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"A STUDY OF CELLULAR BIOCHEMISTRY IN SURGICAL PATIENTS"

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ABSTRACT

1. The technology and interpretation of the measurement of the total exchangeable potassium (K_{te}^{39}) in human beings is described.

2. The K_{te}^{39} is a function of the "lean body mass" or total mass of oxidizing cellular protein.

3. Depleted hospital patients show a reduction in K_{te}^{39} of about 18 percent below the average normal.

4. Erythrocyte potassium has been measured in 67 patients and found to be 7.5 percent below the normal value in depleted patients; a finding which may be applicable to clinical problems.

5. Tissue potassium exchange phenomena continue under investigation in vivo; the conduct of the red cell with respect to potassium exchange in vitro is also being studied at present. Detailed description of this work will be found in the technical report now in preparation and is not described in this status report.

6. An initial exploration of the use of glucose-amino-acid-potassium-insulin combinations intravenously in depleted patients has been started.

The purpose of this infusion is to provide both a stimulus and the raw materials for formation of protoplasm in body tissues.

7. Total Body Water determinations continue as a principal effort of this Task Order and in eight instances gave an average figure for water content in the human being of 70.9 percent of body weight.

8. The technology of deuterium measurement employed in the total body water method, is reviewed.

REPORT PROPER

"A STUDY OF CELLULAR BIOCHEMISTRY IN SURGICAL PATIENTS"

Status Report

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A. POTASSIUM METABOLISM

Introductory note - In the previous progress report dated 30 April 1947 all aspects of the potassium work under way in this Task Order were mentioned. The reader is referred to that report for the early work in urinary excretion of radioactive potassium and for background information relative to other aspects of the research. In this section of the present status report attention is directed chiefly to the measurement of the total exchangeable potassium, and to the intravenous use of potassium-glucose-amino acid-insulin combinations.

In the second section of this report the deuterium work now in progress is described.

In a technical report now in preparation, all aspects of the work of this Task Order are covered in detail.

I. Material

1. Eighty-two patients have received injections of K^{42} (radioactive potassium), with subsequent study of urinary excretion.
2. Thirty-two of these patients have been the subject of study of the penetration of K^{42} into body cells by the direct analysis of tissue and blood cells.
3. Twenty-seven patients have been on complete metabolic balance study to provide a background of information by means of which the isotope studies may be interpreted.

II. Results and Interpretation

1. Using specific activity data (K^{42}/K^{39} ratios) from urine voided 24 to 36 hours after the injection of radioactive potassium (K^{42}), the total exchangeable potassium (K_{te}^{39}) has been measured in 44 patients, both acutely ill and systemically well.

2. These data indicate that the mean K_{te}^{39} for systemically well individuals is 36.0 ± 10.75 milliequivalents per kilogram. In depleted patients this figure is reduced approximately 18 percent to 30.9 ± 7.5 milliequivalents per kilogram. As will be noted from the standard deviations which are large, there is considerable overlap of these curves. Comparing the two means by Snedecor's formula*, the statistical significance (t) for the difference between these means has a value of 8.00; the difference between these means is at least eight times the standard error of the difference. The spread is therefore about three times that required for statistical significance.

3. Modes of expressing this information are still in a formative stage. The K_{te}^{39} is probably proportional to and therefore a measure of the "lean body mass", or mass of oxidizing cellular protein tissues, represented largely by skeletal muscle, liver, kidneys, heart, lungs and other parenchymatous organs. This mass of tissue constitutes the metabolically signifi-

cant fraction of body weight and measurement of its mass has never been available by direct means. The "lean body mass" may be predicted to be highly variable relative to body weight because of intrinsic variability and fat content, and the weight of skeleton and connective tissue. Other modes of expression for K_{te}^{39} such as "milliequivalents per square meter of body surface", "milliequivalents per cubic centimeter of plasma volume",

$$t = \frac{a_1 - a_2}{\sqrt{\left(\frac{\sigma_1}{n_1}\right)^2 + \left(\frac{\sigma_2}{n_2}\right)^2}}$$

"milliequivalents per cubic centimeter of extracellular volume", and milliequivalents per cubic centimeter of total body water" are being explored in hopes of finding a basic reference point against which the $K_{t_e}^{39}$ may be expressed.

