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CHROMIUM (III) IN THE HUMAN BODY

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Tek Hian Lim
(Ph. D. thesis)

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THE KINETICS OF THE TRACE-ELEMENT
CHROMIUM (III) IN THE HUMAN BODY

by

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THE KINETICS OF THE TRACE-ELEMENT CHROMIUM(III)

IN THE HUMAN BODY

Doctor of
Philosophy

Tek Hian Lim

Biophysics

ABSTRACT

This work involved kinetic studies of the trace-element chromium(III) in humans. The proposed model was derived from analysis of digital data acquired by a scintigraphic data analyzer from a whole body gamma-ray scintillation scanner, and from whole-body counts and plasma clearance data. These analyses were performed in vivo after intravenous administration of $^{51}CrCl_3$ to normal subjects and to patients suffering from hemochromatosis.

Rate-constants were calculated by fitting directly experimental data to the proposed kinetic model. Results showed that a linear relation exists between chromium(III) absorbing organs and the blood plasma chromium(III) compartment.

Rapid and slow uptake as well as clearance of chromium (III) were detected, primarily in the liver, spleen, adipose and muscle tissues and some in bone. Approximately 30% of the injected whole body radiochromium(III) was cleared within one day, and approximately 80% of the plasma radiochromium(III) was cleared within the same period.

It was found that blood plasma radiochromium(III) clearance

was faster in patients suffering from hemochromatosis compared to normal subjects; furthermore, a higher percentage of unbound radiochromium(III) in the plasma was found in hemo-chromatosis. A metabolic relationship between the two phenomena is suspected.

A whole-body clearance component with a long half-life of approximately 10 months suggests that chromium(III) is stored in some organ of the body, probably primarily in the liver. The whole-body scans showed the greatest concentration of radiochromium(III) to be in the liver.

At the present time, as far as we know, the chromium (III) kinetic model, presented in this work, is the first model of chromium(III) metabolism of any form to be proposed, and it is based on in vivo metabolism of a metabolically unperturbed human system.

Some researchers have reported that chromium(III) is important for glucose metabolism in humans. In this context, hopefully in the future, the model will be useful in assessing nutritional requirements of chromium(III) in the human diet, and its importance in disease processes such as diabetes.

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Finally, I would like to thank my wife, Laurette, and my children Patricia, Dillana and Roger for their understanding, patience and support.

This work is dedicated to my late parents, Mr. and Mrs. D. K. Lim.

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I. Introduction

I. 1. Background and goal of present research

Chromium deficiency was not recognized until 1959 when chromium(III) was identified as the active component of a dietary ingredient required for optimal glucose utilization in rats[1].

The earliest detectable and most prominent feature of chromium(III) deficiency in rats and other experimental animals is an impairment of glucose tolerance. A more severe degree of chromium(III) deficiency leads to a syndrome indistinguishable from mild diabetes mellitus, including glycosuria and fasting hyperglycemia[2].

It has been shown[3,4] that chromium(III) by itself does not influence glucose uptake in adipose and muscle tissue but by its presence, it makes insulin more effective in facilitating the transport of glucose across the cell membrane. Chromium(III) acts only when insulin is also present.

Recently, there has been increasing interest in the implication of these findings to human nutrition, and several reports suggest[5,6] that chromium(III) is a nutritional requirement for man.

Results of a number of therapeutic trials of dietary supplementation with physiological quantities of chromium(III) to patients with impaired glucose tolerance including mature onset and chemical diabetics, exhibited improvement in an average of four out of ten patients[7].

At the present time, fundamental knowledge concerning the "biological function" of chromium(III) and its critical nutritional level is still in an early stage. However, with the use of tracer techniques a considerable amount of knowledge of the fate and distribution of chromium(III) can be gathered. It is the purpose of this study to apply tracer methods in human subjects and to establish an acceptable chromium compartment model, the solution of which might lead to important information concerning biological function of chromium(III).

The experiments are divided in 5 categories as follow:

- Blood serum chromium level determination (in vitro)
- Blood serum radiochromium(III) clearance (in vivo)
- Radiochromium(III) localization in the body (in vivo)
- Whole-body radiochromium(III) retention (in vivo)
- Modeling and computer analysis

I. 2. <u>Present status of biological function of</u>

<u>chromium(III) in humans and animals</u>

Information contributing to our knowledge of possible biological functions of the trace element chromium(III) falls in five categories which, individually or together, may help define the nutritional and physiological significance of the given element[8].

A. Chromium in soil, water, plants, animal and man[3,5,8,9].

a. Soil chromium levels depend on geographical locations; it is mostly in the form of Cr_2O_3.

b. Water chromium levels are less than 1 ppb; sea water has less chromium than river water; it also depends on geographical locations; and it occurs mostly in chromium(III) form.

c. Plant chromium levels depend on geographical soil chromium concentration, the amount of fertilizer etc. The normal levels vary from 10 to 1000 μg/kg plant on a dry basis, e. g., 390 ppb in potatoes and 590 ppb in hay.

d. Chromium levels in animals, including mammals, range from 20 to several hundred ppb in most mammalian tissue, more concentrated in some tissues such as brain and central nervous system. In some tissues of some sea animals

(clam, crab) it can be up to 100 ppm. Chromium in the human body decreases with age, except in the lungs. Also, body chromium level depends very much on geographical location and diet.*

B. Reaction of chromium with biological materials in vitro

    a. In chromium tanning of skin[5,10], the interaction of chromium(III) with protein probably involves complex formation between polynuclear chromium(III) species and the carboxyl groups of the protein.

    b. Selective affinity of chromium(III) to transferrin in blood plasma and of chromium(VI) to red blood cells was found in vitro as well as in vivo[11].

    c. In a succinate cytochrome dehydrogenase system, addition of ppm of chromium(III), stimulated the enzyme activity twice as much as did an equal amount of aluminum[4,12].

    d. Phosphoglucomutase, which requires magnesium and a second metal, responded to chromium(III) as a second metal with higher activity than Al, Pb, Fe etc.; in the absence of magnesium

*Present findings concerning chromium(III) levels in human blood plasma ranges from 2 to 20 ppb (p.21), and levels in other organs probably also have to be revised downward.

only chromium(III) increased enzyme activity[5,13].

e. Rat liver responded to chromium(III) _in_ _vitro_ and _in_ _vivo_ with an increase of fatty acid and synthesis of cholesterol from acetate[5,14].

f. Chromium(III) has been reported as a constituent of proteolytic enzymes, essential for their function; the activity of trypsin, inactivated by dialysis, was reconstituted by addition of chromium(III)[5,15].

g. Stimulation of growth and biotin synthesis by chromium(III) in Aerobacter Acrogenes (bacteria was observed[5,16].

h. Nanogram quantities of chromium(III) are required for the optimal effect of insulin in every insulin-dependent system that has been investigated[4,5,8], and an uncorrected deficiency of chromium necessitates the addition of unphysiologically large concentrations of insulin to the media to achieve a normal response. In the presence of insulin, addition of chromium(III) to rat epididymal tissue of chromium deficient animal results in increase of[4,17]:

1) the rate of glucose uptake

2) the oxidation of glucose to CO_2

3) the incorporation of glucose carbon into fat. The rate of incorporation of acetate into fat which is not insulin-dependent, is unchanged.

i. The intracellular sites of action of insulin and probably also chromium were reported as follows:

1) ribosome, where chromium(III) probably facilitates the insulin-stimulated incorporation of amino acids into protein[18].

2) mitochondria, where insulin stimulated swelling of mitochondria from chromium-deficient tissues is enhanced by the addition of chromium(III)[19]. Polarographic studies have indicated that chromium(III) initiates the formation of disulfide linkages between the intra-chain disulfide of insulin and sulfhydryl groups of the mitochondrial membrane by participating in a ternary complex. This is believed to be the first step by which insulin increases the flux of glucose through the membrane[20].

j. At the present time it is not possible to postulate a special role of chromium related to the function of DNA and RNA, since chromium has not been studied yet in the field

of DNA and RNA metabolism. However some re-
searchers[5,21,22] reported the following:

1) extremely high concentration of chromium

(III) was found in nucleoprotein (up to

10 times higher than in either nucleic

acid or protein).

2) outstanding in chromium-nucleic acid bond

stability was also reported.

k. Stimulation of fermentation of yeast by chro-

mates [chromium(VI)] in quite high concentra-

tion (125-200 ppm) had been reported 80 years

ago[5].

C. <u>Chromium deficiency in experimental animals</u>

A low-chromium state can be described if an im-

pairment of some function can be prevented or

cured by physiological amounts of this element.

The present known methods to measure chromium

in different tissue can determine only the total

chromium concentration; these analysis do not

always give biologically meaningful results since

these levels do not provide a valid index yet of

chromium nutritional status. Chromium(III) re-

acts differently compared to chromium(VI), <u>in</u>

<u>vivo</u> as well as <u>in</u> <u>vitro</u>. Simple chromium analy-

sis does not tell what its chemical form is.

For these reasons interpretation of the measured

chromium levels in different organs is still not

straightforward.

a. Glucose tolerance

Many simple chromium compounds and synthetic
organic complexes, with the exception of highly
stable complexes, exhibit biological activity
of similar magnitude. However, the biologi-
cal effect achieved with any of these is not
optimal and specifically it is not of the
same magnitude as that achieved with "glu-
cose tolerance factor" (GTF) chromium[1]. GTF
is an organic low molecular weight, dialyz-
alble, naturally occurring chromium(III)-
containing compound. The existence of such
a factor had been suspected on the basis of
observations that brewers yeast and some of
its chemical fractions prevented the impair-
ment of intravenous glucose tolerance develop-
ing in rats on Torula yeast diet, which was
found to be deficient in chromium. Trivalent
chromium as chromium acetate, added at 10 ppm
to the drinking water, improved the impaired
glucose tolerance in five out of five monkeys
23,24.

b. Chromium-insulin

The influence of chromium(III) on glucose
tolerance is closely tied to the action of
insulin[5]. When rats raised on a low chromi-
um diet were subcutaneously injected with

10 mU of insulin/100g of body weight after
18 hours of fasting, their hypoglycemic res-
ponse was significantly less than that of
chromium-supplemented controls. Similarly,
incorporation of glucose carbon into tissue
glycogen 1 hour after injection of 1 mU of
insulin/100g of body weight was much greater
in chromium(III)-supplemented rats than in
their deficient controls. Chromium(III) sup-
plemented rats also maintained a higher gly-
cogen concentration in their tissue after
18 hours of fasting than did low-chromium
controls.

c. Syndrome simulating diabetes melitus

Fasting hyperglycemia and glycosuria did
exist in rats raised under maximal exclusion
of chromium contamination. Thus a diabetes-
like syndrome has been observed in chromium-
deficient rats[2].

d. Protein metabolism

When rats are raised on a low-chromium diet
and are kept in plastic cages, chromium(III)
supplementation in the drinking water (2ppm)
results in a mild stimulation of growth, as
compared with unsupplemented controls. The
increased body weight is not due to water
retention or fat deposit, but reflects a
proportional increase

of tissue protein[5,27].

D. Site of action of chromium(III)

Since glucose tolerance is a complex function
regulated by many diverse factors, the in vivo
experiments did not offer specific information as
to the site or mode of action of chromium(III)
in the organism. Subsequently, it was attempted
to define the site(s) by the use of various in
vitro experiments with chromium-deficient, epi-
didymal adipose, muscle and other tissues or sub-
cellular fractions.

a. Epididymal adipose tissue

The fact that glucose uptake and the utili-
zation of glucose for two different pathways,
incorporation of glucose carbon into lipids
and into CO_2, are affected by chromium(III)
in approximately the same magnitude, and the
ineffectiveness of the element in the absence
of insulin, suggest that the site of action
of chromium(III) is near the site of action
of insulin, close to the first step of glu-
cose metabolism[4,17].

The observed effect of chromium on cell
entry of glucose, established the fact that
the insulin-responsive cell membrane is one
site of action of chromium(III)[17]. Even
though an action at this site can account

for many effects of chromium, and insulin as well, on glucose metabolism, it has become evident that both chromium(III) and insulin must have additional sites of action, probably intracellular.

The mode of interaction between these two agents is not clear, but interaction of GTF with insulin has been suggested by Evans, et al[28]; he believes that the α and ϵ amino groups of insulin are involved in the binding of GTF to insulin. Schwartz, on the other hand, considers this to be merely speculative.*

At least five biochemical possibilities of chromium(III) interaction with insulin have been suggested by Mertz[5,29,30].

1) Chromium(III) may form a complex with insulin in the pancreas or in the blood. The element could serve to maintain the polypeptide chains in an optimal tertiary configuration.

2) A possible role of chromium(III) as an inhibitor of tissue insulinase.

3) Chromium(III) could serve to increase the initial binding of insulin to tissue.

4) Chromium(III) could act as cofactor of a

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*Personal communication.

membrane-carrier structure involved in glucose transport.

5) Chromium(III) could act as a catalyst in the initial reaction between insulin and specific membrane receptor sites.

b. Mitochondria

When it was shown that water uptake by isolated liver mitochondria was significantly increased by the addition of insulin together with certain sulfhydryl compounds, a system of subcellular, insulin-sensitive membranes became available as another model to study the action of chromium. Insulin-stimulated swelling of mitochondria from chromium-deficient tissues is enhanced by the addition of chromium(III)[19].

c. Muscle

The effect of chromium(III) on glucose metabolism in muscle tissue has not been as extensively studied as in fat tissue. The main physiological action of insulin in muscle tissue may be the regulation of an intracellular process, protein synthesis, in which chromium(III) has been shown to play a role. Ribosomes are the suspected site of action[5].

d. Crystalline Lens

The mechanism of action of insulin on the
lens is not known, but it clearly adds an
example of insulin-chromium(III) interaction.
It was demonstrated that consistent insulin
effects on glucose metabolism can be obtained
with rat lens if the donor animals are suffi-
ciently supplemented with chromium(III)[31,32].

E. Role of chromium(III) in human nutrition[3,5,6,33,34,35,36,37,38,39]

The question whether low-chromium states exist
in man is of considerable theoretical and prac-
tical interest. There are a number of syndromes
related to impaired glucose metabolism with un-
known etiology. The most common one is the grad-
ual decline of glucose tolerance with increasing
age which has been observed in samples of the
United States population. Another example is
maturity-onset diabetes which is not caused by
lack of insulin, but has been attributed by dif-
ferent investigators to widely varying causes;
one of these causes is chromium deficiency. A
severe impairment of glucose metabolism in pro-
tein malnutrition in many areas is also well
known, and a high incidence of abnormal glucose-
tolerance tests in obesity and arteriosclerosis
has been described.

The following were some reported clinical results:

a. Significant improvement in glucose tolerance of four out of six maturity onset diabetics was observed following the daily administration of 180 to 1,000 μg chromium as $CrCl_3 \cdot 6H_2O$ for periods of 7 to 13 weeks under strictly controlled conditions. Shorter periods of chromium supplementation did not improve glucose utilization.

b. In another study, 4 of 12 diabetics* treated with 1 mg chromium daily for 6 months had improved glucose tolerance, but 16 treated for a shorter period of time with 150 μg chromium daily did not improve.

c. The majority of elderly subjects over the age of 70 years exhibit chemical evidence of impaired glucose tolerance. Ten such elderly subjects have been treated with 150 μg chromium daily for periods up to 4 months. No improvement was observed in six, but glucose tolerance was restored to within normal limit in the other four subjects.

d. Fifty percent of a group of middle-age subjects with impaired glucose tolerance who

*Not reported fully by the author[3] concerning the type of diabetes.

were treated with 150 µg chromium daily for 6 months, had a marked improvement in glucose tolerance.

e. Trials of chromium-supplementation have also been undertaken with infants suffering from protein-calorie malnutrition, which is frequently complicated by disorders of carbohydrate metabolism manifested by fasting hypoglycemia or impaired glucose tolerance, or both. Hopkins, et al[38] reported that malnourished infants in some areas benefit from chromium treatment, but they also clearly show that not all cases of malnutrition are complicated by low chromium states.

The results of studies in at least four different countries indicate that in some, but not all, geographical regions, protein caloric malnutrition is complicated by chromium deficiency. By analogy with experimental animals, the protein deficiency of these patients probably accentuated the effects of chromium deficiency.

Chromium has been shown to be essential for the maintenance of normal glucose metabolism in several species of experimental animals. Several reports mentioned that man does require chromium but it is still difficult to recommend a specific daily allow-

ance because of the extreme variations of availability among different sources of chromium. If all the dietary chromium were in the form of the biologically active GTF, an intake of 10-30 µg would probably meet the requirement. Chromium in the form of simple salts is much less effective, and probably several hundred micrograms would-be required per day.

II. Experimental methods and results

II. 1. Serum chromium determination

A. Method of analysis

The most widely used method for trace elements determination in biological material in most laboratories is the atomic absorption spectroscopy method. This method is based on measurements of the intensity of radiation absorbed by unexited atoms in the flame. With the use of special burners and by the use of organic solvents, the sensitivity of this method for measurement of chromium levels can be increased to 6 ng[5,40]. The main drawback of this method is the preparation of samples. Both wet- and dry-ashing appear acceptable. Since the wet method is more rapid and leaves the chromium in solution it is used almost exclusively. Even so, sample preparation is not as simple as in neutron activation analysis and contamination of chromium from the environment is not easy to control.

Colorimetric procedure has been used in some laboratories to determine low chromium levels in biological materials. This method is based on the formation of colored complexes with reagent. Using 1.5-diphenyl-carbohydrazide as reagent a limit of 3.5 ng of chromium can be

determined[5,40]. The main drawback of this method
at such low chromium level is the instability
of the color and interferences from other ions.

The use of x-ray fluorescence spectrometry,
for analysis, was found to be deficient in sev-
eral respects - especially the elemental range
for analysis extends down only to an atomic
number of about 25. Chromium, atomic weight
24, is just below this limit[41]. Also the
chances for contamination from the environment
during pre- and post-sample handling is large.

In this experiment neutron activation of
Cr-50 (4.31% abundance) was used to determine
the chromium level in human serum. The basic
principle of neutron activation analysis is
the production of radioactive isotopes of a
desired element by neutron interaction with
stable isotopes of the element, followed by
the measurement of the radiations emitted.
This method is chosen because of its high sen-
sitivity and accuracy in detecting trace amount
of chromium (down to several ppb with an error
of about ±15%) as well as its simplicity in
preparing and handling of samples and because
of the availability of the TRIGA Mark III
Berkeley Research Reactor at the University
of California, Berkeley campus.

B. Sample preparation and neutron irradiation

A sample of approximately 20cc of blood was drawn in a "chromium free"* centrifuge tube through a plastic needle. The sample was then centrifuged for 30 minutes at 3000 rpm. About 8cc of serum was transferred to another "chromium free" centrifuge tube by decanting the top layer of the centrifuged blood. The serum sample was then freeze-dried for approximately 24 hours. Precisely measured dried serum (in the range of 200 mg) was loaded, by direct pouring, into a high purity fused quartz tube and heat sealed.

