earlier results. This has been shown in Fig. 1, where the standard error of the mean has been separately drawn for each time of observation. It may be seen clearly by inspection that no straight line could pass close to all the points; a single exponential course could describe the observations at 15, 45 and 120 min adequately, but deviations arose after 300 min just as they did in the conventional experiments of Goodford and Hermansen[12]. These deviations from a single exponential were not due to variation between guinea pigs since the effect was observed in each individual animal, nor were they artefacts due to the assumption that exchange was complete after 1,440 min. This was, of course, implied in the selection of the logarithmic ordinate scale, but the relative activity could not exceed 1 at equilibrium and any smaller value would only accentuate the curvature in Fig. 1.

It may be concluded from the previous considerations that the potassium in the tænia coli was not uniformly distributed. However, the effect of duration of experiment on rate of exchange was investigated in a further series of experiments in which nine muscles were dissected from each guinea pig and placed in inactive Krebs solution. Three were transferred to the radioactive organ bath after 60 min and were removed for analysis during the next hour (Fig. 2*a*). Three more pieces were transferred after 300 min and similarly observed (Fig. 2*b*), while the remaining three muscles were only transferred to radioactive solution after 1,440 min (Fig. 2*c*). The results in Figs. 2*a*, *b* and *c* are similar, and in particular do not show any progressive change in the initial rate of uptake of potassium-42 after *in vitro* pre-incubation periods as long as 24 h. The later, slower exchange of potassium in the tænia coli[12] is therefore largely, or entirely, due to a lack of uniformity in the tissue, as is commonly assumed, and is not appreciably due to any deterioration of the preparation *in vitro*.

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Effect of Calcium Ions on Melanophore Dispersal

It has been suggested that calcium ions act as the coupling agent in muscle excitation and contraction[1,2], in stimulation of the adrenal medulla and cortex[3], and in vasopressin release from the posterior pituitary[4]. Their basic role in membrane reactions has become obvious from many other observations, including that on which we wish to report here.

Hydrated Na^+ and Ca^{++} ions are of similar size[5], so that cross-action and synergism in biological phenomena may be expected. This applies also to nervous conduction, where calcium behaves similarly to sodium: it enters during excitation and leaves during recovery[6], probably playing a key part in this process[7]. In view of this interrelation, it seemed unlikely that the melanophore dispersing action of melanocyte stimulating hormone should be specific to sodium[8–10].

In a series of 200 examinations the skin from the back of tree frogs (*Hyla arborea*) was removed, halved longitudinally and flattened on filter paper with its green-coloured side facing upwards. The skin was then incubated at room temperature for an hour in frog-isotonic sucrose solution, buffered with 2 mM sodium bicarbonate. This procedure was shown to bring extracellular ions down to inert concentrations without significantly affecting the tissue-bound calcium[9]. After incubation, half the skin was soaked in different mixtures of frog-isotonic sucrose and isotonic cation chloride solutions, both buffered with 2 mM sodium bicarbonate; the other half was kept in buffered sucrose solution for control, the Na^+ of the buffer being ineffective at this concentration (2 mM).

Osmotic activity was checked by the Fiske osmometer. The colour of the skin was recorded every 15 min for 3 h according to the colour scale introduced by us[11]. With this method we found that 10 mM calcium chloride not only supported melanocyte stimulating hormone action, *in vitro*, but also caused considerable darkening by itself. Higher concentrations caused lightening (yellowish colour) or inhibition of action of melanocyte stimulating hormone. Among other bivalent ions, only Mg^{++} behaved like Ca^{++} in supporting action of melanocyte stimulating hormone, but none of them, in concentrations up to isotonicity, led to darkening of the skin.

After soaking the skin in frog-isotonic K^+ solution, the melanophore dispersal could still be induced by 10 mM Ca^{++}, this cation acting as a darkening agent by itself, or in support of action of melanocyte stimulating hormone. Melanophore response to Ca^{++} after depolarization by K^+ solution may thus be comparable with smooth muscle response under similar conditions[2].

We accordingly propose as a working hypothesis that Ca^{++} may be the coupling agent between melanocyte stimulating hormone stimulation and melanophore dispersal, having a coupling function parallel to that postulated for muscle[1] and other systems[3,4].

In conclusion we have found that Ca^{++} alone in proper concentrations causes *in vitro* darkening of the skin of the tree frog (*Hyla arborea*)—even in frog-isotonic potassium chloride. Melanocyte stimulating hormone may be presumed to act primarily on the cell membrane, its melanophoric effect being mediated by Ca^{++}. The melanophore reaction may therefore serve as an easily accessible model reaction for examining coupling phenomena.

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