

## CXXV. THE TRANSMISSION OF VITAMIN A FROM PARENTS TO YOUNG IN MAMMALS.

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THE attack now being made on the important question of the vitamin A reserves of the human organism in relation to health and disease [Wolff, 1929] may be expected to advance considerably our knowledge of the possible benefits of vitamin A therapy; but whatever may be learned of the influence of the vitamin A reserve of the adult upon health, it is unlikely that work with uncontrolled material in the form of available tissues from autopsies can yield much information concerning the importance and effect of the vitamin A supply of the foetus and the growing child before and after weaning. Wolff has noted that, weight for weight, the livers of new-born children examined by him contained much less vitamin A than the livers of adults: 62·5 % contained no vitamin A, 25 % contained up to one-half as much as the average adult, and 12·5 % contained as much as the average adult examined by him. Although this observation was based on the examination of a small number of livers, it does suggest that the infant is normally born with only a small store of vitamin A in its liver and it directs interest to the factors which control the supply of vitamin A to the child up to the time of weaning, knowledge of which will show how the vitamin A store of the infant can best be enriched. As the controlled experimental work which is needed for an investigation of these factors cannot be performed on human beings, the interest is at present transferred to lower mammals, for which the relevant conditions are essentially similar.

There are two ways in which a young mammal may receive an endowment of vitamin A before it is weaned. The vitamin A may pass through the placental barrier into the foetal circulation during gestation, or it may be ingested by the suckling in the colostrum and the milk. Available data concerning this transmission of vitamin A from mother to young are scanty. It has not been recorded that the vitamin can pass the placental barrier of any other mammal than the human, and the factors affecting the passage in the human are nowhere mentioned, so far as the writer is aware. It is known that vitamin A is present in human, cow's and goat's milk, but again the factors which control the amount in the milk are unknown. Drummond, Coward and Watson [1921] claimed that the vitamin A content of cow's milk is closely dependent on the vitamin content of the diet and shows seasonal variation; but

recently MacLeod, Brodie and MacLoon [1932] have found that there is little seasonal variation in the vitamin A content of cow's milk, and inferred that the vitamin content of the diet has little effect on that of the milk. Moore [1932] has calculated that the amount of carotene ingested by the cow in summer pasture is many times greater than that secreted with the milk-fat as vitamin A or unchanged carotene, while much vitamin A is stored in the liver and an even greater amount of carotene excreted unchanged. Drummond, Coward and Watson [1921] also reported that cow's colostrum is richer in vitamin A than cow's milk.

Experiments on the effect of the mother's diet on the vitamin A reserves of the young rat at weaning were performed by Scheunert and Candelin [1925] who claimed that an increase in the mother's intake of vitamin A caused an increase in the vitamin A reserves of the young; but apparently the young rats had access to solid food containing vitamin A after they were able to eat, so that it is doubtful whether the authors were justified in drawing any conclusions from the results of the experiments they described.

The fullest use of the data mentioned does not, however, enable us to gain an insight into the factors controlling the transmission of vitamin A from the mammalian mother to her young, although it does suggest that any such transmission is a strictly regulated process. In the principal experiment described below a quantitative study has been made of the effect of the carotene content of the maternal diet before and during lactation upon the amount of vitamin A received by young rats up to the time of weaning, in order to obtain further information about the vitamin transfer.

#### EXPERIMENTAL.

##### *The presence of vitamin A in the liver of embryo rat and rabbit.*

Before proceeding to quantitative experiments on the transmission of vitamin A it was thought advisable to find whether the liver of the embryo normally contains vitamin A in the species to be used. Two foetuses of the rat (taken at random from females in the stock colony) were killed at birth and the liver was dissected from each, weighed, and dissolved in 5 cc. of 5% aqueous KOH. The solution was extracted with ether, and the ethereal solution dried by filtering through anhydrous sodium sulphate and evaporated. The oily residue was dissolved in 1 cc. of chloroform and 0.5 cc. of the solution treated with 2 cc. of antimony trichloride reagent. A faint blue colour showing absorption at  $620 m\mu$  was produced, indicating the presence of some vitamin A in the tissue. The remaining 0.5 cc. of solution of the liver oil was then treated with antimony trichloride and the depth of colour measured in a Lovibond tintometer. It was thus found that the whole liver of each foetus contained 10 B.U. (blue units) of vitamin A, corresponding closely to 30 B.U. per g. of liver tissue. (All figures recorded for the colorimetric estimation of vitamin A are expressed in Lovibond blue units calculated by the method of Moore [1930].)

