systolic potentiation observed by the latter investigators was not accompanied by a significant change in membrane repolarization.

*Summary.* 1. Cells in the region of the sino-atrial node of rabbit are unable to respond to as high a frequency of stimulation as those cells not included in the pacemaker area. 2. The same phenomenon has been observed during rapid atrial arrhythmias in the absence of electrical stimulation. 3. The magnitude of the contractile tension developed by the atrium is related to repolarization of atrial cell action potentials, being greater for those demonstrating exponential rather than sigmoid curves. This is a consistent finding occurring during periods of electrical stimulation and with the first few spontaneous beats following stimulation, but the tension and action potential repolarization are not related to each other in any simple way.

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Received June 29, 1956. P.S.E.B.M., 1956, v93.

## Effect of DPPD, Methylene Blue, BHT, and Hydroquinone on Reproductive Process in the Rat.* (22656)

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Although several compounds have been found to prevent or cure certain vit. E deficiency symptoms, such as brown discoloration of the uterus and cloudy swelling of the convoluted tubules of the kidney, only 2, diphenyl-p-phenylenediamine (DPPD) and methylene blue, have been reported to substitute for tocopherol in preventing resorption-gestation in vit. E-deficient female rats. In our laboratory also, preliminary experiments indicated that methylene blue and DPPD gave slight positive responses under the conditions of our standard vit. E bioassay procedure(1). Consequently, we were led to investigate this apparent vit. E activity further.

*Methylene blue.* The results of vit. E bioassays in which the responses of 5 levels of methylene blue were compared with five levels of standard, *d*-α-tocopherol. showed median fertility doses (MFD) of 36 mg and 0.29 mg respectively. Thus, methylene blue has less than 1% the activity of α-tocopherol. However, during the course of these experiments, Moore, Sharman, and Ward(2) reported that under the conditions of vit. E bioassay in their laboratory, methylene blue was completely negative. The bioassay procedures used at Cambridge and by us were practically identical except for length of time of depletion. Moore used 5 months and we used about 5 weeks. Consequently, the slight positive response we obtained for methylene blue may have been due to its protective or sparing effect on the small, residual quantities of α-tocopherol still remaining in the tissues of our animals after only 5 weeks deple-

* Communication No. 233.

TABLE I. Effect of DPPD* and *d*-α-Tocopheryl Acetate on Reproductive Performance of Vit. E-Deficient Female Rats.

| | Dietary supplement | | |
|---|---|---|---|
| | Negative control, no suppl. | 0.2% DPPD | 0.002% *d*-α-tocopheryl acetate |
| Fertility index (No. of females pregnant to No. mated) | 3/3 | 9/9 | 9/9 |
| Litter efficiency (% of pregnant animals with at least 1 viable fetus) | 0 | 33 | 100 |
| Mortality (No. of females dying at parturition) | | 2 | 0 |
| Mean length of gestation (days) | | 25 | 22 |
| Mean litter size | | 2.3 | 7.8 |
| Viability index (No. of young alive at 3 days to No. born) | | 0/7 | 63/70 |
| Lactation index (No. of young weaned to No. alive at 3 days) | | — | 61/63 |

* Feed grade DPPD.

tion. Quite recently, Christensen, Dam, and Gortner(3) revised their original conclusion (4) since methylene blue has been found to be ineffective as vit. E in preventing fetal resorption if the depletion diet is rigorously freed of tocopherol, thus eliminating the sparing effect of methylene blue on small, residual amounts of tocopherols in the vit. E-low ration. Therefore, methylene blue is not a vit. E substitute for preventing fetal resorption and, furthermore, should be used with caution, according to Moore(5), since at high levels of 0.252% in the diet it caused growth depression and severe damage (fragmentation) to the spleen of male rats. We also observed that all female rats died when supplemented with 100 mg/day of methylene blue.

*DPPD.* Results of a standard bioassay of DPPD in a Tween-water dispersion for vit. E potency showed that under the conditions of our test (5-12 week depletion period), DPPD possessed 7% the activity of the *d*-α-tocopherol standard on a molar basis. Johnson and Goodyear(6) reported that DPPD (0.385 mg/wk) supported reproduction in female rats maintained on a vit. E-low ration from weaning. Subsequent experiments showed(7) that this vit. E-like effect of DPPD disappeared as soon as the animals' tissue stores of tocopherol were used up.