The samples were then irradiated, together with samples of a known amount of chromium standard, in the TRIGA III reactor central thimble irradiation facility for 8 to 16 hours. The thermal neutron flux is approximately 3.5×10^{13} n/cm^2-sec. A uniform flux was obtained by rotating the sample container periodically.

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*In order to avoid trace-metal contamination of the serum specimen, all glassware including quartz tubes were cleaned with detergent, soaked in a solution of dilute HCl and HNO_3 and finally rinsed several times with distilled water. The distilled water with which the tubes were rinsed, was checked for its chromium content by neutron activation analysis. Within the limits of the ability of neutron activation analysis, no chromium contamination was detected.

Following irradiation the samples were cooled for about 14 days. This allowed all short half-life isotopes to decay. The quartz vial was then washed in boiling distilled water and frozen in liquid nitrogen before being broken open. This last procedure minimizes the escape of chromium to the environment during transfer of the irradiated serum to counting vials. Immediately after transfer the counting vials were sealed.

C. Gamma-ray analysis

The Cr-51, generated from neutron activation of Cr-50, which emits gamma rays of 0.321 MeV, was counted for up to 24 hour with a 35cc Ge(Li) detector (Canberra Inc.) and a 4096 channel pulse-height analyzer (Nuclear Data Inc.). The data collected were then analyzed at the LBL CDC-7600 computer using the program SAMPO. SAMPO is a computer program that analyzes the exact γ energy of any channel location, the area of the photopeak* and statistical and calibration uncertainties of these quantities.

D. Results

Using the above method, blood samples were

---

*The net counts of a specific γ-ray were obtained by integrating the area of the photopeak.

analyzed originating from several random sub-
jects. The serum chromium levels found ranged
from 2 ppb ± 20% to 15 ppb ± 8%. In two out
of two trials, serum chromium levels were found
to be higher after glucose intake relative to
fasting.

II. 2. Serum chromium as a function of blood glucose
and plasma insulin levels

Because serum chromium levels were found to
be related to body glucose metabolism after
glucose intake, the following experiments were
performed to see if there was a consistent re-
lation between serum chromium levels, blood
glucose and plasma insulin values during dif-
ferent stages of the body glucose metabolism.

Three normal subjects and two patients
with impaired glucose tolerance were given
glucose tolerance tests. In addition to blood
glucose, insulin and serum chromium levels
were measured in each sample (table 1, fig. 1).
Insulin was measured by radioimmunoassay and
blood glucose by the modified Hossman method.

It was found that in all three normal sub-
jects and the two patients, the fasting plasma
chromium levels ranged from 2 ppb to 6 ppb.
Peak levels of serum chromium were noted at
90 to 120 minutes after oral administration

BLOOD SERUM CHROMIUM LEVELS
(ng/g blood serum, wet weight)
AND ORAL GLUCOSE TOLERANCE TEST

Subject-Diagnosis Sex - Age	Fasting	30 min.	60 min.	90 min.	120 min.	180 min.
Normal Subject #1 Male - 40 y.	5.8+14%	7.9+9%	12.6+9%	14.9+8%	11.2+9%	8.9+17%
Normal Subject #2 Male - 28 y.	2.2+19%	6.3+10%	11.1+9%	12.0+9%	11.4+9%	6.2+14%
Normal Subject #3 Male - 48 y.	1.9+18%	10.3+13%	10.1+15%	10.6+9%	10.8+11%	6.9+19%
Normal Subject #4	6.1+8%					
Normal Subject #5	8.1+11%					
Acromegalic Diab. Male - 45 y	2.8+17%	4.9+14%	5.4+9%	9.3+14%	12.4+8%	5.2+10%
Mature Onset Diab. Male - 40 y.	4.5+21%	4.4+23%	7.9+11%	9.8+10%	12.6+8%	5.6+18%
Hemochromatotic #1	6.9+18%					
Hemochromatotic #2	4.3+23%					

Table 1

of 100 gm of glucose; the levels ranged from
9 ppb to 15 ppb (fig. 1). Glucose and insulin
peaks in normal subjects were reached almost
at the same time, i. e., at approximately
30 minutes after glucose intake, while in the
patients with impaired glucose tolerance they
occurred after 60 to 90 minutes. Analysis of
the data also showed that in both normals and
patients with impaired glucose tolerance a
delay occurred, the chromium reachings its
peak after glucose and insulin. The differ-
ences noted in the pattern of the serum chro-
mium response curves between normal and im-
paired patients was the difference in the rate
of increase of chromium response during the
first hour after glucose intake (faster in
normals), followed by a peak which was delayed
compared to normals (fig. 1).

II. 3. Serum radiochromium(III) clearance

Serum radiochromium(III) clearance experi-
ments were performed in three normal subjects
and three hemochromatotic patients (Appendix
B). Blood was periodically drawn from patients
starting seconds after 100 μCi of $^{51}CrCl_3 \cdot 6H_2O$
was injected up to a period of 60 days. Inter-
vals between drawing ranged from 3 minutes to
14 days.

BLOOD SERUM CHROMIUM AND INSULIN RESPONSE
AND
ORAL GLUCOSE TOLERANCE TEST
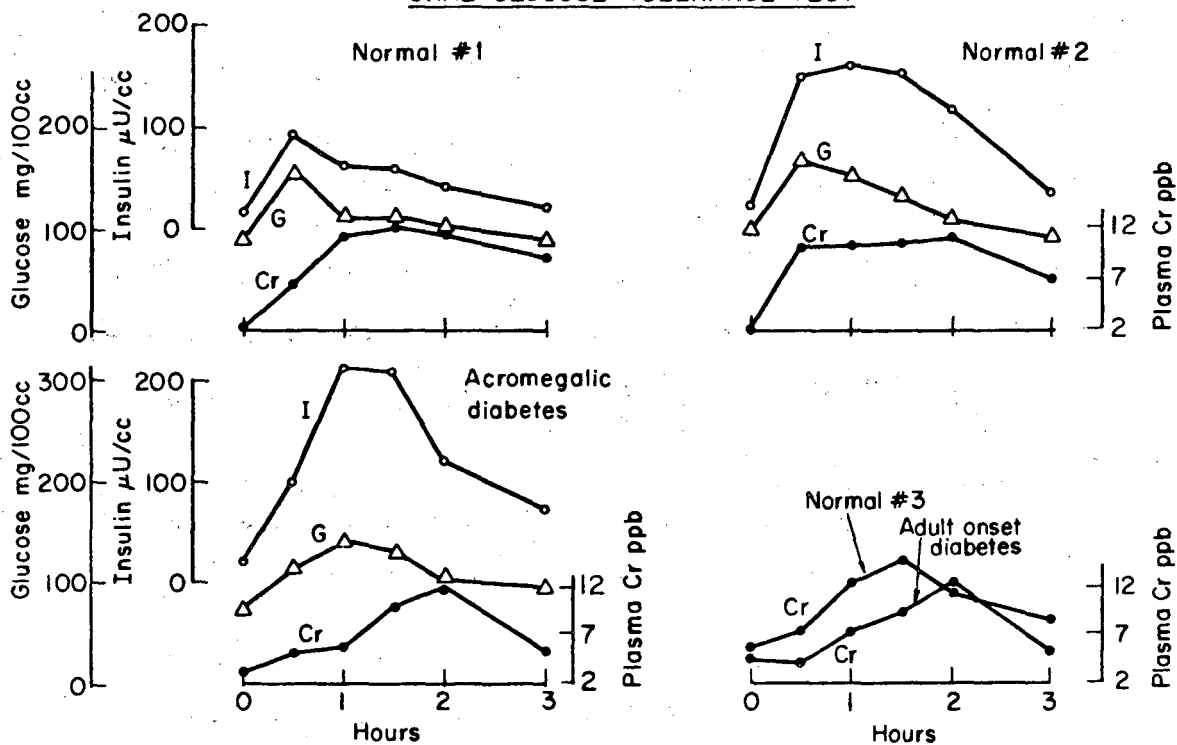
XBL7612-11440

Fig. 1

The blood was centrifuged for approximately
30 minutes at 3000 rpm. Following centrifuga-
tion the plasma was ultracentrifuged for approxi-
mately 24 hours at 114,000g and 17°C. Under
these conditions plasma protein sedimented to
the bottom, leaving a relatively protein-free
supernatant.

During the 60 days the experiment was per-
formed, it was found that most radioactive
chromium(III) is bound to serum protein with
m.w. larger than 90,000. Some unbound radio-
chromium(III) was also found. As is well known,
chromium(III) in blood is bound to transferrin
(m.w. 90,000)[11,42].

During the first minutes post injection
the average free radiochromium(III) portion
ranged from approximately 25% in some patients
with hemochromatosis to approximately 4% in
most normal subjects. After this transient
period the percentage levelled off to approxi-
mately 10% in hemochromatosis compared to
approximately 3% in normals. This number
remains constant for several days, beyond which
the unbound radiochromium(III) could no longer
be determined accurately due to its very low

level of activity (fig. 2,3). Surprisingly, from our in vitro experiment when plasma incubated with physiological amounts of $^{51}CrCl_3 \cdot 6H_2O$ was ultracentrifuged, the same percentages (approximately 25% in hemochromatosis and approximately 4% in normals) of free or unbound radiochromium(III) was found.

Least square fit to the plasma radiochromium(III) clearance data in 3 normals resulted in at least 5 detectable exponential clearance components with respective average half-lives of: 57 minutes*, 4.1 hours*, 18 hours*, 6.7 days*, and 11 months*** (table 2). The first component is believed to be related to the rapid accumulation of radiochromium(III) in the liver, adipose and muscle tissues, the spleen and some in bone.

The second component is probably associated with the clearance of dialyzable chromium(III) through the kidney into the urine.

The third and fourth components might be related to the slower accumulation or uptake by the liver, both adipose and muscle tissues, spleen and bone.

---

* Determined from experimental data
** Based on the final slope calculated from whole body excretion data.

PLASMA ^{51}Cr(III) CLEARANCE HALF-LIVES

SUBJECT # SEX-AGE	Diagnosis	$(T\text{-}1/2)_1^*$	$(T\text{-}1/2)_2^*$	$(T\text{-}1/2)_3^*$	$(T\text{-}1/2)_4^*$	$(T\text{-}1/2)_5^{**}$
N #1 M - 38y	Normal	50m(45%)	3.9h(19%)	17h(19%)	6.2d(17%)	330d(<1%)
N #2 M - 27y	Normal	60m(32%)	3.6h(19%)	16h(26.5%)	7.1d(22%)	230d(<1%)
N #3 M - 40y	Normal	60m(25.5%)	4.8h(26%)	20h(27.5%)	6.8d(21%)	444d(<1%)
H #1 F - 31y	Hemochr.	20m(47%)	1.5h(30.0%)	11.4h(15%)	4.6d(8.0%)	206d(<1%)
H #2 F - 54y	Hemochr.	30m(45%)	2.0h(30.0%)	10h(17.5%)	6.6d(7.5%)	135d(<1%)
H #3 M - 38y	Hemochr. (early stages)	80m(34.0%)	4.8h(23.0%)	18.2h(19.0%)	6.5d(24%)	305d(<1%)

* Least square fit to plasma ^{51}Cr(III) clearance curve
** Determined from the final slope calculated from whole body excretion data
   m: minutes; h: hours; d: days

Table 2
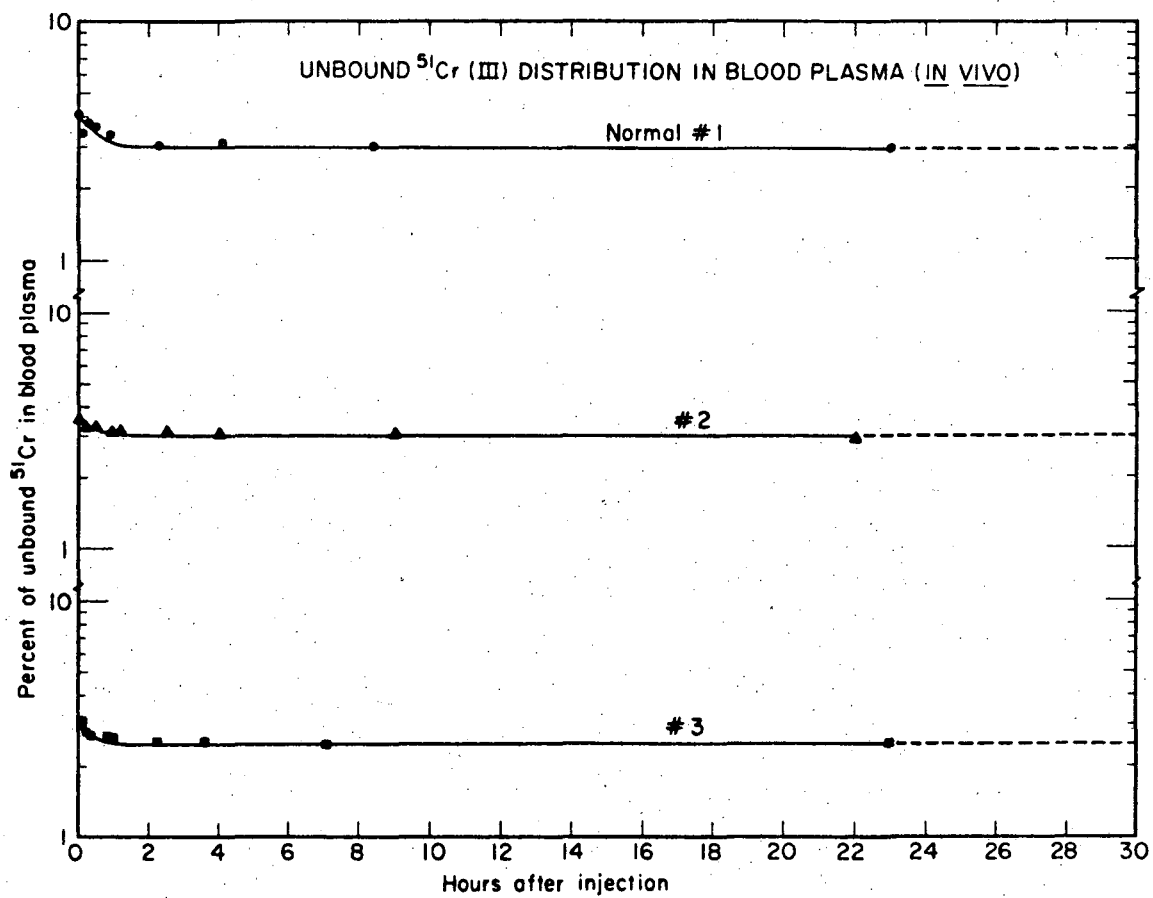
Fig. 2
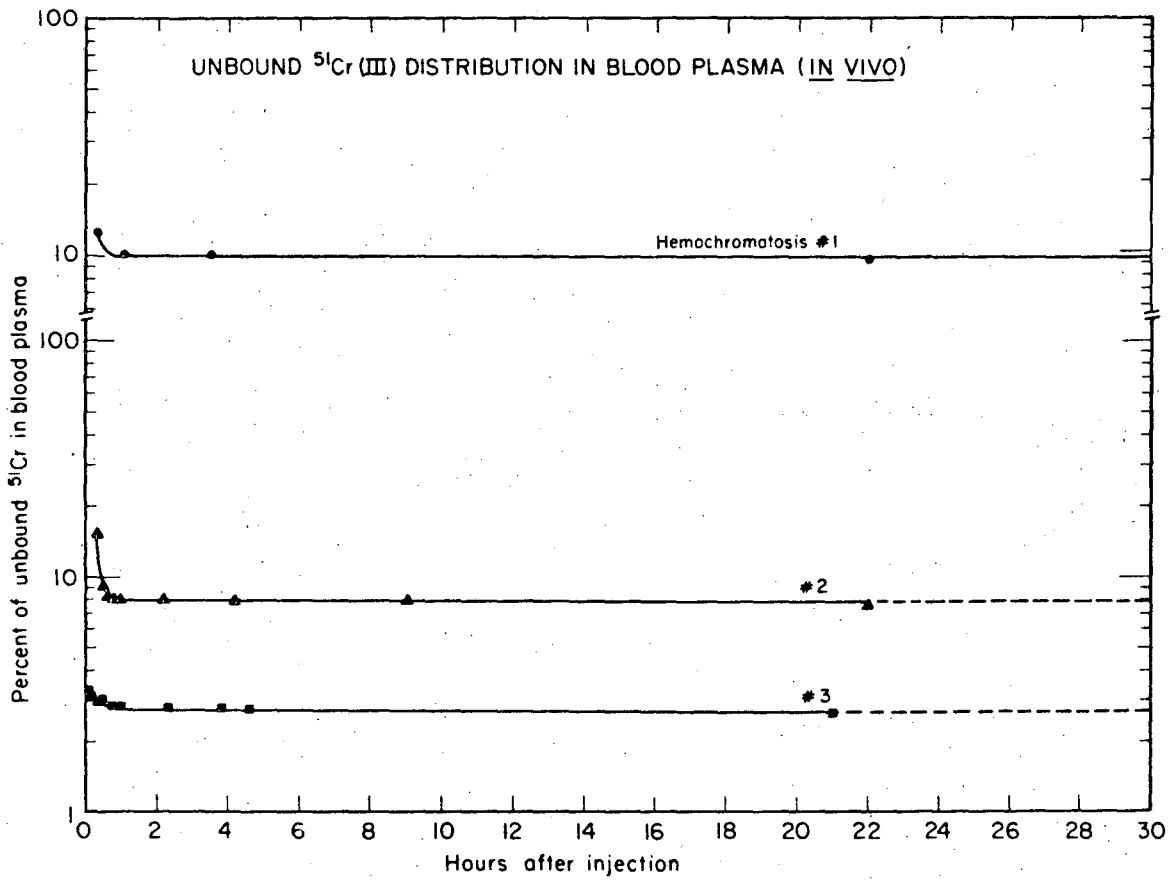
Fig. 3

The last component has its logical origin from the clearance of stored chromium(III) into the urine. The average, clearance in 3 patients with hemochromatosis occurred more rapidly than in normal subjects (table 2).

The fact that *in vitro* incubation of radio-chromium(III) with plasma gave approximately the same free to total plasma chromium(III) ratio compared to *in vivo* and the fact that a quite linear relation exists between free plasma chromium(III) concentration and bound plasma chromium(III) concentration at steady state condition (fig. 2,3), indicated that free chromium is "produced" or at least exists all the time in human blood plasma.

The existence of free chromium(III) at all times can only be explained by assuming that exchange of free and bound chromium(III) takes place; this suggests that the plasma chromium(III) pool consists of a free or unbound or dialyzable chromium(III) subpool and a plasma or transferrin bound chromium(III) subpool. (As is well known and mentioned earlier chromium(III) is primarily bound in blood plasma to transferrin.)