Examination was also made of the liver oils of still-born foetuses, which gave about the same figure for total vitamin A content; and of the whole body fat of the foetus, which had the same total vitamin A content as the liver oils alone. The fat from the foetus from which the liver had been removed was found to contain no vitamin A, showing that the small vitamin A store of the foetus is chiefly contained in the liver—a distribution similar to that in the adult. The presence of vitamin A in the body of the embryo before term was also demonstrated by killing a pregnant rat at 15 days and removing the embryos from the uterus. Two were dissolved whole in 5 cc. of 5 % aqueous KOH and treated as above, when it was found that each embryo contained 5 B.U. of vitamin A.

Similar observations were made with the livers of newborn and embryo rabbits. As an average figure for several newborn rabbits, chosen at random from different stock litters, each whole liver contained 100 B.U. of vitamin A, corresponding to 25 B.U. per g. Two embryos were taken from a doe four weeks pregnant and the liver of each was found to contain 30 B.U. of vitamin A. Thus the embryo receives from the parental organism through the placental barrier either vitamin A or materials from which to make its own vitamin A in the rat and the rabbit as well as the human.

*Transmission of vitamin A to the embryonic and suckling rat.*

The chief experiment was carried out on a single group of females, the controlled variables of the experiment being (a) the vitamin A content of the diet from weaning to the end of pregnancy and (b) the vitamin A content of the diet during lactation. Measurements were made of (a) the vitamin A content of the livers of some of the newborn young from each doe's litter at birth, (b) the vitamin A content of the livers of some young from each doe's litter at weaning, (c) the vitamin A content of the doe's liver immediately after she weaned her litter, and (d) the growth of the remaining young rats from each litter when fed on a vitamin A-free diet after weaning.

The group of females for experiment was chosen from a single litter of Glaxo B stock albinos containing 9 does and 2 bucks. The does were labelled *A* to *I* and separated into three groups at four weeks old, when all nine weighed between 34 g. and 39 g. Does *A* and *B* were placed on a vitamin A-free diet and given no supplement to it until they had ceased to grow; thereafter they received a small daily dose of vitamin A, sufficient to maintain a submaximal growth rate. Does *C*, *D* and *E* were given a diet composed of maize meal 1060, milk powder 400, whole grain wheat 200, dried yeast 200, wheat germ 60, calcium phosphate 60 and salt mixture 20 parts, with a bi-weekly supplement of a little lettuce and milk. Does *F*, *G*, *H* and *I* received a basal diet containing 90 % of the mixture fed to *C*, *D* and *E* and 10 % of red palm oil, with a similar supplement of lettuce and milk. Thus the animals *A* and *B* received a diet very low in vitamin A, while *C*, *D* and *E* received a moderate

