Physiology.— *Diet and reproduction.* III. By G. GRIJNS, K. DE HAAN and J. A. VAN DER LOEFF.

(Communicated at the meeting of June 30, 1928).

Since our former communication [1]) a lot of publications have appeared on this subject and all authors come to the postulation of a special nutritive factor controling reproduction.

EVANS [2]) describes the sterility in male rats on various diets, each of which is able to make young rats grow normally and distinguishes four grades of sterility, based on the microscopical investigation of the testes. Wheat germ oil prevents sterility when added to the deficient diet.

EVANS and BURR [3]) purified the unsaponifiable matter of wheat germ oil to a yellow oily substance, of which a single dose of 5 mg. administered to females in the beginning of pregnancy suffices to enable them to produce vigourous young and to rear them even on an E-poor diet.

BARNETT SURE [4]) confirms the observations of EVANS and BISHOP [5]) about the resorption of fetus in sterile females, giving photograms of the uterus, and also the observation of DANIELS and HUTTON, that rats fed a diet of skimmed milk, dextrin, with yeastvitamin, codliver oil and ethereal wheat embryo extract where sterile, but that the addition of the mixture of potassium aluminium sulfate, sodium fluoride, sodium silicate and manganese sulfate to this diet was able to preserve fertility in four generations.

SURE [6]) also proves, that wheatgerm oil not only prevents sterility, but that it also possesses lactation promoting properties. The lactation factor is destructed by heating till 110° C., the sterility preventing not. The ethereal extract contains the active substances, which are not destroyed bij saponifying the fat in alcoholic KOH [7]).

The butterfat contains much less vitamin E. than wheatembryo, but 10 % butterfat in de dietary will suffise to keep up reproduction [8]). Milksecretion

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1) These Proceedings, Vol. **28**, p. 942 and **29**, p. 873.

2) H. M. EVANS: Invariable occurrence of male sterility with dietaries lacking fatsoluble vitamin E. Proc. Nat. Acad. o. Sciences U. S. A. **2**, p. 373, 1925.

3) H. M. EVANS and C. O. BURR: The antisterility vitamin: fatsoluble E. Proc. Nat. Acad. of Sciences U. S. A. **2**, p. 334.

4) BARNET SURE: Dietary requirements for reproduction VI. J. o. Biol. Chem. **69**, p. 41, 1926.

5) H. M. EVANS and K. S. BISHOP: J. o. Metabol. Research 1923, p. 213, 242.
id. id. J. Amer. Med. Assoc. 1923, **81**, p. 889.

6) B. SURE, Dietary requirements etc. VII. J. o. Biol. Chem. **69**, p. 53.

7) id. „ „ „ VIII. „ „ „ „ **74**. p. 37, 1927.

8) id. „ „ „ XI. „ „ „ „ **74**, p. 71, 1927.

requires considerably more B-vitamin than optimum growth [1]) and codliveroil is but poor in vitamin E [2]).

HOGAN and HARSHAW [3]), who could not confirm the findings of EVANS and his cooperators in his former experiments, stated that after they substituted lard for the "crisco" (a dehydrogenated cottonseed oil product) his rats really grew sterile.

On a ration of casein 20, cornstarch 50, lard 10, codliveroil 5, dried yeast 9, cellulose 2, salts 4, males become sterile and females can not rear their young. After 8 months they too where sterile. B-vitamine gave no improvement. The rats did not suffer from anaemia.

CLAYTON stated, that unsaturated animal fats in the ration seriously interfere with reproduction [4]).

SURE and SHILLING [5]) described beriberi in suckling rats, whose mothers were fed a B-vitamin lacking diet. They conclude that for lactation twice as much B-vitamin is necessary as for growth. The clinical symptoms they described, differ much from what I saw myself of beriberi in man and in avian polyneuritis.

Soon after we had initiated our experiments with yellow fat, we also began such with *Ostelin*, a codliver oil product manufactured by the english company GLAXE.

Young rats got the following mixture direct after weaning.

Casein purified	80	Hydrogenated fat	34
Saltmixture	25	Ostelin 2 % in oliveoil	20
Amylum oryzae	260	Marmite	25
Albumen e sangue	10	Decitrated lemonjuice	25

In the first part of the experiment they grew normally, but than slackened a bit. The males did not surpass 190 gm the females reached 150.

