. Erb et al. (1947) fed rations supplying 2,000 I.U. of vitamin A per day to bulls until clinical manifestations appeared; this level was subsequently raised to 4,000 I.U. and then to 6,000 I.U. per day to permit weight gain and life maintenance. As weight increased, the amount of vitamin A fed daily per pound of body weight declined from about 14 I.U. to 9 I.U. Semen collected from these low level bulls after 16 months of age showed lowered spermatozoan motility, spermatozoan concentration and survival time as compared with controls given 100,000 I.U. of vitamin A daily. Libido was relatively good. In a second trial, male calves were fed either 4,000 or 6,000 I.U. of vitamin A daily until their body weights reached 300 lb.

The vitamin A intake level was then reduced to either 16 or 8 I.U. per pound of body weight per day. When the calves on these dietary regimes reached 13 months of age, semen collection was attempted but only one animal produced satisfactory semen. This bull had received the higher (16 I.U./W_{lb}/day) level of vitamin A supplementation. In ejaculates from other males, spermatozoa were scarce or absent and spermatozoan motility was slight. Semen from two vitamin A deficient beef bulls studied by Madsen et al. (1948) exhibited increased proportions of morphologically abnormal spermatozoa, decreased spermatozoan motility and increased amounts of cellular debris. Decreased sexual desire and ability accompanied the seminal changes. Carotene administration improved both semen quality and libido. The enhancement of bovine semen quality with weekly supplemental doses of 500,000 to 1,000,000 I.U. of vitamin A was demonstrated by De Vuyst et al. (1959). Spermatozoan concentration, per cent live spermatozoa and percentage of non-returns to service were increased, methylene blue reduction time was decreased, fructolysis was improved and ejaculate volume remained unaffected. Roussel et al. (1963) observed increased semen volume and spermatozoan concentration in bulls given supplementary stabilized vitamin A at the level of 45,000 I.U. per day. Methylene blue reduction time was decreased as was the

percentage of abnormal spermatozoa but no significant difference was detected in initial spermatozoan motility. It was suggested by Bratton et al. (1948) that in mature A-avitaminotic bulls, physical inability to mount preceded serious seminal deterioration.

Comparable alterations in testicular structure have been demonstrated in experiments with vitamin A deficient male rats. Early stages of testicular atrophy were characterized by the presence of spermatids with scattered chromatin particles, as well as structureless layers of material probably derived from these cells. The only cells remaining when atrophy was completed were derived from sustentacular cells and these frequently were multinucleated (Wolbach and Howe, 1925). Orderly spermatogenesis did not appear to precede beyond the primary spermatocyte phase but detection of abnormal stages suggested that sporadic but ineffective attempts at spermatogenesis had been made (Sutton and Brief, 1939; Mayer and Goddard, 1951). Similarly, no gametes beyond the spermatocyte level were detectable in the tubules of rats fed a diet containing vitamin A acid rather than vitamin A alcohol or aldehyde (Howell et al., 1963). The seminiferous tubules were frequently filled with Sertoli cells. The acid form of vitamin A was obviously not satisfactory for supplying the reproductive requirements,

as judged by degenerative alterations. Gross examination revealed that the testes were small and pinkish. If the rat had been born with some hepatic vitamin A reserves, the testes were frequently oedematous as well. Testicles of vitamin A deficient rats were heavier than those from controls when both were calculated on a per 200 gram body weight basis (Sampson and Korenchevsky, 1932a,b). Absolute testes weights were, however, lower for the deficient animals than would be expected in view of the testicular atrophy detected histologically. The original weight increase was attributed to oedema present in stages prior to extensive atrophy. Macroscopic examination showed the testes to be soft and semi-transparent, as well as oedematous.

Evans (1932) demonstrated that the testicular lesions associated with hypovitaminosis A occurred even in the presence of adequate vitamin E, thus squelching any suggestion that tocopherol deficiency might be the actual underlying cause of the lesions. Damage sustained as a result of the deficit was detectable earlier in vitamin A than in vitamin E deficiency (Mason, 1930). In addition, A avitaminosis-induced degenerative changes were repairable while those sustained as a result of tocopherol lack appeared essentially irrepairable (Everhart, 1958).

Opinions among investigators have differed as to whether the testicular degeneration in vitamin A deficient males is incurred as the direct result of spermatogenetic tissue disturbances or as the indirect result of hormonal or gonadotropic alterations.

The theory that testicular degeneration in vitamin A deficient males could result from hormonal or gonadotropic changes has received some consideration. Hypotheses and controversies surrounding the role of gonadotropins in spermatogenesis have been discussed by Nalbandov (1964). Both FSH (follicle stimulating hormone) and ICTH (interstitial-cell-stimulating hormone, also known as LH or luteinizing hormone) were necessary for spermatogenesis, with the ICTH apparently being required for completion of the process. Uncertainty existed as to whether ICTH per se affected the spermatogenetic process or whether the gonadotropin exerted its effect indirectly through induction of androgen secretion by Leydig cells. Evidence suggested that final spermatogenetic differentiation might be androgen-dependent. Mayer and Truant (1949) conjectured that vitamin A deficiency interrupted the synthesis or release of androgens but not the ability of target organs to respond to them. It was later established (Grangaud et al., 1961) that the conversion

of transdehydroandrosterone to androstenedione was blocked in vitamin A deficient rats. However, further work by Mayer and Goddard (1951) demonstrated that androgens were produced in the testes of vitamin A deficient rats in response to chorionic gonadotropin administration. This finding suggested that defective pituitary function in the production of gonadotropins might be responsible for the androgen inadequacy encountered in avitaminotic A males. When gonadotropins were administered, however, spermatogenesis failed to advance beyond the spermatocyte stage, indicating that factors other than inadequate gonadotropin production were probably involved in the defective testicular function. FSH and testosterone administration did not prevent the occurrence of testicular degeneration in vitamin A acid-fed rats (Coward et al., 1966), possibly due to lack of ICTH per se as discussed by Nalbandov (1964). Gambal (1966) recently proposed that phospholipids may play a functional role in spermatogenesis. Either vitamin A deficiency or FSH-testosterone administration effected a decrease in testicular phospholipids but an increase in total testicular lipids. Gambal further postulated that altered testicular lipid concentrations might be related to abnormal synthesis or secretion of gonadotropic hormones by the pituitary gland, which in turn might be regulated by the vitamin A level.

Work by Palludan (1966) with A-avitaminotic boars, however, indicated that degenerative spermatogenetic changes were not produced indirectly as a result of hormonal alterations but were instead the direct effect of disturbances in the spermatogenetic tissues brought about by lack of vitamin A. Sutton and Brief (1939) had earlier suggested that increased gonadotropic activity in vitamin A deficient male rats resulted from an attempt of the pituitary to compensate for the gonadal damage that was incurred as a direct result of the absence of vitamin A. They noted that the same type of compensatory response occurred in male rats following castration, with the increase in gonadotropic activity being even greater in the latter case. Spermatogenesis was either absent or very limited. Atrophied testes from deficient rams were consistently lighter in weight than normal testicles from supplemented controls. In one study by Dutt (1959), normal testes from supplemented rams weighed 7 to 10 times as much as those from deficient rams. A-avitaminotic rams showed depression and eventual loss of libido. Lindley et al. (1949) reported that lack of libido was slightly more common in deficient rams than in controls but suggested that once these deficient males demonstrated sexual interest, they maintained libido even when the severity of the deficiency prevented them from mounting or even

standing.

Semen from vitamin A deficient rams showed defects similar to those observed in the semen of cattle. Semen volume, initial spermatozoan motility and spermatozoan concentration were all reduced while the per cent of morphologically abnormal spermatozoa was increased (Lindley et al., 1949; Sosa, 1965). Sapsford (1951) found that the high per cent of abnormal spermatozoa could be reduced by feeding pure carotene to the deficient rams. The ability of ovine spermatozoa to reduce methylene blue was also decreased by the deficiency.

Similar testicular alterations have been reported in vitamin A deficient males of other species. Extensive epithelial desquamation was revealed by histological examination of testes from deficient male mice (Wolfe and Salter, 1931). Structureless material filled the tubular lumen while all pre-spermatozoan forms were absent except for primary germ cells amidst the unaffected interstitial tissue. In a later study by McCarthy and Cerecedo (1952), only seven per cent of 30 deficient male mice studied proved fertile on mating. Seminiferous epithelial degeneration also occurred in guinea pigs which were given vitamin A acid ester (methyl retinoate) as their only source of vitamin A (Howell

et al., 1967). The inadequacy of the acid form of vitamin A for reproduction was again exemplified by this study.

Overall results obtained from various species have clearly indicated that avitaminosis A is detrimental to the well-being of the male genital system. The degenerative lesions, while perhaps less dramatic than those yet to be described in the female, could nonetheless account for at least a part of the poorer reproductive performance associated with vitamin A deficiency.

EFFECT OF VITAMIN A ON FEMALE REPRODUCTION AND ON THE PATHOLOGY OF THE OFFSPRING

The adverse effect of vitamin A deficiency on female reproductive performance has been recognized for several decades. Hart et al. (1924) observed that cows fed on carotene deficient wheat rations gave birth to weak, often premature calves that died soon after parturition. Slow placental clearance and afterbirth retention were characteristic. In other cows, estrus failed to appear. Small, underdeveloped ovaries were noted in deficient bovines with cystic pituitaries by Madsen et al. (1942). Abortions in the second half of gestation (Hart and Guilbert, 1933), as well as oedema of fetal membranes, cotyledons and uterine wall (Payne and Kingman, 1947) were reported from studies with

range cattle. Congenital blindness and other ocular anomalies were noted in Guernsey calves from cows receiving inadequate daily carotene intakes (Ronning et al., 1953).

Guilbert et al. (1940) suggested that the minimal vitamin A level required for successful reproduction in most mammalian species was about three times as great as the minimum level required for normal growth and maintenance. Swanson et al. (1968) obtained no lactational response to increased vitamin A supplementation in cows already on adequate vitamin A levels. He thus concluded that vitamin A levels adequate for successful reproduction were also satisfactory for normal lactation.

In sheep, vitamin A deficient ewes were able to conceive but showed gestational inadequacy as judged by birth of stillborn or weak lambs (Hart and Miller, 1937; Eveleth et al., 1949). Cases of abortion, stillbirth and early lamb mortality were also reported under desert conditions in vitamin A deprived Merino and Barki ewes (Ghanem, 1964). Miller et al. (1942) proposed that a very advanced stage of depletion was required before estrus, ovulation, breeding, fertilization, and implantation were interrupted.

Stillbirths and neonatal mortality were also noted in offspring of vitamin A deficient sows (Goodwin and

Jennings, 1958; Saunders, 1958). Bailey and Nelson (1965) maintained a cross-bred gilt on a vitamin A devoid ration through five gestation periods and noted that there was a progressive reduction in the number of piglets farrowed and weaned as the severity of the deficiency in the dam increased. Histological studies by Goliarkin (1941) showed follicular atrophy and uterine epithelial keratosis in vitamin A deficient sows. The ocular abnormalities classically associated with neonatal offspring from vitamin A deficient dams were first characterized in the porcine species. Hale (1934) noted congenital anophthalmos and other defects in pigs born to vitamin A deficient sows. Numerous later reports, including those of Watt and Barlow (1956) and Goodwin and Jennings (1958), confirmed Hale's observation. Defects in nephritic, pulmonary and urogenital systems, internal hydrocephalus, bone marrow protrusion (Palludan, 1961), oedema (Goodwin and Jennings, 1958) and absence of bristles (Goliarkin, 1941) were among the other malformations detected in vitamin A deficient offspring.

A massive collection of published literature has accumulated on the effect of vitamin A on reproduction in female rats. The scope and volume of this literature makes it impossible to present either intensive or extensive coverage of the topic in this review, but an attempt has been

made to extract the more pertinent points for consideration. Wolbach and Howe (1925) noted stratified squamous keratinization and atrophy of mucus secreting epithelial tissues, including those of the genital tract, in vitamin A deficient rats. Indeed, this and supporting observations led Moore (1957) to state that "keratinisation of vaginal epithelium without significant abnormalities in ovaries appears so early and regularly in animals deprived of vitamin A that it has been used as basis of biological test for vitamin A deficiency." Estrus, ovulation and copulation continued in spite of vaginal cornification but fertilization was impaired (Evans, 1928). Moore (1957) has considered the possible relationship between estrogen, vitamin A and vaginal cornification. Vaginal keratinization could be induced by either the withdrawal of vitamin A or the presence of estrogen. Indeed the normal state of the vaginal epithelium appeared to be one of keratinization with vitamin A being required for mucus formation. The vitamin A probably functions in the synthesis of the mucoid-constituent mucopolysaccharides as has been discussed earlier in this review. Estrogen favoured cornification even in the presence of vitamin A; this observation may partially explain vaginal smear cornification at the point in the estrus cycle where

estrogenic levels were highest. Progesterone might be conducive to mucus formation where vitamin A supplies were adequate. The uterine epithelium was also susceptible to vitamin A deficiency changes (Moore, 1957) but metaplasia was observed only in intact rats on vitamin A devoid diets and not in similarly depleted castrated rats unless the latter were treated with estrogen (Bo, 1957). Disturbance of estrogen-vitamin A balance was held responsible for this keratinizing action. Uterine epithelial stratification did not represent an appreciable spread of vaginal keratinization (Popova, 1958). Similarly uterine horn epithelial stratification was not continuously linked with that in the neck of the uterus or in the vagina. Wolbach and Howe (1925) reported that keratinization appeared in uterine glands before it was discernible on surface epithelium. The mucosa of the uterus was replaced by stratified, keratinizing epithelium before the oviducts were affected.

Few ovarian alterations have been noted in vitamin A deficient animals (Wolbach and Howe, 1925; Moore, 1957). According to Truscott (1947), ovaries from deficient rats weighed less, on both a wet and dry basis, than those from controls of comparable body weight. Burkel and Taylor (1963) noted changes in the nuclei of the theca interna

cells of atretic follicles and in the interstitial cells. The chromatin pattern of these nuclei resembled those of plasma cell nuclei. Lipid and carbohydrate contents of various ovarian constituents were also reported for the deficient ovaries in their study.

The classical work on rat reproduction per se was conducted by Mason (1935). Pregnant, vitamin A deficient females frequently experienced resorption, premature appearance of the "placental sign," fetal death, prolonged gestation and labor, uterine bleeding, and the accumulation of fetid fluid indicative of genital tract infection. The placenta was affected before the fetus. Weakness and lactational failure took a heavy toll in the neonatal young; xerophthalmia and subnormal weaning weights were characteristic in survivors. Direct disturbances in the epithelial tissues of the genital tract, attributable to vitamin A inadequacy, were blamed for the gestational failure since there were no apparent abnormalities in either the ovaries or the ova produced by them. Implantation was also apparently normal and there was no indication that the function of the anterior hypophysis was defective.

Similar vitamin A deficiency symptoms were observed in female rats by Tansley (1936). Congenital-abnormalities

in offspring of vitamin A deficient rats, as reported by Warkany and Roth (1948), included ocular anomalies, oedematous swellings, subcutaneous hemorrhages, inward turning and puffiness of the feet, diaphragmatic hernia and defects in kidneys, heart, lungs, ureters and testes.

In the female rabbit, most of the studies on reproduction, as related to vitamin A deficiency, followed in the wake of a report (Kendall et al., 1950) of gestational failure, hemorrhage and reduced litter size in New Zealand White rabbits fed a diet containing 49.5 per cent soybean hay. This forage was assumed to be adequately supplied with provitamin A. However, the similarity of the symptoms observed in these rabbits to those reported earlier by Mason (1935) in vitamin A depleted rats led workers to postulate that vitamin A deficiency, perhaps due to the lipoxidase-catalyzed oxidative destruction of carotene, could conceivably be responsible for the pregnancy breakdown. The possibility that other nutritive factors were involved in the etiology of the condition was not entirely eliminated, however. Subsequent studies on vitamin A per se in rabbit reproduction were thus undertaken with a view to clarifying the cause and interpretation of this phenomenon. One of these (Lamming et al., 1954a,b) was designed to demonstrate the effect of incipient vitamin A deficiency on various

phases of gestation in the doe following copulation. Their results showed a 14 per cent decrease in the number of deficient rabbits that mated normally as compared with controls. Similarly, conception rates were 18 per cent lower in deficient does. Premature ova degeneration was indicated by the fact that the number of eggs recovered was smaller than the number of corpora lutea counted; in addition, the number of cleaved ova observed 40 hours and 96 hours after coitus was lower in the deprived females. Embryos from deficient does examined 16 or 28 days post coitum were neither as heavy nor as numerous as those from controls. The reduction in litter size at 16 days was attributed to ova degeneration and infertility while that discerned at 28 days reflected abortion and resorption losses as well. Decidual formation, uterine proliferation and corpora lutea numbers were not affected. Mottled placentas, suggestive of reduced vascularity, lent support to the idea that vitamin A deficiency affected the decidua before the fetus itself. One hypothesis suggested that abortion and resorption resulted from progesterone lack, brought about by placental degeneration owing to vitamin A deficiency. In this respect, Hays and Kendall (1956) found that progesterone, administered to does that had been fed a deficient diet for 12-13 weeks prior to mating, brought about an increase in both the total

number and the number of live offspring. It was thus suggested that progesterone improved the ability of the does to carry young for extended periods. Whether progesterone synthesis was directly inhibited by vitamin A deficiency, whether progesterone was involved in a physiological process essential for pregnancy maintenance, or whether the hormone acted non-specifically, to enable the mobilization of nutrients not already present in quantities sufficient for pregnancy continuum, was not known. The latter possibility was favoured by the results of rat studies conducted by Kendall and Hays (1960). Reviews on the work of Grangaud and Conquy (1958a,b,c), however, suggested the involvement of vitamin A in progesterone biosynthesis since progesterone relieved some of the deficiency symptoms in vitamin A deprived rats, while its precursor pregnenolone did not. This indicated a possible conversion impairment related to the deficiency. Johnson and Wolf (1960) observed that progesterone biosynthesis from cholesterol was inhibited only in severe deficiency; this might explain the observation that progesterone did not noticeably improve reproductive performance in does fed the deficient diets for only four to eight weeks prior to mating (Hays and Kendall, 1956). These does were perhaps not severely deficient.

Fetal ocular abnormalities were observed in rabbits by Lamming et al. (1954b). Millen et al. (1953) and Lamming et al. (1954c) were the first to consider hydrocephalus as a characteristic symptom of vitamin A deficiency in the offspring of vitamin A deprived does. Hydrocephalus appeared in a high proportion of the young 14 to 74 days post-parturition when the dams had been deprived of vitamin A for 14 weeks prior to mating. Congenital hydrocephalus in stillborn and late fetal rabbits was demonstrated when does were subjected to deficient diets for periods of up to 38 weeks before breeding (Millen et al., 1954). Millen and Woollam (1956) reported a higher proportion of hydrocephalic young from does deprived 24 to 28 weeks precoitum as compared with those depleted for only 12 to 15 weeks. The hydrocephalus was initially attributed to a stenosis of the cerebral aqueduct (Millen et al., 1953) but it was later suggested that the primary etiological factor was an over-production of cerebrospinal fluid by the choroid plexuses (Lamming et al., 1954c; Millen, 1956). Witzel and Hunt (1962) confirmed that the volume of cerebrospinal fluid was increased in vitamin A deficient rabbits; they simultaneously proposed a possible explanation for its function in hydrocephalus production. Perhaps related to this

finding was the observation of elevated intraventricular pressures in hydrocephalic (Millen and Dickson, 1957) and in some non-hydrocephalic (Millen and Woollam, 1956) offspring born of deficient does. Anterior fontanelle persistence, calvarian expansion (Millen, 1956), dome shaped heads and roughened fur (Witzel and Hunt, 1962) were prominent features in the hydrocephalic young.

GENERAL OBSERVATIONS ON VITAMIN A

Overall results of studies in various species have clearly indicated that growth, health and reproduction are detrimentally affected by a deficiency of vitamin A. The present concept of vitamin A as a vital factor in sulfated mucopolysaccharide synthesis (Moore, 1957; Wolf and Johnson, 1960; Sundaresan and Wolf, 1963; Thompson, 1965) is a useful tool in attempting to explain many of the symptoms associated with avitaminosis A. Female reproductive defects may be attributed to epithelial changes that occur when constituent mucopolysaccharide synthesis is inhibited by a lack of vitamin A. Degeneration in male reproductive organs (Palludan, 1966) may also be related to deterioration in epithelial tissues concurrent with absence or inadequacy of vitamin A. Growth depression associated with avitaminosis

A is frequently confounded by pathological lesions which probably result, at least partially, from the increased susceptibility of the deteriorating epithelial tissues to bacterial and parasitic invasions. The possibility has also been advanced that vitamin A deficiency may cause interference with the synthesis or release of hormones that are essential for the fulfilment of reproductive function in animals of both sexes (Mayer and Truant, 1949; Hays and Kendall, 1956; Grangaud and Conquy, 1958a,b,c; Grangaud et al., 1961).

While feasible mechanisms for the role of vitamin A in its various functions have thus been tentatively established, and while minimal vitamin A requirements for most mammalian species have been determined (Guilbert et al., 1940), no specific recommendations have been made concerning the minimal vitamin A requirements for rabbits per se (Branion, 1966; N.A.S.-N.R.C., 1966). Work in other species has suggested that the minimum vitamin A requirement for growth and well-being in most mammals is 4 to 6 $\mu g/W_{kg}/$ day, with minimal reproductive requirements being about thrice that level (Guilbert et al., 1940). The limited information available on the vitamin A requirements of rabbits has been reviewed but none of the studies described therein were involved with the direct ascertainment of minimum

vitamin A levels for growth, maintenance or reproduction. The minimal vitamin A requirements of the rabbit thus represent a missing link in the lengthy chain of established quantitative vitamin A levels for other species. The need for establishing minimal requirements for rabbits becomes more apparent when one considers the growing importance of commercial rabbit rearing. It has been estimated that in the United States alone, domestic rabbit meat consumption ranges between 25 and 30 million pounds annually (N.A.S.-N.R.C., 1966). Rabbits are rapid, efficient meat producers with only eight to nine weeks being required for the young rabbit to reach five to six pound market weight. Carcasses from meat type breeds have dressing percentages up to 60 per cent of liveweight (C.D.A., 1966). In order to maximize productivity in both the does and their growing offspring, it is vital to feed all required nutrients at levels compatible with optimal performance. The need for quantitatively defining these requirements is obviously in keeping with this goal of maximum productivity.

The literature reviewed has thus amply verified the underlying need of all animals, including rabbits, for a level of vitamin A that is compatible with the attainment of normal growth, health and reproduction. Although

quantitative requirements may vary slightly with species, the qualitative requisite for vitamin A remains paramount throughout.

III. GENERAL MATERIALS AND METHODS

COMPOSITION OF THE DIETS

The four experimental rations were prepared at Macdonald College in a pellet mill (Superior Templewood Junior Provender Press) with a physical capacity of 17 kg. Thus, each individual batch of a particular ration was quantitatively restricted to 17 kg. The composition of the basal diet used in the preparation of all four experimental diets appears in Table 1.

Table 1. COMPOSITION OF BASAL RATION

Ingredient	Per cent in Ration
Wheat	37.0
Soybean meal	8.0
Brewer's yeast	6.0
Molasses	7.0
Salt	0.5
Chromic oxide	0.5
Dicalcium phosphate	1.0
Heated grass and alfalfa meal	40.0
Total	100.0

In addition, a premix consisting of 17.5 g of vitamin D (1,654 I.U./g), 12.5 g of vitamin E (4.41 I.U./g) and a variable amount of vitamin A (Table 2) was also added for each 17 kg of ration produced.

Table 2. AMOUNTS OF VITAMIN A ADDED TO THE BASAL RATION IN THE PREPARATION OF THE FOUR EXPERIMENTAL DIETS (LEVELS OF VITAMIN A) AND THE RESULTANT VITAMIN A CONTENT OF EACH OF THESE DIETS

Level of vitamin A provided by diet ($\mu g/W_{kg}/day$)	0	3	6	12
Vitamin A premix[ab] added per 17 kg ration (g)	0	0.467	0.934	1.868
Vitamin A present per g ration (μg)	0[c]	0.10	0.20	0.40
Vitamin A present per g ration (I.U.)[b]	0[c]	0.29	0.58	1.16
Vitamin A provided by diet (I.U./W_{kg}/day)[b]	0[c]	8.72	17.44	34.89

[a]Vitamin A premix had activity of 10,583 I.U./g.

[b]Conversions of μg to I.U. were based on 0.344 μg vitamin A (acetate) = 1 I.U. vitamin A.

[c]Any traces of carotene contained in basal diet constituents were so minute as to be negligible.

Since vitamin A was the only variable dietary constituent,

the four experimental rations prepared are hereafter referred to throughout the text as the 0, 3, 6 and 12 μg levels of vitamin A. The amount of vitamin A that had to be added to 17 kg of the ration to supply levels of 0, 3, 6 and 12 μg/kg body weight/day was calculated on the premise that the rabbit's daily feed requirement was equal to about three per cent of its body weight. A four kg rabbit, for instance, would theoretically consume 120 g of feed daily. Vitamin A was then added in quantities such that the total daily required amount of the vitamin would be supplied in the aliquot of ration normally consumed by the rabbit to satisfy its other nutritive needs. In the four kg rabbit, for example, the total designated daily supply of vitamin A would have to be contained in the 120 g of ration which the animal normally consumed to meet its other nutrient requirements.

PREPARATION OF HEATED GRASS-ALFALFA MEAL

Flat metal pans were filled to a depth of about three inches with finely powdered, sun-cured grass and alfalfa meal (guaranteed minimum 11% protein and maximum 33% fiber). The trays and their contents were then heated in a Despatch oven at an internal temperature of approximately

100 to 120°C for periods of 90 hours or longer to destroy the carotene present in the forage meal. The material was usually heated continuously for the first 24 to 36 hours and then removed for an 8 to 10 hour period to permit cooling, prior to resumption of heating. Ten to 14 hour daytime heating periods were normally alternated with overnight cooling intervals for the remaining duration of the carotene destruction process. Periodic cooling prevented the combustion that occurred during continuous heating.

CAROTENE DETERMINATION

At the conclusion of the preparative process, a composite sample of the heated material was selected. Four grams of this well-mixed composite sample were analyzed for carotene content according to the Bailey-Walker extraction and the column chromatography procedures outlined by A.O.A.C. (1960). The chromatographic procedure was modified slightly in that Celite Analytical Filter Aid (Johns-Manville) was used as the source of diatomaceous earth and magnesium oxide (Baker brand) as the adsorbent in the preparation of the column. A 5 ml aliquot of the diluted eluate was transferred to a half inch colorimeter test tube (Bausch & Lomb) and its transmittance was recorded in a Bausch & Lomb

Spectronic 20 Colorimeter/Spectrophotometer at wavelength 436 mμ. The carotene concentration of the same was then determined directly from a standard curve prepared initially by plotting per cent transmittance against concentration for standard solutions of known carotene (100% beta) concentration. Acetone-hexane (1:9) was used as solvent in the standard solutions and for dilutions.

Carotene destruction was judged to be satisfactorily completed when a 99.5 to 100 per cent transmittance was recorded for the eluate from the heated grass-alfalfa samples. Traces of carotene (up to maximum of 0.25 μg/g) could possibly have been present at 99.5% transmittance but quantitatively these traces were considered to be of little significance, particularly in view of the extensive heating to which the material had been subjected. Percentage transmittance for most batches was 100%. Where percentage transmittance was less than 99.5%, further heating of the grass-alfalfa meal was carried out until the resultant eluate met the required percentage transmittance specification. The heated, essentially carotene-free material was then introduced into the basal ration as 40% of its composition. The other major basal dietary constituents were assumed to contain nil or negligible quantities of carotene based on

either initial chemical analyses carried out according to the A.O.A.C. (1960) method or on information supplied from the literature (Hart et al., 1916; Morrison, 1956).

IV. EXPERIMENTS AND RESULTS

GENERAL OUTLINE OF EXPERIMENTS

A total of three experiments were carried out to study the specific effects of vitamin A level on growth of weanling rabbits (Experiment 1), on semen production of male rabbits (Experiment 2) and on reproductive performance of female rabbits (Experiment 3). It was anticipated that the information provided by these three studies could be used in predicting the minimal vitamin A requirements for growth and reproduction of rabbits.

EXPERIMENT 1. EFFECT OF 0, 3, 6 and 12 μg LEVELS OF VITAMIN A ON THE GROWTH PERFORMANCE OF WEANLING RABBITS

Twenty-four New Zealand White rabbits were obtained from six litters weaned at four weeks of age in the rabbit colony at Macdonald College. Of these, three males and three females were randomly allotted to each of the four experimental treatments (0, 3, 6 and 12 μg levels). Individual rabbits were identified by code numbers formulated on the basis of treatment level, sex and individual (Table 3).

Table 3. CODED IDENTITIES[a] OF INDIVIDUAL WEANLING RABBITS IN EACH OF THE FOUR VITAMIN A LEVEL GROUPS

Level of Vitamin A ($\mu g/W_{kg}/day$)[b]			
0	3	6	12
0M1	3M7	6M13	12M19
0M2	3M8	6M14	12M20
0M3	3M9	6M15	12M21
0F4	3F10	6F16	12F22
0F5	3F11	6F17	12F23
0F6	3F12	6F18	12F24

[a]The first digit(s) (0, 3, 6 or 12) in the coded number refers to the vitamin A level that the rabbit received. The letter (M or F) indicates sex. The final digit(s) (1-24) identify rabbits as individuals.

[b]$\mu g/W_{kg}/day$ = micrograms per kilogram of body weight per day.

The weanling rabbits were assigned to individual cages so that all vitamin A levels were represented by equal numbers of animals in upper and lower sections of the batteries. Three rabbits from each of the four treatments were assigned to the 12 end cages where drafts were more likely to be encountered. These measures were designed to eliminate environmental differences resulting from cage location. Within any particular vitamin A level, rabbits were

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0	3	6	12
0M1	3M7	6M13	12M19
0M2	3M8	6M14	12M20
0M3	3M9	6M15	12M21
0F4	3F10	6F16	12F22
0F5	3F11	6F17	12F23
0F6	3F12	6F18	12F24

[a]The first digit(s) (0, 3, 6 or 12) in the coded number refers to the vitamin A level that the rabbit received. The letter (M or F) indicates sex. The final digit(s) (1-24) identify rabbits as individuals.

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allotted to cages completely at random.

Animals were placed in the cages in May, 1967. Following a two day adaptation period, initial body weights were determined and the experimental diets were then introduced. The pelleted ration was fed ad libitum. Body weights and feed consumptions were recorded weekly for the 24-week duration of the test period. In the subsequent "post-growth" phase, body weights were measured at irregular intervals, with a terminal weight being taken at the time of death. In this period, males and females were considered separately for comparison purposes. Reproductive studies (Experiments 2 (Trial 1) and 3) were carried on simultaneously with the "post-growth" study, utilizing the same animals for each.

