

portance to pure physiology of studying the influence of the environmental temperatures and humidities existing in the tropics upon normal heat production and heat loss.

(The details of this investigation will be reported in a forthcoming number of the *American Journal of Physiology*.)

¹ Benedict and Collins, *Boston Med. and Surg. Journ.*, **183**, 449 (1920).

² Benedict, Miles, and Johnson, *Proc. Nat. Acad. Sci.*, **5**, 218 (1919).

INVARIABLE OCCURRENCE OF MALE STERILITY WITH DIETARIES LACKING FAT SOLUBLE VITAMINE E*

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It has already been possible to show¹ that in the female, sterility may be produced by dietary regimes which nevertheless contain the known vitamins and are adequate for growth. We have reported the cure of such sterility by a variety of natural foods and by small doses of alcoholic and ethereal extracts of those foods. The evidence at hand is thus conclusively in favor of the existence of a new vitamin or food accessory to which the designation of fat soluble E^{1a} may be given.

When male rats from mothers on natural foods are weaned on the twenty-first day of life and then reared upon a basal or "pure" food ration, they are usually at first fertile but when from ninety to one hundred and fifty days of age (usually at the close of the fourth month) become sterile. In a large group we have encountered one or two instances of retention of this initial fertility for a period of seven months, but this is exceptional. The sterility which supervenes in animals held upon these rations, while variable in the exact time of its appearance, is invariable in its ultimate occurrence. At the present time, though many experiments are in progress, completed studies may be summarized as follows: A total of one hundred and eighty males (rats) have been maintained on such a basal diet or modification of it and submitted to repeated functional tests, several thousand "positive"² matings with females of proven fertility having been carefully followed. The distribution of animals with reference to the dietaries employed may be seen from the following table. The animals were invariably sterile.

We have studied fifteen "second and third generation" males. Their mothers had been reared on the basal ration, fertility having been induced by the administration of minute amounts of highly concentrated extracts

of vitamine E. The sons were now similarly reared. *Such males are sterile from the beginning of sexual maturity.*

EXISTENCE OF STERILITY IN MALE RATS, REARED AND HELD ON THE BASIC RATION OR MODIFICATIONS OF IT

DIET	NUMBER OF MALES BRED	NUMBER OF MALES STERILE
B.R. (Basic Ration)—Casein 18, Cornstarch 54, Lard 15, Milkfat 9, Salts 4, Yeast 0.4–0.6 gram daily	52	52
B.R. No. 60—Casein (extracted) 18, Cornstarch 54, Lard 22, Cod Liver Oil 2, Salts 4, various levels yeast (0.2 to 1.6 gram daily)	46	46
B.R. No. 51—Casein 18, Cornstarch 54, Lard 22, Cod Liver Oil 2, Salts 4, Yeast 0.4–0.6 gram daily	32	32
B.R. No. 51—Yeast 0.05 gram daily	2	2
B.R. No. 55—Casein (extracted) 18, Cornstarch 54, Lard 23, Cod Liver Oil 2, Salts 4, Yeast 0.4–0.6 gram daily	7	7
B.R. No. 59—Casein (extracted) 18, Cornstarch 54, Lard 15, Milkfat 9, Salts 4, Yeast 0.4–0.6 gram daily	4	4
F.F.P. 23—Casein 23, Cornstarch 73, Salts 4, Yeast 0.4–0.6 gram daily plus 2 drops Cod Liver Oil	5	5
B.R. No. 17—B.R. 67, Merrill Soule Whole Milk Powder 33	5	5
B.R. No. 27—B.R. 67, Skim Milk Powder 33	9	9
F.F.P. 18—Casein (extracted) 18, Cornstarch 76, Agar 2, Salts 4, Yeast 0.4–0.6 gram daily plus 5 drops Cod Liver Oil	3	3
F.F.P. 18 High Calcium—Casein 18 (extracted), Cornstarch 73, Agar 2, Salts 4, Calcium Lactate 3, Yeast 0.4–0.6 gram daily plus 5 drops Cod Liver Oil	4	4
F.F.P. 18 plus 1 drop Cod Liver Oil	6	6
B.R. No. 88—B.R. No. 51 with 5% yeast in Cup	5	5
TOTALS	180	180

