may supply a good deal of the daily caloric requirement (*1, 8, 12*). The animal then rapidly drifted into negative balance with concomitant increased excretion of urinary nitrogen and destruction of body protein to yield much needed energy. This rapid loss in body protein represents the ultimate response to a caloric deficit, and in the present experiments occurred after the animals had been on a restricted diet for 32 days. The differential changes of body tissues, however, after a period of caloric restriction demonstrates the lability of some organs and the stability of others to deprivation in calories.

Literature Cited

(1) Addis, T., Poo, L. J., Lew, W., *J. Biol. Chem.* **115,** 111 (1936).
(2) Allison, J. B., J. AGR. FOOD CHEM. **1,** 71 (1953).
(3) Allison, J. B., *Vme Congr. Intern. Médicine Tropicale et Paludism* **2,** 465 (1954).
(4) Allison, J. B., Anderson, J. A., *J. Nutrition* **29,** 413 (1945).
(5) Allison, J. B., Anderson, J. A., Seeley, R. D., *Ann. N. Y. Acad. Sci.* **47,** 245 (1946).
(6) Calloway, D. H., Spector, H., *Am. J. Clin. Nutrition* **2,** 405 (1954).
(7) Calloway, D. H., Spector, H., *J. Nutrition* **56,** 533 (1955).
(8) Elman, R., Heifetz, C. J., *J. Exptl. Med.* **73,** 417 (1941).
(9) Howe, P. E., *J. Biol. Chem.* **49,** 109 (1921).
(10) Hsaio, S. C., *Science* **107,** 24 (1948).
(11) Johnson, R. M., Deuel, H. J., Jr., Morehouse, M. G., Mehl, J. W., *J. Nutrition* **33,** 371 (1947).
(12) Kosterlitz, H. W., Campbell, R W., *Nutrition Abstr. Revs.* **15,** 1 (1945-46).
(13) McCoy, J. R., Allison, J. B., Crossley, M. L., Wannemacher, R. W., Jr., *Am. J. Vet. Research,* in press.
(14) Munro, H. N., *Physiol. Rev.* **31,** 449 (1951).
(15) Robinson, H. W., Price, J. W., Hogden, C. G., *J. Biol. Chem.* **120,** 481 (1937).
(16) Robscheit-Robbins, F. S., Whipple, G. H., *J. Exptl. Med.* **89,** 339 (1949).
(17) Rosenthal, H. L., *J. Nutrition* **48,** 243 (1952).
(18) Rosenthal, H. L., Allison, J. B., *Ibid.*, **44,** 423 (1951).

Received for review October 3, 1955. Accepted March 5, 1956. Presented in part before the Division of Biological Chemistry, 128th Meeting, ACS, Minneapolis, Minn., September 1955.

FEED ANTIOXIDANTS

Inhibitory Effect of Feed Grade Diphenyl-*p*-phenylenediamine (DPPD) on Parturition in Rats

BERNARD L. OSER and MONA OSER

Food Research Laboratories, Inc., 48-14 Thirty-Third St., Long Island City 1, N. Y.

Feed grade diphenyl-*p*-phenylenediamine (DPPD), at dietary levels only several times in excess of those used for the prevention of encephalomalacia in chicks, induces prolonged gestation in rats, associated with high mortality both of overdeveloped pups at birth and of the mothers during or after parturition.

THE DEMONSTRATION THAT CHICK ENCEPHALOMALACIA resulted from a deficit of vitamin E relative to the unsaturated fatty acids in the ration (*1*), directed attention to the protective effect of antioxidants on tocopherols, which are themselves antioxidants against rancidity and vitamin A oxidation. Diphenyl-*p*-phenylenediamine (DPPD) has proved to be the most effective of the antioxidants studied (*4, 5*) and, until recently, without objection from the Food and Drug Administration, it has been used extensively in poultry rations. Though it is an aromatic amine, its low solubility, as well as results of toxicological feeding tests, have militated against the possibility of hazard from this use. It should be emphasized that the commercial grades of DPPD contain from 5 to 10% of unidentified impurities.