As may be seen from table 1 out of 9 females only one had a litter; she died in parturition. The males on the contrary showed reproduction when mated with normally fed females.

It is impossible to explain this experiment and those with maïze-foods by a single fertility controling factor.

For if we would try to explain the maizefood experiments, by assuming that the males are more sensible to the lack or the scantity of a fertility controling factor in the maize than the females are, in the case of the ostelin diet, where the females are sterile, the males not, we should come to the

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[1]) BARNET SURE Dietary requirements etc. X. J. o. Biol. Chem. **74**, p. 55, 1927.

[2]) id. „ „ „ IX. „ „ „ „ **74**, p. 45, 1927.

[3]) A. G. HOGAN and H. M. HARSHAW: Some relations between fertility and the Composition of Diet. Research Bulletin Univ. Missoury N^{0}. 94, 1926.

[4]) MAY M. CLAYTON: The influence of diets containing unsaturated animal fats on reproduction and lactation in the rat. Journ. o. Biol. Chem. **74**, Proc. Am. Soc. of Biologist 21th meeting, p. LXXIV, 1928.

[5]) B. SURE and S. J. SCHILLING: The production of beriberi in the nursing young of the albinorat on diets entirely satisfactory for growth. J. o. Biol. Chem. **74**, LXXIV, 1927.

TABLE 1. Ration XVI (Osteline).

Male	Female	Young	Weaned	Male	Female	Young	Weaned
(500)	519	0		(609)	646	6 [1]	0
(517)	521	0		640	641	0	
518	(542)	13	8	643	634	0	
520	(542)	9	0	"	639	2	0
526	521	0		"	639	0	
527	519	0		"	634	0	
"	533	0		644	645	0	
"	(537)	9	7	"	638	0	
528	(543)	9	0	(770)	638	0	
(584)	519	0		"	641	0	
"	521	0		"	645	0	
(609)	641	0		"	(634)	4 [2]	0

opposite view, for there we should suppose the females to be the more sensible.

In our second communication we already showed, that male fertility and the milksecretion are controled by different factors. That milksecretion and female fertility also depends on different factors is not yet proven by our experiments, but we think it probable. One might think, that from the same factor milksecretion required more than gestation. But if we consider the velocity of growth we are inclined to hold the opposite view.

The curves of growth as they are ordinarily given are simple weight curves. They show the total of weight of the animal on a given time.

But it seems more likely, that the required concentration of a food factor in a diet depends on the *intensity* of growth. Therefore we must consider the increase of weight with regard to the weight reached in every moment. If we suppose the growth-constant as invariable during a short time between two weighings, we come to a formula $K = (ln\,.\,G_1 - ln\,.\,G_2) : (t_1 - t_2)$ [3]. Now K is not invariable, but we come to an approximate diagram, if we take the intervals of observation short. We therefore plotted out our weights on several ages, and calculated the average value of K for every two weeks.

[1]) Mother died in parturition; found in utero.

[2]) Died in pregnancy.

Figures in parenthesis mean rats on normal rations.

[3]) Cf. SAMUEL BRODY: Growth and development. Univ. Missouri Agric. Exper. Station. Research Buletin. **97**, 1927.

In chart 1 the growthcurve of males and females (A) and the intensity curve (B) are given. We see, that the growth-intensity (percentage rate)