Statistical Procedures

At the end of week 17, data for the parameters studied (body weight, cumulative rate of gain, cumulative feed intake and cumulative feed efficiency) was subjected to analyses of variance for groups with equal replication, while treatment mean differences were compared by Duncan's Multiple Range Test (Steel and Torrie, 1960). Week 17 represented the last week in which all 24 animals were alive and subjected to experimental measurements. This was the main reason for conducting the analyses at that point in the

growth study.

In the "post-growth" period, data for body weights of males at 24 weeks, 33 weeks and death were subjected to analyses of variance for groups with unequal replication and the differences between treatment means were compared by Kramer's modification of Duncan's Multiple Range Test (Steel and Torrie, 1960). The same analyses were applied to body weights of females recorded at 24 weeks and at death.

Results

Complete absence of dietary vitamin A was markedly detrimental to overall growth performance (Table 4). After 17 weeks of dietary treatment, rabbits fed the 0 μg level of vitamin A had gained less weight ($P < 0.01$), consumed less feed ($P < 0.01$) and shown poorer feed conversion efficiency than the rabbits that received the 3, 6 or 12 μg levels of vitamin A.

Weight gain, feed consumption and feed efficiency did not vary significantly among the 3, 6 and 12 μg level groups but numerical comparisons of treatment means for these parameters did suggest the presence of treatment differences. As the duration of the growth period increased beyond 17 weeks, the magnitude of numerical differences among treatment means also increased so that the overall

Table 4. EFFECT OF LEVEL OF VITAMIN A ON GROWTH PARAMETERS IN YOUNG RABBITS THAT HAD CONSUMED THE EXPERIMENTAL DIETS FOR SEVENTEEN WEEKS POST-WEANING

Level of vitamin A ($\mu g/W_{kg}$/day)	0	3	6	12
Total no. rabbits observed	6	6	6	6
Means for:				
Body weight* (g)	2386[a]	3287[b]	3401[b]	3482[b]
Cumulative rate of gain+ (g/day)	12.8[a]	20.5[b]	19.7[b]	22.5[b]
Cumulative feed consumption[x] (g/day)	97.7[a]	120.2[b]	128.0[b]	134.4[b]
Cumulative feed efficiency[ø] (g feed/g gain)	7.6[a]	5.9[a]	6.5[a]	6.0[a]

Different superscripts within the same row indicate differences significant at $P < 0.05$.

*See Appendix Table 1 for mean body weights in the other 23 weeks.

+See Appendix Table 2 for mean cumulative rates of gain in the other 23 weeks.

[x]See Appendix Table 3 for mean cumulative feed consumptions in the other 23 weeks.

[ø]See Appendix Table 4 for mean cumulative feed efficiencies in the other 23 weeks.

pattern of treatment effect became appreciably more obvious (Figures 2, 3 and 4 and Appendix Table 4). In general each successive increase in the level of dietary vitamin A supplementation (over 3 to 12 μg level range) resulted in a corresponding additive increment of improvement in overall growth performance.

As the level of vitamin A in the ration increased, the amount of ration consumed also increased. There was a slight complication arising from the magnitude of this intake increase in the 12 μg level group. It had been assumed that each rabbit would consume a daily aliquot of ration quantitatively equal to about 3% of its body weight. Vitamin A addition to the basal diet had been based on this premise. It was obviously impractical to attempt to "limit feed" young rabbits whose body weights were changing daily so ad libitum consumption of rations was adopted. Since rabbits were fed ad libitum, they were not restricted to consuming only 3% of their body weight in ration and, in actual fact, all rabbits in the 12 μg level group did ingest the diet in quantities greater than 3% of body weight. This increase in feed intake meant that rabbits on the 12 μg level, over the overall 24 weeks of the experiment, probably consumed, on the average, closer to 14 μg of vitamin A/W_{kg}/day than 12 μg/W_{kg}/day. This discrepancy has been kept in

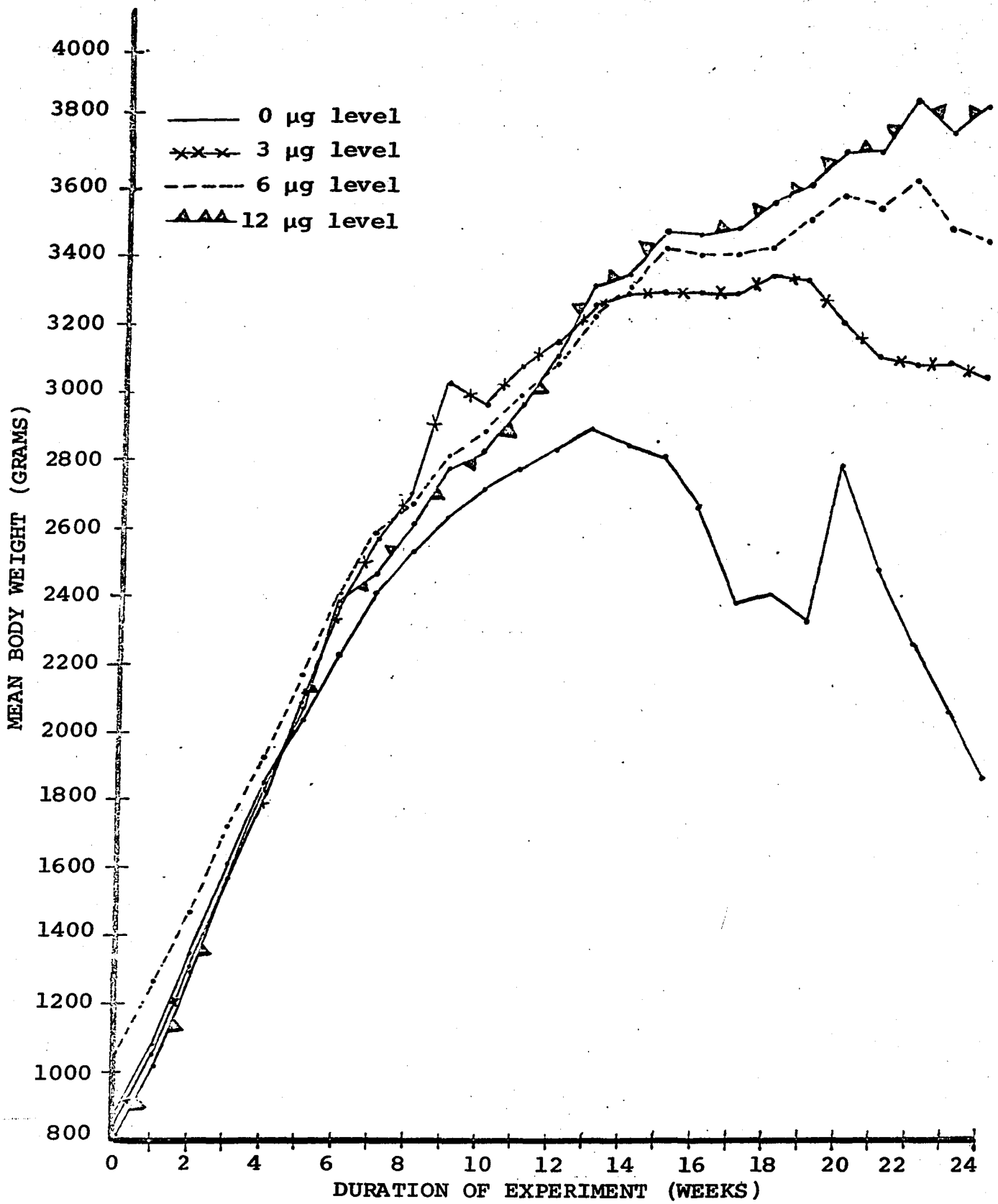

Figure 2. Effect of level of vitamin A on body weight of weanling rabbits over a 24-week period.[a]

[a]This graph is based on the data that appear in Appendix Table 1.

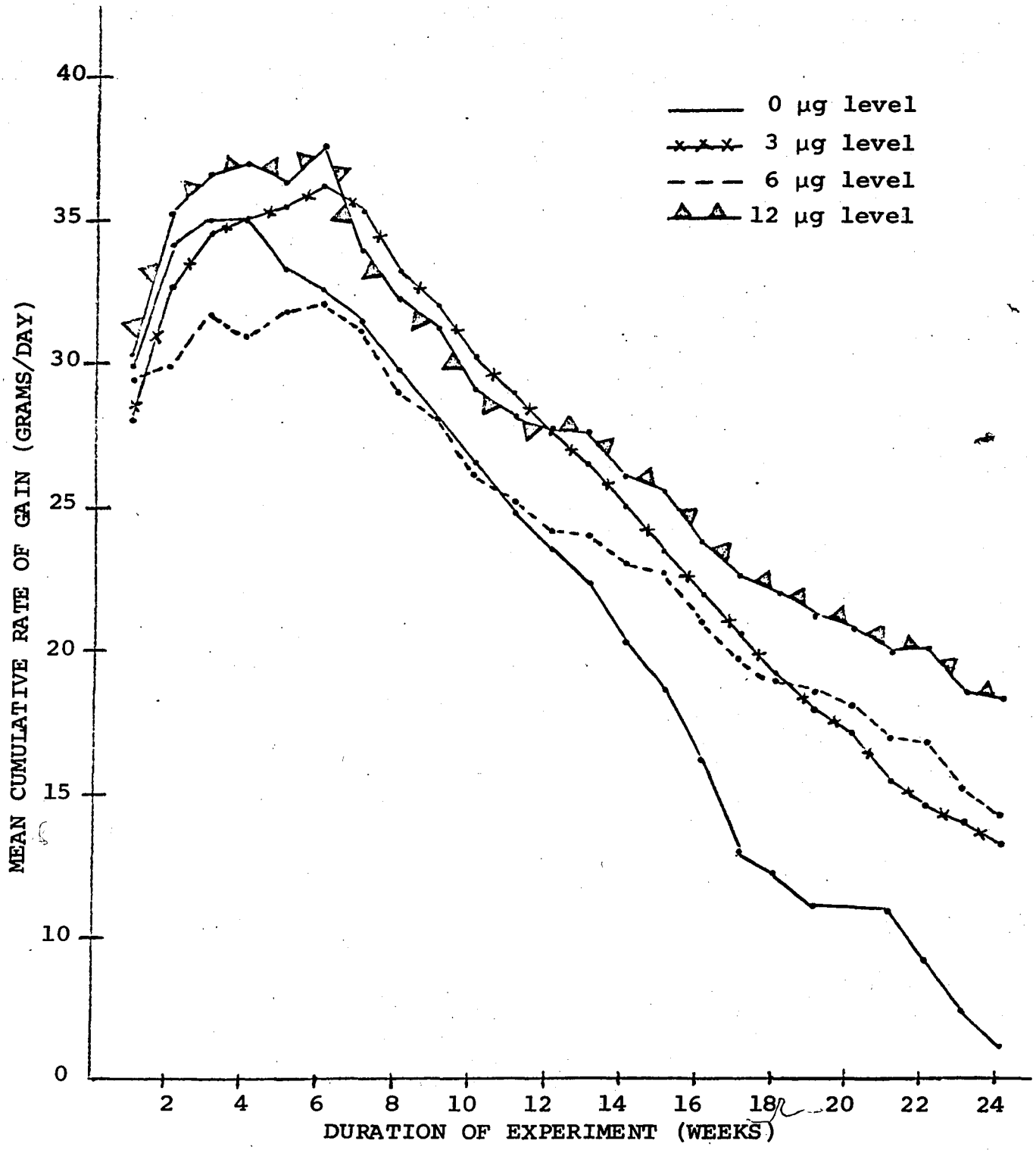

Figure 3. Effect of level of vitamin A on cumulative rate of gain of weanling rabbits over a 24-week period.[a]

[a]This graph is based on the data that appear in Appendix Table 2.

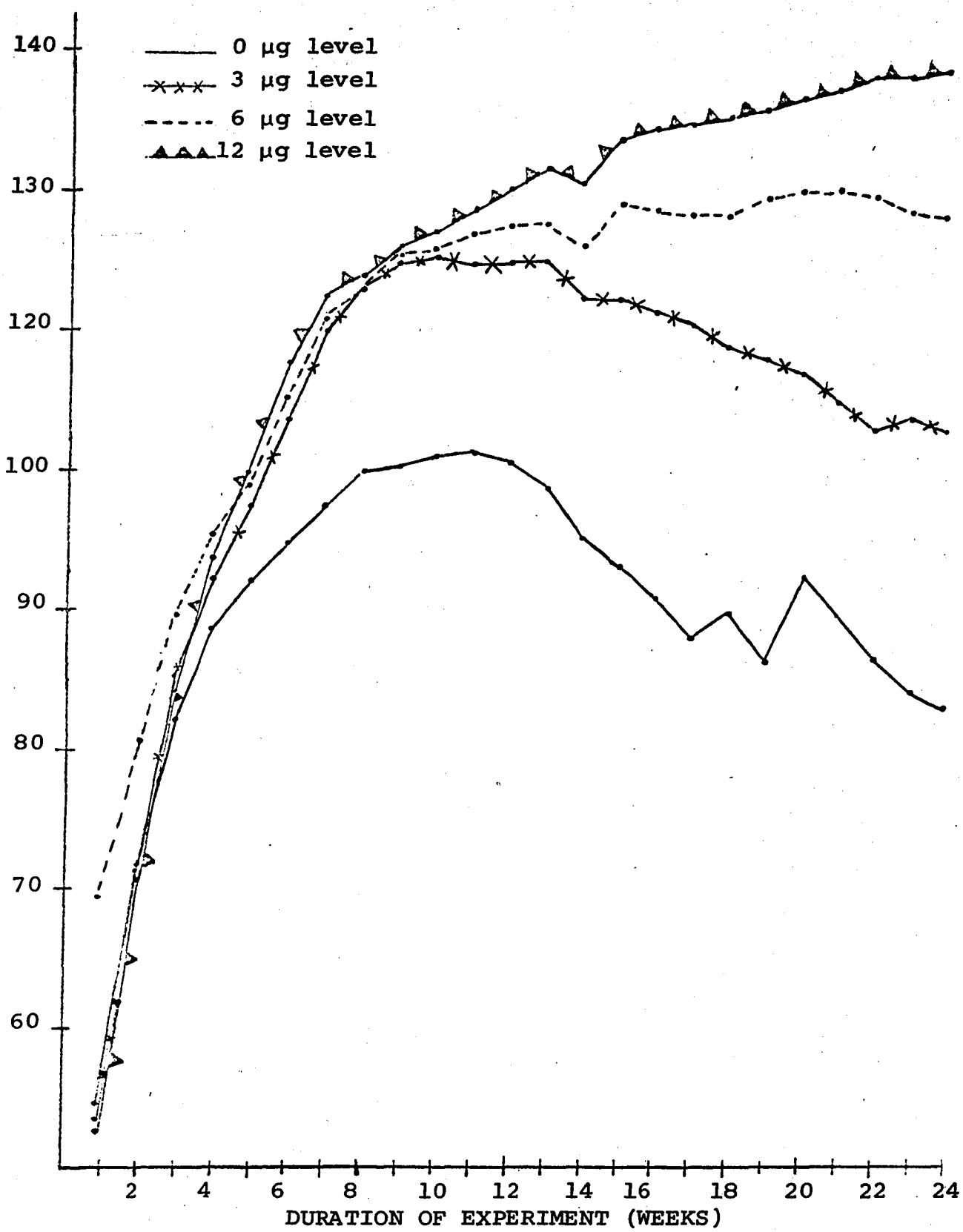

Figure 4. Effect of level of vitamin A on cumulative feed consumption of weanling rabbits over a 24-week period.[a]

[a]This graph is based on the data that appear in Appendix Table 3.

mind in interpreting and discussing the results but for convenience and consistency in the remainder of the text, the group of rabbits receiving the highest level of vitamin A supplementation continues to be referred to as the 12 μg level group. It is also possible that certain rabbits in the 6 μg level group may have consumed, on the overall average, closer to 7 $\mu g/W_{kg}/day$ since their feed intakes were also somewhat higher than was originally expected. The feed intakes of individual rabbits on the 6 μg level varied considerably, the range for week 24 being 114 to 150 g/day. However, differences between actual and theoretical intakes of vitamin A appear much less definite in the 6 μg level group than in the 12 μg level group.

Rabbits on the 12 μg level consumed more feed than those on the 6 μg level. In turn, rabbits receiving the 3 μg level ate less feed than did those on the 6 μg level. This pattern was well defined at week 17 of the growth period and prevailed throughout the remaining seven weeks of that period (Figure 4). The faster rate of gain observed in the 12 μg level rabbits (Figure 3) was undoubtedly related to their higher feed consumption. At the end of 17 weeks of treatment, however, mean growth rate for the 6 μg level group was slightly inferior to that for the 3 μg level group, in spite

of the fact that feed consumption was higher in the 6 μg level group. By week 19, however, this trend had reversed and in the five weeks thereafter (Weeks 20-24), the 6 μg level group held a slight advantage over the 3 μg level group with respect to growth rate. At the end of week 17, feed efficiencies for 3 and 12 μg level groups were about equal while feed conversion efficiency for the 6 μg level group was inferior to both (Table 4). By week 19, however, the 12 μg level group had gained superiority in the conversion of feed to flesh and the margin of this superiority continued to widen in the following weeks (Appendix Table 4). Feed conversion efficiency of the 6 μg level group remained slightly below that of the 3 μg level group, even at the end of the growth trial (Week 24). Feed consumption, rate of gain, and feed conversion efficiency did not vary significantly ($P > 0.05$) between male and female rabbits on the same dietary treatment.

Differences in post-weaning growth rates were reflected in the mean body weight differences that existed among the four treatment groups. At week 17, rabbits in the 12 μg level group were, on the average, both heavier and faster growing than those on the other three vitamin A levels (Table 4). However, mean body weight for the 6 μg level group was higher than that for the 3 μg level group

in spite of the fact that cumulative rate of gain appeared to be lower in 6 μg level group. The cause of this apparent contradiction was traced to the fact that rate of gain was expressed on a cumulative period basis, rather than on a non-cumulative, weekly basis. Rate of gain after week 13 was actually lower in 3 μg level group than in the 6 μg level group, when observed on a weekly weight gained basis (Figure 2), but this difference was not detectable in the cumulative rate of gain value based on growth rate over the whole period.

Initial deceleration of growth rate in 0 μg level rabbits commenced at week 5, two weeks before a similar decline in growth rate was first noted in rabbits on the 3, 6 and 12 μg levels (Figure 3). As early as week 3 of the growth period, the mean feed consumption of rabbits on 0 μg level was lower than that of the other three groups (Figure 4). Cumulative feed consumption declined steadily from week 12 to the end of the growth period except for two rises at weeks 18 and 20 when feed consumptions for rabbits with very poor appetites were removed from the mean by the deaths of these individuals. Decrease in feed consumption by the 0 μg level group was accompanied by a sharp, continuous decline in growth rate that was finally climaxed by a decrease in mean body weight in week 14 (Figures 2 and 3). A similar

decline in cumulative feed consumption in week 14 preceded initial body weight loss at week 17 in the 3 μg level group, while a comparable, but much less severe drop was noted in the 6 μg level group at week 22, one week prior to initial body weight decrease. Feed consumption and body weight means for the 12 μg level group were both still increasing at the end of the growth period. In the 0 μg level group, there appeared to be a particularly close relationship between time on treatment prior to initial body weight decline (98 days) and time on treatment prior to ocular lesion onset ($\bar{x}$ = 99 days) (Figure 2 and Table 5).

Onset of ocular lesions, like body weight decline, was progressively delayed with each successive increase in vitamin A level. While all animals on the 0 and 3 μg levels developed ocular lesions (Figure 5) before the end of the growth period, only three of the rabbits on the 6 μg level and one of the rabbits on the 12 μg level developed eye lesions, and even in these four animals the ocular abnormalities did not appear until the post-growth period, frequently following some form of stress. At various stages of the growth period, young rabbits on the 0 and 3 μg levels of vitamin A showed other characteristic symptoms of vitamin A deficiency besides ocular lesions. In most cases the dramatic appearance of corneal opacity was preceded by varying

Table 5. EFFECT OF LEVEL OF VITAMIN A ON INCIDENCE AND TIME OF ONSET OF OCULAR LESIONS AND DEATH IN YOUNG RABBITS

Level of vitamin A ($\mu g/W_{kg}/day$)	0	3	6	12
Total no. rabbits observed	6	6	6	6
Total no. rabbits with ocular lesions	6	6	3	1
Per cent of rabbits with ocular lesions (%)	100	100	50	16.7
Total no. rabbits that died	6	5	1	0
Per cent of rabbits that died (%)	100	83.3	16.7	0
Mean time on diet prior to onset of ocular lesions $\pm$ S.E. (days)	99 ± 1.4^{a}	138 ± 5.7^{a}	248 ± 41.0^{b}	336 ± 0.0^{b}
Mean time on diet prior to death $\pm$ S.E. (days)	135 ± 8.7^{a}	233 ± 30.9^{b}	223 ± 0.0^{ab}	A*

Different superscripts within the same row indicate differences significant at $P < 0.05$.

*A = All 6 rabbits on 12 μg level were still alive at termination of the experiment, 336 days after initial introduction of dietary treatments.

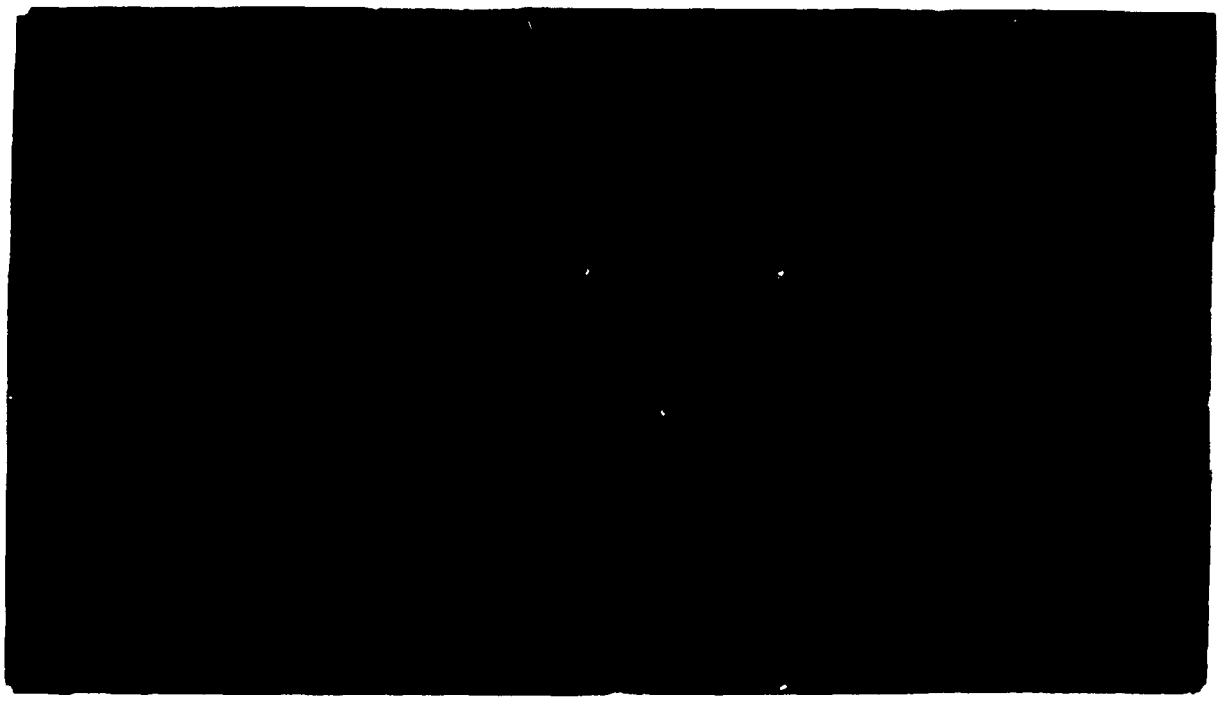

Figure 5. An early stage of ocular lesion development in a young rabbit on the 0 μg level of vitamin A. Note the opaque layer of cells partially covering the cornea.

degrees of ataxia. In some animals "snuffles" occurred. As the duration of the deficiency progressed, the severity of the ocular lesions increased with both eyes usually being affected. Eyelids of three rabbits were dry, crusty and inflamed while in another, mucous exudate caused complete eye closure. Respiration and coordination continued to deteriorate. Some rabbits exhibited a "star-gazing" stance, characterized by a continuous upward tilting of the head; less extreme variations of this syndrome were noted in other rabbits. Listlessness, inappetence, scouring, emasciation and shallow breathing were noted with varying frequency in individual rabbits.

All six rabbits receiving the 0 μg vitamin A level died but the percentage mortality decreased progressively as the vitamin A level increased until, at the highest level of vitamin A supplementation, all six animals were still alive at the conclusion of the experiment, 46-48 weeks after introduction of the experimental diets (Table 5). In animals that died, lifespans were significantly longer for rabbits on the 3 μg vitamin A level than for those on the 0 μg level ($P < 0.05$). Only one animal on the 6 μg level succumbed and the lifespan of this animal was 10 days less than the mean value for the 3 μg level group.

Post-mortem examination of the six 0 μg level rabbits

and the five 3 μg level rabbits that died showed that secondary infections, manifested chiefly in respiratory, hepatic and intestinal disorders, were the direct cause of most deaths. Suppurative pneumonia with lung atrophy or consolidation characterized the most severe cases, while less dramatic pulmonary lesions were discernible in other rabbits. Nodular lesions on the surface of the liver suggested hepatic coccidiosis in one rabbit while intestinal coccidiosis appeared to be the precipitating cause of death in certain other cases. Only one of the 12 rabbits receiving the two higher vitamin A levels died, although ocular lesions developed in three others. One male in the 12 μg level group showed extreme incoordination throughout his lifetime but this did not appear to be related to vitamin A deficiency since no ocular lesions were evident. It was presumed that some form of physical trauma (i.e. a blow or fall) could have been responsible for this condition. Snuffling, persistent scouring and emasciation preceded death in the single succumbing 6 μg level female. Papillary lesions in the anal region and bleeding from the pads of the feet, coupled with the previous infirmities, prevented the breeding of this female in the post-growth period prior to her death. Post-mortem examination revealed extensive pulmonary lesions.

In an attempt to evaluate the adequacy of the 12 μg

level diet as a normal growth promoting ration, average daily gain for rabbits receiving that level in the present study was compared with gains observed in rabbits fed more conventional diets by other investigators. Average daily gains for comparable stages of growth in these independent investigations are presented in Table 6.

Table 6. COMPARISON OF THE WEIGHT GAINS OF RABBITS FED THE TWELVE MICROGRAM LEVEL DIET IN THE PRESENT STUDY WITH THE WEIGHT GAINS OF RABBITS FED CONVENTIONAL DIETS AS REPORTED BY CASADY (1961), MACARTNEY (1966), AND N.A.S.-N.R.C. (1966)

Stage of growth period[a]	Average daily gain (g/day) as reported by			
	Casady	Macartney[b]	N.A.S.-N.R.C.[c]	Present Worker
3 or 4 to 8 weeks[d]	41.5	36.5	-	36.8
8 to 14 weeks	33.2	-	31.8	23.8
14 weeks to 5 months	16.5	-	-	13.2

[a]Stage of growth period is based on age of rabbits.

[b]Macartney's study was restricted to 4-8 week stage of growth.

[c]N.A.S.-N.R.C. value for daily gain was calculated for the period in which body weight fell between 4-7 pounds. This undoubtedly included the 8 to 14 week period, but may in some cases have extended into the 14 week to 5 month period.

[d]Initial body weights were recorded at 3 weeks of age in Casady's study and at 4 weeks of age in Macartney's and the present studies.

Eight of the original 12 male rabbits were still alive at the end of the 24 week growth trial. Dietary affliations of the 8 males appear in Table 7.

Table 7. EFFECT OF LEVEL OF VITAMIN A ON BODY WEIGHT OF MALE RABBITS, AS MEASURED AT END OF GROWTH PERIOD (WEEK 24), AT MIDPOINT OF POST-GROWTH PERIOD (WEEK 33), AND AT DEATH (WEEKS 38-46)

Level* OF VITAMIN A ($\mu g/W_{kg}/day$)	3	6	12
Total no. males observed	2	3	3
Mean body weight ±S.E. for:			
Week 24 (g)	3109 ± 319^{a}	3275 ± 38^{ab}	3856 ± 199^{b}
Week 33 (g)	3325 ± 246^{a}	3463 ± 49^{a}	4092 ± 88^{b}
Weeks 38-46 (g)	3039 ± 626^{a}	3249 ± 98^{a}	3980 ± 153^{a}

Different superscripts within the same row indicate differences significant at $P < 0.05$.

*All males on 0 μg level died prior to this time.

Mean body weight for the 12 μg level males was heavier than that for the 3 or 6 μg level males in all three comparative periods (Week 24, Week 33 and at death). The small number of animals observed in each treatment prevented

statistical significances from being obtained in all cases, but the large numerical differences indicated unquestionably that there were treatment effects. In all three comparisons, mean body weight did not differ significantly between 3 and 6 μg level groups, but in each case the 6 μg level group held the numerical advantage.

A total of 8 females out of the original 12 survived the initial 24 week growth period. The number of females in each treatment is shown in Table 8.

Table 8. EFFECT OF LEVEL OF VITAMIN A ON BODY WEIGHT OF FEMALE RABBITS, AS MEASURED AT END OF GROWTH PERIOD (WEEK 24)* AND AT DEATH (WEEKS 32-48)+

Level[x] of vitamin A ($\mu g/W_{kg}/day$)	3	6	12
Total no. females observed in Week 24	2	2[ø]	3
Mean body weight ± S.E. for Week 24	3339±115[a]	3948±330[a]	3835±190[a]
Total no. females killed or that died from Weeks 32-48	2	3	3
Mean body weight ± S.E. at death	2955±235[a]	3470±591[a]	3612±250[a]

Different superscripts within the same row indicate differences significant at $P < 0.05$.
*No unbred females were included in this comparison.
+None of the females were pregnant at the time of death.
[x]All females on 0 μg level died prior to this time.
[ø]Body weight of one unbred female on 6 μg level was excluded from this mean.

All but one of the females weighed at the end of Week 24 had been bred. The pathological lesions that prevented the breeding of this 6 μg level female have already been described. Because of this difference in reproductive state, the body weight of this female was excluded from the mean calculated for the 6 μg level group at the end of Week 24. Mean body weight for females on the two higher vitamin A levels (6 and 12 μg) appeared heavier than that for the 3 μg level group, although statistical significances were lacking. Mean body weight of 6 μg level females was slightly higher than that for the 12 μg level group, presumably due to the fact that one bred female on the 12 μg level was not pregnant. However, non-pregnancy undoubtedly also lowered the mean body weight value for 3 μg level females as well.

