

Summary. A combined operation of bilateral vagotomy 4 cm above the diaphragm and girdling of the esophagus at the same level is described as a means of producing in dogs an experimental condition comparable to clinical cardiospasm. In the majority of cases the regurgitation of food can be shown to be accompanied by a failure of receptive relaxation of the cardiac orifice of the stomach. Since neither bilateral vagotomy in the thorax^{1,4} nor girdling the esophagus above the diaphragm is sufficient in itself to produce these results, it may be concluded that some but not all of the fibers responsible for receptive relaxation of the cardia of the dog course downward within the wall of the esophagus.

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Failure of Maternal Vitamin A Depletion to Produce Congenital Anomalies in the Young of Rats.

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Attention recently has been directed to possible dietary causes of congenital anomalies by Hale's observations^{1, 2, 3} on the occurrence of microphthalmia, hare lip, cleft palate, and the failure of the kidneys to leave their embryonic position in the young of vitamin A-deficient sows. Others who have investigated reproduction in vitamin A-deficient hogs have reported abortion, resorption or the birth of dead fetuses. In some instances prolonged labor was observed, but congenital anomalies in the young were not described.^{4, 5}

Hart, Meade and Guilbert⁶ made no mention of congenital defects in calves born to cows showing night blindness at the time of parturition, nor were anatomical abnormalities recorded by Hart and Miller⁷ among lambs from ewes kept on vitamin A-low rations for nearly a year and night blind at the time of lambing.

¹ Hale, Fred, *J. Heredity*, 1933, **24**, 105.

² Hale, Fred, *Am. J. Ophthal.*, 1935, **18**, 1087.

³ Hale, Fred, *Texas State J. Med.*, 1937, **33**, 228.

⁴ Hughes, J. S., Auel, C. E., and Lienhardt, H. F., *Kansas Agric. Exp. Sta. Tech. Bull.*, 1928, **23**, 1.

⁵ Hughes, E. H., *J. Agric. Res.*, 1934, **49**, 943.

⁶ Hart, G. H., Mead, S. W., and Guilbert, H. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1230.

⁷ Hart, G. H., and Miller, R. F., *J. Agric. Res.*, 1937, **55**, 47.

Sherman and MacLeod,⁸ Evans,⁹ and Batchelder¹⁰ all found reduced reproductive capacity in vitamin A-deficient rats. Sure¹¹ was the first to show that lack of vitamin A results in resorption of fetuses, even if abundant vitamin E is provided. This work was confirmed by Mason,¹² who showed that vitamin A deprivation also caused appearance of the "placental sign" (red blood cells in the vaginal smear) one to 4 days early, prolonged gestation, and difficult parturition with all or part of the young born dead. Mason makes no mention of congenital anomalies, although he states that all young born to his large series of vitamin A-deficient mothers were routinely examined. Tansley¹³ and Newton,¹⁴ who have confirmed Mason's work, observed no anomalies in the young.

Hale⁸ states that he has observed 2 cases of congenital blindness in rats from vitamin A-deficient mothers, but mentions that hereditary factors are not ruled out. Browman,¹⁵ investigating the reproductive performance of rats receiving adequate vitamin A but which had had a previous history of vitamin A depletion, encountered microphthalmia in one percent of the young born to these mothers. However, 0.7% of the young from the stock colony showed this defect.

In an extensive study of congenitally malformed children, Murphy and Bowes¹⁶ have judged the maternal diet to have been inadequate in 40% of the cases. They do not, however, present control studies on the incidence of inadequate diets among mothers of normal infants.

We have studied the effects of maternal vitamin A deprivation upon the rat fetus, placing particular emphasis upon the question of congenital anomalies.

Two A-low diets were employed:

	A	B
Casein (hot alcohol extracted)	24	22
Sucrose	20	64
Hydrogenated cottonseed oil (Crisco)	22	—
Salt mixture No. 185 ¹⁷	4	4
Yeast (ether extracted)	10	10

⁸ Sherman, H. C., and MacLeod, F. L., *J. Am. Chem. Soc.*, 1925, **47**, 1658.

⁹ Evans, H. M., *J. Biol. Chem.*, 1928, **77**, 651.

¹⁰ Batchelder, E. L., *Am. J. Physiol.*, 1934, **109**, 430.

¹¹ Sure, B., *J. Agric. Res.*, 1928, **37**, 87.

¹² Mason, K. E., *Am. J. Anat.*, 1935, **57**, 303.

¹³ Tansley, K., *Biochem. J.*, 1936, **30**, 839.

¹⁴ Newton, W. H., *J. Physiol.*, 1938, **92**, 32.

