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New Dietary Deficiency with Highly Purified Diets.* II. Supplementary Requirement of Diet of Pure Casein, Sucrose, and Salt.

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In a recent preliminary report[1] it was shown that female rats could attain normal adult weight and exhibit normal ovulation on the highly purified diet 519† only when a small amount of lettuce or liver was added to the daily supplement of yeast and cod liver oil. There remained some doubt as to whether Vitamine B as found in yeast (antineuritic B, plus growth B) was capable of making up the growth deficiency if the daily dose were somewhat increased. There was also naturally some question as to whether there might not be possible mal-effects in attempting to satisfy the entire energy requirements of mammals throughout the life cycle with sugar.

Vitamine B Requirements. In this paper we shall employ the term Vitamine B to signify both antineuritic B and purely growth promoting B, both being found in yeast and wheat germ. Eight groups of 3 or 4 animals each have been reared on diet 519† with daily doses of dried yeast or wheat germ as supplements for their Vitamine B requirements. It is especially important to note that we have reared all animals without access to faeces or bedding, *i. e.*, on wire bottoms. The brief summary of results given in Table 1 brings out several interesting facts. 1. The growth was strikingly uniform in all groups and was subnormal, 180 to 190 gm. being the approximate

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†Ingredients	Diet 519	Diet 520	Diet 521	Diet 522
Casein, highly purified	25.0	—	—	30.0
Casein, commercial	—	27.5	27.5	—
Sucrose, recrystallized	75.0	—	75.0	46.0
Sucrose, commercial	—	75.0	—	—
Lard, commercial	—	—	—	20.0
Salts, 185	4.0	4.0	4.0	4.5

Note: The slight increase in the amount of commercial casein is to offset its moisture and impurities. In all four diets, the nutritive ratio is approximately constant and approximately 1:4.

normal weight at 90 days of age in females from our colony. 2. Animals receiving 1.0 gm. of yeast daily were not superior in growth or ovulation to those receiving only 0.7 gm. yeast. This would appear to establish that stunted growth of animals on these diets is not due to a shortage of Vitamine B, both components of which are normally abundant in our yeast. 3. The age of maturity and early ovulation history varied greatly, and unaccountably in the different groups. In some groups (4, 8) after a fair beginning they became markedly subnormal; in others (2, 3) they were strikingly regular in spite of subnormal growth.

The Necessary Supplement for Diet 519. Our early work indicated that diet 519 was deficient in some substance other than Vitamine A, B, D, or E and that this deficiency was overcome by the addition of 0.5 gm. fresh beef liver or 10 gm. fresh lettuce daily.

Repetition of the work has fully confirmed the above findings. The number of animals has been left small because of the expense of the diet, but the results are so uniform that there is no doubt as to the validity of our early conclusions. Table 2 summarizes the results of tests on the following supplements: liver, lettuce, lard and the impurities in commercial casein.

Groups 4 and 5, with commercial casein, show little or no improvements over the 3 control groups (groups 1, 2, and 3), and it is evident that the various milk ingredients other than protein, which are in considerable amount in commercial casein, do not supplement diet 519. Groups 6, 7, 8, and 9 show the value of liver, lettuce and lard as supplements to the pure diet. In these groups, the animals all grew uniformly to the normal 90 day weight of 190 grams and showed excellent ovulation histories, whereas in some groups their litter mate sister controls exhibited very defective ovulation. The remarkable effect of lard has become the subject of special further study and is being reported at this time under separate title.[2] It seemed desirable to attempt a preliminary fractionation of lettuce leaves. The leaves were slowly dried in a warm room so that the cured product retained all of the natural green color and a fine odor. The powdered leaves were extracted with pure ether in a Soxhlet. The ether extract was evaporated to a small volume so that 1 cc. represented 1.0 g. of the dried leaf and this was evaporated on the yeast dose daily. In other cases, unextracted dry leaf powder was fed with the yeast dose. The subnormal growth of the animals receiving these supplements (Table 3) indicates a considerable destruction of the active substance present in fresh lettuce leaf had resulted from the drying process.

Reproduction and Lactation on the Pure Diets. Animals reared on diets 519 or 520 are invariably sterile by the 90th day of life, as was shown by breeding them at the occurrence of the preoestrous stage with fertile males and observing resorption gestations. But that sterility is due to low E is shown by the production of good litters when wheat germ oil is administered in sufficient dosage.

In most of our tests on reproduction and lactation on the "pure" diets, the effort to study these phenomena was always made somewhat beyond the close of the third month of life and consequently at a time when, as has just been explained, the ovulation history was poor. In these animals, consequently, oestrous had to be induced. We accomplished this by subcutaneous injection of 15 mg. of a placental extract.

