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Vitamin E Research.

By P. Schoorl.

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In honorem Prof. Grijns.

A. *Improved Vitamin E deficiency.*

While we were determining the biological value of oatmeal, nine female rats were found to be completely sterile on a diet consisting of:

		Parts	
Diet No. 69	Amylum Solani	100	None of these ingredients were purified.
	Casein (technical)	60	
	Dextrin (technical)	130	
	Salt mixure	15	
	Yeast (brewers')	20	
	Cod liver oil	2	
	Butterfat	13	

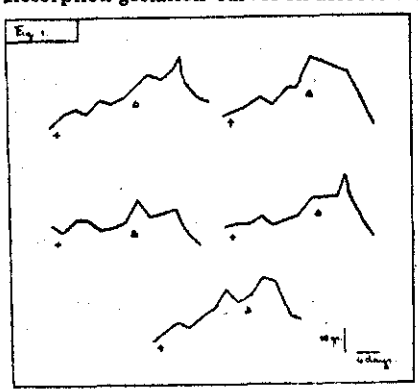
As we suspected a Vitamin E deficiency, but had not controlled insemination, implantation, weight increase till the 20 th day of gestation and subsequent resorption, we then examined regularly the vaginal smears of these nine rats. Oestrous cycles appeared to be quite normal and after insemination a resorption gestation was seen, similar to those described by Evans and Burr[1]. (See fig. 1, numbers 3293, 3299, 3296, 3298 and 3301.) Now our conclusion was of course that lack of Vitamin E was the cause of this sterility. We than placed a new series of female rats on the same diet and controlled them from the beginning of mating with males reared on a normal stock diet. The 12 rats under observation

immediately showed normal resorption gestations. There was no first litter fertility of the kind often described with diets lacking in Vitamin E (Evans and Burr[1]), Waddell and Steenbock[2]), Grijns[3]).

The corn diets for Vitamin E-deficiency used in our laboratory up till that time mostly showed the first generation to be fertile and the second sterile. With this procedure it was always an extremely time-consuming matter to secure in the rats sterility and resorption gestation. It now seemed that we could follow a much quicker procedure, for on diet No. 69 not single litter has been born of 29 female rats that have been mated several times[4]).

These results are, in our opinion, inconsistent with the views of Evans and Burr[1] that female rats ordinarily become sterile after having littered once or twice on a vitamin E-free diet, because all animals start life with a natural store of vitamin E. The iron treated diets of Waddell and Steenbock[2]), which destroy the vitamin E in the body, support this. Probably Evans's diets for vitamin E research still contained more vitamin E than ours, or our ration also prossesses a vitamin E-destroying factor, for *all* our female rats were steril already at the first gestation.

Resorption gestation curves on diet No 69.



As we neither used a diet treated with an ether solution of ferric chloride nor one with an important percentage of lard, as Evans advises, we recognise this property of our vitamin E deficient diet No 69 as a new feature in vitamin E research.

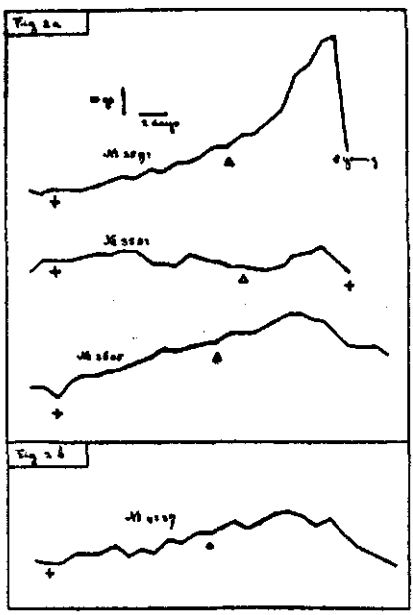
Since we published our earlier results with diet No 69[4]) we have reared some 125—150 female rats on it and we have found that it induces 100 per cent sterility. Evans' view that dietaries still lower in vitamin E may cause earlier resorption, is difficult to accept, for in our experiments resorption begins normally the 20th day after insemination. This is just the same period as mentioned by Evans[1]) and also by Waddell and Steenbock[2]), with the iron treated diet that also causes 100 per cent sterility in the first gestation.