II. 4. Serum radiochromium(III) response and glucose
      tolerance test

On several occasions, 14 days after admin-
istration of 100 µCi of $^{51}CrCl_3 \cdot 6H_2O$ intra-
venously, a gluscose tolerance, insulin and radio-
chromium(III) response test was performed.
Blood was drawn at 30 minute intervals for
3 hours. Radioactivity was found to decrease
gradually during the whole test (fig. 4).
No increase in radiochromium(III) levels
was noted during the 3 hours the glucose toler-
ance test was performed (both, in patients
suffering from hemochromatosis and normal
subjects). It is important to note that in
the separate experiment described in II.2 the
total plasma chromium was higher after glucose
administration relative to fasting while in
this test only injected radiochromium(III) was
measured.

II. 5. Radiochromium(III) localization, whole body
      scanning and whole body counting

At the present time, the whole body scan-
ner is the best instrument available for vis-
ualization of radiochromium(III) in the human
body. Localization of radiochromium(III) in
the human body was performed at LBL Donner
Laboratory of the University of California
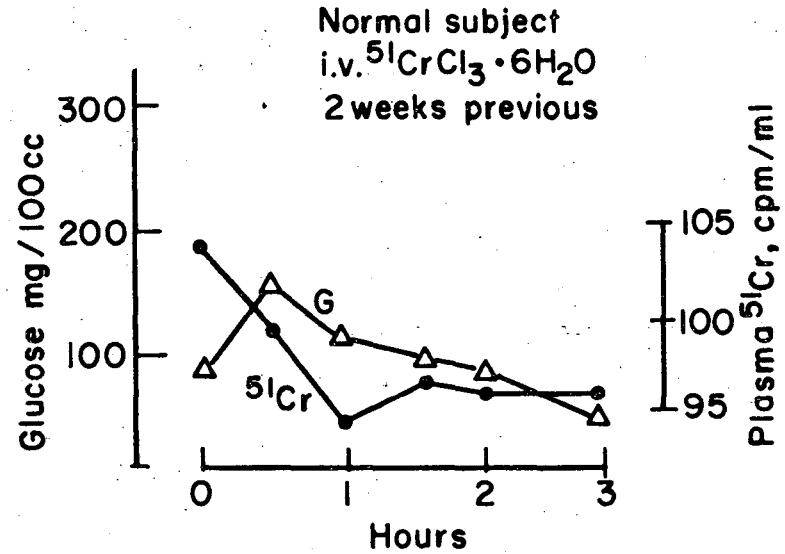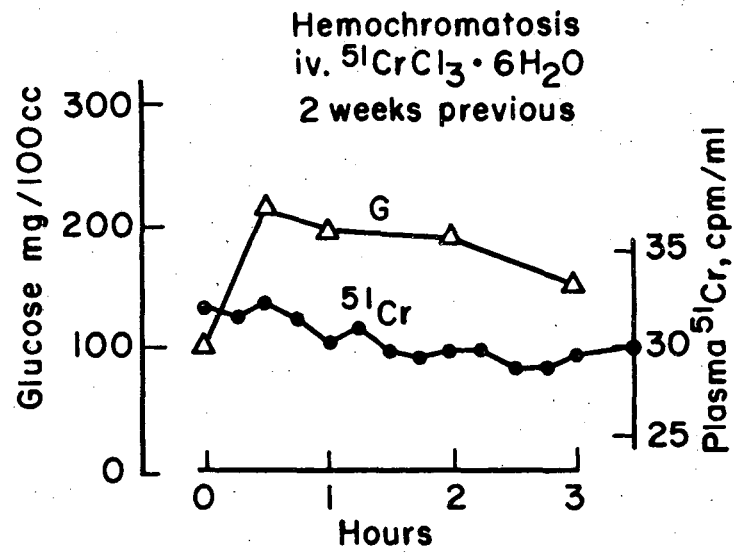
Glucose Tolerance and Serum Radiochromium (III)

Response Tests

Fig. 4

XBL771-3010

in 3 normal and 3 hemochromatotic subjects
(appendix B).

The Mark II computerized rectilinear whole
body scanner was used. The scanner has recen-
tly been interfaced to a Hewlett Packard 5407A
Scintigraphic Data Analyzer, which allows acqui-
sition of data for further analysis[43]. The
scanner operates by moving the patient over an
array of 4 rows of 16 2.5 x 2.5 cm (NaI(Tl)
crystal detectors (fig. 5). Since there were
no previous experience in the conduct of this
experiment, especially to what extent the
physical preparation of the subjects has to be,
subjects were asked to follow their regular
routine activities prior to and during the
course of the experiment.

Subjects were periodically scanned starting
seconds after 100 µCi of $^{51}CrCl_3 \cdot 6H_2O$ was
administered intravenously, up to a period of
90 days. Scan duration varied from 3 to 44
minutes, both anterior and posterior. Inter-
vals between scanning ranged from 3 minutes
to 14 days.

From scan images, radiochromium(III) was
localized primarily in the liver, body soft
tissue, spleen, and bones (figs. 6,7,8,9,10,11).

Magnetic discs

Removable cartridge

Fixed disc

Magnetic tape

Display

Display controller

HP 2100 A Computer

Teleprinter

4 Rows of 16 Nal crystals
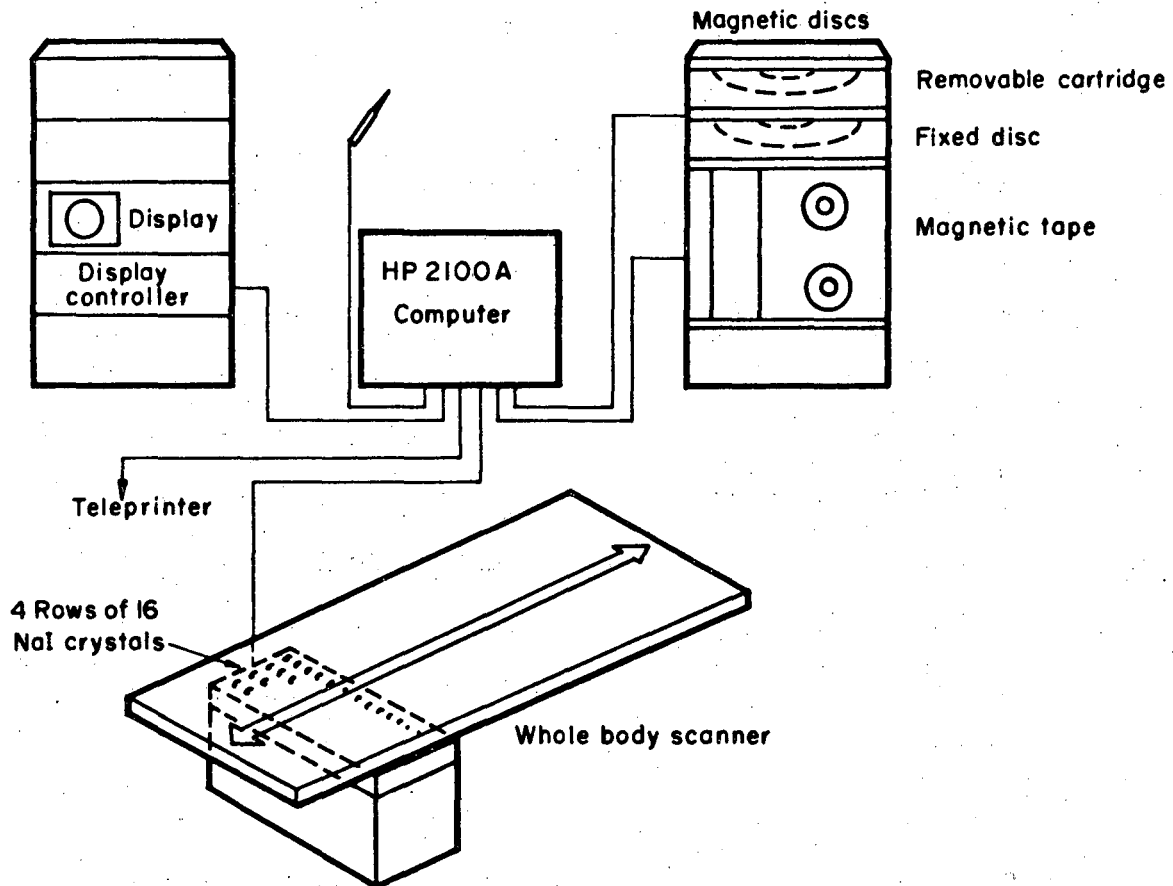
Whole body scanner

THE MARK II COMPUTERIZED RECTILINEAR
WHOLE-BODY SCANNER

XBL771-3061

Fig. 5

For a period up to 6 months the total radio-chromium remaining in the body as a function of time was determined with the Donner Laboratory whole body counter.

A. Liver Chromium(III) (fig. 10,11)

Concentration of radiochromium(III) in the liver was found to be the highest among all of the body organs. Examination of a semi-log plot of data points (fig. 12), suggest that the liver radiochromium(III) clearance curve (blood activity subtracted) consists of at least a fast* and slow* exponential component. Absorption also occurred through both rapid and slow exponentials. Rapid absorption and clearance suggest the existence of a labile chromium(III) subpool in the liver. Slow absorption and clearance of chromium(III) suggest the existence of a chromium(III) storage subpool.

It was also found that clearance of radio-chromium(III) from this organ occurred more rapidly in patients with hemochromatosis (fig. 13, 14).

---

*"fast", "medium" and "slow" clearance or uptake is related to half-lives within a range of respectively 0.5-12 hours, 1-14 days, and 3-12 months.

Cr^{51} Distribution (Anterior)
NORMAL MALE SUBJECT #2
Cr^{51} Cl$_3$ Intravenously
Mark II Whole Body Scanner
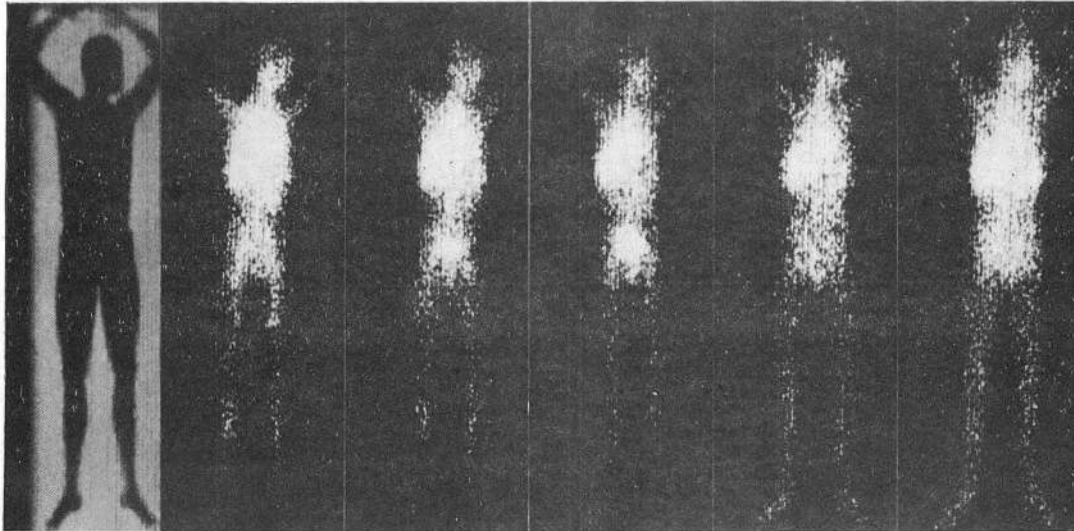
Am 241 Transm   3 min   1 h   4 h   1 d   5 d
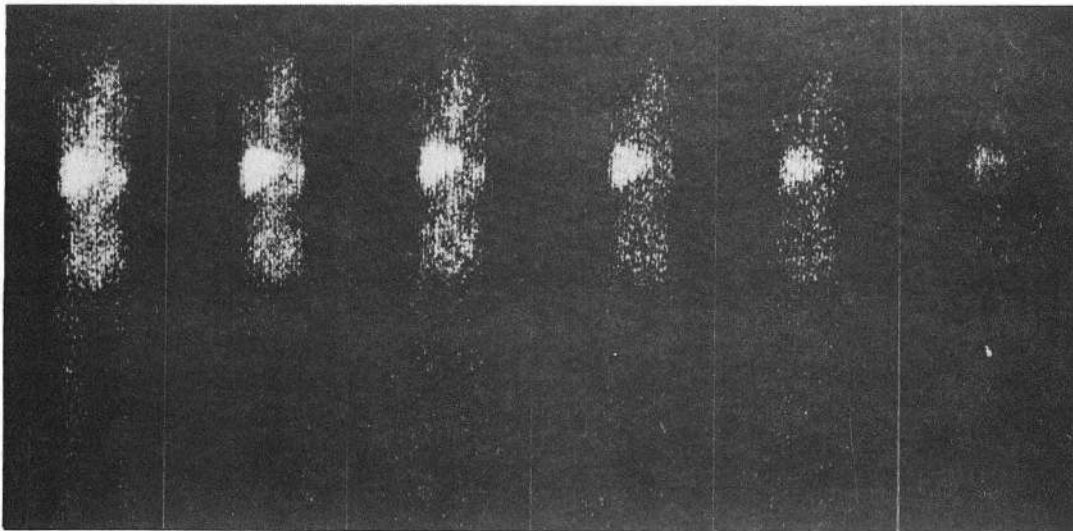
15 d   28 d   46 d   56 d   70 d   84 d

XBB 768-7589

Fig. 6

Cr^{51} Distribution (Posterior)
NORMAL MALE SUBJECT #2
Cr^{51} Cl$_3$ Intravenously
Mark II Whole Body Scanner
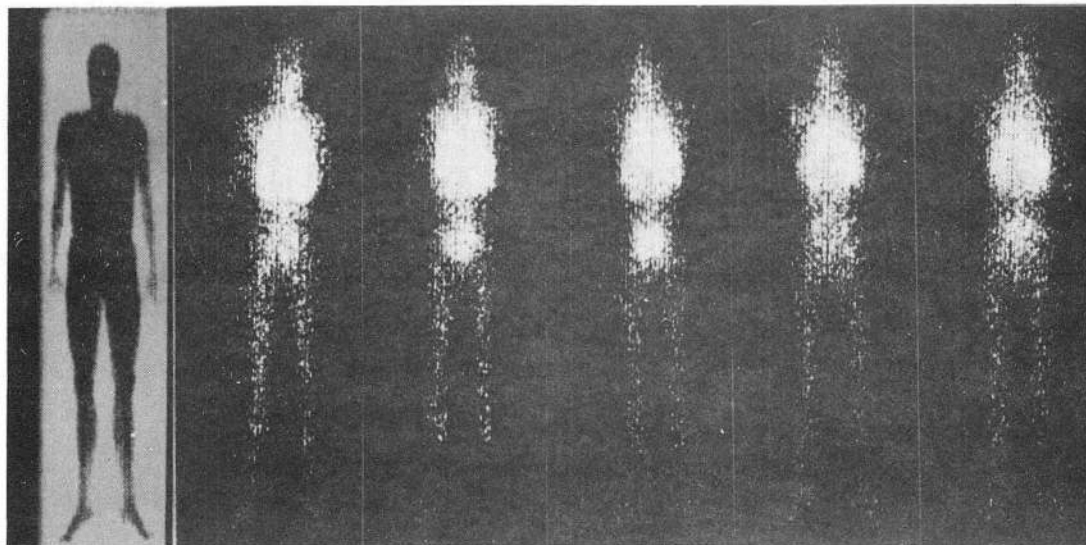
| Am 241 Transm | 20 min | 1 h | 4 h | 8 h | 1 d |

| 2 d | 5 d | 15 d | 28 d | 56 d | 84 d |
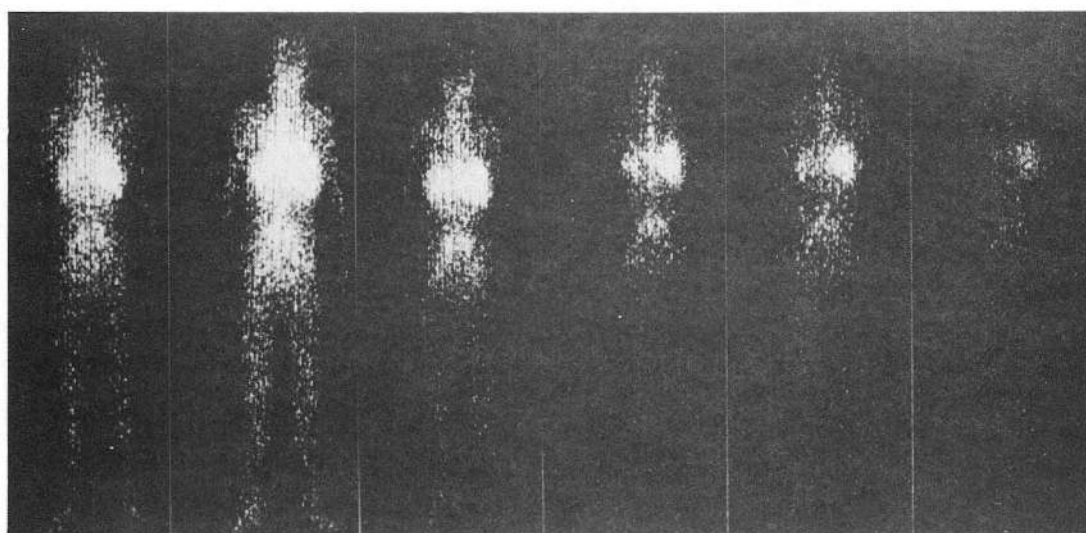
Fig. 7

XBB 768-7590

37

Cr^{51} DISTRIBUTION
(Anterior)
EARLY ENDOGENOUS HEMOCHROMATOSIS
Cr^{51}Cl$_3$ INTRAVENOUSLY
Mark II Whole Body Scanner