4. The lack of large differences in the values of $K_{t_e}^{39}$ in sick as versus well patients, is traceable to this difficulty in finding a constant reference point. This will be made clear by considering, for example, a patient who has lost weight largely at the expense of body fat. This patient, though ill and depleted, may be found to have a $K_{t_e}^{39}$ increased in relation to his total body weight. On the other hand in a patient whose metabolic deficit has occurred at the expense of body cellular protein stores, the $K_{t_e}^{39}$ may be expected to be low relative to body weight. Further exploration of these phenomena is in progress.

5. The measurement of $K_{t_e}^{39}$ in rabbits followed by an analysis of the entire carcass for potassium is under way at the present. This will yield a check on the accuracy of $K_{t_e}^{39}$ measurement and the relation of this entity ($K_{t_e}^{39}$) to the actually analyzed total body potassium. Many analytic problems present themselves in the chemical analysis of an entire ashed carcass.

~~Progress in accuracy and reproducibility is being attained at present.~~

6. The erythrocyte as an indicator of cellular K stores continues to occupy attention. Erythrocyte potassium determinations have been carried out in triplicate and quadruplicate in 67 carefully studied individuals. These individuals comprise three groups: depleted hospital patients, well hospital patients and normal ambulatory professional staff. These data reveal the following information:

Table I

Erythrocyte Potassium
mg./100 cc. packed cells

Group	No. of Patients	No. of Determinations	Average	Standard Deviation
Normal Ambulatory	24	24	408.5	±23.2
Hospital Well	15	33	395.0	±23.0
Hospital Depleted	28	121	378.0	±30.0

$$t = \frac{408.5 - 378.0}{\sqrt{\left(\frac{23.2}{24}\right)^2 + \left(\frac{30.0}{121}\right)^2}} = 31$$

7. Statistical significance(t) gives a value of 31 in comparing the averages for normal ambulatory individuals and depleted hospital patients. This indicates that the difference in these means is approximately 30 times the standard error of the difference and is strongly significant. This fact gives us for the first time a simple clinical blood determination which may indicate the extent of cellular depletion.

8. It is difficult to assign the precise biochemical mechanism which is operative in alteration of erythrocyte potassium content. We find little correlation between erythrocyte potassium content and plasma base. The only significant correlation we have observed is that patients who have a low erythrocyte potassium also have a relatively low erythrocyte potassium content per unit of erythrocyte hemoglobin suggesting that in the presence of depletion, more K leaves the cell than does hemoglobin.

9. Potassium exchange curves in tissue have been further documented in surgical patients. With the exception of the red cell all tissues studied are in physical equilibrium with reference to the exchange of K^{42} for K^{39} approximately 24 hours after a single injected dose of K^{42} . The urine K^{42}/K^{39} ratios reflect specific activity conditions in all tissues studied

at equilibrium; the K presented to the kidney for excretion shows the same isotopic constitution as that observed within cells. The urine K^{42}/K^{39} ratio can therefore be used as an indicator for the calculation of K_{te}^{39} .

10. The erythrocyte has peculiar properties with reference to K^{42} exchange, reaching equilibrium only at 72 hours after injection; in some cases equilibrium has not been attained even at that time. This raises the question of the presence of a "non-exchangeable" fraction of K in the red cell or a "slowly exchangeable" fraction of K. Attention has therefore been shifted to the in vitro behavior of the red cell membrane with reference to K^{42} exchange, to determine if this phenomenon can be produced outside the living organism and therefore independent of hematopoietic processes. We have found in one experiment (which needs further corroboration) that exchange is essentially complete at 40 hours. The biological details of this experiment bear further development; the avoidance of hemolysis in red cell-plasma systems maintained at +37 degrees Centigrade for 96 hours with constant agitation, is difficult. Any hemolysis occurring alters permeability rates and casts some doubt on the reliability of the result. Technical perfection of this mode of study is under way at present.

11. ~~Clinical studies continue as the basis for interpretation of this~~ program of investigation of potassium metabolism. Patients receiving K^{42} for the calculation of K_{te}^{39} are on complete balance study whenever feasible. The study of post-operative changes remains a feature of the research in many cases. The negative nitrogen balance and negative potassium balance accompanied by positive sodium balance which have previously been described as occurring in post-operative patients have been observed in virtually all our cases. The resumption of positive K balance antedates the resumption of positive N balance in many instances.

