The absence of closed-ring figures at synapsis, such as are characteristic of secondary trisomics in *Datura*,[6] suggests that none of the mouse trisomics are of the secondary or isochromosomal type. Similarly, no evidence has been found, in the form of alternative synaptic partners of different morphology, which should characterize tertiary or translocation-type trisomics.

The ever-increasing use of human karyotypes in clinical investigations and the rapidly progressing cytogenetic studies on the mouse may be expected eventually to indicate which members of the respective chromosome complexes may become involved in viable aneuploidy. It will be of particular interest to learn whether chromosomes other than the smaller ones may be involved.

Summary.—The discovery of three different autosomal trisomics, which are classed as the primary or whole-chromosome type, indicates that autosomal aneuploidy occurs in the mouse. Two of the trisomic individuals were sterile and the third semisterile; no external deviations from the normal phenotype were observed. In each case the trisomy involves members of the smaller chromosome classes.

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*PARATHYROID HORMONE, ION EXCHANGE, AND MITOCHONDRIAL SWELLING**

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The addition of parathyroid hormone to isolated liver mitochondria produces an increased accumulation of magnesium and phosphate,[1] a release of calcium and hydrogen ions,[2] and a stimulation of respiration.[3] These changes are similar to those produced by the hormone on renal tubular function:[4] the retention of calcium and hydrogen ion and the excretion of phosphate. In addition, the hormone promotes the excretion of potassium.[5] For this reason it became of interest to study the effect of parathyroid hormone upon potassium exchange in isolated mitochondria, and to correlate changes in potassium movements with respiration, magnesium and phosphate uptake, hydrogen ion evolution, and mitochondrial swelling.

Experimental.—Mitochondria were prepared from rat liver and suspended in 0.4 *M* sucrose to give 2.5 ml of suspension per gram of liver.[1] They were used im-

mediately, and no experiment was conducted with mitochondria kept for more than 45 min on ice.

The swelling experiments were conducted at 24°C by measuring the optical density at 520 mμ in a Zeiss spectrophotometer. Two-tenths ml of mitochondrial suspension were added to 2.5 ml of medium. Hydrogen ion evolution was measured at 24°C in a Radiometer titrograph by maintaining a pH of 6.9. The pH of all reagents was adjusted to pH 6.9 before addition to the mixture.

Magnesium, phosphate, and potassium uptakes were measured at 24 and 30°C. Phosphate was measured either by chemical means,[6] or by measuring the content of radioactive phosphate, P^{32}. The uptake of potassium was measured either by isotopic means employing K^{42}, or by measuring potassium by a flame photometric procedure employing a Zeiss spectrophotometer. Magnesium was determined by flame absorption spectroscopy utilizing a Perkin-Elmer atomic absorption spectrometer, model 214. In all cases the basic incubation medium contained 270 mM sucrose, 20 mM Tris hydrochloride, pH 6.9, 10 mM succinate, 5 mM magnesium either as acetate or chloride, 0.5 per cent bovine serum albumin, and mitochondria equivalent to 0.2 gm of fresh rat liver in a total volume of 3.5 ml. Potassium was added either as the acetate or chloride salt. Phosphate was added in the form of sodium phosphate buffer, pH 6.9, usually to a final concentration of 5 mM. After addition of the appropriate isotope, K^{42} or P^{32} 0.01 μc per 3 ml, the incubations were carried out as previously described.[1] In all cases the mitochondria from a 2-ml aliquot of incubation mixture were separated from the medium by rapid filtration through pads of celite[7] which were then washed immediately with 5 ml of cold 0.4 *M* sucrose. The pads were then dried and counted directly or extracted with 5 ml of 3 per cent perchloric acid, and the ion content of the extract was determined.

Results.—Potassium uptake: The addition of parathyroid hormone to isolated mitochondria led to a marked increase in potassium accumulation (Fig. 1*A*). This was true whether radioactive or stable potassium was measured. The uptake required substrate and was blocked by antimycin A (Fig. 1*A*). In the presence of antimycin A, ATP, and magnesium, parathyroid hormone did not stimulate potassium uptake (Fig. 1*A*). The substrate-supported uptake of potassium was dependent upon the nature of the anion added. It was observed with potassium acetate but not with potassium chloride (Fig. 1*B*). The uptake occurred in the absence of either magnesium or ATP. In fact, potassium uptake was greater in both control and hormone-treated mitochondria in the absence of magnesium (Fig. 1*C* compared to 1*A*). However, the hormonal effect upon potassium accumulation was greater in the presence of 5 mM magnesium (or greater) than in its absence (Fig. 2*B*). The hormonally stimulated uptake of potassium was a function of potassium concentration (Fig. 2*A*) being maximal at approximately 60 mM K^+. Of considerable significance was the fact that parathyroid hormone stimulated substrate-supported potassium uptake even at 4°C (Fig. 2*C*). This hormonal effect was blocked by antimycin A.

