magnitude larger than that supposed for frog muscle fibres. Meanwhile, the distance between the thick and thin filaments at L_0 was nearly the same in both crayfish and frog fibres[15].

To examine the anatomical origin of the above highly compliant SEC, fine carbon particles were firmly attached to the fibre surface, and the length changes of the fibre segments divided by the particles were recorded during the course of a quick release of a tetanized fibre with a 35 mm ultra high-speed cine-camera (Beckman, Inc., Model 165) at 40,000–50,000 frames/sec (figure 2B). As shown in figure 2A, the time course of length change of each fibre segment was fairly uniform until the total fibre length was shortened by 2%, i.e. the magnitude of quick release required to reduce the isometric tension from P_0 to zero (figure 1). This implies a uniform distribution of the highly compliant SEC along the entire fibre length. Microscopic observation (up to $\times 400$) and cinematographic recording (Redlake Corp., Locam, 500 frames/sec) of isometrically contracting fibres excluded the possibility that the SEC originates from extremely stretched sarcomeres distributed along the fibre length; though some fibre segments shortened by stretching others during the development of isometric tension, the resulting variation of sarcomere length along the fibre length was not very large.

The present results strongly suggest that the highly compliant SEC in crayfish muscle fibres results not only from the elastic elongation of the cross-bridges but also from the elongation of some other structures in each sarcomere such as the thick and thin filaments and the Z-disc. Much more experimental work is needed on the origin of the SEC which may be essential for the understanding of muscle contraction.

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The fate of choline in the circulating plasma of the rat

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Summary. Labelled free choline injected into the peritoneum failed to enter the brain but preferentially entered the liver. Subsequently labelled phospholipid was found in the plasma with a concurrent increase in the brain. This labelled plasma injected by cardiac puncture caused a rapid incorporation of the choline labelled phospholipid into the brain.

Diamond[1] has shown that a pulse label of radioactive choline could within a short time label the ACh pool in the brain. However some authors have argued that the choline found in the brain is supplied under normal conditions in the blood not as free choline but as a choline containing phospholipid. (For a recent review see Ansell and Spanner[2].) This paper reports the fate of labelled choline injected into the peritoneal cavity.

Methods. Injection of [methyl-^{3}H] choline: 50 µCi of [^{3}H] choline were injected into the peritoneal cavity of male rats, which were then killed at intervals of 7.5 to 120 min following the initial injection. Plasma samples were prepared by centrifuging heparinized blood at 3000 rpm for 5 min in a bench centrifuge. Tissue samples were homogenized in a 1% solution of Triton X-100 and the radioactivity calculated in dpm/g fresh tissue.

Labelled free choline in the plasma was separated from the labelled phospholipid by passing a sample of plasma through a 5×0.63 cm column of Amberlite CG50 resin in the sodium form. The compounds having a net neutral or negative charge passing through the resin were identified as phospholipids[3], the free choline was eluted from the column with 0.1 M HCl.

Preparation of plasma containing labelled phospholipid: Female rats were injected with 200 µCi of [methyl-^{3}H] choline. After 4–5 h the blood was removed by cardiac puncture and the labelled plasma obtained. Choline free plasma was prepared by running the plasma through columns of Amberlite CG50 resin. The plasma containing labelled phospholipid was then injected into male rats of 300 g, again by cardiac puncture.

Extraction of labelled choline, ACh, and phosphatidyl choline from brain: The labelled products in the forebrain 60 min after an i.p. injection of 200 µCi of [^{3}H] choline were examined in rats weighing 300 g. The cerebrum was removed by the freeze blow method and the material obtained extracted with absolute ethanol at −70 °C for 20 h to reduce post mortem release of choline[4].

The extract was run on a 12×1 cm column of Amberlite IRF97[5] in 0.02 M Na_2HPO_4-citric acid buffer at pH 4.3. Under these conditions neutral and negatively charged molecules came through the column with the void volume while choline and ACh followed later.

Results. Choline injected into the peritoneal cavity finds its way into the blood, liver and brain[6]. The distribution of isotope in the tissues with time shows a rapid uptake into the liver (figure 1) but very little isotope is found in the brain (the concentration is only 0.5% of that in the liver). When the total weight of the tissue is taken into consideration it was found that 50% of the injected isotope is in the liver but less than 0.05% in the brain. The isotope level in the kidney reached a peak at 15 min and was accompanied by an increase of isotope in the urine.

By separating the free choline in the plasma it was found that the amount of labelled free choline in the plasma falls steadily, while the labelled phospholipid in the plasma

increases. The isotope content of the brain shows a close association with this increase in plasma lipid.

Injection of plasma containing [^{3}H] labelled phospholipids: Labelled plasma free of choline was injected into male rats by cardiac puncture. The injection (approximately 0.5 µCi) compares with the 50-µCi injection of free choline. There was a significantly higher percentage of the injected isotope in the brain after 20 min (figure 2) than following similar injections of choline. Free choline appeared in the plasma of the injected animal after 20 min, although none was injected and it is probably the appearance of this plasma free choline that causes the increase in concentration of isotope in the liver and kidney at 60 and 120 min after the initial injection.