None of the females were pregnant when terminal body weights were recorded at death (Weeks 32-46). Mean body weight at this time was heaviest in the 12 μg level group, followed by the 6 and 3 μg level groups, respectively.

In general, post-growth performance of both male and female rabbits supported the trend established in the growth period toward increased body weight with each successive elevation in dietary vitamin A level over the range studied.

EXPERIMENT 2. EFFECT OF 0, 3, 6 and 12 μg LEVELS OF VITAMIN A ON THE REPRODUCTIVE PERFORMANCE OF MALE RABBITS

Trial I. Effect of vitamin A level on the reproductive performance of young male rabbits that were introduced to the experimental rations prior to puberty.

The eight young bucks observed in the post-growth phase of Experiment 1 were simultaneously used as subjects to study the effect of vitamin A level on semen quality. The numerical distribution of these males among the 3, 6 and 12 μg levels of vitamin A is indicated by Table 7. Each male was housed in an individual cage and fed the experimental ration ad libitum.

One male on the 3 μg vitamin A level showed testicular retention and absence of libido which prohibited his use in all but the histological portion of this study. In the other seven bucks, libido, semen quality and fertility were evaluated, as well as histological testicular condition.

Collection of Semen

Semen was collected from the seven young males over a six week period from February to March, 1968. Three collections per day were made once a week for each rabbit, except where refusals occurred.

Semen was collected in an artificial vagina (A.V.) in which a graduated centrifuge tube cut off at the 6 ml mark was used as the collection receptacle. The A.V. was filled with hot water and a small amount of K-Y lubricating jelly was smeared over the open end just prior to use. A live doe served as the teaser animal.

Libido Determination

Sexual interest or libido was rated on the following scale:

Rating
0 - Refused to mount female and serve A.V.
1 - Mounted female and served A.V. very slowly
2 - Mounted female and served A.V. after extensive courtship
3 - Mounted female quite readily and served A.V.
4 - Mounted female at once and completed ejaculation almost immediately.

Each male was evaluated on this basis for each individual semen ejaculate collected from him.

Semen Evaluation Procedure

Each individual ejaculate of semen was evaluated on the basis of the criteria outlined below. These parameters were examined, on the days of collection, in the sequence in which they are discussed.

1. Ejaculate volume

Semen volume was read directly from the graduated

scale on the 6 ml centrifuge tube used for collection.

2. Per cent spermatozoa showing motility

A small drop of fresh semen was placed on a prewarmed microscope slide and an equal volume of warm (37°C) physiological saline was added. A coverslip was placed over the diluted semen drop and the slide was subjected to microscopic examination. The percentage of motile spermatozoa present in the observed field was estimated to the nearest 5 per cent. The numerical range of the evaluation scale used was zero to 100 per cent.

3. Progressive motility of spermatozoa

Progressive motility was assessed using the same slide as that used to determine percentage motility. Forward motility was rated on a 0 to 4 scale, according to the criteria laid down by Trimberger (1962). These ratings were further broken down into intervals of 0.5 and recordings were made at that level.

4. Per cent dead spermatozoa

One drop of freshly ejaculated semen was mixed with a single drop of eosin-nigrosin stain on a prewarmed slide in accordance with the stain concentrations suggested in Herrick and Self (1962). A second slide was drawn over the

first to form an even smear which was dried in about a minute by warming over the flame of an alcohol burner. Slides were examined immediately or shortly after preparation. One hundred spermatozoa in each of three separate fields were differentiated as live or dead. Dead spermatozoa readily took up the pink stain while live spermatozoa remained colourless. The average of the number of dead spermatozoa counted in the three fields was expressed as a per cent and taken as the mean value for that ejaculate. In later stages of the study the number of observed fields was reduced from three to two without any apparent impairment of consistency or accuracy. The scale for recording per cent dead spermatozoa covered the range of zero to 100.

5. pH

Hydrogen ion concentration or pH was recorded to the nearest 0.1 on a Fisher Accumet pH meter Model 210 equipped with a single glass electrode.

6. Per cent abnormal spermatozoa

The three ejaculates from an individual rabbit were combined to provide a composite semen sample. A portion of this composite sample was suitably diluted with 5% sodium citrate and the diluted sample was then examined for

morphologically abnormal spermatozoa. The differential examination of 200 spermatozoa was carried out in the counting chambers of a Spencer Brightline Hemocytometer, with 100 spermatozoa being examined in each of two fields. The average number of defective spermatozoa in the two fields studied, when expressed on a percentage basis, represented the per cent abnormal spermatozoa for that sample of semen. The range for expression of per cent abnormal spermatozoa was zero to 100. The following changes were considered indicative of morphological spermatozoan abnormality:

a) Tailless heads;
b) Bent midpieces;
c) Small, enlarged or abnormally shaped heads;
d) Double headed spermatozoa;
e) Detached or detaching acrosomes;
f) Abnormal fusion of head and midpiece;
g) Retained cytoplasmic droplets;
h) Coiled or broken tails;
i) Double or triple flagellated spermatozoa.

7. Concentration of spermatozoa

Spermatozoan concentration per c.c. of semen was measured in the same hemocytometer as was used in the per cent abnormal spermatozoan determination. Appropriate aliquots of individual composite semen samples were diluted at rates of 1:50 or 1:100 with 5% sodium citrate, to which had been added a few drops of 10% formalin to inactivate the spermatozoa. Occasional dilutions of 1:20 or 1:200 were

used for samples of very low or very high spermatozoan concentrations.

Spermatozoa were counted in the five large squares across the top and in the five across the bottom in one of the counting chambers. The procedure was repeated in the second counting chamber and the average of the four readings was taken as the number of spermatozoa present in the unit area. This value was multiplied by the dilution factor and again by an area factor of 5 x 10,000 to give the number of spermatozoa present per cubic centimeter of semen.

8. Total spermatozoa per ejaculate

Spermatozoan concentration per c.c. of semen was multiplied by the corresponding ejaculate volume to give total spermatozoan production per ejaculate.

Breeding Performance or Fertility Evaluation

Each male was mated with two females during the two week interval just prior to the start of the semen collection period. At each individual mating, the doe was twice force bred to a particular male. The only functional male on the 3 μg vitamin A level had been mated on seven additional occasions prior to this breeding period in an attempt to accurately assess his fertility before serious health impairment occurred. Of the seven bucks studied, this 3 μg

level male (3M8) appeared the most likely to show seminal deterioration since he was on the lowest level of vitamin A supplementation.

Post-Mortem Procedures

Surviving males were killed by cardiac puncture at the conclusion of the experiment. Testes were excised, weighed and fixed in Bouin's solution for subsequent histological examination. Each testicle was divided into two or three sections with a sharp scalpel blade prior to placement in the fixative. Testes from males that died during the growth or reproductive periods were treated in the same manner.

Histological Procedures

1. Fixing

Sections were placed in Bouin's fixative for two to seven days and were then transferred to 50% ethanol for a day or overnight. On removal, they were stored in 70% ethanol until processed.

2. Dehydration and infiltration

All steps in the dehydration and infiltration processes were carried out automatically in an Elliot Tissue Processor (Table 9).

Table 9. SUMMARY OF STEPS IN THE DEHYDRATION AND INFILTRATION OF TESTICULAR SECTIONS IN THE AUTOMATIC TISSUE PROCESSOR

Medium	Duration of Time (hours)
Water	1.0
Ethanol (50%)	1.0
Ethanol (70%)	2.0
Ethanol (70%)	2.0
Ethanol (85%)	2.0
Ethanol (95%)	1.0
Absolute alcohol	2.0
Absolute alcohol & toluene (50:50)	1.0
Toluene	1.5
Toluene	1.5
Melted Paraplast (60°C)	1.0
Melted Paraplast (60°C)	1.0

3. Embedding and sectioning

Processed samples were embedded in Paraplast using plastic moulds. Embedded samples were sectioned on a rotary microtome (Jung Ag Heidelberg 20364) to thickness of 7 to 10 microns (μ). Most sections were cut at 7 μ. Random individual sections were selected for mounting.

4. Affixing sections to slides

The affixative medium (Table 10) was placed in a water bath maintained at about 48°C. Sliced sections were placed on the liquid surface from whence they were removed directly to the surface of the slide. For each specimen

examined, four sections were selected and affixed to a single slide.

Table 10. COMPOSITION OF AFFIXATIVE MEDIUM[a]

Solution	Component	Quantity
A	Distilled water	1500 c.c.
	Glycerine	50 c.c.
	Phenol	7 g
B[b]	Distilled water	500 c.c.
	Egg whites	3

[a]Solutions A and B were prepared separately and then combined to form the affixative medium.

[b]The egg whites were shaken with 50 c.c. of distilled water prior to adding the remaining 450 c.c. of water. The resulting solution was then filtered until clear. The filter consisted of two layers of cheesecloth placed between four layers of filter paper on the top and three layers of filter paper on the bottom.

5. Staining

After drying, slides were stained with Heidenhain's iron hematoxylin according to the following format (Table 11).

6. Mounting of coverslips

Stained slides were permitted to dry before coverslips

Table 11. SUMMARY OF STEPS INVOLVED IN THE STAINING OF TESTICULAR SECTIONS WITH IRON HEMATOXYLIN

Medium	Duration of Time
Toluene	6 min.
Toluene	4 min.
Absolute alcohol	3 min.
Ethanol (95%)	3 min.
Ethanol (70%)	3 min.
Distilled water	Rinse
Distilled water	Rinse
Ferric ammonium sulfate[a] (2%)	3 hr.
Distilled water	30 sec.
Running tap water	3 min.
Hematoxylin[b]	2 hr.
Distilled water	Rinse
Running tap water	5 min.
Iron alum[c]	5 ± 1 min.
Running tap water	30 min.
Distilled water	Rinse

[a]Ferric aluminum sulfate acts as mordant.

[b]Hematoxylin is the staining agent.

[c]Iron alum serves as a differentiating agent.

were applied with Permount. Excess mounting medium was removed with xylene.

7. Interpretation of sections

Specimens were coded numerically prior to histological procedures. Stained sections were examined for evidence of spermatogenetic arrest or disturbance. Spermatogenesis

was considered to be impaired when numerous seminiferous tubules showed few potential gametes developing beyond the spermatogonial or primary spermatocyte stage or when there was absence of pre-spermatozoan forms with evidence of spermatogenetic disorganization. Decreased diameter of seminiferous tubules and excessive desquamation of epithelial and gametic cell layers within these tubules were also regarded as abnormal. Lumenal occlusion by desquamated cells was also noted as an irregularity.

Statistical Procedures

Data for all semen variables except those expressed on a percentage basis were subjected to analyses of variance for groups with unequal replication. Treatment means were subsequently compared using Kramer's modification of Duncan's Multiple Range Test, provided the F values in analyses of variance proved significant ($P < 0.05$). Libido data were treated in the same fashion. Percentage data for the remaining semen variables were transformed by the arcsin $\sqrt{\text{percentage}}$ method before being subjected to these same two statistical procedures.

Male reproductive efficiency was calculated by the following ratio:

$$\% \text{ Reproductive Efficiency} = \frac{\text{Total number of resulting pregnancies}}{\text{Total number of matings}} \times 100\%$$

An analysis of variance for groups with unequal replication was conducted on the transformed data corresponding to the calculated % reproductive efficiency values.

Testicular weights were expressed as a percentage of body weight in mg-%. These weights were also subjected to analysis of variance for groups with equal replication while treatment mean differences were tested by Duncan's Multiple Range Test. Details of all statistical procedures here employed were found in Steel and Torrie (1960).

Results

Semen from males that were fed on the two higher vitamin A levels was generally superior in all characteristics studied to that produced by the single functional male that consumed the 3 μg vitamin A level (Table 12). However, libido appeared to be slightly stronger in the 3 μg level buck than in males on the 6 μg ($P < 0.05$) and 12 μg levels. By contrast, the second surviving male on the 3 μg level was so deficient in sexual desire that he was eliminated from both semen collection and breeding studies. Libido differences may, therefore, have been more influenced by individual animal variations than by any treatment effect *per se*. The raw data suggested that the lower mean libido ratings in the 6 and 12 μg level groups were attributable

Table 12. EFFECT OF LEVEL OF VITAMIN A ON THE CHARACTERISTICS OF SEMEN AND ON THE LIBIDO RATING OF YOUNG MALE RABBITS

Level of vitamin A ($\mu g/W_{kg}/day$)	3	6	12
No. of males studied	1	3	3
Means for:			
Libido rating	4.0^{a}	3.6^{b}	3.8^{ab}
Volume (ml)	0.28^{a}	0.95^{b}	1.39^{c}
pH	7.3^{a}	7.1^{ab}	7.0^{b}
Per cent motility (%)	3^{a}	49^{b}	29^{c}
Progressive motility	0.36^{a}	2.07^{b}	1.61^{c}
Per cent dead spermatozoa (%)	88^{a}	33^{b}	47^{c}
Per cent abnormal spermatozoa (%)	53^{a}	41^{b}	35^{b}
Spermatozoan conc./cc., x 10^6	5^{a}	146^{b}	134^{b}
Total spermatozoa/ejaculate x 10^6	1.5^{a}	124^{b}	178^{c}

Different superscripts within the same row indicate differences significant at $P < 0.05$.

to the lower libido ratings of isolated individuals within those groups rather than to any overall group response to treatment.

With each successive increase in the level of vitamin A supplementation, there was a significant ($P < 0.01$) rise in ejaculate volume. Mean ejaculate volume for males on the 12 μg level was nearly five times as great as the mean volume for the 3 μg level male. Similarly, the quantity of semen produced per ejaculate by the 6 μg level male was, on the average, more than three times as great as the mean volume ejaculated by the 3 μg level buck.

Semen from the 3 μg level male contained significantly ($P < 0.01$) fewer motile spermatozoa than did semen from 6 or 12 μg level males. The limited number of motile spermatozoa that were present in the semen of the 3 μg level male showed a less ($P < 0.01$) vigorous rate of forward propulsion than did those in the semen of males on the two higher vitamin A levels. In keeping with the same general trend, semen from the low vitamin A level male also contained greater percentage of dead ($P < 0.01$) and abnormal ($P < 0.05$) spermatozoa than did semen from the more highly supplemented males. Both per cent spermatozoan motility and forward motility were significantly higher ($P < 0.01$) in semen from males on the 12 μg level than in semen from bucks on the 6 μg level. The mean dead spermatozoan content of the semen was also higher ($P < 0.01$) in the 12 μg level group. The apparent inferiority of the 12 μg level

group in these instances was largely attributable to the extremely poor quality of semen produced by one individual male within that treatment group. This same male (12M20), however, exhibited excellent libido even though he showed severe physical incoordination.

Semen from the 3 μg level male contained a higher percentage of morphologically abnormal spermatozoa than did semen from either the 6 μg ($P < 0.05$) or 12 μg ($P < 0.01$) level groups. Males on the 12 μg level appeared to produce somewhat fewer abnormal spermatozoa than did males on the 6 μg level but these differences were not significant ($P > 0.05$). The overall data suggested that the percentage of abnormal spermatozoa was reduced as the level of dietary vitamin A supplementation was increased.

Spermatozoan concentration per c.c. of semen was lower in the 3 μg level male than in bucks that received the 6 μg ($P < 0.01$) or 12 μg ($P < 0.05$) vitamin A levels. Mean spermatozoan concentrations for the 6 and 12 μg level groups did not differ significantly ($P > 0.05$), although the 6 μg level group did enjoy a slight numerical advantage. The lower mean value for the 12 μg level group again reflects the poor semen quality of individual male 12M20. As the level of vitamin A supplementation increased, the total number of spermatozoa produced per ejaculate also increased.

Ejaculates from the 3 μg level male contained significantly fewer ($P < 0.01$) spermatozoa than did ejaculates from 6 and 12 μg group males. This observation reflects the fact that both ejaculate volume and spermatozoan concentration per c.c. were also significantly lower in the 3 μg level group. Although mean spermatozoan concentration per c.c. of semen was lower in the 12 μg group than in the 6 μg level males, the total spermatozoan content per ejaculate was higher ($P < 0.05$) in the 12 μg level group. This difference undoubtedly reflected the greater mean volume of semen produced by males on the 12 μg level.

Differences in hydrogen ion concentration (or pH) among groups appeared to be related to variations in spermatozoan concentrations. Seminal pH was significantly higher ($P < 0.01$) for the 3 μg level male than for the 12 μg level group. Other differences were not statistically significant ($P > 0.05$) but numerical values suggested that the acidity of the semen increased as the level of vitamin A supplementation was elevated. This decrease in pH at higher vitamin A levels was undoubtedly a reflection of the rise in total spermatozoan concentration that also occurred at these levels. Fructolysis would be enhanced by the greater number of spermatozoa present and the greater resultant production of lactic acid would lower the seminal pH.

Semen from all seven males studied was fertile as indicated by the fact that each male sired at least one litter in the breeding period that preceded the semen collection interval. There was, however, considerable variation in the degree of fertility both within and among treatment groups as evidenced by numerical differences in per cent reproductive efficiency (Table 13).

Table 13. EFFECT OF LEVEL OF VITAMIN A ON REPRODUCTIVE EFFICIENCY OF YOUNG MALE RABBITS[a]

Level of vitamin A ($\mu g/W_{kg}/day$)	0	3	6	12
No. of males studied	0[b]	1[c]	3	3
Total no. of matings	-	9	6	6
Total no. of resulting pregnancies	-	1	4	5
Reproductive efficiency (%)	-	11	67	83

Reproductive efficiency did not vary significantly ($P > 0.05$) among the four groups.

[a]See Appendix Table 5 for data on individual males.

[b]All males on 0 μg level died prior to breeding age.

[c]One male on 3 μg level died prior to breeding age while a second was eliminated from the study because of libido deficiency.

Reproductive efficiency appeared to improve as the level of dietary vitamin A intake increased, although statistical significances were absent ($P > 0.05$) due to the limited number of individuals observed. The single functional male on the 3 μg vitamin A level sired only one litter out of nine matings whereas the three males on the highest vitamin A level sired five litters out of six matings. Per cent reproductive efficiency for the 6 μg level males was intermediate between those of the other two groups.

Treatment vitamin A level also contributed significantly ($P < 0.05$) to the variation observed among mean group testicular weights (Table 14). Mean testicular weight expressed on a mg % basis for the 12 μg level group was significantly heavier than that for the 0 μg ($P < 0.01$), 3 μg, or 6 μg ($P < 0.05$) level groups. Although mean testicular weights did not vary significantly among the 0, 3 and 6 μg level groups, the testes from males on the 6 μg level group did appear to be heavier than those from bucks on the two lower levels. Mean testicular weight per 100 g of body weight appeared higher in the 0 μg level group than in the 3 μg level group but this difference may have been at least partially due to differences in average age at death for males on these two levels. The age at death factor may also have influenced within treatment variation in testicular

Table 14. EFFECT OF LEVEL OF VITAMIN A ON TESTICULAR WEIGHTS OF YOUNG MALE RABBITS*

Level of vitamin A ($\mu g/W_{kg}$/day)	0	3	6	12
No. of males observed	3	3+	3+	3+
Mean time on diet prior to death ± S.E. (days)	136±15	251±81	322±0	321±0
Means for combined left and right testicular weights ± S.E. (mg %)[x]	93±15[a]	64±18.5[a]	106±38.2[ab]	184±13.3[b]

Different superscripts within rows indicate differences significant at $P < 0.05$.

*See Appendix Table 6 for data on individual males.

+One male in 3 μg level group and all animals in 6 and 12 μg level groups were killed at end of experiment by cardiac puncture. The other two males on 3 μg level and all three males on 0 μg level died during experimental period.

[x]mg % = mg of testicular weight per 100 g of body weight.

weights, particularly within 0 and 3 μg level groups.

Differences in mean testicular weights among the four vitamin A level groups were frequently explained by corresponding histological testicular differences. Histopathological alterations were fairly similar in the testes of all three males that received the 0 μg level of vitamin A.

Spermatogenesis was not active, with spermatogonia and primary spermatocytes being the most advanced stages that were commonly detected (Page 102, Figure 6). Mature spermatozoa appeared absent. Tubules almost all showed extensive desquamation, fragmentation and presence of structureless cytoplasmic material in the lumen. Dark staining nuclei were frequently observed in the degenerate fragments. Vacuoles were frequently formed in the lumen amidst the degenerate material. Tubular membrane withdrawal, tubular diameter reduction and complete tubular denudation were discernible in portions of the sections. There was no overall evidence suggestive of orderly spermatogenesis. It was expected that rabbits that died at an early age would normally show less testicular development than those that survived to well beyond sexual maturity. However, the extensiveness of the testicular disorganization observed in one of the 0 μg level males that died at 146 days of age appeared to be much in excess of that normally expected in a five month old buck. This suggested that vitamin A deficiency was probably more responsible for the observed testicular disorganization than was the tender age of the rabbit.

Testicular changes in two of the males on the 3 μg level differed from those observed in the 0 μg level bucks in that limited spermatogenesis was detected in the former

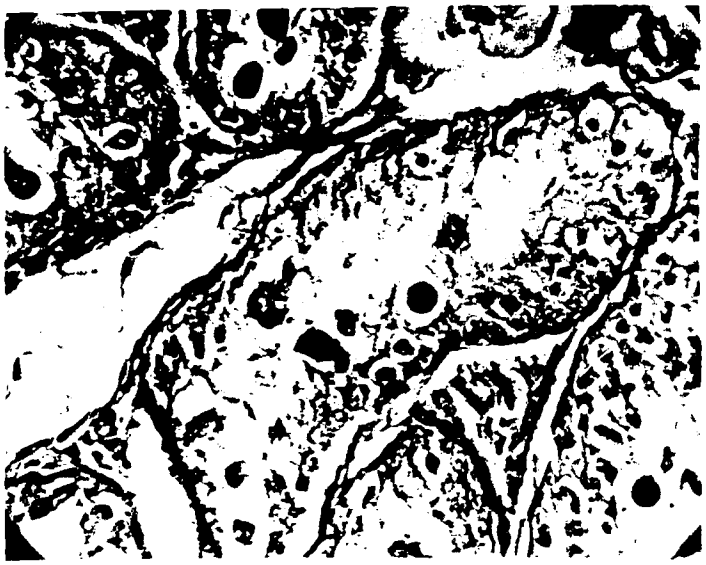

Figure 6. Phase-contrast photomicrograph showing desquamation and fragmentation in seminiferous tubules of a male that died after receiving the vitamin A deficient diet (0 µg level) for 127 days. Note the absence of pre-spermatozoan forms other than spermatogonia (Iron hematoxylin x 400).

Figure 7. Phase-contrast photomicrograph showing very limited spermatogenesis in seminiferous tubules of a male that received 3 µg level of vitamin A for 322 days. Note the presence of a single mature spermatozoan in the lumen of one tubule (Iron hematoxylin x 400).

group. Limited numbers of mature spermatozoa, immature spermatozoa and spermatids were present, although they were frequently mixed with desquamated cells in the tubular lumen or surrounded by the structureless cytoplasmic material that filled the tubule. Many tubules contained only spermatogonia and primary spermatocytes with desquamation and vacuolization being prominent features of their general disorganization (Page 102, Figure 7). In other tubules even more extensive desquamation occurred and the exfoliated fragments frequently possessed dark staining nuclei. Normal, degenerating and degenerate tubules were all discernible within the testes of 3 μg level males, but the latter two types definitely predominated. The third rabbit on the 3 μg level exhibited cryptorchidism. Histological examination of his testes revealed cellular desquamation and absence of spermatogenesis beyond the spermatogonium or primary spermatocyte level (Page 104, Figure 8). Tubule size was small. Vacuoles present frequently contained dark staining fragments. Cytoplasmic material occluded the tubular lumen in some instances. Almost every tubule appeared disorganized with no evidence of active spermatogenesis. These degenerate changes are in keeping with the cryptorchid nature of the male studied.

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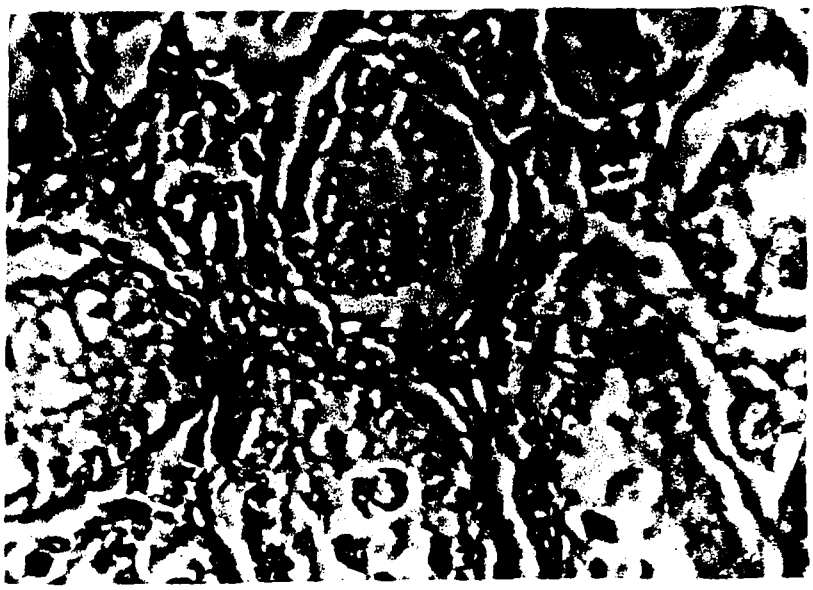

Figure 8. Phase-contrast photomicrograph of seminiferous tubules in cryptorchid rabbit fed the 3 μg level of vitamin A for 267 days. Note the absence of spermatogenesis, the presence of dark-staining fragments in the tubular lumina and the extensive desquamation (Iron hematoxylin x 400).

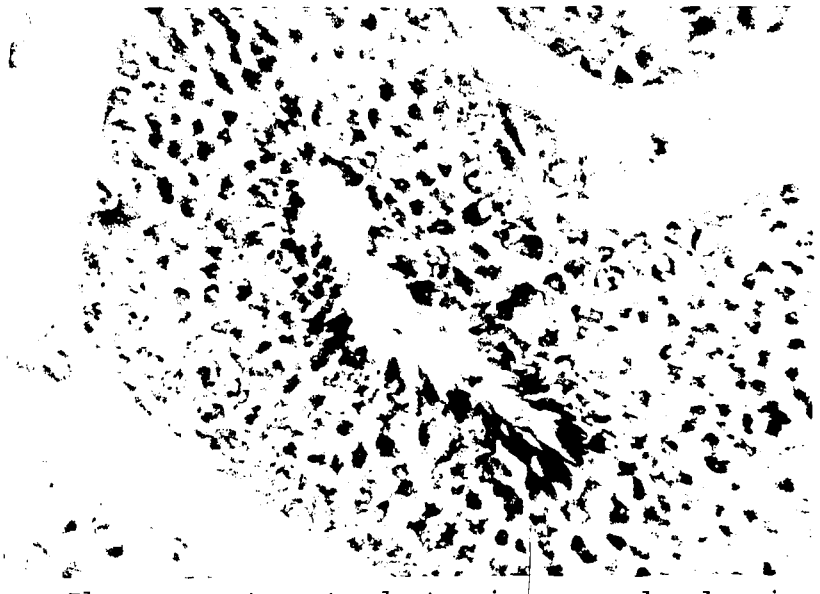

Figure 9. Phase-contrast photomicrograph showing active spermatogenesis in seminiferous tubule of a male rabbit fed the 12 μg level of vitamin A for 321 days. Note the abundance of mature spermatozoa in the lumen (Iron hematoxylin x 400).

Completed spermatogenesis, as evidenced by the presence of spermatids and mature spermatozoa, was observed in all three rabbits receiving the 6 μg level of vitamin A. All stages of spermatogenesis were detected in the seminiferous tubules of these rabbits. Slight desquamation was noted in the seminiferous tubules of two of the males on the 6 μg level but the most extensive and severe exfoliation occurred in the third member of the treatment group (Male 6M15). This observation was of special interest since 6M15 was the only male on the 6 μg vitamin A level to develop ocular lesions during the experimental period. Some of the seminiferous tubules in the testicles of male 6M15 contained mature spermatozoa while others appeared to have pre-spermatozoan forms, but many of the tubules were completely inactive. These degenerate tubules were characterized chiefly by the presence of necrotic structureless material in the lumen and by the absence of all pre-spermatozoan forms except for a layer of spermatogonia on the basement membrane. In some tubules even the basement membrane was denuded or ruptured. Dark-staining desquamated fragments were frequently contained in the vacuoles that were present. Mature spermatozoa were less numerous in the tubules of male 6M15 than in those of his two group mates. Even though excessive desquamation was noted in the

tubules of one individual male fed the 6 μg vitamin A level, the overall spermatogenetic performance of bucks in that group was considerably superior to that observed in the males that received vitamin A supplementation at the 0 or 3 μg levels.

Active spermatogenesis was well defined in the seminiferous tubules of males that received vitamin A supplementation at the 12 μg level (Page 104, Figure 9). Mature spermatozoa and spermatids were present in abundance, as were pre-spermatozoan forms. Limited desquamation was observed in some tubules but the extent of the exfoliation was probably not greater than that expected under normal circumstances. Basement membrane dissociation also occurred in a few tubules. Although male 12M20 had shown seminal abnormalities during life, his seminiferous tubules appeared essentially normal, with abundant spermatozoa and spermatids being detected in them at the time of histological examination. Spermatogenetic prolificacy and seminiferous tubule integrity generally appeared more satisfactory in males receiving the 12 μg vitamin A level than in those fed on the 0, 3, or 6 μg levels.

Trial II. Effect of vitamin A level on the reproductive performance of <u>mature</u> male rabbits that were introduced to the experimental rations after the onset of puberty.

Eight sexually mature male rabbits of the New Zealand White breed were used to study the effect of vitamin A level on semen quality. Rabbits were housed in individual cages that were equipped with detachable waterers and feeders. The ration allotment for each individual rabbit was weighed out daily in an amount equal to three per cent of the rabbit's body weight. Rabbits were normally weighed at weekly intervals, at which time feed requirements for the following week were calculated on the basis of the new body weights.

All bucks had become accustomed to the procedure of semen collection for a considerable time period prior to initiation of dietary treatment. The method of collecting semen was identical to that described for the young males. Regular semen collection and examination procedures were carried out during a four week pre-treatment period while the males were still being fed a normal commercial rabbit diet. Three ejaculates from each rabbit were collected and examined weekly with all collections being made on a single day. This four week pre-treatment interval constituted the control period of the experiment.

At the end of the control period, two rabbits were randomly allotted to each of the four dietary vitamin A levels (0, 3, 6 and 12 μg). Individual rabbits on the four

treatments were identified by code numbers (Table 15).

