The Uptake by Skeletal Muscle of Tritium Labeled Decamethonium and Dimethyltubocurarine[1]

Stable depolarizing neuromuscular blocking agents, like carbachol and decamethonium (C-10), produce a characteristic dual block upon isolated preparations (JENDEN, KAMIJO and TAYLOR[2]; JENDEN[3]). The rapid initial block, phase I, recovers spontaneously and then passes slowly into a second block, phase II. Phase I block is characterized by depolarization (THESLEFF[4]), while phase II block appears to have very similar pharmacological characteristics to D-tubocurarine (curare) block (JENDEN, KAMIJO and TAYLOR[2]). It has been suggested that the progress of phase II block is related to the slow penetration of the depolarizing drug into the receptor mass (JENDEN, KAMIJO and TAYLOR[2]) and possibly into the muscle fiber (TAYLOR[5]; TAYLOR and NEDERGAARD[6]). Support for the latter was obtained with an I-131 labeled depolarizing drug, 1,10-*bis*-(dimethyliodoethylammonium)-decane dichloride (iodocholinium) (CREESE, TAYLOR and TILTON[7–9]; TAYLOR, CREESE and SCHOLES[10]). Unfortunately the iodocholinium used was impure, its stability of some concern and a biphasic block was not observed consistently. To overcome these difficulties we used tritium labeled decamethonium (C-10-H^3) in the present study. In contrast to the depolarizing neuromuscular blocking agents, the anti-depolarizing drugs, like D-tubocurarine (curare), produce a single block upon isolated preparations presumably by reacting with surface receptors at the motor end-plate (HOLMES, JENDEN and TAYLOR[11]; WASER[12,13]; WASER and LÜTHI[14]). Hence it is possible that the anti-depolarizers do not enter the muscle fiber and that they can prevent the uptake of the depolarizing agents. In order to test these possibilities we have examined the skeletal muscle uptake of C-10-H^3 and tritium labeled dimethylether-H^3 of D-tubocurarine (dimethyltubocurarine-H^3) as well as the ability of curare to interfere with the uptake of C-10-H^3.

Methods. C-10-H^3 dichloride and dimethyltubocurarine-H^3 dichloride were obtained commercially[15]. The purity of the drugs was assessed by paper chromatography. Slips of guinea-pig diaphragm were placed in a tissue bath filled with physiological salt solution at 37 °C containing the labeled drug. After appropriate incubation periods the muscles were removed and digested by a modification of the method of GJONE, VANCE and TURNER[16] and the radioactivity determined with a Packard Tri-Carb liquid scintillation spectrometer.

Results. A subparalytic concentration, $6 \cdot 10^{-6} M$, of C-10-H^3 was continually taken up by the muscle during 5 h as shown in the Figure. The uptake by resting muscle approximately followed the progress of phase II block. After 5 h the uptake greatly exceeded that which could be accounted for by the extracellular space, the latter being less than 0.3 ml/g (CREESE, TAYLOR and TILTON[7]). In contrast very little dimethyltubocurarine-H^3 in a subparalytic concentration was taken up during an incubation period of the same duration (Figure).

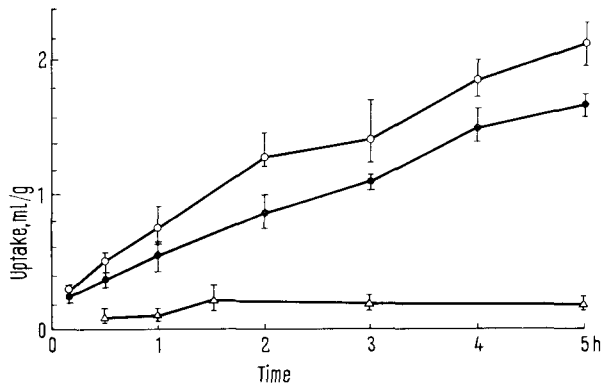
Curare markedly decreased the uptake of C-10-H^3, as shown in the Table. When supraparalytic concentrations, $5 \cdot 10^{-6} M$ to $10^{-5} M$, of curare were used, the depression of C-10-H^3 uptake reached asymptotic values. The Figure also shows the ability of a just subparalytic concentration of curare to decrease the uptake of C-10-H^3.

Discussion. The results obtained are consistent with the hypothesis that depolarizing neuromuscular blocking agents, like C-10, are taken up by the muscle fiber whereas the antidepolarizing agents, like dimethyltubocurarine, are not. The uptake of C-10-H^3 presumably represents penetration of the drug into the intracellular compartment. The uptake of dimethyltubocurarine-H^3 can be accounted for by the extracellular space and further con-

C-10-H^3 uptake and curare concentration

Curare concentration $\cdot 10^{-6} M$	Mean ml/g	Range	No. of experiments
0	2.98	2.21–3.57	6
1	2.14	1.91–2.55	3
2	1.76	1.56–2.13	7
5	1.06	1.05–1.07	3
8	1.03	0.92–1.14	3
10	1.18	1.02–1.38	3

The tabulated values represent ml of physiological salt solution which have been cleared of $6 \cdot 10^{-6} M$ C-10-H^3/g resting diaphragm after 5 h. The muscles were pretreated with curare for 30–45 min.