In the above studies all criteria known to us have been employed. We have emphasized the fact that the normality of the females chosen had been proven in each individual instance. Matings took place on the occurrence of a precise stage in the oestrous cycle and the fate of "positive" matings followed by examination of the female for detection of the implantation sign (occurrence of erythrocytes in the upper vaginal canal from the fourteenth to the seventeenth day of gestation). Impairment in the spermatozoa does not in our experience lead to a transitory period characterized by their ability to fertilize yet with intrauterine failure of the young. Intrauterine failure is characteristic of the female sterility disease, but when sterility supervenes in the male there is no evidence of implantation in the female.

We have also studied the sex responses of males and analyzed the bouchon vaginale within a maximum of five minutes after its formation for the presence or absence of sperm and the detection of motility in the latter. These methods of study have enabled us to recognize broadly four stages

in the development of male sterility which may be enumerated as follows: (1) Normal abundance of sperm in the bouchon vaginale; loss of fertilizing power of the sperm;³ approximately normal sex responses. Toward the end of this stage abnormal spermatozoa and instances of fused spermatozoa may be found. (2) Complete loss of sperm from the bouchon vaginale; sterility. Toward the end of this period our statistics show that less than half of all matings with oestrous females lead to a "positive" mating, whereas normally the proportion is over seventy per cent. (3) Loss of power to form the vaginal plug; there may or may not be a retention of sex responses. (4) Loss of all sex interest.

Male sterility is sooner or later characterized by grave changes in the seminiferous epithelium and in advanced cases by complete loss of that epithelium. For a long period of time, Leydig tissue is abundant.

Male sterility can be prevented by feeding from the day of weaning, in addition to the basic ration, certain single natural foods which have proven curative for female sterility. We have thus successfully employed a very potent substance, lettuce leaves, and two less potent ones, milk-fat and beef musculature. In the case of males born from mothers held upon the basic ration, the prevention of sterility may be similarly accomplished by the prophylactic use of foods. In these cases as well as in others we may also accomplish the same end by the prophylactic use of alcoholic or ethereal extracts of certain beneficial foods. We have employed exclusively the ether extract of wheat germ. Six animals, for instance, have recently been studied with wheat germ oil, three receiving one drop daily and three six drops daily.⁴ In the case of the single drop doses, fertility was retained for a period of about ten months, when sterility supervened. They were killed when thirteen months old and remarkably normal looking seminiferous epithelium still found. The animals receiving six drops daily preserved their fertility until the time they were sacrificed at thirteen months of age. The testes were normal. All of the brother littermate controls were sterile by the close of the fifth month and at thirteen months the seminiferous epithelium had practically disappeared.

We have found male sterility, once positively detected, difficult to cure, although proper prophylactic experiments are invariably successful. In our earlier work, cures were not usually attempted until from the fifth to the eighth month, at a time therefore when sterility was well advanced. The subjoined table will show that the administration of wheat germ oil at this time resulted in decisive cures, however, in about one-fifth of the cases. The animals were chosen from a large group and were in all cases controlled by brothers allowed to remain on the basic ration, in which group no instance of spontaneous recovery of fertility has been known to us. It is of interest that the cures were only secured after prolonged administration of the substance containing vitamine E, administration usually

for a period of three months; in one instance, indeed, fertility has first manifested itself after fourteen months of such curative regime.