B. *The Vitamin E content of royal jelly of the honey bee.*

In continuation of the work of Hill and Burdett[5]) we tried to determine the vitamin E content of the royal jelly of the honey bee. Hill and Burdett[5]) suggested the possibility that

the difference in fertility between the queen and the worker bee might be due to a difference in the vitamin E content of the food of the larvae. All the larvae start life equal, for both queen and worker larvae are incubated from fertilised eggs, and it is known that a worker larva not more than three days old can be converted into a queen. The suggestion was made by Hill and Burdett[5]) that the larvae destined to be a queen was given a diet rich in vitamin E, while this vitamin was withheld from the worker larvae.

Gestation curves on diet 69 with supplement of royal jelly.



+ = insemination
△ = erythrocyt sign

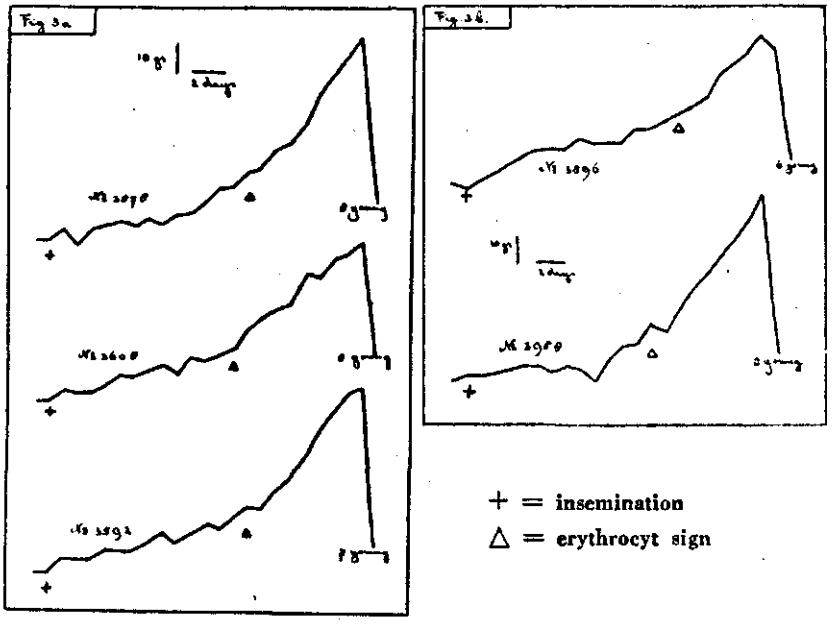
In cooperation with Mr. L. v. Giersbergen, bee-keeping expert, we were able to test the royal jelly. We are much oblidged to Mr. v. Giersbergen for his assistance in collecting this are material.

In the summer of 1933 we arranged our first experiment; we then had 5 gr. of the royal jelly at our disposal. As we did not know anyting about the vitamin E potency of this material, we made a trial with three rats (No 3591, 3581 and 3605). One of them (see fig. 2 a) No 3591 produced a normal litter of 8 young. The daily dose fed was 0,05 gr. during the 20 days after insemination. The second rat No 3581 showed what was probably a resorption gestation while she was receiving 0,05 gr. daily for 2 weeks before insemination and for the three weeks of gestation. Rat No 3605 showed the normal curve of a resorption gestation. The result of this first experiment is in any case not very clear, for No 3591 received less royal jelly than Nos. 3581 and 3605, which showed resorption gestations. It is possible that No 3591, which had been placed in a cage with two other experimental rats, had secured some wheat germ oil from the mouths of her partners, for these others were receiving doses of 0,5 gr. wheat germ oil daily.

We repeated this experiment in the autumn of 1934 with a much larger amount of royal jelly. It was Mr. v. Giersbergen again who was so kind as to gather the material for our experiments.

Normal gestation curves with 20 doses of 0,5 cc wheat germ oil.

Normal gestation curves with 20 doses of 0,2 cc wheat germ oil.

