mitochondria was also absent. Under the conditions in which the aforementioned tumour mitochondria failed, the mitochondria from this tumour showed sometimes the same O_2 uptake with *dl*-BHB as liver mitochondria.

Four other tumours (three transplanted hepatomas and one testis tumour) yielded mitochondria which oxidized various amounts of *dl*-BHB, in general a little less than half the amount oxidized by the liver mitochondria. These mitochondria showed insignificant DPNase and no ATPase activities. The present results, as far as the ATPase activities are concerned, confirm the earlier data[2]. The conditions governing the DPNase activities in relation to the ATPases of the tumour mitochondria are being studied further. A full account of our work will be published later.

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Inhibition of immune hemolysis by di*iso*propyl fluorophosphate

The demonstration that Ca^{++} and Mg^{++}, or other divalent cations, such as Co^{++} or Ni^{++}, are essential[1,2] for hemolysis of sheep erythrocytes (*E*) by rabbit antibody (*A*) and guinea pig complement (*C'*) suggests an enzymic mechanism, the postulate reaction scheme[3] being as follows:

$$E + A \rightleftharpoons EA \qquad (1)$$

$$EA + C'1,4 \xrightarrow{Ca^{++}} EAC'1,4 \xrightarrow[37^\circ]{\text{slow}} \text{inactive} \qquad (2)\ (2')$$

$$EAC'1,4 + C'2 \xrightarrow[(Co^{++} \text{ or } Ni^{++})]{Mg^{++}} EAC'1,4,2 \xrightarrow[37^\circ]{\text{rapid}} \text{inactive} \qquad (3)\ (3')$$

$$EAC'1,4,2 + C'3 \longrightarrow E^* \qquad (4)$$

$$E^* \longrightarrow \text{ghost} + \text{hemoglobin} \qquad (5)$$

where E^* refers to a damaged cell which undergoes further transformation with release of hemoglobin and *C'*1,2,3 and 4 refer to components of *C'*.

In the course of an investigation of chemical inhibitors of immune hemolysis, it was found that di*iso*propyl fluorophosphate (DFP) inhibited the immune hemolytic reaction. Inhibitory concentrations of DFP were less than the concentrations of DFP shown to be without effect on nonesterolytic enzymes[4,5].

Sheep erythrocytes, *A*, *C'*, *EA*, *EAC'*1,4, *EAC'*1,4,2, *C'*2,3, veronal buffer, and ethylenediaminetetraacetate (EDTA)-veronal buffer, used as diluent, were prepared as described in previous papers[6,7]. DFP was first diluted in *iso*propyl alcohol and kept as a stock solution. Aliquots were emulsified in 0.12 *M* sodium bicarbonate to a concentration of 0.125 *M* DFP and subsequent dilutions of the 0.125 *M* DFP were made in veronal buffer. All DFP emulsions were made from the stock DFP-*iso*propyl alcohol (2.5 *M* DFP) just before addition to the reaction mixtures. Control experiments showed that the alcohol itself and the alcohol-bicarbonate solution in the amounts used, did not affect the hemolytic reaction.

The effect of DFP on reaction steps (3) and (4) and on the overall reaction, *EA* + *C'*, was determined in the following manner: DFP was added to reaction mixtures containing *EA* or *EAC'* 1,4 in veronal buffer, or *EAC'*1,4,2 in EDTA-veronal buffer at 0°C immediately followed by the

addition of C', $C'2,3$, EDTA-C' ($C'3$) respectively to a total volume of 7.5 ml. The reaction mixtures were incubated at 37° C for one hour, centrifuged, and the hemolysis determined by measuring oxyhemoglobin in the supernatant fluid in the Beckman Spectrophotometer at 541 mμ.

Fig. 1 shows that DFP inhibits the overall reaction; *i.e.*, $EA + C'$, and reaction step (3), but has little effect on (4). This indicates that the inhibition is occurring in the reaction between EAC' 1,4 and $C'2$. The possibility that DFP is exerting its inhibition by binding Mg^{++} was eliminated by demonstrating that the degree of inhibition was not altered when the reactions were performed in the presence of varying Mg^{++} concentrations. In addition, others[5] have shown that DFP is without effect on enzymes that require Mg^{++} as an ionic cofactor. NaF did not exert comparable inhibition to immune hemolysis even at a concentration of 10^{-2} *M*. DFP at a concentration of $3 \cdot 10^{-3}$ *M* did not inhibit reaction (5).

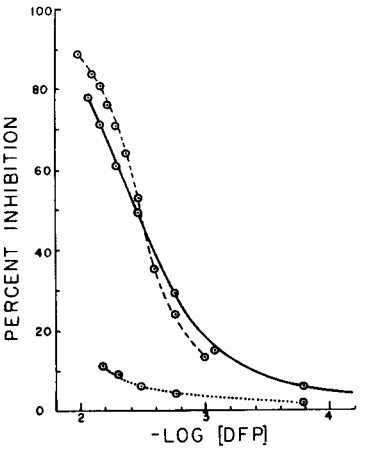

Fig. 1. Effect of DFP on $EA + C'$ (dash line), $EAC'1,4 + C'2,3$ (solid line), $EAC'1,4,2 + C'3$ (dotted line).

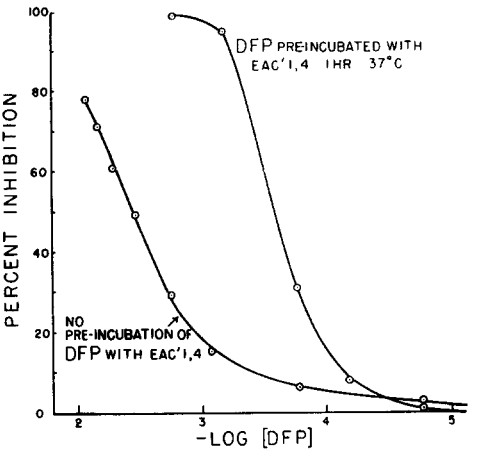

Fig. 2. Effect of incubation of DFP with EAC' 1,4 for one hour at 37°C.

Since DFP appeared to exert its inhibition on either $C'2$ or $EAC'1,4$, a 20% inhibitory concentration of DFP was incubated with $C'2,3$ and $EAC'1,4$ for 1 hour at 37°C. Whereas the degree of inhibition remained constant when DFP was pre-incubated with $C'2$, pre-incubation of DFP with $EAC'1,4$ resulted in a marked increase in inhibition when $C'2,3$ was added. Fig. 2 shows that incubation of DFP with $EAC'1,4$ for 1 hour at 37°C increased the sensitivity of $EAC'1,4$ to DFP 10-fold. This effect was pronounced even with incubation at 0°C. In contrast to this, preliminary incubation of a 20% inhibitory concentration of DFP with EA or C' at 37°C or with $EAC'1,4,2$ or $C'3$ in the presence of EDTA at 0°C did not increase the degree of hemolysis inhibition.

The significance and mechanism of inhibition of immune hemolysis by DFP, a substance reported to act on esterases, will be described in detail elsewhere.

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