chromatoelectrophoretic method in which only an aliquot of the radioactivity in each tube is counted.

Summary. I^{125}-labeled hormones have been satisfactorily used in the double antibody radioimmunoassay technique for insulin, human growth hormone and rat growth hormone. The longer half-life isotope offers the important advantage of decreasing the need for frequent iodinations of these hormones as is necessary with I^{131}.

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Calcium and Potassium Uptake from Sodium Free Media by Frog Stomach Muscle.* (31028)

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When longitudinal strips of frog stomach muscle are immersed overnight in phosphate Ringer solutions containing 6 mEq potassium per liter and in which sodium is completely replaced by lithium, they lose the greater part of their potassium and virtually all their sodium. The sodium and potassium ions lost from the fibers are replaced by lithium. On reimmersion, at room temperature, in Ringer solutions containing 106 mEq sodium and 10 mEq potassium per liter the muscles readily accumulate potassium ions, but, when sodium is replaced by lithium in the reimmersion fluid no net uptake of potassium occurs. Partial replacement of lithium by sodium in the reimmersion fluid results in net potassium uptake in amounts roughly proportional to the external sodium concentration(1). Thus it appears that sodium plays an essential role in the uptake of potassium by this tissue.

These results are compatible with a direct stimulatory effect of external sodium on potassium uptake by frog stomach muscle(1,2). However, they do not exclude the possibility that the effects observed are due to other causes. One alternative explanation is suggested by the work of Judah and his associates(3,4). These workers found that rat liver slices immersed in low sodium media take up calcium. This uptake of calcium has marked effects on cellular metabolism, the turnover of ATP and of phosphoprotein being greatly reduced. These effects are readily reversed by sodium. Somewhat earlier, Cosmos and Harris showed that, in frog skeletal muscle exposed to low sodium media, there is an enhanced uptake of calcium by the fibers(5).

On the basis of Judah's observations, the failure of isolated strips of frog stomach muscle to accumulate potassium in the absence of sodium ions(1) might be explained as follows. Since all the immersion fluids contained the normal amount of calcium (1.8 mEq per liter) it is possible that, during overnight soaking in sodium free Ringer solutions, the muscles so treated took up relatively large amounts of calcium. During reimmersion in sodium free media the internal calcium concentration would be expected to remain high, with a consequent disruption of ATP production and

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TABLE I. Effect of Ca^{++} on Uptake of Potassium by Isolated Strips of Frog Stomach Muscle.

Set	Group	1st immersion fluid	2nd immersion fluid	% Wt change during 2nd immersion	K^+ content following 2nd immersion
1	1	110 Li^+ : 6 K^+ : 1.8 Ca^{++}	106 Li^+ : 10 K^+ : 1.8 Ca^{++}	+ 5.0 ± 6.9*	18.6 ± 5.8*
	2	" " "	" " : 0 Ca^{++}	+ 1.8 ± 4.9	21.2 ± 4.9
	3	" " "	106 Na^+ : " : 1.8 Ca^{++}	+ 1.9 ± 4.1	48.8 ±10.1
2	4	110 Li^+ : 6 K^+ : 1.8 Ca^{++}	106 Li^+ : 10 K^+ : 1.8 Ca^{++}	− 2.2 ± 2.5	18.9 ± 6.6
	5	" " "	106 Na^+ : " "	+ 1.2 ± 4.0	47.3 ± 8.3
	6	" " : 0 Ca^{++}	106 Li^+ : " : 0 Ca^{++}	+ 1.7 ± 4.5	12.8 ± 4.3
3	7	116 Na^+ : 0 K^+ : 1.8 Ca^{++}	106 Na^+ : 10 K^+ : 1.8 Ca^{++}	+10.4 ± 6.3	61.9 ± 5.0
	8	" " "	" " : 5.4 Ca^{++}	+ 9.0 ± 6.8	63.1 ± 6.9
	9	" " : 5.4 Ca^{++}	" " "	+14.8 ± 6.8	59.1 ± 6.8

Average K^+ content in mEq/kg wet weight of 6 individual muscle strips is shown for each group. Cation contents of immersion fluids in mEq/liter. In addition to the cations listed the immersion fluids contained 113.6 mEq Cl^-, 2.15 mEq $-HPO_4^{2-}$, and 0.85 mEq $-H_2PO_4^-$ per liter (8).