A further test of the apparent anti-sterility potency of DPPD was conducted in which DPPD was fed mixed into the vit. E-low diet and fed to vit. E-deficient female rats beginning 7 days prior to mating. In Table I are shown the results obtained in comparison with a control group treated identically except that the diet was supplemented with 0.002% *d*-α-tocopheryl acetate instead of DPPD. DPPD in the diet (0.2%) was less effective than *d*-α-tocopheryl acetate (0.002%) in preventing typical vit. E-deficiency gestation resorptions, as evidenced by "litter efficiencies" of 33% and 100%, respectively. But more striking was the effect of DPPD on the duration of gestation and on mortality of young. Gestation was prolonged to 25 days from a normal of 22 days, and all of the newborn rats died before 3 days of age.

Other experiments with a vit. E-free diet or a stock diet confirmed these adverse effects of DPPD. Typical results are shown in Table II. Butylated hydroxytoluene (BHT) and hydroquinone (HQ) were also fed to determine whether these antioxidants would show similar toxicity in pregnant animals. Neither BHT nor HQ at the 0.3% level induced the prolonged gestation and mortality characteristic of DPPD. BHT at the 1.55% level caused a drastic weight loss in the pregnant females which was associated with fetal deaths. This response was not unexpected since at this level of feeding the rats were receiving each day about half the single dose $LD_{50}$ of BHT as reported by Deichmann *et al.* (8).

The DPPD effect is more pronounced in animals fed the supplemented "stock diets" 10 days before insemination than in those fed the supplemented "vit. E-low diets fortified with *d*-α-tocopheryl acetate" starting on the day of insemination. Since these experiments were conducted at different times, comparisons should be made with caution. The most

TABLE II. Effect of DPPD* on Reproduction in Female Rats.

| Group No. | Supplement and level in diet (%) | Fertility index | Litter efficiency | Mortality index | Mean length of gestation | Mean litter size | Viability index | Lactation index |
|---|---|---|---|---|---|---|---|---|
| | Vit. E-low diet† + .001% *d*-α-tocopheryl acetate (supplemented diets fed starting on day of insemination) | | | | | | | |
| 1 | Negative control | 14/17 | 100 | 0/17 | 22 | 7.7 | 73/ 85 | 71/73 |
| 2 | DPPD .0125 | 8/11 | 60 | 1/11 | 23 | 6.2 | 18/ 31 | 18/18 |
| 3 | " .0625 | 11/11 | 88 | 0/11 | 24 | 5.1 | 8/ 51 | 6/ 8 |
| 4 | " .313 | 10/11 | 90 | 2/11 | 25 | 6.0 | 0/ 54 | |
| 5 | " 1.55 | 8/11 | 50 | 1/11 | 26 | 7.3 | 0/ 29 | |
| 6 | BHT .0125 | 9/11 | 100 | 0/11 | 22 | 8.7 | 71/ 78 | 70/71 |
| 7 | " .0625 | 11/11 | 100 | 0/11 | 22 | 7.5 | 73/ 82 | 59/73 |
| 8 | " .313 | 11/11 | 91 | 0/11 | 22 | 8.7 | 72/ 87 | 69/72 |
| 9 | " 1.55 | 9/11 | 0 | 0/11 | 22 | | | |
| | Stock diet‡ (supplemented diets fed 10 days prior to insemination) | | | | | | | |
| 1 | Negative control | 16/17 | 88 | 1/17 | 23 | 10.6 | 71/104 | 69/71 |
| 2 | DPPD .0125 | 10/12 | 83 | 0/12 | 24 | 7.9 | 4/ 79 | 3/ 4 |
| 3 | " .0625 | 15/17 | 70 | 5/17 | 25 | 4.9 | 0/ 49 | |
| 4 | " .313 | 8/10 | 60 | 5/10 | 25 | 5.3 | 0/ 16 | |
| 5 | " 1.55 | 11/13 | 33 | 7/13 | 25 | 4.7 | 0/ 14 | |
| 6 | HQ .003 | 10/10 | 100 | 0/10 | 22 | 8.1 | 79/ 84 | 78/79 |
| 7 | " .3 | 10/10 | 100 | 0/10 | 23 | 7.8 | 65/ 76 | 62/65 |

* Feed grade DPPD. † Diet #301 of Harris and Ludwig(9). ‡ Diet of Ames *et al.*(10) except with linseed meal replacing soybean meal and partially hydrogenated vegetable oil (Primex) replacing margarine oil.