The identity of the isotope in the brain following i.p. injection of [^{3}H] choline: Extracts of brain following i.p. injection of choline were examined and the results obtained showed that approximately 40% of the total isotope in the brain after 1 h was extractable into ethanol. The remainder was precipitated with the protein by the ethanol. Of the label in the ethanol soluble fraction most was accounted for by phospholipid but there were significant amounts present as free choline (approximately 8%) and ACh (6–7%), the remainder as phospholipid.

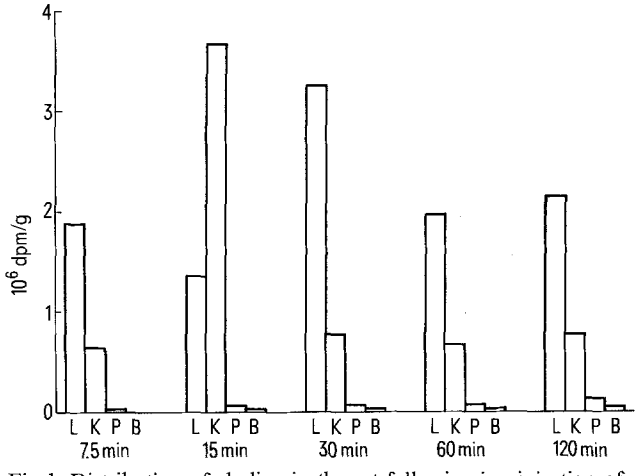

Fig. 1. Distribution of choline in the rat following i.p. injection of ^{3}H choline (50 µCi). The histograms from left to right show the distribution of isotope after 7.5, 15, 30, 60 and 120 min. L: liver, K: kidney; P: plasma; B: brain.

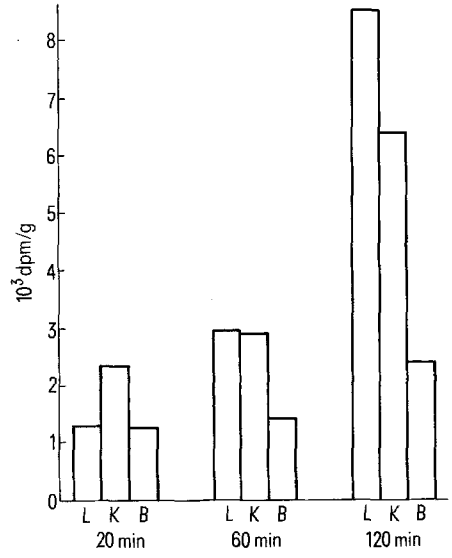

Fig. 2. Distribution of isotope following the intracardiac injection of labelled plasma (i.e. containing labelled phospholipid 0.5 µCi). The histograms show the amount of isotope in tissues 20, 60 and 120 min after injection. L: liver; K: kidney; B: brain.

Discussion. It is now accepted that the stepwise methylation of phosphatidylethanolamine which results in the synthesis of choline in the liver does not occur in the brain[7–9]. The arteriovenous differences which were first reported by Dross and Kewitz[10] have been confirmed by Spanner, Hall and Ansell[11] and Aquilonius et al.[12] among others. From the results we have reported here, it appears that phospholipid enters the brain and other tissues from the plasma and that during the subsequent metabolism of this phospholipid choline is released. The loss of choline from the brain has been shown following injection of labelled choline either into the cerebral ventricles or the cortex[13,14].

The results reported here confirm that it is the phospholipid formed in the liver which enters first the plasma and subsequently the brain. Haubrich, Wang and Wedeking[15] have reported that after i.v. injection the concentration of [^{3}H] choline in the brain reached a peak after 2 min and then began to decline rapidly. The subsequent fall would, on the basis of the results reported here, be due to incorporation of the circulating choline into the liver followed by some excretion. These obervations are in agreement with those of Illingworth and Portman[16] although the amount of ACh-synthesis from lipid choline obtained by these authors is surprisingly high when compared with the present results. The synthesis of ACh in peripheral tissues which do not have a barrier such as that found between the blood and brain may still depend on the plasma choline but values for free choline in plasma are now thought to be below 1 nmole/ml[11]. Although it is possible that choline is also supplied to ganglia as phospholipid it must be remembered that choline has been shown to be an essential constitutent of the perfusion medium if ACh-synthesis is to be maintained in the perfused superior cervical ganglia[17]. Choline uptake mechanisms play an important part in the conservation of choline for ACh-synthesis but it now seems that this free choline originates from phospholipid.

Pathways for the release of choline from phospholipid are at present being investigated and the enzyme glycerophosphorylcholine diesterase has already been reported in a nerve ending rich fraction prepared from rat brain[18].

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