gave secondary reactions which were undesirable and caused inconsistent results. The autolysis devised by Reisser which takes place for 72 hours at 2° has not been attempted in this laboratory since it was desired to take all possible precautions against contamination of the extracts. However, this method of extraction should cause a 4-fold increase in the recoverable units.

The isolation of callicrein as a chemical entity would appear to depend on the finding of a method for stabilizing this hypotensive substance. The instability encountered in our preparation at around 400 Frey units per mg N and those reported by other authors(5,7) has hampered further attempts to characterize this enzyme.

Summary. A method has been devised for preparation of highly purified pancreatic callicrein. The fractionation procedure involves three ethanolic fractionations at pH 5.5, 7.5, and 6.5 followed by one ammonium sulfate precipitation. Solutions of hypotensive enzyme at this level of purity proved highly unstable and further purification was abandoned. Preliminary purification of urinary callicrein revealed that the method could be adapted to this source.

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Tolerance of the Rat for N,N'-Diphenyl-P-Phenylenediamine. (22702)

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The demonstration that N, N'-diphenyl-p-phenylenediamine (dppd) is effective in the prevention of encephalomalacia in chicks fed vit. E-low diets containing fish oils, and in the stabilization of carotene and vit. A in practical rations, has led to its widespread use in commercial mixed feeds. The concentration commonly employed is 0.025% of the diet. Matterson *et al.*(6) have reported that chicks which received a diet containing 1% dppd throughout a 12-week growth trial remained free of serious lesions and maintained a normal growth rate. Draper and Johnson (1) observed no adverse effects from administering dppd to lambs at a level of 0.1% for 7 weeks, and Jensen and coworkers* reported that 0.025% dppd improved reproductive performance in turkey hens fed a tocopherol-low ration.

The present experiments were designed originally to determine the degree of replace-

* Abstract published.

TABLE I. Reproductive Performance of Rats Receiving a Vit. E-Free Diet Supplemented with dppd from Weaning Age.

Treatment	No. females	No. conceptions	No. pups born— Total	Per conception	% born alive
0	25	0	0	0	—
Vit. E (30 mg/wk)	19	17	155	9.1	57
.1% dppd	10	7	21	3.0	19
.025% "	14	10	71	7.1	7
.005% "	16	16	108	6.8	14
.385 mg " /wk*	10	9	69	7.7	88

* Approximately .0006% of diet.

ability of vit. E by dppd in the rat; but following the observation that small concentrations of dppd fed continuously through growth and gestation produced adverse effects upon reproduction, a study of the long-term consequences of dppd feeding was conducted.

Methods. Weanling female rats of the Sprague-Dawley strain were maintained throughout growth and reproduction on a diet of the following composition (%): Cerelose 64.6, casein 20, tocopherol-low lard 10, salts(5) 4, choline 0.4, vitamin premix (in cerelose) 1. Vit. A and D were administered weekly by dropper. The premix provided the following concentrations of vitamins in μg per gram of diet: Thiamin 100, riboflavin 15, nicotinic acid 100, pyridoxine 6, calcium pantothenate 40, biotin 0.5, menadione 4, folic acid 4 and vit. B_{12} 0.05. Comparable groups of rats were fed the basal diet alone or supplemented with alpha-tocopheryl acetate (approximately 30 mg/wk placed on the diet), or dppd (0.1%, 0.025%, or 0.005%). Other animals received .385 mg dppd per week in 3 equal oral doses (equivalent to approximately 0.0006% of the diet eaten).

Results. During the growing period of 6 weeks all animals gained normally and remained in healthy condition with no manifestations of dppd intoxication or vit. E deficiency. The females, weighing 175-200 g, subsequently were mated to healthy males which had been maintained on a stock diet.

During gestation the females receiving 0.1% dppd exhibited anorexia, rough haircoat and anemia. At parturition gross hemorrhages from the vagina were observed and, upon autopsy, hemorrhages were located in the uterus. Of 10 females, 3 succumbed to hemorrhages and the remainder were in semimoribund condition. The hemorrhagic animals were acutely anemic but the prothrombin times were normal. Seventeen dead and 4 live pups were obtained from this group.

Improvement in reproductive performance was observed with lower concentrations of dppd in the diet. These results are summarized in Table I. Litter size was comparable among the groups receiving the 3 lowest levels, but the proportion of live pups was subnormal except at the lowest concentration. No abnormalities were found in the hemoglobin values, prothrombin times or white blood cell counts during gestation. However, the feeding of dppd appeared to be associated with an increase in the gestation period, the average for the females receiving 0.025% dppd being 25.4 days and that for the 0.005% level being 23.8 days, compared with 22.8 days for the positive controls.

The efficacy of dppd in reversing sterility in females maintained on a vit. E-low diet was also examined. The females were depleted of vit. E using a diet similar to that outlined but containing 4% fish liver oil as a source of fat. Methyl linoleate was given orally twice per week at a rate equivalent to 0.4% of the diet. All the females exhibited reproductive failure during the first cycle. Supplementation was begun one week before remating. The results are shown in Table II.

It has been reported previously that dppd fails to regenerate vit. E-deficient female rats fed a diet essentially devoid of this vitamin, although, at a level of .385 mg per week given prophylactically, dppd can delay the appearance of resorption-gestation for one or two cycles(2,3). The regenerative effect observed

TABLE II. Efficacy of dppd in Restoring Fertility of Female Rats Previously Maintained on Vit. E-Low Diet.