After the birth of the young, the yeast dose was trebled because we had previously shown that at least thrice the maternal requirement for B is essential for the proper lactation of 6 young. To prevent the young from falling through the wire bottom, solid sheets of tin flooring were employed. No bedding of any sort was allowed, but the cages were not allowed to get cold, being held at 24° C. by thermostatic control. Although great care was exercised by us, all but 3 of the 71 young given for lactation in these 13 gestations, perished during the lactation period. (Table 4.) Evidently, then, the deficiency of the "pure" diets is shown in an especially magnified way by attempted lactations on those diets.

Reproduction and Lactation on the Pure Diet 519 Supplemented by Lettuce. The females in Table 2, group 7, had received lettuce all their lives and were hence not sterile. They were mated when about 4 months old and good litters were produced. A brief record of their lactation performance is given in Table 5. Here also, during lactation, Vitamine B was increased by feeding 3 times the usual dose of yeast. The lactation performance may be said to be far from perfect, but the mortality was not high and the young are still in fine health, though small. These second generation young, therefore, repeat nicely our experience with their parents. They are small, glossy coated, active individuals with every appearance of health, save again for the two striking features of sexual subnormality and incomplete growth.

Summary. 1. The "pure" diet of casein (Van Slyke), sucrose (recrystallized) and salts 185, is not adequate for normal growth although supplemented by very high levels of vitamines A, B, D, and E. In some cases, with the defective growth, ovulation is strikingly regular though in other cases it becomes defective after a month or two. 2. Lactation fails with the "pure" diet. 3. The growth and

ovulation deficiencies are corrected by small daily doses of lettuce or liver, or by the inclusion of lard in the diet. 4. With the additional supplement of lettuce, females produce good litters and they successfully wean them if the yeast dose is adequate. 5. The second generation animals thus successfully weaned, repeat the performance of their parents. Though in perfect health on the "pure" diets, they are always greatly retarded in growth. 6. These experiments, twice repeated, appear to leave no room for doubt that the particular "pure" diet in question either demands an extraordinary and unique amount of some one of the known vitamines or else another and unknown member (F or H) of the vitamine class. Although care to detect them was exercised, the specific criteria for belief in inadequacy in A, D, and E were not encountered. The absence of any change with increased B dosage appears to speak decisively against the complicity of B and to hence leave the second hypothesis as the only alternative.

TABLE 1.

Showing the lack of any appreciable effect of various sources and levels of Vitamine B on the constant subnormal growth produced by Diet 519.

Group	Diet	Age at opening	Age at first estrus	Ovulation cycles	Wt. at 90 days
	519	days	days	days	gm.
2	0.7 g. yeast (Fleishman)	43	43	30, 6, 5, 5, 5, 5, 6, 5, 6, 5, 7	131
	3 drops cod liver oil	39	39	20, 15, 6, 6, 7, 10, 18, 6	124
	(Patch)	69	69	6, 5, 5, 5, 4, 6, 4, 4, 6	138
		38	38	12, 3, 7, 9, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5	144
	519				
3	0.7 g. yeast (N'western)	46	51	5, 5, 5, 5, 5, 4, 6, 5, 5, 5, 4, 5, 6, 5, 5	152
	3 drops cod liver oil	41	47	10, 6, 6, 4, 4, 7, 5, 5, 5, 5, 4, 6, 5, 4	152
	(Patch)	47	47	6, 5, 6, 8, 5, 11, 6, 5, 5, 6, 5, 5	157
	519				
4	1.0 g. yeast (Fleishman)	44	48	5, 8, 7, 6, 13, 8, 5, 7, 8, 13, 34	132
	3 drops cod liver oil	45	45	5, 5, 5, 4, 6, 5, 5, 6, 5, 7, 6, 6, 10, 10, 25	136
	(Patch)	42	42	10, 6, 23, 8, 7, 5, 61	142
	519				
6	0.6 g. fresh wheat germ	60	69	6, 10, 11, 7, 9, 17, 23, 10	107
	3 drops cod liver oil	51	51	5, 5, 5, 5, 5, 5, 6, 6, 5, 6, 6, 7, 7, 34	120
	(Patch)	46	57	5, 5, 7, 6, 6, 6, 6, 8, 6, 24, 10	150

TABLE II.

Showing the value of various materials as a supplement to diet 519. (The first three groups constitute controls on the unmodified diet 519 and consist of litter mate sisters of animals distributed in other groups.)

