

taurine or sodium sulfate. When diets containing diphenyl were supplemented with l-cystine or dl-methionine, however, the deficiency imposed by the diphenyl was promptly removed and growth was resumed. The results indicate that diphenyl is handled in the organism of the rat by some mechanism which involves the sulfur of sulfur-containing amino acids ordinarily available for growth.

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**Prolonged Maintenance of a Mammary Carcinoma in Vitamin E-Deficient Rats.\***

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Observations concerning a possible relationship between vitamin E and the incidence and growth of neoplasms are fragmentary, and are characterized by wide differences in the type of animal, neoplasm, and experimental method used. Adamstone<sup>1</sup> has described the occurrence of a transplantable lymphoblastoma in young chicks fed a mixed diet treated with ferric chloride in ether to destroy vitamin E. It may be noted, however, that the domestic fowl is prone to spontaneous manifestation of leucemic disease. Furthermore, our own experiences in experiments with rats indicate that ferric chloride treatment of mixed diets does not consistently destroy all traces of vitamin E present, especially when the fat content of the diet is low. The experiments of Davidson<sup>2, 3</sup> suggest that an excess of vitamins, especially liberal additions of vitamin E, decreases the incidence of tar carcinoma in mice. However, in repeating these studies Cameron and Meltzer<sup>4, 5</sup> observed only a delay in the appearance of the growth, which they attributed to the larger size and increased resistance of the vitamin-fed animals rather than to a specific vitamin effect. These

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<sup>1</sup> Adamstone, F. B., *Am. J. Cancer*, 1936, **28**, 540.

<sup>2</sup> Davidson, J. R., *Canadian Med. Assn. J.*, 1934, **31**, 486.

<sup>3</sup> Davidson, J. R., *Canadian Med. Assn. J.*, 1935, **32**, 364.

<sup>4</sup> Cameron, A. T., and Meltzer, S., *Am. J. Cancer*, 1937, **30**, 55.

<sup>5</sup> Cameron, A. T., and Meltzer, S., *Am. J. Cancer*, 1937, **30**, 70.

conclusions are in accord with the observations that tumors produced in susceptible mice by painting the skin with 3-4 benzopyrene<sup>6</sup> or with methylcholanthrene<sup>7</sup> are in no way influenced by the vitamin E content of the diet. Furthermore, the development of the Ehrlich carcinoma in mice is not influenced by frequent subcutaneous injections of wheat germ oil<sup>8</sup> and neither deficiency nor excess of vitamin E in the diet of the rat bears any relationship to development of the Jensen sarcoma.<sup>9, 10</sup> It should be emphasized that the studies reported have been concerned only with the influence of the absence or presence of vitamin E upon a single generation of the neoplasm concerned. It was felt that prolonged maintenance of a neoplasm in vitamin E-deficient rats, by successive implantations, would afford more convincing evidence concerning any possible relation of this vitamin to tumor growth; and perhaps test the validity of the hypotheses that vitamin E may be essential to the maintenance of nuclear function and structure in rapidly dividing cells<sup>11, 12</sup> or related to the control of mitotic activity of the cell nucleus.<sup>13</sup>

The Walker 256 mammary carcinoma was maintained by successive implantations, at intervals of 30 to 45 days,† in the following groups of rats: (1) male and female rats fed a stock diet of commercial dog biscuit, (2) female rats fed an E-deficient diet supplemented with 10 drops of wheat germ oil, daily, beginning at the time of tumor inoculations, and (3) female rats fed an E-deficient diet and proven to be suffering from sterility due to lack of vitamin E. The vitamin E-deficient diet contained casein (22%), starch (54%), lard (20%), salts (4%), supplemented with 1 g of brewer's yeast and 6 drops of cod liver oil, daily. A total of 98 E-deficient and 54 control rats was used.

Tumor tissue obtained from the donor rat while under light anesthesia was cut into small fragments and transplanted into the subcutaneous tissue of the abdominal wall of the lightly anesthetized recipient. Aseptic technic was used throughout the operative procedure. During the first few series of transplants the trochar-

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<sup>6</sup> Haddow, A., and Russell, H., *Am. J. Cancer*, 1937, **29**, 363.

<sup>7</sup> Carruthers, C., *Am. J. Cancer*, 1939, **35**, 546.

<sup>8</sup> Engel, P., *Z. f. Krebsfor.*, 1933, **39**, 148.

<sup>9</sup> Marchesi, F., *Riv. di Patol. Sper.*, 1933, **11**, 396.

<sup>10</sup> Zagami, V., *Riv. di Patol. Sper.*, 1933, **11**, 381.

<sup>11</sup> Mason, K. E., *Am. J. Anat.*, 1933, **52**, 153.

<sup>12</sup> Juhász-Schäffer, A., *Ergeb. d. Inn. Med. u. Kinderh.*, 1933, **45**, 129.

<sup>13</sup> Adamstone, F. B., *Science*, 1934, **80**, 450.

† Depending upon the duration of growth and onset of central necrosis in the tumors of donor rats.

needle method was used. Subsequent implants were made by inserting a small piece of the inoculum into the subcutaneous tissue through a small skin incision subsequently closed with a metal skin clip. The latter method gave a decidedly higher percentage of "takes" than the former, which may have been due in large measure to other improvements in the technic of inoculation. The size of the inoculum was kept as constant as possible. Schrek<sup>14</sup> has observed that variation in the size of the inoculum produces a significant effect upon the percentage of "takes" and the length of the "latent periods", but has little effect upon the growth-constant of the tumor.