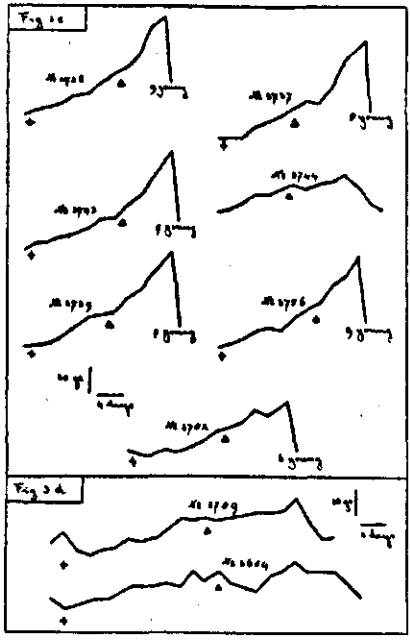


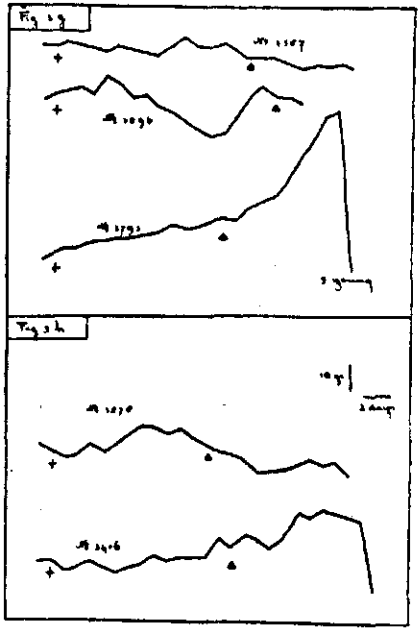
We now mixed 1,5 gr. of the royal jelly with 45 gr. of diet No 69. In this way the experimental animals recrived a much greater dose than in the first experiment (0,4 gr.). Again three rats were chosen for this experiment. (Nos. 4169, 4237 and 3903. See fig. 2 b.) No. 4169 was inseminated, but no implantation sign was seen. In stead of that, oestrus returned regularly. No. 3903 behaved in the same manner. She was inseminated three times at intervals of 18,16 and 30 days respectively, but no implantation signs were observed. Rat No. 4237 showed a normal resorption gestation, so that our experiment again failed to prove that royal jelly contains vitamin E. We did not, however, prove that it does not contain vitamin E, for it is always possible that the ration itself destroyed the vitamin E, when we mixed the royal jelly with the ration in the second experiment. Further work is required to settle the question.

The work of Hill and Burdett[8]) did not actually prove that vitamin E is present in royal jelly, for their experiments were not controlled by the vaginal smear tests, and no observations of insemination, implantation or resorption were made.

C. *What is the minimum dose of wheat germ oil needed for fertility and when do the female rats need it.*

In our first experiments on vitamin E research we gave a rather big dose of wheat germ oil to be sure that fertility would return after a resorption gestation (0,5 cc daily during the three weeks





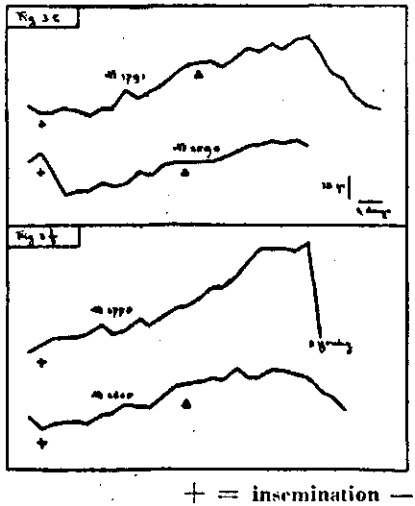


Fig. 3 c.
Gestation curves with doses of 0,1 cc wheat germ oil from weaning till pregnancy 3 times a weak.

Fig. 3 d.
Resorption gestation curves with one dose of 0,5 cc wheat germ oil.

Fig. 3 e.
Resorption gestation curves with 2 doses of 0,5 cc wheat germ oil.

Fig. 3 f.
Gestation curves with 3 doses of 0,5 cc wheat germ oil.

Fig. 3 g.
Gestation curves with 4 doses of 0,5 cc wheat germ oil.

Fig. 3 h.
Gestation curves with 5 doses of 0,5 cc wheat germ oil.