The uptake of $6 \cdot 10^{-6} M$ C-10-H^3 (open circles) and of $2 \cdot 10^{-6} M$ dimethyltubocurarine-H^3 (open triangles). The uptake of $6 \cdot 10^{-6} M$ C-10-H^3 (closed circles) in the presence of $2 \cdot 10^{-6} M$ curare is also shown. Curare was added 30–45 min before C-10-H^3. The uptake is expressed in ml of bath fluid cleared per g tissue. Each point is the average of 3 single determinations. The bars represent the ranges.

[1] Supported in part by U.S.P.H.S. Grant No. B-738.

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firms that it acts extracellularly. The predominant site of uptake of C-10 and binding of curare is most likely the motor end-plate, as shown by autoradiography (WASER[12,13]; WASER and LÜTHI[14]). Studies on the distribution of C-10-H^3 in sections of diaphragm muscle (TAYLOR, CREESE, NEDERGAARD and CASE[17]) also suggest that C-10-H^3 is taken up through the motor end-plates.

The uptake and phase II block of C-10 appears to be related since their time course is approximately the same. This is supported also by the finding that curare can prevent the onset of phase II block of depolarizers (NEDERGAARD and TAYLOR[18]) and decrease the uptake of C-10-H^3 [19].

Résumé. Le décaméthonium, une substance dépolarisante, est absorbé par le muscle squelettal tandis que la substance non-dépolarisante, le diméthyltubocurarine, ne l'est pas. L'absorption du décaméthonium se rapporte peut-être à ce bloque de deuxième phase qui se fait voir avec les substances dépolarisantes.

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Stability of the Colloidal Chromic Radiophosphate (^{32}P) to the Isotopic Exchange

The relatively high percentage of ^{32}P accumulation observed in bone (especially bone marrow) after the injection of colloidal solutions of chromium phosphate, has been explained by several authors[1,2] as being due to the liberation of phosphate ions in the organism mainly because of the instability of the chemical bond in the chromic phosphate molecule.

This work has been done to test the chromium phosphate stability to the isotopic exchange with the ionic phosphate, normally present in the organic fluids.

Two different types of colloidal chromic phosphate (^{32}P) have been assayed: a true colloidal solution (type B) and one with a larger particle suspension (type F). The latter is currently used as a therapeutic agent.

Type B[3] was prepared by heating at 70–80 °C a mixture of 1.5 ml of H_3PO_4 solution (10 mg/ml), 1.8 ml of CrO_3 solution (10 mg/ml) and the ^{32}P activity (1 mc of carrier-free ^{32}P) in 2 ml of distilled water. Then, stirring continuously, 100 mg of Na_2SO_3 dissolved in 3 ml of 2% gelatin solution were added. After being boiled for a few minutes and then cooled to room temperature, the almost clear blue-green solution was dialysed against distilled water until no activity was detected in the water. The radioactive yield was 40–50%.

Type F[3] was prepared by mixing 4 ml of H_3PO_4 solution (10 mg/ml) with 5 ml of CrO_3 solution (10 mg/ml) and the ^{32}P activity incorporated. After heating for 15 min in a boiling water bath, 2 ml of Na_2SO_3 solution (200 mg/ml) and, immediately, 2 ml of 6% gelatin solution were added. The heating was continued another 10 min and then the excess of ionic phosphate was eliminated by dialysis as described before. The radioactive yield was 75%.

In both preparations, the final concentrations were chromic phosphate 3 mg/ml and gelatin 6 mg/ml.

The isotopic exchange was studied by incubation, at 37 °C, of 10 μC of colloid (tested phosphate ion-free by electrophoresis) with an isotonic phosphate solution at pH 7.2 (1 vol 2.1% KH_2PO_4 + 3 vol 2.2% $Na_2HPO_4 \cdot 2H_2O$). The incubation was carried out under sterile conditions and samples were taken at different intervals: 1, 2 and 6 h; 1 day, 2, 5, 7, 9 and 12 days. The activity as ionic phosphate was determined by electrophoresis using buffer veronal-sodium veronal pH 8.6 for 1 h at a voltage gradient of 15–20 V/cm.

The distance of migration of both ionic phosphate and colloidal chromic radiophosphate was determined by

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Percentage of ^{32}P as chromic phosphate after incubation

Time	1 h	2 h	6 h	24 h	2 days	5 days	7 days	9 days	12 days
Type B	98.3 ± 0.5[a]	98.3 ± 0.5	98.3 ± 0.5	97.6 ± 0.4	97.9 ± 0.1	97.0 ± 0.2	96.7 ± 0.0	96.6 ± 0.4	96.6 ± 0.4
Type F	95.8 ± 0.2	95.8 ± 0.2	95.8 ± 0.4	94.2 ± 1.4	94.6 ± 1.4	93.5 ± 0.3	93.7 ± 0.7	93.4 ± 0.1	93.4 ± 0.2

[a] Standard deviation.