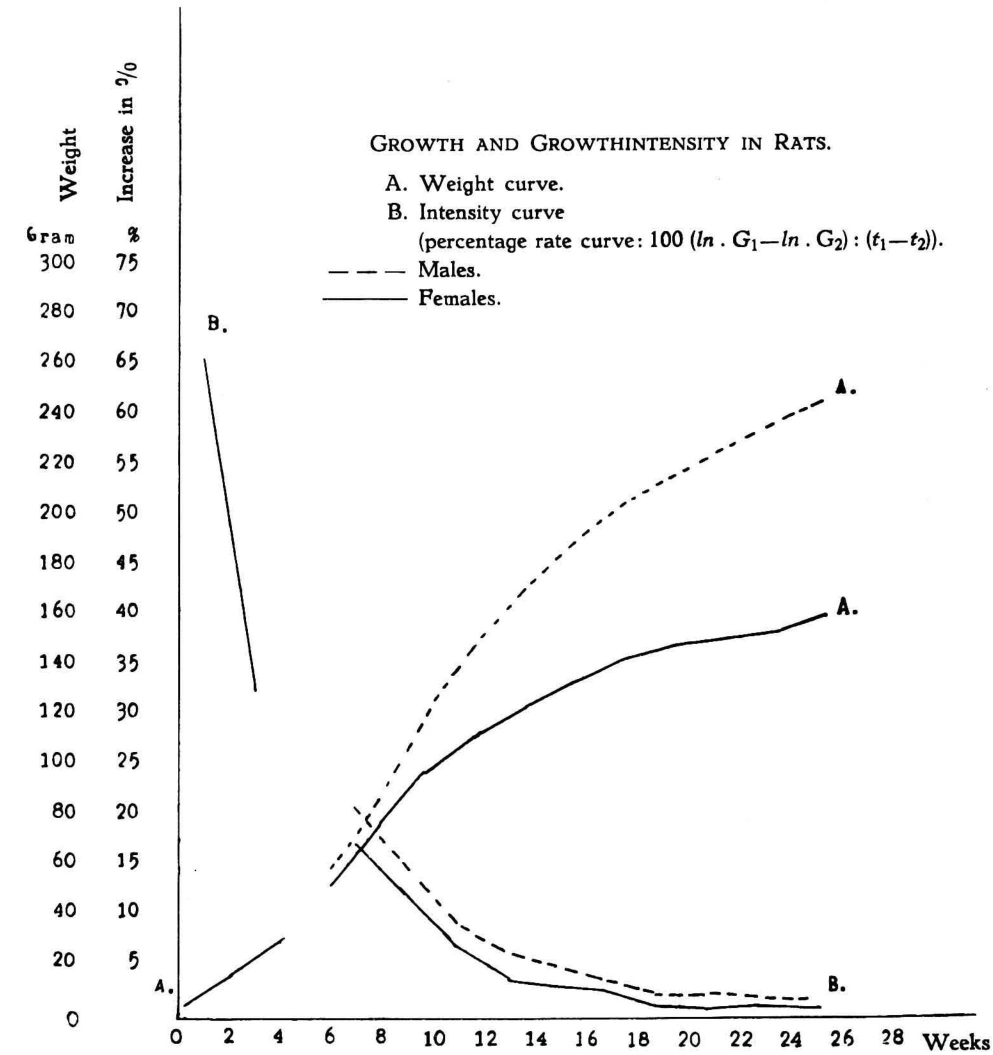

Fig. 1.

is diminishing very rapidly after birth. The curve becomes less and less steep with age.

The gap in the diagram between 4 and 6 weeks is caused by the fact, — that for the litters we had only weights of rats on experimental diets while the rats older than 5 weeks were all stockrats. So the figures of both series were not fully comparable, but as the curve shows the error is not great.

If we remember, that a rats gestation time is three weeks, and that a young rat weights about $4\frac{1}{2}$ gr. while the ovum weighs only 25×10^{-4} m.gr.

and the secundinae have to be formed too, we see, that in utero the intensity of growth is still much greater.

From a number of females we microscopised the ovaria. They always contained Graafian folliculae and ovula. In two cases we found dead foetus in the whomb.

We have tried to get further information on the question of the number of fertility factors by investigating several diets. Firstly we sought for the constituent of normal food, in which the reproduction controling factors might be hidden. To that purpose we substituted in an experiment the suet by hardened fat. The results in table 2 show that it is not the suet that carries the fertilising factors.

TABLE 2. Stockration with hardened fat for suet.

First generation				Second generation			
Males	Females	Young	Weaned	Males	Females	Young	Weaned
813	851	5	4	936	964	9	8
	852	8	8		968	1	0
847	808	7	6	940	957	12	11
	807	11	0		958	5	0
Third generation				963	937	8	0
					999	10	10
.1126	1130	8	7	1001	998	6	2
					1000	8	0

In a second experiment we gave water in the place of the milk, and withdrew clover and carrots. The young grew well; only the males did not grow as heavy as normal ones. One male mated with 2 females on stockdiet begot 17 young, 13 of which were weaned (77 %). Out of 7 females in 9 matings, 3 bore young, together 25 but none were weaned (table 3). The dropping of the milk from our normal ration diminished female fertility to the half, and made rearing impossible. Males showed fertile.

Maizefood I—III kept females fertile and permitted weaning; thus we should suppose the milkfactor either in coconutmeal and peanutmeal or in maize.