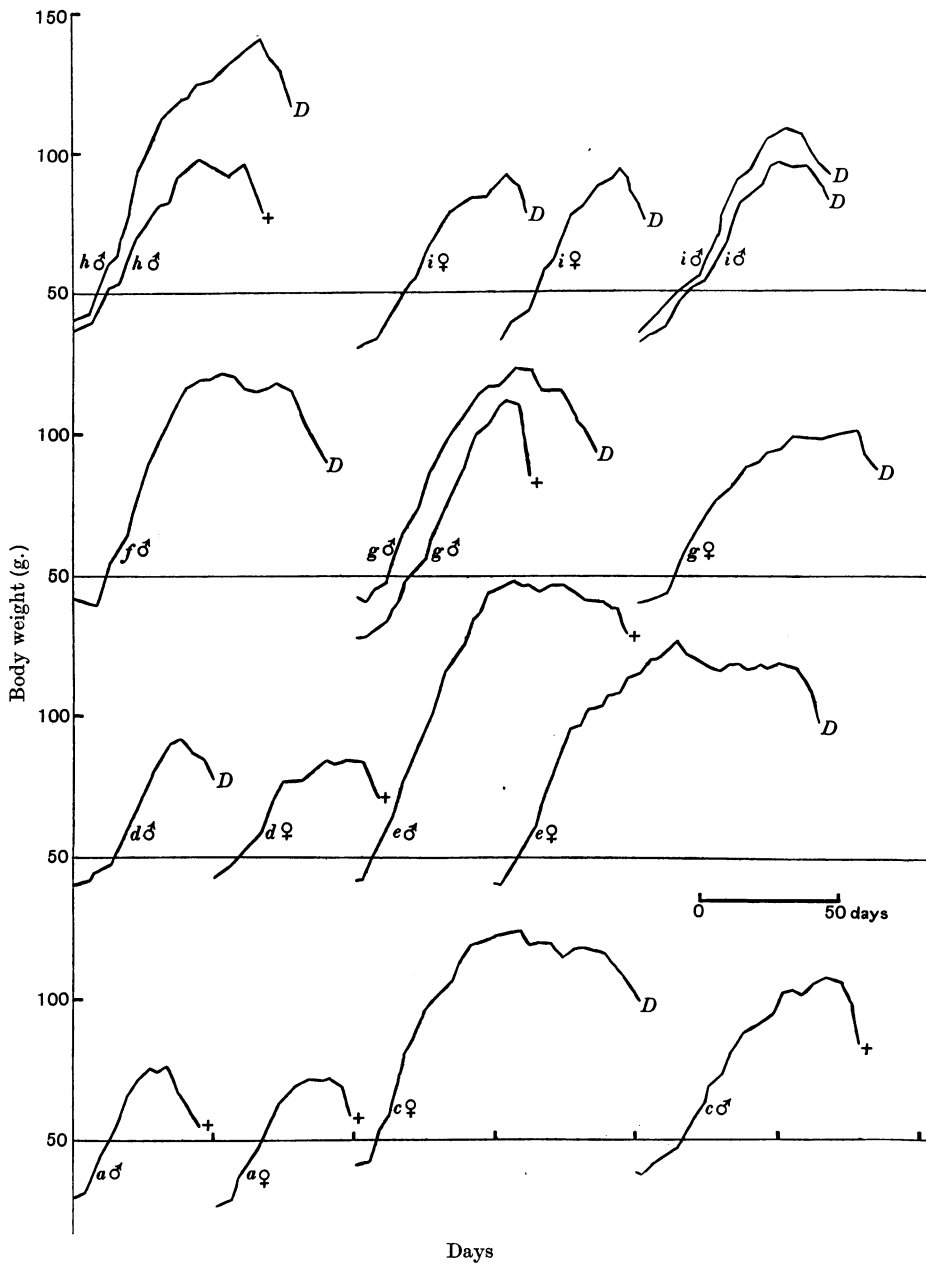


Fig. 1. Growth curves of young rats from does fed with different amounts of carotene during pregnancy and lactation. (For diet of parents compare Table I.) + signifies died. D signifies discontinued. a, c, d, etc. signify offspring of doe A, C, D, etc., respectively.

amount, and *F*, *G*, *H* and *I* a large amount of carotene. When the does were three months old a buck was introduced to the cage housing each group, and each doe when seen to be pregnant was removed to a separate cage but kept on the same diet until the birth of her litter. All the does except *A* and *B* grew fairly uniformly and weighed between 220 g. and 250 g. when they produced litters; doe *B* died and doe *A* grew more slowly than the others and bore a litter when weighing 180 g. After the birth of the litters the diets were rearranged as follows: the does *A*, *C*, *D*, *F*, *G* received the vitamin A-free diet and the does *E*, *H*, *I* received the red palm oil diet rich in carotene. To ensure that none of the young should ingest vitamin A except in the milk, all does receiving the red palm oil diet were fed in a cage apart from the litter and brushed before being returned to the litter cage.

At the birth of each litter two young were killed (only one if the litter was small) and the liver of each dissected out; the liver oils were examined for vitamin A as described above. Each litter was weaned at the age of 21 days and two young were killed in order to examine the liver oils for vitamin A; the remaining animals were given a diet of white bread moistened with water for one week and afterwards a synthetic vitamin A-free diet. Their weight curves were plotted until they were depleted of the vitamin. After the weaning of the litter the doe was generally killed also in order to measure the store of vitamin A in her liver. These measurements are collected in Table I and the growth curves of the young rats weaned from the experimental does are shown in Fig. 1.