Table 15. CODED IDENTITIES[a] OF INDIVIDUAL MATURE MALE RABBITS IN EACH OF THE FOUR VITAMIN A LEVEL GROUPS

Level of Vitamin A ($\mu g/W_{kg}/day$)			
0	3	6	12
0M25	3M27	6M29	12M31
0M26	3M28	6M30	12M32

[a]The first digit(s) (0, 3, 6 or 12) in the coded number refers to the vitamin A level that the rabbit received. The letter (M or F) indicates sex. The final digits identify rabbits as individuals.

A two week adaptation period was allowed to permit the rabbits time for adjustment to their new diets. During this period rabbits were fed a mixture of commercial and experimental diets, with the proportion of the latter increasing until, at the end of the two weeks, all rabbits were being fed completely on the experimental rations. These diets were maintained throughout the 26-week semen collection period, during which ejaculates were collected and examined with the same frequency as in the control and adaptation periods. The 26-week duration of this semen

collection period was not continuous, however. Semen was collected and examined for an initial 17-week period referred to hereafter as semen collection period I. Regular semen collections were then suspended for a 16-week period, although rabbits continued to consume the experimental diets. Within this 16-week interval each male was mated to four different females as a means of checking seminal fertility. This 16-week period is hereafter denoted as the breeding period. Semen collection was resumed for a further nine weeks following the breeding interval (semen collection period II). The overall duration of Experiment 2, Trial II was 48 weeks (Table 16).

Table 16. A SUMMARY OF THE SEQUENCE AND DURATION OF INDIVIDUAL PERIODS WITHIN EXPERIMENT 2, TRIAL II

Experimental Period	Duration of Period (Weeks)
Control	4
Adaptation	2
Semen collection I[a]	17
Breeding	16
Semen collection II[a]	9
Total Experimental Period	48

[a]Semen collection periods I and II are considered in the text to cover weeks 7-23 and 24-32 respectively, even though there was a 16-week breeding period interposed between them. This was done so that the semen collection period might be more readily considered as a single unit to facilitate data analyses.

Libido Determination

Libido in each male was recorded for every individual ejaculate collected. The basis of evaluation was the same as that employed in the study with the young males.

Semen Evaluation Procedures

The parameters studied were identical with those examined in the young males. The procedures used were also the same with two notable exceptions:

1. Per cent abnormal spermatozoa was determined from the same stained slide as was used to determine the per cent dead spermatozoa. A separate determination of per cent abnormal spermatozoa was made on each ejaculate collected, rather than a single determination being made on the composite semen sample as was the case with the young males.

One hundred spermatozoa were examined in each of five fields on the stained slide and the number of morphologically defective spermatozoa was recorded. The number of fields examined was later reduced to two with no evidence of impaired detection efficiency. The average number of defective spermatozoa in the two (or five) fields studied, when expressed on a percentage basis, represented the per cent abnormal spermatozoa for that ejaculate. The criteria

used for detecting abnormal spermatozoa were the same as those used in the young males. There was some hazard with this method that the artifactual occurrence of "tail coiling" might be increased as a result of the slight warming of the slide during the smear drying process.

2. Spermatozoan concentration per c.c. of semen was determined for each individual ejaculate of semen collected rather than for the composite semen sample as was the case with the young males.

Post-Mortem Procedures

Testes from the two mature males that died during the course of the experiment and from the six that were killed by cardiac puncture at the end of the experiment were excised, weighed, and fixed in Bouin's solution. Histological and staining procedures for the testes from mature males were identical with those described for young males. The basis of interpretation was also the same.

Statistical Procedures

Statistical procedures used were the same as those employed in Trial I, except for the examination of the libido and semen data. The libido and semen data was analyzed in a 1620 Computer at Macdonald College, utilizing a program written by Dr. J.E. Moxley. Each ejaculate of semen

collected constituted an individual observation. The raw data collected in each week of the semen collection period was adjusted to eliminate the effect of pre-treatment individual animal variability. The adjustment factors were calculated from data collected during the 4-week pre-treatment or control period. Data collected in the adaptation period and in the first two weeks of semen collection period I was not included in the analyses. Analysis of variance was carried out on the adjusted data for each of the semen variables observed over a 24-week period (weeks 9-23 and 24-32, Table 16). Libido rating was handled in the same fashion. For purposes of analysis, the total 24-week semen collection interval was subdivided into six 4-week periods in order that the effect of duration of dietary treatment (i.e. the effect of period) on semen variables could be studied in addition to the effect of treatment per se. Effects of ejaculation sequence and period x treatment interaction were also examined in the analyses of variance. Where F values for treatment, period or ejaculation sequence were significant ($P < 0.05$), Kramer's modification of Duncan's Multiple Range Test was applied to detect differences among individual means.

Results

Table 17. EFFECT OF LEVEL OF VITAMIN A, PERIOD OF COLLECTION AND SEQUENCE OF EJACULATION ON THE CHARACTERISTICS OF SEMEN AND ON THE LIBIDO RATING OF MATURE MALE RABBITS

Level of Vitamin A ($\mu g/W_{kg}$/day)	Libido Rating	Ejaculate Volume (ml)	pH	Per cent Motility (%)	Per cent Progressive Motility	Per cent Dead Sperm (%)	Per cent Abnormal Sperm (%)	Concentration of Sperm per cc x 10^6	Total Sperm per Ejaculate x 10^6
0	3.8^a	$.52^a$	7.1^a	54^a	2.1^a	27^a	27^a	342^a	282^{ab}
3	3.6^b	$.88^b$	7.3^b	46^b	1.8^b	40^b	26^{ab}	401^b	318^a
6	3.9^c	$.37^c$	6.9^c	56^a	2.2^c	31^c	25^b	478^c	248^{bc}
12	3.7^{ab}	$.65^d$	7.1^a	63^c	2.5^d	30^{ac}	18^c	285^d	216^c
Period of Collection									
1	3.7^a	$.73^a$	6.9^a	58^a	2.3^a	33^{ab}	18^a	370^a	293^a
2	3.6^a	$.68^{ab}$	6.9^a	56^{ab}	2.3^a	34^{ab}	21^b	385^a	284^a
3	3.4^b	$.62^{bc}$	7.1^b	51^b	2.0^b	35^a	21^b	369^a	278^a
4	3.6^a	$.53^{cd}$	7.1^b	52^b	2.0^b	32^{abc}	20^b	401^a	301^a
5	4.0^c	$.58^{bcd}$	7.2^b	55^{ab}	2.2^{ab}	29^c	27^c	326^a	217^b
6	4.0^c	$.48^d$	7.2^b	54^{ab}	2.2^{ab}	29^{bc}	35^c	361^a	187^b
Sequence of Ejaculation									
1	3.8^a	$.80^a$	7.0^a	54^a	2.1^a	33^a	26^a	482^a	400^a
2	3.7^a	$.57^b$	7.1^b	55^a	2.2^a	32^a	23^b	339^b	228^b
3	3.7^a	$.45^c$	7.1^b	54^a	2.1^a	32^a	23^b	285^c	153^c

Different superscripts within columns of each section indicate differences significant at $P < 0.05$.

The libido rating earned by male rabbits at the first ejaculation did not appear to vary appreciably at either the second or third ejaculation (Table 17). Libido did vary significantly, however, with dietary treatment and with period of collection ($P < 0.01$). There was also a significant ($P < 0.01$) period x treatment interaction contributing to the observed variation.

Males receiving the 6 μg vitamin A level were sexually more aggressive than those that consumed the 0, 3, or 12 μg vitamin A levels ($P < 0.05$). Mean comparisons showed that libido was stronger in the 0 μg level group males than in those on the 3 μg level, but differences in sexual interest between other groups were not significant. The overall effect of vitamin A level (treatment) on male libido appeared very inconsistent. The only really clear-cut relationship between libido rating and dietary vitamin A level is not shown by the statistical analysis. Buck 0M25, on the vitamin A deficient basal diet, developed clinical manifestations of vitamin A deficiency and died in week 29 of the semen collection period, 41 weeks after initial introduction of the deficient experimental diet. In the semen collection period just prior to death, this buck appeared emasciated and too weak and incoordinated to ejaculate, yet he twice attempted to mount the teaser doe. This observation suggested

that libido in mature males was retained even during severe physical incapacitation.

A comparison of libido means for the six collection periods showed a progressive decrease in libido for the first three periods, with the lowest rating for the whole experiment being observed in period 3 ($P < 0.05$). Sexual interest increased slightly in period 4 and rose even more in periods 5 and 6. The "rejuvenation" of male sexual vigor in the latter three periods may be a result of the lessened sexual activity which occurred in the 16-week period that was interposed between weeks 3 and 4 of period 4.

It was also observed that libido for the 0 μg level group was not significantly lower ($P > 0.05$) in any of the six individual periods studied than was the libido recorded for the most aggressive treatment group within that period.

A significant amount of the variation observed in all eight of the semen characteristics studied (Table 17) was attributable to treatment ($P < 0.01$). Period of collection contributed significantly ($P < 0.01$) to the variation observed in all semen variables except in spermatozoan concentration per cc of semen. Variations observed in per cent motility, progressive motility and per cent dead spermatozoa were not significantly ($P > 0.05$) influenced by the sequence of ejaculation. However, ejaculation sequence was found to

be a significant source of variation ($P < 0.01$) in the other five semen variables. Interaction between period and treatment was noted for all semen characteristics studied except for per cent dead spermatozoa.

Volume of semen varied significantly between ejaculates, regardless of the treatment imposed or the collection period observed. Mean semen volume for the first ejaculation was nearly twice as great as that for the third ejaculation, with volume for the second ejaculation being intermediate between the other two.

Although the mean volume of semen produced by each of the treatment groups was significantly different ($P < 0.01$) from that produced by each of the other treatment groups, there did not appear to be any consistent relationship between dietary vitamin A level and mean ejaculate volume. Mean ejaculate volume was highest in the 3 μg level group, followed by the 12, 0 and 6 μg level groups, respectively.

Semen production, considered over all treatments, was quantitatively greatest in period 1 and lowest in period 6. Period 1 represents an early portion of the experiment (8-12 weeks after introduction of experimental diets) while period 6 includes the final four weeks of the study (weeks 28-32). There appeared to be a relatively steady decline in semen production as the duration of the experiment

(number of periods) increased except in the case of periods 4 and 5. This may reflect the fact that period 4 included data from the first week in which semen collection was resumed following the interval of lessened sexual activity in the breeding period (i.e. the first week of semen collection period II).

Motile spermatozoan content of semen and progressive motility rating both increased significantly ($P < 0.01$) with each successive elevation of dietary vitamin A level from 3 to 6 to 12 μg. However, motile spermatozoa were less numerous ($P < 0.01$) in the semen of males on the 3 μg vitamin A level than in semen of bucks on the 0 μg vitamin A level. The progressive spermatozoan motility was correspondingly also lower in the 3 μg than in the 0 μg level group. One male in the 0 μg level group failed to show severe deficiency symptoms even at the end of the experimental period and this fact may account, in part, for the lesser rate of decline in seminal quality, as evidenced by the higher motility ratings.

Neither per cent nor progressive motility was appreciably affected by ejaculation sequence but both of these characteristics varied significantly ($P < 0.01$) with the period of collection. Per cent and progressive spermatozoan

motilities were lower in periods 3 and 4 than in the initial period but increased again in periods 5 and 6, following the breeding interval. The early trend toward decreased spermatozoan motility with increased duration of experimental treatment thus appeared to be somewhat counterbalanced by the period of reduced sexual activity (i.e. the breeding period). The lower spermatozoan motility in the semen of the 3 μg level group was reflected in the higher percentage of dead spermatozoa recorded for that group. There did not appear, however, to be any consistent relationship between level of dietary vitamin A and the dead spermatozoan content of the semen. It was of interest to note that dead spermatozoa were numerically least abundant in semen from the 0 μg level group. Statistical analysis revealed, however, that semen from 0 and 12 μg level groups did not vary significantly in dead spermatozoan content ($P > 0.05$). A similar absence of significance was noted for differences in dead spermatozoan content of semen from 6 and 12 μg level males. The dead spermatozoan content of semen reached its peak in collection period 3 and then declined, with the lowest levels being recorded in periods 5 and 6, just after the breeding period.

Males on the 12 μg level produced fewer abnormal

spermatozoa than did bucks on the lower levels of vitamin A supplementation. This suggests that a decrease in rate of abnormal spermatozoan production may be related to an increase in the level of dietary vitamin A consumed. This idea is consistent with the observation that morphologically abnormal spermatozoa were most numerous in the semen of 0 μg level males. The rate of abnormal spermatozoan production increased as the duration of the experiment progressed, with the lowest number of aberrant cells being observed in the initial period of the experiment (Period 1). The number of abnormal spermatozoa in the semen increased progressively in the subsequent periods, reaching a maximum in the final period (6) when the percentage of abnormal spermatozoa was nearly twice as great as that recorded for the initial period. Examination of data for all treatments in all periods indicated that initial semen ejaculates contained significantly ($P < 0.01$) more abnormal spermatozoa than did second or third ejaculates.

Spermatozoa concentration per cc of semen increased significantly ($P < 0.05$) with each successive rise in vitamin A intake from 0 to 3 to 6 $\mu g/W_{kg}/day$. The consistency of this pattern was marred by the fact that males on the highest level of vitamin A supplementation produced fewer spermatozoa per cc of semen than did any of the other

groups. However, it should be noted that one of the males in the 12 μg level group suffered from coccidiosis and bleeding feet during part of the semen collection period. It is quite possible that these afflictions were detrimental to the semen quality of this male. The lower spermatozoan concentration of the 12 μg level group could thus perhaps be attributed more to the poor physical condition of this rabbit than to dietary treatment. Spermatozoan concentration per cc of semen was observed to decrease progressively with successive ejaculations, regardless of the treatment imposed. The second quantitative measure of spermatogenesis was provided by calculating total spermatozoa per ejaculate, which represented the product of ejaculate volume and spermatozoan concentration per cc of semen. Since ejaculate volume was not consistently related to dietary vitamin A level and since spermatozoan concentration per cc was lowest in the 12 μg level group, it was not surprising that ejaculates from males on the 0 and 3 μg levels of vitamin A supplementation contained more spermatozoa than did ejaculates collected from bucks on the 6 and 12 μg vitamin A levels. The absence of any consistent relationship between total spermatozoa per ejaculate and dietary vitamin A level thus appeared to be due to the fact that variation in ejaculate volume was relatively independent of vitamin A level. The total

ejaculate content of spermatozoa did not vary appreciably in the first four periods studied but did decrease significantly ($P < 0.05$) in the last 8 weeks of the experiment (Periods 5 and 6). Examination of data for all treatment groups within all periods suggested that the total number of spermatozoa decreased progressively in successive ejaculations.

It was anticipated that there might be a relationship between spermatozoan concentration and hydrogen ion concentration (pH) since the acidity of semen is dependent upon the amount of fructolysis and lactic acid production that is carried on by the spermatozoa. Presumably this acid producing activity would be greatest in the semen samples that contained the highest numbers of spermatozoa. The lowest mean pH recorded for semen in the present experiment was a 6.9 reading for the 6 μg level group, the same group that showed the highest mean spermatozoan concentration per cc of semen. It is probable that dietary vitamin A level did not influence hydrogen ion concentration directly but rather exerted an indirect effect on pH through inducement of alterations in spermatozoan concentration. pH values for the other three treatment groups showed a less consistent relationship to spermatozoan concentration, with the highest pH actually being recorded for the group that had the second highest spermatozoan concentration per cc of

semen. As the duration of the experiment increased, a slight rise in seminal pH was observed to occur concurrently with a decrease in total spermatozoa per ejaculate, especially in periods 5 and 6. Initial ejaculates were slightly more acidic than those collected at second and third ejaculations, regardless of treatment or period involved. This finding was consistent with the previous observation that spermatozoan concentration was greatest in the first ejaculate.

Overall differential response to dietary vitamin A level appeared to be most consistent for the concentration of spermatozoa per cc of semen and for the percentage of morphologically abnormal spermatozoa. Responses to differential levels of vitamin A supplementation appeared more variable for the other seminal parameters studied.

The fertility of semen from all eight males studied was demonstrated by the fact that each male sired at least one litter (Table 18). Reproductive efficiency did not vary significantly among the four groups ($P > 0.05$), but did appear to be lower in the 12 μg level group than in any of the other three. It has already been mentioned, however, that one male on the 12 μg level was in poor physical condition during much of the experimental period. The poor health of this animal undoubtedly contributed to the poorer breeding performance of the overall 12 μg level group.

Table 18. EFFECT OF LEVEL OF VITAMIN A ON REPRODUCTIVE EFFICIENCY OF MATURE MALE RABBITS[a]

Level of vitamin A ($\mu g/W_{kg}/day$)	0	3	6	12
No. of males studied	2	2	2	2
Total no. of matings	8[b]	8	6[c]	8
Total no. of resulting pregnancies	7	6	5	4
Reproductive efficiency (%)	88	75	83	50

Reproductive efficiency did not vary significantly ($P > 0.05$) among the four groups.

[a]See Appendix Table 7 for data on individual males.

[b]The first four matings were carried out prior to the appearance of ocular lesions in the males while the last four were carried out after the initial onset of eye symptoms.

[c]One male (6M29) on 6 μg level died prior to the third mating.

Testicular weights generally provided a fairly accurate assessment of the spermatogenetic activity occurring within the testes, as verified by the later histopathological studies. Comparisons of treatment means suggested that testicular weight, as a percentage of body weight, increased with each successive elevation in the level of vitamin A intake (Table 19). The absence of statistically significant differences was not surprising in view of the limited number

Table 19. EFFECT OF LEVEL OF VITAMIN A ON TESTICULAR WEIGHTS OF MATURE MALE RABBITS[a]

Level of vitamin A ($\mu g/W_{kg}$/day)	0	3	6	12
No. of males observed	2	2	2	2
Mean time on diet prior to death ±S.E. (days)	286±14	300±0	230±67	297±0
Means for combined left and right testicular weights ±S.E. (mg %)[b]	131±60	143±38.4	158±2.5	175±4.1

Testicular weight did not vary significantly ($P > 0.05$) among the four groups.

[a]See Appendix Table 8 for data on individual males.

[b]mg % = mg of testicular weight per 100 g of body weight.

of animals observed and the wide within group variation noted. An excellent example of this variability between individual animals was provided by comparing the testicular weights of the two males in the 0 μg level group. Male 0M25 had the lightest testes of any rabbit in the experiment while male 0M26, by contrast, had the heaviest testicles of any rabbit in the study. Histological examination confirmed that the two males differed in their testicular response to the deficiency regime. Buck 0M25 died after 272

days on the experimental diet and exhibited severe atrophic testicular lesions (Page 126, Figure 10). Although occasional isolated spermatids and spermatozoa were detected, there was a general lack of organized spermatogenesis with spermatogonia frequently being the only detectable gametogenetic stage present in the degenerating tubules. Seminiferous tubules appeared reduced in size and their lumina were frequently filled with structureless, cytoplasmic material. Vacuoles were prevalent. Multi-nucleated, dark-staining fragments were regularly detected in the empty or debris-filled lumina. Occasionally the lumina were occluded by desquamated cells. Extensive degeneration of the germinal cell layer, disintegration of the tubular structure and disruption of spermatogenesis were all apparent in the seminiferous tubules of male 0M25. The second mature male in the 0 μg level group (0M26) failed to show these testicular lesions. Most of the seminiferous tubules appeared relatively normal in size and function (Page 126, Figure 11) when the animal was killed after 300 days on the deficient diet. All stages of spermatogenesis were present, with late spermatids and mature spermatozoa being abundant. Lumina were well defined in many tubules. Some desquamation of intermediary stage spermatozoan precursors was detected

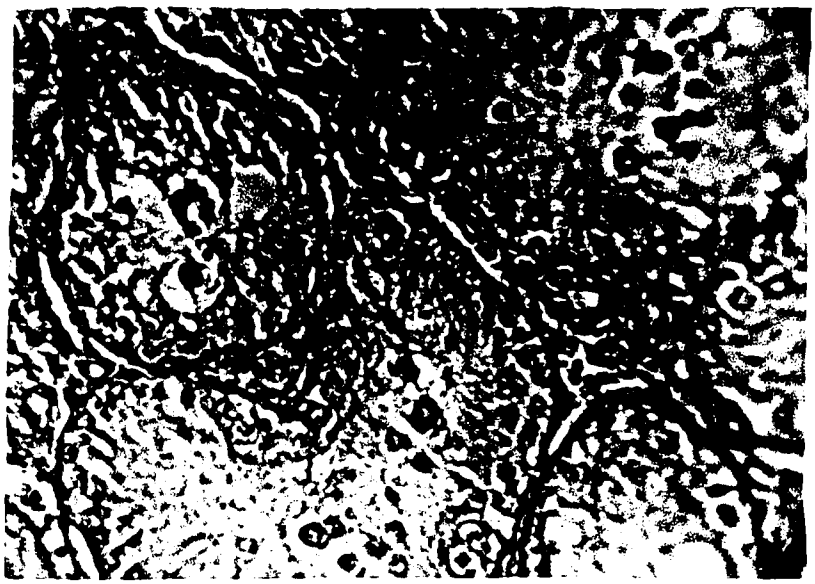

Figure 10. Phase-contrast photomicrograph showing tubular degeneration in the testes of mature male 0M25 after 272 days of vitamin A deprivation (Iron hematoxylin x 400).

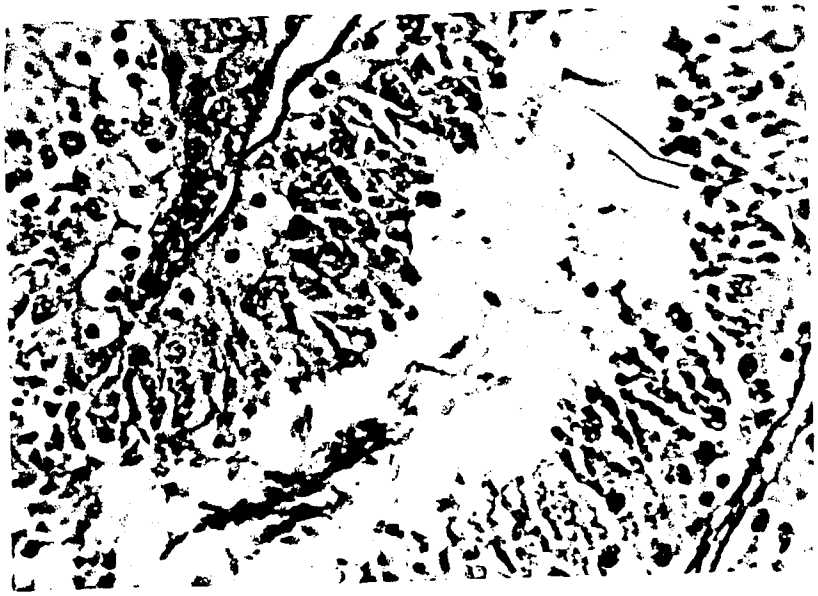

Figure 11. Phase-contrast photomicrograph showing relatively normal spermatogenesis in a seminiferous tubule from the testes of mature male 0M26 after 300 days of vitamin A deprivation (Iron hematoxylin x 400).

but this was probably within the normal expected range. Degenerate nuclei and ruptured basement membranes were detected infrequently. However, the majority of tubules appeared normal, with spermatogenesis being relatively complete.

Variability in the testicular weights of individual animals was also demonstrated in the 3 μg level group but the extent of the variation was less than that observed in the 0 μg level group. The similarity of the histopathological lesions observed in the testes of the two males also attested to the fact that individual animal variability was lower within the 3 μg level group. The chief testicular alterations are illustrated in Figure 12 (Page 128). Mature spermatozoa, spermatids, and pre-spermatozoan stages were all detected but in certain tubules there appeared to be an excessive desquamation of pre-spermatozoan cells, leading in some instances to partial occlusion and in other cases to enlargement of the tubular lumina. Desquamation of pre-spermatozoan stages is not in itself abnormal but the extensiveness of the exfoliation observed in the present study suggests that desquamation may exceed the normal level. Tubules were regular in size but occasionally contained degenerate fragments within the lumina. Membranal rupture and vacuole formation were also discernible in certain

Figure 12. Phase-contrast photomicrograph showing an excessive amount of desquamation in seminiferous tubules of a male fed the 3 μg level of vitamin A supplementation. Exfoliation is most pronounced in the two outer tubules. Note the dark staining desquamated fragment in the right tubule. Mature spermatozoa are detectable in the tubular lumina (Iron hematoxylin x 400).

Figure 13. Phase-contrast photomicrograph showing a "normal" rate of desquamation in other seminiferous tubules of the same male (as in Figure 12). Mature spermatozoa are abundant in the lumina (Iron hematoxylin x 400).

tubules. Aside from possible excessive desquamation, the seminiferous tubules appeared to be functionally normal (Page 128, Figure 13).

Seminiferous tubules of normal size were present in testicular sections from both males on the 6 μg level of vitamin A supplementation. Spermatogenesis was complete, as evidenced by the presence of mature spermatozoa in the tubular lumina. Limited desquamation was noted in testicular sections from one of the males (0M30) but it is doubtful if this exfoliation exceeded the normal level. Occasionally luminal occlusion or enlargement resulted from this desquamation. Vacuolization and membranal rupture also occurred but were infrequent. Pre-spermatozoan stages appeared abundant. Much more extensive desquamation was observed in testicular sections from the second male on the 6 μg vitamin A level (6M29). This animal died some hours previous to tissue fixation and consequently the degree of desquamation may have increased as a result of post-mortem autolytic changes. The desquamated cells that commonly occluded the tubular lumina were frequently characterized by dark-staining nuclei. There was considerable intertubular variation with respect to the degree of desquamation. Mature spermatozoa were abundant and frequently appeared to be mixed with the exfoliated cells in the

tubular lumina.

Testicular sections from rabbits on the 12 μg vitamin A level demonstrated relatively normal spermatogenesis, with all stages being represented in some of the seminiferous tubules (Page 131, Figure 14). Tubules were of normal size although the lumina were frequently enlarged, particularly in male 12M31. Limited desquamation was again noted but appeared to be within the normal range of expectation. Lumina were occasionally occluded by the desquamated cells. There appeared, however, to be little detectable functional difference between the seminiferous tubules studied in the 12 μg level males and those observed in male 6M30 of the 6 μg level group. In both groups, satisfactory testicular function was indicated by active and complete spermatogenesis.

EXPERIMENT 3. EFFECT OF 0, 3, 6 and 12 μg LEVELS OF VITAMIN A ON THE REPRODUCTIVE PERFORMANCE OF FEMALE RABBITS AND ON THE PATHOLOGICAL DEVELOPMENT OF THEIR OFFSPRING

Seven of the does observed in the post-growth phase of Experiment 1 were simultaneously used as subjects to study the effect of vitamin A level on female reproduction. The rabbits continued to consume the experimental diets ad

Figure 14. Phase-contrast photomicrograph of a functional seminiferous tubule from the testis of a mature male fed the 12 μg level of vitamin A supplementation. Active spermatogenesis is indicated by the abundance of pre-spermatozoan stages and by the presence of mature spermatozoa in the lumen (Iron hematoxylin x 680 (approx.)).

libitum throughout this period of reproductive study. Two does on the 3 μg vitamin A level, two on the 6 μg level and three on the 12 μg level were included in the total of seven females observed. One additional surviving female in the 6 μg level group was excluded from the study because of her extremely poor physical condition.

The seven does were bred at about six and one half months of age. Each doe was force-mated at least twice at each breeding period, often to two different males. Nest boxes and nesting materials were provided a few days prior to the anticipated parturition date. Females that failed to kindle were rebred promptly as were does that lost their first litter. In subsequent gestation periods, however, does were not rebred until two weeks after the loss of the litter. Surviving litters were generally weaned at four weeks of age. Dams of these weaned litters were then permitted a two week "rest" period prior to remating.

Over the whole duration of the experimental period, each doe was mated on at least two separate occasions. Several does were bred three times and one individual was actually mated on five different occasions. Variation in the number of breedings for individual does resulted from differences in their individual conceptual and lactational performances. Doe reproductive performance was evaluated

on the basis of the following parameters:

1) Total number of young born.
2) Average number of offspring per parturition.
3) Total number of young alive at birth.
4) Total number of young alive at 14 days.
5) Total number of young alive after 21 days.
6) Condition of the young at time of death or at 21 days of age.
7) Per cent reproductive efficiency (total no. times kindled/total no. times bred).

Post-Mortem Procedures

At the conclusion of the study, surviving females were killed by cardiac puncture. Ovaries and uteri were excised and weighed. Whole ovaries and sections of the uterine horns were fixed in Bouin's solution for histological examination. Organs from females that died in either the growth or reproductive phases of this study were treated in the same manner.

Histological Procedures

Ovarian and uterine cross sections were subjected to the same preliminary histological procedures as have been described for the testicular sections. Both ovarian and uterine sections were stained with Heidenhain's iron hematoxylin. Uterine sections were counterstained with eosin (Table 20) immediately following the staining process.

Table 20. SUMMARY OF STEPS INVOLVED IN THE COUNTERSTAINING OF UTERINE SECTIONS WITH EOSIN YELLOW

Medium	Duration of Time
Tap water (with lithium carbonate and ammonia)	5 min.
Acid alcohol	Rinse
Tap water (with lithium carbonate and ammonia)	5 min.
Ethanol (70%)	1 min.
Ethanol (95%)	1 min.
Eosin Yellow	12 sec.
Ethanol (95%)	Rinse
Ethanol (95%)	Rinse
Ethanol (95%)	Rinse
Absolute alcohol	1 min.
Xylene	10 min.
Xylene	10 min.

Interpretation of Sections

1. Ovaries

Ovarian sections were examined for presence and stage of follicular development. Corpora lutea were noted when present.

2. Uteri

Uterine sections were interpreted on the basis of endometrial changes. Individual sections were examined for evidence of:

a) keratinization;
b) metaplasia of the tall columnar cells lining the endometrial surface and uterine glands, to a squamous or cuboidal type of cell which might be suggestive of early keratinizational changes;
c) hyperplasia of the cells lining the endometrial surface and particularly of those lining the uterine glands;
d) desquamation of cells lining the uterine glands either on the surface or in the internal portion of the endometrium;
e) occlusion of uterine glands resulting from (c) and (d);
f) excessive mucus secretion, possibly resulting from irritation due to the deficiency, or complete absence of exudate, possibly associated with keratinization.

The presence of these changes was considered to be suggestive of vitamin A inadequacy. The does were assessed on the basis of the changes observed in the 16 to 24 sections studied from each of the individual females.

Statistical Procedures

The various parameters used to assess female reproductive performance were all compared on a percentage basis. The transformed data (arcsin $\sqrt{\text{percentage}}$ transformation) for each of these parameters was subjected to an analysis of

variance for groups with unequal replication (Steel and Torrie, 1960).

