clude definitively that no significant difference in incidence in neoplasia occurred among our test and control groups. Grace's data, by contrast, do not permit a definitive appraisal of the differences which were recorded, but leave them highly suspect of spontaneous mammary carcinoma influence.

Summary. Mice of the ICR/Ha strain were sex-segregated at 21 days of age and were followed for development of neoplasia for a period of 650 days in comparison with groups which were not sex-segregated. There was a high incidence of spontaneous neoplasia in the non-segregated mice and this was principally mammary carcinoma. Incidence of mammary carcinoma was drastically reduced by sex segregation but incidence of other malignancies of minor occurrence was not affected. Injection of extracts of human malignant materials apparently did not influence occurrence of malignancy either in non-segregated or in sex-segregated mice. These findings are discussed in relation to those of Grace *et al* who reported a high incidence of mammary carcinoma in ICR/Ha mice given human malignant tissue but not in control groups.

We wish to acknowledge the technical assistance of E. A. Ridley and T. Beddow, and the statistical analysis by Mr. A. Itkin.

1. Girardi, A. J., Hilleman, M. R., Zwickey, R. E., PROC. SOC. EXP. BIOL. AND MED., 1962, v111, 84.

2. Grace, J. T., Jr., Mirand, E. A., Mount, D. T., *A.M.A. Arch. Int. Med.*, 1960, v105, 482.

3. Grace, J. T., Jr., *Surg. Clin. N. Am.* W. B. Saunders Co., 1962, v42, 273.

4. Bittner, J. J., *Pub. Health Rep.*, 1939, v54, 1113.

5. ———, *Cancer Res.*, 1948, v8, 625.

6. ———, *J. Nat. Cancer Inst.*, 1958, v21, 631.

Received September 3, 1963. P.S.E.B.M., 1963, v114.

Influence of Antioxidants on Myoglobin Concentrations in Vitamin E-Deficient Guinea Pig Skeletal Muscle.* (28748)

R. D. BUCKLEY†, D. D. SCHOTTELIUS AND B. A. SCHOTTELIUS
Department of Physiology, State University of Iowa, Iowa City

The absence of an antioxidant, possibly vitamin E, is implicated in many of the symptoms of nutritional muscular dystrophy in animals(1). Both methylene blue (MB) and N, N′-diphenyl-p-phenylene-diamine (DPPD) have been used to protect experimental animals against the consequences of Vit. E deficiency. This role of MB, DPPD, and other antioxidants, was reviewed by Dam(2). Muscular dystrophy in calves is prevented by MB administration(3). DPPD given to lambs on a Vit. E-deficient diet delays the onset of dystrophy symptoms(4). Draper and Csallany(5) noted that DPPD brings about a rapid remission of gross dystrophic symptoms in rabbits maintained on a tocopherol-deficient diet from which all lipoid material is absent. Thus, this antioxidant possesses a curative activity with respect to muscular dystrophy in the rabbit which is independent of the presence of Vit. E in the diet. The augmented cholesterol in dystrophic rabbit muscle declines very slowly in response to DPPD treatment(6). In the guinea pig, DPPD only delays the appearance of dystrophic symptoms, and does not prevent a rise in muscle cholesterol or total lipid(7,8). Because of the differences between rabbit and guinea pig in regard to experimental results and interpretation, we sought to determine the effect of MB and DPPD supplementation on myoglobin concentration in skeletal muscles of guinea pigs fed a Vit. E-deficient diet. In these experimental animals, the myoglobin concentration is markedly in-

* Supported by a grant from Muscular Dystrophy Associations of America, Inc.

† Present address: Dept. of Biochemistry, School of Medicine, Univ. of Southern California, Los Angeles.

TABLE I. Guinea Pig Gastrocnemius Myoglobin Concentrations and Standard Errors of These Values in Vit. E-Deficient Animals (Group A), Vit. E-Supplemented Animals (Group B), Methylene Blue-Supplemented (Group C) and N,N'-diphenyl-p-phenylenediamine-Supplemented Animals (Group D) After 15, 21 and 30 Days on Respective Dietary Regimens. Myoglobin concentrations are given in mg/g of fresh muscle tissue.

	Group A (E-def)	Group B (E-suppl)	Group C (MB-suppl)	Group D (DPPD-suppl)
15 days				
No. of animals	9	9	14	15
Myoglobin	1.31 ± .04	1.12 ± .05*	1.27 ± .09	1.08 ± .08*
21 days				
No. of animals	10	12	8	10
Myoglobin	1.54 ± .06	1.26 ± .08*	1.47 ± .18	1.29 ± .09*
30 days				
No. of animals	10	9	11	11
Myoglobin	.91 ± .06	1.56 ± .18*	1.07 ± .08†	1.14 ± .08*†

* Significantly different than E-def value ($p \leq .05$).
† Significantly different than E-suppl value ($p \leq .05$).

creased after 15 days on the deficiency diet (9), then falls to subnormal levels by the 21st day(10).