12. Intravenous therapy directed toward nutrition of the cell is being cautiously attempted, based on our findings concerning potassium exchange.

Our assumption has been that the formation of intracellular material (protoplasm) requires carbohydrate (glycogen), amino acids (for protein synthesis), electrolyte, and vitamin-enzyme materials plus a "stimulus". The latter has been provided by the intravenous administration of insulin, basing this on the proposition that the formation of glycogen will require the laying down of protein matrix and, possibly, the entire pattern of intracellular material.

In vivo glucose-insulin titration curves have been studied to determine the amount of insulin which must be administered in order to produce maximum

utilization of intravenously administered glucose. We have found that glucose infusions at the rate of 0.75 grams per kilogram per hour are most efficiently utilized with the concomitant administration of 0.5 units of regular insulin

per gram of glucose. This provides a minimal blood sugar elevation and virtually no urinary loss. It is of interest that in fresh post-operative

states this combination does not produce maximal utilization of carbohydrate to the extent observed in stabilized patients. This infusion has been con-

tinued for two hours at the standard rate. This provides an insulin dose in the neighborhood of 30 to 60 units of regular insulin. To this solution is

added KCl at a final concentration of 20 milliequivalents per liter and amino acid at 0.5 grams per gram of glucose or 0.375 grams per kilogram per hour.

Suitable administration of the B vitamins is maintained. This entire mixture is well tolerated without local or systemic reaction. To avoid a transient

period of hypoglycemia following the intravenous administration of glucose-insulin combination a "chaser" of 5 percent dextrose in water is administered

for a half hour. The balance effects of this nutrition are being analyzed at the present time.

B. STUDY OF DEUTERIUM EXCHANGE

1. The measurement of total body water ($H_{t_0}^1$) remains a major effort of this Task Order. This is done by measuring the dilution in the patient of measured infusion of heavy water (D_2O).

2. Eight measurements of total body water in seven human patients have been completed to date yielding an average total body water of 70.9 percent of body weight.

3. Technical problems of great interest attached to the analytic parameters of the method. Measurement of 0.1 atom percent deuterium with 3 percent accuracy is our objective for this work.

4. For the past two years our attention has been focused on refinements of the falling drop method of deuterium measurement with a resultant 10-fold increase in accuracy.

5. Temperature control of the water bath thermostat to .0005 degrees Centigrade is desirable and has been attained for short periods of time (1-3 hours); daily fluctuations up to .008 degrees Centigrade are observed during operation of our equipment, due to alterations in line temperature of the cooling water.

6. Measurement of the time of drop fall may be carried out accurately to .02 seconds with the electric clocks employed. Using a temperature for the orthofluorotoluene tube of 27.06 degrees Centigrade and a drop size which has a falling time (for distilled water) of 90 seconds, this timing apparatus gives us a thousand measurable intervals for the increment of concentration between distilled water and 0.1 percent D. Stated otherwise our time accuracy in terms of deuterium concentration is equivalent to .0001 atoms percent D. This is approximately three times the desired accuracy and is not a limiting factor.

7. Drop size reproducibility must be held to close tolerances. This depends on the actual size of the drop as released from the micro pipette, and the "reproducible geometry" of the entire system. At the present time averages of three drops which total in the neighborhood of 90 seconds may be held to .2 seconds. This gives one hundred measurable intervals between distilled water and 0.1 percent D and an accuracy of .001 percent D, a loss of a factor of 10 over the accuracy obtained by the time measurement alone.

8. Water purification remains an outstanding problem in accuracy and reproducibility. Employing urine as the starting material, our distilling apparatus did not obtain constant density results using vacuum distillation. An element of light density either escaped removal or was introduced in the course of multiple distillations by this technique first from acid and then from base. Care is taken not to introduce hydrogen by acidifying with P_2O_5 and alkalizing with Na_2O_2 .

