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Mechanisms of Ion Transport at the Subcellular Level with
Special Emphasis on the Action of Parathyroid Hormone

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MASTER

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During the 8 months that the present contract has been in effect,
the following has been accomplished:

Studies on the interrelationship between vitamin D, parathyroid hormone and thyrocalcitonin in the control of serum calcium and Phosphate concentrations (reprints included). It could be demonstrated that in extremely vitamin D deficient rats that thyroparathyroidectomy failed to change the already low serum calcium concentration while serum phosphate ran to high values. Administration of parathyroid hormone (PTH) failed to change serum calcium and was only partially effective in preventing the rise in serum P. On the other hand, thyrocalcitonin (TCT) markedly reduced the serum calcium concentrations from 4.0 mg % to 2.5 mg % in the vitamin D deficient animal thus demonstrating its independence of vitamin D for its action. Serum P response (lowering) by either PTH or TCT was independent of the vitamin.

The low serum calcium which resulted from TCT administration remained low in thyroparathyroidectomized (TPX) rats but rapidly returned to normal values in intact rats. Furthermore, administration of PTH antagonized the TCT action on serum calcium in both normal and vitamin D deficient rats.

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The above results taken in conjunction with present knowledge of vitamin D action have led to the suggestion that vitamin D induces the formation of a calcium transport system which moves calcium and phosphate from bone mineral to blood across osteoclasts and osteocytes. Parathyroid hormone and thyrocalcitonin compete for membrane sites on these cells controlling the release of calcium and phosphate into the extracellular fluid. With this type of model, the data obtained could be explained and it provides a working hypothesis for experimental examination to be described in the renewal request.

Still other studies were initiated on the relationship of the two hormones under conditions of hypervitaminosis D. Preliminary results revealed that if vitamin D deficient rats were given 40,000 i.u. of vitamin D daily for 2 days and then thyroparathyroidectomized, they would die within 24 hours. Death resulted from hypercalcemia and hyperphosphatemia. Removal of only the parathyroids did not result in death and thus the primary gland of importance to protect against hypercalcemia and perhaps hyperphosphatemia is the thyroid. This appeared to be accounted for by thyrocalcitonin. However, it was found that the hyperphosphatemia resulting from thyroparathyroidectomy of hypervitaminotic D rats could not be reversed by either PTH or purified TCT. However, crude extracts appeared able to reverse the hyperphosphatemia. These studies are currently being confirmed and if they are reproducible, it appears as if a new hormone which controls serum phosphate may exist in the thyroid glands. This exciting possibility is placed high on the priority list for next year's investigation.

Experiments relating to ion transport actions of the parathyroid hormone. As described in the original proposal some work is continuing on the effects of the parathyroid hormone on mitochondrial ion transport. The most significant new findings are embodied in the attached preprint of a paper which will be submitted to Biochimica et Biophysica Acta. By supplying energy by either respiration or ATP, mitochondria can be made to reverse the flow of electrons such that electrons from succinate can be made to reduce NAD or further acetoacetate to β -hydroxybutyrate. This reversal takes place by the following set of reactions:

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where A is the NAD and B is the flavoprotein. $\text{X} \sim \text{Y}$ is the high energy non-phosphorylated intermediate generated during oxidative phosphorylation or from ATP. It is also the intermediate believed to be the energy donor for ion transport. If these facts are true then energy linked reduction of NAD and hence acetoacetate reduction should compete with PTH induced transport of magnesium phosphate. This was readily demonstrated as shown in the attached preprint. As might be expected the reduction of acetoacetate inhibited the PTH induced ion transfer. In this system, the PTH effect could be shown in the presence of P_i but not Mg^{++} alone but could be demonstrated again in the presence of both ions. This indirect measure of hormone action then suggests that perhaps P_i is the

primary ion translocated and that one should look for a PTH stimulated carrier of P_i in mitochondria. However, other interpretations are possible as suggested in the attached preprint.

Additional experiments have shown that PTH dependent reactions of mitochondria also compete with ATP dependent acetoacetate reduction. This reaction is strongly inhibited by high concentrations of Mg^{++} necessary to demonstrate ion transport. Thus it seems likely that the earlier failure to demonstrate ATP dependent magnesium phosphate translocation in response to PTH may be the result of the high concentrations of magnesium ions used. Clearly there is much that remains to be learned concerning the PTH dependent ion translocations in the mitochondria as will be outlined in the renewal proposal.

The major question surrounding the mitochondrial experiments is its physiologic significance. In short, how can it be rationalized or tied to the PTH induced phosphate diuresis? At the present time this question cannot be answered, but the question itself forced us to take a broader view of the phosphate diuresis and the experimental approach to its elucidation. For this reason we have begun to look for either a PTH stimulated, P_i dependent ATPase in kidney subcellular fractions. The reasoning behind these two apparently opposite measurements is if PTH stimulates an active secretion then a stimulation of the ATPase would be expected. If, on the other hand, inhibition of P_i reabsorption takes place then inhibition of an ATPase would be expected. These studies are currently in progress and thus far no data are available on this point.

Finally, in the search for an ion transport system which can be approached technically and which might respond to PTH and TCT, a small intestinal perfusion system has been developed and is currently under study. An attempt will be made to establish an effect of PTH and of TCT on the intestinal transport of calcium and phosphate where various diffusion layers imposed by in vitro gut sac preparations are not present. Thus far, the surgical and mechanical techniques have been mastered and we are currently developing the best medium for perfusion of the blood vessels and of the intestinal lumen. It seems likely that during the next year we should have been able to test the effect of PTH and TCT in this system.

It should be noted that during the current contract grant, a shift in emphasis away from some of the mitochondrial work described in the original proposal was made. Firstly, the original proposal was written one year before it was funded. Some of the work therein proposed was carried out in another laboratory (H. Rasmussen working with B. Chance). Furthermore some question of the physiologic significance of the mitochondrial systems exists. For these reasons we have placed much less emphasis on the mitochondrial system and favor instead a broader approach to the action of the parathyroid hormone. Therefore, a more thorough understanding of the hormones action at the physiologic level appeared needed especially in regard to other actions of vitamin D and parathyroid hormone. These studies are well on the way to being completed. Ion transport under the influence of the hormones and vitamin D at the cellular level will next be emphasized which will then lead to the concentration of effort at the subcellular level.

Work Published on the Present Contract

Morii, H. and H. F. DeLuca. Relationship Between Vitamin D Deficiency, Thyrocalcitonin, and Parathyroid Hormone. Am. J. Physiol. 213, 358-362 (1967).

By the completion of the work on the present contract, the \$19,080 which includes indirect costs will have been spent according to the original contract budget. A number of equipment items needed to study ion transport in isolated mitochondria have not yet been purchased. However, we plan to do so in the next six months. In addition, a recorder is not required as originally indicated because one became available from another project. Instead we must replace a worn out fraction collector for the preparation of both PTH and TCT.