Table I. *Vitamin A transfer from mother to young in the rat.*

Amounts of vitamin A are expressed in Lovibond blue units (see text).

Parent doe	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>	<i>H</i>	<i>I</i>
Carotene content of parent's diet from weaning to birth of litter	V. low	Medium	Medium	Medium	V. high	V. high	V. high	V. high
Carotene content of parent's diet while suckling litter	None	None	None	V. high	None	None	V. high	V. high
Amount of vitamin A in liver of young at birth	0	5	10	5	10	10	0	10
Amount of vitamin A in liver of the young at weaning	0	30	10	75	35	30	66	25
Amount of vitamin A in liver of parent after weaning young	20	—	1125	—	—	22,000	20,000	10,000
Average weight of young at weaning (g.)	28	40	40	42	42	35	40	32
Maximum weight attained by young on A-free diet after weaning (g.)	74	118	89	140	122	112	122	98
Days on A-free diet taken to deplete vitamin A store of young	50	77	50	78	79	62	66	55
Number of young weaned	4	4	5	4	2	5	4	6

*Transmission of vitamin A to the embryonic rabbit.*

An observation was made upon the effect of the carotene content of the diet of the rabbit during pregnancy on the amount of vitamin A stored in the liver of the young at birth. From a breeding colony two does were selected of similar age, weight and dietetic history. After mating, one of them received

the ordinary diet of the colony; the other was fed on bran, which had been rubbed with red palm oil, and occasional meals of cabbage to sustain its appetite. During pregnancy it consumed about 3 kg. of bran and 300 g. of red palm oil.

At the birth of the litters two young were removed from each and their livers dissected out. The liver oils were examined in the usual way for vitamin A and it was found that the vitamin A content of each whole liver was about 100 B.U. There was no additional amount of vitamin A in the livers of the young whose parent had received the additional carotene. From this finding it is concluded that the rabbit resembles the rat in that the amount of vitamin A in the liver of the young at birth is not dependent on the amount fed to the mother, provided that she has some reserve.

#### DISCUSSION.

##### *Deductions from the quantitative data on vitamin A transmission in the rat.*

From the figures given in Table I the following conclusions may be drawn concerning vitamin A transmission to the young rat.

1. The amount of vitamin A in the liver of the rat at birth is small and subject to little variation. It cannot be increased by giving large amounts of carotene to the mother before or during pregnancy.

2. The amount of vitamin A in the liver of the young rat when it is weaned is generally larger than the amount in its liver at birth, and is more variable. It is dependent on the carotene content of the mother's diet during lactation, which is evidently a factor in determining the amount of vitamin A in the milk. Thus, under the conditions described, the vitamin A store in the liver of the young rat increased on the average during suckling by 50 B.U. when the lactating mother received a diet rich in carotene, but only by 12 B.U. when the mother received a diet devoid of carotene and vitamin A. When the mother had a negligible reserve of vitamin A in her liver and received a diet free from carotene during pregnancy and lactation, no measurable amount of vitamin A could be found in the liver of the young at weaning (see figures for doe A in Table I). It is probable that the vitamin A reserve of the mother at parturition also exerts an effect on the vitamin A content of the milk but it must be considerably smaller than the effect due to carotene in the diet during lactation, and is not large enough to show up unequivocally in the work described.

3. The weight of the young rat at weaning does not appear to be greatly influenced by the amount of carotene received by the mother at any time, with the exception that the offspring of a doe almost depleted of vitamin A may be stunted (see doe A, Table I).

4. The maximum weight attained by the young and the period for which they can subsist when placed on a vitamin A-free diet after weaning do not appear to depend on the amount of carotene received by the mother before or during lactation; again with the exception that if the mother is almost depleted

and receives a vitamin A-free diet during lactation the growth and the period will both be restricted.

It must be emphasised that for the present purpose whenever vitamin A was found in the foetal liver the assumption was made that this vitamin was obtained from the mother by transmission through the placental barrier. Two other possibilities demand attention. First, it is possible that carotene and not vitamin A passes from the mother to the foetus; a possibility which cannot at present be tested with the rat as the carotene would presumably be transformed into vitamin A in the foetal liver. It may be remarked that the liver oils of the foetus are almost completely colourless and can therefore contain no carotene. Secondly, there is a possibility that the foetus may at some stage of its development be able to synthesise vitamin A from simpler substances. This, however, would appear to be improbable in view of the observation that the foetus is generally born with little vitamin A in its liver and sometimes with none at all.

