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MASTER

SELECTIVE ISOTOPIC LABELING OF THE

PARATHYROID GLAND

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AIMS

The aims of this project are unchanged from previous progress reports, in that the major effort has been directed towards the development of a clinically reliable technique for the anatomic identification and functional evaluation of the human parathyroid glands. This has resulted in considerable "spin-off" into other areas of related investigation. These include (1) an assessment of the intermediary metabolism of selenomethionine, (2) the analysis of changing blood level following injection of labeled amino acids, (3) the control mechanisms of thyroid and parathyroid amino acid acretion.

EXPERIMENTS AND RESULTS:

The major effort during the past year has been directed toward obtaining a more thorough understanding of the mechanisms controlling thyroid and parathyroid amino acid acretion in an effort to develop a clinically reliable test by augmenting parathyroid and suppressing thyroid amino acid uptake. The results to date have been encouraging.

The ability to suppress the thyroid has been well documented, the fact that the suppression is maximal at 20% of the control values suggests a residual thyroid amino acid acreting potential not dependent upon the pituitary thyroid axis. While this may in part, represent thyrocalcitonin, experiments directed at manipulating the calcium milue and studying changing thyroid uptake have not been definitive. The specific residual thyroid amino acid acretion, therefore, is as yet unexplained.

A. Studies directed at enhancing parathyroid selenomethionine acretion:

The parathyroid uptake has been studied in considerable detail in the rat. This study necessitated the development of a reliable technique for measuring the small nitrogen content of the rat parathyroid. A publication titled "Simplified Method for Tissue Nitrogen Content", (25) resulted from our attempts to develop a reliable means of assessing rat parathyroid activity.

The current technique involves the tail vein injection of selenomethionine, surgically removing the glands, and determining the parathyroid activity as the per cent of injected dose per microgram nitrogen. The nitrogen technique is reliable to the range of 1-50 micrograms of tissue nitrogen. The average rat parathyroid contains approximately 6-10 micrograms nitrogen.

The ability to enhance the parathyroid uptake was initially demonstrated by a low calcium diet for twenty-one days. Experiments on the relation of the serum calcium, phosphorus, and magnesium levels to detailed manipulation of the calcium diet have been undertaken during the past year (Table 1,2) The dietary enhancement of parathyroid amino acid acretion appears earlier than the changes in systemic calcium content, which may well reflect compensatory parathyroid hyperactivity to compensate for the low dietary intake of calcium (Figure 1,2) The parathyroid activity can be progressively enhanced by a low calcium diet of fifteen to twenty days. This study documented that it was possible to enhance

parathyroid selenomethionine accretion in the normal rat. However, it does not appear to be of clinical practicality because of the unpalatable diet and the time necessary to develop these changes. Further investigations directed toward phosphate deprivation using standard clinical procedures will be undertaken.

Other means of enhancing parathyroid uptake was investigated in the experimental animal. EDTA was used to lower serum calcium and, thereby, stimulate parathyroid amino acid accretion. Initial experiments were frustrating in that we were unable to demonstrate amino acid accretion if the selenomethionine was given fifteen minutes prior to intravenous EDTA. The EDTA injection reliably suppressed the serum calcium at twenty-five minutes which rebounded to normal levels at two hours. The magnesium was decreased slightly in these experiments and it also came back to normal at two hours. The phosphate levels did not change remarkably. (Table 3) In these experiments, there was no evidence of enhancement of selenomethionine uptake by the parathyroid gland at either twenty-five minutes or two hours. (Figure 3) Despite these discouraging results, additional experiments were undertaken in which selenomethionine was injected fifteen minutes after the EDTA at the time of the low serum calcium content. In these experiments there was minimal increased uptake of selenomethionine at twenty-five minutes, but no evidence of an improved uptake at two hours. (Figure 4) These results were also frustrating in that the changes seen were not significant.

Therefore, additional experiments were undertaken injecting the selenomethionine at the same time as the EDTA in groups of at least six animals each. Again the calcium level decreases reliably at twenty-five minutes from the control animals rebound to normal at two hour and four hour levels. (Table 5) At twenty-five minutes, there was no evidence of an enhanced parathyroid amino acid accretion, however, at two hours after injection the parathyroid uptake was increased by twenty-five per cent without an overlap between the controls and experimentals and a statistically valid stimulation of parathyroid uptake. (Figure 5) The four hour animals showed a progressive increase in parathyroid uptake in the experimentals over the controls documenting the ability to selectively enhance parathyroid selenomethionine accretion without significantly influencing the other neck organs using EDTA. The dosage used in EDTA is practical for human in that the serum calcium level dropped twenty per cent at twenty-five minutes but returned to normal in two hours similar to the changes seen in the clinical diagnostic tests.

It is apparent, the input function of selenomethionine upon the parathyroid is rather short in that the time relation between the amino acid injection and the parathyroid stimulating EDTA is critical. This is consistent with previous experiments which demonstrated a short serum half life of TCA soluble label in both the rat and in man. (Ref 10) Further studies to selectively enhance the parathyroid amino acid accretion are in progress, and will be undertaken in the renewed proposal. (See enclosed graphs.)