+ = insemination — △ = erythrocyt sign.

of pregnancy). This dose of 0,5 cc. daily after insemination proved to be sufficient. See fig. 3a, Nos. 3578, 3608 and 3592. The pregnancy curves are surprisingly normal in comparison with weight curves of normal pregnant rats. When given during pregnancy, 10 cc. wheat germ oil is sufficient.

We wished to know if it was possible to secure fertility with a much smaller dose. For this purpose we gave, 0,2 cc. daily instead of 0,5 cc. over the same period after insemination. Of the 3 rats

under observation 2 produced normal litters (see fig. 3 b, Nos. 3896 and 3958). The tird, No. 3955, showed red blood corpuscules in the vaginal smear ten days after insemination and oestrus cycle did not return for 11 days, while no increase of weight occured to. Such behaviour is often met in our experiments and we are not sure if it is a sign for vitamin E deficiency or not. In general a dose of 0,2 cc. for 20 days after insemnation (a total of 4 cc. wheat germ oil) is sufficient for fertility. We then decreased the dose once more, to 0,1 cc. daily, but gave it three times a week from weaning till pregnancy. This period lasted almost 3 months so that they got not more than $13 \times 3 \times 0{,}1$ cc. $=$ 4 cc. wheat germ oil. Nine female rats were treated in this way. This dose appeared to be still sufficient, because 6 of the 9 rats littered normally (see fig. 3 c, Nos. 3736, 3737, 3743, 3739, 3756 and 3752). Two did not show any sign of pregnancy (Nos. 3748 and 3730), while one did not receive enough vitamin E and showed a resorption gestation (No. 3744).

We then tried to establish whether the minimum dose is very small or not. Two inseminated rats received 0,5 cc. wheat germ oil on the day of insemination. Both remained sterile (see fig. 3 d, Nos. 3789 and 3654). For thwo others we raised the dose to two doses of 0,5 cc., one on the day of insemination and one the following day. Both remained sterile (see fig. 3 e, Nos. 3791 and 3590). We then gave a dose of 0,5 cc. wheat germ oil 3 times, during the first three days of gestation. One littered and the other showed a resorption gestation (see fig. 3 f, Nos. 3778 and 3605). With four daily doses of 0,5 cc. by 3 rats, we observed one almost normal litter; the two other rats were abnormal and decreased in weight after insemination (see fig. 3 g, Nos. 3575, 3596 and 3793). A trial with 5 daily doses of 0,5 cc. directly after insemination did not give a satisfactory result (see fig. 3 h, Nos. 3578 and 3416). It is possible that number 3416 had littered, but in that case she devoured her litter immediatly, for we found no young, while she lost 27 gr. in weight within 24 hours.

It is our opinion that it is possible to get normal fertility with a quantity of about 2 cc. wheat germ oil when it is given in daily doses of 0,2 in stead of doses of 0,5 cc. Large doses given only during the first 3—5 days of pregnancy are not as effective as smaller doses over a longer period after insemination.

D. *The vitamin E content of Vibeta — a vitamin E concentrate for medical purposes.*

According to an information in Ars Medici [6]), Vibeta is a vitamin E concentrate made from wheat germ oil by the firm of Dr. Georg Henning at Berlin.

We tried to determine the potency of the tablets purchased by a chemist. The results of our tests are to be found in table 1.

The tablets were ground in a mortar and mixed with the food (diet No. 69). In this way we gave 3 experimental animals 1 tablet during the period of pregnancy. Four other animals received resp. 2, 3, 6 and 8 tablets. All the 7 animals showed resorption gestations[7]). Our conclusion is that this concentrate has a very low vitamin E potency, if any ; it is also possible that the alleged vitamin E destroying property of diet 69 has influenced the experimental results. Further experimental work must be arranged to settle this.

Table 1.

