# Effect of Cholinesterase Inhibitors on Electrolyte Distribution *in vivo*. (22140)

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Mechanisms governing the metabolism of electrolytes at the cellular level remain obscure. It is not known how cells maintain high concentrations of potassium in the face of constant interchange of potassium between intracellular and extracellular compartments. Potassium metabolism may be related to both carbohydrate and acetylcholine metabolism. Observations concerning potassium and acetylcholine have been made *in vitro* on red cells, nerve and muscle. Greig and Holland reported increased permeability of dog erythrocytes due to cholinesterase inhibitors(1). They suggested that cholinesterase activity maintained the selective permeability(2). Other investigators found no direct correlation between inhibition of cholinesterase activity and breakdown of the cellular potassium concentration gradient in the human red cell(3). Recently it has been shown that this erythrocyte synthesizes insufficient acetylcholine to provide energy for accumulation of potassium(4). The relationship between cholinesterase and potassium during neural conduction or muscular contraction would appear to be even more complex. Nachmansohn concluded that acetylcholine increased permeability of nerve membranes (5). Cholinesterase inhibitors increase the rate of entry of sodium into squid axon and decrease that of potassium(6,7). Holland, Dunn and Greig found that acetylcholine caused a loss of potassium from auricular muscle which is reversed by physostigmine (8). They felt that the metabolism of acetylcholine may be correlated with the transfer of sodium and potassium. However, Fenn and co-workers found that acetylcholine caused a small liberation of potassium from muscle, probably due to contraction, and concluded that acetylcholine did not control potassium transfer(9). Little information is available concerning *in vivo* interrelationships of cholinesterase and potassium. In acute experiments cholinesterase inhibitors in lethal doses cause hyperkalemia(10). However, this occurs in association with anoxemia and acidosis. It was felt that repeated sublethal doses of cholinesterase inhibitors would obviate these alterations and, at the same time, induce chronic disturbance of acetylcholine metabolism. It was not known if the loss of cellular potassium due to cholinesterase inhibitors *in vitro* could be reproduced *in vivo*. If this occurred a diet deficient in potassium would impose an impediment to the replenishment of cellular potassium. The following studies were carried out to determine the effect of cholinesterase inhibitors on tissue electrolytes *in vivo*.

*Methods.* Male Wistar rats weighing 100 g were given a low potassium diet (0.5 mM potassium per 100 g diet) composed of casein, 24%; glucose, 62%; corn oil, 8%; and salt mixture, 6%(11), plus a daily vitamin supplement of 1/100th of a capsule of Tycopan (Lilly). Distilled water was partaken *ad lib*. Additional rats were given sufficient supplementary potassium (as KCl) to bring the potassium content of their diet to 0.4%; these are referred to as normal potassium rats. Anticholinesterase compounds were administered to potassium deficient and normal potassium rats as follows: Group A1 was composed of control rats injected with 0.5 ml isotonic saline and rats injected with 0.05 mg diisopropylfluorophosphate (DFP) in 0.5 ml isotonic saline; and Group B was composed of potassium deficient controls injected with 0.5 ml propylene glycol and rats on both types of diet injected with 0.15 mg p-nitrophenyldiethylthionophosphate (Parathion) in 0.5 ml propylene glycol. In these groups subcutaneous injections were given twice weekly starting 27th day of diet. Rats were sacrificed on 44th, 49th and 71st days. An addi-

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tional series of potassium-deficient rats (Group A2) was composed of controls given 0.5 ml isotonic saline and rats given 0.05 mg DFP twice weekly for 8 weeks, then 0.10 mg twice weekly for 2 weeks, then a final dose of 0.25 mg. The last injection was followed immediately by muscular tremors. This group of rats was injected starting the 5th day of diet and sacrificed on 76th day. No rat was sacrificed prior to 24 hours after an injection. Rats were weighed weekly. Red cell cholinesterase determinations were done by the method of Michel(12) on blood obtained from the tip of tail. Rats were killed by a blow on head, blood obtained by cardiac puncture, and samples of thigh muscle and liver were removed. The hematocrit; plasma water, sodium, potassium and chloride; and skeletal muscle and liver water, fat, potassium, sodium and chloride were determined according to previously described methods (13), except that sodium and potassium were determined by flame photometry.

*Results. Body weight.* After the first 10 days of potassium-deficient diet the rats had a decreased rate of gain in weight compared with those on normal potassium diet. Administration of DFP and Parathion did not alter rate of gain.