9. Introduction of a combustion train into the preparation of these water samples for density measurement has evidently removed this "lightness factor" yet water purification still involves an error which in terms of dropping time is equivalent to approximately 2 seconds or .01 percent D (about 10 parts per million of density). It will be noted that .01 percent

D constitutes a 10 percent error when dealing with total concentrations of 0.1 percent D. In practical terms this means that if approximately 50 cubic centimeters of 100 percent D_2O (obtained from the Atomic Energy Commission) is injected into a patient of normal body weight, and the final concentration obtained is approximately 0.1 percent D, then the total body water (total exchangeable hydrogen) calculates out to 50,000 cubic centimeters of water. Ten percent error therefore assumes the magnitude of 5 liters. The D_2O space

as measured in this patient therefore may fall anywhere between 45 and 55 liters. Since significant changes in total body water, clinically, may involve only 2 to 4 liters it is clear that this method does not yet yield as accurate a result as appears desirable. Our accuracy must be increased by a factor of 10; it would also be possible to increase the total dose of D_2O by a factor of 5, an increase which is almost prohibitively expensive.

10. At the present time we are of the opinion that the desired accuracy can be obtained with the oxidation train and falling drop apparatus as now operated in this laboratory, after further refinements have been introduced. It is worthwhile to examine critically other methods of measuring deuterium.

11. The mass spectrometer may be used to measure deuterium concentration in four ways: 1) by the measurement of the mass of H_2O vapor, 2) by the measurement of the mass of electrolytically produced H_2 , 3) by the measurement of the mass of H_2 produced by catalytic reduction of H_2O and 4) by the measurement of the mass of H_2 physically equilibrated with H_2O-D_2O mixtures. Experience with all four of these applications of the mass spectrometer has been obtained in various laboratories at present working in this country.

~~The responsible investigators of Task Order XIII have maintained contact with~~
other workers in this field. One laboratory which has had experience in deuterium measurement for approximately 10 years uses H_2 produced by the reduction of H_2O over zinc in a micro combustion train and measured in a 180 degree mass spectrometer. Accuracy by this method theoretically could be obtained to .002 percent D. However, data from this instrument display variations of .02 to .03 percent D not infrequently. This variation is as large or larger than that now obtained with the falling drop apparatus.

12. It is important to bear in mind that the analytic requirements for various workers in this field will vary with the biological problem under investigation. In our work large quantities of distilled water can be produced from urine or plasma, for the end point determination. Quantity is therefore not a limiting factor. Many workers (such as the group mentioned in paragraph 11 above) who are studying the synthetic incorporation of deuterium into organic molecules employ the products of combustion of small quantities of organic solids. In their work small quantities of H_2 are produced and the use of the mass spectrometer is virtually essential because of the small quantities involved. It should also be noted that in much synthetic work accuracy of 3 percent at the level of 0.1 percent D is not required, whereas this accuracy is a fundamental requirement in our measurement of total body water.

13. Deuterium analyses on the mass spectrometer by the direct analysis of the density of H_2O vapor involves many pitfalls which have been investigated and have not been surmounted at the present time.

14. It is possible to equilibrate gaseous hydrogen with H_2O-D_2O mixtures and attain a DH ratio in the supernatant gas which is smaller than the DH ratio in the underlying liquid by a factor of 3.7. This "equilibrated hydrogen" as opposed to electrolytically produced hydrogen or reduced hydrogen may then be run into the mass spectrometer for analysis. Experience has not been gained sufficient to make any statement regarding the accuracy of this method.

15. The gradient tube as developed by Linderstrom-Lang and others may be used for deuterium concentration measurement. We have had experience with this method in our laboratory and at concentrations of 0.1 percent D three percent accuracy is virtually unobtainable. Microflotation apparatus has been designed for determination of small concentrations of deuterium. This method

may yield a high order of accuracy but is very time-consuming for the measurement of each sample. We have not had experience with this in our laboratory.

16. In conclusion, the technology of deuterium measurement at low concentration with high orders of accuracy is largely limited by purification methods. The use of the mass spectrometer holds great promise for sample measurements but, pending the elaboration of technical details in other laboratories, this Task Order will continue to direct its efforts toward perfection of the falling drop method, an effort in which large strides have been made during the last six months of 1947.

TECHNICAL REPORT STATUS FOR PERIOD

1. Intracellular Biochemistry in Surgical Patients by Francis D. Moore - seminar delivered at Department of Biochemistry , Harvard Medical School, 20 October 1947. Not published.
2. The Use of Isotopes in Surgical Research by Francis D. Moore - Surg. Gyn. & Obs., 86, 129, 1948.
3. A Study of Cellular Biochemistry in Surgical Patients - Technical Report - In preparation.