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STERILITY IN MINK INDUCED EXPERIMENTALLY BY DEFICIENCY OF VITAMIN B_6

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The special sexual cycle of mink, with mating season once in the year, means that great demands must be made to the fertility of the breeding animals. Reduced breeding performance in either males or females will under such circumstances have far-reaching consequences. Sterility is thus also a considerable minus factor in mink-breeding. Despite its great extent, the etiologic factors are little known. *Onstad* (1961) has reported testicular hypoplasia in mink as the cause of reduced fertility and aspermia. It was thought that there existed a congenital hypoplasia of one or both testicles.

Knox (1962) has reported the occurrence of infectious abortion in female mink. *Ender & Helgebostad* (1943, 1959) have drawn attention to alimentary factors as a cause of poor breeding results in foxes and with special view to a lack of one or more vitamins within the B complex. Vit. B_6-deficiency is reported as cause of sterility in rats. A preliminary report on deficiency of vit. B_6 in mink has recently been published (*Helgebostad* 1961).

Testicular Hypoplasia in Mink.

Testicular hypoplasia has been produced by experimental deficiency of vit. B_6. Deficiency of vit. B_6 was established by incorporation of the anti B_6 factor: desoxypyridoxine in the feed. The experiments comprised six standard mink born in 1961, each animal was given daily doses of 8 mg desoxypyridoxine.

The feed was in other respects an all-rounded one, consisting mainly of boiled, gutted fish, supplemented with carbohydrates, dried skimmed milk, beef tallow, peanut oil and doses of vitamins, sufficient to cover the daily need for vit. A. D. E. and the various B vitamins, except B_6.

Three animals served as controls. They were given the same feed, but instead of desoxypyridoxine they received appropriate doses of vit. B_6 in the feed. The experiment started the 26. Nov. 1961, at which date the testicles were found to be normally developed.

RESULTS

All experimental animals behaved normally and had good appetite during the first eight weeks of the experiment. One of the males died on the 58th day of the experiment after having eaten poorly a couple of days. This animal showed symptoms of pneumonia in addition to signs of B_6 deficiency. Weight gain was about normal. Three male mink died at the end of February and beginning of March with symptoms of deficiency of vit. B_6. Another male succumbed on March the 5. after puncture of the heart was undertaken, whilst two males, nos. 200 and 416 survived the mating season until July, when they were taken out of the experiment. Those two were both willing to mate. Male no. 416 mated once and was in other respects interested in the females it came into contact with. No sperms could be found in the vaginal smears from the mated female. Male no. 200 mated tree times. In one of the vaginal smears a few sperms were found after mating. After two successive matings with the same male, no sperms could be discovered. The male behaved otherwise normally and was willing to mate. Clinical-chemical analyses of samples of blood and urine revealed distinct symptoms of vitamin B_6 deficiency.

None of the females which were mated with the two B_6 deficient males, became pregnant. In the middle of the mating season, March the 17., the two test males, a control male, and in addition two other males which had received the normal ration of the Experimental Station, were castrated. The castration was performed under ether narcosis. Immediately after the castration, the testicles were fixed in formalin and in Bouin's fluid.

The testicles from the B_6 deficient test animals which died or were killed immediately before the mating season, and from