Ovarian and uterine weights for the four treatment groups were expressed on a mg % basis but were not compared statistically since there was wide variation in the reproductive status of the females involved.

Results

Table 21. EFFECT OF LEVEL OF VITAMIN A ON PARAMETERS OF REPRODUCTIVE PERFORMANCE IN FEMALE RABBITS[a]

Level of vitamin A ($\mu g/W_{kg}$/day)	0	3	6	12
No. of females studied	0[b]	2	2	3
Total no. of times bred	-	8	7	9
Total no. of times kindled	-	2	6	7
Total no. of young born	-	11	41	49
Average no. of offspring/ parturition	-	5.5	6.8	7.0
Total no. of young alive:				
at birth	-	3 (27%)	33 (81%)	44 (90%)
at 14 days	-	0 (0%)	6 (15%)	29 (59%)
after 21 days	-	0 (0%)	5 (12%)	27 (55%)
Reproductive efficiency (%)	-	25	88	78

Differences among treatment means were not significant ($P > 0.05$) for any of the parameters studied.

[a]See Appendix Table 9 for data on individual females.
[b]All females on the 0 µg level succumbed prior to breeding age.

The overall reproductive performance of female rabbits appeared to improve progressively as the level of vitamin A supplementation was increased (Table 21). Since the number of females that comprised each treatment group was small, significant differences among treatment means for the various parameters were not observed. Nevertheless, existing numerical differences were quite well-defined and suggestive of trends.

Reproductive efficiency was lowest (25%) in the 3 μg level group but did not vary markedly between the 6 and 12 μg level females (88% and 78%, respectively). Female 3F10, in the 3 μg level group, was bred five times and kindled twice. Of the five young born in the first litter, two were dead and the other three succumbed shortly thereafter. The doe died just after giving birth to six offspring in her second litter. These, too, were dead when first observed. Nest making was not attempted in either instance and the young were consequently born on the wire floor of the cage. Most of the young appeared hydrocephalic and two showed cranial hemorrhage. Ocular abnormalities were not confidently detectable at this early stage of development. The second doe in the 3 μg level group (3F11) failed to produce any young, living or dead, although she was bred on three separate occasions.

The two does in the 6 μg level group (6F16 and 6F18) each kindled three times after being mated four and three times, respectively. Of the 20 young born to 6F16, 12 were alive at birth while only one survived longer than two weeks. Doe 6F18 gave birth to 21 young, all living. Of these, only five were alive at two weeks of age and four at three weeks of age. Only two offspring from dams on the 3 μg level survived until either weaning or slaughter. Hydrocephalus and ocular abnormalities (e.g. corneal opacity, microphthalmia and anophthalmia) were the most consistent findings in the young rabbits. Ocular anomalies were not conclusively discernible in the very young rabbits. "Dome-shaped" heads were frequent, ostensibly due to increased cerebrospinal fluid pressure in the hydrocephalic rabbits. In some cases this pressure was sufficient to cause apparent "flattening" of the cranial lobes. Retraction and partial paralysis of forelimbs, poor coordination and convulsions were observed as the condition of the young rabbits deteriorated. Hemorrhages in cranial, nasal and peritoneal cavities occurred. Enteritis, pulmonary lesions, abdominal distension, and petechial nephritic hemorrhages were detected in individual animals. Extensive yellowish spotting on the liver of one rabbit suggested hepatic coccidiosis.

The three does in the 12 μg level group (12F22, 12F23

and 12F24) were each bred on three separate occasions. Females 12F23 and 12F24 each kindled twice while 12F22 bore litters thrice. Of the 49 young born to these three rabbits, 44(90%) were alive at birth. The lifespan of young rabbits increased progressively as the level of vitamin A supplementation was elevated. Fifty-nine per cent of the young born to dams on the 12 μg vitamin A level were still alive at 14 days of age, as compared with 15% of the young from dams that received the 6 μg level. The percentage of offspring alive at 21 days of age was similarly higher for the 12 μg level group (55% versus 12%). None of the offspring from females on the 3 μg vitamin A level survived to two weeks of age. Deaths after 21 days of age claimed three of the five surviving rabbits in the 6 μg level group and six of the 27 living offspring in the 12 μg level group.

Young rabbits from dams receiving the 12 μg level of vitamin A grew more rapidly than those whose mothers received the 6 μg vitamin A level (Page 140, Figure 15). All offspring that lived long enough to be examined showed various ocular lesions. Corneal opacity was common. In some rabbits the eyes appeared glassy with pearl-like lenses while in others, the eyes showed a distinct greenish tinge, suggestive of ocular hemorrhage. In addition, exophthalmia,

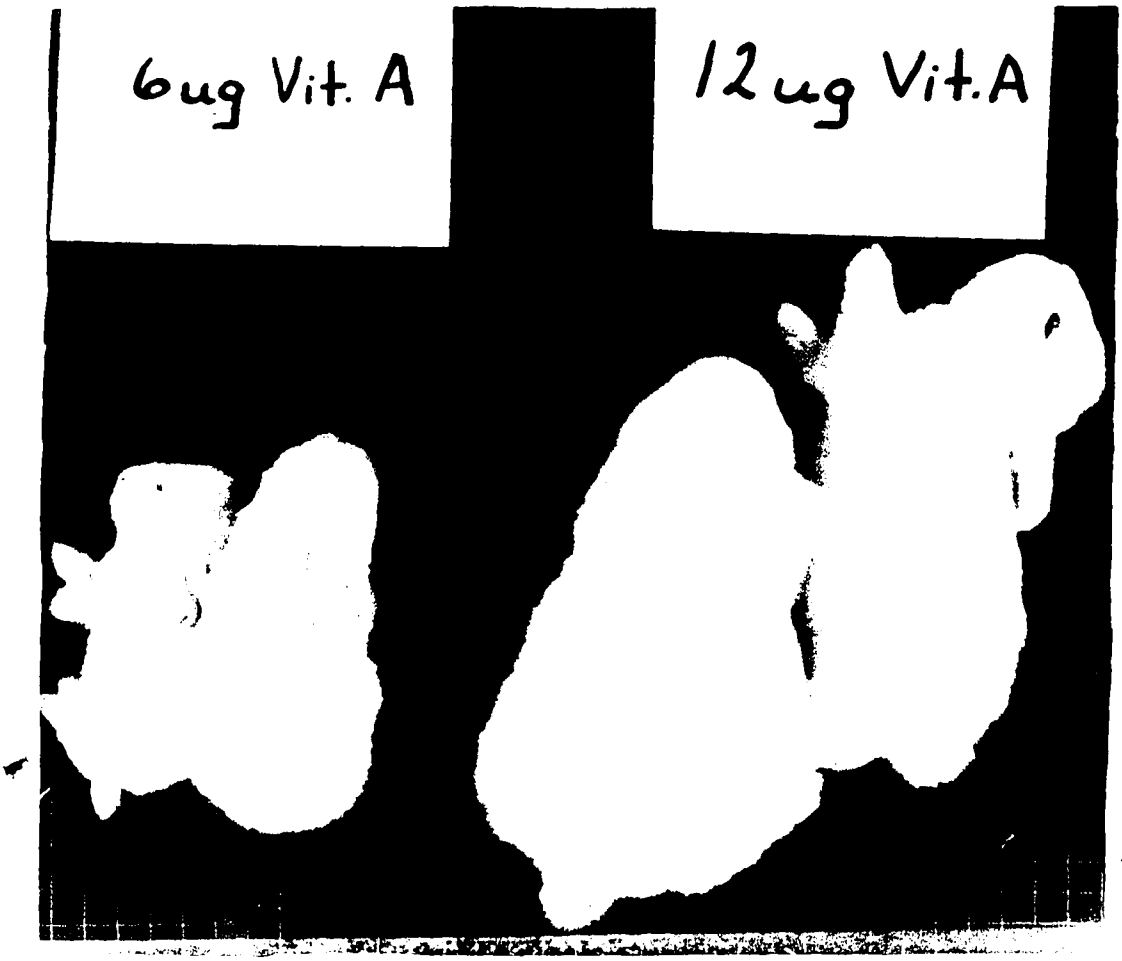

Figure 15. Differential growth of young rabbits whose dams received the 6 and 12 μg levels of vitamin A supplementation. The four rabbits shown are all of the same age. Note the "dome-shaped" head and ocular abnormality in the rabbit on the extreme left. One rabbit in each group showed severe head retraction and incoordination and thus had to be placed in a prostrate position for photographing.

microphthalmia and anophthalmia were all observed in individual instances. Hydrocephalus was evident in many of the offspring. Scours, hemmorrhage of the cranial and peritoneal cavities, incoordination, partial paralysis, and pulmonary ecchymoses were also observed in the offspring. The appearance of pathological abnormalities in these young rabbits provided strong evidence that the highest level of vitamin A supplementation used in the present experiment was inadequate to support satisfactory reproductive performance in female rabbits.

Mean ovarian weights, recorded at death, did not vary appreciably among the 3, 6 and 12 μg level groups (Table 22). Ovarian weight appeared lower in the 0 μg level group but this was probably due to the fact that females in this group died at an earlier age and had not been bred at the time of death. Mean uterine weights appeared to be heavier for the two groups on the higher levels of vitamin A supplementation but this again was probably a reflection of the differences in reproductive status that existed among the treatment groups.

Histological examination of ovarian sections revealed that all stages of follicular development were present in does from each of the four treatment groups studied.

Table 22. EFFECT OF LEVEL OF VITAMIN A ON OVARIAN AND UTERINE WEIGHTS OF FEMALE RABBITS[a]

Level of vitamin A ($\mu g/W_{kg}$/day)	0	3	6	12
No. of females studied	3	3	3	3
Age at death (days)	164	275	329	366
Combined ovarian weight±S.E. (mg %)	7.8±2.0	17.8±9.2	13.7±6.9	16.0±4.5
Uterine weight ± S.E. (mg %)	125±24.1	113±25.8	186±83.2	180±69.7

[a]See Appendix Table 10 for additional data on individual females.

Primary and secondary follicles, as well as mature or Graafian follicles were observed (Page 143, Figure 16). Intermediate maturing follicles were present in other sections. The vitamin A level did not, therefore, appear to affect any particular stage of follicular development. Corpora lutea (Page 143, Figure 17) were also observed in does on all levels of vitamin A supplementation. Follicular atresia (Page 144, Figure 18) was suspected in animals on all dietary regimes but the thinness of the sections made it difficult at times to distinguish true degenerative changes from those occurring as artifacts of the sectioning. Comparisons based on the relative frequency of the various ovarian

Figure 16. Phase-contrast photomicrograph of a cross section of ovary from a young female that died after 130 days on the 0 µg level of vitamin A. Primary and secondary follicles are present. A large mature follicle with ovum is shown in centre (Iron hematoxylin x 400).

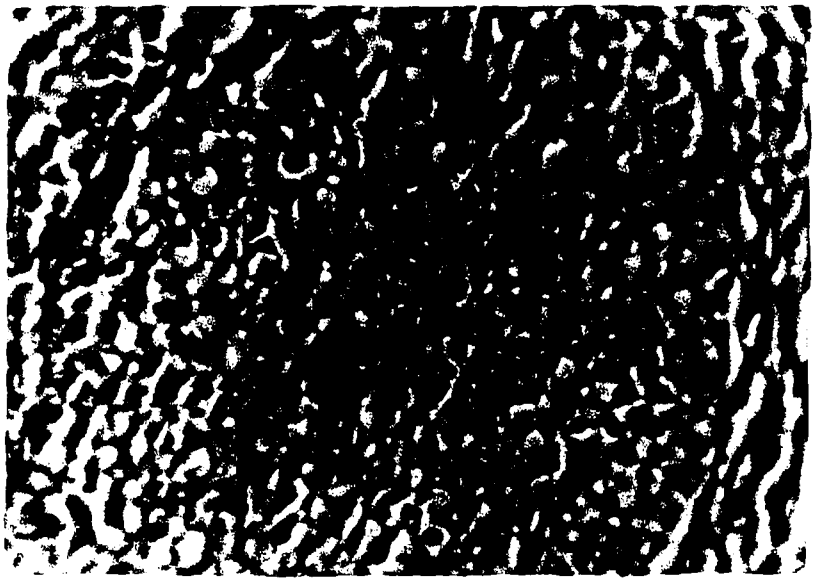

Figure 17. Phase-contrast photomicrography of a regressing corpus luteum from the ovary of a female that died after 223 days on the 6 µg level of vitamin A (Iron hematoxylin x 400).

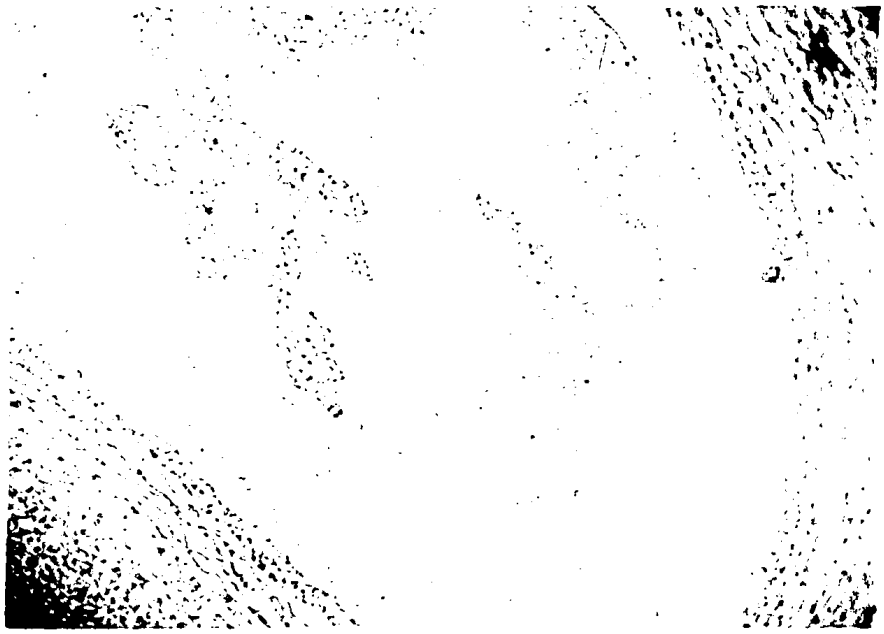

Figure 18. Phase-contrast photomicrograph of an atretic follicle from the ovary of a female that died after 334 days on the 3 μg level of vitamin A (Iron hematoxylin x 100).

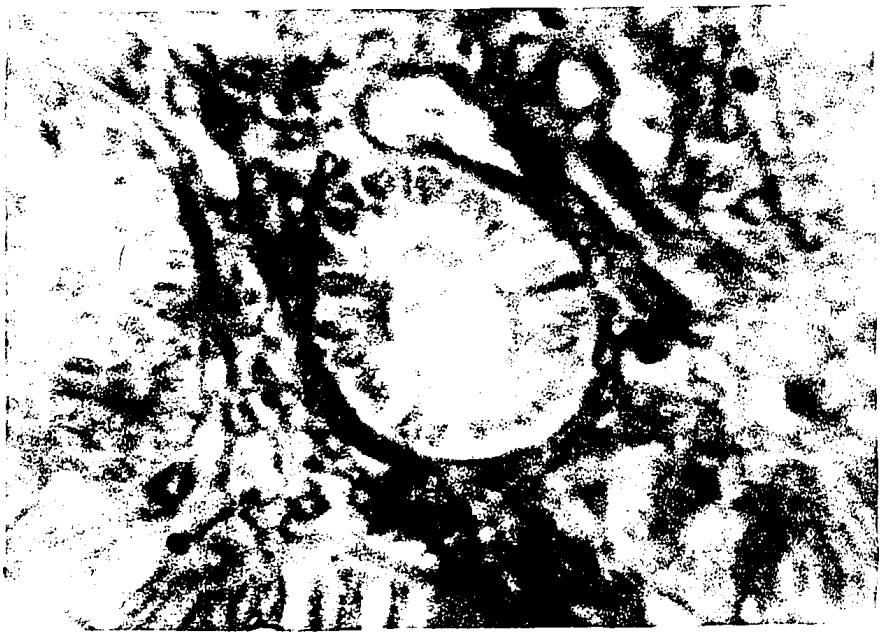

Figure 19. Phase-contrast photomicrograph of a cross section of uterine horn (cervical end) from a female fed the 6 μg level of vitamin A for 366 days prior to death. Note the normal ciliated columnar cells at the bottom left hand edge of the photo. There is also evidence of limited desquamation in the internal uterine glands (Iron hematoxylin and Eosin x 630).

follicular types in females on the four dietary regimes were not made but might have proved useful in detecting quantitative dissimilarities. However, age differences at the time of death and resultant variations in ovarian development might nullify much of the benefit to be derived from this procedure.

There was no evidence of extensive uterine keratinization in any of the does studied, regardless of their dietary affiliations. Sections of uterine horn from the three females in the 0 μg level group showed extensive desquamation of the cells lining the endometrial surface and the uterine glands. Some glands were completely occluded by the exfoliated cells while in others only the mouths were occluded, with the inner glandular lumina remaining essentially intact. Other cells showed complete cellular denudation. Excessive mucus production was evidenced as a solid pink staining mass in some sections, perhaps suggesting a cellular attempt to compensate for irritation, possibly induced by lack of vitamin A. Early stages of cornification or isolated patches of keratinization were discerned in uterine sections from two of the females in the 0 μg level group (Page 146, Figure 20). In other spots, cellular metaplastic alterations appeared to be occurring under the surface layer of epithelial cells. Some cells appeared light

Figure 20. Phase-contrast photomicrography of a cross section of uterine horn (mid-portion) from a young female fed the 0 μg level of vitamin A for 130 days prior to death. Note the patch of keratinization between the arrows (Iron hematoxylin and Eosin x 1050 (approx.)).

staining and it was conjectured that they might be changing away from the columnar, ciliated type of cell which normally lines the endometrial surface and glands. It must be emphasized that keratinization and cellular changes were scattered and occurred only at isolated points with no evidence of extensive overall uterine keratinization. Some of the other described changes may, however, have suggested a predisposition toward cornification.

Uterine sections from the three individual rabbits in the 3 μg level group were not strictly comparable since two of the rabbits were much older when examined than was the third. The young doe had fewer internal uterine glands and showed less uterine proliferation than did her older group mates but the histopathological alterations appeared much the same in both age groups. Cellular desquamation was prominent both in surface and in internal uterine glands. Occlusion of uterine glands occurred, sometimes due to desquamation and at other times due to apparent metaplastic or hyperplastic changes. A few of the lining cells appeared cuboidal or squamous rather than columnar but the distinction was not nearly as clear-cut as that observed in the 0 μg level group (Page 146, Figure 20). Mucus production was particularly evident in sections from the uterus of the younger doe in the 3 μg level group.

Tall, ciliated columnar-type cells (Page 144, Figure 19) lined the surface and glands of the endometrium in most of the uterine sections taken from females that received the 6 μg level of vitamin A supplementation. Occasional light staining cells were detected and in isolated spots cuboidal or squamous cells appeared to replace their columnar counterparts but in most instances cells were columnar in type. Desquamation of internal uterine gland cells was detected. In some uterine glands, partial or complete occlusion was noted while in others, the lining cells were columnar and orderly. In a few places, cells appeared heaped up into small piles but this state was usually associated with exfoliation or proliferation, and even then the outer lining cells were frequently of the columnar type.

The histological uterine picture was essentially similar for all three rabbits on the highest level of vitamin A supplementation (12 μg level). The vast majority of the endometrial surface and internal uterine glands were lined by tall columnar, ciliated cells. Limited desquamation was in progress in some internal and surface uterine glands. Other internal glands were lined by a regular layer of columnar cells. Light staining cells were occasionally detected and a heaping up of cells was also noted in rare

cases. Mucus production appeared limited. Overall changes observed were not different from those observed in females of the 6 μg level group.

There was thus little overall histological evidence to suggest that poor female reproductive performance might be attributed to uterine keratinization since even the vitamin A deficient does of the present study exhibited only isolated patches of uterine keratinization.

V. DISCUSSION

INTRODUCTION

Although the literature is well documented with reports on the biochemical mode of action of vitamin A, on the pathology of vitamin A deficiency and on the minimal vitamin A requirements of other mammalian species, there does appear to be a lack of information on the minimal vitamin A requirements of the rabbit. In view of the growing importance of the commercial rabbit industry, it is rather surprising that systematic investigations designed to ascertain minimum vitamin A levels have not been carried out previously in rabbits. The only early rabbit study that was directly pertinent to this undertaking was that of Phillips and Bohstedt (1938). Their work showed that growth and reproduction were satisfactory in rabbits fed β-carotene at the rate of 50 $\mu g/W_{kg}/day$. However, Phillips and Bohstedt were more interested in obtaining an adequate level of vitamin A than a minimum one. Since species vary in their ability to convert carotene to vitamin A, there was an additional problem in trying to determine what level of vitamin A was equivalent to a β-carotene level of 50 $\mu g/W_{kg}/day$ in rabbits.

Guilbert et al. (1940) have suggested that a vitamin A level of 4-6 μg/W_{kg}/day is minimal for growth and well-being in most mammalian species. Their studies further indicated that the minimum vitamin A requirement for reproduction was about three times as great as that for growth. The present work was undertaken with an aim towards establishing specific minimum vitamin A requirements for growth, health and reproduction in rabbits of both sexes. It would thus be possible to determine whether the vitamin A requirements of rabbits were consistent with those recommended for other mammalian species.

GROWTH

Any comprehensive evaluation of overall growth performance necessitates the examination of two variables - rate of gain and feed consumption.

Overall growth performance in rabbits on the vitamin A depleted ration (0 μg level) was generally inferior to that of rabbits on the vitamin A supplemented levels (3, 6 and 12 μg levels). In most instances these differences were apparent by week 17 when statistical analyses were performed but they became more pronounced in the following weeks. Because of the small number of animals in each treatment group and the wide within-treatment variation in some cases,

statistical significances were frequently lacking, even though clear cut numerical mean differences were obvious.

It is possible that the lower rate of gain observed in the 0 μg level group may be at least partially attributable to the lower feed consumption of that group. This would support the proposal of Orr and Richards (1934) that growth depression in vitamin A deficiency was the result of depressed appetite rather than of any direct anabolic effect of the vitamin inadequacy. It is not possible to determine from the present study whether the observed growth depression was entirely due to lowered feed intake resulting from inappetence or whether a direct depressant effect of the vitamin A inadequacy was also involved. The fact that the feed consumption of 0 μg level rabbits was lower than that of 3, 6 and 12 μg level rabbits as early as week 3 of the growth period appears to indicate that there was at least some depression of appetite in rabbits fed the deficient ration. Regardless of the mechanism involved in bringing about this growth retardation, the paramount fact remains that growth depression and subsequent weight loss did occur in the vitamin A deficient rabbits of the present study, just as it had occurred in the A-avitaminotic rabbits of previous investigations. Since body weight is dependent upon rate of gain, the lower mean body weight for the 0 μg

level group was actually a reflection of the lower rate of gain observed in that group of rabbits.

For each successive increase in vitamin A supplementation above the deficiency level, there was an observable increase in feed consumption. This may indicate a successive decline in appetite depression as the vitamin A level rises progressively above the deficiency range. The practice of ad libitum feeding in this study removed any restriction on the voluntary intake of feed by the rabbits. As a result the feed consumption of rabbits in both the 6 and 12 μg level groups was greater than that theoretically anticipated at the time when the vitamin A contents of the diets were originally calculated. This greater feed consumption meant that rabbits in the 12 μg group actually consumed vitamin A at a level higher than the intended 12 μg/W_{kg}/day. Over the total 24 week period, these rabbits probably received closer to an average level of 14 μg/W_{kg}/day, depending on the amount of feed that they actually consumed. Rabbits on the 6 μg level may have received closer to 7 μg/W_{kg}/day since their feed consumption, as a group, was also slightly in excess of expectation. However, there were wide individual rabbit differences with respect to feed intake in the 6 μg level group.

As feed consumption increased, rate of gain and body weight also increased, with the degree of response being proportional to the level of vitamin A in the ration consumed. It thus appeared that body weight variations in rabbits receiving different levels of vitamin A might be explainable on the basis of differential feed consumptions. This observation suggests that there may be some effect of vitamin A level on either the suppression or stimulation of appetite in rabbits on the 3, 6 and 12 μg levels. Coward et al. (1931) have suggested that the growth response of rats is directly related to the vitamin A level provided. Body weight data for rabbits in both growth and post-growth phases of the present experiment appear to support this hypothesis that weight increase is proportional to dietary vitamin A level. It is possible that the rate of vitamin A absorption from the intestinal tract of the rabbit may be directly proportional to the concentration of vitamin A administered as has been suggested by Reifman et al. (1943) in rats.

Feed efficiency may be defined as the number of units of feed consumed per unit of body weight gained. It is therefore clearly dependent upon both feed consumption and rate of gain. Cumulative feed efficiency at the end of week

17 was poorer in the 0 μg level group than in the 3, 6 or 12 μg level groups, although differences did not reach significance. The poorer feed efficiency of the 0 μg level group was at least partially attributable to one individual rabbit that consumed over 15 grams of feed for each gram of weight increase. This one animal raised the group mean considerably, although even among the other five animals there was some variation. This decrease in feed efficiency must be attributed to reduced rate of gain and eventual loss of weight in the period just prior to week 17, since feed intake appeared to be voluntarily reduced at this time. Mayer and Krehl (1948) observed that decreased feed efficiency was the earliest indication of avitaminosis A in rats. With rabbits in the present study, weight loss was first observed in the 0 μg level group at week 14 while mean cumulative feed efficiency did not drop below that of the other three treatment groups until week 16. Prior to week 16, all groups had shown a progressive decrease in feed efficiency, but this decrease was a natural consequence of growth stage advancement. At first glance, it would appear that weight decreased before feed efficiency declined. This sequence would be contrary to the findings of Mayer and Krehl. However, their calculations of feed efficiency were based on four day

periods and hence were non-cumulative, whereas those in the present study were expressed on a cumulative basis, embracing the whole period. It is thus conceivable that if feed efficiencies had been calculated for individual weeks on a non-cumulative basis in the present study, then decreased feed conversion efficiency for the 0 μg level group might have been detected earlier and perhaps even have been the initial indicator of avitaminosis A in the rabbits studied.

Although there were no significant differences among feed efficiencies for the 3, 6 and 12 μg level groups at the end of week 17, it was of interest that the numerical feed efficiency for the 3 μg level group was superior to that of the 6 μg level group and about on par value with that of the 12 μg level group. This observation may be explained by the fact that true vitamin A deficiency was not established in the 3 μg level group until the latter part of the 17-week period considered in analyses. Growth rate was definitely depressed once the deficiency was established, but since growth rate evaluation was on a cumulative basis covering the whole 17-week period, the actual drop in growth rate toward the end of the period was overshadowed in the cumulative mean by the relatively superior growth rate that prevailed in earlier weeks of the period. Thus growth rate was dropping at week 17 but the

cumulative growth rate mean for the whole 17-week period failed to show the actual magnitude of this late period drop. This factor, coupled with the lower cumulative feed consumption of the 3 μg level group, resulted in feed conversion efficiency being higher in the 3 μg level group than in the 6 μg level group at the end of week 17 and even at the end of the whole 24-week growth period.

Since initial continuous growth deceleration occurred two weeks earlier in the 0 μg level group than in the 3, 6 and 12 μg level groups, it is not surprising that body weight decline occurred earliest in the 0 μg level group. Initial body weight loss in the 0 and 3 μg level groups was frequently accentuated by sequential pathological lesions. In the 0 μg level group, ocular lesion appearance and initial weight loss closely parallelled one another on a time sequence basis. In none of the six rabbits studied was there more than a two-week period separating the initial appearances of these two parameters in any individual animal. Within the 0 μg level group, there was remarkable uniformity among animals in the time of onset of ocular lesions (95-105 days) and in the time of initial body weight loss (14-16 weeks). The overall group values for the 0 μg level show that growth inhibition was first noted at week 14 (98 days) while ocular lesions initially appeared, on the average, 99 days after the

introduction of the vitamin A deficient diet. These values are in good agreement with the periods of time observed by Hetler (1934), Mellanby (1935), Rao (1936), Phillips and Bokstedt (1938) and Pirie and Wood (1946). The present period was longer than the 2 month periods of Nelson and Lamb (1920) and Mann et al. (1946) but shorter than the 4-8 months observed by Perlman and Williard (1941). The breed of rabbit, age at initiation of diet, vitamin A activity of previous dietary regime and the nature and purity of the deficient diet may all be factors contributing to these differences.

Onset of ocular lesions, like body weight decline, was delayed as the level of vitamin A supplementation increased from 0 to 3 $\mu g/W_{kg}/day$. In the 3 μg level group, ocular lesions first appeared at 126-161 days ($\bar{x}$ = 138), following initial body weight decline which was detected for the 3 μg level group at 17 weeks (119 days). Time of ocular lesion appearance was far less uniform in the 3 μg level group than in the 0 μg level group. These differences are in line with the individual within-treatment variation observed by Williams and Pelton (1966) in rats. These workers suggested that individual differences in absorption, storage or retention of the vitamin could be responsible for the response differences noted. It would appear from this study

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that low level vitamin A supplementation resulted in a greater individual animal variability within treatment than did the complete absence of such supplementation.

Although the time of ocular lesion onset was delayed by increasing the level of vitamin A from 0 to 3 $\mu g/W_{kg}$/day, the incidence of eye lesions was not reduced since all 12 animals on 0 and 3 μg levels eventually exhibited ocular abnormalities of varying severity. While ocular lesions in the present study were prominent, they were probably less severe than those reported in some of the previous investigations where extreme xerophthalmia and conjunctivitis were noted. It is speculated that the eye lesions of the rabbits in the present study would have progressively intensified if the animals had survived for longer periods of time.

The percentage of animals exhibiting eye lesions decreased progressively as the vitamin A supplementation was raised from 3 $\mu g/W_{kg}$/day to the 6 and 12 μg levels. Of the four rabbits on the two higher levels that did show ocular lesions in the post-growth period, three developed symptoms only after extended periods of stress - successive pregnancies in two instances and severe illness in the third.

In addition to growth depression, weight loss, inappetence and ocular lesions, the deficiency syndrome in rabbits on 0 and 3 μg levels of vitamin A was also characterized

by varying degrees of incoordination, as well as by respiratory disorders. These symptoms were essentially similar to those reported in vitamin A deficient rabbits by other workers. Whether the nervous lesions observed in the present work were attributable to bone overgrowth (Mellanby, 1941, 1947), to bone undergrowth (Aberle, 1934; Wolbach and Bessey, 1940) or to increased cerebrospinal fluid pressure (Jubb and Kennedy, 1963) was not determined.

