sis of choline. It is suggested that this role of methionine is related in some as yet unknown manner with the action of androgens.

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Cholinesterase Activity and Potassium Permeability in Human Erythrocytes. (24023)

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Since Dale's(1) suggestion that an enzyme was present in blood which brought about destruction of acetyl choline, and subsequent work which led to clarification of the nature of this enzyme as a specific acetyl cholinesterase(2-5), its exact role in metabolism of the erythrocyte has remained obscure. Lindvig, Greig, and Peterson(6) found that acetyl choline decreased the net exchange of sodium and potassium of human erythrocytes suspended in bicarbonate buffer, and also delayed hemolysis which was apparently due to increased intracellular cation and water. These processes were reversed by physostigmine. Production by normal erythrocytes of a substrate for cholinesterase, demonstrated by Holland and Greig(7), could necessitate a means for its removal upon completion of its function. Thiele and Pennell(8) confirmed the production of cholinesterase substrate in erythrocytes, which, at –1.5 - 0°C, greatly exceeded its hydrolysis. They postulated a cholinesterase-substrate-dextrose relationship important in synthetic and hydrolytic functions, and suggested also a contractile function of acetyl choline, alternately contracting and relaxing the fibrous structural proteins of the cell as a means of regulating membrane permeability. Taylor, Weller and Hastings(9), using radioactive potassium and calculating its concentration as the resultant of 2 exchange rates, showed a loss of the ion from human red cells when exposed to cholinesterase inhibitors in concentrations approximately 100 times that necessary to inactivate completely the enzyme. The relationship between enzyme inhibition and potassium loss was not readily apparent. Goodman, Marrone and Squire(10) found that the *in vivo* potassium gradient was unrelated to erythrocyte cholinesterase activity.

The following report describes our attempts to demonstrate altered permeability to potassium in human erythrocytes in the presence of acetyl choline, and cholinesterase inhibitors diisopropylfluorophosphate and physostigmine

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TABLE I. Serum and Cell Potassium Concentrations during Storage with Physostigmine, DFP and DFP + AcCh. Concentration of physostigmine = 5×10^{-6} molar; concentration of DFP = 0.01 mg/ml; concentration of AcCh = 3.6×10^{-3} molar. Values are in milliequivalents/l of serum or packed cells.

		Days								
		0	2	3	4	7	8	10	12	15
Blood + physostigmine	Cells	109.0		101.8		85.0		80.6		83.9
	Serum	4.3		8.5		10.9		13.1		16.0
Control	Cells	105.3		91.8		87.2		77.7		82.6
	Serum	4.2		9.0		11.5		13.8		16.9
Blood + DFP	Cells	91.9			84.3		77.9		70.9	67.8
	Serum	4.5			13.3		20.2		26.8	29.6
Control	Cells	93.4			86.3		81.2		72.4	70.6
	Serum	4.0			12.2		17.9		25.4	27.4
Blood + DFP + AcCh	Cells	111.3	102.8							
	Serum	4.1	7.8							
Control	Cells	106.5	100.3							
	Serum	4.4	8.6							

sulfate at 4° and 37.5°C.

Materials and methods. Normal blood was drawn aseptically from donors into either acid-citrate-dextrose (ACD) solution (20 ml/100 ml blood) or heparin sodium (2000 USP units 100 ml blood). Two separate aliquots of 250 ml each were drawn from each donor for storage experiments in ACD at 4°C. Acetyl choline chloride (Merck) (AcCh) was dissolved in sterile Parpart's buffered saline. pH 7.4(11). Physostigmine sulfate (Merck) solution was prepared in Parpart's saline and filtered through a Seitz filter. Diisopropyl-fluorophosphate (Technical Operations. Inc.) (DFP) was autoclaved in sealed capillary tubes; solution was effected by crushing capillaries in sterile Parpart's saline. Drugs in 5 ml saline were added to blood with sterile. cotton-plugged pipettes under ultraviolet hood. Sterile saline was added to control bottles. Aliquots for analyses were removed aseptically with an 18 gauge needle and 20 ml syringe. Cultures of stored blood at conclusion of each experiment showed no growth in 48 hours on beef infusion agar and broth with 5% dextrose, thioglycollate broth, or trypticase soy broth. Cholinesterase activities and AcCh concentrations were determined by the method of Hestrin(12). Potassium analyses were done on Beckman model DU spectrophotometer with flame attachment. Experiments at 37.5°C were done in 25 ml Erlenmeyer flasks with 10 ml heparinized blood and the drug(s) added in 0.53 ml 5% dextrose. Flasks were covered with Parafilm (Marathon Corp.) with pinhole to allow gas exchange but retard evaporation, and shaken gently at 90 oscillations/minute on a rack mounted in Warburg apparatus. Hematocrit values were determined by the method of Wintrobe. These values did not change significantly in any sample during the experiment.