Relationship between potassium uptake and the accumulation of magnesium-phosphate: The hormonally stimulated uptake of magnesium and phosphate occurred in the absence of added potassium, and, contrariwise, potassium accumulation occurred in the absence of magnesium and phosphate. However, the addition of increasing amounts of potassium acetate led to a progressive decline in the hor-

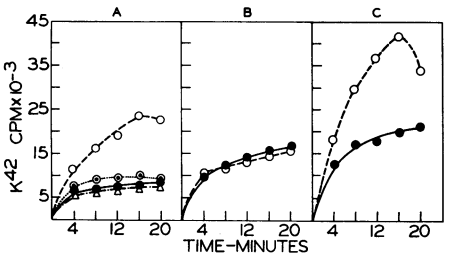

FIG. 1.—(*A*) K^{42} uptake as a function of time in the absence (●—●) and presence (○- - -○) of 2×10^{-6} *M* parathyroid hormone, in the presence of hormone plus antimycin A 2.5 μg/ml (△- · -△), and in the presence of hormone, antimycin A, and 5.0 mM ATP (⊙- - -⊙). The incubation medium contained 20 mM Tris-HCl buffer pH 6.9, 270 mM sucrose, 10 mM succinate, 40 mM potassium acetate, 0.01 μc of K^{42}, 1% bovine serum albumin, 5 mM magnesium chloride, and 0.5 ml of mitochondrial suspension per flask in a total volume of 3.5 ml. (*B*) The uptake of K^{42} in the absence (●—●) and presence (○- - -○) of parathyroid hormone when the potassium (40 mM) was added as KCl rather than potassium acetate. (*C*) K^{42} uptake in magnesium-deficient medium in absence (●—●) and presence (○- - -○) of parathyroid hormone. The medium was the same as employed in (*A*) except that $MgCl_2$ was omitted, and 5 mM ATP was present.

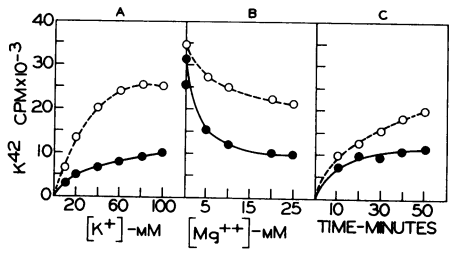

FIG. 2.—(*A*) The uptake of K^{42} after 15 min incubation as a function of potassium concentration. Medium as in Fig. 1*A* except for varying amounts of potassium acetate. Uptake in the absence (●—●) and presence (○- - -○) of 2×10^{-6} *M* parathyroid hormone. (*B*) K^{42} uptake after 10 min incubation as a function of magnesium concentration in the absence (●—●) and presence (○- - -○) of parathyroid hormone. Medium as in Fig. 1*C* except for varying amounts of magnesium acetate. (*C*) K^{42} uptake as a function of time at 4°C in the absence (●—●) and presence (○- - -○) of parathyroid hormone. Conditions as in Fig. 1*C*.

monally produced accumulation of magnesium and phosphate (Fig. 3). No accumulation of magnesium or phosphate was observed when the potassium concentration was greater than 60–70 mM. This observation, plus the fact that potassium uptake was greater in the absence of magnesium (Figs. 1*C* and 2*B*), suggested that potassium and magnesium might compete for a similar carrier or transport site. This supposition was not borne out. Increasing the magnesium concentration to 25 mM did not overcome the inhibition of phosphate uptake produced by 60 mM potassium. A reciprocal plot of 1/phosphate uptake versus 1/magnesium concentration as a function of potassium concentration from 0 to 30 mM K^+ indicated that K^+ was a noncompetitive inhibitor of phosphate uptake. The K_I for potassium was estimated to be 1.8 mM.

Ion accumulation and hydrogen ion release: It is known that the uptake of magnesium-phosphate under the influence of hormone is accompanied by a release of hydrogen ion although the exact mechanism has not been studied.[4, 10] Likewise, the uptake of calcium by mitochondria is accompanied by hydrogen ion evolution.[8, 11] Most recently, Moore and Pressman have reported hydrogen-potassium exchange in mitochondria under the influence of the antibiotic valinomycin.[9] For these reasons it was deemed necessary to determine the relationship between ion accumulation and hydrogen ion evolution.

Potassium-hydrogen exchange did not occur in the presence of parathyroid hormone even though potassium was accumulated.