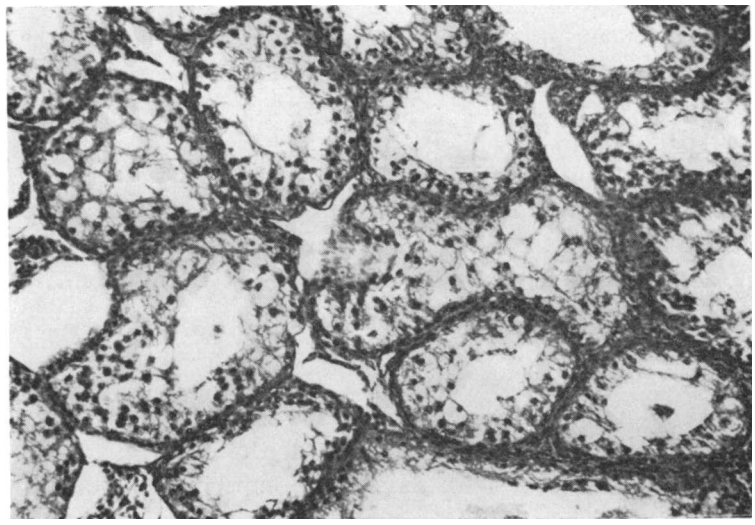


Fig. 1. Testis from mink No. 416 B with B_6 deficiency, castrated March the 17. Epithelium is low and consists of Sertoli cells, spermiogonia and some spermiocytes. Distinct vacuolation and pyknosis. No spermiogenesis. HE. 120 ×.

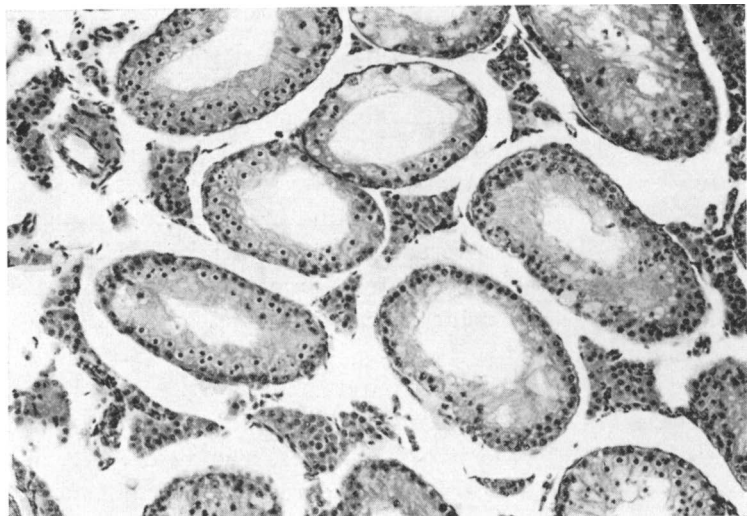


Fig. 2. Testis from mink No. 200 B with B_6 deficiency, castrated March the 17. In this case the vacuolation is not so pronounced. The pyknotic nuclei lie in a granular, eosinophil mass. No spermiogeensis. HE. 120 ×.

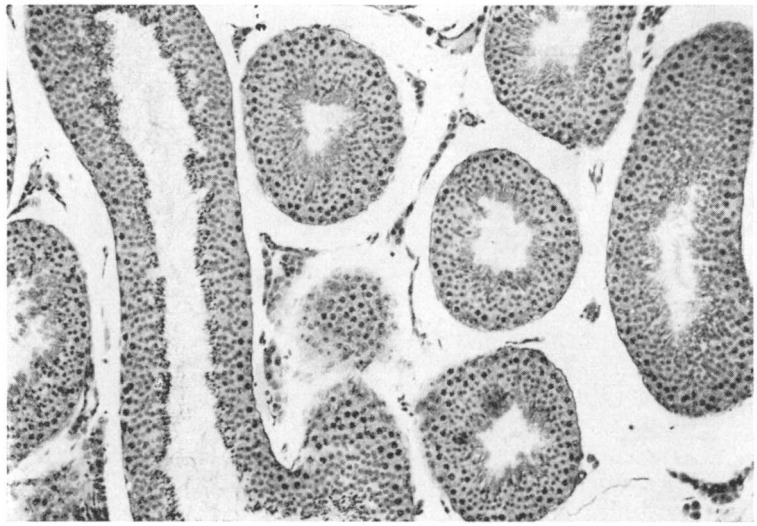


Fig. 3. Testis from control mink No. 143 B, castrated March the 17. High epithelium with normal development of sperms. HE. 120 ×.

those which were castrated, appeared atrophic and were considerably smaller than normal. Each of them weighed singly 0.7—1.7 g, whilst the testicles from the three control mink weighed on an average 2.6 g each.

By histological examination of testis from the B_6 deficient animals the epithelium of tub. contorti was found to be low and there were practically no signs of spermiogenesis. In many ducts only Sertoli cells and spermatogonia were found. In some places spermiocytes and a few spermides were visible. Sperms were difficult to find, in some of the animals they were completely lacking. In most cases the cells which were present showed degenerative changes, they were unduly granular or could be highly vaculated with more or less distinct nuclear changes, usually in the form of pyknosis. The duct of the epididymis contained no or very few sperms. The interstitial tissue in the testicles seemed to be quite normal (see Figs. 1 and 2).