*Cholinesterase activity.* Fig. 1 shows red cell cholinesterase activity. Rats given Parathion had a moderate reduction in cholinesterase activity and those given DFP had a marked reduction. There was no apparent difference between rats on a potassium deficient diet and those on normal potassium intake. Relative cholinesterase activity of intercostal muscle was estimated histochemically in Group A2(14). Cholinesterase activity at the motor endplate of potassium deficient rats showed no detectable difference from that of normals, but was definitely less in rats given DFP.

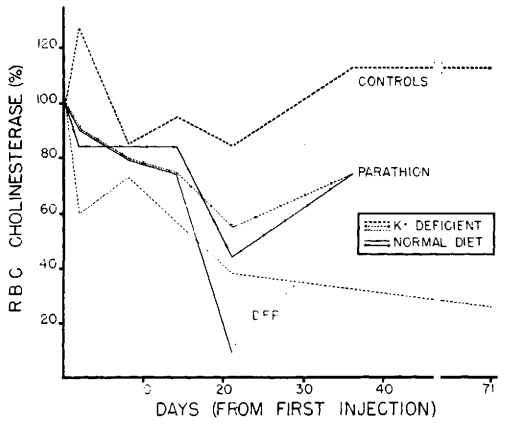


FIG. 1. Cholinesterase levels of red cells in rats given repeated injections of cholinesterase inhibitors (values expressed as % of the mean of 11 determinations on control potassium-deficient rats).

*Hematocrits.* There were no consistent differences among hematocrit values.

*Plasma electrolytes.* The potassium deficient diet produced marked hypokalemia and hypochloremia (Table I). There were no significant differences in the plasma electrolytes as a result of the administration of cholinesterase inhibitors.

*Muscle water and electrolytes.* Skeletal muscle of all groups showed essentially the same water content (Table I). Potassium deficient rats had a marked reduction of potassium in their muscles and a less marked increase in sodium concentration. No differences are found in muscle water or electrolytes between control rats and those given DFP or Parathion.

*Liver water and electrolytes.* Livers of potassium deficient rats tended to have a higher water content than did the normals (Table I). The reduction in liver potassium in potassium deficient rats was much less than the reduction in muscle potassium and the increase in sodium was slight. There were no apparent differences in liver water and electrolytes between control rats and those intoxicated with anticholinesterase compounds.

*Derived data.* Extracellular water phase, intracellular water phase, and intracellular sodium concentration are also shown in Table I. These have been calculated as presented by Cotlove *et al.* Changes in skeletal muscle due to potassium depletion, namely, increase in extracellular water, decrease in intracellular phase, and a marked gain in intracellular sodium concentration are similar to those reported by these authors(15). These changes do not occur in the liver. None of these derived values for muscle and liver discloses

TABLE I. Water and Electrolyte Values in Plasma, Muscle and Liver of Control Rats and Rats Subjected to Cholinesterase Inhibitors.

| Group | Plasma Determined | | | | Muscle | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Determined | | | | Derived | | |
| | $(H_2O)$ g/l | $(K^+)$ | $(Na^+)$ | $(Cl^-)$ | $(H_2O)$ g | $(K^+)$ | $(Na^+)$ | $(Cl^-)$ | $(H_2O)_E$ g | $(H_2O)_C$ g | $(Na^+)_C$ mEq |
| | | mEq/kg $H_2O$ | | | | mEq | | | | | |
| K deficient | | | | | | | | | | | |
| Saline control | 923 | 2.34 | 161 | 104 | 764 | 57.0 | 52.7 | 13.3 | 112 | 652 | 36.5 |
| S.E. | 3 | .09 | 2 | 4 | 1 | 1.7 | 3.2 | .3 | 3 | 1 | 4.3 |
| | (6) | (5) | (6) | (6) | (3) | (6) | (5) | (5) | (3) | (3) | (3) |
| DFP | 925 | 3.09 | 156 | 101 | 768 | 63.7 | 48.1 | 13.1 | 118 | 646 | 29.4 |
| S.E. | 1 | .49 | 2 | 3 | 2 | 2.6 | 3.9 | .8 | 10 | 5 | .8 |
| | (6) | (6) | (6) | (6) | (3) | (6) | (6) | (6) | (3) | (3) | (3) |
| Normal K | | | | | | | | | | | |
| Saline control | 919 | 6.51 | 158 | 111 | 763 | 103 | 17.6 | 11.9 | 102 | 671 | .5 |
| S.E. | 3 | .70 | 4 | 5 | — | 4 | .9 | .5 | 8 | 18 | 1.7 |
| | (4) | (4) | (3) | (4) | (1) | (3) | (4) | (4) | (3) | (3) | (3) |
| DFP | 919 | 6.45 | 154 | 117 | 764 | 98.3 | 18.7 | 10.0 | 92 | 672 | 4.8 |
| S.E. | 2 | .31 | 2 | 2 | 3 | 3.8 | .5 | .9 | 7 | 7 | 2.9 |
| | (4) | (4) | (3) | (4) | (3) | (4) | (4) | (4) | (3) | (3) | (3) |