B. Clinical studies with data handling and subtraction techniques:

A second major avenue of investigation during the past year has been the use of combined label techniques at a clinical level and computer programming to bring out the parathyroid from the changing background of blood and tissue. The problem of defining a persistent focus of radioactivity against a changing background is common to many forms of scanning (e.g. brain scans).

The localization of parathyroid adenomata with selenomethionine involved the estimation of the localization of this material in the parathyroid in the presence of a blood background of the same material which is changing with respect to time. Since the material remains in the parathyroid for only a few hours, it is not possible to utilize the normal technique of waiting for the blood activity to decrease and hence, the blood to gland ratio to increase. By making a series of photoscans sequential in time, and then optically integrating these, we have shown in certain circumstances, that it is possible to delineate an active parathyroid nodule which is not apparent on a single scan.

In an attempt to quantitate, and improve this approach, a digital data acquisition system based on an incremental magnetic tape transport has been constructed for use in conjunction with rectilinear scanners. The equipment which is at present under test, provides for the direct recording of two separate channels of digital pulse information, positional coordinates of the detector, and lapsed time from the commencement of the scan. (Ref 26, 27)

Programs have been written for the manipulation of this data by the IBM 360/50 computer which provide for;

(a) Correction of recorded count rate for the decay of the isotope used.

(b) Dual isotope scan measurements with simultaneous recording and printing of separated scans.

(c) Dynamic background using two isotopes.

Dynamic background subtraction involves the use of two isotopes, one of which is selected because it remains in the blood stream during the course of the study (e.g. ^{99}Tc Albumin). The time rate of change of selenium activity in the blood stream is measured and this data is used to modify the matrix of data for the isotope selected to remain in the blood stream. This data matrix, which has been modified for the time dependence of blood activity of selenomethionine now represents the blood background produced by the selenomethionine. Subtraction of this modified matrix from the matrix of data recorded for the distribution of selenomethionine should result in a third matrix which represents only selenomethionine retained in the parathyroid tissue.

Initial experiments and tests with this equipment show that this may prove a useful technique. This program has now been worked out and evaluated at a clinical level. In three patients to date there was one case where the computer localization of parathyroid was superior to the previous photoscan approach. The data obtained include, (1) the total selenomethionine activity as seen in the photo-

scans (with or without superimposition), (2) the distribution of blood throughout this activity, and (3) the tissue distribution where there is no blood containing isotope. If it is successful, of course, this approach has a wide application to many other techniques, where the problem is one of defining specific uptake in a tumor or an organ against a changing background of blood or tissue activity of the same radioactive tracer.

The subtraction of the changing blood background in conjunction with suppressed thyroid uptake and pre-scanning with I-125 to demonstrate persistent foci of thyroid amino acid accretion adds great potential to the clinical utility of parathyroid scintiscanning. In addition, during the forthcoming year, we hope to gain insight into experimental means of enhancing the parathyroid amino uptake for subsequent clinical application.

TABLE I

SERUM VALUES: FIVE DAY NO CALCIUM DIET

No calcium diet obtained from Nutritional Biochemistry. Control animals received same diet supplemented with Calcium Lactate (1.5%).

TIME AFTER SELENOMETHIONINE	25 Minutes		Two Hour		Four Hour	
	Control	Experimental	Control	Experimental	Control	Experimental
1. Per cent Se^{75} as protein	20.66 $\pm$ 1.38	27.59 $\pm$ 3.29	84.70 $\pm$ 1.32	85.56 $\pm$ 5.6	89.80 $\pm$.91	94.84 $\pm$ 1.65
2. mEq Ca	5.02 $\pm$.15	4.64 $\pm$.10	5.15 $\pm$.06	4.62 $\pm$.05	5.14 $\pm$.04	4.98 $\pm$.05
3. mEq mg	3.09 $\pm$.22	3.33 $\pm$.18	2.08 $\pm$.12	2.33 $\pm$.19	2.38 $\pm$.17	2.63 $\pm$.18
4. mg% Po_4	7.96 $\pm$.48	8.47 $\pm$.60	7.64 $\pm$.52	9.04 $\pm$ 1.00	10.21 $\pm$.66	10.16 $\pm$.71

TABLE II

SERUM VALUES: TEN DAY NO CALCIUM DIET

No calcium diet obtained from Nutritional Biochemistry. Control animals received same diet supplemented with Calcium Lactate (1.5%).

TIME AFTER SELENOMETHIONINE	25 Minutes		Two Hour		Four Hour	
	Control	Experimental	Control	Experimental	Control	Experimental
1. Per cent Se^{75} protein	21.96 $\pm$ 2.22	29.70 $\pm$ 1.74	94.55 $\pm$.96	93.08 $\pm$ 3.53	102.7 $\pm$ 5.66	96.42 $\pm$ 3.13
2. mEq <u>Ca</u>	4.90 $\pm$.07	4.83 $\pm$.06	4.82 $\pm$.04	4.97 $\pm$.05	5.06 $\pm$.07	5.03 $\pm$.07
3. mEq mg	2.56 $\pm$.16	2.52 $\pm$.18	2.48 $\pm$.13	2.65 $\pm$.17	2.57 $\pm$.35	2.54 $\pm$.41
4. mg% Po_4	6.56 $\pm$.36	7.63 $\pm$.53	10.40 $\pm$.22	10.21 $\pm$.29	10.21 $\pm$.12	7.96 $\pm$.36

TABLE III
SERUM VALUES - ^{75}Se 15 minutes before EDTA

Each figure represents the mean of at least six animals $\pm$ standard error. 33.5026 mg/ml EDTA given i.p. 15 minutes prior to i.v. 50uc ^{75}Se -selenomethionine.