Histological examination of testicles from the animals in the control group showed normal conditions (Fig. 3).

ABSORPTION STERILITY IN FEMALES

Deficiency of vit. B_6 in females was produced in the same manner by incorporating desoxypyridoxine, the anti B_6 factor, in the basal feed, which had the following percentual composition:

Boiled, gutted coalfish	80
Dried, skimmed milk	2.6
Beef tallow	3.1
Peanut oil	0.5
Sugar	2.6
Whole wheat	11.2

Addition of vitamins was given in the following daily doses in mg:

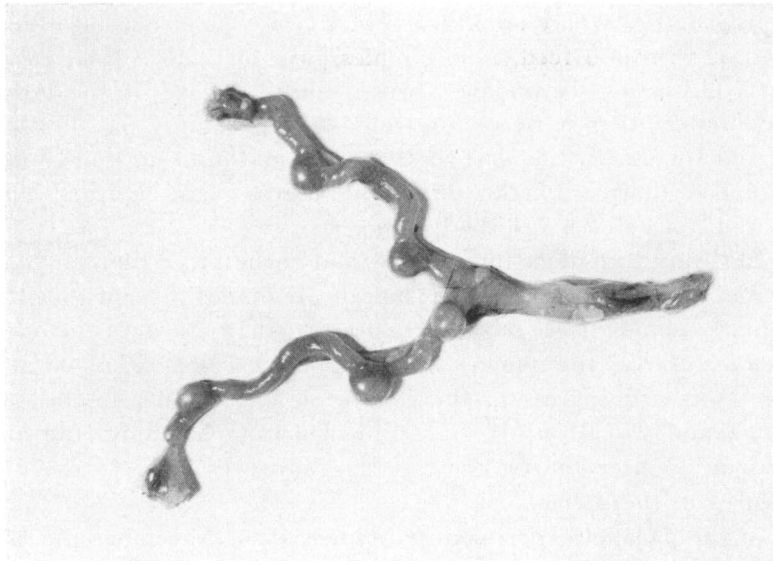
Aneurin	0.8
Riboflavin	1.4
Calcium pantothenat	2.7
Nicotinic acid	3.4
Folic acid	0.07
Biotin	0.01
Vit. B_{12}	0.005
Paraminobenzoic acid	10.0
Inositol	8.0
Ascorbic acid	10.0
Vit. A	3000 I.U.
Vit. D	300 I.U.
Vit. E	5.0 mg.

All rations were supplemented with iron and copper in quantities corresponding to 20 mg $FeSO_4 \cdot 7H_2O$ and 0.3 mg $CuSO_4 \cdot 5H_2O$, three times weekly.

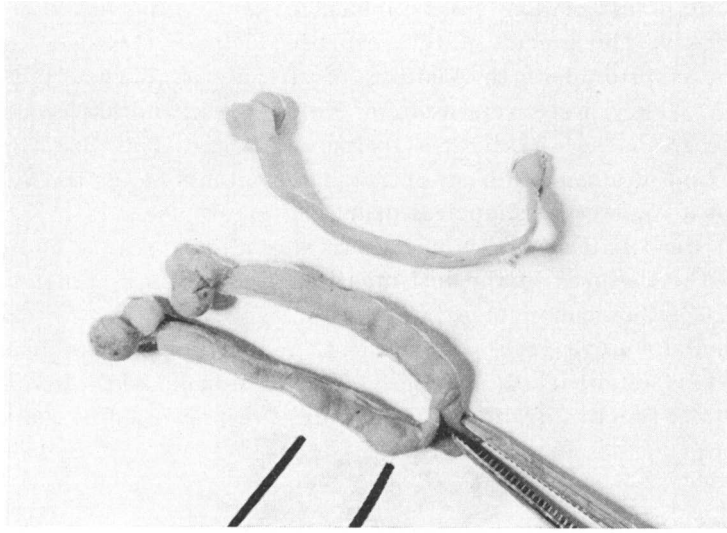
The experiments started on December the 18. 1961 and were terminated after delivery of kits. The test animals were devided into three groups (Group A, B and C). Animals belonging to group A received the basal feed without addition of vit. B_6, whereas those belonging to group B received the same feed with addition of 8 mg desoxypyridoxine (the anti B_6 factor) from March the 20. Daily doses of desopyridoxine were increased to 12 mg from April the 30.