Am^{241} trans.   3 min   1 hr   5 hr   1 day   3 days

8 days   20 days   34 days   48 days   55 days   76 days
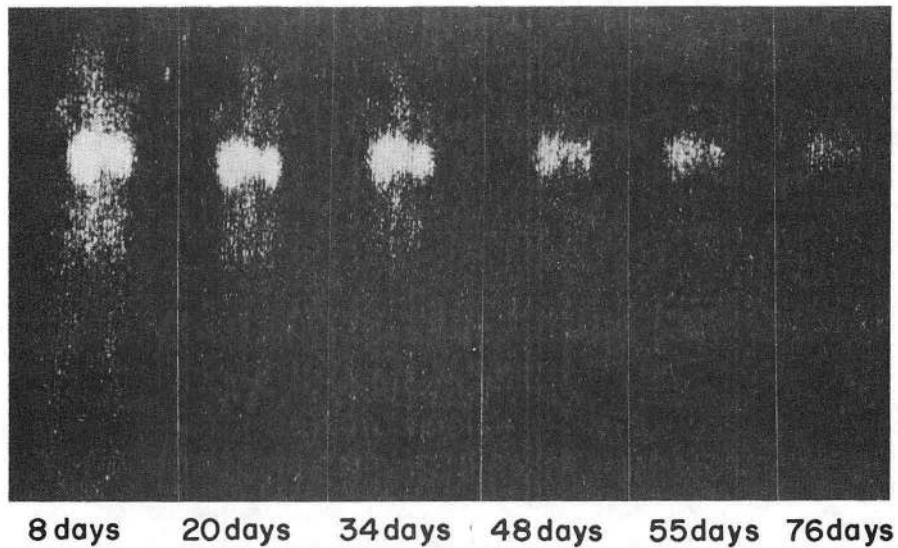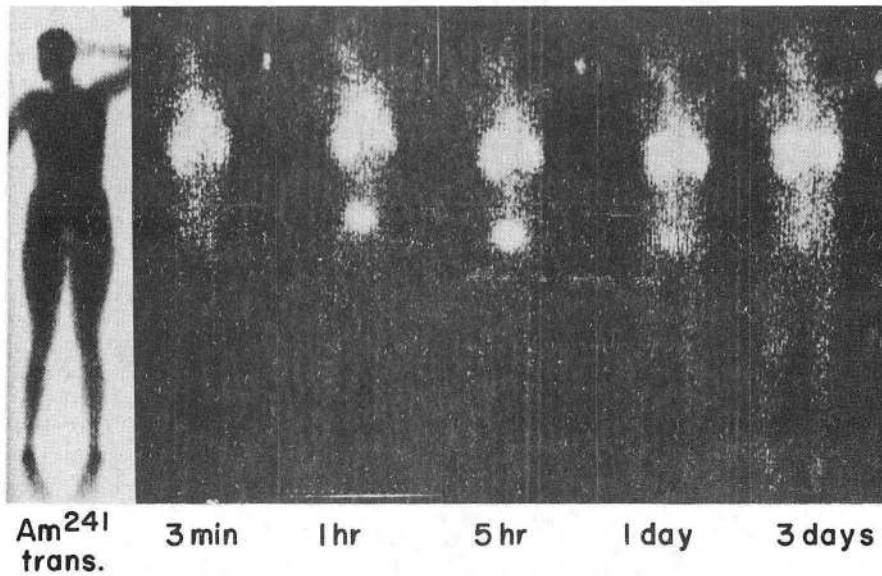
XBB 765-4027

Fig. 8

Cr⁵¹ DISTRIBUTION
(Posterior)
EARLY ENDOGENOUS HEMOCHROMATSIS
Cr⁵¹Cl₃ INTRAVENOUSLY
Mark II Whole Body Scanner
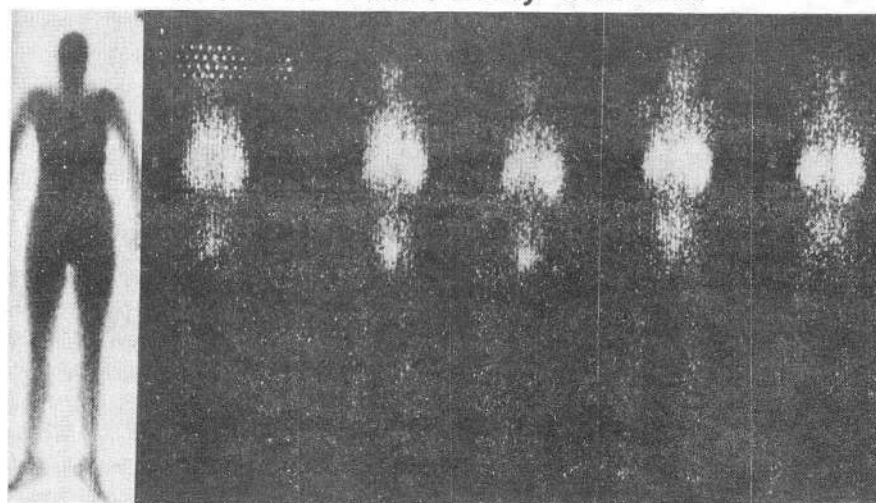
Am²⁴¹ trans.   8 min   2 hr   8 hr   2 days   8 days
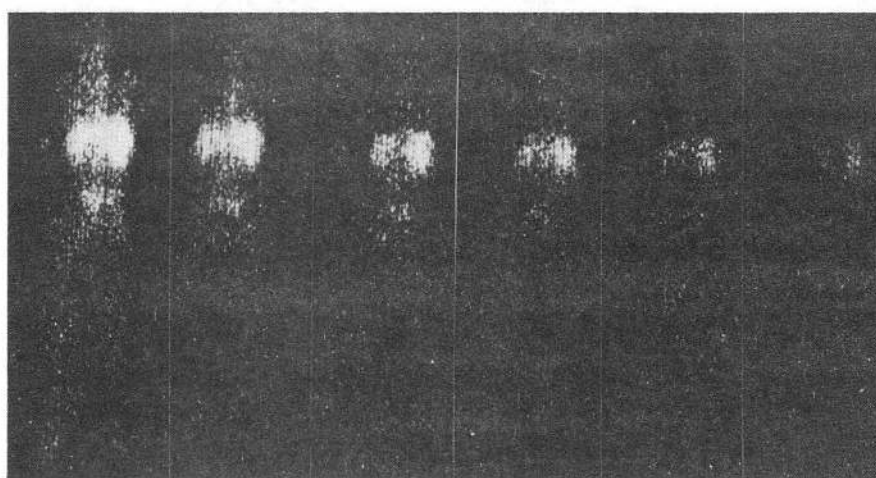
20 days   34 days 48 days   55 days   69 days 76 days

XBB 765-4028

Fig. 9

^{51}Cr DISTRIBUTION IN DIFFERENT ORGANS

Anterior   Posterior
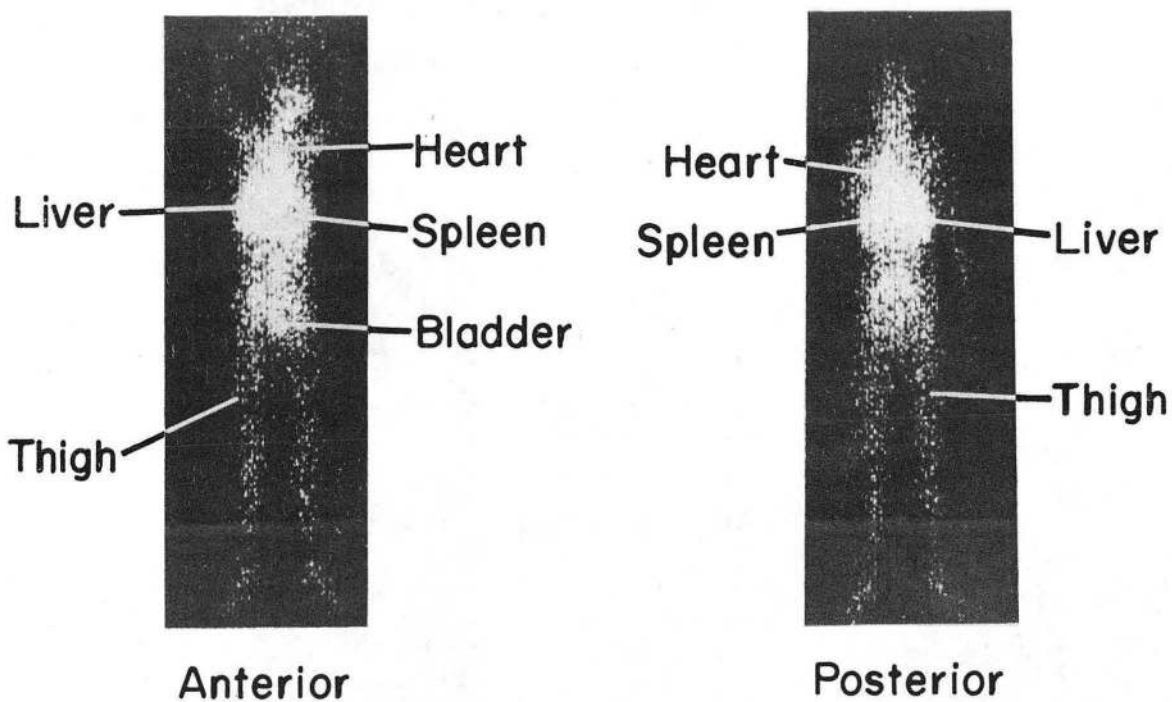
Normal Subject
2 Days post – injection

XBB 760-11069

Fig. 10

^{51}Cr DISTRIBUTION IN DIFFERENT ORGANS
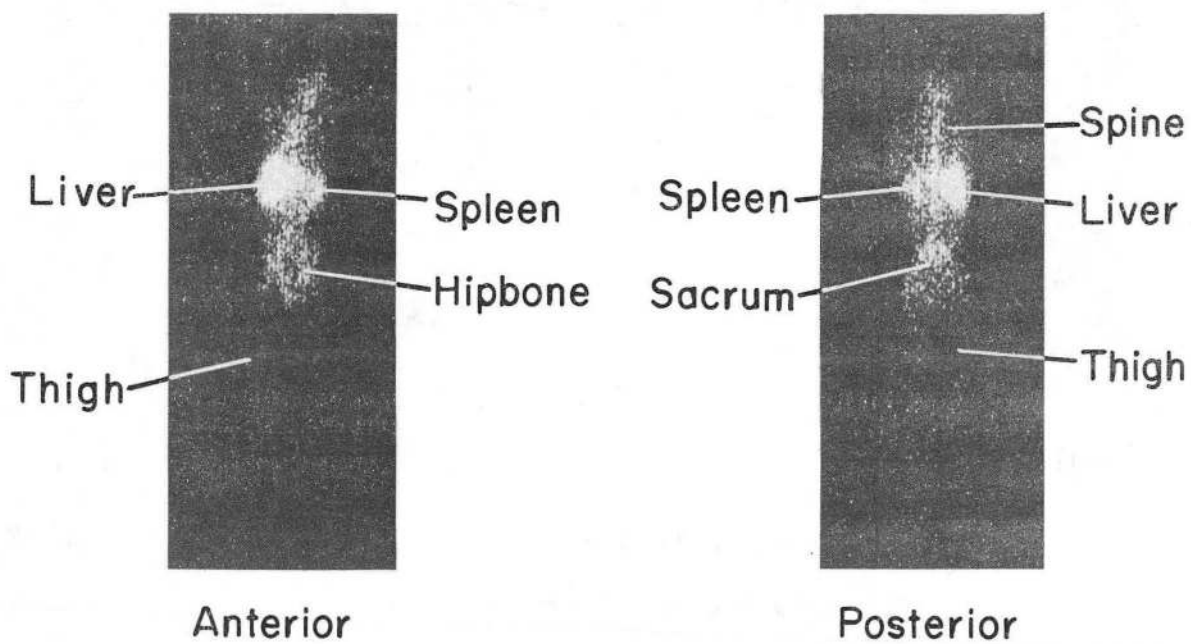
Normal Subject
28 Days post - injection

XBB 760-11070

Fig. 11
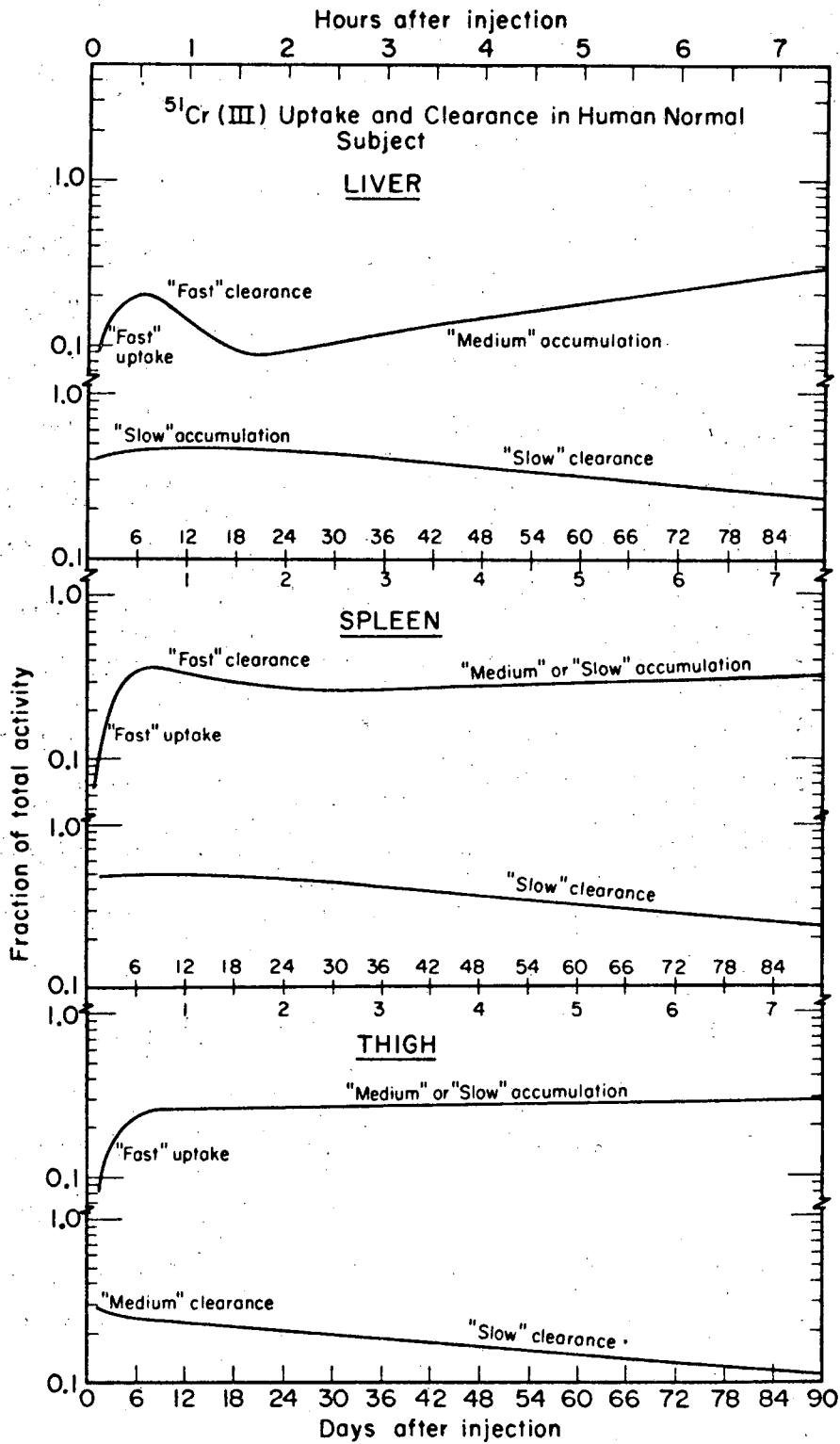
Fig. 12

B. Adipose and muscle tissue chromium(III)

   (fig. 10,11)

Examination of radiochromium(III) clearance curve from the thigh (fig. 12) suggests that this organ cleared radiochromium(III) through at least a rapid and slow exponential. Examination of radiochromium(III) clearance curve (blood activity subtracted) and scan-images suggest that a medium* exponential absorption component existed besides the slow. Accumulation of chromium(III) in adipose and muscle tissues is described by ref. (17) as primarily related to the transport of glucose across cell membranes. Furthermore, it is suspected that chromium(III) is involved in lipid and protein metabolism[17].

C. Spleen chromium(III) (fig. 10,11)

Absorption and clearance of radiochromium (III) in the spleen followed a combined pattern as does absorption and clearance in the liver and soft tissues. Clearance occurred through exponentials with approximately the same half-lives as the liver (fig. 12).

D. Bone (fig. 10, 11)

Radiochromium(III) can be considered as

---

* See footnote on page 34.