¹⁵ Browman, L. G., *Am. J. Physiol.*, 1939, **125**, 335.

¹⁶ Murphy, D. P., and Bowes, A. D., *Am. J. Obstet. and Gynec.*, 1939, **37**, 460.

¹⁷ McCollum, E. V., Simmonds, N., and Pitz, W., *J. Biol. Chem.*, 1916, **27**, 33.

Diet B was supplemented by 0.2 cc of cottonseed oil 3 times weekly.

Since performance on these 2 diets does not appear to differ in any important respect, the results will be discussed without reference to the diet used.

Females previously fed Purina dog chow were placed on the described diets at approximately 30 and 60 days of age. They were placed with males at varying periods after the vaginal smears had shown cornified cells continuously.

Of 36 females, mated as proven by the demonstration of sperm in the vagina, 2 failed to conceive, 11 resorbed their fetuses completely,* and 23 carried one or more young to term. These results are tabulated in more detail in Table I.

In our experience, females so depleted as to show xerophthalmia or weight loss generally will not mate. Of the animals mated, the most severely depleted resorbed their fetuses. Those rats which gave birth to young showed gestation periods from 23 to 25 days, with normal labor in some instances, and in others, with labor extending over 12 hours. This confirms the observation of Mason, Tansley, and Newton. It is noteworthy that a similar prolongation of pregnancy with failure of the birth mechanism is induced by the injection of anterior pituitary preparations into pregnant rats.¹⁸ In this latter instance, prolongation of gestation and failure of the

TABLE I.

Age when placed on A-low diet, days	Days on A-low diet	Matings	♀ failing to conceive	♀ which resorbed completely	♀ which carried 1 or more young to term	Young born or carried to term	% of young alive
60-70	60-70	7	0	1	6	52	76
	100-120	12	0	1	11	81	51
25-30	60-70	7*	2	4	1	3	100
	85-90 } †	7	0	2	5	38	52
	100 }	3	0	3	0	—	—
Controls: A-low diet and 40 γ carotene 3x weekly bred when 90-110 days old							
		3	0	0	3	23	100
Controls: Purina dog chow and lettuce							
		11	0	0	11	68	100

*Received 0.25 cc cod liver oil daily after 9-11 day of pregnancy.

†Refused to mate and lost weight. Given 120 γ carotene between 60 and 75 days on A-low diet.

* That they had conceived and implanted fetuses was proved by the occurrence of the placental sign.

¹⁸ Teel, H. M., *Am. J. Physiol.*, 1926, **79**, 170.

birth mechanism are associated with persistence of abnormal lutein tissue in the ovaries and failure of a new crop of follicles to ripen.

Young born normally or obtained by Caesarian section were minutely examined for abnormalities. The following specific points were checked: ears, eyes, nostrils, lips, roof of mouth, limbs and feet, tail, vertebral column, anus and genital papilla. Except when it was desired to rear the young, the presence of eyeballs was unequivocally established by dissection, and in most instances the viscera, especially the kidneys, were examined. All of the young from A-low mothers appeared anatomically normal except for one which had only the stump of a tail. About half the young were born dead. Resorption sites and embryos in the process of resorption were found even in mothers who bore mature young. The macerated fetuses represented various stages of development, but no anomalies were found where sufficient structure remained to permit of satisfactory examination.

The young reared by A-low mothers (only the less deficient animals would nurse young) were essentially normal in appearance, although somewhat underweight. No evidence of impaired vision was noted. All the young ceased to grow and developed severe xerophthalmia by the time they were 5 weeks old.

Control mothers on dog chow and on the A-low diet plus 40 μg of carotene 3 times weekly gave birth to living young in every instance after gestation periods of 21 to 23 days. Two of the young from control mothers on dog chow were hydrocephalic.

From these observations, as well as from the work of others already mentioned, it may be seen that, if rat females have sufficient vitamin A to enable them to bear any young, their offspring are anatomically normal. Sheep and cattle appear to behave similarly. Hale emphasized the severity of depletion of his sows, and the possibility should not be overlooked that swine may be able to mate, conceive and carry fetuses when more severely depleted than can rats, sheep, or cattle. If this is true, it is possible that this larger store of vitamin A in rats, necessary for conception, permits the early critical stages of the embryo's development to proceed unaffected.

Summary. 1. Lack of vitamin A failed to induce congenital anomalies in the young of rats. 2. Very severely depleted female rats refuse to mate. In order of decreasing severity of depletion, they will: mate but fail to conceive; conceive but resorb their fetuses; give birth to dead fetuses with difficult and protracted labor after a prolonged gestation; show only prolonged gestation.