The animals were observed daily after the inoculation to determine the earliest signs of growth. The number of days elapsing between the time of the inoculation and the day on which a nodule was first palpable was considered the latent period, in accordance with the definition of Ottensooser.<sup>15</sup> The latent period was further determined by the method of Schrek,<sup>16</sup> whereby the interception of the linear growth curve on the abscissa determines the duration of the latent period. Both methods gave essentially the same result, except that the linear curve intercepted the abscissa about one day before a nodule was palpable. The latter observation remained constant throughout these studies. Measurements of the tumors were made every 3 or 4 days. The rate of growth of the tumor was evaluated by the method of Schrek,<sup>16</sup> modified to the extent of using the arithmetic mean of 2 or more diameters of the tumor instead of the geometric mean of 3 diameters. Both criteria were carefully compared and were found to give essentially the same results. From the data obtained it was possible to express the growth rate of the tumor in centimeters per day.

During 8 months maintenance in control and vitamin E-deficient rats through 6 successive transplantations the tumor exhibited no changes in its rate of growth. The percent of takes and the latent periods of the tumor were essentially the same in the 2 groups of animals (Table I). The tumor was subsequently maintained only in the E-deficient rats. However, at the 12th successive implantation the tumor was successfully inoculated into 14 deficient rats, 7 of which were given moderately large doses of vitamin E beginning at the time of inoculation. The growth rate and other characteristics of the tumor were practically identical in the two groups, and indistin-

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<sup>14</sup> Schrek, F., *Am. J. Cancer*, 1936, **28**, 364.

<sup>15</sup> Ottensooser, R., *Z. f. Krebsfor.*, 1930, **30**, 553.

<sup>16</sup> Schrek, F., *Am. J. Cancer*, 1935, **24**, 807.

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TABLE I.  
Growth of the Walker 256 Mammary Carcinoma When Maintained for 8 Months  
in Control and Vitamin E-deficient Rats.

	Dietary treatment of rats		
	Stock diet (Controls)	E-deficient diet: supple- mented with vitamin E (Controls)	E-deficient diet
Successive transplantations	6	6	6
No. of inoculations	44	54	61
No. of "takes"	19	19	28
% of "takes"	43	35	46
Latent period (avg) in days	4.5	4.25	4.3
Growth-constant (avg) in cm per day	0.130	0.135	0.133

guishable from those observed during the first 6 transplantations. Nor was any significant change observed after the tumor was carried through another 10 successive implantations in E-deficient rats, at the end of which time it had been maintained for 2½ years in E-deficient rats by means of 22 successive implantations.

Several pieces of the tumor from each rat were fixed in Zenker and prepared for routine histological study. Representative tumors from each series were fixed in corrosive-sublimate-acetic and stained according to the method of Ludford<sup>17</sup> for special study of the nuclear chromatin in normal and malignant cells. No significant differences were observed in either the histological organization or the cytological characteristics of the tumors removed from control and E-deficient rats.

In an additional series of studies an attempt was made to produce the transplantable intraabdominal tumors observed by Rowntree and his associates<sup>18</sup> in rats fed an ether-extracted wheat germ oil subjected to treatment with sodium hydroxide. Daily doses of 1 cc of an oil prepared according to the method used by the latter investigators were fed to normal stock rats for periods of 128 to 214 days with no result. Bioassay of the oil revealed an exceedingly low concentration of vitamin E. Nor were any neoplasms observed in rats killed from 5 to 7 months after having received, over a period of 7 weeks, a daily intake of vitamin E equivalent to more than 2000 times the minimal daily requirement of the female rat.‡ These observations are in ac-

<sup>17</sup> Ludford, R. J., *Proc. Roy. Soc. London, Ser. B*, 1928, **102**, 397.

<sup>18</sup> Rowntree, L. G., Steinberg, A., Dorrance, G. M., and Ciccone, E. F., *Am. J. Cancer*, 1937, **31**, 359.

‡ In the form of a molecular distillate of wheat germ oil, for which we are indebted to Dr. R. C. Sherwood, Research Laboratories, General Mills, Inc., Minneapolis.

cord with those of numerous other investigators.<sup>19-26</sup>

The experimental data presented offer rather convincing evidence that vitamin E is in no way related to the induction of neoplastic growths, or to the growth and morphologic characteristics of the Walker 256 tumor in the rat. The fact that the latter tumor is not affected by prolonged existence in an environment devoid of vitamin E does not necessarily invalidate the hypothesis that vitamin E may be peculiarly necessary for maintenance of nuclear structure and function in rapidly proliferating tissues, but suggests that if any such relationship does exist it must be concerned with the more complex processes of cellular maturation and differentiation rather than with simple mitotic activity which represents the predominant type of cellular activity in the growth of neoplasms.

*Summary.* Maintenance of the Walker 256 mammary carcinoma in vitamin E-deficient rats for a period of 2½ years, by 22 successive implantations, produced no demonstrable alteration in the rate of growth, transplantability, histologic organization, or cytologic characteristics of the tumor.

Normal rats fed concentrates of wheat germ oil failed to show the neoplastic growths observed by Rowntree and his associates.

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<sup>19</sup> Bacharach, A. L., *Nutr. Abst. and Rev.*, 1938, **7**, 811.

<sup>20</sup> Day, H. G., Becker, J. E., and McCollum, E. V., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 21.

<sup>21</sup> Carruthers, C., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 107.

<sup>22</sup> Halter, C. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 257.

<sup>23</sup> Demole, V., *Z. f. Vitaminforsch.*, 1939, **8**, 341.

<sup>24</sup> Evans, H. M., and Emerson, G. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **41**, 318.

<sup>25</sup> Dingemans, E., and van Eck, W. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **41**, 622.

<sup>26</sup> Auchincloss, R., and Haagensen, C. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 703.