cles of clay or pyrophyllite in the mixture so that the outer surface of a large fraction of the particles in the mixture would be composed of bauxite.


### References

1. BÖNING, P. *Z. tech. Physik*, 1927, **8**, 385.
2. FRENKEL, J. *J. Phys.* (USSR), 1941, **5**, 25.
3. ISRAEL, H. *Z. tech. Physik*, 1928, **9**, 289.
4. KNOBLAUCH, O. *Z. phys. Chem.*, 1901, **39**, 225.
5. LOEB, L. B. *Science*, 1945, **102**, 573.
6. MACLEOD, G. F., and SMITH, L. M. *J. agric. Res.*, 1943, **66**, 87.
7. RICHARDS, H. F. *Phys. Rev.*, 1923, **22**, 122.
8. RUDGE, W. A. D. *Proc. roy. Soc. Lond.*, 1914, **A90**, 256,
9. SCHNURMANN, R. *Proc. phys. Soc. Lond.*, 1941, **53**, 547.
10. WILSON, H. F., JANES, R. J., and CAMPAU, E. J. *J. econ. Entomol.*, 1944, **37**, 651.


## The Effect of α-Tocopheryl Phosphate on Diphosphopyridine Nucleotidase


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We have previously reported (*2*) that the inhibition of E-deficient heart-muscle succinoxidase and the stimulation of lactic dehydrogenase from the same source by α-tocopheryl phosphate probably are best explained by the assumption that the compound (α-TPh) inhibits the enzyme diphosphopyridine nucleotidase (DPNase). The preservation of DPN thereby would produce inhibition of succinoxidase by the promotion of oxalacetate formation by the DPN-requiring malic dehydrogenase and would produce stimulation of lactic dehydrogenase, since this enzyme requires DPN.

Ames (*1*) recently has concurred that inhibition of succinoxidase by α-TPh is due to DPNase inhibition and has amplified this concept to show that α-TPh can form an insoluble salt with calcium, thus removing the latter ion so that it does not activate DPNase.

Neither our previous investigation nor that of Ames was based on direct measurements of DPNase activity in the presence of tocopherol. The present paper presents such data, which confirm the hypothesis.

Normal guinea pig hearts were homogenized in distilled water, a 10% suspension being used as a source of DPNase. Sodium-*dl*-α-tocopheryl phosphate was prepared by E. M. Schultz, of the Department of Organic Chemistry of this Division. DPN was prepared by the method of Williamson and Green (*5*) and was estimated in the reaction mixtures by the fluorometric method of Levitas, *et al.* (*3*).

The experiments were set up in 15-ml graduated centrifuge tubes, each of which contained 1 ml of M/5 phosphate buffer, pH 7.2, 0.5 ml of homogenate, and 0.5 ml of buffer containing 500γ of DPN. $CaCl_2$ and α-TPh were dissolved in water and added in the order described below. The volumes were made to 3.0 ml with distilled water. After completion of additions, the contents of the tubes were mixed, and the tubes were incubated at 37° for 0 time, 15, 30, and 45 min, and 1 hr, after which times the enzymes were inactivated by the addition of 0.2 ml of 20% trichloracetic acid. The incubation was carried out without stirring, in order to minimize solubility product effects. After filtration, the filtrates were analyzed for DPN, and the results were plotted against time.

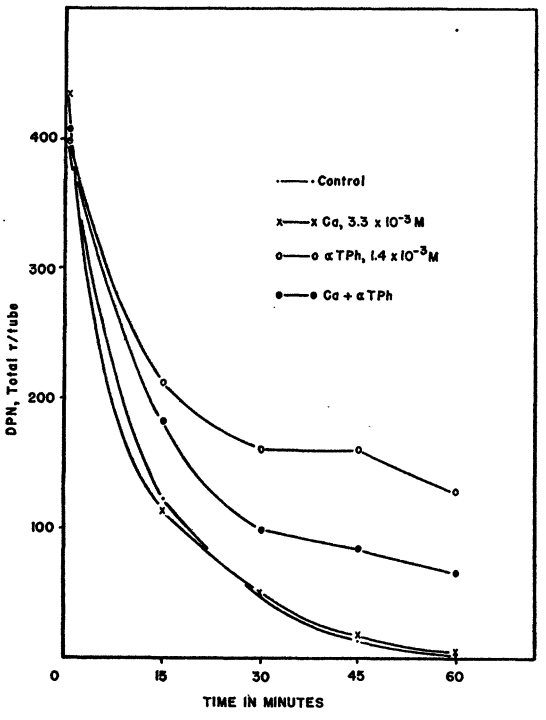


FIG. 1

Fig. 1 demonstrates that the rate of breakdown of DPN is inhibited markedly by α-TPh. In this experiment α-TPh was present in the concentration of $1.4 \times 10^{-3}$ M and $CaCl_2$, $3.3 \times 10^{-3}$ M. The calcium relieved the α-TPh inhibition but did not increase the rate of DPNase activity when α-TPh was not added. In view of the data of Ames (*1*), showing the effect of varying concentrations of α-TPh and of calcium on DPN breakdown, it was thought desirable to carry out experiments similar to his by the above techniques. Table 1 shows the results of such experiments. The data agree very well with those of Ames, as to the effect not only of varying concentrations but also of the order of addition of α-TPh and calcium. Percentage inhibition has been calculated on the basis of the 1-hr values.

The fact that calcium has less effect in relieving the tocopherol inhibition when added before the α-TPh is explained by Ames as being due to the removal of some of the calcium as calcium phosphate, thus allowing the α-TPh to remove the residual calcium more efficiently.