* ± S.D.

utilization(3,4). This inhibition of ATP turnover might in turn interfere with the mechanisms governing potassium accumulation by the cells(6). On the other hand, when muscles, previously soaked in lithium Ringer, are transferred to normal Ringer containing sodium, the effects of calcium might be reversed, permitting potassium uptake to occur. The experiments described herein were designed to test this hypothesis.

Materials and methods. Longitudinal strips of freshly excised stomach muscle, prepared as previously described(1) and weighing about 30-50 mg were used in these experiments. Three strips of muscle from a single stomach were taken for each experiment. Following excision the muscle strips were immersed overnight at 5°C in the first immersion fluids shown in Table I. They were then carefully blotted, weighed, and transferred for 2 hours at room temperature (25°C) to the second immersion fluids listed in Table I. During both procedures individual strips were immersed in 10 ml aliquots of the appropriate medium.

At the end of the second immersion the muscles were blotted, weighed, and analyzed for potassium as follows. Following oxidation in concentrated HNO_3 and evaporation to dryness as described elsewhere(1) the dry residue was dissolved in 10 ml deionized water and the potassium content of the resulting solution was determined using a Beckman DU flame photometer.

Results and discussion. Three sets of experiments were carried out, each set consisting of 6 individual experiments using 3 strips of muscle taken from the same animal. In the first 2 sets the effect of removing calcium from the medium on the uptake of potassium by muscles immersed in sodium free solutions was investigated. In the third set the effect of increased calcium levels in the bathing fluid on the uptake of potassium stored in media containing normal amounts of sodium was studied. The results are summarized in Table I. The K^+ contents listed in this table are based on the average weights of the muscles following the first and second immersions. It is seen that, in most cases, the weight change that occurred during the second immersion was relatively small.

Considering first the results included in set 1 of Table I, it is seen by comparing Groups 1 and 3 that, as previously reported (1), replacement of sodium by lithium in a second immersion fluid containing the normal amount of calcium (1.8 mM) markedly inhibits the uptake of potassium by the muscles during the second immersion. Removal of calcium from the second immersion fluid did not result in any significant increase in potassium content of muscles kept in a sodium free medium throughout ($P>0.4$ for Group 2 compared to Group 1 of Table I).

Turning next to set 2 of Table I it is evident from a comparison of Groups 4, 5, and 6 that omission of calcium from both

first and second immersion fluids did not prevent the inhibition of potassium uptake observed when sodium is absent from the second immersion fluid. On the contrary, muscles kept throughout the experiment in sodium free media without calcium had a significantly lower potassium content at the end of the second immersion than muscles immersed in sodium free solutions containing the normal amount of calcium ($P<0.001$ for Group 4 compared to Group 6 of Table I).

Finally, it is seen from Groups 7, 8 and 9 of Table I that addition of extra calcium either to the second immersion fluid or to both immersion fluids did not significantly decrease the uptake of potassium by muscles immersed in media containing sodium ($P>0.8$ for Group 8 compared to Group 7, and $P>0.5$ for Group 9 compared to Group 7 in Table I).

It may be inferred from these results that the inhibition of potassium uptake observed in isolated frog stomach muscles when sodium is replaced by lithium in the bathing fluid (1,2) is not due to increased amounts of calcium in the muscles and consequent disruption of their metabolic activity. Thus, if the failure of muscles soaked overnight in the cold in a lithium medium to accumulate potassium on transfer to a second lithium medium is due to an enhanced internal calcium content, then, since these muscles readily accumulate potassium when reimmersed in a medium containing the normal amount of sodium, it must be inferred that transfer to solutions containing sodium results in a reduction of the internal calcium level with a consequent reversal of the inhibitory effects of this ion and a restoration of the ability of the fibers to accumulate potassium. On this basis it might be expected that reimmersion of the muscles in a calcium free lithium solution would result in at least a partial restoration of their ability to accumulate potassium since calcium would be expected to diffuse out of the muscles under the influence of the concentration gradient imposed by its absence from the the external solution. However, in the present experiments, no significant increase in potassium content of the muscles was observed as a result of reimmersion in a calcium free solution (compare Groups 1 and 2 of Table I).