The addition of magnesium to mitochondria in the presence of substrate and hormone led to no H^+ evolution. The subsequent addition of phosphate led to hydro-

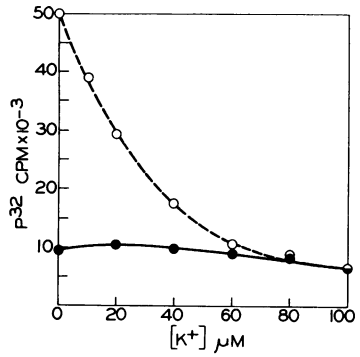

FIG. 3.—The uptake of P^{32} after 15 min incubation as a function of potassium concentration in the absence (●—●) and presence (○- - -○) of 2×10^{-6} *M* parathyroid hormone. The incubation mixture contained 270 mM sucrose, 20 mM Tris-HCl pH 6.9, 5 mM magnesium acetate, 10 mM sodium phosphate pH 6.9, 1% bovine serum albumin, 10 mM sodium succinate, varying concentrations of potassium acetate, 0.6 μc of P^{32}, and 0.5 ml of mitochondrial suspension in a final volume of 3.5 ml. A similar pattern was obtained when magnesium rather than P^{32} was measured.

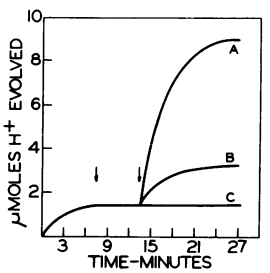

FIG. 4.—Hydrogen ion evolution in the presence of parathyroid hormone. The initial medium (6 ml) contained 270 mM sucrose, 10 mM sodium succinate, 2×10^{-6} *M* parathyroid hormone, and 0.5% bovine serum albumin. It was adjusted to pH 6.9, then 1.0 ml of mitochondrial suspension was added at time zero. After 5 min no further H^+ evolution took place. At 7.5 min 35 μmoles of magnesium acetate, pH 6.9, were added. No H^+ was evolved. At 13.5 min 70 μmoles sodium phosphate pH 6.9 (*A* and *B*), or sodium sulfate, pH 6.9 (*C*), were added. The difference between (*A*) and (*B*) was that 280 μmoles of potassium acetate were added with the magnesium acetate at 7.5 min to incubation (*B*).

gen ion evolution (Fig. 4*A*). Approximately one hydrogen ion was evolved for every phosphate accumulated. Conversely, if phosphate was added in the absence of Mg^{++}, no H^+ was evolved but production commenced with the addition of magnesium. If sulfate replaced phosphate, no H^+ was evolved (Fig. 4*C*) even though magnesium sulfate accumulation is known to occur under these circumstances.[10] Increasing the potassium concentration led to a diminished accumulation of magnesium-phosphate (Fig. 3*A*) and a diminished evolution of hydrogen ion (Fig. 4*B*).

Potassium accumulation and mitochondrial swelling: The addition of potassium acetate but not sodium acetate to mitochondria, in the presence of hormone and magnesium, led to prompt swelling (Fig. 5*A*). The extent of swelling was proportional to the potassium concentration. The time course of swelling and potassium accumulation were similar. Potassium chloride was not accumulated (Fig. 1*B*) nor did it promote swelling (Fig. 5*B*). Accumulation was greater in the absence of magnesium (Fig. 1*C*) and so was swelling (Fig. 5*C*). Both accumulation (Fig. 1*A*) and swelling (Fig. 5*D*) were blocked by antimycin A. Likewise, both accumulation and swelling occurred at low temperature, and both were blocked by antimycin. It should be noted that potassium acetate-induced swelling occurred in the absence of parathyroid hormone (Fig. 5*F*) but was considerably greater in the presence of hormone (Fig. 5*A*). The addition of ATP had little effect upon swelling in the absence of hormone but increased the extent of swelling in the presence of hormone. At low concentrations of potassium (2 mM), parathyroid hormone promoted K^{42} uptake without any increase in swelling. Hence it appears that hormone promotes potassium accumulation, which when significant leads to mitochondrial swelling.