The animals in the control group received the same feed as those in group A, with addition of 0.6 mg vit. B_6 daily. Doses of vit. B_6 was increased to 1.2 mg per animal from April the 3.

Groups A and B each comprised 10 standard mink, whilst the control group included 18 females. They were all mated in the period March the 9.—22. using normally fed males. Investigation of semen fluid from those males revealed normal contents of sperms. In a parallel test (see page 1) which started November the 26. 1961, six males and six females were used. Three of the females survived the mating season and were mated. The other three females died before the mating season.



F i g. 4. Uterus of mink which died on 38th day of pregnancy through lack of vit. B_6. Marks of six resorbed foetuses, like pea-sized prominences. The contents were shapeless, gruellike.



F i g. 5. Bottom, uterus of mink on which laparatomy was performed on May the 15., 58th day of pregnancy. We see marks of resorbed foetuses (five in all) like small prominences in the horns. Above, for comparison, uterus horn of non-pregnant mink at same time. We see clearly the difference in development. The organs have been fixed in formalin.

Results

In group A, where no addition of vit. B_6 was given, but otherwise an all-round feed, four females gave birth to a total of 20 pups. The rest (six females) were "emtpy". Two of the latter were under ether narcosis subjected to laparatomy on May the 15. and the 22. In the first of the two was found an empty undeveloped uterus. In the other the uterus was enlarged and showed marks of 6 resorbed foetuses.

In Group B none of the females had borne kits as late as May the 15., nor did they show any sign of pregnancy, except that the mamary glands were larger thanthey usually are in juvenile animals. Five of the females in this group were one year old and were thus primiparae. In the course of May the 14. to the 18., eight females in Group B were subjected to laparatomy, and one of them to uterotomy. They all presented marks of resorbed foetuses in the uterus.

In the parallel experiment, commenced on November the 26., one of the females died. Post mortem examination revealed six resorbed foetuses. Another female in the same experiment was subjected to uterotomy and there was found evidence of a foetus. The uterus was larger in females with resorbed foetuses than in juvenile females. The horns measured 4—6 mm in diameter externally. The marks of the resorbed foetuses revealed themselves as prominent thickenings of the uterus horns (Figs. 4 and 5). They were yellowish or rather dark and different in colour from the slightly rose-coloured uterus. The size varied from that of a pea to a bean or nut. The contents of the thickened uterus areas were a shapeless gruel.

In the smallest thicknings there was a very scanty content. Thus the foetuses in the last-mentioned thickenings must have died at a very early stage.

In the control group, 15 of the 18 mated females got whelps and they obtained alltogether 83 kits of normal size. Breeding results of females in the various groups are presented in Table 2.

Table 2.

Group	Number of females	Number of females which mated	Number of females which bore whelps	Total number of kits	Empty females
A	10	10	4	21	6
B	10	10	0		10
Control	18	18	15	83	3

DISCUSSION

According to Norway's official statistics relating to furred animals, 17.2 % of the mated mink females are emtpy. *Johansson* (1961) gives corresponding figures for mink in Sweden and Denmark.

The sterility may be due to pathological conditions in one of the sexes or in both sexes simultaneously. Deficiencies of B vitamins are a frequent ailmant in the breeding of furred animals. Where deficiency of one of the B factors occurs it must often be reckoned that there is a deficit of several of them.

The authors have previously shown how sterility in female foxes and reduced vitality in the pups may be due to deficiencies of vit. B_1 or Biotin (*Ender & Helgebostad,* 1943, 1959).

In the experiments now carried out, sterility has been shown to be due to lack of vit. B_6 in both males and females. In male mink atrophy of the testicles, aspermia and marked degenerative changes of the testiclar tissue were found.