CURES OF DIETARY STERILITY IN THE MALE WITH SIX DROPS OF WHEAT GERM OIL DAILY

DIET	CON- TROLS	NUMBER OF CURES AT- TEMPTED	SUC- CESS- FUL	AGE OF RAT WHEN WHEAT GERM OIL WAS STARTED	TIME ON WHEAT GERM OIL BEFORE CURE WAS EFFECTED	NOTES
B.R. Yeast 0.4-0.6 gram daily	17	17	0	5-8 mo.		
B.R. No. 60 Yeast 0.2 gram daily	4	8	4	8 mo.	3-4 mo.	
B.R. No. 60 Yeast 0.4- 0.6 gram daily	4	8	3	5-6 mo.	4-6 mo.	
B.R. No. 60 Yeast 0.8 gram daily	4	10	2	5-8 mo.	4-6 mo.	
B.R. No. 60 Yeast 1.6 gram daily	2	4	*1	8 mo.	14 mo.	*Only one litter to date
F.F.P. 18 plus 5 drops Cod Liver Oil	1	*2	0	7-8 mo.		
F.F.P. 18 High Calcium plus 5 drops Cod Liver Oil	1	*3	0	7-8 mo.		
F.F.P. 18 plus 1 drop Cod Liver Oil	1	*5	2	7-8 mo.	*1-3½ mo.	*Male sired one litter just before wheat germ oil was given although the 3 preceding positive matings were in- fertile
B.R. No. 51 Yeast 0.4- 0.6 gram daily	2	*4	*0	5 mo.	*4 mo.	*To date
B.R. No. 88 (20 drops wheat germ oil)	2	*3	*1	4 mo.	*6 mo.	*To date
TOTALS	38	64	13	4-8 mo.	1-14 mo.	

* Male given wheat germ oil as soon as sterility was established.

In many cases before the initiation of the attempted cure, one testis was removed in order to ascertain the histological state of the organ and to make a comparison with the condition of the remaining organ when fertility had been induced. We attribute success in the restoration of fertility to the sporadic retention of bits of seminiferous epithelium in some tubules, although the majority of tubules were devoid of epithelium. The cure of dietary sterility was in all instances accompanied by an improvement in the sex response as well as by the regeneration of the epithelium in some tubules and development of normal fertilizing power of the sperma-

tozoa. It is interesting that the administration of the vitamine in the form of wheat germ oil has also resulted in a longer retention of normal sex responses in animals where the restoration of fertility was not accomplished. It has also led to superior growth, usually changing a growth plateau to the slow rise characteristic of the growth of males on natural foods. This general improvement could hardly be due to the "nutritional" value of the insignificant added substance. Since it was also present in cases which were not cured and in which the testicular epithelium had disappeared, it would seem a constitutional effect not mediated through the sex glands.

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¹ Evans and Bishop, "On the Existence of a Hitherto Unrecognized Dietary Factor Essential for Reproduction," *Science*, December 8, 1922, see also *J. Metabolic Res.*, 3, No. 2, February, 1923; and the *J. Amer. Med. Assoc.*, 81, September 15, 1923.

^{1a} We have adopted the letter E as the next serial alphabetical designation, the antirachitic artanine now being known as D.

² "Positive," used only for instances in which the occurrence of sexual congress was positively ascertained by the finding of sperm or the copulatory plug (bouchon vaginale) within eighteen hours after pairing such males with females of proven fertility and at the pro-oestrous or early oestrous stage.

³ Such cases as we have been able to examine by the "fresh" method in this period (i.e., the period characterized by abundance of sperm with sterility) have always shown absence of motility in the sperm.

⁴ In conjunction with Dr. George O. Burr, I shall later report upon the successful prophylactic employment of minute traces of a very concentrated fraction out of wheat germ oil, namely, the sterol-free or red oil portion of the non-saponifiable part of the original oil.

A HISTOLOGICAL STUDY OF STERILITY IN THE ALBINO RAT DUE TO A DIETARY DEFICIENCY

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In the winter of 1921-22, at the request of Dr. Osborne and Dr. Mendel, a thorough study was begun to determine the histological condition of the reproductive organs of rats fed artificial diets of known composition, adequate for growth but not for reproduction.

So far as we can find C. B. Paul¹ was the first to intimate that fertility is affected by diet. He states that "an excessive meat diet interferes with the development of the male reproductive organs" and that "the defective development is most pronounced in the seminal vesicles and prostate