TIME AFTER SELENOMETHIONINE	25 Minutes		Two Hour		Four Hour	
	Control	Experimental	Control	Experimental	Control	Experimental
1. Per cent Se^{75} as protein	14.08 $\pm$ 1.38	41.24 $\pm$ 1.39	97.79 $\pm$ 3.55	98.44 $\pm$ 4.12	91.02 $\pm$ 3.34	89.17 $\pm$ 5.59
2. mEq <u>Ca</u>	4.78 $\pm$.11	3.88 $\pm$.16	5.20 $\pm$.14	5.05 $\pm$.27	4.96 $\pm$.11	4.75 $\pm$.05
3. mEq <u>mg</u>	1.84 $\pm$.17	1.50 $\pm$.2	2.89 $\pm$.24	2.85 $\pm$.12	2.48 $\pm$.16	2.38 $\pm$.19
4. mg% Po_4	4.12 $\pm$.29	4.25 $\pm$.19	8.63 $\pm$.53	5.27 $\pm$.20	9.88 $\pm$.23	8.61 $\pm$.42

TABLE IV

SERUM VALUES - ^{75}Se 15 Minutes After EDTA

Each figure represents the mean of at least six animals $\pm$ standard error. 33.5026 mg/ml EDTA was given i.p. prior to 50uc i.v. injection of ^{75}Se -selenomethionine.

TIME AFTER SELENOMETHIONINE	5 Minutes		25 Minutes		2 Hour	
	Control	Experimental	Control	Experimental	Control	Experimental
Per cent ^{75}Se as protein	19.36 $\pm$ 1.17	18.34 $\pm$ 1.17	25.06 $\pm$ 2.73	52.4 $\pm$ 9.48	85.38 $\pm$ 3.35	96.53 $\pm$ 5.53
mEq Ca	5.30 $\pm$.15	3.62 $\pm$.25	5.08 $\pm$.17	3.59 $\pm$.36	4.34 $\pm$.08	4.54 $\pm$.42

No mg or Po_4 done at this time.

TABLE V

SERUM VALUES - ^{75}Se - EDTA Simultaneous

Each figure represents the mean of at least six animals $\pm$ standard error. 33.5026 mg/ml EDTA was given i.p. 15 minutes prior to 50uc i.v. injection of ^{75}Se -selenomethionine.

TIME AFTER SELENOMETHIONINE	25 Minutes		Two Hour		Four Hour	
	Control	Experimental	Control	Experimental	Control	Experimental
1. Per cent ^{75}Se as protein	41.9 $\pm$ 1.97	42.41 $\pm$ 4.70	99.21 $\pm$ 3.62	100.21 $\pm$ 2.24	91.10 $\pm$ 1.39	98.46 $\pm$ 2.68
2. mEq <u>Ca</u>	5.05 $\pm$.16	4.03 $\pm$.16	5.72 $\pm$.19	5.67 $\pm$.28	4.87 $\pm$.15	4.95 $\pm$.13
3. mEq <u>mg</u>	1.97 $\pm$.22	1.55 $\pm$.08	2.19 $\pm$.18	2.43 $\pm$.32	2.01 $\pm$.03	1.93 $\pm$.13
4. mg% Po_4	6.14 $\pm$.47	6.34 $\pm$.62	3.12 $\pm$.73	8.19 $\pm$.20	7.31 $\pm$.61	8.04 $\pm$.31

FIGURE I

^{75}Se -activity as % ID / $\mu\text{gN} \times 10^6$ or / ml blood $\times 10$

50 μc I.V. selenomethionine after 5 days on a no calcium diet.

Each point represents mean of 6 animals $\pm$ s.e.

— = control
 - - - = experimental

FIGURE II

^{75}Se -activity as % ID / $\mu\text{gN} \times 10^6$ or / ml blood $\times 10$

50 μc I.V. selenomethionine after
10 days on a no calcium diet. Each
point represents mean of 6 animals
 $\pm$ s.e.

FIGURE III

^{75}Se -activity as (% ID / $\mu\text{gN} \times 10^6$ or ml blood $\times 10$)

FIGURE IV

^{75}Se -activity as (% ID / $\mu\text{gN} \times 10^6$ or ml blood $\times 10$)

50 μc I.V. selenomethionine
15 min.

after i.p. EDTA (33.5 mg)

— = control

- - - = experimental

(Each point represents mean of
6 animals $\pm$ s.e.)

FIGURE V

^{75}Se -activity as (% ID) / $\mu\text{gN} \times 10^6$ or ml blood $\times 10$

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