As the vitamin A store of the young rat cannot be raised by feeding large amounts of carotene to the mother during pregnancy and lactation, there must be some factor at work limiting quite strictly the amount of vitamin A which can pass through the placental barrier or into the milk. Gunderson and Steenbock [1932] have found that "increasing the vitamin B intake both absolutely and in relation to the amount of milk secreted had no discernible effect on the vitamin B content of cow's or goat's milk" and concluded that "the maximum vitamin B content of milk is under definite physiological control." Their finding appears to be an exact parallel with that now reported for vitamin A, but one hesitates to postulate a "physiological control" of transmission to the foetus and into the milk. In the first place, it has been emphasised by Moore [1932] that there is a similar "control" of the maximum vitamin A content of cow's milk, and that while the amounts of carotene and vitamin A passing into the milk daily are very small compared with the intake of carotene, large amounts of vitamin A are stored by the liver. A simple explanation of this would be that the carotene after entering the blood-stream is nearly all absorbed in its passage through the liver, so that little would be found in the general circulation.

A point which emerges from the data of Table I and requires further investigation is that there is no simple proportionality between the vitamin A store of the young at weaning and the length of time they can subsist on a vitamin A-free diet after weaning: thus a rat having a vitamin A store of about 70 B.U. was able to live for 70 to 80 days, one with a store of 25-30 B.U. for about the same time, while one with no measurable store of vitamin A lived for 50 days before declining in weight. It is known that the vitamin A store in the liver of the young rat has a considerable effect on the length of time required to deplete its store on a vitamin A-free diet, but the form of the relationship is by no means clear. It is receiving attention in further work.

In the work described no attempt was made to distinguish between the

colostrum and milk of the rat, so that all references to transmission of vitamin A in the milk include the colostrum; it must be borne in mind that the colostrum may differ from the milk in vitamin A content.

*Application of findings to other species.*

It is not to be expected that the same findings would necessarily be obtained for other species of mammals. The complexity of the placenta varies from species to species so that in the more highly developed forms, as for example in man and the rat (haemochorial placenta), the maternal and foetal bloods come into much more intimate contact than in less highly developed forms such as those of the cow or goat (syndesmochorial placenta) or the pig (epitheliochorial placenta). When the contact between the two circulations is more intimate more substances are transmissible through the placental barrier than when the contact is less intimate. Whether carotene or vitamin A would pass with equal facility through all types of placenta is unknown and must remain for the present an open question.

Needham [1931, p. 1497] has pointed out a relationship between the placenta and the colostrum—namely that in species with more highly developed placentas the colostrum has less importance and is more like the later milk than in species with less highly developed placentas. From this it would appear to be likely that, although the colostrum of the cow is richer in vitamin A than its milk, the same may not hold true for man or the rat. A further investigation of this point is in progress.

Since man and the rat both possess placentas of the same type it may be assumed that the relative importance of the placenta, colostrum and milk for the transmission of vitamin A from mother to young will be similar for the two species. On the basis of this assumption the quantitative results described above suggest that the best way of applying vitamin A therapy with the object of giving the child a reserve of vitamin A is to dose it directly. Failing this, the best results may be expected to follow from giving the mother regular fairly high doses of vitamin A during the period of suckling. To give the mother vitamin A during pregnancy will not affect the vitamin store of her child to any noticeable extent.

SUMMARY.

1. It has been shown that vitamin A is normally found in the liver of the rat and the rabbit at birth, but only in small amount which cannot be increased by giving the mother a diet rich in carotene during gestation.

2. The store of vitamin A in the liver of the young rat increases two- or three-fold during suckling, but the increase may be greater when the lactating parent receives a diet rich in carotene. There appears to be a limit to the amount of this increase, due in turn to a limitation of the amount of vitamin A which can pass into the milk.



3. The significance of these results for other species is discussed and the conclusion reached that in human beings direct feeding of the vitamin to the infant must be employed to build up its reserve of vitamin A.

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