Treatment	No. females	No. conceptions	No. pups born— Total	No. pups born— Per conception	% born alive
0	22	0	0	0	—
.1% dppd	19	15	71	4.7	3
.01% "	17	17	109	6.4	5
Vit. E	23	19	161	8.5	71

in terms of pups born in this experiment is probably attributable to its protective action on the small quantity of vit. E contributed by the fish liver oil. The oil, upon assay by the procedure of Tosic and Moore(4) was found to contain 8 mg 100 g, and contributed, therefore, about 3.2 μg g of diet. These data indicate that the dietary requirement of the rat for vit. E may be considerably reduced when an effective antioxidant is supplied simultaneously, and support the concept of a dual role for vit. E in the body, including a general antioxidant function and another indispensable role in whch it cannot be replaced by other antioxidants. The inferior survival *in utero* on both concentrations of dppd in this regeneration experiment further substantiates the long-term toxic effect of this compound at levels well below those used in commercial feeds. However, only slight indications of hemorrhaging at parturition were observed in the animals receiving 0.1% dppd, possibly because of the shorter period of administration. The possibility that levels below .01% might exert a regenerative effect is being investigated.

Weanling rats showed no adverse effects from receiving 0.5% dppd in the diet for 12 weeks, but subsequently developed "porphyrin" staining of the whiskers and haircoat over the head and neck. This condition was accompanied by a rough haircoat and moderate depression of weight gain. Determinations of hemoglobin values and prothrombin times on blood from these animals have revealed no significant changes. Food consumption and growth rate during the early part of the growing period indicate that palatability was not a problem.

In the majority of these experiments the dppd employed was a feed grade product containing approximately 95% active material. However, part of the growth and reproduction studies were conducted using pure dppd, and indicate that the toxicity also is encountered after prolonged feeding of the pure compound and is not attributable to a contaminant in the feed grade product.

The results of this study show that prolonged continuous oral administration of dppd in a vit. E-low diet at levels substantially below those used commercially in mixed feeds is toxic for the rat. The symptoms do not appear during growth, except at very high concentrations, but occur at parturition, and are characterized mainly by vaginal hemorrhages in the female and a high incidence of stillbirths. The possibility that this toxicity may be significantly modified by the presence of vit. E in the diet is worthy of investigation but may be regarded as unlikely.

Summary. Inclusion of N, N'-diphenyl-p-phenylenediamine in a vit. E-low diet fed to female rats through growth and reproduction has resulted in varying degrees of reproductive failure. Toxicity was encountered during growth at a level of 0.5% but not at 0.1% or below. Rats receiving 0.1% dppd exhibited uterine and vaginal hemorrhages at parturition accompanied by anorexia, anemia and emaciation. Concentrations of 0.025%, 0.01% and 0.005% resulted in a high incidence of stillbirths. No adverse effects were encountered from feeding 0.0006% dppd in the diet. The data show that dppd elicits symptoms of toxicity in the rat at concentrations below those used in commercial mixed feeds.

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Metabolic Properties of Quinidine; Effects of Quinidine Sulfate on Anaerobic Carbohydrate Metabolism of Rat Diaphragm.* (22703)

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Several papers have appeared recently concerning the effect of quinidine on various aspects of carbohydrate metabolism(1,2,3). The influence of this compound on the anaerobic phase of carbohydrate metabolism has not been thoroughly explored, and results which appear to be conflicting have been reported. Following the work of Furman and Howard(4) confirmed by Fajans and his group(5) who showed that quinidine decreases glucose tolerance in human subjects, we decided to investigate the influence of this drug on the anaerobic metabolism of rat diaphragm in an attempt to gather information on the mechanism of this effect.

The combined results of these experiments on the effect of quinidine on endogenous glycolysis, on glycolysis in the presence of glucose and fructose and on anaerobic consumption of glucose and fructose, point to the conclusion that utilization of these hexoses is impaired at one or more steps close to their entry into the Embden-Meyerhof sequence.

Materials and methods. Albino rats (Holtzman) weighing from 90 to 130 g were used. Following a 24-hour fast the rats were killed by a blow on the head and the diaphragms quickly removed and placed in Krebs-Ringer's bicarbonate solution at 1°C. The diaphragms were cut into either 2 or 4 pieces, weighed and used as soon as possible. All experiments were carried out under anaerobic conditions using the standard Warburg procedure. Incubation was carried out at 37°C in Krebs-Ringer's bicarbonate solution with or without quinidine sulfate (U.S.P., Merck). The flasks were gassed one-half hour with 95% nitrogen-5% carbon dioxide. After an equilibration period of 15 minutes glucose (Baker) or fructose (Nutritional Biochemicals) dissolved in Krebs-Ringer's bicarbonate solution was added from the side bulb to give a final total volume of 3.0 ml. Glucose or fructose was determined by the Somogyi-Nelson method (6).

Results. Effect of quinidine on anaerobic uptake of added glucose and fructose. Glucose (4 mg/ml) and fructose (5 mg/ml) uptake was determined, each in a series of 5 experiments, with or without 1 x 10^{-3} M quinidine. The following mean values and standard errors were obtained: glucose consumption without quinidine, 2.74 ± 0.21 mg/g (wet wt) tissue/hr; with quinidine, 1.44 ± 0.16 mg/g/hr; fructose consumption without quinidine, 2.70 ± 0.27 mg/g/hr; with quinidine, 1.28 ± 0.08 mg/g/hr. The inhibition of glucose or fructose uptake produced by 1 x 10^{-3} M quinidine is significant at the 0.01 level. In 2 experiments in which glucose consumption was measured for a 30-minute pe-

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