It would seem that another interpretation of these

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results would also be possible. If one examines the data with the hypothesis that α-TPh has a direct inhibitory action on DPNase, one might consider that, if calcium were partially removed by phosphate, there would be less residual calcium available to α-TPh, more α-TPh uncombined with calcium, and a resulting greater DPNase inhibition. Conversely, if α-TPh were added to the buffer first, followed by calcium, the α-TPh would compete with phosphate for calcium and thus would have a better chance of reacting with calcium ions; the precipitation of α-TPh would be more complete, and less DPNase inhibition would result. Consequently, the direct inhibition of DPNase by α-TPh seems to us to be a valid possibility.

TABLE 1

INHIBITION OF DPNASE BY α-TPH AND EFFECT OF CALCIUM*

| | | Calcium added before α-TPh | | | Calcium added after α-TPh | |
|---|---|---|---|---|---|---|
| Molarity of α-TPh | | $5.6 \times 10^{-4}$ | $1.4 \times 10^{-3}$ | $2.8 \times 10^{-3}$ | $1.4 \times 10^{-3}$ | $2.8 \times 10^{-3}$ |
| Molarity of $CaCl_2$ | 0 | 1.0 | 26.1 | 42.7 | 28.8 | 54.1 |
| | $3.3 \times 10^{-4}$ | 1.0 | 28.7 | | 26.4 | |
| | $3.3 \times 10^{-3}$ | | 15.5 | 41.4 | 3.5 | 8.8 |
| | $3.3 \times 10^{-2}$ | | 10.0 | | 4.0 | |

* Figures represent percentage inhibition of DPNase.

In the great majority of these experiments (10 out of 11) the addition of calcium to the control tubes, without α-TPh, produced no acceleration of DPN breakdown in the time intervals used. Swingle, *et al.* (*4*) have reported activation of DPNase in rat liver by calcium in experiments of 20-min duration. Since our first readings were made at 15 min, we may have failed to see the phenomenon. Of course, it is possible that heart-muscle DPNase does not require calcium for activation or, more probably, that sufficient calcium already is present. However, experiments using washed homogenates showed a great decrease in DPNase activity but no activation of the DPNase remaining was observed following the addition of calcium.

In conclusion, it may be said that α-tocopheryl phosphate has been shown by direct DPN estimation to inhibit DPNase. The observations of Ames that calcium relieves this inhibition have been confirmed. In our opinion, there are still bases for the possibility that α-TPh has a direct inhibitory effect on DPNase.

### References


1. AMES, STANLEY R. *J. biol. Chem.*, 1947, **169**, 503.
2. GOVIER, WM. M., YANZ, N., and GRELIS, M. E. *J. Pharm. exp. Therap.*, 1946, **88**, 373.
3. LEVITAS, N., ROBINSON, J., ROSEN, FRED, HUFF, J. W., and PERLZWEIG, W. A. *J. biol. Chem.*, 1947, **167**, 169.
4. SWINGLE, K. F., AXELROD, A. E., and ELVEHJEM, C. A. *J. biol. Chem.*, 1942, **145**, 581.
5. WILLIAMSON, S., and GREEN, D. E. *J. biol. Chem.*, 1940, **135**, 345.


# The Sex of the Host as a Factor in *Plasmodium gallinaceum* Infections in Young Chicks


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In chicks inoculated with *P. gallinaceum* Brumpt, the percentage of parasitized erythrocytes, the rate of endothelial invasion, and the therapeutic efficacy of quinine have been observed to differ significantly with the sex of the host.

Fourth-day parasite counts[1] were accumulated on 449 White Rock chicks inoculated at 6–8 days of age with

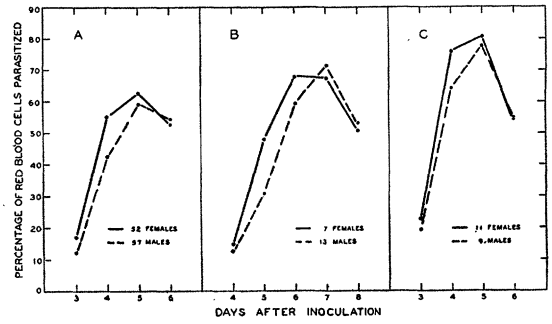


FIG. 1. Average daily parasite counts of male and female chicks infected with *P. gallinaceum*: (A) 109 White Rock chicks inoculated at 8 days of age with $16 \times 10^6$ parasitized red cells; (B) 20 White Rock chicks inoculated at 8 days of age with 1,000,000 parasitized red cells; (C) 20 Rhode Island Red chicks inoculated at 7 days of age with $16 \times 10^6$ parasitized red cells.

$16 \times 10^6$ parasitized red cells (*1*). Analysis by sex showed that for 222 females the counts averaged 62.3% (S.E. = 1.2), whereas for 227 males the counts averaged 53.8% (S.E. = 1.2). Comparable sex differences were also found in the daily parasite counts in two different breeds of infected chicks (Fig. 1).

To determine whether the sex difference in parasite counts could be experimentally increased, male and female sex hormones of proven activity in chicks (*6*) were administered. Ninety infected chicks were divided into 3 equal groups. Each chick in group A was injected intramuscularly with 0.1 mg of testosterone propionate in 0.1 cc of corn oil daily for 6 days, beginning on the day before the inoculation. Chicks in group B were similarly injected with 0.1 mg of α-estradiol benzoate, and each of the controls in group C received 0.1 cc of corn oil alone daily for the same period. The difference in parasite counts in the two sexes was not significantly increased by the administration of male or female sex hormones under the conditions of these experiments.

[1] Smears of peripheral blood were air dried, fixed with methyl alcohol, and stained with Giemsa. When the parasite count was 30% and above, it was obtained by counting the parasitized cells in 100 erythrocytes. When lower counts were found, the number of erythrocytes examined was increased so that the probable error remained under 10% (*4*).