	1 tablet No. 4421	1 tablet No. 4294	1 tablet No. Z	2 tablets No. A	3 tablets No. 4237	6 tablets No. A	8 tablets No. 4421
1	+161	+148	+201	+154	+182	+173	+166
2	—	150	205	155	186	178	168
3	165	153	209	162	186	180	168
4	165	152	209	164	187	183	169
5	165	154	203	167	190	184	168
6	164	152	203	162	190	181	172
7	167	151	208	162	191	182	173
8	169	152	206	164	192	180	—
9	170	151	205	165	184	188	178
10	164	154	201	168	190	188	177
11	168	152	205	170	187	△193	179
12	167	155	△207	△172	188	△190	△183
13	172	158	212	175	191	193	△183
14	△177	△159	△210	177	186	192	186
15	△177	158	207	△176	△192	—	186
16	176	160	207	180	△188	195	189
17	178	164	208	180	194	191	192
18	175	162	210	180	190	193	189
19	178	165	216	181	192	195	192
20	178	154	218	185	192	194	193
21	179	152	208	188	—	193	192
22	178	147	207	—	188	188	192
23	172	147	205	164	176	190	183
24	172	145	206	158	178	174	180
25	167	—	—	155	—	171	175

Summary.

A. One of the experimental diets (No. 69) used on the Laboratory of Animal Physiology at Wageningen (Holland) has a high percentage of Amylum Solani and dextrin, together with casein; it shows improved vitamin E deficiency compared with the ordinary vitamin E-deficient corn diets, which mostly give one or two litters before sterility is seen. The improved diet is not treated

with an ether solution of ferric chloride and does not contain any lard. Not a single litter has been born to 150 female rats on this diet.

B. Royal jelly (the food of the larvae of the honey bee destined to be a queen) did not show clear evidence of vitamin E potency. Possibly a vitamin E-destroying property of diet 69 is responsible.

C. Daily wheat germ oil doses of 0,5 cc. during pregnancy proved to be sufficient for fertility. Daily doses of 0,2 cc. over the same period are also sufficient. When 0,1 cc. is given 3 times a week from weaning till pregnancy, one gets also good results, but 1 or 2 doses of 0,5 cc. directly after insemination are insufficient. With 3—5 daily doses of 0,5 cc. directly after insemination 2 litters outof 7 gestations resulted.

D. The vitamin E concentrate Vibeta did not show any anti-sterility potency. The considerations mentioned unter B are also relevant here.

ZUSAMMENFASSUNG.

Vitamin E-Untersuchungen. Von P. Schoorl.

Im veterinär-physiologischen Institut Wageningen wird für Vitamin E-Untersuchungen eine Vitamin E-freie Nahrung No. 69 verwendet, bestehend aus Kartoffelmehl, Dextrin und Casein, Hefe, Salzmischung, Lebertran und Butterfett. Während der Schwangerschaft genügen 0,5 ccm Weizenkeimöl für die Fortpflanzung, sonst reichen auch 0,2 ccm aus. Das Vitamin E-Konzentrat Vibeta hat keine Antisterilitätswirkungen. Mit dem Fruchtsirup, wie er für die Bienenkönigin verwendet wird, wurde keine Vitamin E-Wirkung erzielt.

CONCLUSION.

Recherches sur la Vitamine E. Par Schoorl.

L'Institut de physiologie vétérinaire de Wageningen a utilisé un régime exempt de vitamine E, No 69, se composant essentiellement de fécule de pomme de terre, de dextrine et caséine, avec de la levure, du mélange salin, de l'huile de foie de morue et de la graisse de beurre. Ce régime s'est révélé très efficace. Des quantités de 0,5 cm^3 d'huile de germe de froment administrée pendant la période de gestation suffisent pour assurer la fertilité. Autrement une quantité de 0,2 cm^3 est même encore suffisante. Le concentré de Vitamine E, Vibeta, n'a pas d'effets contraires à la stérilité. Le sirop de jus de fruits préparé pour la reine des abeilles n'exerce aucun effet caractéristique de la vitamine E.

LITERATURE.

1. *Evans and Burr, Armin O. Leuschner*, 1927. — 2. *Waddell and Steenbock*, J. Nutrit. 1931. 4. 79. — 3. *Gryns*, Proceed. R. Soc. Amsterdam, 1925, 31 October. — 4. *Schoorl*, Arch. Neerl. Physiol. Homme et Anim. 1934. 19. 403. — 5. *Hill and Burdett*, Nature, 1932. 130. 540. — 6. Ars Medici 1934. 24. 348. — 7. *Schoorl*, Nederl. Tijdschr. v. Geneesk. 1935. 79. 2086.