^{51}Cr(III) Retention in Normal Subject
LIVER

SPLEEN

THIGH

Fraction remaining in organ

Days after injection
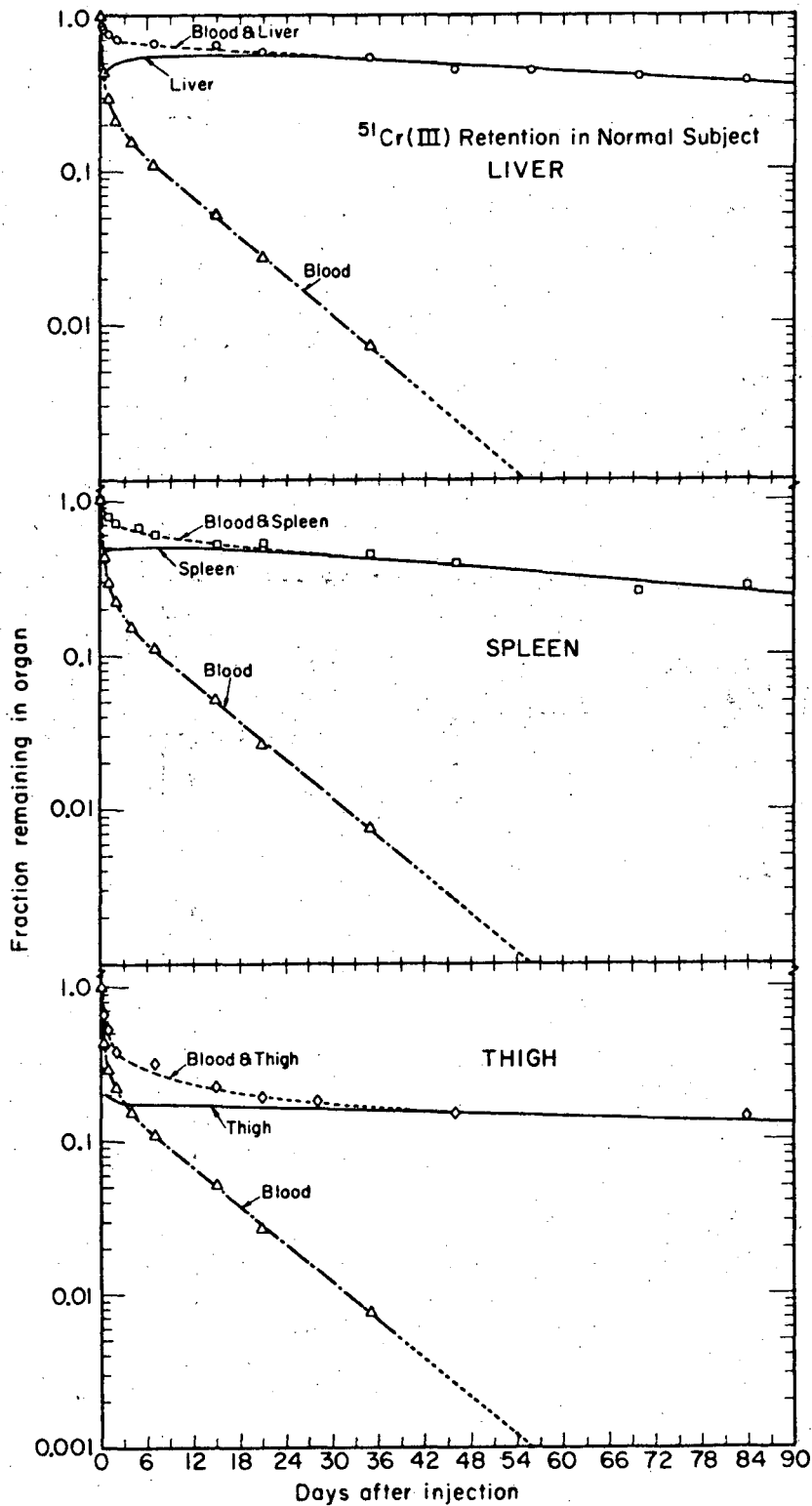
XBL7610-9412

Fig. 13
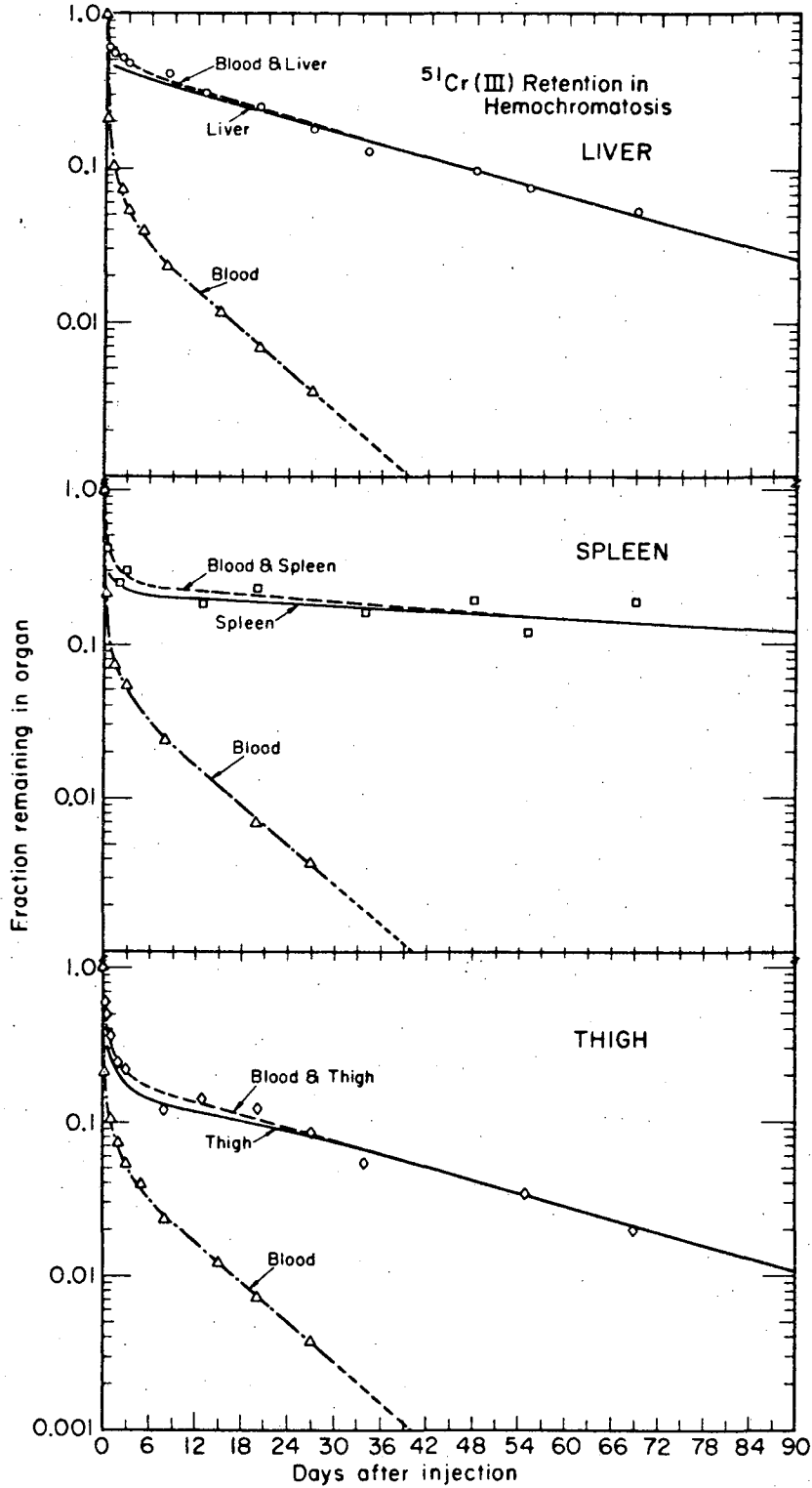
Fig. 14

an inferior boneseeker*; accumulation in the
joints was detected in a patient suffering
from arthritis (fig. 15). Fast accumulation
of radiochromium(III) in bones cannot be
detected successfully probably due to the high
activity of radiochromium(III) in the blood
and soft tissues.

E.   Other organs (fig. 10,11)

Accumulation of radiochromium(III) in
other organs of the body, such as the kidneys,
lungs, central nervous system and the heart
cannot be detected with the present experimen-
tal techniques. This is primarily due to the
high activity of the circulating blood compared
to the probably low concentration of radio-
chromium(III) accumulated in the above mentioned
organs especially during the first day of the
experiment.

F.   Whole body chromium(III) (fig. 6,7,8,9,
     10,11)

In general body tissues absorbed radio-
chromium(III) very rapidly. More than 50% of
blood plasma chromium(III) was absorbed by
different body organs within hours after
intravenous administration. Clearance was

---

*visually the concentration in bone is rather small
 compared to F-18.

^{51}Cr (III) DISTRIBUTION IN HEMOCHROMATOSIS
SUFFERING FROM ARTHRITIS

Enlarged liver

Joints

Anterior
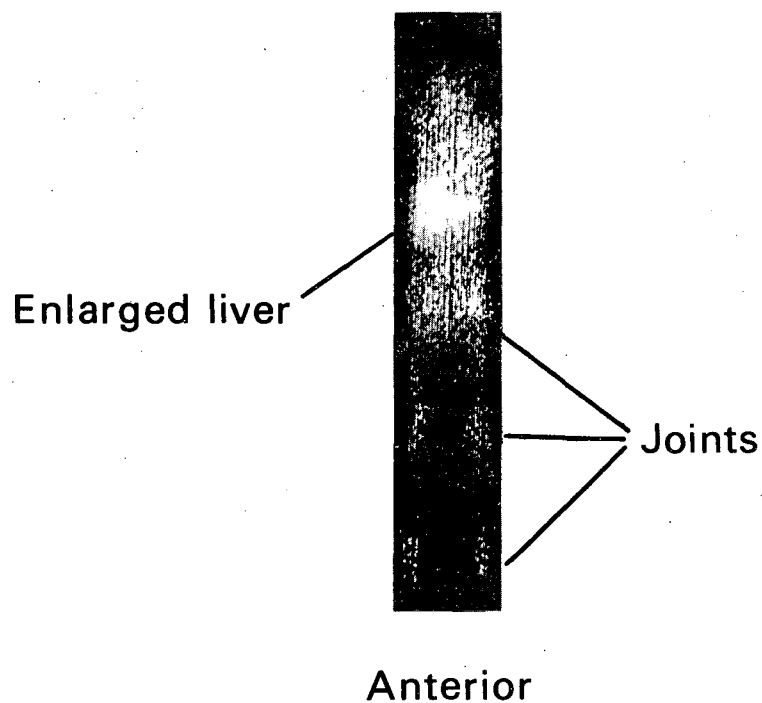
4 DAYS POST-INJECTION

Fig.15   XBB 771-50

more rapid in patients suffering from hemochromatosis compared to normals. Rapidity of clearance from the body depends on the concentration of free chromium(III) in blood plasma.

Radiochromium(III) 6 months after administration is most concentrated in the liver, about half of it was estimated, being in this organ and the remainder distributed in other body tissues. The excretion at this time is dominated by the slow rate component.

II. 6. Data analysis

A. Serum radiochromium(III)

Blood was drawn seconds after intravenous administration of $^{51}CrCl_3 \cdot 6H_2O$, up to a period of 2 months. Intervals between drawing varied from 3 minutes to 2 weeks. During the first 24 hours approximately 10 blood samples of approximately 20cc each were drawn. The plasma was then separated from the blood cells and ultracentrifuged for approximately 24 hours at 114,000g and 17°C. The portion of unbound or free radiochromium(III) was then determined by counting the top two-thirds (supernatent) of the ultracentrifuged plasma separately. The bottom part contained the plasma bound radiochromium(III). After several days the unbound portion of radiochromium(III) cannot

be determined any longer accurately, unless at
least several hundred cc's of blood are drawn,
since the activity is below the detection limit
of our 4 x 9" NaI(Tl) crystal detector. The
total plasma radiochromium(III) activity was
determined by counting 20cc or more (up to
500cc in hemochromatosis obtained in the
course of phlebotomy therapy) of whole blood
directly; the plasma portion of the blood
was determined from the hematocrit.

The data points obtained from both free
and plasma bound radiochromium(III) are esti-
mated to be accurate to within counting statis-
tics and pipetting errors only(<5%).

B. Localization of radiochromium(III)

Determination of radiochromium(III) locali-
zation in different human organs was performed
using the Mark II computerized rectilinear
scanner. From scan images, concentrations of
radiochromium were detected in the liver, soft
tissue, spleen and bones.

The assumption was made that counts over
the liver, spleen or bone includes counts from
radioactivity within the liver, spleen or bone
cells and counts from blood circulating in the
organ. Counts over the body soft tissue
include counts from radioactivity within the

soft tissue cells and the circulating blood only. Correction for the activity from circulating blood is made by subtracting the circulating blood counts in that region of the organ of interest from the total counts (fig. 13,14). Also the assumption was made that at time t=o, the total counts were contributed by the circulating blood alone, and that none had accumulated in the cells of the organ of interest. Other corrections include background and radioactive decay.

As mentioned above, absolute determination of radiochromium(III) in any organ of interest is quite complicated and with the present available techniques is far from accurate. Also, during the first several minutes post injection rapid accumulation and excretion of radiochromium occurred. Successive prone and supine scans cannot be performed synchronously with the Mark II scanner. Since the minimum scanning time for this experiment is 3 minutes, successive scans have a minimum time difference of at least 3 minutes. Applying a geometric mean and source thickness correction by using two conjugate views[43,44,45] leads to a rather large error. For this reason source thickness and geometric mean corrections were not applied.

The total counts originating from activity
present in any organ of interest, were obtained
by integrating the area of the scan-image of
the organ of interest[43,44].

Since the activity of the organ of interest
was obtained on a semi-quantitative basis and
cannot be used as an accurate input to match
a proposed compartment model, other more ac-
curate input data were chosen. These are
the total body radiochromium(III) retention
and plasma radiochromium(III) clearance data.

III. Chromium(III) compartment model and kinetics

III. 1. Schematic of proposed pathway

On the basis of data obtained from radiochromium(III) localization in different body organs, whole body counting, plasma radiochromium(III) clearance experiments and ultracentrifugation of labeled blood plasma, a schematic diagram showing chromium(III) pathway in the human body was proposed (fig. 16).

Transportation of chromium(III) is by the plasma protein, transferrin. Chromium(III) is believed to be stored primarily in the liver. Utilization of chromium(III) is suspected to be primarily by body soft tissue[17]. Excretion of dialyzable chromium(III) is through the kidney into the urine.

III. 2. Proposed physiological compartment model

The proposed chromium(III) pathway in the human body combined with results obtained from plasma radiochromium(III) clearance, suggest a multicompartment physiological model for chromium(III) kinetics as show in fig. 17.

The model considered blood plasma chromium(III) as the "central compartment." The plasma chromium(III) compartment con-

GI Tract

<u>Absorbtion</u>   →   Cr (III)

↓ ↓ ↓ ↓ ↓ ↓   Cr (III)

<u>Transport</u>   Blood Plasma

BB ⇌ BF

<u>Storage and</u>
<u>Transformation</u>   BB ↓   ↑ BB+BF

Liver + Spleen ?

BB   BF (Dialyzable)

Membrane

<u>Utilization</u>   CELL

Intra

(Liver, Adipose, Muscle
tissue and Bone)

Dead cell   Kidney

<u>Excretion</u>   Urine, Faeces,   Urine
Bile, Sweat,   (Rapid clearance)
etc.

BB: Blood Plasma   BF: Unbound or Free
Bound   Chromium (III)
Chromium (III)
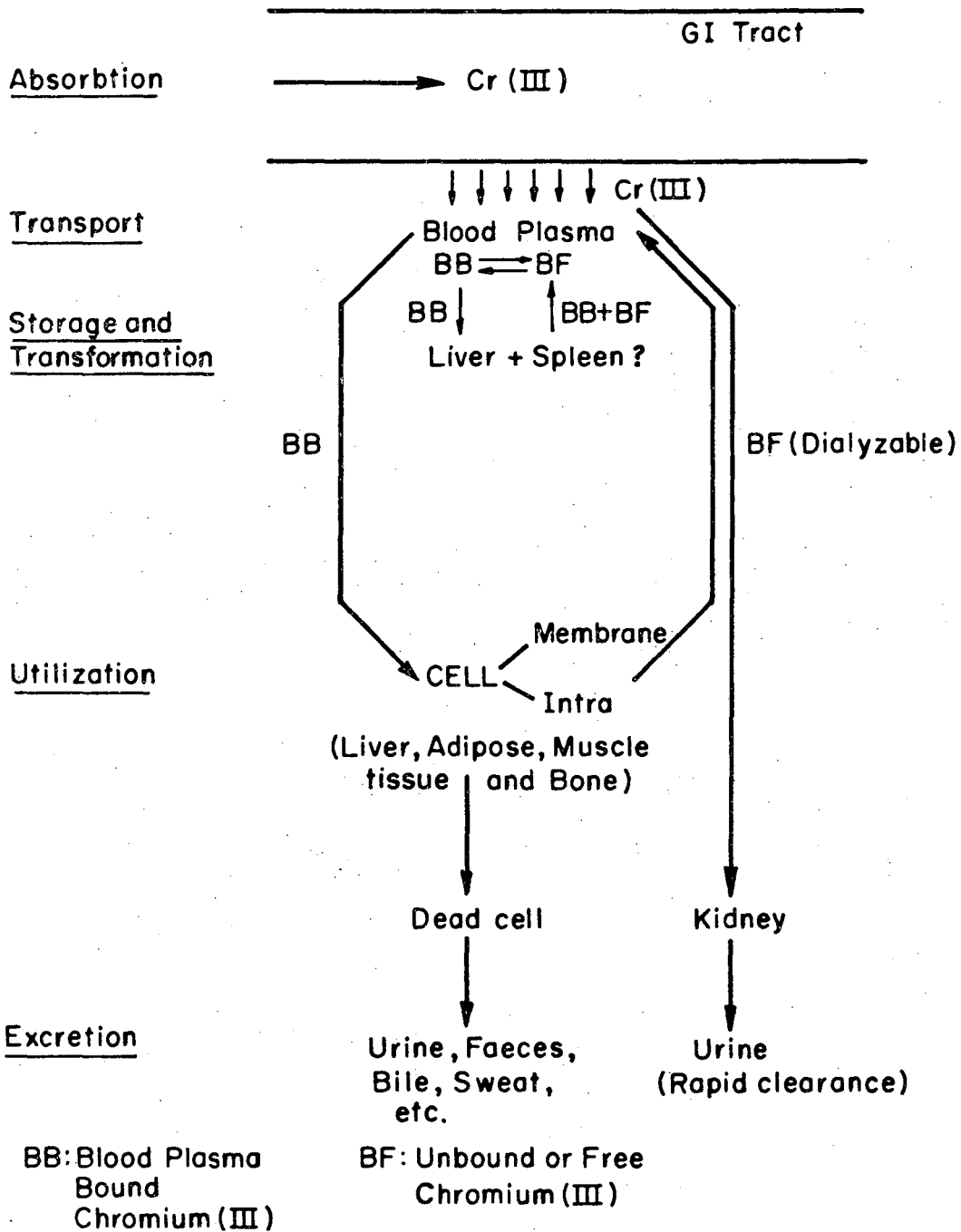
PROPOSED CHROMIUM (III) METABOLIC PATHWAY

XBL7612-10739

Fig. 16

sists of two subcompartments, the free or
unbound chromium(III) and the protein bound
chromium(III) subcompartment. Exchange of
chromium(III) exists between the two subpools.
The soft tissue chromium(III) pool probably
consists of primarily adipose and muscle
tissue. Possible tissue chromium(III) sub-
pools would include cell membrane and inner
cell organelles.

The liver consists also of two subcom-
partments. The first subcompartment has a
fast turn-over and is called the "labile"
subcompartment. The second subcompartment
stores chromium(III) and releases it back
to the blood plasma probably upon demand;
this is called the storage subcompartment.
The spleen has a clearance characteristic
which suggest that it also stores radiochro-
mium(III). The half-life of storage of
radiochromium(III) in the spleen is approxi-
mately the same as the liver storage.

A bone chromium(III) compartment was
added to the model since scan images indi-
cated accumulation of radiochromium in the
bones. To complete the model a hypotheti-
cal compartment consisting of organs that
accumulate or absorb chromium(III) but can-

not be detected with the present imaging
techniques was added.

III. 3. Mathematical model and computing method

A. Mathematical model

Results obtained from various experi-
ments mentioned earlier provided some pre-
sumptive information on the physiological
model and how the compartments are inter-
connected. The model consists of at least
6 compartments as described in fig. 17. Some
compartments have subcompartments.

The five detectable exponential com-
ponents of plasma radiochromium(III) clear-
ance compared to at least six detectable
different organs which accumulate radiochro-
mium(III) can only be explained by assuming
that some organs metabolize chromium(III)
with approximately the same time constant[46].
Estimates of liver, spleen and thigh accumu-
lation of radiochromium(III) confirmed this
statement (fig. 12,13,14) .