Improvement in survival and longevity accompanied consecutive increases in dietary vitamin A level. All six animals on the 0 μg level were dead by the end of the growth period (Week 24), whereas all six animals on the 12 μg level were still alive at the conclusion of the post-growth period (Weeks 46-48). The slight decrease in mortality observed as vitamin A level increased from 0 to 3 $\mu g/W_{kg}/day$ was accompanied by a significant increase in lifespan.

A further increase in vitamin A level from 3 to 6 $\mu g/W_{kg}/day$ was accompanied by an improvement in viability of nearly 70%. These observations are in keeping with those of Paul and Paul (1946) who demonstrated that ocular lesion incidence was reduced and lifespan increased in rats when the level of vitamin A supplementation was increased.

There did not appear to be any consistent relationship between length of time on dietary treatment prior to ocular

lesion onset and the duration of time on treatment prior to spontaneous death, within individual rabbits. Within the 0 μg level group, the first two rabbits to exhibit ocular lesions (at 95 and 98 days) were also the first to succumb at 116 days post-weaning. On the other hand, the last rabbit to show ocular anomalies (at 105 days) died at 130 days post-weaning which was earlier than the times of death of two other rabbits which showed eye lesions after 98 and 99 days on the diet. These differences are again probably attributable to individual variations, perhaps in original resistance of the animal to secondary infections.

Deaths in vitamin A deficient rabbits were most frequently attributable to secondary bacterial or parasitic invasions, manifested particularly in pneumonia and coccidiosis. This increased susceptibility to bacterial and parasitic infection probably reflects impairment in the integrity of the epithelial membranes, particularly of the respiratory and intestinal tracts. It has been postulated that vitamin A is required, probably as an enzyme cofactor, for the synthesis of mucopolysaccharides which are important constituents of mucus membrane epithelia. Depression of constituent mucopolysaccharide synthesis, attributable to the absence or inadequacy of the vitamin A cofactor, could thus result in disruption of the integrity of the epithelial

membranes, rendering them accessible to secondary parasitic and bacterial invasions. This theory supports the hypothesis of DeRuyter and Rosenthal (1936) that primary manifestations of vitamin A deficiency arise from atrophic alterations in mucus membrane epithelia.

Overall growth performance and health pattern were generally better in rabbits receiving vitamin A at the 12 μg level than in those receiving 0, 3 or 6 $\mu g/W_{kg}/day$. A valid question arises from this observation - Was the growth response of rabbits on the 12 μg level ration as satisfactory as the response that would have been obtained with the feeding of a stock colony diet? This question cannot be fully answered on the basis of the present evidence but it is worthy of some speculation.

Growth rate for the 4-8 week period in rabbits on the 12 μg level ration was essentially identical with that observed by Macartney (1966) in a previous study at Macdonald College (Table 6). Macartney fed a diet of similar composition to that in the present study with the notable exception that 40% dehydrated alfalfa meal was used in place of the carotene-devoid, heated alfalfa and grass meal that constituted 40% of the ration in the present study. The two diets were pelleted in the same fashion so that consumption differences attributable to texture and particle size should have

been minimal. The only major factor that prevents the indiscriminate use of Macartney's value as a valid comparison index in the present experiment is the fact that his study included only four animals. Rate of gain in the rabbits studied by Casady (1961) over a comparable period (3-8 weeks) was higher than that observed in either the present study or that of Macartney (1966). However, Casady's rabbits were initially weighed at 3 weeks of age rather than at 4 weeks as was the case in the present study. While rabbits in the present experiment were weaned at 4 weeks of age, those studied by Casady were left with the doe until 8 weeks of age. It is thus possible that the rate of gain in the period from 3 to 4 weeks may have improved the overall period growth rate for Casady's rabbits, making it higher than those in the present and Macartney's studies. However, rates of gain in the two subsequent periods (8-14 weeks and 14 weeks-5 months) were also higher in Casady's rabbits than in those of the present study. The wisdom of accepting the growth rates calculated by Casady as standard values for comparison with the present work is very questionable since the management practices and environmental conditions under which the two diets were fed varied widely. Differences in the strain of New Zealand White rabbits used in the two studies could also contribute somewhat to response differences.

According to the estimates of the N.A.S.-N.R.C. (1966), the expected rate of gain for rabbits weighing 4-7 lb is .07 lb/day (31.8 g/day). Rabbit weights in the present study were in this range mainly during the 8-14 week stage of growth, although there was some overlap with the 14 week-5 month period. In both periods the average daily gain for the 12 μg level group was lower than the value projected by the N.A.S.-N.R.C. (1966).

The validity of any conclusions drawn from comparisons among experiments conducted under different environmental conditions with different populations of rabbits is subject to challenge. However, these comparisons suggested that the growth rate of rabbits on the 12 μg level was probably somewhat lower than that of rabbits receiving the stock colony diets of Casady (1961) and the N.A.S.-N.R.C. (1966). Whether these growth differences were attributable to vitamin A levels *per se* or to variations in other nutrient levels is not clear. It would be expected that the ration used by Macartney (1966) was rich in the vitamin A precursor, carotene since dehydrated alfalfa meal constituted 40% of the dietary composition. Growth for the 4-8 week period in rabbits fed this diet was essentially identical with the growth rate observed in the 12 μg level group whose members actually received slightly more than 12 μg of vitamin A/W_{kg}/

day because of their high feed intake. This may suggest that a vitamin A level slightly in excess of 12 $\mu g/W_{kg}/day$ is adequate for optimal growth. If this is true, then the relatively superior growth rates of the rabbits utilized by Casady (1961) and N.A.S.-N.R.C. (1966) can perhaps be attributed to quantitative dietary differences in nutrients other than vitamin A. Undoubtedly these differences were considerably enhanced by environmental variations as well. It is unfortunate that Macartney's limited study was not extended to include the 8-12 week and 8 week-5 month stages of growth, so that a more accurate appraisal of the growth performance of the 12 µg level group could be obtained through comparisons with his growth rate values.

An overall examination of growth and pathology in rabbits of the present study suggests that a vitamin A level of 3 $\mu g/W_{kg}/day$ is not adequate to permit normal growth or to maintain health. The minimal vitamin A level for growth and well-being, under normal circumstances, appears to lie between 3 and 6 $\mu g/W_{kg}/day$ and present observations would tend to support a minimal level very close to the upper limit of that range. This range is in good agreement with the vitamin A level of 4-6 $\mu g/W_{kg}/day$ proposed by Guilbert et al. (1940) as the minimal requirement for most mammalian species. Under stress conditions, however, the 6 µg vitamin

A level was not adequate to prevent weight loss and pathological lesion onset in rabbits of the present study. Growth in rabbits fed the present basal diet appeared to be optimal or nearly optimal when a vitamin A supplementation level slightly in excess of 12 $\mu g/W_{kg}/day$ was used.

Growth rate in rabbits fed the 12 μg level ration may have been lower than that observed in rabbits fed stock colony diets but there are indications that deficiencies in dietary nutrients, other than vitamin A, may have been involved in this apparent inferiority. The fact that one female in the 12 μg level group developed ocular lesions after successive pregnancies tends to suggest that a vitamin A level slightly in excess of 12 $\mu g/W_{kg}/day$ may be inadequate to satisfy reproductive requirements, even though this same level appears to be nearly optimal for growth.

REPRODUCTIVE PERFORMANCE OF THE MALES

The most reliable index of fertility in any particular male is his actual breeding performance. In the present study breeding performance was evaluated on the basis of reproductive efficiency defined according to the following ratio and expressed in per cent.

$$\% \text{ Reproductive Efficiency} = \frac{\text{Total number of resulting pregnancies}}{\text{Total number of matings}} \times 100\%$$

Failure of statistical analyses to indicate any significant effect of dietary vitamin A level on the reproductive efficiencies of either young or mature male rabbits is not surprising in view of the small number of observations in each treatment and the frequently large individual variation within treatments. It would have been desirable to increase the number of individual males in each treatment but the limiting factor was the total quantity of dried alfalfa meal that could be heat-processed at any one period for incorporation into the experimental rations. In addition only seven of the original 12 males placed on the dietary treatments prior to puberty were alive and functional at breeding age. With these limiting factors in mind, it appeared valid and justifiable to numerically assess the relative magnitudes of the treatment means in an attempt to detect established trends that might suggest a relationship between dietary vitamin A level and reproductive efficiency.

Data for the mature male rabbits showed no consistent relationship between level of dietary vitamin A consumption and per cent reproductive efficiency. Males that received the highest level of vitamin A (12 $\mu g/W_{kg}/day$) showed the lowest overall mean reproductive efficiency of the four treatment groups. This fact appears to oppose the

theory that increased vitamin A supplementation has a beneficial effect on fertility. However, it should be noted that the poor overall group performance was mainly attributable to the poor breeding record of buck 12M31 who suffered from coccidiosis and extensive lesions on pads of the feet. These inflictions had an adverse effect upon his reproductive efficiency. While the level of dietary vitamin A did not appear to influence reproductive efficiency in mature males, it did seem to exert an effect on the reproductive efficiencies of young male rabbits that had been placed on the dietary treatments prior to the attainment of sexual maturity. Three young males receiving no vitamin A supplementation (0 μg level) and one young buck receiving the 3 μg level failed to survive to breeding age. A second male was eliminated from the breeding study because of testicular retention and lack of libido while the third member of the 3 μg level group was included as part of the male breeding population since his libido was satisfactory. Since 3M8 was the only surviving functional male on the 3 μg level at the time of breeding, all group mean values for the 3 μg treatment throughout the breeding and seminal studies of this experiment are based on data from this rabbit alone. Overall group reproductive efficiencies of 11.1%, 66.7% and 83.3% were recorded for males on the 3, 6 and 12 μg levels respectively, indicating that the

reproductive efficiency of young male rabbits improved as the dietary vitamin A supplementation level increased.

The question arises as to why a trend toward improved reproductive efficiency with increasing vitamin A supplementation is discernible in males placed on dietary treatments prior to sexual maturity (i.e. "young males") but not in males placed on treatments after the onset of sexual maturity (i.e. "mature males"). It is conceivable that mature males on the 0 μg level had not reached a stage of deficiency sufficiently severe to seriously impair their seminal quality at the time of breeding, particularly since their hepatic vitamin A stores at the start would be considerably higher than those of the young animals. This hypothesis seems feasible since ocular lesions, an early indication of vitamin A deficiency, had just appeared in the two mature males prior to the final two mating periods. Chevrel and Cormier (1948) reported that testicular degeneration in pubescent vitamin A deficient male rabbits could occur prior to the appearance of ocular lesions but observed that the testicular atrophy was more marked and extensive in the rabbits exhibiting ocular lesions. However, these two workers did not examine the seminal quality or fertility of the deficient rabbits so it is not certain whether the testicular degeneration at the time of ocular lesion appearance

was sufficiently severe so as to actually impair the males' breeding capacity. It thus appears probable that, in the present experiment, severe deterioration in seminal quality and resultant reduction in fertility did not occur until considerably after the initial appearance of somatic deficiency lesions. Indeed, Bratton et al. (1948) have reported that mature vitamin A deficient bulls lose their physical ability to mount before there is any serious deterioration in their seminal quality.

A second possible explanation for the response difference is the fact that the young male rabbits were placed on the four dietary regimes at weaning and hence were exposed to the effects of the various vitamin A levels prior to the onset of puberty. The mature male rabbits, on the other hand, were introduced to the experimental diets only after sexual function had already been established. It is possible that avitaminosis A incurred in male rabbits at or prior to puberty is more detrimental to reproductive performance than that sustained after the attainment of sexual maturity. This theory has been advanced by Hodgson et al. (1946) and by Erb et al. (1947) based on their studies with A-avitaminotic bulls. The feeding of marginal or submarginal vitamin A levels to pubertal and prepubertal male rabbits could conceivably influence the pattern of

reproductive efficiency differences in the same way but to a less marked extent than is the case where no vitamin A supplementation is provided.

It is interesting to note that all bucks used in the two breeding trials were fertile since each individual male, both young and mature, sired at least one litter. Hodgson et al. (1946) contended that poor quality in semen did not necessarily result in reduced fertility of bulls. This conclusion was based on the fact that cows were successfully impregnated with semen from these vitamin A deficient bulls. The present work in rabbits lends support to this concept. Males 3M8 and 12M20 consistently produced semen of poor quality. In spite of this, each of the males successfully impregnated one doe, indicating that poor semen quality was not synonymous with semen infertility. The fact that only one litter resulted from nine matings of 3M8 indicates that while poor seminal quality does not eliminate fertility, it does substantially reduce it. Thus poor seminal quality appears to impair fertility without completely annihilating it. It was therefore considered expedient to compare certain parameters in the semen of males on the four dietary treatments in an attempt to establish which seminal characteristics most consistently reflected changes in the level of dietary

vitamin A intake. Differences in sexual interest or libido were examined at the same time. It was anticipated that the information gained might prove useful in explaining differences in reproductive efficiencies among treatment groups as well as in verifying or interpreting the results of testicular weight measurements and histopathological studies.

No consistent relationship between dietary vitamin A level and libido rating was discernible in male rabbits, whether introduced to the experimental diets before or after puberty. The great individual variability in libido, that occurs even within treatments, is well illustrated by comparing bucks 3M7 and 3M8, both fed on the 3 μg level of vitamin A since weaning. Male 3M7 was so lacking in sexual interest that he was eliminated from the breeding study while 3M8 exhibited libido that was stronger than the mean libido rating for either 6 or 12 μg level groups. However, the fact that libido was completely inhibited in one male on the 3 μg level may suggest that severely inadequate vitamin A intake depresses sexual interest. Loss or reduction of libido has previously been noted in bulls (Erb et al., 1947) and rams (Lindley et al., 1949; Dutt, 1959) that incurred vitamin A deficiency prior to puberty. However, Lindley et al. (1949) observed that once the deficient rams became initially accustomed to serving the artificial vagina, their

sexual interest was maintained throughout later collection periods, even when they were too weak to stand or to mount a ewe.

Irregular variability in libido rating among the four groups of mature males may be attributed to individual animal differences since libido patterns in these rabbits were already well established prior to the introduction of dietary treatments. It has been noted that OM25 twice attempted to mount the teaser doe, even when he was emasciated, incoordinated and too weak to ejaculate. Bratton et al. (1948) have reported a similar tenacious retention of libido in physically incoordinated vitamin A deficient bulls. Bulls maintained on low levels of vitamin A following initial induction of clinical vitamin A deficiency by Erb et al. (1947) showed acceptable libido but the two deficient beef bulls studied by Madsen et al. (1948) did not.

In general, semen from males that were placed on dietary treatments prior to puberty showed a more consistent pattern of quality improvement with increasing level of vitamin A intake than did semen from bucks that were placed on the various levels of vitamin A after the onset of puberty. This further adds to the evidence supporting the earlier contention (Hodgson et al., 1946; Erb et al., 1947) that the stage of sexual maturity at which vitamin A deficiency is incurred determines the severity of the male's reproductive

impairment. This inconsistency between mature and young males is reflected in several of the semen characteristics studied.

Volume of ejaculates from males placed on experimental treatments prior to puberty appeared to increase as dietary vitamin A level rose; volume of ejaculates from male rabbits placed on experimental treatments after puberty, on the other hand, showed no consistent relationship to dietary vitamin A level consumed. Reports from the literature fail to agree on whether or not the level of vitamin A influences the volume of semen produced in other species. It is evident from the data that mature male rabbits produce the greatest volume of semen at the first ejaculation, irrespective of which dietary treatment is being imposed. There was also a progressive decrease in semen volume over the 24-week semen collection period with mean ejaculate volume in period 6 being considerably lower than that in period 1.

The fact that changes in per cent spermatozoa showing motility were always paralleled by similar changes in progressive motility of spermatozoa for all four treatments in both young and mature rabbit studies suggests that there is a very close relationship between these two semen variables. The spermatozoan motility of semen from young males increased progressively as the level of dietary vitamin A increased

from the 3 to 12 μg level. Mature males followed this trend only to the extent that those fed the 12 μg level showed spermatozoan motility significantly superior to that of the other three groups. At the 6 μg level, spermatozoan motility was slightly, but not significantly, higher than at 0 and 3 μg levels. The fact that males on the 0 μg level did not show the lowest spermatozoan motility is probably due to the fact that male 0M26 on the 0 μg level treatment failed to show serious deterioration in physical condition or seminal quality even at the end of the experiment. This male probably had large initial liver reserves of vitamin A, although he did eventually exhibit ocular lesions. After the death of male 0M25, mean values for the 0 μg treatment were based entirely on the semen data for male 0M26. The fact that his semen quality was good undoubtedly contributed to the apparent superiority of the 0 μg treatment. The present results therefore support the general consensus of opinion expressed in the literature that spermatozoan motility decreases as vitamin A deficiency progresses.

The per cent dead spermatozoa present in the semen of males placed on the experimental treatments before and after sexual maturity differed considerably. In young males, the per cent dead spermatozoa was highest at the lowest vitamin

A level (3 μg) but semen from rabbits on the highest vitamin A level (12 μg) contained more dead spermatozoa than did semen from those on the intermediate vitamin A level (6 μg). This apparent inconsistency can probably be attributed to the extremely high dead spermatozoan numbers in the semen of male 12M20, on the 12 μg level, rather than to any overall treatment effect. Male 12M20 was peculiar in that his semen frequently contained dark-pigmented granular material. Microscopic examination of this unidentified material revealed concentric, laminated layers that appeared to center around a single crystal as the nucleus. Incoordination accompanied poor seminal quality in this male yet his libido remained exceptionally good and he actually sired one litter. It is likely that the incoordination in this rabbit was incurred as the result of some unknown physical trauma since no ocular lesions or other vitamin A deficiency symptoms were discernible. This theory does not, however, explain the poor seminal quality. At one point in the experiment there appeared to be scrotal infection but seminal deterioration was noted prior to this development. With this explanation clearly in mind, it seems justifiable to conclude that a decrease in per cent dead spermatozoa accompanies an increase in dietary vitamin A level. The study of semen from mature males, however, revealed no consistent relationship between per cent

dead spermatozoa and vitamin A level. This could indicate that vitamin A deficiency incurred prior to puberty was more detrimental to spermatozoan viability than that sustained after the onset of puberty. However, the apparent absence of treatment effect on per cent dead spermatozoa could also indicate that mature males on the 0 μg level had not yet reached the stage of deficiency where spermatogenesis was seriously affected. That vitamin A supplementation increases the per cent live spermatozoa in bovine semen has been shown by De Vuyst (1959) but vitamin A levels used were much higher than those in the present study.

Present results indicate that the incidence of morphologically abnormal spermatozoa increased as the level of dietary vitamin A decreased in male rabbits, regardless of whether the experimental diets were introduced prior to or after puberty. Whether the increased number of aberrant forms observed is due to disturbances in the spermatogenetic tissues or to alterations in the balance of hormones that regulate the normal completion of spermatogenesis remains a subject for speculation. The increased incidence of abnormal spermatozoa in vitamin A deficient rabbits substantiates similar observations reported in the literature for other species. Further examination of the rabbit data suggested that abnormal spermatozoa were most abundant in initial semen

ejaculates, irrespective of which dietary treatment group was being considered. An overall increase in abnormal spermatozoan production during the semen collection period was indicated by the fact that aberrant forms were nearly twice as prevalent in period 6 as in period 1.

Previous studies in other species have shown that seminal spermatozoan concentration is reduced in avitaminotic A males. The present results are in general agreement with this fact. Young males on 6 and 12 μg vitamin A levels produced more spermatozoa per cc of semen than did the male receiving the 3 μg level. The fact that spermatozoan concentration for the 12 μg level group was slightly, but not significantly, lower than that of the 6 μg level group is probably a reflection of the poor spermatozoan concentration of individual male 12M20 described previously. Spermatozoan concentration per cc in the older males rose significantly as the level of dietary vitamin A increased through the 0, 3 and 6 μg range of treatment levels. This finding is in agreement with the trend suggested by the young male data. The obvious exception to this otherwise orderly pattern is the fact that mean spermatozoan concentration per cc of semen for the 12 μg level group is significantly lower than those for the 0, 3 and 6 μg levels. This apparent lack of conformity may be explainable on the basis that male 12M31

on the 12 μg level of vitamin A showed very poor physical condition and soreness of the hind feet during the latter part of semen collection period I. Spermatozoan concentration per cc of semen dropped somewhat in this period up to week 14 when 12M31 was physically unable to serve the artificial vagina. The introduction of a more solid surface than the wire flooring of the cage apparently helped this male, enabling collections to be resumed. Spermatozoan concentration for this male improved following the breeding period but did not again reach the high level observed in week 10 of semen collection period I. Because of physical and labor limitations in the overall preparation of rations, only two males were included in each of the four treatment groups in Experiment 2 (Trial II). A decline in spermatozoan concentration for one member of the pair would therefore be strongly reflected in the treatment mean value for that group. Statistical analyses were unable to separate mean differences due to treatment from those due to physical incapacitation of the rabbit. As a consequence, the spermatozoan concentration changes were attributed to treatment when in actual fact the poor physical condition of the rabbit, unrelated to treatment, was probably the more influencing factor. This may explain why spermatozoan concentration for the 12 μg level group was inconsistent with

the suggested trend towards higher spermatozoan concentration with increased level of dietary vitamin A.

Overall spermatozoan production is best assessed on the basis of total spermatozoa per ejaculate, which represents the product of two other semen variables - ejaculate volume and concentration of spermatozoa per cubic centimeter of semen. Total spermatozoan production increased progressively as the dietary vitamin A level was elevated in male rabbits placed on treatment prior to puberty. There was no such discernible relationship between total spermatozoan production and vitamin A intake level in males placed on treatments after puberty, however. In fact, total production of spermatozoa was lower in males receiving 6 and 12 μg levels of vitamin A than in those receiving 0 and 3 μg levels. This trend probably reflects treatment group differences in ejaculate volume as well as the lower spermatozoan concentration per cubic centimeter of semen in the 12 μg level group.

According to Anderson (1942) spermatozoan concentration is negatively correlated with pH. Mann (1964) explained that semen containing a lower concentration of spermatozoa was more alkaline because of the lesser amount of fructolysis and lactic acid accumulation that occurred. Increased fructolysis and lactic acid accumulation was responsible for the

lower pH observed in semen which contained a high spermatozoan concentration. Semen from young males observed in the present study decreased in pH as the level of dietary vitamin A supplementation was increased. This increase in acidity is probably a reflection of the fact that spermatozoan concentration also increased. Examination of the semen from mature male rabbits on 0, 3, 6 and 12 μg levels failed to reveal any consistent pattern between hydrogen ion concentration and vitamin A level. Mature males on the 6 μg level had the lowest seminal pH and highest spermatozoan concentration per cc of semen but the other groups failed to show any consistent relationship between pH and spermatozoan concentration. Mean pH values for all treatment groups in the present study were well within the 6.6 to 7.5 range, suggested as normal for rabbit semen by Spector (1956).

It was interesting to note the effect of ejaculation sequence on the hydrogen ion and spermatozoan concentrations of semen samples from the eight mature bucks. Spermatozoan concentration/cc of semen was highest in the initial ejaculate, lower in the second ejaculate and lowest in the third ejaculate collected. Since both spermatozoan concentration and semen volume decreased significantly at the second and third ejaculations, it was expected that total spermatozoan

content would be greatest in the first ejaculate. Confirmatory results indicated that the mean total spermatozoan content of the first ejaculate was actually more than one and one-half times as great as that of the second ejaculate. Semen collected at first ejaculation tended to be slightly more acidic than that obtained at later ejaculations. It appears probable that this lower pH is the result of the higher spermatozoan concentration of the initial ejaculate.

Semen data for the young males were collected over an uninterrupted six-week period so no consideration of period effect was necessary. Data for mature males, on the other hand, were accumulated in a four-week pre-treatment period, followed by two semen collection periods, which were interrupted by a 16-week breeding period. The extent of the vitamin A depletion at different stages of the semen collection period also varied, depending upon the length of time the males had received the experimental diets up to that particular point. These factors made it desirable to carry out statistical analyses in order to determine whether mean values for semen characteristics varied significantly among the six 4-week periods studied. Indeed, the period of semen collection did contribute significantly to the variation in all seminal characteristics examined except for spermatozoan concentration/cc of semen. In many instances this

variation did not show a consistent pattern from period to period, but a few of the differences were worthy of note. At the end of the semen collection study (Period 6), semen volume and total spermatozoan production were significantly lower than they had been originally (in Period 1), while pH and abnormal spermatozoan production had increased significantly above their initial levels. On the other hand, neither spermatozoan motility nor spermatozoan mortality was significantly higher or lower in Period 6 than it had been in Period 1. An examination of the statistical results suggests that interjection of a 16-week breeding period between semen collection periods I and II may have temporarily altered the trends that had been developing at the end of semen collection period I in some of the semen variables. However, it should be pointed out that after 23 weeks on the experimental diets, no real trends were discernible from the raw unanalysed data and thus this was deemed an appropriate time to check male fertility through actual matings. Frequency of mating during the 16-week breeding period was considerably lower than that of normal semen collections so that there may have been some tendency towards rejuvenation of declining testicular function during this period of lessened sexual activity. Data collected in the

first week of semen collection period II constituted week 4 of period 4, while that collected in the other 8 weeks made up periods 5 and 6. By having the immediate post-breeding data fall within period 4, some reflection of the post-breeding period trend is injected into treatment means for period 4, making the remaining post-breeding data in periods 5 and 6 a more accurate indicator as to whether the later changes in semen variable means actually result from treatment or whether they reflect the lessened sexual activity of the breeding period.

The effect of lessened sexual activity in the breeding period may be reflected in the fact that all semen variables examined showed a slight improvement in period 4 or 5 followed by a decline again in period 6, except for spermatozoan concentration per cubic centimeter which appeared to rise slightly in period 6. The death of male 0M25 in the first week of period 6 may have influenced this slight increase in spermatozoan concentration. The overall picture thus suggests that semen quality may have improved slightly after the breeding period, during which time sexual demands were somewhat lessened. However, this improvement seems to represent only a short-lived rejuvenation, with reversion back to the original pattern of seminal deterioration occurring in the subsequent period (6). It is speculated that

seminal quality would have continued to decrease if semen collections had been continued beyond the 24-week period. If the duration of the experiment had been extended, it is likely that males on 0 μg and possibly 3 μg levels would have become progressively more vitamin A deficient until testicular function was ultimately disturbed to the point where semen quality deteriorated. Period means are based on observations over all treatments and hence would reflect this deterioration.

The quality of semen presumably reflects the condition of the testicular environment in which it was produced. It should thus be possible to ascribe poor semen quality to impaired or aberrant testicular function. In the present study, the effect of vitamin A level on the testes per se was evaluated on the basis of testicular weight determinations and histopathological examinations.

Since rabbits died at varying ages and weights, the recorded testes weights were expressed as a percentage of body weight in mg % in order to provide a kind of "common denominator" for comparison purposes. Testes from young male rabbits fed low levels of vitamin A (0, 3 μg) since weaning were lighter than those from young bucks receiving higher levels of vitamin A supplementation (6, 12 μg). These results are consistent with reports of testicular weight

decreases in vitamin A deficient boars (Palludan, 1966) and rams (Lindley et al., 1949; Dutt, 1959; Sosa, 1965). The validity of this relationship might be questioned on the basis that males in the 0 μg level group had heavier testes than those in the 3 μg level group. However, it must be kept in mind that rabbits on the 3 μg level were, on the average, 114 days older at the time of death than were those on the 0 μg level. It might therefore be postulated that males that were subjected to the low vitamin A levels for the longer periods of time underwent more extensive testicular atrophy than did those which died at a relatively earlier age. The lower testes weights observed in the 3 μg level group could thus be a direct reflection of the greater testicular atrophy resulting from a more prolonged exposure to the low vitamin A level. It is interesting, in this respect, to compare testes weights (in mg %) of two individual males (0M1 and 3M9) that died after comparable periods of time (166 and 163 days) on 0 and 3 μg vitamin A levels. This comparison illustrates that at a comparable stage of development testes from male 3M9 were heavier than those from 0M1 when both were expressed as a percentage of body weight. This evidence lends further support to the concept that low dietary vitamin A levels depress testicular weights. Testes from males placed on dietary treatments after puberty showed the same pattern

of weight increase response as did those from the young males. It is interesting that within 0 and 3 μg groups respectively, the testicular weights of males 0M25 and 3M28 appeared to decrease in response to the vitamin A inadequacy whereas those of their group-mates (0M26 and 3M27) did not seem to reflect the deficient treatment levels, even at slaughter 300 days after introduction of the experimental diets and at a time after ocular lesions had appeared in male 0M26. This pattern may indicate individual differences in hepatic vitamin A stores and hence variation in the time required for depletion of the vitamin A reserves. Whether the dietary treatments were imposed prior to or after sexual maturity, however, did not appear to appreciably affect the observed trend toward heavier testicular weights with increased level of vitamin A intake.

Reduction in testicular weights of males receiving low levels of dietary vitamin A may be explained on the basis of the testicular atrophy that was observed during histopathological examinations. In general, spermatogenetic prolificacy and seminiferous tubule organization were more satisfactory in males receiving the 12 μg vitamin A level than in those receiving 0, 3 or 6 μg levels. The lack of marked histological differences between testes of mature males on 6 and 12 μg levels provides the only exception to this generalization.

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Testicular lesions in male rabbits suffering from severe vitamin A deficiency (0 μg level) were characterized by a cessation or retardation of active spermatogenesis beyond the spermatogonium or primary spermatocyte stages, with an absence or near absence of mature spermatozoa. Destruction of the organization of the seminiferous tubules accompanied the spermatogenetic inhibition. The fact that the young male rabbits were only about five months old at the time of death might suggest that lack of sexual development at that age was responsible for lack of spermatogenesis and seminiferous tubule development. However, there should have been some clear cut indications of spermatogenetic initiation by five months of age if testicular function and development were normal. Further evidence that the lesions described are characteristic of vitamin A deficiency is provided by the occurrence of almost identical testicular lesions in mature male 0M25 who developed ocular lesions and eventually died after consuming the 0 μg level for a prolonged period. The only discernible difference in testicular lesions between young and mature males was the occasional appearance of mature spermatozoa in isolated tubules of testes of mature rabbits, whereas such spermatozoa were virtually absent in testes of young males. It would thus appear that vitamin A deficiency incurred after the onset of puberty

retards and eventually stops active spermatogenesis, whereas the deficiency sustained prior to puberty would seem to inhibit the initiation of spermatogenesis. If spermatogenesis in mature male rabbits declined gradually as the severity of the deficiency syndrome intensified, it would be possible to have both active and inactive seminiferous tubules within the same testis at the same time. Since individual testicular tubules may be at different stages of spermatogenesis at any one time, the pre-spermatozoan forms present in one tubule may be more detrimentally affected by the deficiency than are earlier stages present in adjacent tubules. This theory is backed by the fact that Chevrel and Cormier (1948) observed an orderly pattern of testicular atrophy with spermatozoa, spermatids, and spermatocytes being preferentially affected by the deficiency in that sequence. Their description of the final degenerative changes in the seminiferous tubules could, with equal accuracy, be applied to the atrophic alterations in the present study. The presence of dark staining nuclei amidst structureless cytoplasmic masses formed from desquamated cells characterized the tubular lumina of each. Earlier testicular weight measurements and semen studies have indicated that mature male 0M26 on the 0 μg level was less severely affected by the feeding of the 0 μg level than was his group mate 0M25. This indication is supported by the

apparent absence of serious testicular lesions and the presence of all stages of spermatogenesis, including abundant mature spermatozoa in testes of male OM26. However, the presence of degenerate nuclei and ruptured membranes in certain tubules of OM26 may suggest a very early stage of testicular deterioration. The appearance of the histological lesions observed in testes of the deficient males in this study appear to be in general agreement with those described by other workers in rats, mice, rams, boars and bulls.