Subsequent studies designed to establish the quantitative relationship between minimal tocopherol levels and concentrations of DPPD required for protection have revealed a toxic manifestation in pregnant rats—viz., delay or complete failure of parturition with attendant mortality of both the mother and litter. This effect has been observed in both synthetic and natural type diets. The following experiment will illustrate the nature of the phenomenon and the levels of DPPD required to induce it.

Five groups of 10 healthy female rats were selected from the Food Research Laboratories' breeding colony, each having previously produced and weaned a normal litter. They were placed in mating cages and fed *ad libitum* the following diet (Table I). This diet was estimated to provide 18.6 mg. of α-tocopherol per 100 grams from natural sources, of which (cf. footnotes to table) 12 mg. were added in the form of distilled mixed tocopherols, N. F. (Distillation Products Industries, Rochester, N. Y.). DPPD [Good-rite DPPD feed grade antioxidant (95% minimum *N,N'*-diphenyl-*p*-phenylenediamine) a product of the B. F. Goodrich Co.] was added at four levels, 0.025, 0.10, 0.40, and 1.60%, the lowest being about twice the concentration employed in practical broiler rations. It was incorporated into the vegetable fat before mixing in the final diet.

After 2 weeks on this diet, vaginal smears were made daily, and at proestrus one breeder male of known fertility was placed in the cage with the female. The male was allowed to remain for not less than 17 nor more than 24 hours—i.e., through stages I and II of the estrus cycle (*2*). Examinations were made for the vaginal plug and the smears were examined for the presence of spermatozoa. In addition smears were made daily to confirm cessation of the cycle. When a mating was unsuccessful, another male was provided at the next proestrus period. Records were kept of body weight, duration of pregnancy, number and weight of pups cast or found *in utero*, and mortality up to the end of a normal gestation and lactation period. The results of this experiment are shown in Table II.

All females were initially fertile, although in some cases more than one mating was necessary for conception to

occur. The gestation period was normal in the control group [cf. 22.6 days as given by Farris (*2*)] but was significantly prolonged even in the group receiving the lowest level (0.025%) of DPPD in the diet (t = 2.45). At higher dosages parturition was delayed in some cases as much as 6 days, the average gestation period being 12 to 14% longer than in the control group. In no case was resorption observed.

That fertility was not affected was shown by the normal proportion of pregnancies resulting in fully developed litters either born or found *in utero*. However, associated with the delay in parturition was an increase in mortality of the pups at birth. These pups appeared larger than normal. Moreover, in most instances, autopsy of the females that died while casting revealed overgrown but otherwise seemingly normal fetuses *in utero*. The opposite was true in one unborn litter at the 0.4% level found at autopsy on the 29th day. The proportion of stillbirths in the control group was 18/107 or 16.8%, whereas in the 0.025% DPPD group it was 42/79 or 53.2%; at higher levels only a few pups were born alive. Two thirds of the live births in the control group survived the 21-day lactation period, whereas less than half of those in the 0.025% DPPD group survived. However, owing to the smaller size of the litters in the latter group, their weights at weaning were considerably greater than those of the controls.

The pups born dead or found in the uterus of females who died at parturition were about 10 to 20% heavier than the controls. However, those born alive were of normal weight except at the 0.025% level, in which group they were heavier than the controls.

The post-partum survival rate of the females diminished significantly at levels of DPPD above 0.1%.

Table I. Composition of Basal Ration

Ingredient	%	Ingredient	%
Whole wheat	39	Pork liver[h]	0.48
Yellow corn	19	Celluflour[a]	1
Nonfat dry milk	20	Vitamin B complex mix[b]	1
Meat meal[c]	10	Vitamins in oil[d]	1
Hydrogenated cottonseed oil[e]	5	Sodium chloride	0.5
Alfalfa[f]	2	Manganese sulfate.H_2O	0.02
Yeast[g]	1	Total	100.0