The chromium(III) pool in adipose as
well as in muscle tissues cannot be deter-
mined by the experimental techniques used.
Quantitative data for the liver, spleen and
bone cannot be obtained as accurately as
the whole body retention and plasma radio-
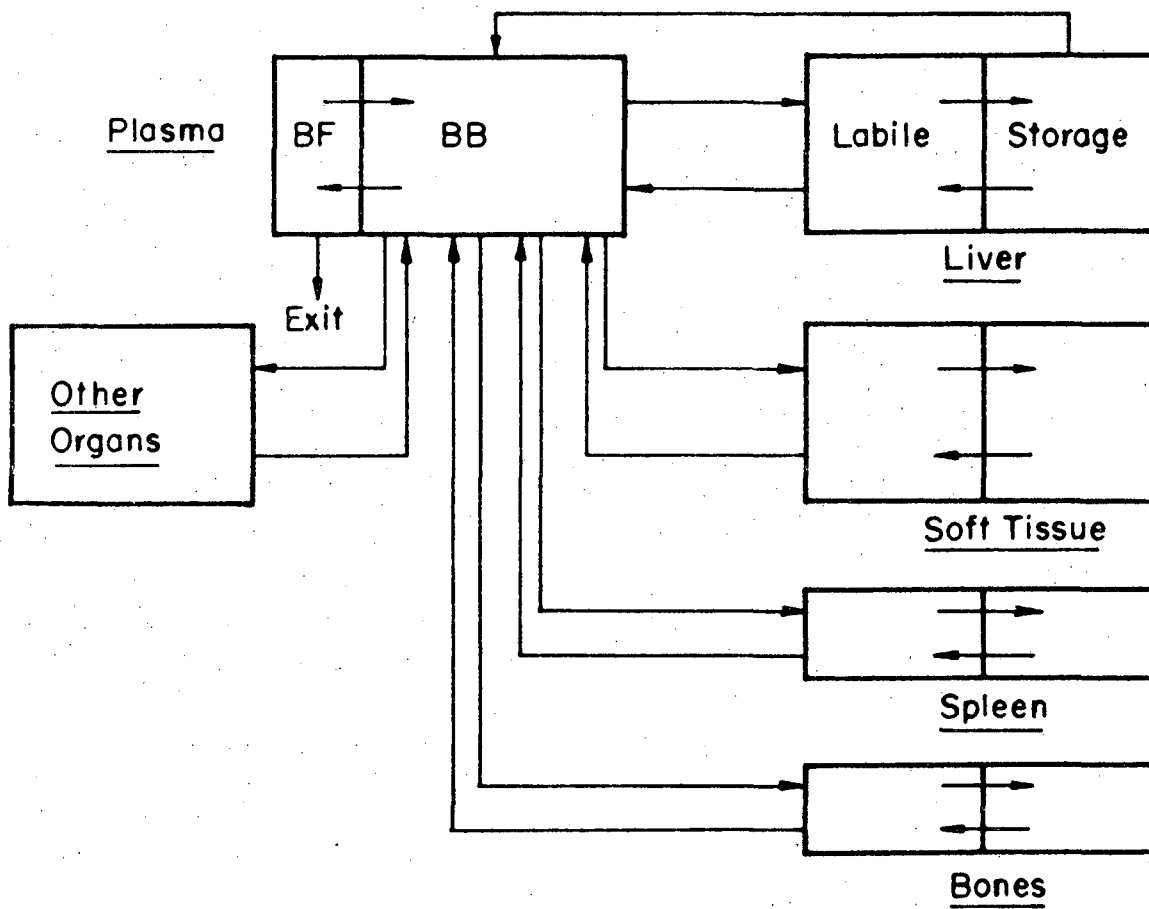
PROPOSED CHROMIUM (III) PHYSIOLOGICAL MODEL

XBL7612-10738

Fig. 17

chromium(III) clearance data points. For
this reason a mathematical model was devel-
oped which grouped all physiological compart-
ments accumulating chromium(III) with the
same or almost the same time constant into
one particular compartment. Since analysis
of the plasma chromium(III) clearance data
yields 5 detectable time constants with res-
pective average half-lifes (in 3 normals) of
∿60 minutes, ∿4 hours, ∿18 hours, ∿7 days,
and ∿11 months, the different hypothetical
compartments in the mathematical model can
at least be characterized by Fast*, Medium*
and Slow*. Other intermediate compartments
such as Fast-Medium or Medium-Slow can be
added if necessary (fig. 18). All hypotheti-
cal compartments are assumed to be related
linearly to the central compartment. The
Fast compartment includes the fast or labile
subcompartments of the bone, muscle, and
adipose tissues, the spleen, and the liver.
The Medium compartment represents the slower
accumulation of chromium(III) in the spleen
and the slower absorbtion of chromium(III)
in either the liver, soft tissue and/or bone.

---

* See footnote on page 34.

Blood Plasma — BF | BB → Fast

Exit
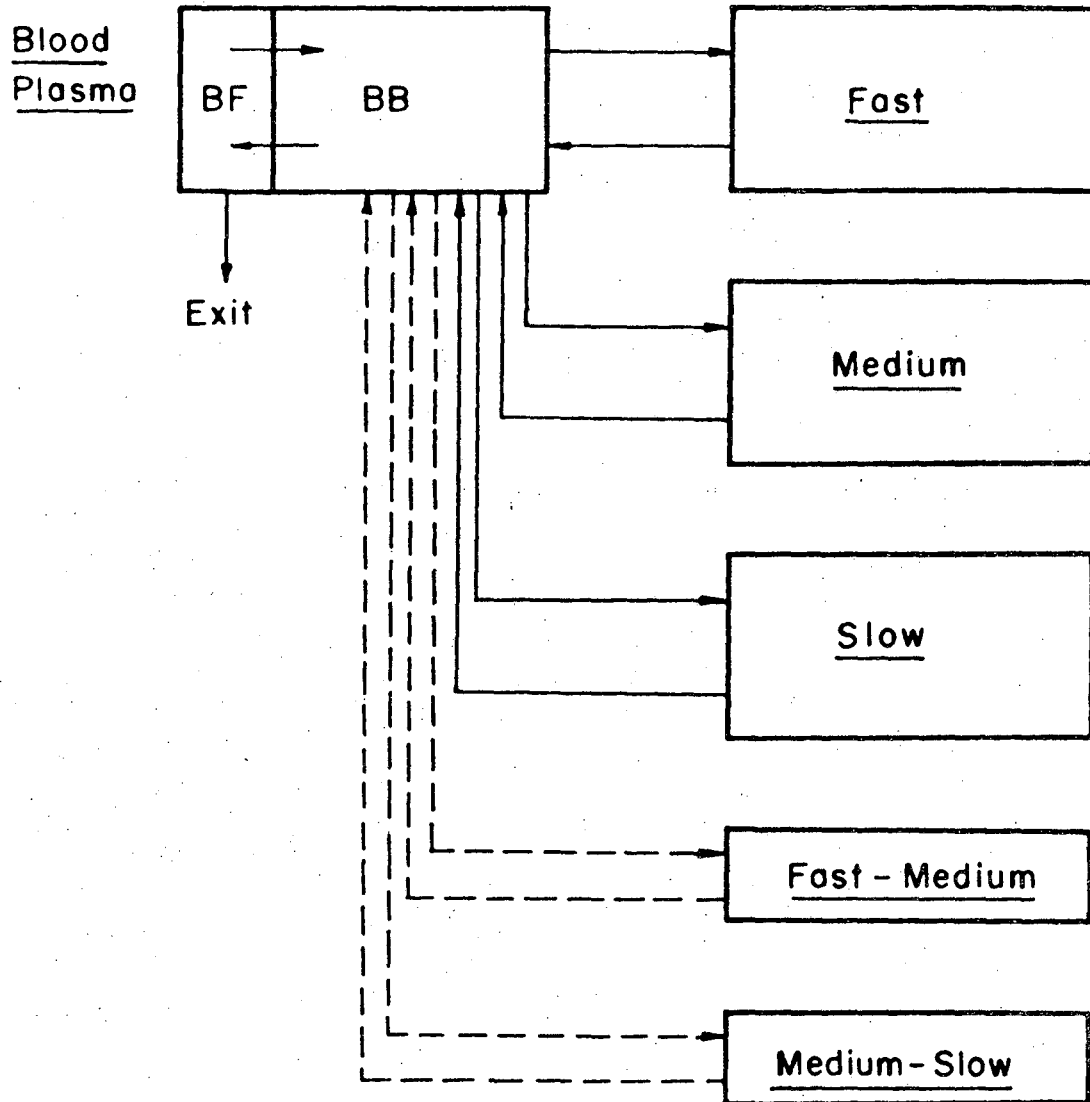
MATHEMATICAL MODEL FOR CHROMIUM (III)

XBL7612-10737

Fig. 18

The slow compartment represents the chromium (III) pool that "stores" chromium(III) primarily in the liver and bones. The highly accurate whole body radiochromium(III) retention and plasma radiochromium(III) clearance data points were then fitted directly to the mathematical model[47,48,49,50,51,52].

However one still needs initial estimates for the values of the parameters $k(I)$'s in order to proceed with an iterative convergence to a least square solution. These initial estimates of the parameters were also obtained from experimental results.

B. Computing methods

The computation of the mathematical model is divided into two stages as follow:

    a. Solution of the differential equations, given a set of values of the parameters $k(I)$'s and initial conditions.

For this purpose the "collapse"*

---

* By collapse is meant the incorporation of one particular compartment with the central compartment to form a single new compartment. This can be justified if the effect or influence of the compartment mentioned first, is assumed negligible, at the time of collapsing, compared to other compartments of the system.

compartment method was used. The
computation is divided into several
steps. Except for the first step,
at each step two or more compartments
"collapse" to form a system of a
maximum of 3 compartments. Hereby,
we reduced the 6 or more compartments
of the system to only 3 at each step.
Collapsing of one or more artificial
compartments to a central compart-
ment was done at times estimated
from experimental data. The detailed
procedure of the method is described
below. First, we introduced and
analyzed, the Free-Bound-Fast, three
compartment system. As initial con-
ditions, the feedback from the Medi-
um, Slow and other compartments (if
any) to the plasma serum bound chro-
mium(III) pool is taken as zero; as
mentioned earlier the transfer rates
to the Fast, Medium, Slow and other
compartment (if any) are assumed to
have initial estimated values.

For a general linear steady
state 3 compartment system with
constant coefficient (fig. 19a) the
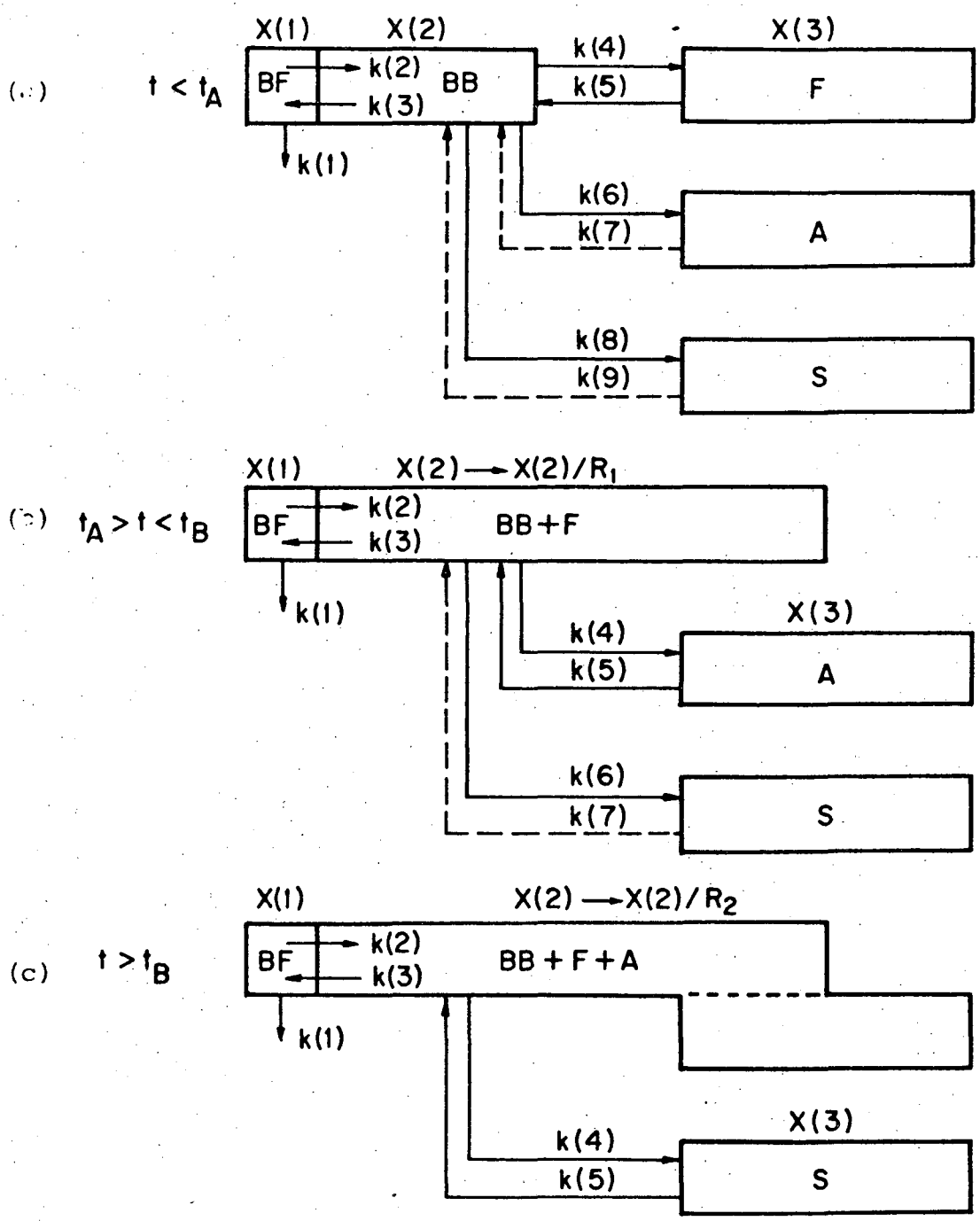
THE "COLLAPSED COMPARTMENT" METHOD MODEL

XBL7612-10736

Fig. 19

Free, Bound and Fast chromium(III)

pools as function of time [BF(t),

and BB(t) and F(t)] can be calcu-

lated from the differential equa-

tions:

$$\frac{dX(1)}{dt} = -[k(1) + k(2)]X(1) + k(3)X(2)$$

$$\frac{dX(2)}{dt} = k(2)X(1) - [k(3) + k(4) + k(6)]X(2) + k(5)X(3)$$

$$\frac{dX(3)}{dt} = k(4)X(2) - k(5)X(3)$$

where

$X(1) \equiv BF$, relative amount of free or

unbound chromium(III) in

the blood [µg or µCi]

$X(2) \equiv BB$, relative amount of serum

protein bound chromium(III)

in the blood [µg or µCi]

$X(3) \equiv F$, relative amount of chro-

mium(III) in artificial

Fast compartment [µg or

µCi]

$k(I) \equiv$ transfer rate [day^{-1}]

and k(6) in this equation represents

k(6) and k(8) in fig. 19a.

The amount of chromium(III) in the

Medium[A(t)] and Slow [S(t)] com-

partments as well as $U(t)$, which is the excretion of free chromium(III) through the kidneys into the urine, are calculated from the linear approximations:

$$A(t)=A_R(t)+k(6)\times UI$$

$$S(t)+S_R(t)+k(8)\times UI$$

$$U(t)=U_R(t)+k(1)\times UI$$

where, $k(I)UI=k(I)\int_{t_{j-1}}^{t_j}B(t)dt$

is a portion of chromium(III) excreted from the blood compartment during the time interval $t_{j-1}-t_j$* and A_R, S_R and U_R are estimated values assuming no backflow of the remainder at t_{j-1} and has zero value at the beginning of the experiment. We then modify the $BB(t)$ pool to include the average backflow from each of the Medium and Slow compartment as follows:

$$BB(t)\rightarrow BB(t)+[k(7)(A+A_p)/2+k(9)$$
$$(S+S_p)/2]t_a$$

---

* In this analysis the time interval $|t_{j-1}-t_j|=t_a$ is the period between two successive times of input data.

and $A(t)$ and $S(t)$ become respectively:

$$A(t) \rightarrow A(t) - [k(7)(A+A_p)/2]t_a$$

and

$$S(t) \rightarrow S(t) - [k(9)(S+S_p)/2]t_a$$

where P stands for previous (before backflow). This process is repeated for all values of t up to the "collapse" point t_A.

The next artifical compartmental system to be analyzed is the Free-(Bound + Fast)-Medium system. Here the Fast compartment is incorporated in the Bound chromium(III) compartment to form a new (BB+F) compartment as drawn in fig. 19b. Again, the BF(t), the new BB(t) (to include the Fast compartment) and the A(t) compartment can be calculated from the differential equations:

$$\frac{dX(1)}{dt} = -[k(1)+k(2)]X(1)+k(3)X(2)$$

$$\frac{dX(2)}{dt} = R(1)[k(2)X(1)-[k(3)+k(4) +k(6)]X(2)+k(5)X(3)]$$

$$\frac{dX(3)}{dt} = k(4)X(2)-k(5)X(3)$$

From fig. 19b,

X(3) now represents the Medium (A) compartment

$X(2) \equiv X(2)/R(1)$ represents the combined (BB+F) compartment and

$R(1) = X(3)/X(2)$

$X(3)$ represents the slow compartment and $k(4)$ in fig. 19b represents $k(6)$ in fig. 19a.

$k(6)$ in fig. 19b represents $k(8)$ in fig. 19a.

$k(5)$ in fig. 19b represents $k(7)$ in fig. 19a.

Once more, $S(t)$ and $U(t)$ are calculated from the linear approximations:

$S(t) = S_R(t) + k(8) \times UI$

$U(t) = U_R(t) + k(1) \times UI$

where as before:

$$k(I)UI = k(I) \int_{t_{j-1}}^{t_j} B(t)dt$$

We then again modify $BB(t)$ as follows:

$BB(t) \rightarrow BB(t) + [k(7)(S+S_p)R(1)/2]t_a$

and

$S(t) \rightarrow S(t) - [k(7)(S+S_p)R(1)/2] t_a$

This process is repeated for all values of t between t_A and a new second "collapse" point t_B. Finally to calculate $S(t)$ the same proce-

dure applies.

b. Data fitting and "adjustment" of parameters.

The whole body radiochromium(III) retention and plasma radiochromium (III) clearance data points were fitted directly to the model. The parameters $k(I)$'s were then "adjusted" to look for the "best" fit of calculated versus experimental data as well as for the functions $BF(t)$, $BB(t)$, $F(t)$, $A(t)$ and $S(t)$.