The present study has not attempted to resolve the controversy as to whether spermatogenetic inhibition in vitamin A deficient males is directly attributable to a disturbance in the spermatogenetic epithelial tissues (Palludan, 1966) or indirectly related to alterations in synthesis or release of androgens (Mayer and Truant, 1949; Grangaud et al., 1961) or gonadotrophic hormones (Mayer and Goddard, 1951). Speculation on this point is pertinent to the discussion, however. Nalbandov (1964) has advanced the theory that androgens may be required for final spermatogenetic differentiation and that induction of androgen secretion by Leydig cells requires gonadotrophic LH or ICSH (interstitial-cell-stimulating hormone). The fact that androgens were produced in testes of vitamin A deficient rats in response to HCG administration (Mayer and Goddard, 1951) suggests that

the testes are capable of producing androgens even in vitamin A deficiency provided the proper gonadotrophic stimulus is available. This strongly suggests that the gonadotrophin ICSH is not produced in the pituitary of the deficient male at a sufficiently high rate to stimulate testicular androgen production by Leydig cells and hence the final spermatogenetic differentiation is blocked. It is possible that spermatogenetic differentiation may also be inhibited to some extent by blockage of androgen synthesis (Grangaud et al., 1961) but the gonadotrophic inadequacy appears to be the more probable cause of the inhibition. Palludan (1966) appeared to offer the most feasible explanation for spermatogenetic blockage when he postulated that the testicular manifestations of vitamin A deficiency result from a direct disturbance of spermatogenetic tissue function. This theory was based on the observation that vitamin A injected into specific portions of the testis of an avitaminotic A boar effected improvement in spermatogenesis in the treated portion of the testis but left the untreated portions unaffected. The maintenance of epithelial tissue integrity is dependent upon sulfated mucopolysaccharide synthesis. In view of the demonstrated requirement for vitamin A in mucopolysaccharide synthesis (Moore, 1957) and in light of the reduced synthesis of mucopolysaccharides during vitamin A

deficiency (Wolf and Johnson, 1960), it does appear likely that testicular deterioration in avitaminotic A males does result from a direct disturbance of spermatogenetic tissue function. Regardless of the mechanism of action involved, however, the fact remains that testicular degeneration does accompany severe vitamin A deficiency whether induced in male rabbits before or after puberty.

When the dietary vitamin A level was increased from 0 to 3 $\mu g/W_{kg}/day$, the severity of the testicular lesions and the inhibition of spermatogenesis were reduced slightly in the young males and considerably in the older bucks. While normal seminiferous tubules were discernible in testes of young males on the 3 μg level, degenerate and degenerating tubules were much more predominant. Limited numbers of mature spermatozoa and spermatids were present in individual tubules but spermatogenesis in most instances did not surpass the spermatogonium or primary spermatocyte stages. Other features of seminiferous tubular deterioration were not different from those observed in the 0 μg level group. The histopathology of young male 3M7 in the 3 μg level group was of particular interest since this buck had shown testicular retention and lack of libido throughout his lifetime. Seminiferous tubules in this male were extremely disorganized and active spermatogenesis was absent. These observations are in

good agreement with the testicular changes described by Nalbandov (1964) as characteristic of cryptorchids. Whether testicular retention in this male was related to level of dietary vitamin A cannot be said with assurance but this observation, together with the evidence of inferior testicular function in the other surviving male in the 3 μg level group, does suggest that 3 μg vitamin $A/W_{kg}/day$ is inadequate for satisfactory reproductive performance in young male rabbits.

In contrast to the severe degeneration observed in testes of young males on the 3 μg level, testicles from mature males on the same diet were structurally normal except for possible excessive cellular exfoliation in certain tubules. Extensive desquamation in the seminiferous tubules of male 3M28 especially could be indicative of testicular degeneration since testes weights in that individual were relatively low. This weight decrease could be explained on the basis of testicular deterioration, characterized by excessive cellular desquamation, and resulting from the consumption of sub-minimal vitamin A levels. Swierstra and Foote (1963) have indicated that in the rabbit there is a considerable loss of spermatogenic cells during normal spermatogenesis, with most of this loss appearing to occur during and immediately after two maturation divisions. Therefore

all normal seminiferous tubules undergo some degree of desquamation as a result of this loss of pre-spermatozoan stages. Exfoliation of cells in excess of this normal loss, however, could reflect testicular degenerative changes, possibly related to low level of vitamin A intake. Whether the desquamation observed in 0M28 is actually extensive enough to be considered abnormal could be contested, but the underlying theory, nevertheless, remains as a possible explanation for the relatively low testicular weights observed in this male on the 3 μg level.

At the 6 μg level of vitamin A supplementation, spermatogenesis was essentially active and complete in both young and mature males. The only young male on the 6 μg level to show excessive tubular desquamation was also the only male to develop ocular lesions indicative of vitamin A deficiency. Testicular histology showed seminiferous tubules at varying stages of spermatogenesis, ranging from complete to inactive. It should therefore not be surprising to note that this buck also had the lowest spermatozoan concentration and the lowest testicular weights of any of the 6 μg level males. The fact that only one of the three animals in the 6 μg level group exhibited deficiency lesions may indicate individual animal variability as to vitamin A requirement. Testes from one of the mature males also showed excessive

desquamation but this was probably due to post-mortem autolytic changes that occurred prior to tissue fixation. This buck succumbed during the night and was not discovered until the following morning, during which time interval autolysis undoubtedly had commenced. Death was attributed to suppuratory pneumonia and was probably sequential to a previous prolonged bout with snuffles. However, no distinct deficiency symptoms were observed that would implicate vitamin A inadequacy as a primary factor in the death.

There appeared to be little structural or functional difference in the seminiferous tubules of mature males receiving 6 or 12 μg vitamin A levels. Again this may be related to the relatively high initial reserves of vitamin A in the livers of males placed on treatment after attainment of sexual maturity and the subsequent long period of time required for these stores to become depleted to the point where male performance would be influenced only by the actual vitamin A intake level, uncomplicated by carry over from initial hepatic reserves. In young males, however, the presence of active spermatogenesis in relatively normal seminiferous tubules suggested that the 12 μg vitamin A level was the most satisfactory of the four levels examined for testicular function. This is in good agreement with the heavier testicular weights and generally superior semen

quality observed in the 12 μg level group. However, the histological study failed to explain why semen from young male 12M20 was so consistently abnormal when, in fact, the testicular seminiferous tubules in this buck appeared essentially normal with abundant mature spermatozoa and spermatids present. Nevertheless, the absence of both testicular degeneration and ocular lesions offers further evidence that physical and seminal inadequacies of this male were probably not attributable to vitamin A deficiency, although the actual cause of these problems remains inapparent.

Overall results of the male reproductive study would appear to indicate that males placed on vitamin A treatments prior to puberty responded unfavourably to both 0 and 3 μg levels, performed moderately well at 6 μg level but showed the best overall performance at 12 μg level. This trend could suggest that in young male rabbits, the 6 μg vitamin A level is minimal for satisfactory reproductive performance whereas the 12 μg level is nearer to the optimum for reproductive function. In males placed on vitamin A treatments after the onset of puberty, the 0 μg level again appeared inadequate, the 3 μg level was questionable but probably unsatisfactory over a prolonged period, while both 6 and 12 μg levels appeared to give acceptable performance, with the 12 μg level being slightly superior to the 6 μg level. It is probable that

a longer duration in time of experiment would have resulted in a more complete exhaustion of the hepatic vitamin A reserves in the mature males and thus allowed a more accurate appraisal of the differences in male response, particularly to 3, 6 and 12 μg levels.

For the 0 - 12 μg range of vitamin A levels considered, it would appear that overall male reproductive performance generally improves as the level of dietary vitamin A intake increases. Since male rabbits are subject to less stress during reproduction than are female rabbits, it seems reasonable to suggest that their minimum vitamin A requirement for satisfactory reproduction may be lower than that of their female counterparts.

REPRODUCTIVE PERFORMANCE OF THE FEMALES

In order for reproductive performance to be considered satisfactory, female rabbits must produce litters containing "normal" numbers of viable, healthy offspring. The fact that offspring from all does on all treatments exhibited some form of ocular abnormality provided strong evidence that even the highest level of vitamin A supplementation used in the present experiment was inadequate to support satisfactory reproductive and lactational performance in breeding does.

Pathological abnormalities detected in the young rabbits of the present study were generally similar to those described by previous workers. Ocular lesions and hydrocephalus were the most consistent lesions observed in the young rabbits from does on all levels of vitamin A in the present study. The cause of the hydrocephalus was not investigated but it has been postulated (Lamming et al., 1954c; Millen, 1956) that an excess production of cerebrospinal fluid may be responsible for the condition. A similar increase in cerebrospinal fluid pressure could perhaps explain the development of exophthalmia and dome-shaped heads in some of the young rabbits.

Females on the 3 μg level of vitamin A reproduced less efficiently than those on the 6 and 12 μg levels. It is not known whether this poorer reproductive efficiency is attributable to ovulation failure, to early ova degeneration or to later fetal resorption. Ovulation failure appears to be the least likely explanation for the lower reproductive efficiency since histological examination showed no apparent effect of vitamin A level on ovarian follicular development. Because females varied widely in age and reproductive status at the time of ovarian examination, it was impossible to accurately test the hypothesis that ovarian weight decreases in vitamin A deficiency (Truscott, 1947). While there were

no external indications of abortion, it is possible that fetal resorption could have occurred, presumably due to impaired progesterone synthesis (Grangaud and Conquy, 1958a,b,c). The poorer reproductive efficiency of the 3 μg level group could also be a reflection of ova degeneration occurring prior to implantation as has been previously observed in does during incipient vitamin A deficiency (Lamming et al., 1954a). It was also demonstrated that female rabbits produced fewer offspring during the period of incipient vitamin A deficiency (Lamming et al., 1954b). Present results show a similar, but less dramatic, decrease in average litter size for females on the 3 μg vitamin A level. It is notable that one female in the 3 μg level group was mated three times without producing a single offspring. It might be speculated that either ova degeneration prior to implantation or fetal resorption after implantation is responsible for the absence or reduced number of offspring. Even at the highest level of vitamin A supplementation, average litter size in the present study did not exceed seven. This is slightly lower than the 8.3 value projected by Kendall et al. (1950) as a "normal" litter size for does of the New Zealand White breed. However, it is suggested that litter size in the 6 and 12 μg level groups was not subnormal but merely reflected variability within the small population of does observed.

The first litter produced by any doe usually contains fewer offspring than do subsequent litters. Initial litters were included in the treatment means calculated for the present experiment but their inclusion may have resulted in lower numerical mean values for average litter size.

Even though the 12 μg level of vitamin A was not adequate to meet all reproductive and lactational needs, it did appear to be closer to the minimum requirement than either of the other levels. This was suggested by the fact that offspring mortality was lowest in the 12 μg level group. The effect of vitamin A level on offspring survival was most pronounced at 21 days post-parturition when survival rates of 0, 12 and 55% were recorded for offspring of females fed the 3, 6 and 12 μg levels of vitamin A, respectively. It is possible that hepatic vitamin A stores were initially greater in the young rabbits from the more highly supplemented dams. This greater storage would enable growth and life to be maintained in the young rabbit for a longer period of time. It is equally feasible to suggest that offspring survival may be related to the amount of vitamin A which the young rabbit receives from its dam during suckling. Does that received the highest level of vitamin A would theoretically transmit more of the vitamin to their offspring through the milk during lactation than would females that received the lower

vitamin A levels. This greater vitamin A supply could perhaps be responsible for the longer life and better growth of the young rabbits whose dams received the highest vitamin A level. Lactational requirements thus appear to be more nearly satisfied by the 12 μg level of vitamin A supplementation than by either the 3 or 6 μg level, but the development of pathological lesions in the offspring clearly indicates that even this level (12 μg) is not fully adequate to satisfy the reproductive and lactational requirements of the dam.

The fact that sections of uterine horn from vitamin A deficient females showed only isolated patches of keratinization was surprising in view of the demonstrated requirement of epithelial tissues for vitamin A (Wolf and Johnson, 1960). It may be that uterine epithelium does not undergo keratinizing changes as early as the corneal and vaginal epithelia. Popova (1958) has indicated that uterine horn stratification in rats is not consistently linked with the stratification observed in the vagina but it is not possible to tell from the present work whether this relationship also applies to rabbits, since no vaginal sections were examined.

The absence of pronounced uterine keratinization may reflect the relatively early age at which vitamin A deficient does died. This may suggest that there was simply not enough time prior to death for uterine stratification to become

widespread. Since none of the females on the 0 μg level survived to breeding age, it was not possible to determine from the present study whether or not epithelial tissue changes occurred in the developing fetuses "in utero."

Mucus formation and secretion are characteristically reduced in the epithelial membranes of vitamin A deficient animals, yet mucus production was apparently unimpaired in the uteri of does on the 0 μg level of vitamin A supplementation at the time of death. This observation supports the previous contention that death intervened before the manifestations of vitamin A deficiency were well established in the uterus. It is possible, but not probable, that excess mucus production represents a tissue response to irritation incurred during the very early stages of avitaminosis A. This could occur only in the very initial stages of deficiency since mucus production thereafter would be severely decreased. The abundant mucus secretion and the limited extent of the uterine keratinization together suggest that does on the deficient diet died prior to the appearance of severe deficiency lesions in the uteri. Questionable patches of keratinization were also observed in isolated sections of uterine horn from females on the 3 μg level but there was again no evidence of extensive cornification. It would thus appear unreasonable to suggest that the lower reproductive efficiency

of the 3 μg level group was attributable to uterine epithelial changes.

Female rabbits obviously require more vitamin A to satisfy their reproductive needs than do their male counterparts. While a vitamin A level of 6 $\mu g/W_{kg}/day$ was adequate to permit satisfactory spermatogenetic function in male rabbits (Experiment 2), a level slightly in excess of 12 $\mu g/W_{kg}/day$ was still not adequate to meet the minimum requirements for reproduction and lactation in breeding females (Experiment 3). It has been noted in other species that the minimum vitamin A level for reproduction is about three times as great as the minimum level required for growth (Guilbert *et al.*, 1940). Since the minimum vitamin A requirement for growth in rabbits has been established at about 6 $\mu g/W_{kg}/day$ (Experiment 1), it seems reasonable to speculate that the minimum level required for satisfactory reproduction and lactation in breeding does is probably not less than 18 $\mu g/W_{kg}/day$.

VI. SUMMARY AND CONCLUSIONS

The present study was undertaken in an attempt to determine the minimum levels of vitamin A required for satisfactory growth and reproduction in rabbits of both sexes. Growth of weanling rabbits, semen production of male rabbits and reproductive performance of female rabbits were the parameters used in making the determinations.

Rations designed to provide vitamin A at the rate of 0, 3, 6 or 12 $\mu g/W_{kg}/day$ were fed to 12 male and 12 female weanling rabbits, as well as to eight mature male rabbits. Since feed consumption for all groups of weanling rabbits was greater than anticipated, the vitamin A levels actually fed were slightly higher than intended. The amount of feed consumed by the rabbits increased with each additional increment of vitamin A supplementation.

Neither growth nor health was satisfactorily maintained in the weanling rabbits that received the 0 or 3 μg levels of vitamin A supplementation. The minimal level of vitamin A that was adequate to sustain growth, in the absence of stress, appeared to be about 6 $\mu g/W_{kg}/day$ while the vitamin A requirement for optimum growth appeared to lie closer to 12 $\mu g/W_{kg}/day$. Feed conversion was most efficient in

rabbits that received this optimum vitamin A level.

Rabbits on the 0 and 3 μg levels developed characteristic deficiency symptoms (weight loss, ocular lesions, ataxia and increased susceptibility to secondary infections) with much greater frequency than did those on the 6 and 12 μg levels of vitamin A. Ocular lesion onset was delayed, mortality was reduced and lifespan was lengthened with each successive increase in the level of vitamin A supplementation. It appeared unlikely that satisfactory health could be maintained on a vitamin A level much below 6 $\mu g/W_{kg}/day$.

Vitamin A level appeared to be more critical for the initiation than for the maintenance of spermatogenesis. Semen quality of young males improved markedly as the level of vitamin A supplementation increased; however, improvement in the quality of semen from mature males was much more limited, with increased spermatozoan concentration and decreased abnormal spermatozoan content being the only consistent alterations attributable to elevation in vitamin A level. The beneficial effect of vitamin A supplementation on reproductive efficiency was also more pronounced in young than in mature male rabbits. Libido rating in both young and mature males was subject to much individual variation but there was limited evidence that mature males retained libido even when physically unable to ejaculate. The absence of sexual

interest in one young buck (on 3 μg level) further suggested that libido might be inhibited in male rabbits that were subjected to hypovitaminosis A prior to puberty.

Testicular weights were heaviest in males on the higher levels of vitamin A. The lighter testicular weights of deficient males were undoubtedly attributable to the degenerative changes that were observed on histological examination.

Overall reproductive performance appeared to be generally satisfactory in males that received vitamin A at either the 6 or 12 μg levels. The minimum vitamin A requirement for satisfactory testicular function would thus appear to be about 6 $\mu g/W_{kg}/day$.

Because of the additional stresses imposed upon them by pregnancy and lactation, female rabbits required a higher level of vitamin A to meet their reproductive needs than did male rabbits.

Females fed 3 μg of vitamin $A/W_{kg}/day$ showed poorer reproductive efficiency than those on 6 and 12 μg levels but the cause of this poorer performance was not ascertained. Litter size increased slightly and offspring mortality decreased substantially as the level of vitamin A supplementation was increased from 3 to 6 to 12 μg. Hydrocephalus and ocular lesions were frequently observed in the offspring of

all females studied, regardless of which vitamin A level they received. Although isolated patches of keratinization were detected in the uterine horns of vitamin A deficient females (0 and 3 μg levels), there was no histological evidence of extensive uterine keratinization. This made it appear unlikely that uterine stratification was responsible for the lower reproductive efficiency of the 3 μg level group or for the pathological anomalies observed in the offspring.

The presence of characteristic pathological abnormalities in the offspring of females that had received the highest level of vitamin A since weaning indicated that a vitamin A level slightly in excess of 12 $\mu g/W_{kg}/day$ was still inadequate to satisfy the vitamin A requirement of the female for reproduction and lactation. Since minimum vitamin A requirements for reproduction are generally about three times as great as the minimum levels for growth, it is speculated that not less than 18 $\mu g/W_{kg}/day$ will be required to meet the reproductive needs of female rabbits. Further experimental work is required to confirm the validity of this extrapolation however.

LITERATURE CITED

Aberle, S.B.D. 1934. Neurological disturbances in rats reared on diets deficient in vitamin A. J. Nutr. 7: 445-461.

Aitken, F.C. and W.K. Wilson. 1962. Rabbit Feeding for Meat and Fur (2nd ed.). Technical Communication No. 12, Commonwealth Bureau of Animal Nutrition, Bucksburn, Aberdeen, Scotland.

Almquist, H.J. 1953. Evaluation of vitamin requirement data. Poultry Sci. 32: 122-128.

Alvarez, J.G. 1947. Vitamin A deficiency of cattle in Columbia, South America. J.A.V.M.A. 111: 216-217.

Anderson, J. 1942. The hydrogen ion concentration of the semen of the bull. J. Agr. Sci. 32: 298-307.

A.O.A.C. 1960. Official Methods of Analysis (9th ed.). Association of Official Agricultural Chemists, Washington, D.C.

Bailey, J.H. and L.F. Nelson. 1965. Reproductive performance of sows on a ration devoid of carotene and vitamin A. J.A.V.M.A. 147: 1387-1392.

Bassett, C.F., L.E. Harris, S.E. Smith and E.D. Yeoman. 1946. Urinary calculi associated with vitamin A deficiency in the fox. Cornell Vet. 36: 5-16.

Bierer, B.W. and C.L. Vickers. 1958. Effect of pelletizing on vitamin A and E. J.A.V.M.A. 133: 228.

Blakemore, F., C.W. Ottaway, K.C. Sellers, E. Eden and T. Moore. 1957. The effects of a diet deficient in vitamin A on the development of the skull, optic nerves and brain of cattle. J. Comp. Path. and Therap. 67: 277-288.

Bo, W.J. 1957. Relation of vitamin A deficiency and estrogen to induction of keratinizing metaplasia in the uterus of the rat. Amer. J. Clin. Nutr. 5: 666-673. (Nutr. Abstr. Rev. 28: No. 3499).

Braman, W.W., A. Black, O.J. Kahlenberg and L. Vores. 1935. The utilization of energy-producing nutriment and protein in white and yellow corn and in diets deficient in vitamins A, D and G. J. Agr. Res. 50: 1-37.

Branion, H.D. 1966. Utilization of carotene and vitamin A by other species, p. 67-88. In Biological values of provitamins A for man, domestic mammals, and birds - A review. Can. Dept. Agr. Publ. 1238. Queen's Printer, Ottawa.

Bratton, R.W., G.W. Salisbury, T. Tanabe, C. Branton, L. Mercier and J.K. Loosli. 1948. Breeding behaviour, spermatogenesis, and semen production of mature dairy bulls fed rations low in carotene. J. Dairy Sci. 31: 779-791.

Brenner, S., M.C.H. Brookes and L.J. Roberts. 1942. The relation of liver stores to the occurrence of early signs of vitamin A deficiency in the white rat. J. Nutr. 23: 459-471.

Burkel, W. and J.J. Taylor. 1963. Changes in the ovary caused by vitamin A deficiency. Anat. Rec. 145: 311. (Abstr.).

C.D.A. 1966. Raising rabbits. Canada Dept. Agr. Publ. 1200. Queen's Printer, Ottawa.

Casady, R.B. 1961. Private communication. Cited from Aitken, F.C. and W.K. Wilson (1962).

Chevrel, M.L. and M. Cormier. 1948. Effets de la carence en vitamine A sur le système génital mâle du lapin. C.R. Acad. Sci. 226: 1854-1855.

Coplan, H.M. and M.M. Sampson. 1935. The effects of a deficiency of iodine and vitamin A on the thyroid gland of the albino rat. J. Nutr. 9: 469-487.

Coward, K.H., K.M. Key, F.J. Dyer and B.G.E. Morgan. 1931. Caseinogen for vitamin tests. Biochem. J. 25: 551-560.

Coward, K.H. 1932. Variation in growth response of rats in vitamin A tests compared with the variation in rats growing normally. Biochem. J. 26: 691-703.

Coward, K.H. 1935. The biological standardisation of vitamins. Nutr. Abstr. Rev. 4: 705-714.

Coward, K. 1953. The Vitamin Requirements of Laboratory Animals. Cited from Worden, A.N. and W. Lane-Petter (1957). The U.F.A.W. Handbook on the Care and Management of Laboratory Animals (2nd ed.). Courier Printing and Publishing Co. Ltd., Kent, England.

Coward, W.A., J.M. Howell, G.A.J. Pitt and J.N. Thompson. 1966. Effects of hormones on reproduction in rats fed a diet deficient in retinol but containing methyl retinoate (vitamin A acid methyl ester). J. Reprod. Fert. 12: 309-317.

Crampton, E.W. 1956. Applied Animal Nutrition. W.H. Freeman and Co., San Francisco.

Crampton, E.W. and L.E. Lloyd. 1959. Fundamentals of Nutrition. W.H. Freeman and Co., San Francisco.

Creech, G.T. and H.R. Seibold. 1943. Histopathology of anasarcous lesions observed in natural cases of vitamin A deficiency in cattle. Am. J. Vet. Res. 4: 353-359.

DeRuyter, Th.H. and O. Rosenthal. 1936. Primary, secondary and non-specific effects in experimental vitamin A deficiency in rats. Ztschr. Vitaminforsch, 5: 169-185 (Nutr. Abstr. Rev. 6: No. 3116).

DeVuyst, A., L. Henriet and C. Meeus. 1959. Vitamin A and fertility. Zootechnia, 8: 49-50 (Nutr. Abstr. Rev. 30: No. 5521).

Diehl, J.F. 1960. Effect of hepatic coccidiosis infection in rabbits on tissue levels of vitamin A and E. J. Nutr. 71: 322-326.

Dutt, B. 1959. Effect of vitamin A deficiency on the testes of rams. Brit. vet. J. 115: 236-238.

Eaton, H.D., C.F. Helmboldt, E.L. Jungherr, C.A. Carpenter and L.A. Moore. 1951. Effect of vitamin A depletion on liveweight, plasma and liver levels of vitamin A and microanatomy in young dairy calves. J. Dairy Sci. 34: 386-395.

Elder, C. 1935. Avitaminosis A in swine. J.A.V.M.A. 87: 22-30.

Emmel, M.W. 1940. The hematology of avitaminosis A in dogs and rabbits. J.A.V.M.A. 97: 145-148.

Erb, R.E., F.N. Andrews, J.F. Bullard and J.H. Hilton. 1944. A technique for the simultaneous measurement of semen quality and testis histology in vitamin A studies of the dairy bull. J. Dairy Sci. 27: 769-772.

Erb, R.E., F.N. Andrews, S.M. Hauge and W.A. King. 1947. Observations on vitamin A deficiency in young dairy bulls. J. Dairy Sci. 30: 687-702.

Evans, H. 1928. The effects of inadequate vitamin A on the sexual physiology of the female. J. Biol. Chem. 77: 651-654.

Evans, H.M. 1932. Testicular degeneration due to inadequate vitamin A in cases where E is adequate. Am. J. Physiol. 99: 477-486.

Eveleth, D.F., D.W. Bolin and A.I. Goldsby. 1949. Experimental avitaminosis A in sheep. Am. J. Vet. Res. 10: 250-255.

Everhart, E.L. 1958. Vitamin A in nutrition. J.A.V.M.A. 132: 344-347.

Frape, D.L., V.C. Speer, V.W. Hays and D.V. Catron. 1959. Thyroid function in the young pig and its relationship with vitamin A. J. Nutr. 68: 333-341.

Gambal, D. 1966. Effect of hormones on the testicular lipids of vitamin A-deficient rats. J. Nutr. 89: 203-209.

Ghanem, Y.S. 1964. The effect of vitamin A deprivation on the reproductive performance of Merino and Barki ewes under desert conditions. Proc. 5th Internat. Cong. Animal Reprod. and A.I. 3: 284-287.

Ghannam, S., H. Al-Alily and S. Deeb. 1966. The effect of different levels of vitamin A on the reproductive organs of young bulls. Int. J. Fert. 11: 306-317.

Goliarkin, F.E. 1941. Influence of vitamin A on appearance of sexual desire and on development of embryo in sows. Proc. Lenin Acad. Agric. Sci. No. 9: 33-36 (Nutr. Abstr. Rev. 12: No. 3141).

Goodwin, R.F.W. and A.R. Jennings. 1958. Mortality of new born pigs associated with a maternal deficiency of vitamin A. J. Comp. Path. and Therap. 62: 82-95.

Grangaud, R. and T. Conquy. 1958a. Algérie méd. 62: 987-1002. Cited from Johnson and Wolf (1960).

Grangaud, R. and T. Conquy. 1958b. C.R. Acad. Sci. 246: 3274-3277. Cited from Johnson and Wolf (1960).

Grangaud, R. and T. Conquy. 1958c. C.R. Soc. Biol. 152: 1230-1234. Cited from Johnson and Wolf (1960).

Grangaud, R., T. Conquy and M. Nicol. 1961. Vitamin A and androgenic steroids. Arch. Sci. physiol. 15: 263-275 (Nutr. Abstr. Rev. 32: No. 1948).

Guilbert, H.R. and G.H. Hart. 1935. Minimum vitamin A requirements with particular reference to cattle. J. Nutr. 10: 409-427.

Guilbert, H.R., C.E. Howell and G.H. Hart. 1940. Minimum vitamin A and carotene requirements of mammalian species. J. Nutr. 19: 91-103.

Guilbert, H.R. and J.K. Loosli. 1951. Comparative nutrition of farm animals. J. Animal Sci. 10: 22-41.

Hale, F. 1934. The relation of vitamin A to the eye development in the pig. Proc. Amer. Soc. Anim. Prod. 27: 126-128.

Hart, E.B., W.S. Miller and E.V. McCollum. 1916. Further studies on the nutritive deficiencies of wheat and grain mixtures and the pathological conditions produced in swine by their use. J. Biol. Chem. 25: 239-261.

Hart, E.B., H. Steenbock, G.C. Humphry and R.S. Hulce. 1924. New observations and a reinterpretation of old observations on the nutritive value of the wheat plant. J. Biol. Chem. 62: 315-322.

Hart, G.H. and H.R. Guilbert. 1933. Vitamin A deficiency as related to reproduction in range cattle. Cal. Agric. Exp. Stat. Bull. 560.

Hart, G.H. and R.F. Miller. 1937. Relation of certain dietary essentials to fertility in sheep. J. Agr. Res. 55: 47-58.

Hays, R.L. and K.A. Kendall. 1956. The beneficial effect of progesterone on pregnancy in the vitamin A deficient rabbit. J. Nutr. 59: 337-341.

Heaton, F.W., J.S. Lowe and R.A. Morton. 1955. The vitamin A deficiency syndrome in the rat. Biochem. J. 60: xviii (Abstr.).

Helmboldt, C.F., E.L. Jungherr, H.D. Eaton and L.A. Moore. 1953. The pathology of experimental hypovitaminosis A in young dairy animals. Am. J. Vet. Res. 14: 343-354.

Hentges, J.F., (Jr.), R.H. Grummer, P.H. Phillips, G. Bohstedt and D.K. Sorenson. 1952. Experimental avitaminosis A in young pigs. J.A.V.M.A. 120: 213-216.

Herrick, J.B. and H.L. Self. 1962. Evaluation of Fertility in the Bull and the Boar. Iowa State University Press, Ames, Iowa.

Hetler, R.A. 1934. The development of xerophthalmia and the keratinization of epithelial tissue on withdrawal of vitamin A from the diet of the monkey (Macacus Rhesus), guinea pig, rabbit and adult albino rat. J. Nutr. 8: 75-103.