In the same time we continued our experiments on maize. We tried to find out firstly if the infertility of the males in the second generation was due to a faulty formation of the testes, or to a degeneration from long abstinence of indispensable foodstuff.

From 6 females and 3 males on maizefood I with codliveroil 2 % we had

TABLE 3. Stockdiet without milk.

Female	Male	Number of young	Weaned
806	836	0	—
„	800	0	—
809	836	10	0
„	800	4	0
816	836	4	0
823	836	0	—
825	833	0	—
„ [1])	914	5	0
826	914	0	—
(814)	800	9	5
(828)	800	8	8
(908)	914	7	0

20 male young and 15 female. (Communication I. p. 879) 4 males and 4 females kept the same diet as their eldren. The four males proved unfertile, their testes remained far below normal weight and in the epididymis no spermatoids were found (table 4 Nos 716, 750, 757, 762). This confirms our previous result (I p. 882). From the 4 females 2 were mated with males on stockrations and reared young. 10 males and the other 11 females got stockrations after weaning. 3 of the males were not regarded further. 3 (Nos 693, 701 and 702) were mated with several females and proved fertile (table 4). In the epididymis we found motile spermatozoids. The same was found in 3 others Nos 756, 749 and 760.

The remaining 6 males Nos 706, 708, 712, 713, 714, and 715 were fed after weaning maizefood I with codliveroil and 2 % from an acetone extract of unbolted wheatmeal. They were mated with normal fed females and all showed infertile. They had atrophy of testes and no spermatozoids in the epididymis. From the females used 4 procreated with normal males (table 5).

In a second series the elder rats already received maizefood I with cod liveroil and acetonextract. Here all males also proved sterile with atrophical testes (table 6, Nos 879, 883, 884, 895). In this case the females were less fertile than normal fed ones.

In the aceton extract of unbolted wheatmeal a fertility promoting factor could not be discovered.

[1]) From 3—5—'27 on stockration. 9—5 killed with gas. 5 foetus found in utero.

TABLE 4. Maizefood I with 2 % codliveroil.

Second generation						Remarks
Male	Female	Young	Weaned	Weight of testes	Spermato-zoids	
716	705	0	—	0.73	—	
750	709	0	—	0.68	—	
757	710	0	—	0.92	—	Single tails?
762	711	0	—	1.20	—	
693	(692)	6	0	3.32	++	This and next two males got stockration after weaning.
	(692)	2	0			
	(631)	1	0			
	(705)	0	—			
701	616	9	9	1.48	++	
	792	0	—			
702	704	8	6	2.56	++	
	608	8	0			
	658	5	0			
	799	5	0			
(783)	710	5	5		++	
	709	?				Abortus?

TABLE 5. Maizefood I with 2 % codliveroil and acetone extract of whole wheat meal.

Male	Female	Young	Weight of testes	Spermatozoids
706	(717)	0	0.27	—
708	(700)	0	1.12	—
712	(965)	0	0.68	—
714	(696)	0	0.75	—
714	(698)	0	0.79	—
715	722	0	0.84	—
(699)	772	11		
(785)	(700)	0		
„	(717)	8		
„	722	9		

TABLE 6. Same ration as table 5.

Male	Female	Young	Weaned	Weight of testes	Spermatozoids
879	902	0	—	not investigated	
883	(905)	0	—	1.04	—
884	882	0	—	0.87	—
„	885	0	—		
895	878	0	—	1.20	—
„	880	0	—		
(904)	882	0	—	3.25	++
„	885	0	—		
(922)	902	7	7		
(920)	878	3	0	3.09	++
„	880	0	—		

TABLE 7. Maizefood I with 2 % codliveroil and 5 % marmite.

First generation			Weaned	Weight of testes	Spermatozoids
Male	Female	Young			
697	737	4	4	well developed	
„	738	8	0		
„	745	0	—		
„	738	5	0		
„	738	4	0		
„	738	5	0		
„	(723)	9	9		
„	(726)	9	8		
779	741	7	7		
„	745	5	0		
910	827	0	—	3.15	++
Second generation					
868	906	0	—	1.21	—
„	906	0	—		
869	872	0	—	1.36	—
870	907	0	—	1.71	+
871	911 [1])	0	—	1.21	+
876	913 [1])	0	—	1.14	—

[1]) 911 and 913 had earlier youngs.

TABLE 8. Maizefood I with 3 % butterfat substituted for hardened fat.