C. The FOCAL Program MAMC5C

Preliminary analysis using the general "collapsed" method Focal program, suggested that the BF, BB and the F compartment could be collapsed to one compartment after just the first "collapse" point t_A. This simplified and shortened the original program considerably. Also, one "collapse" point, t_A, was sufficient. This program gave acceptable results for both normal subjects and hemochromatosis. The lowest standard deviation of the difference between calculated and experimental data using logarithmic units was approximately 25%. The mathematical model for this modified program is described in

$t < t_A$

X(1) X(2)   X(3)

BF k(2) BB k(4) F
    k(3)   k(5)

k(1)

k(6) A
k(7)

k(8) S
k(9)

$t > t_A$

X(2)

BF BB F

k(1)

k(6) X(1)
k(7) A

k(8) X(3)
k(9) S
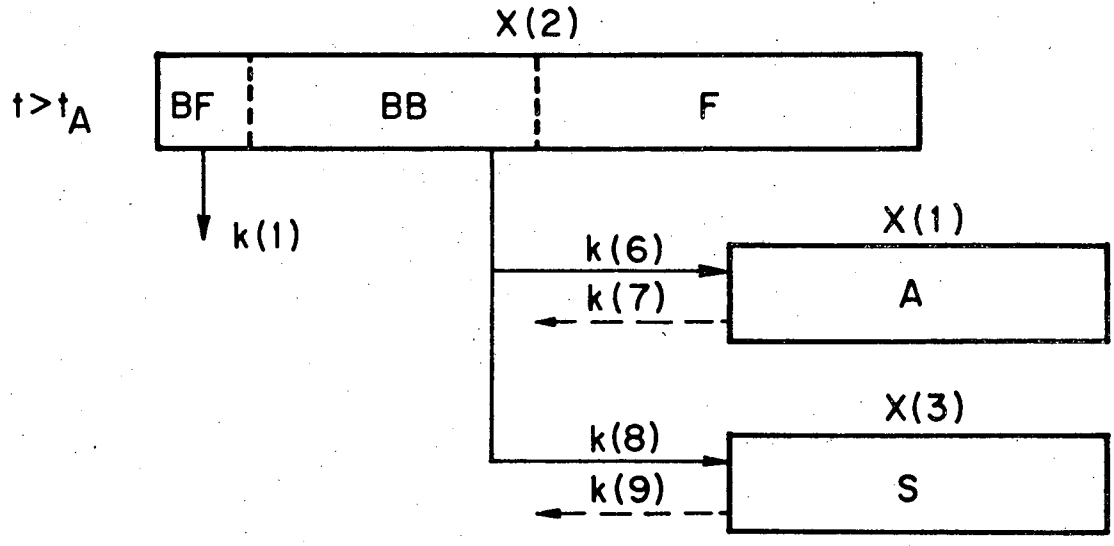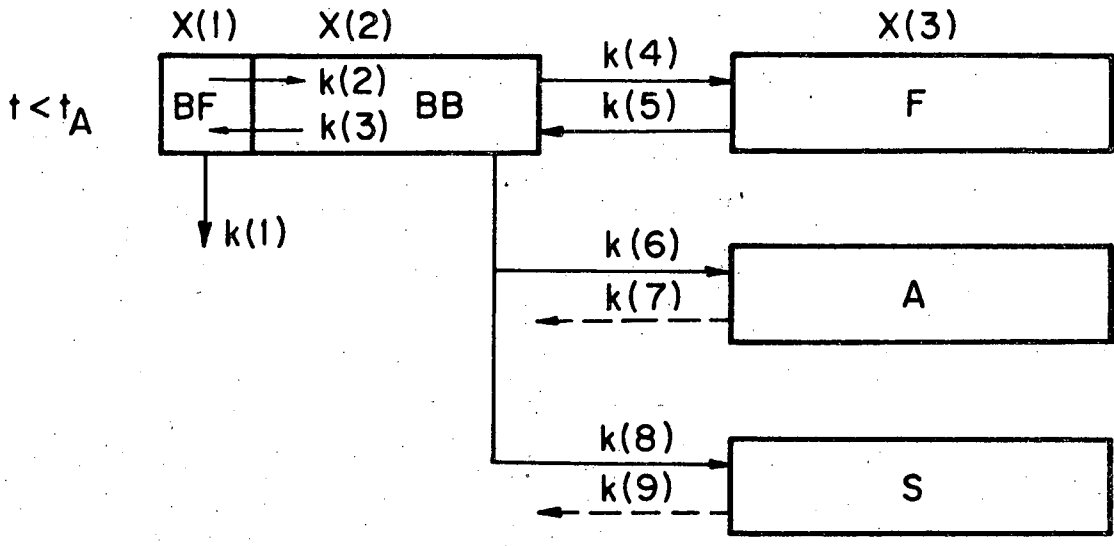
MODEL MAMC5C

XBL7612-10735

Fig. 20

fig. (20). The program in named MAMC5C.
Twenty-two data points were provided for the
input and the program computed nine para-
meters.

Results obtained from this program, al-
though acceptable, are not too satisfactory;
especially during the first period of the
experiment. The errors were rather large.
Preliminary results show that the parameter
k(9) is not sensitive in controlling the
solution. To improve these deficiencies,
26 data points and an additional parameter
k(10) were introduced. k(10) represents the
release of chromium(III) from the body by
some route other than the kidney. The modi-
fied program was MMC5CII.

D. The modified MAMC5C program or the

Focal program MMC5CII

As was mentioned earlier this program
has 26 data points of which the longest time
for some subjects goes beyond 100 days.*
The addition of 2 data points to the whole
body retention input data made k(9) more
sensitive. These additions improved the

---

* Blood radiochromium(III) clearance input data beyond the
50 days period are extrapolated values. For this reason
the two last blood radiochromium(III) clearance data
points in some subjects were ommitted in the calculation.

$t < t_A$

X(1) X(2)   X(3)

BF   →k(2) BB   k(4)→   F
     —k(3)←   ←k(5)

↓k(1) ↓k(10)

k(6)→   A
←k(7)

k(8)→   S
←k(9)

$t > t_A$

X(2)

BF BB   F

↓k(1) ↓k(10)

k(6)→   X(1)
←k(7)   A

k(8)→   X(3)
←k(9)   S
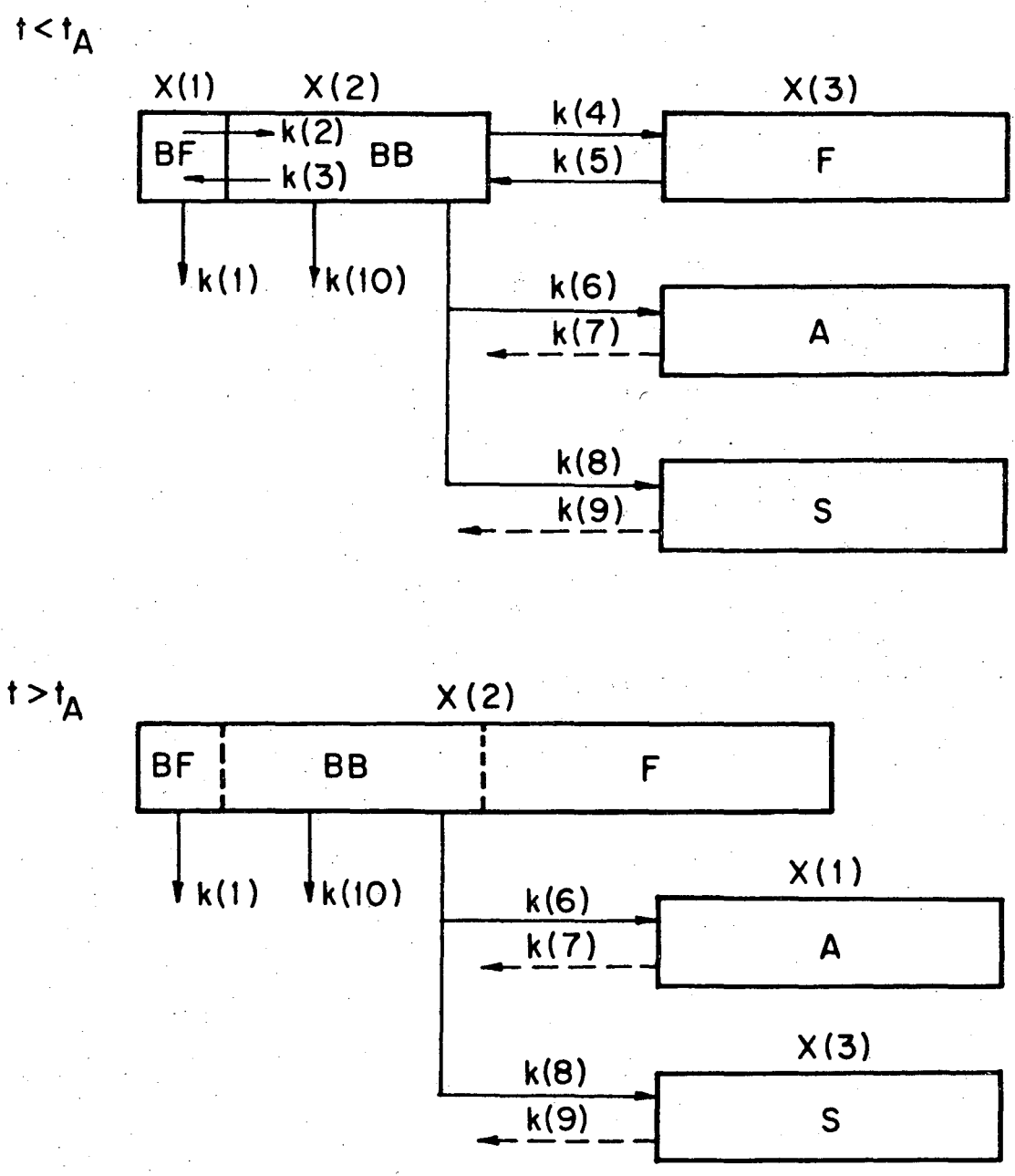
MODEL MMC5C II

XBL7612-10734

Fig. 21

accuracy of the model especially at the early

stages (first hours) of the experiment.

Using this program the standard deviation of

the difference between calculated and experi-

mental data using logarithmic units, for nor-

mals and hemachromatosis averaged around 20%.

Fig. (21) shows the detail of the model

both before and after the "collapse" time t_A.

The complete program is listed in

Appendix (A).

The calculated parameters of 6 subjects

are listed in table (3) and the amount of

radiochromium(III) in different hypothetical

compartments at various times is listed in

tables (4-9).

III.   4.   Summary and Discussion

Although only 3 subjects of each category,

normals and hemochromatosis, were analyzed

in this work, differences in chromium(III)

metabolic parameters and patterns between the

two groups were found.   Except for the first

several minutes post-injection, all experi-

mental data was found within acceptable sta-

tistical error.   The balance error for all

the calculation was not to exceed 0.03%.

Inspection and comparison of experimen-

tal data, different calculated parameters

CALCULATED RATE-CONSTANTS USING THE FOCAL PROGRAM "MMC5CII"

SUB-JECT*	k(1)**	k(2)**	k(3)**	k(4)**	k(5)**	k(6)**	k(7)**	k(8)**	k(9)**	k(10)**	S.D.***
N#1	5.295	247.3	16.000	77.55	124.5	0.871	0.269	0.1894	0.0021	0.1402	0.2230
N#2	5.206	172.0	8.118	44.28	145.0	0.900	0.356	0.1400	0.0030	0.0845	0.2078
N#3	4.000	238.1	12.180	126.50	303.6	0.716	0.305	0.1707	0.0016	0.0881	0.1841
H#1	28.500	292.0	11.000	123.30	53.3	0.807	0.127	0.3050	0.0034	0.0630	0.1734
H#2	18.840	84.88	4.243	41.60	42.4	2.097	0.368	0.2123	0.0051	0.0651	0.2956
H#3	6.772	243.2	8.810	133.4	399.9	0.774	0.287	0.1497	0.0023	0.0731	0.2257

   * For more information concering the subjects, see Appendix B
  ** in [day^{-1}]
*** Standard deviation of the differences of calculated and experimental data
    in logarithmic unit

Table 3

CALCULATED ^{51}Cr(III) DISTRIBUTION IN THE FAST, MEDIUM AND SLOW COMPARTMENT
(% OF TOTAL BODY)--SUBJECT: N#1

T (Days)	F-^{51}Cr Calc.	T-^{51}Cr Calc.	T-^{51}Cr Exp.	Fast Calc.	Medium Calc.	Slow Calc	Excr. Calc.	Excr. Exp.	SD	%BL
0.014	6.5962	67.376	69.50	29.49	0.639	0.139	2.356	2.10	0.2230	0.00
0.027	3.7637	60.789	60.50	34.85	1.298	0.283	2.784	2.40	0.2230	0.00
0.047	3.5315	59.072	57.80	34.83	2.267	0.496	3.332	3.50	0.2230	0.00
0.100	3.3618	57.311	52.70	33.21	4.719	1.040	4.723	4.00	0.2230	0.00
0.324	2.7602	46.209	32.00	27.26	13.52	3.076	9.929	18.00	0.2230	0.00
0.917	1.7087	28.605	13.00	16.88	27.81	6.924	19.78	31.00	0.2230	0.00
3.920	0.4758	7.9647	10.60	4.699	34.09	14.34	38.91	43.0	0.2230	0.00
14.000	0.1625	2.7197	3.380	1.605	12.99	22.33	60.36	56.4	0.2230	0.00
28.000	0.0445	0.7450	0.970	0.440	3.445	25.38	69.99	63.0	0.2230	0.00
36.000	0.0230	0.3848	0.390	0.227	1.705	25.72	71.96	66.0	0.2230	0.00
49.000	0.0101	0.1681	0.140	0.099	0.660	25.61	73.46	70.0	0.2230	0.00
80.000	0.0052	0.0862	0.200	0.051	0.271	24.58	75.01	74.3	0.2230	0.00
135.000	0.0045	0.0759	0.160	0.045	0.233	22.63	77.01	80.0	0.2230	0.00

F-^{51}Cr: % of free ^{51}Cr in blood plasma; T-^{51}Cr: total ^{51}Cr in blood plasma (% of total in the body); EXCR: excretion of ^{51}Cr from the body (% of total in the body); SD: Standard Deviation of the differences of calculated and experimental data; %BL: % balance error.

Table 4

CALCULATED ^{51}Cr(III) DISTRIBUTION IN FAST, MEDIUM, AND SLOW COMPARTMENT
(% OF TOTAL BODY)--SUBJECT: N#2

T (Days)	F-^{51}Cr Calc.	T-^{51}Cr Calc.	T-^{51}Cr Exp.	Fast Calc.	Medium Calc.	Slow Calc.	Excr. Calc.	Excr. Exp.	SD	%BL
0.022	5.0984	75.696	77.60	19.77	1.153	0.180	3.203	2.64	0.2078	0.00
0.045	3.2066	72.109	71.40	21.13	2.582	0.405	3.777	3.60	0.2078	0.00
0.091	3.0297	68.851	65.30	20.22	5.304	0.838	4.782	4.70	0.2078	0.00
0.166	2.8119	64.008	55.00	18.77	9.388	1.503	6.324	8.00	0.2078	0.01
0.356	2.3511	53.518	45.00	15.69	18.02	2.991	9.768	15.4	0.2078	0.01
0.938	1.4547	33.114	30.00	9.710	33.55	6.264	17.36	27.0	0.2078	0.01
1.960	0.8215	18.700	21.60	5.484	41.10	9.595	25.12	34.3	0.2078	0.01
7.000	0.3758	8.5530	10.90	2.508	28.23	17.30	43.41	44.9	0.2078	0.01
15.000	0.1775	4.0394	5.200	1.185	13.26	23.24	58.27	53.4	0.2078	0.01
28.000	0.0563	1.2808	1.700	0.376	4.089	26.40	67.84	62.0	0.2078	0.01
42.000	0.0205	0.4656	0.390	0.137	1.384	26.77	71.24	65.8	0.2078	0.01
84.00	0.0077	0.1743	0.300	0.051	0.429	24.91	74.43	72.1	0.2078	0.01
112.000	0.0071	0.1604	0.200	0.047	0.391	23.53	75.87	75.4	0.2078	0.01

F-^{51}Cr: % of free ^{51}Cr in blood plasma; T-^{51}Cr: total ^{51}Cr in blood plasma (% of total in the body); EXCR: excretion of ^{51}Cr from the body (% of total in the body); SD: Standard Deviation of the differences of calculated and experimental data; %BL: % balance error.

Table 5

CALCULATED ^{51}Cr(III) DISTRIBUTION IN FAST, MEDIUM, AND SLOW COMPARTMENT
(% OF TOTAL BODY)--SUBJECT: N#3

T (Days)	F-^{51}Cr Calc.	T-^{51}Cr Calc.	T-^{51}Cr Exp.	Fast Calc.	Medium Calc.	Slow Calc.	Excr. Calc.	Excr. Exp.	SD	%BL
0.004	39.036	88.543	86.90	10.31	0.088	0.021	1.043	0.90	0.1841	0.00
0.045	3.2721	68.096	69.90	27.07	1.966	0.472	2.397	1.80	0.1841	0.00
0.156	2.9969	62.405	59.20	24.81	6.748	1.648	4.394	7.50	0.1841	0.00
0.96	1.6986	35.370	29.80	14.06	27.92	7.801	14.85	23.10	0.1841	0.00
4.00	0.5787	12.050	14.00	4.790	34.94	17.24	30.98	36.30	0.1841	0.00
10.00	0.3041	6.3329	6.800	2.517	20.39	25.47	45.30	44.70	0.1841	0.00
14.00	0.2098	4.3685	4.500	1.736	14.03	78.73	51.13	48.60	0.1841	0.00
21.00	0.1108	2.3073	2.460	0.917	7.350	32.07	57.36	53.40	0.1841	0.00
29.00	0.0550	1.1458	1.360	0.456	3.586	33.81	61.01	55.60	0.1841	0.00
35.00	0.0337	0.7023	0.740	0.279	2.150	34.37	62.50	58.40	0.1841	0.00
49.00	0.0133	0.2771	0.230	0.110	0.773	34.62	64.22	61.60	0.1841	0.00
63.00	0.0079	0.1635	0.210	0.065	0.407	34.34	65.02	65.20	0.1841	0.00
78.00	0.0063	0.1312	0.200	0.052	0.304	33.90	65.62	67.50	0.1841	0.00

F-^{51}Cr: % of free ^{51}Cr in blood plasma; T-^{51}Cr: total ^{51}Cr in blood plasma (% of total in the body); EXCR: excretion of ^{51}Cr from the body (% of total in the body); SD: Standard Deviation of the differences of calculated and experimental data; %BL: % balance error.

Table 6

CALCULATED ^{51}Cr(III) DISTRIBUTION IN FAST, MEDIUM, AND SLOW COMPARTMENT
(% OF TOTAL BODY)--SUBJECT: H#1

T (Days)	F-^{51}Cr Calc.	T-^{51}Cr Calc.	T-^{51}Cr Exp.	Fast Calc.	Medium Calc.	Slow Calc.	Excr. Calc.	Excr. Exp.	SD	%BL
0.0066	13.450	63.407	57.00	28.33	0.214	0.081	7.972	9.00	0.1734	0.00
0.0930	0.8704	26.194	30.80	59.20	2.163	0.822	11.62	11.0	0.1734	0.00
0.1975	0.8146	24.518	21.20	55.40	4.187	1.603	14.29	22.5	0.1734	0.00
1.9200	0.2932	8.8241	7.400	19.94	21.34	9.273	40.63	50.2	0.1734	0.00
2.8340	0.1842	5.5453	5.400	12.53	23.70	11.13	47.09	53.0	0.1734	0.00
8.0	0.0491	1.4762	2.350	3.336	19.50	14.91	60.78	61.0	0.1734	0.00
20.0	0.0199	0.5981	0.700	1.351	8.237	17.58	72.18	69.0	0.1734	0.00
27.0	0.0125	0.3769	0.380	0.852	5.069	18.15	75.56	72.0	0.1734	0.00
34.0	0.0081	0.2446	0.270	0.553	3.148	18.35	77.71	76.1	0.1734	0.00
48.0	0.0039	0.1177	0.110	0.266	1.312	18.19	80.12	76.8˙	0.1734	0.00
69.0	0.0020	0.0607	0.060	0.137	0.502	17.44	81.86	79.0	0.1734	0.00
94.0	0.0015	0.0454	0.040	0.103	0.304	16.40	83.15	82.6	0.1734	0.00
227.0	0.0010	0.0306	0.010	0.060	0.191	11.64	88.07	89.3	0.1734	0.00

F-^{51}Cr: % of free ^{51}Cr in blood plasma; T-^{51}Cr: total ^{51}Cr in blood plasma (% of total in the body); EXCR: excretion of ^{51}Cr from the body (% of total in the body); SD: Standard Deviation of the differences of calculated and experimental data; %BL: % balance error.