Group	Diet	Age at opening	Age at first estrus	Ovulation cycles	Wt. at 90 days
	519	days	days	days	gm.
1	0.8 g. yeast	43	72	No cycles	130
	3 drops cod liver oil	47	48	19, 22, 35	143
		53	57	4, 5, 7, 90	150
4	521 (identical with 519 except for the employment of commercial casein)				
	0.8 g. yeast	43	50	6, 5, 5, 6, 13, 6, 4, 6, 5, 5, 16, 35+	148
	3 drops cod liver oil	52	56	6, 6, 5, 5, 5, 5, 5, 5, 5, 5, 5, 14, 34+	164
		61	61	16, 16, 13, 56+	149
5	520 (identical with 519 except for the employment of commercial casein and sucrose)				
	0.7 g. yeast	78	80	9, 18, 20	162
	3 drops cod liver oil	71	72	7, 4, 6, 5, 11, 23	132
		78	80	6, 40	138
		68	72	7, 5, 17, 6, 8, 12	138
		76	—	— no ovulations	138
		63	63	5, 6, 6, 4, 5, 5, 7, 6, 6	156
6	519				
	0.8 g. yeast	54	55	7, 8, 9, 22	190
	3 drops cod liver oil	41	42	6, 4, 5, 4, 6, 5, 5, 5, 5, 4, 4, 5, 5, 4	189
	½ g. liver	Wheezy 56th d. Killed. Wt. 122g. Growing rapidly			
7	519				
	0.8 g. yeast	43	43	7, 6, 6, 5, 6, 4, 6, 5, 5, 5, 5, 5, 6, 5	190
	3 drops cod liver oil	43	43	11, 5, 7, 5, 4, 6, 6, 5, 8, 11, 5	194
	10 g. fresh lettuce	49	49	12, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5	179
9	522 (identical with 519 save for the decrease in sugar and the addition of commercial lard)				
	1.0 g. yeast	41	42	5, 5, 6, 5, 6, 5, 5, 6, 6, 6, 8, 5, 5	189
	3 drops cod liver oil	41	48–	6, 7, 5, 5, 5, 7, 5, 7	182
		43	43	4, 8, 5, 7, 6, 5, 5, 5, 5, 6, 18	174

TABLE III.

Showing partial efficacy of dried lettuce and lettuce leaf oil as a supplement to diet 519.

Group	Diet	Age at opening	Age at first estrus	Ovulation Cycles	Wt. at 90 days
	519	days	days	days	gm.
1	0.7 g. yeast	46	46	8, 7, 7, 6, 6, 6, 5	151
	3 drops cod liver oil	58	62	10, 7, 7, 7	150
	dried lettuce	34	34	5, 13, 5, 4, 6, 10, 5, 5, 5, 4	173
	519				
2	0.7 g. yeast	67	71	4, 7, 5, 7, 5	158
	3 drops cod liver oil	36	36	11, 26, 5, 6, 5	129
	ether extract of 1 gr.	39	39	33, 8, 6, 9	147
	dry lettuce	41	70	5, 5, 7, 6	137

TABLE IV.

Showing the completion of gestation but failure in lactation of mothers on pure diets (519, 520, 521).

Wheat germ oil (10 drops daily) was given during gestation and the yeast dose trebled during lactation.

No. of young born		Average birth wt. of living	No. of young given for lactation	No. of young weaned	Notes
Alive	Dead				
		gm.			
9	0	3.5	6	0	Destroyed by mother on 3rd day
3	0	5.0	3	0	Found dead by 13th day
8	0	4.5	6	0	Found dead by 13th day
7	0	6.0	6	0	All destroyed by 6th day
8	0	4.5	6	0	All dead by 6th day
7	0	5.0	6	0	All destroyed by 2nd day
8	0	5.4	6	0	All destroyed by 2nd day
8	0	5.0	6	0	All gone by 5th day
5	0	5.2	5	2	3 gone by 16th day
5	0	5.0	5	1	4 gone by 16th day
7	0	4.7	6	0	All gone by 7th day
8	0	5.0	6	0	All gone by 10th day
4	0	5.5	4	0	All gone by 6th day

TABLE V.

Showing the Beneficial Effect on Lactation of Adding a Small Amount of Fresh Lettuce Leaf (10 grams) daily to the Pure Diet 519.

(Casein pure 25, sucrose pure 75, salts 4; separately, 3 drops of cod liver oil on 2.1 grams whole dried yeast.)

No. of young born living	Average birth weight	No. of young given for lactation	No. of young weaned	Average wt. on 21st day of life
	gm.			gm.
7	5.0	6	3	30.0
4	4.8	4	4	24.0
5	6.0	5	5	28.0

TABLE VI.

Showing the growth of second generation males and females on diet 519 plus 0.7 g. yeast daily and 3 drops cod liver oil. (Always on wire bottom.)