Comparison of effects of parathyroid hormone and valinomycin: Some of the effects

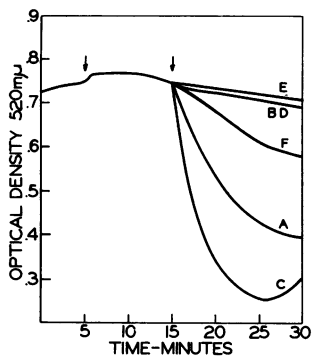

FIG. 5.—Mitochondrial swelling produced by parathyroid hormone and potassium in the absence of phosphate. The incubation medium for (*A*), (*B*), (*D*), (*E*), and (*F*) contained 5 mM $MgCl_2$, 10 mM sodium succinate, 20 mM Tris-HCl pH 6.9, 1% bovine serum albumin, and 270 mM sucrose. In (*C*) $MgCl_2$ was omitted. At time zero, 0.2 ml of mitochondrial suspension were added to 2.5 ml of media. At 5 min 100 μg of bovine parathyroid hormone were added to all but (*E*) and (*F*). At 15 min 40 μmoles of potassium acetate were added to (*A*), (*C*), (*D*), and (*F*), and 40 μmoles of KCl to (*B*). Antimycin A, 10 μg, was added to (*D*) just before parathyroid hormone addition. No potassium was added to (*E*).

of parathyroid hormone upon ion translocations are similar to those of valinomycin as reported by Moore and Pressman.[9] However, there are striking differences. For example, the effects of valinomycin upon phosphate transport require the presence of potassium, whereas those of parathyroid hormone do not. Likewise, K^+ uptake in the presence of valinomycin can be supported by energy derived either from ATP or substrate, whereas only substrate oxidation will serve in the presence of parathyroid hormone.

Discussion.—The effects of parathyroid hormone upon isolated mitochondria are complex. It seems evident from the present studies that whatever the fundamental reaction regulated by the hormone, its activation can bring about the accumulation of potassium in the absence of phosphate and magnesium, and conversely the accumulation of phosphate and magnesium in the absence of potassium. High potassium completely suppresses the accumulation of magnesium-phosphate but does not completely inhibit the hormonally stimulated phosphate-dependent respiration. These effects of potassium are apparently related to its causing or being accompanied by mitochondrial swelling which leads in turn to a more rapid magnesium-phosphate efflux (nonenergy-dependent) from, and a less rapid influx into, the mitochondria thereby reducing net accumulation.

It is quite clear that the hormonal effects upon both potassium and magnesium accumulation are coupled to the activity of the electron transport and phosphorylative reactions in the mitochondria. Our previous evidence with metabolic inhibitors[3] and the present data with potassium are all consistent with coupling either directly between electron transport and ion accumulation or between ion accumulation and one of the nonphosphorylated high energy intermediates in oxidative phosphorylation. It is pertinent to re-emphasize that hormone stimulates K^+ uptake in the absence of phosphate and magnesium. Furthermore, in contradistinction to other studies on ion transport in mitochondria, it has not been possible to demonstrate an hormonal stimulation of ion accumulation in the presence of ATP and antimycin A.

It is also necessary to emphasize that with magnesium-phosphate accumulation there is a clear relationship between ion uptake and respiration. The same is not true for potassium. At low levels of K^+, there is no stimulation of respiration, and at high levels the stimulation is small and not critically related to K^+ concentration, nor influenced by hormone. It does seem clear that K^+ and Mg^{++} do not compete for the same transport site or carrier.

The data in this paper indicate that the expression of an inherent ion transport property of a biological membrane is determined by the ionic environment in which the membrane is placed. In physiological terms, this means that a membrane with the same functional capabilities will nevertheless exhibit different functional properties if present at the cell surface as compared to a completely intracellular location, e.g., as a mitochondrial membrane. It is becoming increasingly apparent that the exchanges of various ions across the mitochondrial membranes are complexly interrelated.

It is noteworthy that the present results constitute one of the few observations of a correlation between ion accumulation and mitochondrial swelling. If our conclusion as to the nature of the relationship is correct, i.e., ion accumulation, by increasing intramitochondrial solute concentration, leads to H_2O uptake, then the mitochondrial membranes are freely permeable to water. If this is the case, then in some instances swelling promoted by other agents may be due to this type of mechanism rather than the relaxation of a contractile mechanism. Swelling and ionic transfers are both closely related to the metabolic state and activity of the mitochondria. A change in this state will undoubtedly lead in many instances to changes in ion binding and translocation, hence to changes in intramitochondrial solute concentrations. The variety and complexities which can be encountered are no better illustrated than in the present instance where potassium acetate, but not potassium chloride, induced swelling under the influence of parathyroid hormone. Neither of these salts is the predominant anion of the natural milieu of mitochondria, but of course few studies upon isolated mitochondria have been carried out in media which in composition approach the intracellular solution in which they normally exist. However, in studies concerned with relating mitochondrial activities *in vitro* to their physiological function *in vivo*, it becomes necessary to pay more attention to this experimental detail.

Summary.—Parathyroid hormone stimulates potassium uptake in mitochondria. Associated with this uptake is a swelling of the mitochondria. Both responses require a functioning electron transport chain.

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