Procedure. In the first experiment, the basic Vit. E-deficient diet(11) was fed to 128 English strain male guinea pigs, 33 days of age at the start, for periods of 15, 21, or 30 days. Four dietary regimens were employed (Groups A-D). The diet of animals in Group A was unsupplemented; Groups B, C and D were supplemented, respectively, every third day with dl-α-tocopherol (17 mg), MB (40 mg) or DPPD (10 mg) per animal. These levels are approximately those used in previous studies(7,9,12). All 128 animals received 25 mg sodium ascorbate and 0.5 ml of cod-liver oil on days when antioxidant supplements were not administered. Cod-liver oil in addition to its Vit. A and D content is rich in unsaturated fatty acids and, although it may contain some Vit. E (12), the net effect is to hasten depletion of tocopherol stores from the animal's tissues (13).

In the second experiment, 19 guinea pigs were separated into 3 groups (E, F and G) and maintained for 15 days on the Vit. E-deficient diet. Subsequently, these groups were supplemented, daily until the 21st day, with dl-α-tocopherol (E), MB (F) or DPPD (G) at the same dosage as used in the first experiment.

On the designated day, the gastrocnemius muscles were removed under ether anesthesia and myoglobin analyses were performed according to the technique previously reported (9). The data were analyzed by Student's small sample "t" test, and the null hypothesis was rejected at the 5% confidence level ($p \leq 0.05$).

Results. Data from the first experiment are summarized in Table I where myoglobin concentrations are expressed on the basis of mg per g of fresh muscle tissue. The pigment concentration in the animals of Group A increased from day 15 to day 21, but had declined strikingly by day 30. There was a steady increase in myoglobin content of Group B between days 15 and 30. When the pigment concentrations of Group C are compared to those of Group A, no significant alteration due to the MB supplement is noted at any dietary duration. The DPPD supplemented animals (Group D) had normal (equivalent to Group B) myoglobin levels through 21 days; but the efficacy of the antioxidant was reduced by day 30.

Table II presents results of intensive tocopherol and antioxidant treatment of animals previously kept on a Vit. E-deficient diet for 15 days. Myoglobin concentrations in muscles of animals from Groups E, F and G are significantly lower than those found in muscles from Group A animals. Comparison of Groups E and B data and Groups G and D data from Tables I (21 days) and II reveals no significant difference attributable to the manner of supplementation with tocopherol

TABLE II. Guinea Pig Gastrocnemius Muscle Myoglobin Concentrations and Standard Errors of These Values. Group A was maintained on a Vit. E-deficient diet for 21 days. Groups E, F and G were maintained on a Vit. E-deficient diet for 15 days and then supplemented on days 16 through 21 with dl-α-tocopherol, methylene blue or N, N'-diphenyl-p-phenylenediamine, respectively. Myoglobin concentrations are expressed as mg/g of fresh muscle tissue.

	Group A (E-def)	Group E (E-suppl)	Group F (MB-suppl)	Group G (DPPD-suppl)
21 days				
No. of animals	10	5	7	7
Myoglobin	1.54 ± .06	1.06 ± .16*	.93 ± .05*	1.09 ± .09*

* Significantly different than E-def value ($p \leq .05$).

or DPPD. The response to MB administration in the second experiment contrasted sharply with that in the first experiment; the difference between values for Groups C and F is significant.

Discussion. Myoglobin content of muscles from the Vit. E-deficient animals we studied exhibited an abnormal rise and then a decline between day 21 and 30. These observations are in reasonable agreement with results of several investigations(9,10,14,15). Although Bender and colleagues(9,10) reported that the initial rise in pigment concentration was maximal at 15 days, the seeming discrepancy is more apparent than real because of minor differences in initial age of the animals and preliminary feeding protocol. The rate of increase in myoglobin concentration with age in muscles from Vit. E-supplemented animals compares favorably with other studies(9,16).

The protocol of our first experiment was devised to provide a protective or maintenance level of MB or DPPD in the diet, providing these antioxidants were adequate replacement for dietary tocopherol. Thus, they might serve as a substitute or act to spare tissue stores of the vitamin. The latter is the role assigned to DPPD by Shull *et al* (7), on the basis of studies where the supplementation was continuous. In our experiment, DPPD delayed the typical myoglobin changes of the deficiency regimen, while MB was totally ineffective. Our second experiment showed that both MB and DPPD caused a remission of the augmented pigment concentration due to an established tocopherol deficiency. This response is similar to that observed by Draper and Csallany after DPPD was administered to rabbits(5). The difference in response to MB supplementation seen in Tables I and II is probably explicable by an additional observation. Animals in Group C were the only ones in either experiment that did not exhibit a gain in body weight during the first 15 days. Evidence from the body weight data suggests that MB was toxic to the young animals on a tocopherol deficient diet, but seemingly tolerated by the older animals. The total quantity of MB administered in the 2 experiments was not too different, and does not appear to be a factor in the quality of the responses. We feel our data on myoglobin changes are more in accord with the concept that the antioxidants have an independent rather than tocopherol sparing effect(5); however, this independent effect may be of decreasing importance in regard to myoglobin when the Vit. E deficiency is prolonged, since DPPD was less effective in the 30 day regimen.