In the control groups, where an all-round feed including vit. B_6 was given, we found normal conditions in the males. With respect to the females a good breeding result was obtained (see Table 2). The same is not the case with Group A, which received the same feed as the control group, but without addition of vit. B_6. In this group there were six empty out of ten mated females.

In Group B, where the animals, besides a feed poor in vit. B_6, received an addition of desoxypyridoxine, no kits were borne. Nine females were subjected to laparatomy and in all were found marks of resorbed foetuses. The uterus was visibly enlarged and bore marks of absorbed foetuses, like oval prominent areas (Figs. 4, 5). Absorption must have occurred at an early stage of the foetus development, as no parts of foetuses were found. Absorption sterility as result of lack of vit. B_6 has previously been reported in rats by Ross & Pike (1956).

In view of the great part pyridoxine plays in protein metabolism it is understandable that inadequate supply of this vitamin leads to reproductive disturbances in breeding animals. The mink feed is very rich in protein and the need for vit. B_6 is for this reason increasing. The protein feed comes for the most part from raw fish and fish products which are relative poor in available vit. B_6 (*Rosenberg* 1945). These facts make it advisable that the feed of furred animals should be supplemented with vit. B_6, especially in the breeding period.

Addendum

The described symptoms of experimental vitamin B_6 deficiency in adult mink was reproduced in 1963 using 2 males and 47 females. The experiment confirmed the results obtained in 1962.

In a separate experiment 2 males and one female were fed the desoxypyridoxine containing diet to which large amounts of pyridoxine were added. Both males showed normal conditions and the female came down with full-borne kits.

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SUMMARY

Testicular hypoplasia in mink has been developed using a vit. B_6 poor diet mixed with desoxypyridoxine.

The diet was supplemented with appropriate doses of vit. A.D.E. and the various B vitamins, except vit. B_6. The testicles appeared small and atrophic. Histological examination showed aspermia and distinct degeneration.

Absorption sterility was induced on a similar diet in mink females. Absorption of the foetuses was verified by laparatomy and uterotomy in the middle of May, 55 to 60 days after mating.

In the control groups where the rations were supplemented with vit. B_6 the animals showed normal conditions.

ZUSAMMENFASSUNG

Sterilität beim Nerz, durch Vitamin B_6-Mangel experimentell hervorgerufen.

Beim Nerz wurde Hodenhypoplasie experimentell durch Vitamin B_6-armes Futter hervorgerufen, dem Desoxypyridoxin zugesetzt worden war.

Dem Futter waren gleiche Mengen von A.D.E.-Vitamin und die verschiedenen B-Vitamine zugesetzt, mit Ausnahme von Vitamin B_6. Die Hoden waren klein und atrophisch. Die histologische Untersuchung derselben ergab Aspermie und deutliche Degeneration des spermiogenetischen Gewebes. Bei Nerzweibchen trat Resorptionssterilität nach Verabreichung des gleichen Futters ein. Eine Resorption der Fötusse wurde durch Laparotomie und Uterotomie Mitte Mai, 55 bis 60 Tage nach der Paarung, bestätigt.

In der Kontrollgruppe, bei der das Futter durch Vitamine einschliesslich des Vitamins B_6 ergänzt worden war, zeigten die Tiere normale Verhältnisse.

SAMMENDRAG

Sterilitet hos mink, fremkalt eksperimentelt ved vitamin B_6 mangel.

Testikkelhypoplasi hos mink er fremkalt eksperimentelt på et vitamin B_6-fattig for, tilsatt desoksypyridoksin.

Foret var tilsatt adekvate mengder vit. A.D.E. og de forskjellige B vitaminer, unntatt vit. B_6. Testiklene var små og atrofiske. Histologiske undersökelser av disse viste aspermi og tydelig degenerasjon av det spermiogenetiske vev. Resorpsjonssterilitet hos minktisper fremkom på et lignende for. Resorpsjon av fostrene ble verifisert ved laparatomi og uterotomi i midten av mai, 55 til 60 dager etter paring.

I kontrollgruppen, hvor foret var supplert med vitaminer inkludert vit. B_6, viste dyrene normale forhold.

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