Male	Female	Young	Weaned	Weight of testes	Spermatozoids
First generation					
606	618	9	6		
„	622	10	10		
607	617	5	5		
„	621	9	0		
609	620	8	7		
Second generation					
720	740	0	—	2.59	+
721	743	0	—	0.75	—
„	744	0	—	1.22	—
727	735	0	—	1.46	—
„	739	0	—		
742	736	0	—		
(777)	735	5	5		
„	739	0	—		
„	740	3	0		
(779)	743	0	—		
„	744	0	—		
„	735	4	0		
(898)	735	6	5		
„	739	0	—		
(697)	723	9	9		
„	726	9	8		
(699)	731	12	12		
„	732	12	12		
Young of females from 2d generation with males from 1st one					
932	930	0	—		
„	931	0	—		
933	934	6	0		
(923)	930	8	0		
„	931	6	4		

To decide the question whether in the experiments with maizefood after all still a lack of B-vitamin might interfere, we fed a number of rats with a maizefood I to which was added 5 % of marmite. The results are led down in table 7 from which we see, that not a single male of the second generation succeeded in propagating. The sterility of the males in this and in former experiments is not explainable by lack of B-vitamin.

Finally we added butterfat to a maize-peanut-codliveroil-food as it

TABLE 9. Ration XIIIa.

First generation			Weaned
Females	Males	Young	
804	838	5	5
821	840	8	6
824	838	6	0
	838	5	0
853	837	8	0
854	840	7	5
856	837	3	3
		Total 42	19 or 45 %

TABLE 10. Ration XIIIa.

Second generation			Weaned	Weight of testes	Spermatozoids
Female	Male	Young			
966	987	0	—	2.12	++
	(1003)	2 [1])	0		
973	985	0	—	1.15	++
977	987	0	—		
	(991)	9	0		
981	986	9	2	2.27	++
982	986	0	—		
988	985	0	—		
	(1003)	4	4		
	Total 24		6 or 25 %		

[1]) Found in whomb after death.

seemed to me, that in the long run rats on artificial diets did better on butterfat than on codliveroil. The first generation showed normal fertility, in the second all males were sterile (table 8).

In our second communication we saw that male rats whose elders had lived on ration XIII (aceton extracted whole wheat meal, wheat starch, hardened fat, butterfat, casein, saltmixture and decitrated lemon juice) and after weaning got unextracted wheat meal, were infertile. We then investigated the case that the first generation got unextracted wheatmeal too. The results are colleced in table 9 and 10. In the first generation 45 % of the young were weaned ; in the second only 25 %.

TABLE 11. Ration XIIIa with albumen e sangue.

First generation			Weaned	Weight of testes	Spermatozoids
Female	Male	Young			
818	835	8	3		
820	835	8	8		
843	817	3	0		
846	817	8	5		
992	994	7	7		
993	994	9	0		
		Total 43	23 or 53 %		
Second generation.					
941	946	8	8	not investigated	
943	946	8	8		
945	953	0	—	2.35	+
945	(994)	0	—		
947	944	10	10		
949	944	4	0		
952	942	0	—	2.62	+
952	(995)	0	—		
954	953	0	—		
954	(994) [1]	0	—		
956	942	0	—		
956	(995) [1]	2	0		
		Total 32	26 or 81 %		

[1]) 994 and 995 had proved fertile with stockfed females.

The fertility of the females diminished as well. As albumen starvation or the use of an uncomplete albumen may be the cause of sterility, we tried an addition of albumen to the former diet. We chose albumen e sangue from BROCADES and STHEEMAN, that had prowed in other experiments to contain but very little vitamin A and B. We added 2 % of the dry feed. Table 11 gives the dates. Reproduction is a little hampered, as out of 12 matings only 5 succeeded, but of the 32 young born 26 were raised or 81 % that is little less than with stockrats.

We see thus a remarkable improvement of milksecretion. It is still dubious, wether this is due to the albumen itself or to impurities of vitamin character. For the albumen contains a little ether extractable matter. In rat 952 the autopsy discovered pathological alterations of uterus and ovaria that explain for the sterility.

From the discussed investigations we may conclude, that so for the production of fertilising spermatozoids as for normal gestation specifical fertility factors are indispensable in the nutrition, that may be compared with the other vitamins. For an efficient milksecretion also a specific factor is necessary, but it is not yet sure, that this is not an albumenfraction.

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