Summary. The rapid increase and subsequent decline in myoglobin concentration which is characteristic of the guinea pig after 15, 21 and 30 days on a Vit. E-deficient diet is largely prevented by a thrice-weekly administration of DPPD. Under the same regimen, MB is not effective and exhibits considerable toxicity for young animals. Both MB and DPPD, when given in 6 daily doses to animals previously kept on a tocopherol-deficient diet for 15 days, bring about a remission of abnormal changes in myoglobin concentration.

1. Horwitt, M. K., *Borden's Rev. Nutr. Res.*, 1961, v22, 1.

2. Dam, H., *Pharm. Rev.*, 1952, v9, 1.

3. Blaxter, K. L., Brown, F., Wood, W. A., *Brit. J. Nutr.*, 1953, v7, 337.

4. Draper, H. H., Johnson, B. C., *J. Animal Sci.*,

1956, v15, 1154.
5. Draper, H. H., Csallany, A. S., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 739.
6. Draper, H. H., *ibid.*, 1959, v102, 737.
7. Shull, R., Alfin-Slater, R. B., Duell, H. J., Jr., Ershoff, B. H., *ibid.*, 1957, v95, 173.
8. Shull, R., Ershoff, B. H., Alfin-Slater, R. B., *ibid.*, 1958, v98, 364.
9. Bender, A. D., Schottelius, D. D., Shottelius, B. A., *Am. J. Physiol.*, 1959, v197, 491.
10. Schottelius, B. A., Schottelius, D. D., Bender, A. D., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 581.
11. Schottelius, B. A., Schottelius, D. D., *Am. J. Physiol.*, 1958, 193, 219.
12. Moore, T., Sharman, I. M., Ward, R. J., *Brit. J. Nutr.*, 1959, v13, 100.
13. Forbes, R. M., Draper, H. H., *J. Nutr.*, 1958, v65, 535.
14. Indovina, I., *Am. J. Physiol.*, 1951, v65, 352.
15. Perkoff, G. T., Tyler, F. H., *Metabolism*, 1958, v7, 751.
16. Scow, R. O., Roe, J. H., *Am. J. Physiol.*, 1953, v173, 22.

Received September 3, 1963. P.S.E.B.M., 1963, v114.

Characteristics of Hypertensive Disease Evoked in Rats by Single or Multiple Injections of Polyvinyl Alcohol.* (28749)

C. E. HALL AND O. HALL
Carter Physiology Laboratory, University of Texas Medical Center, Galveston

It has been shown that administration of certain polyvinyl alcohol (PVA) solutions to various animals causes uptake of the polymer by many tissues and organs(1-3). More recently it has been found that daily subcutaneous administration of PVA solutions to rats causes the development of hypertensive cardiovascular disease, macromolecular hypertension(4-7), which is aggravated by a high NaCl intake(5), and by unilateral nephrectomy(8).

No information is available to indicate whether such prolonged treatment is essential or whether the response might be elicited by small quantities of material, but requires a certain "latent period" to become manifest. Accordingly the present experiment was designed to determine whether hypertension could be induced by brief acute treatment, followed by a period during which its effects were assessed.

Materials and methods. Thirty-three young female rats of the Holtzman strain were unilaterally nephrectomized (day 1) and divided into 4 groups. Rats of Groups 1, 2 and 3 each received respectively 2.5, 5.0 and 7.5 ml of 5% PVA.† Since the material is more effective subcutaneously than intraperitoneally(8) the former route was employed. Animals of the 3 groups each received 2.5 ml on day 2, but those of Groups 2 and 3 received a second injection on day 6, and those of Group 3 yet a third injection on day 9. Animals of Group 4 served as noninjected controls. All animals received a 1% NaCl solution *ad lib* to drink and Purina Laboratory Chow. Blood pressures were measured periodically by a tail plethysmograph and values in excess of 150 mm Hg systolic were regarded as hypertensive.

On the 170th day of the experiment a microhematocrit and hemoglobin (Cyanmethemoglobin method) measurement and a serum protein determination (Goldberg Refractometer) were made on each animal from a sample of tail blood. The survivors were killed on the 171st day. Various tissues were placed in 10% neutral formalin for fixation. Organs to be weighed were dissected free of extraneous material, dried on filter paper and weighed on an analytical balance. Sections were stained with Congo red and with hematoxylin and phloxine.

Results. It was assumed that the group

* This study was supported by grants from USPHS.

† Elvanol, grade 71-30, I. E. du Pont de Nemours & Co., Inc.