Age at opening	Age at 1st estrus	Ovulation Cycles	Wt. at 45th day	Wt. at 60th day
days	days	days	gm.	gm.
55	—	None at 65 days	83	108
59	—	" " " "	78	114
48	—	" " " "	67	102
48	—	" " " "	62	106
51	—	" " " "	66	108
54	—	" " " "	60	102
48	48	8	62	108
53	53	No more at 65 days	63	88
—	—	———	79	126
—	—	———	64	100
—	—	———	59	86
—	—	———	72	132

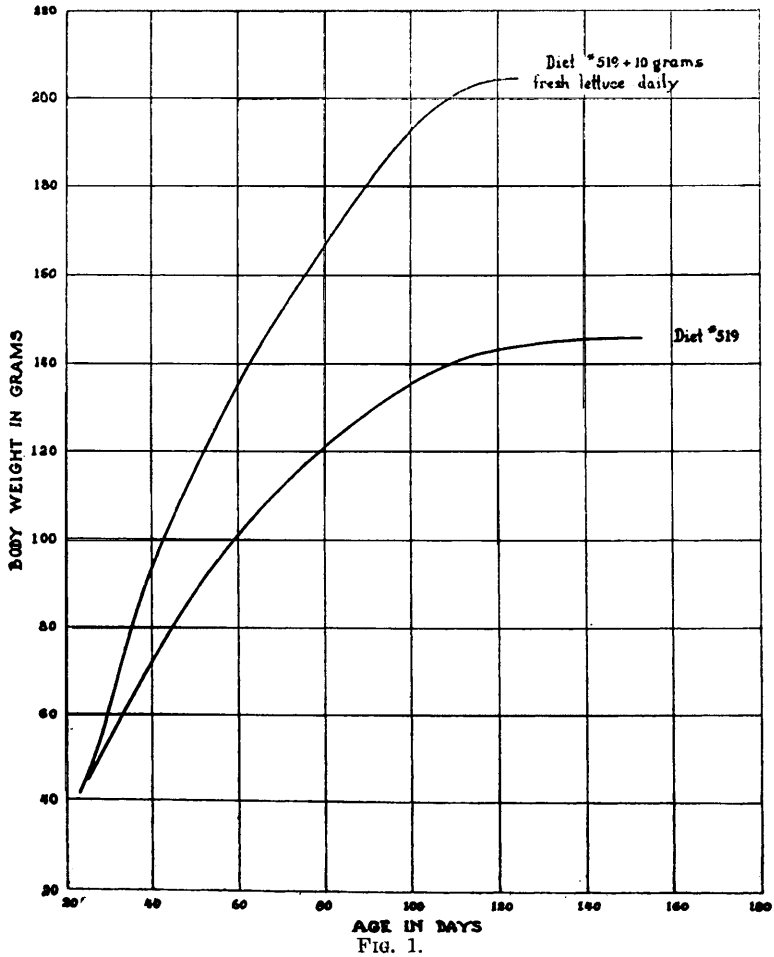

FIG. 1.

Composite growth curves of females showing the invariable stunting which oc-

curs with the highly purified Diet 519 and generous supplies of the known vitamines contrasted with the normal growth produced in litter mate sisters held on the same diet supplemented only with 10 gm. daily of fresh lettuce leaf substance.

Diet 519—Casein, purified, 25; sucrose, recrystallized, 75; salts 4; yeast or wheat germ daily, 0.7 grams; cod liver oil daily, 3 drops.

[1] Evans, H. M., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 740.

[2] Evans and Burr, The Beneficial Effect of Fat in the Diet and the Evidence for a New Dietary Factor for Growth and Ovulation.

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A Powerful Bacteriophage Against Hemolytic Streptococci of Erysipelas Origin.

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Attempts by several authors to obtain an active lytic principle against streptococci were without definite results. Successful attempts with single strains were reported by Piorkowsky,[1] Dutton,[2] Hadley and Dabney,[3] Clark and Clark.[4] Only two strains were of human origin (Piorkowsky, Dutton). It remains yet to decide as to whether streptococci as a genus are susceptible to the bacteriophage phenomenon, or whether the few strains of streptococci, in which this phenomenon was observed, carried a factor, not common to other streptococci as a genus, which enabled them to be affected by the phage. In this work attempts were made to obtain a lytic principle against human pathogenic streptococci by means of various lytic principles, namely: anti-*B. coli* phage; anti-*staphylococcus* phage; lytic principle isolated by Clark and Clark from activated sludge against a strain of rabbit *streptococcus hemolyticus*. Numerous human strains proved to be entirely resistant towards these phages. One hundred and two strains were tested against Clark and Clark's phage. These human strains also completely lacked the power of regenerating these principles. Since the power of regeneration is essential for obtaining classical bacteriophage phenomenon, various resistant strains were placed under supposedly favorable conditions to enable them to perform this function. All these attempts failed. An effort was then made to "train" the pathogenic strains to regenerate bacteriophage by "adapting" the microorganisms to the lytic principles, instead of the usual method, of adapta-