[a] Chicago Dietetic Supply House, Inc., Chicago, Ill.
[b] In Celluflour carrier, thiamine HCl 600 γ, riboflavin 1200 γ, pyridoxine 400 γ, calcium pantothenate 4 mg., niacin 5 mg., choline chloride 200 mg., inositol 100 mg., p-aminobenzoic acid 2.5 mg., biotin 1 γ, pteroylglutamic acid 1 γ, cyanocobalamin 1 γ, liver concentrate 1 to 20 25 mg.
[c] 65% protein, Swift & Co., St. Joseph, Mo.
[d] Vitamin A 200 U.S.P. units, vitamin D 20 U.S.P. units, α-tocopherol 12 mg., menadione 100 γ in cottonseed oil ($\approx$ 450 mg. linoleic acid).
[e] Primex, Procter & Gamble Distribution Co., Richmond Hill, N. Y.
[f] Alfalfa meal, Andrew Goetz's Sons, Inc., Brooklyn, N. Y.
[g] Type C1, Pabst Brewing Co., Chicago, Ill.
[h] Vacuum dried, Valentine Co., Inc., Richmond, Va.

Table II. Effect of DPPD on Parturition

	DPPD, % in Diet				
	0.0	0.025	0.10	0.40	1.60
Females per group	10	10	10	10	10
Matings	19	20	15	20	26
Pregnancies	10	10	10	10	10
Duration of pregnancy, days					
Range	21–23	22–24	22–25	23–29	22–27
Mean	22.1	22.9	24.1	25.2	24.7
Standard error	0.23	0.23	0.30	0.68	0.54
Litters born					
Complete	10	9	7	6	4
Partial[a]	...	...	...	1	3
Pups born					
Dead	18	42	21+	18+	20
Alive	89	37	14	2	4
Pups alive at					
4 days	65	20	6	0	3
21 days	60	16	6	0	3
Mean weight of pups, g.					
Born dead or *in utero*	5.5	6.6	6.3	6.6[b]	5.9
Born alive	5.5	6.2	5.4	5.0	5.0
Females died					
During parturition	0	1	3	3	5
Post-partum (day)	2 (2, 13)	0	0	1 (13)	2 (1, 1)

[a] Litters of which one or more pups were cast but mother died during parturition.
[b] Omitting two autolyzed fetuses at 29 days, weighing 3.0 grams each.

These observations indicate an inhibitory effect of DPPD on parturition, resulting in prolonged gestation and continued growth of the fetuses. Whether death of the females resulted from the primary defect or was incidental to the effort syndrome of casting overgrown pups is not known. The normal appearance of the fetuses suggests that hormonal failure may have been responsible for delaying or preventing uterine contractions, although a direct effect of DPPD on smooth muscle cannot be ruled out. On the hypothesis that the posterior pituitary might be involved, histopathological examinations were made of this organ in five rats that died in parturition after gestation periods of 24 and 25 days' duration as well as of normal rats sacrified on the 22nd day of pregnancy. These preliminary studies give no evidence of organic injury to the pituitary but further investigation is indicated. It is conceivable that functional impairment of the oxytocin-secreting process or a direct chemical antioxytocic action may have inhibited uterine contraction even in the absence of anatomic damage to the pituitary gland.

Inasmuch as these experiments were conducted on a commercial grade of DPPD, the possibility that the offending factor is an impurity rather than diphenyl-p-phenylenediamine itself cannot be discounted. The evidence here presented should not be construed to signify that the retarding effect on parturition is shared by antioxidants other than DPPD; in fact, preliminary studies with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) exonerate these compounds (*3*). Whether DPPD might manifest this pharmacodynamic effect in some way in the avian species remains to be determined.

The interesting possibility that compounds exerting such action may have clinical value in the prevention of premature birth requires further exploration.

Literature Cited

(1) Dam, H., *Proc. Soc. Exptl. Biol. Med.* **52,** 285 (1943).
(2) Farris, E. J., Griffith, J. Q., "The Rat in Laboratory Investigation," J. B. Lippincott, Philadelphia, 1949.
(3) Harris, P. L., Distillation Products Industries, Rochester, N. Y., personal communication.
(4) Singsen, E. P., Bunnell, R. H., Matterson, L. D., Kozeff, A., Jungherr, E. L., *Poultry Sci.* **34,** 262 (1955).
(5) Singsen, E. P., Potter, L. M., Matterson, L. D., Bunnell, R. H., Kozeff, A., Jungherr, E. L., *Ibid.*, **34,** 1075 (1955).

Received for review April 24, 1956. Accepted May 24, 1956.