and amino-acids into the intact levator ani preparation. In a first series of experiments, insulin (0·001 I.U./ml.) and D-xylose-^{14}C were added to the medium and the percentage distribution of the pentose in the total tissue water was determined after 2 h of incubation. In control preparations incubated without insulin this distribution was 65·4 ± 4·1 per cent; the corresponding value with insulin in the medium was 102·5 ± 3·8 per cent.

In another series of experiments, the same concentration of insulin was added to the medium together with α-aminoisobutyric acid-^{14}C (0·1 mM). The distribution ratio (= c.p.m. in 1 ml. intracellular water: c.p.m. in 1 ml. medium) of this non-utilizable amino-acid was determined after 2 h of incubation. It was found to be 6·22 ± 0·42 for the preparations incubated with insulin in the medium but only 2·06 ± 0·02 for the control preparations incubated without insulin.

We think that these results show that the levator ani muscle from the rat can be prepared as an intact *in vitro* preparation and that this preparation is suitable for investigations of transport mechanisms over the cell membrane.

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Effect of *d*-Tubocurarine on Uptake of Labelled Carbaminoyl Choline in Brain Slices of the Rat

The presence of neurones in the cerebral cortex which are sensitive to acetylcholine has been well established[1]. The stable substance carbaminoyl choline (carbachol) is also effective[2], and we have measured the uptake of carbaminoyl choline chloride (*N*-methyl-^{3}H) in slices of cerebral cortex from the rat.

Slices the mean thickness of which was less than 0·4 mm were obtained from rat brains. The heads were removed by a guillotine and the cerebral hemispheres were sectioned by means of a stainless steel blade mounted in a frame, together with a plastic guide which contained a shallow recessed trough[3]. Two rectangles of tissue were obtained from each slice, and one was used as a control. The tissues weighed 20–30 mg and these were floated in tubes containing 10 ml. physiological saline[4] at 37° C, the solution being gassed with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide by means of a fine spray from thin plastic tubes the ends of which were plugged with fluted glass. Labelled carbachol was added to give a concentration of 0·15 μg/ml. The slices were later weighed, transferred to counting vials and dissolved in 0·5 ml. methanolic potassium hydroxide (N/2) at 70° C with shaking. To the vials were added 18 ml. of a mixture prepared from adding 42 ml. of 'Liquifluor Scintillator' (Pilot Chemicals, Ltd.) to 300 ml. methanol and diluting to 1 l. with toluene. The radioactivity was measured by conventional liquid scintillation methods with an efficiency of 10 per cent. A suitable dilution of the saline in methanolic potassium hydroxide was also counted.

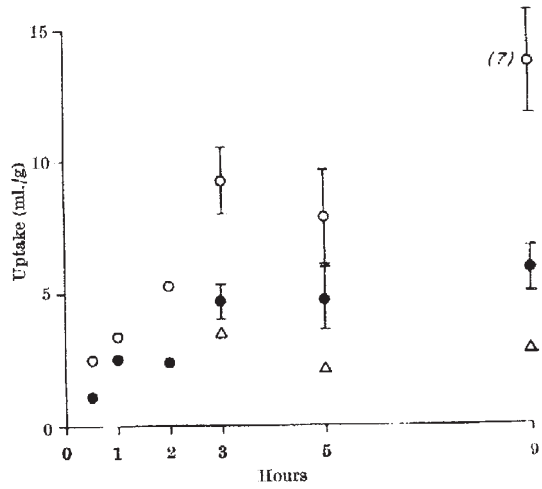

Fig. 1. Uptake of labelled carbachol in rat brain slices. Open circles give mean values, and the limits show the standard deviation. Closed circles show uptake in presence of *d*-tubocurarine (5 μg/ml.). Triangles show uptake in the presence of strychnine (10 μg/ml.). Each point at 0·5, 1, 2 h gives mean of 2 experiments. Each point at 3, 5, 9 h gives mean of 4 except for one point where the number of experiments (7) is indicated by the figure in parentheses

Fig. 1 shows the uptake of labelled carbachol. The results have been expressed as ml./g, obtained from the ratio (activity per g)/(activity per ml. solution). It can be seen that the activity is comparatively high, and after 3 h an amount of compound contained in more than 9 ml. saline has been concentrated in each g of tissue.

The closed circles in Fig. 1 show the uptake in the presence of *d*-tubocurarine (5 μg/ml.), which was applied at least 30 min before the addition of labelled carbachol. In each of 18 pairs the uptake of carbachol was markedly reduced in the presence of *d*-tubocurarine. The triangles show that the uptake of carbachol in the presence of strychnine (10 μg/ml.) was also reduced in the 12 slices which were investigated.

The action of *d*-tubocurarine in brain slices resembles the effect in skeletal muscle where *d*-tubocurarine, which acts initially as a pharmacological antagonist, has been found to decrease the uptake of a labelled compound resembling decamethonium[5,6].

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