Two objections may be made to the validity of this experiment as a test of the possible role of calcium in uptake of potassium by frog stomach muscle. In the first place, the change in the membrane potential of the fibers induced by removing calcium from the external medium(7) might tend to reduce outward diffusion of this ion. Secondly, a large proportion of the intrafiber calcium is presumably in a bound state and, while it might exchange readily with sodium ions entering from the outside, it might not be greatly affected, within the duration of the reimmersion process (2 hours) by mere removal of external calcium. In either case it might be argued that, in the muscle strips included in Group 2 of Table I, the internal calcium remained sufficiently high throughout the second immersion to prevent any significant uptake of potassium.

Both these objections seem to be sufficiently answered by the results obtained with the muscle strips included in Groups 4-9 of Table I. For example, in Group 6, where the muscles were maintained throughout both the first and second immersions in calcium free lithium solutions, no increase in their potassium content, compared with muscles kept in similar solutions containing the normal amount of calcium (Group 4) was observed, despite the fact that, as in the other two sets of experiments, these muscles readily accumulated potassium when transferred to solutions containing sodium (Group 5). There was, on the contrary, a highly significant decrease in potassium content of the muscles of Group 6 when compared with those of Group 4 ($P<0.001$). Similarly, the results obtained with Groups 7, 8 and 9 clearly show that the presence of increased external calcium concentrations, and thus, presumably, of increased internal calcium levels, did not significantly reduce the uptake of potassium by muscles immersed throughout the experiment in solutions containing sodium.

Summary and conclusions. Isolated strips of frog stomach muscle, previously immersed overnight at 5°C in Na^+ free Ringer solutions containing 110 mEq Li^+, 6 mEq K^+,

and 1.8 mEq Ca^{++}/l accumulated K^+ when reimmersed in a solution containing 106 mEq Na^+, 10 mEq K^+ and 1.8 mEq Ca^{++}/l. When Na^+ was replaced by Li^+ in the second immersion fluid, K^+ accumulation was inhibited. Removal of Ca^{++} from the Li^+ Ringer used for the second immersion did not significantly increase the uptake of K^+ by the fibers. Muscles kept in Ca^{++} free Li^+ Ringer during both immersions had a significantly lower K^+ content than muscles immersed throughout in Li^+ Ringer containing 1.8 mM Ca^{++}. Increasing the Ca^{++} content of the fluid used for the second or for both immersions had no significant effect on the final K^+ content of muscles kept throughout the experiment in media containing normal concentrations of Na^+. It is concluded that the inhibition of K^+ uptake by frog stomach muscle observed when Na^+ is replaced by Li^+ in the second immersion medium does not arise from metabolic disturbances caused by an increased entry of Ca^{++} into the fibers.

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Chick Edema Factor: Some Tissue Distribution Data and Toxicologic Effects in the Rat and Chick.* (31029)

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The chick edema factor (CEF), responsible for a large number of deaths in the broiler industry in the fall of 1957, was traced to the unsaponifiable matter (unsap) of the fat used in the broiler rations. It since has been crystallized(1,2,3) and its structure proposed as that of a hexachlorohexahydrophenathrene(*2*). It is known that in the toxic fat, a mixture of related compounds can be found, some toxic and some relatively nontoxic.

To lay the groundwork for a study of the specific physiological effects of pure CEF compounds, a few short studies have been completed to learn the distribution of the toxic material in the body and which organs were primarily affected.

Experimental. Adult rats and day-old White-Rock chicks were used. Because the pure material was not available in sufficient quantity, we used, from the toxic fat, the unsap which represented 38% of the original toxic fat and was estimated to contain at least 10 ppm CEF. The unsap was force-fed because the animals' food intake was drastically curtailed when it was mixed in the diet. All animals were offered water and commercial feed *ad libitum.*

Table I shows the experimental plans and dosage levels employed for all 3 Trials. Feed consumption and fecal and urinary excretion were measured for the rats. Body weights were recorded in all experiments. All animals, upon sacrifice, were examined grossly for pathology and selected organs were weighed and frozen. Hydropericardial fluid (HPF) volume was measured in the chicks.

In addition to the examination for gross effects, the presence of CEF material in various tissue was determined. Adrenals, kidneys, and livers were assayed in the rats; livers only

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