Results. Table I shows serum and cell potassium concentrations during storage at 4°C with physostigmine, DFP and DFP + AcCh. Periodic cholinesterase assays showed the enzyme to be completely inhibited by DFP, and about 80% inhibited by physostigmine, at concentrations used. This latter figure is, however, probably lower than degree of inhibition actually existent in stored blood, due to dilution (100 fold) of the enzyme during assay with concomitant partial dissociation of the enzyme-drug complex. The degree of inhibition remained essentially constant during the study. The experiment with DFP + AcCh was terminated at 48 hours due to non-enzymatic hydrolysis of 90% of the added AcCh at that time.

Table II shows the effect of AcCh, DFP, and DFP + AcCh on re-entry of potassium into cells which had been stored for 24 hours in heparin, prior to incubation at 37.5°C, to permit leakage of potassium into the serum. Table III shows the effects of the same drugs and combinations of drugs on fresh heparin-

TABLE II. Effects of DFP and DFP + AcCh on Re-entry of Potassium into Erythrocytes Stored 24 Hr at 4°C. Temperature of incubation, 37.5°C. Concentration of DFP, 0.01 mg/ml; concentration of AcCh, 3.6 × 10^{-3} molar. Values are in milliequivalents/liter of serum or packed cells.

		0 hr	3 hr	6 hr
Blood only	Cells	112.5	120.6	123.4
	Serum	9.2	6.2	6.6
Blood + DFP	Cells	112.5	124.1	123.0
	Serum	9.2	6.2	6.4
Idem + AcCh	Cells	117.5	123.5	123.2
	Serum	8.8	6.2	6.0
Blood + AcCh	Cells	117.5	125.6	124.1
	Serum	8.4	6.2	6.0

ized cells.

Discussion. The suggestion(6) that erythrocyte cholinesterase may be actively engaged in AcCh hydrolysis for maintenance of normal cell fragility implies a function of the enzyme similar to its role in neural transmission. AcCh produced intracellularly or on the cell surface could be responsible for maintaining a certain status of the membrane with the resultant cation partitioning. In view of the known extracellular site of action of AcCh at the synapse and myoneural junction, if the 2 processes were similar, one would expect a significant decrease in cation concentration gradient upon either inhibition of the enzyme (assuming continued production of cholinesterase substrate) or addition of exogenous substrate, or both. The fact that no changes were apparent over and above control changes in the potassium gradient under these conditions indicates that this cation is not the one primarily involved in the suggested synthetic-hydrolytic system.

TABLE III. Effects of DFP and DFP + AcCh on Escape of Potassium from Fresh Erythrocytes. Temperature of incubation, 37.5°C. Concentration of DFP, 0.01 mg/ml; concentration of AcCh, 3.6 × 10^{-3} molar. Values are milliequivalents/liter of serum or packed cells.

		0 hr	3 hr	6 hr
Blood only	Cells	114.5	115.9	116.0
	Serum	3.0	4.6	5.3
Blood + DFP	Cells	114.8	116.6	115.9
	Serum	2.9	4.6	5.3
Idem + AcCh	Cells	116.0	118.6	117.8
	Serum	2.9	4.5	5.0
Blood + AcCh	Cells	117.5	118.2	119.5
	Serum	2.9	4.7	5.3

Summary. Inhibition of human erythrocyte cholinesterase with or without exogenous AcCh had no effect on loss of potassium from cells during storage at 4°C or during incubation at 37.5°C. The re-entry at 37.5°C of potassium into cells previously stored for 24 hours at 4°C to permit its leakage into the serum was also unaffected.

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