Hodgson, R.E., S.R. Hall, W.J. Sweetman, N.G. Wiseman and H.T. Converse. 1946. The effect of vitamin A deficiency on reproduction in dairy bulls. J. Dairy Sci. 29: 669-689.

Hogan, A.G. and J.W. Hamilton. 1942. Adequacy of simplified diets for guinea pigs and rabbits. J. Nutr. 23: 533-543.

Howell, J.McC., J.N. Thompson and G.A.J. Pitt. 1963. 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. Fert. 5: 159-167.

Howell, J.McC., J.N. Thompson and G.A.J. Pitt. 1967. Changes in the tissues of guinea pigs fed on a diet free from vitamin A but containing methyl retinoate. Brit. J. Nutr. 21: 37-44.

Johnson, R.M. and C.A. Baumann. 1948. Relative significance of growth and metabolic rate upon the utilization of vitamin A by the rat. J. Nutr. 35: 703-715.

Johnson, B.C. and G. Wolf. 1960. The function of vitamin A in carbohydrate metabolism; its role in adrenocorticoid production. Vitamins & Hormones, 18: 457-483.

Jubb, K.V.F. and P.C. Kennedy. 1963. Pathology of Domestic Animals. Vol. I. Academic Press, New York.

Jungherr, E.L., C.F. Helmboldt and H.D. Eaton. 1950. Parotid gland lesions in experimental bovine vitamin A deficiency. J. Dairy Sci. 33: 666-675.

Kendall, K.A., G.W. Salisbury and N.L. Van Demark. 1950. Sterility in the rabbit associated with soybean hay feeding. J. Nutr. 42: 487-500.

Kendall, K.A. and R.L. Hays. 1960. Maintained pregnancy in the rat as associated with progesterone administration and multiple-nutrient deficiency. J. Nutr. 70: 10-12.

Lamming, G.E., G.W. Salisbury and K.A. Kendall. 1954a. The effect of incipient vitamin A deficiency on reproduction in the rabbit. I. Decidua, ova and fertilization. J. Nutr. 52: 217-225.

Lamming, G.E., G.W. Salisbury and K.A. Kendall. 1954b. The effect of incipient vitamin A deficiency on reproduction in the rabbit. II. Embryonic and fetal development. J. Nutr. 52: 227-239.

Lamming, G.E., D.H.M. Woollam and J.W. Millen. 1954c. Hydrocephalus in young rabbits associated with maternal vitamin A deficiency. Brit. J. Nutr. 8: 363-369.

Langham, R.F., L.B. Sholl and E.T. Hallman. 1941. The pathology of the bovine kidney in vitamin A deficiency. Am. J. Vet. Res. 2: 319-323. Cited from Helmboldt et al. (1953).

Lewis, J.M., O. Bodansky, K.G. Falk and G. McGuire. 1942. Vitamin A requirements in the rat. The relation of vitamin A intake to growth and to concentration of vitamin A in the blood plasma, liver and retina. J. Nutr. 23: 351-363.

Lindley, C.E., H.H. Brugman, T.J. Cunha and E.J. Warwick. 1949. The effect of vitamin A deficiency on semen quality and the effect of testosterone and pregnant mare serum on vitamin A deficient rams. J. Animal Sci. 8: 590-602.

Lucas, A., C. Quinchon, C. Feugeas, M. Henry, J.M. Gordon, R. Gourdon and L. Toucas. 1955. Kérato-conjonctivite, manifestation d'avitaminose A dans un troupeau de moutons. Rec. Méd. Vét. 131: 665-669.

Macartney, R.E. 1966. The optimal level of alfalfa in the diet of the growing rabbit. 4th Year Project. Macdonald College, McGill University.

Madsen, L.L., S.R. Hall and H.T. Converse. 1942. Cystic pituitary in young cattle with vitamin A deficiency. J. Nutr. 24: 15-24.

Madsen, L.L., I.P. Earle, R.E. Davis, H. Bastron, C.W. Thies, C.A. Cabrell and H.D. Ray. 1947. Some observations on beef cattle affected with generalized edema or anasarca due to vitamin A deficiency. J. Nutr. 34: 603-619.

Madsen, L.L., O.N. Eaton, L. Heemstra, R.E. Davis, C.A. Cabell and B. Knapp (Jr.). 1948. Effectiveness of carotene and failure of ascorbic acid to increase sexual activity and semen quality of vitamin A deficient beef bulls. J. Animal Sci. 7: 60-69.

Mann, I., A. Pirie, K. Tansley and C. Wood. 1946. Some effects of vitamin A deficiency on the eye of the rabbit. Amer. J. Ophthalmol. 29: 801-815.

Mann, T. 1964. The Biochemistry of Semen and of the Male Reproductive Tract. Methuen and Co. Ltd., London.

Mason, K.E. 1930. The specificity of vitamin E for the testis. I. Relation between vitamins A and E. J. Exp. Zool. 55: 101-122.

Mason, K.E. 1935. Foetal death, prolonged gestation and difficult parturition in the rat as a result of vitamin A deficiency. Am. J. Anat. 57: 303-349.

Mayer, J. and W.A. Krehl. 1948. Influence of vitamin A deficiency on the gross efficiency of growth of rats. Yale J. Biol. Med. 20: 403-405.

Mayer, J. and A.P. Truant. 1949. Effect of administration of testosterone on vitamin A-deficient rats. Proc. Soc. Exp. Biol. Med. 72: 436-438.

Mayer, J. and J.W. Goddard. 1951. Effects of administration of gonadotropic hormone on vitamin A deficient rats. Proc. Soc. Exp. Biol. Med. 76: 149-151.

McCarthy, P.T. and L.R. Cerecedo. 1952. Vitamin A deficiency in the mouse. J. Nutr. 46: 361-376.

Mellanby, E. 1934. Xerophthalmia, trigeminal degeneration and vitamin A deficiency. J. Pathol. Bacteriol. 38: 391-407.

Mellanby, E. 1935. Lesions of the central and peripheral nervous systems produced in young rabbits by vitamin A deficiency and a high cereal intake. Brain, 58: 141-173.

Mellanby, E. 1941. Skeletal changes affecting the nervous system produced in young dogs by diets deficient in vitamin A. J. Physiol. 99: 467-486.

Mellanby, E. 1947. Vitamin A and bone growth: the reversibility of vitamin A-deficiency changes. J. Physiol. 105: 382-399.

Millen, J.W., D.H.W. Woollam and G.E. Lamming. 1953. Hydrocephalus associated with deficiency of vitamin A. Lancet, 265: 1234-1236.

Millen, J.W., D.H.W. Woollam and G.E. Lamming. 1954. Congenital hydrocephalus due to experimental hypovitaminosis A. Lancet, 267: 679-683.

Millen, J.W. 1956. Experimental hydrocephalus. Proc. Roy. Soc. Med. 49: 980-982.

Millen, J.W. and D.H.W. Woollam. 1956. The effect of the duration of vitamin A deficiency in female rabbits upon the incidence of hydrocephalus in their young. J. Neurol. Neurosurg. Psychiat. 19: 17-20.

Millen, J.W. and A.D. Dickson. 1957. The effect of vitamin A upon the cerebrospinal fluid pressures of young rabbits suffering from hydrocephalus due to maternal hypovitaminosis A. Brit. J. Nutr. 11: 440-446.

Miller, R.F., G.H. Hart and H.H. Cole. 1942. Fertility in sheep as affected by nutrition during the breeding season and pregnancy. Cal. Agr. Exp. Sta. Bull. 672.

Moore, L.A., C.F. Huffman and C.W. Duncan. 1935. Blindness in cattle associated with a constriction of the optic nerve and probably of nutritional origin. J. Nutr. 9: 533-551.

Moore, L.A. 1939. Carotene intake, level of blood plasma carotene, and the development of papillary edema and nyctalopia in calves. J. Dairy Sci. 22: 803-812.

Moore, L.A. 1941. Some ocular changes and deficiency manifestations in mature cows fed a ration deficient in vitamin A. J. Dairy Sci. 24: 893-902.

Moore, T. 1957. Vitamin A. Elsevier Publishing Co., New York.

Morrison, F.B. 1956. Feeds and Feeding (22nd ed.). The Morrison Publishing Co., Ithaca, N.Y.

Muelder, K.D. and E. Kelly. 1941. The effect of the quantity of basal food intake upon the utilization of vitamin A. J. Nutr. 21: 13-24.

Nalbandov, A.V. 1964. Reproductive Physiology (2nd ed.). W.H. Freeman and Co., San Francisco.

N.A.S.-N.R.C. 1966. Nutrient Requirements of Domestic Animals, No. 9. Nutrient Requirements of Rabbits. National Academy of Sciences-National Research Council Publ. 1194. Washington, D.C.

Nelson, E.C., B.A. Dehority, H.S. Teague, V.L. Sanger and W.A. Pouden. 1962. Effect of vitamin A intake on some biochemical and physiological changes in swine. J. Nutr. 76: 325-332.

Nelson, V.E. and A.R. Lamb. 1920. The effect of vitamine deficiency on various species of animals. I. The production of xerophthalmia in the rabbit. Am. J. Physiol. 51: 530-535.

Nielsen, S.W., J.H.L. Mills, J.E. Rousseau (Jr.) and C.G. Woelfel. 1966a. Parotid duct metaplasia in marginal bovine vitamin A deficiency. Am. J. Vet. Res. 27: 223-233.

Nielsen, S.W., J.H.L. Mills, C.G. Woelfel and H.D. Eaton. 1966b. The pathology of marginal vitamin A deficiency in calves. Res. vet. Sci. 7: 143-150.

O'Donoghue, J.G. 1954. Vitamin A deficiency in beef cattle in Alberta 1953. Can. J. Comp. Med. & Vet. Sci. 18: 141-147.

O'Donoghue, J.G. 1955. Vitamin A deficiency in beef cattle. Can. J. Comp. Med. & Vet. Sci. 19: 367-372.

Orr, J.B. and M.B. Richards. 1934. Growth and vitamin A deficiency. Biochem. J. 28: 1259-1273.

Palludan, B. 1961. Teratogenic effect of vitamin A deficiency in pigs. Ugeskr. Loeger, 123: 21-24; 25-29. (Nutr. Abstr. Rev. 31: No. 3732).

Palludan, B. 1966. Direct effect of vitamin A on boar testis. Nature, 211: 639-640.

Paul, H.E. and M.F. Paul. 1946. The relation of vitamin A intake to length of life, growth, tooth structure and eye condition. J. Nutr. 31: 67-78.

Payne, M.G. and H.E. Kingman. 1947. Carotene blood levels and reproductive performance in range Hereford cattle. J. Animal Sci. 6: 50-55.

Perlman, H.B. and J. Williard. 1941. The ear in experimental vitamin A deficiency. Ann. Otol. Rhinol. Laryngol. 50: 349-362.

Phillips, P.H. and G. Bohstedt. 1938. Studies on the effects of a bovine blindness-producing ration upon rabbits. J. Nutr. 15: 309-319.

Pirie, A. and C. Wood. 1946. Effects of vitamin A deficiency in the rabbit. 1. On vitamin C metabolism. 2. On power to use preformed vitamin A. Biochem. J. 40: 557-560.

Popova, E.A. 1958. Variability of uterine and vaginal epithelium in vitamin A deficiency. Arh. Patol. 20: 59-68 (Nutr. Abstr. Rev. 29: No. 3772).

Rao, M.V.R. 1936. Studies on vitamin A deficiency. 1. Xerophthalmia and trigeminal nerve degeneration. Indian J. Med. Res. 24: 439-457.

Rao, M.V.R. 1940. Studies on vitamin A deficiency. 4. The effect of carotene on the peripheral nerve lesions produced by vitamin A deficiency. Indian J. Med. Res. 27: 731-734.

Reifman, A.G., L.F. Hallman and H.J. Devel (Jr.). 1943. The effect of concentration on the absorption of vitamin A. J. Nutr. 26: 33-42.

Ronning, M., E.R. Berousek, A.H. Kuhlman and W.D. Gallup. 1953. The carotene requirements for reproduction in Guernsey cattle. J. Dairy Sci. 36: 52-56.

Roussel, J.D., T.E. Patrick, H.C. Kellgren, P.F. Randel, and L.L. Rusoff. 1963. Influence of high level vitamin A supplement on semen characteristics and blood composition of breeding bulls. J. Dairy Sci. 46: 583-585.

Safarov, Ju.B. and S.A. Dzul'faev. 1961. An eye disorder in rabbits and guinea pigs. Krolik. Zver. 2: 21. (Nutr. Abstr. Rev. 31: No. 5356).

Salley, J.J., W.F. Bryson and J.R. Eshleman. 1959. The effect of chronic vitamin A deficiency on dental caries in the Syrian hamster. J. Dent. Res. 38: 1038-1043.

Sampson, M.M. and V. Korenchevsky. 1932a. The influence of vitamin A deficiency on male rats in paired feeding experiments. Biochem. J. 26: 1322-1339.

Sampson, M.M. and V. Korenchevsky. 1932b. Changes in the testes of rats kept on a diet deficient in vitamin A. J. Pathol. and Bacteriol. 35: 875-887.

Sandford, J.C. 1957. The Domestic Rabbit. Crosby Lockwood & Son, Ltd., London.

Sapsford, C.S. 1951. Seasonal changes in spermatogenesis in rams: their relation to plane of nutrition and to vitamin A status. Austral. J. Agric. Res. 2: 331-341.

Saunders, C.N. 1958. Abortions and stillbirths in pigs: an analysis of 67 outbreaks. Vet. Rec. 70: 965-970.

Seshan, P.A. and K.C. Sen. 1942. Studies on carotene in relation to animal nutrition. Part III. Stability of carotene in plant material with special reference to hay making and storage. J. Agr. Sci. 32: 275-285.

Sherman, H.C. and E.L. Batchelder. 1931. Further investigation of quantitative measurement of vitamin A values. J. Biol. Chem. 91: 505-511.

Slanetz, C.A. 1943. The adequacy of improved stock diets for laboratory animals. Am. J. Vet. Res. 3: 182-189.

Smith, H.A. and T.C. Jones. 1957. Veterinary Pathology. Lèa & Febiger, Philadelphia.

Sosa, E. 1965. The effects of a vitamin A deficiency on the reproductive capacity of rams. Diss. Abstr. 25 (10): 5473.

Spector, W.S. (ed.). 1956. Handbook of Biological Data. W.B. Saunders Co., Philadelphia.

Spratling, F.R., P.S. Bridge, K.C. Barnett, J.T. Abrams, A.C. Palmer and I.M. Sharman. 1965. Experimental hypovitaminosis-A in calves: Clinical and gross post-mortem findings. Vet. Rec. 77: 1532-1542.

Spratling, F.R. and P.S. Bridge. 1966. Vitamin A deficiency in young cattle. Agriculture, 73: 558-561.

Steel, R.G.D. and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc., N.Y.

Steenbock, H. and K.H. Coward. 1927. Fat soluble vitamins. The quantitative determination of vitamin A. J. Biol. Chem. 72: 765-779.

Sundaresan, P.R. and G. Wolf. 1963. Evidence for the participation of a vitamin A-derivative in ATP-sulfurylase action. Fed. Proc. 22: 293 (Abstr. 792).

Sutton, T.S. and B.J. Brief. 1939. Anatomical and physiological changes in the pituitary glands of vitamin A deficient rats. Ohio Agric. Exp. Stat. Bull. 610.

Swanson, E.W., G.G. Martin, F.E. Pardue and G.M. Gorman. 1968. Milk production of cows fed diets deficient in vitamin A. J. Animal Sci. 27: 541-548.

Swierstra, E.E. and R.H. Foote. 1963. Cytology and kinetics of spermatogenesis in the rabbit. J. Reprod. Fert. 5: 309-322.

Swingle, K.F. and H. Marsh. 1956. Vitamin A deficiency and urolithiasis in range cattle. Am. J. Vet. Res. 17: 415-424.

Tansley, K. 1936. The effect of vitamin A deficiency on the development of the retina and on the appearance of visual purple. Biochem. J. 30: 839-844.

Thompson, J.N. 1965. Some aspects of the metabolism and biochemistry of vitamin A. Proc. Nutr. Soc. 24: 160-166.

Thorp, W.T.S., H.A. Keener, S.I. Bechdel and N.B. Guerrant. 1942. Observations on the pathology of dairy calves on low vitamin A diets. Am. J. Vet. Res. 3: 27-31.

Trimberger, G.W. 1962. Artificial insemination, p. 144-162. In E.S.E. Hafez, (ed.), Reproduction in Farm Animals. Lea & Febiger, Philadelphia.

Truscott, B.L. 1947. Ovarian weight and density in vitamin A-deficient rats. Anat. Rec. 98: 111-126.

Turner, C.D. 1955. General Endocrinology (2nd ed.). W.B. Saunders Co., Philadelphia.

Turner, R.G. and E.R. Loew. 1933. Infection of accessory sinuses in vitamin A deficiency. The role of carotene in infection of the upper respiratory tract. J. Infect. Dis. 52: 102-120.

Warkany, J. and C.B. Roth. 1948. Congenital malformations induced in rats by maternal vitamin A deficiency. J. Nutr. 35: 1-11.

Watt, J.A. and R.M. Barlow. 1956. Microphthalmia in piglets with avitaminosis A as the probable cause. Vet. Rec. 68: 780-783.

West, E.S. and W.R. Todd. 1963. Textbook of Biochemistry (3rd ed.). The Macmillan Co., N.Y.

Williams, R.J. and R.B. Pelton. 1966. Individuality in nutrition effects of vitamin A-deficient and other deficient diets on experimental animals. Proc. Nat. Acad. Sci. 55(1): 126-134.

Witzel, E.W. and G.M. Hunt. 1962. The ultra structure of the choroid plexus in hydrocephalic offspring from vitamin A deficient rabbits. J. Neuropathol. Exp. Neurol. 21: 250-262.

Woelfel, C.G., R.C. Hall (Jr.), M.C. Calhoun, J.E. Rousseau (Jr.), H.D. Eaton, S.W. Nielsen, E.J. Kersting and J.J. Lucas. 1965. Volume and osmolarity of urine of hypovitaminotic A Holstein heifers. J. Dairy Sci. 48: 1346-1352.

Wolbach, S.B. and P.R. Howe. 1925. Tissue changes following deprivation of fat soluble A vitamin. J. Exp. Med. 42: 753-778.

Wolbach, S.B. and O.A. Bessey. 1940. Relative overgrowth of the central nervous system in vitamin A deficiency in young rats. The explanation of the neurological lesions occurring in this deficiency. Science, 91: 599-600.

Wolf, G. and B.C. Johnson. 1960. Vitamin A and mucopolysaccharide biosynthesis. Vitamins and Hormones, 18: 439-455.

Wolfe, J.M. and H.P. Salter (Jr.). 1931. Vitamin A deficiency in the albino mouse. J. Nutr. 4: 185-192.

Wooley, J.G. and W.H. Sebrell. 1945. Niacin (nicotinic acid) an essential growth factor for rabbits fed a purified diet. J. Nutr. 29: 191-199.

Woollam, D.H.M. and J.W. Millen. 1956. The relationship between hypovitaminosis A and the cerebrospinal fluid pressure in the chick: an experimental study. Brit. J. Nutr. 10: 355-363.

Wright, Sampson. 1965. Sampson Wright's Applied Physiology (11th ed.). Oxford University Press, London.

APPENDIX

Appendix Table 1. EFFECT OF LEVEL OF VITAMIN A ON BODY WEIGHT OF RABBITS OVER A TWENTY-FOUR WEEK PERIOD (GRAMS)

Week	Levels of vitamin A ($\mu g/W_{kg}/day$)			
	0	3	6	12
0	872[a]	850[a]	1057[a]	809[a]
1	1081	1046	1262	1020
2	1350	1306	1474	1300
3	1606	1573	1721	1575
4	1848	1826	1921	1838
5	2033	2085	2167	2075
6	2232	2364	2402	2380
7	2408	2573	2581	2465
8	2536	2703	2674	2613
9	2639	3027	2818	2768
10	2717	2962	2883	2837
11	2774	3078	2987	2967
12	2836	3155	3085	3118
13	2896	3247	3223	3305
14	2853	3293	3302	3347
15	2812	3297	3425	3472
16	2673	3297	3402	3467
17	2386	3287	3401	3482
18	2410[b]	3244	3425	3561
19	2335[b]	3233	3512	3610
20	2788[c]	3215	3581	3710
21	2481[c]	3118	3548	3719
22	2266[c]	3085	3625	3868
23	2066[c]	3094[e]	3489	3776
24	1877[d]	3047[e]	3449	3846

[a]Initial mean body weights.

[b]Mean based on 4 rabbits.

[c]Mean based on 2 rabbits.

[d]Mean based on 1 rabbit.

[e]Mean based on 5 rabbits.

Deaths of rabbits caused the number of individuals contributing to the means to be reduced in each of the above.

Appendix Table 2. EFFECT OF LEVEL OF VITAMIN A ON CUMULATIVE RATE OF GAIN OF RABBITS OVER A TWENTY-FOUR WEEK PERIOD (GRAMS PER DAY)

Week	Levels of vitamin A ($\mu g/W_{kg}$/day)			
	0	3	6	12
1	29.9	28.0	29.4	30.1
2	34.1	32.6	29.8	35.1
3	34.9	34.4	31.6	36.5
4	34.9	34.9	30.9	36.8
5	33.2	35.3	31.7	36.2
6	32.4	36.1	32.0	37.4
7	31.4	35.2	31.1	33.8
8	29.7	33.1	28.9	32.2
9	28.0	31.9	28.0	31.1
10	26.4	30.2	26.1	29.0
11	24.7	28.9	25.1	28.0
12	23.4	27.4	24.1	27.5
13	22.2	26.3	23.8	27.4
14	20.2	24.9	22.9	25.9
15	18.5	23.3	22.6	25.4
16	16.1	21.8	20.9	23.7
17	12.8	20.5	19.7	22.5
18	12.2[a]	19.0	18.8	21.8
19	11.0[a]	17.9	18.5	21.1
20	13.7[b]	16.9	18.0	20.7
21	10.9[b]	15.4	16.9	19.8
22	9.1[b]	14.5	16.7	19.9
23	7.4[b]	13.9[d]	15.1	18.4
24	6.1[c]	13.2[d]	14.2	18.1

[a]Mean based on 4 rabbits.

[b]Mean based on 2 rabbits.

[c]Mean based on 1 rabbit.

[d]Mean based on 5 rabbits.

Deaths of rabbits caused the number of individuals contributing to the means to be reduced in each of the above.

Appendix Table 3. EFFECT OF LEVEL OF VITAMIN A ON CUMULATIVE FEED CONSUMPTION OF RABBITS OVER A TWENTY-FOUR WEEK PERIOD (GRAMS PER DAY)

Week	Levels of vitamin A ($\mu g/W_{kg}$/day)			
	0	3	6	12
1	64.8	63.6	79.5	62.9
2	81.6	81.3	90.6	80.7
3	92.1	95.4	99.7	94.3
4	98.6	102.3	105.4	103.7
5	102.0	107.2	108.9	109.9
6	104.7	113.5	115.2	117.6
7	107.4	119.9	120.9	122.2
8	109.8	122.9	123.0	123.8
9	110.2	124.6	125.3	125.8
10	111.0	125.0	125.6	126.8
11	111.1	124.5	126.8	128.4
12	110.4	124.6	127.2	129.9
13	108.7	124.8	127.4	131.2
14	105.0	122.1	125.9	130.1
15	103.0	122.0	128.8	133.3
16	100.7	121.2	128.4	134.1
17	97.7	120.2	128.0	134.4
18	99.7[a]	118.6	128.0	134.9
19	96.2[a]	117.8	129.2	135.4
20	102.2[b]	116.6	129.7	136.2
21	99.5[b]	114.7	129.9	136.8
22	96.2[b]	112.9	129.3	137.8
23	94.0[b]	113.5[d]	128.4	137.9
24	92.7[c]	112.5[d]	127.9	138.1

[a]Mean based on 4 rabbits.

[b]Mean based on 2 rabbits.

[c]Mean based on 1 rabbit.

[d]Mean based on 5 rabbits.

Deaths of rabbits caused the number of individuals contributing to the means to be reduced in each of the above.

Appendix Table 4. EFFECT OF LEVEL OF VITAMIN A ON CUMULATIVE FEED EFFICIENCY OF RABBITS OVER A TWENTY-FOUR WEEK PERIOD (GRAMS OF FEED PER GRAM OF GAIN)

Week	Levels of vitamin A ($\mu g/W_{kg}/day$)			
	0	3	6	12
1	2.2	2.3	2.7	2.1
2	2.4	2.5	3.0	2.3
3	2.6	2.8	3.2	2.6
4	2.8	2.9	3.4	2.8
5	3.1	3.0	3.4	3.0
6	3.2	3.1	3.6	3.1
7	3.4	3.4	3.9	3.6
8	3.7	3.7	4.3	3.8
9	3.9	3.9	4.5	4.0
10	4.2	4.1	4.8	4.4
11	4.5	4.3	5.1	4.6
12	4.7	4.5	5.3	4.7
13	4.9	4.7	5.4	4.8
14	5.2	4.9	5.5	5.0
15	5.6	5.2	5.7	5.2
16	6.3	5.6	6.1	5.7
17	7.6	5.9	6.5	6.0
18	8.2[a]	6.2	6.8	6.2
19	8.8[a]	6.6	7.0	6.4
20	7.5[b]	6.9	7.2	6.6
21	9.1[b]	7.4	7.7	6.9
22	10.6[b]	7.8	7.8	6.9
23	12.7[b]	8.2[d]	8.5	7.5
24	15.2[c]	8.5[d]	9.0	7.6

[a]Mean based on 4 rabbits.

[b]Mean based on 2 rabbits.

[c]Mean based on 1 rabbit.

[d]Mean based on 5 rabbits.

Deaths of rabbits caused the number of individuals contributing to the means to be reduced in each of the above.

Appendix Table 5. EFFECT OF LEVEL OF VITAMIN A ON REPRODUCTIVE EFFICIENCY OF INDIVIDUAL YOUNG MALE RABBITS

Level of Vitamin A ($\mu g/W_{kg}/day$)	Rabbit No.	Total No. Matings	Total No. Resulting Pregnancies	Reproductive Efficiency (%)
3	3M8	9	1	11
6	6M13	2	2	100
	6M14	2	1	50
	6M15	2	1	50
12	12M19	2	2	100
	12M20	2	1	50
	12M21	2	2	100

Appendix Table 6. EFFECT OF LEVEL OF VITAMIN A ON TESTICULAR WEIGHTS OF INDIVIDUAL YOUNG MALE RABBITS

Level of Vitamin A ($\mu g/W_{kg}/day$)	Rabbit No.	Time on Diet Prior to Death (days)	Final Body Weight (g)	Combined Weight of Left and Right Testes (mg)
0	0M1	166	1877	1168
	0M2	127	1908	2086
	0M3	116	1157	1245[b]
3	3M7	267	2414	1278
	3M8[a]	322	3663	1410
	3M9	163	2340	2327
6	6M13[a]	322	3001	2756
	6M14[a]	322	3377	6436
	6M15[a]	322	3370	2199
12	12M19[a]	321	4237	6875
	12M20[a]	321	3706[c]	7698
	12M21[a]	321	3999	7301

[a]Rabbit was killed by cardiac puncture.

[b]A small portion of the epididymis may have been weighed with testicle.

[c]40 c.c. of blood was removed prior to weighing.

Appendix Table 7. EFFECT OF LEVEL OF VITAMIN A ON REPRODUCTIVE EFFICIENCY OF INDIVIDUAL MATURE MALE RABBITS

Level of Vitamin A ($\mu g/W_{kg}$/day)	Rabbit No.	Total No. Matings	Total No. Resulting Pregnancies	Reproductive Efficiency (%)
0	0M25	4	3	75
	0M26	4	4	100
3	3M27	4	3	75
	3M28	4	3	75
6	6M29	2	1	50
	6M30	4	4	100
12	12M31	4	3	75
	12M32	4	1	25

Appendix Table 8. EFFECT OF LEVEL OF VITAMIN A ON TESTICULAR WEIGHTS OF INDIVIDUAL MATURE MALE RABBITS

Level of Vitamin A ($\mu g/W_{kg}/day$)	Rabbit No.	Time on Diet Prior to Death (days)	Final Body Weight (g)	Combined Weight of Left and Right Testes (mg)
0	0M25	272	2475	1777
	0M26[a]	300	4206	8032
3	3M27[a]	300	3483	6329
	3M28[a]	300	4310	4530
6	6M29	163	4690	7310
	6M30[a]	297	3923	6290
12	12M31[a]	297	3965	6885
	12M32[a]	297	3608	6367

[a]Rabbit was killed by cardiac puncture.

Appendix Table 9. EFFECT OF LEVEL OF VITAMIN A ON PARAMETERS OF REPRODUCTIVE PERFORMANCE IN INDIVIDUAL FEMALE RABBITS

Level of Vitamin A ($\mu g/W_{kg}/day$)	Rabbit No.	Total No. Times Bred	Total No. Times Kindled	Total No. Young Born	Average No. Offspring per Parturition	Total No. Young Alive at Birth	Total No. Young Alive at 14 days	Total No. Young Alive after 21 days	Reproductive Efficiency (%)
3	3F10	5	2	11	5.5	0	0	0	40
	3F11	3	0	0	-	-	-	-	0
6	6F16	4	3	20	6.7	12	1	1	75
	6F18	3	3	21	7.0	21	5	4	100
12	12F22	3	3	20	6.7	15	7	7	100
	12F23	3	2	16	8.0	16	10	8	66.7
	12F24	3	2	13	6.5	13	12	12	66.7

Appendix Table 10. EFFECT OF LEVEL OF VITAMIN A ON THE OVARIAN AND UTERINE WEIGHTS OF INDIVIDUAL FEMALE RABBITS

Level of Vitamin A ($\mu g/W_{kg}/day$)	Rabbit No.	Age at Death (days)	Reproductive Status at Death	Combined Ovarian Weights (mg %)	Uterine Weight (mg %)
0	0F4	146	Unbred, prepubertal	10.3	159
	0F5	187	Unbred	5.3	112
	0F6	160	Unbred, prepubertal	7.8	105
3	3F10	364	Post-parturient	8.3	-[a]
	3F11	278	Bred, non-pregnant	14.8	139
	3F12	184	Unbred	30.2	88
6	6F16	367	Lactating	21.7	277
	6F17	253	Unbred	4.8	76
	6F18	366	Lactating	15.0	207
12	12F22	366	Lactating	16.0	143
	12F23	366	Bred, non-pregnant	21.5	281
	12F24	366	Lactating	10.6	117

[a]This doe died just after kindling while the uterus was still in extremely proliferated condition so the weight was excluded from the mean.