sensitive criterion of DPPD feeding, a high incidence of early mortality of young, occurred in both experiments in groups fed 0.0125% DPPD, the lowest level tested. In another experiment, supplementation of the diet with 400,000 I.U./kg of vit. A or 0.1% of *d*-α-tocopheryl acetate failed to alleviate the toxicity. Those young rats which survived this early period of life (3 days) were able to suckle successfully, as evidenced by a normal lactation index. Considerable maternal mortality occurred at levels of 0.0625% DPPD and higher in the stock diet. The abnormality leading to death seemed to be largely mechanical. Gestation was prolonged, in some instances as much as 36%. Probably the fetuses continued to grow *in utero* until, when parturition did occur, the birth process was exceedingly difficult and prolonged. Many of the young were born dead and most of the mothers that died did so during parturition. Other symptoms occasionally observed were vaginal bleeding and prolapse of the uterus. The physiological defect caused by DPPD is probably failure of "the trigger mechanism" which starts the birth process at the proper time.

*Summary and conclusions.* 1) Vit. E. bioassays (prevention of resorption-gestation) of both methylene blue and DPPD indicated a low order of activity. However, this apparent tocopherol-like potency was due probably to a protective or sparing effect on small residual quantities of vit. E in the diet and in the body tissues. Under conditions where the diet was rigorously freed of tocopherol and the depletion period was extended, methylene blue and DPPD were found by other investigators to be completely devoid of vit. E activity. 2) At higher levels of supplementation, both methylene blue and DPPD were toxic. Methylene blue in doses of 100 mg/day killed pregnant female rats. Feeding pregnant rats diets containing DPPD at a level of 0.0125% in the diet resulted in mortality of their young. Higher levels of DPPD induced prolonged gestation and maternal mortality. Neither BHT (0.313%) nor hydroquinone (0.3%) produced these symptoms of toxicity, but BHT at the 1.55% level in the diet resulted in drastic loss of weight and fetal deaths.

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Received July 2, 1956. P.S.E.B.M., 1956, v93.

## Mouse Pathogenicity with Gastric Mucin as an Index of Staphylococcus Virulence. (22657)

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Bacterial virulence, defined broadly as the capacity of an organism to invade and multiply in tissues and produce disease or death, is best measured by observing pathological effects induced by the microorganism in a suitable host. Animal virulence tests, although employed for a number of other bacterial species, have not been applied to staphylococci chiefly because of their inconstant and irregular pathogenicity for laboratory animals. Such biochemical properties as coagulase, pigment and hemolysin production as well as mannite fermentation are utilized instead with the greatest reliance being placed on the first, but the validity of these criteria as indices of virulence in all instances has been subject to serious question by many investigators. Lack and Wailling(1), as one example, in a study on the factors contributing to pathogenicity of staphylococci, state, "While there is strong evidence that the absence of coagulase production is associated with nonpathogenicity, there is no evidence that all coagulase-producing strains are pathogenic."

Hog gastric mucin has found wide application as an adjuvant in converting infections caused by organisms considered of low virulence for mice on intraperitoneal injection into rapidly lethal ones. Among the numerous bacterial species reported amenable to its action in this regard are some strains of staphylococci(2-4). Ercoli *et al.*(5) have shown that staphylococci injected intraperitoneally into mice with mucin remain viable and spread from the abdominal cavity, while without it they are rapidly destroyed. It was believed of interest to ascertain whether the mouse could be converted from a relatively resistant host to staphylococcus infection by the intraperitoneal route into one readily susceptible, by means of the adjuvant, and if so, to determine whether this procedure could be adapted to the differentiation of virulent from avirulent members in the same manner that animal inoculations are employed with corynebacteria, clostridia, mycobacteria, etc. To facilitate this evaluation, the *in vitro* presumptive biochemical reactions of a series of strains of staphylococci were compared with their intraperitoneal pathogenicity for mice with mucin.

*Materials and methods.* 60 strains of staphylococci recently isolated from a variety of such clinical sources as suppurative lesions, blood, nose and throat, urine and skin were employed. 5% hog gastric mucin (Type 1701-Wilson) was prepared according to Miller(6). Ten albino Swiss mice, weighing approximately 15-20 g, were used for each bacterial strain, of which 5 received 0.5 ml of 5% hog gastric mucin and the remainder 0.5 ml of physiological saline intraperi-