| Group | Liver | | | | | | |
|---|---|---|---|---|---|---|---|
| | Determined | | | | Derived | | |
| | $(H_2O)$ g | $(K^+)$ | $(Na^+)$ | $(Cl^-)$ | $(H_2O)_E$ g | $(H_2O)_C$ g | $(Na^+)_C$ mEq |
| | | mEq | | | | | |
| K deficient | | | | | | | |
| Saline control | 734 | 72.9 | 24.5 | 20.0 | 177 | 557 | −4.0 |
| S.E. | 4 | 4.8 | 1.4 | 1.4 | 11 | 9 | 1.5 |
| | (4) | (5) | (6) | (6) | (4) | (4) | (4) |
| DFP | 735 | 80.7 | 23.2 | 20.5 | 172 | 568 | .5 |
| S.E. | 4 | 7.0 | .7 | .8 | 21 | 22 | 1.4 |
| | (6) | (4) | (5) | (4) | (4) | (4) | (4) |
| Normal K | | | | | | | |
| Saline control | 719 | 83.4 | 21.8 | 23.1 | 199 | 532 | − .3 |
| S.E. | 3 | 8.8 | 1.9 | 1.7 | 10 | 21 | 8.2 |
| | (3) | (3) | (4) | (4) | (4) | (4) | (4) |
| DFP | 720 | 85.9 | 19.0 | 20.8 | 185 | 522 | −6.8 |
| S.E. | 4 | 3.0 | 1.7 | 1.9 | 10 | 15 | 1.8 |
| | (4) | (4) | (4) | (4) | (3) | (3) | (3) |

Muscle and liver data/kg fat-free whole tissue.
S.E. = Stand. error of mean. Numbers in parentheses indicate No. of observations.
Data presented for Group A1 rats only.

any difference between animals receiving and those not receiving cholinesterase inhibitors.

*Histologic study.* Cardiac muscle revealed myodegeneration typical of potassium deficiency(16) and renal tubular changes of uncertain origin. No differences were noted between control animals and those given Parathion and DFP.

*Discussion.* Alterations reported here as the result of potassium depletion are similar to those found by Heppel(17) and Yannet and Darrow(18). Changes in water and electrolyte composition of plasma, skeletal muscle, and liver could not be related to repeated administration of Parathion or DFP. Lack of evidence in these experiments that shifts of water and electrolytes occur as a specific result of cholinesterase inhibition suggests that hyperkalemia produced by acute lethal doses of anticholinesterases is unlikely to be related to changes in cellular permeability directly as a result of excessive concentrations of acetylcholine. The possibility remains that disturbance of cellular permeability may be too transient to have been encountered in these studies.

*Summary.* Normal rats and potassium deficient rats were injected with DFP and Para-

thion over many weeks. Electrolytes of plasma, skeletal muscle and liver of the potassium deficient rats showed the effects of potassium depletion. Studies of cholinesterase disclosed reduction in activity of this enzyme in rats receiving cholinesterase inhibitors. No effects due to cholinesterase inhibition were found upon either concentrations or distributions of water, potassium, sodium or chloride in plasma, skeletal muscle and liver.

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## Effects of Adrenalectomy on Specific Activity of Deoxyribonuclease in Rat Spleen After Total Body X-Irradiation.* (22141)

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In view of the fact that specific activity of deoxyribonuclease II (DNase II)(1) in rat spleen is more than doubled after total body x-irradiation(2), it has been of interest to determine the role of adrenal cortex in this phenomenon. Leblond and Segal(3) recorded involution of the spleen as a result of localized x-irradiation after adrenalectomy. Prolonged administration of corticotropin to rats decreases cellularity of the spleen, and therefore the amount of deoxyribonucleic acid (DNA) present(4). Reduction in tissue mass of the organ has been attributed to lymphocytolysis and the inhibition of mitosis resulting from adrenocortical steroids(5).

We have measured the DNase II activity in spleens of control, x-irradiated intact, adrenalectomized and x-irradiated-adrenalectomized rats.

*Methods.* Twenty-four young adult male rats of the Sprague-Dawley strain were used. They were divided into 4 groups of 6 animals each. The groups were treated as follows: Group I—control, Group II—x-irradiated-intact, Group III—adrenalectomized, Group IV—x-irradiated-adrenalectomized. All were housed in metal cages in an air conditioned room at 80°F, and fed a diet of Purina Lab-

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