Table 7

CALCULATED ^{51}Cr(III) DISTRIBUTION IN FAST, MEDIUM AND SLOW COMPARTMENT
(% OF TOTAL BODY)--SUBJECT: H#2

T (Days)	F-^{51}Cr Calc.	T-^{51}Cr Calc.	T-^{51}Cr Exp.	Fast Calc.	Medium Calc.	Slow Calc.	Excr. Calc.	Excr. Exp.	SD	%BL
0.0154	21.483	67.645	61.10	16.51	1.059	0.108	14.68	7.20	0.2956	0.00
0.036	4.1177	45.881	49.40	32.23	2.977	0.303	18.61	12.1	0.2956	0.00
0.086	1.4741	36.523	37.50	35.32	6.811	0.700	20.65	23.4	0.2956	0.00
0.178	1.2579	31.874	28.00	30.82	12.77	1.336	23.19	31.6	0.2956	0.01
0.383	0.9375	23.971	18.00	22.97	22.80	2.487	27.75	46.3	0.2956	0.03
0.910	0.4988	12.755	11.20	12.22	35.51	4.360	35.12	57.4	0.2956	0.03
1.920	0.2512	6.4237	6.500	6.156	39.07	6.136	42.19	63.9	0.2956	0.03
3.920	0.1703	4.3539	5.400	4.173	32.96	8.144	50.35	68.9	0.2956	0.03
9.10	0.0994	2.5424	2.580	2.437	19.33	11.43	64.24	74.8	0.2956	0.03
23.0	0.0255	0.6507	0.840	0.624	4.820	14.38	79.51	79.1	0.2956	0.03
51.0	0.0040	0.1029	0.110	0.099	0.634	13.81	85.33	83.5	0.2956	0.03
86.0	0.0025	0.0641	0.005	0.062	0.357	12.04	87.45	86.6	0.2956	0.03
149.0	0.0019	0.0491	0.004	0.047	0.272	9.331	90.27	89.7	0.2956	0.03

F-^{51}Cr: % of free ^{51}Cr in blood plasma; T-^{51}Cr: Total ^{51}Cr in blood plasma (% of total in the body); EXCR: excretion of ^{51}Cr from the body (% of total in the body); SD: Standard Deviation of the differences of calculated and experimental data; %BL: % balance error.

Table 8

CALCULATED ^{51}Cr(III) DISTRIBUTION IN FAST, MEDIUM, AND SLOW COMPARTMENT
(% OF TOTAL BODY)--SUBJECT: H#3

T (Days)	F-^{51}Cr Calc.	T-^{51}Cr Calc.	T-^{51}Cr Exp.	Fast Calc.	Medium Calc.	Slow Calc.	Excr. Calc.	Excr. Exp.	SD	%BL
0.00278	50.381	92.275	91.00	6.297	0.053	0.010	1.365	1.00	0.2257	0.00
0.04653	2.418	70.788	72.00	22.860	2.317	0.451	3.583	3.80	0.2257	0.00
0.10625	2.292	67.123	65.00	21.680	5.328	1.046	4.826	6.60	0.2257	0.00
0.17222	2.163	63.341	55.00	20.460	8.412	1.668	6.124	9.70	0.2257	0.00
0.87500	1.231	36.033	31.00	11.640	29.490	6.538	16.310	25.20	0.2257	0.00
2.0	0.639	18.700	22.80	6.039	39.510	10.700	25.050	38.70	0.2257	0.00
7.0	0.269	7.886	11.40	2.547	29.360	18.510	41.700	46.70	0.2257	0.00
14.0	0.147	4.302	5.10	1.389	16.000	24.140	54.170	49.50	0.2257	0.00
21.0	0.082	2.387	2.70	0.771	8.799	27.010	61.030	55.90	0.2257	0.00
28.0	0.046	1.356	1.38	0.438	4.922	28.410	64.870	60.00	0.2257	0.00
36.0	0.026	0.745	0.81	0.241	2.627	29.060	67.320	61.20	0.2257	0.00
49.0	0.012	0.339	0.35	0.109	1.100	29.150	69.300	64.10	0.2257	0.00
64.0	0.007	0.198	0.18	0.064	0.575	28.710	70.450	67.60	0.2257	0.00

F-^{51}Cr: % of free ^{51}Cr in blood plasma; T-^{51}Cr: total ^{51}Cr in blood plasma (% of total in the body); EXCR: excretion of ^{51}Cr from the body (% of total in the body); SD: Standard Deviation of the differences of calculated and experimental data; %BL: % balance error.

Table 9

and hypothetical compartments in normal sub-
jects and hemochromatotic patients lead to
the following comments:

A. The turnover rate of free chromium(III)
in the blood is 3 to 6 times higher in hemo-
chromatosis compared to normals. The fact
that the amount of free chromium(III), in
vitro as well as in vivo, is also 3 to 6
times higher in hemochromatosis compared to
normals, indicates that the transfer rate
k(1) is linearily dependent on the amount
of free chromium(III). The higher percen-
tage of free chromium(III) in hemochroma-
totic patients compared to normal subjects
can be explained by the fact that the plasma
protein transferrin in hemochromatosis is
more saturated with iron (>70%) compared to
normals, (<50%). This is presumably, because
more binding-sites for chromium(III) are
available in normals compared to patients
suffering from hemochromatosis.

B. The much larger k(8) (absorbtion in the
slow compartment) compared to k(9) (release
from the slow compartment) in both normals
and hemochromatosis suggest the existence
of chromium(III) storage in the hypothetical
slow compartment.

C. This model assumed that some chromium (III) in the plasma bound pool is excreted from the body by some route other than the kidney with a rate of k(10). The physiological significance of such excretion is not understood. However, we believe that there may be a relationship between k(10) and the involvement of chromium(III) in cell organelles, so loss of dead cells from the body would result in a loss of chromium(III). This route of loss (approximately 2% of k(1), the loss through the kidney into the urine), is a reasonable non-urinary mechanism to which k(10) may be attributed. For all subjects, k(10) has a rather constant value, compared to k(1), which has much larger values in subjects suffering from hemochromatosis.

D.   Further analysis of the liver, spleen
and thigh clearance curves*, resulted in the
following relative compartment sizes of the
Fast, Medium and Slow compartment:

	F.	M.	S.
Liver N#2	30%+27%	23%+27%	47%+27%
Spleen N#2	47%+ 2%	18%+ 2%	35%+ 2%
Thigh N#2	52%+11%	20%+11%	28%+11%
Liver H#1	28%+13%	47%+13%	25%+13%
Spleen H#1	25%+ 1%	22%+ 1%	53%+ 1%
Thigh H#1	39%+10%	36%+10%	25%+10%

Table 10

* in one normal and one patient suffering
  from heomchromatosis

E.   The model assumed that all parameters
had constant values during the course of the
experiment.   However, during the first day
post injection further inspection of calcu-
lated versus experimental whole body and
blood plasma radiochromium(III) clearance
data, indicated that a large deviation
occurred (up to 40%) every time data was
collected after food intake.*   The large
deviation can probably be associated with
the increase in body metabolic activity

* approximately 3 hours post injection

during that time; probably either a variation
occurred in the parameters or a nonlinear
relation existed that leads to the large
difference between calculated and experimen-
tal data. This phenomena deserves further
investigations by future researchers.

F. The sensitivity to a change of the para-
meters k(I)'s was measured by calculating
the resultant fractional change in the
standard deviation of the differences of
calculated and experimental values in log-
arithmic unit ($\Delta SD/SD \times 100$) for a 20%
increase in each k(I) independently.

The values are listed in table 11.
The parameters k(1), k(2) and k(3) were
found most sensitive to the change. The
most insensitive parameters were k(5) and
k(9).

G. It is important to note that subject H#3
is a 38 year old male in the early stages of
hemochromatosis*. His plasma, liver, thigh
and spleen radiochromium(III) clearance and
uptake pattern (fig. 22) as well as the cal-
culated parameters** (tables 3,9) can be

---

* his brother died of hemochromatosis and his mother has
an elevated serum iron.
** calculated from the chromium(III) kinetic model.

Table of $\frac{\Delta SD}{SD} \times 100$

for an increase of 20% in k(I)

|	k(1)	k(2)	k(3)	k(4)	k(5)	k(6)	k(7)	k(8)	k(9)	k(10)
N#1	16.6	15.7	11.0	2.8	1.3	5.3	7.7	5.3	1.5	1.7
N#2	17.7	16.1	13.1	2.0	1.9	5.1	6.5	5.3	1.3	2.1
N#3	21.3	18.0	13.7	2.9	0.3	9.1	11.8	11.8	1.8	2.3
H#1	52.1	29.9	54.1	1.0	2.6	0	8.2	7.2	2.0	1.6
H#2	13.0	11.0	9.5	0.7	0.2	2.2	2.2	2.1	0.7	0.1
H#3	14.6	6.7	8.0	0.6	0.6	2.4	3.3	3.3	0	1.2
Average	22.6	16.2	18.2	1.7	1.2	4.0	6.6	5.8	1.2	1.5
	±14.75	±7.85	±17.7	±1.0	±.96	±3.18	±3.49	±4.54	±0.75	±0.79

Table 11

considered to be within the range of normal

subjects. Laboratory tests indeed showed

60% saturation of iron binding capacity

(30-50% in normals). Scan-images (fig. 23)

did not show any enlargement of the liver,

and the concentration of radiochromium(III)

in the liver and thigh were found slightly

higher than in the average normal. Anterior

scan-images of the body did not clearly show

the spleen, although the spleen chromium(III)

concentration was almost as high as in the

average normal (fig. 22). His transfer-

rates $k(4)$ and $k(5)$ are higher than in the

average normal with a high $k(5)/k(4)$ ratio.

H. At the present time the chromium(III)

kinetic model, presented in this work, is

the only model which is based on results ob-

tained from experiments with human subjects.

As far as we know, it is in fact the first

model of chromium(III) metabolism of any

form to be proposed, and it is based on in

vivo metabolism of a metabolically unperturbed

system. It was reported earlier that

chromium(III) is important for glucose

metabolism in humans. In this context,

hopefully in the future, the model will be
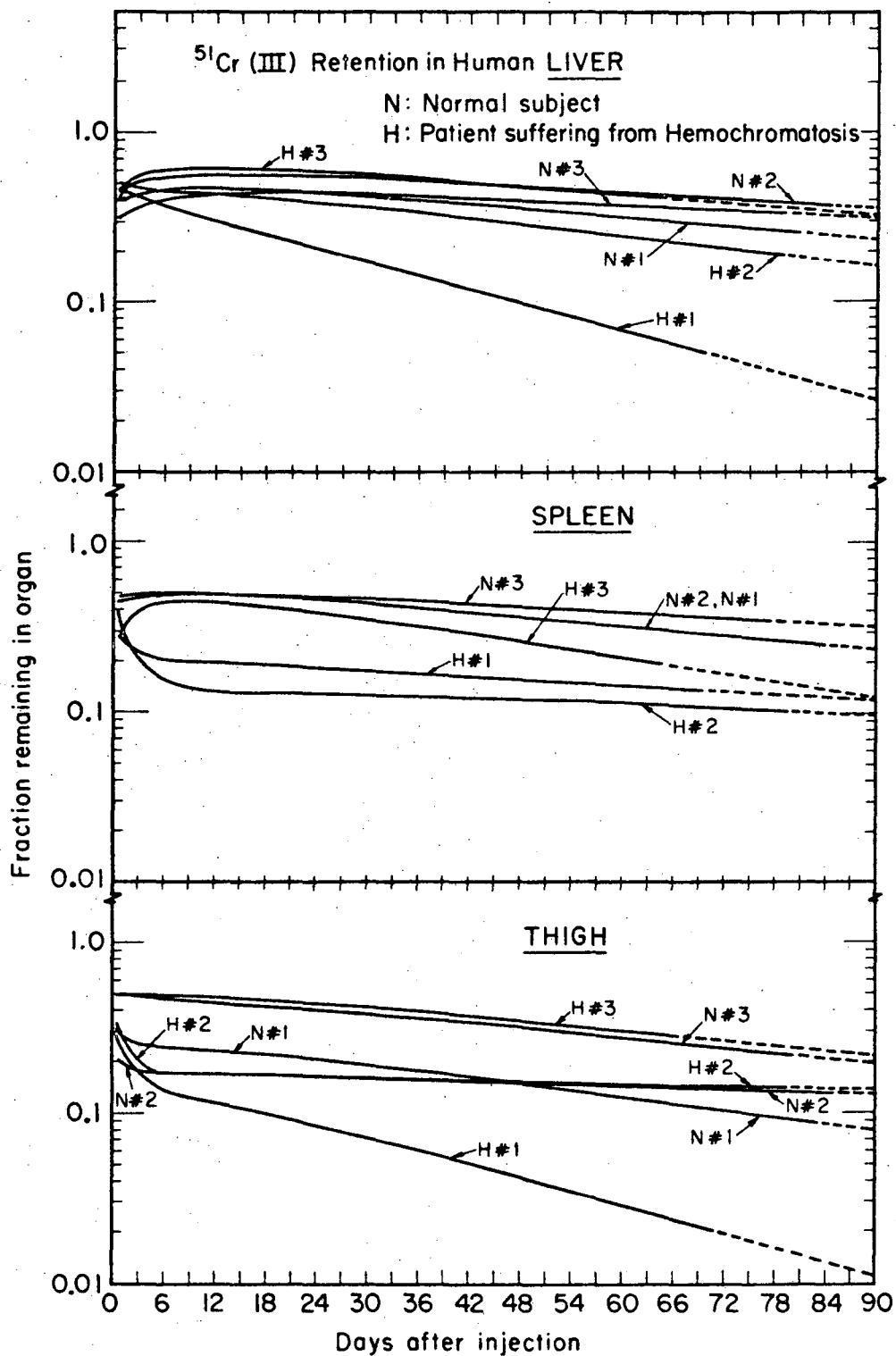
Fig. 22

^{51}Cr (III) DISTRIBUTION
IN DIFFERENT ORGANS

Liver
(not enlarged)

Spleen
(not clearly visible)

Spine

Liver

Hipbone

Thigh

ANTERIOR
28 Days
Post injection

POSTERIOR
7 Days
Post injection
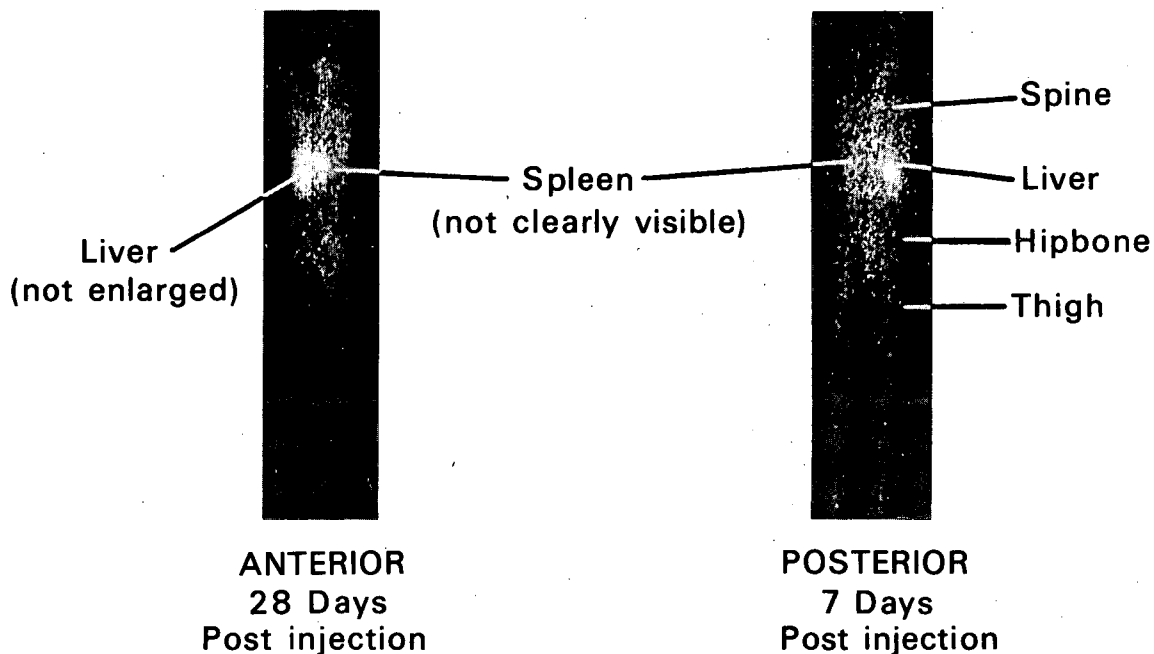
SUBJECT H #3
EARLY STAGE HEMOCHROMATOSIS

XBB 771-155

Fig.23

useful in assessing nutritional requirements
of chromium(III) in the human diet, and its
importance in disease processes such as
diabetes.

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APPENDIX A

```
C-PS/8 FOCAL, 1971

01.01 T "MMC5C2: MAMM 5 CMPTS; LA1<<LA2..<<LA5",!
01.09 E
01.30 D 21; D 10.2; D 10.21; T !!
01.40 G 10.1


03.10 S P1=1/(K(1)+K(2));S TA=1/(K(3)+K(4)); T "T1,T2,T3"
03.20 T 3*P1,3*TA,3/K(5); S TA=5*(P1+TA+1/K(5)); T " TA",TA
03.30 F I=1,NP; D 19
03.40 T "  P1",P1,!


05.01 S A=0; S S=0; S U=0; S ST=0; S SS=0; F I=1,3; S X(I)=0
05.05 S II=1; S X(1)=100; S R(1)=0; S R(2)=0
05.10 S K1=K(1);S K2=K(2);S K3=K(3);S K4=K(4);S K5=K(5);S K6=K(6)+K(8)+█
05.11 D 14
05.20 F J=1,P1-1; S TI=T(J)-T(J-1); D 6; D 8
05.25 S TI=TA-T(P1-1); D 6
05.30 S R(1)=X(1)/X(2); S R(2)=X(3)/X(2); S X(1)=A; S X(3)=S; S II=2
05.32 S K1=0; S K2=K(7); S K3=K(6); S K4=K(8); S K5=K(9); S K6=K(1)*R(1█
05.33 D 14
05.34 S TI=T(P1)-TA; D 6; D 8
05.35 F J=P1+1,NP;S TI=T(J)-T(J-1); D 6; D 8
05.70 S SS=SS-(FLOG(B/BX(NP)))↑2-(FLOG(BL/BX(NP-1)))↑2
05.80 S SQ=FSQT(SS/(NP*2-2))
05.85 T %2.00,JJ," L SD",%5.04,SQ,!; R
05.87 T %5.04,"R",R(1)*K(2)/K(3),R(2)*K(5)/K(4),K(7)*X(1)/(K(6)*X(2)),!


06.05 S BL=B
06.10 S AO=A; S SO=S; D 15
06.25 I (II-2) 6.3,6.55,6.55
06.30 S A=AO+K(6)*YI; S CA=K(7)*(A+AO)*TI/(2*A+K(7)*(AO+A)*TI)
06.32 S A=A*(1-CA)
06.35 S S=SO+K(8)*YI; S CS=K(9)*(S+SO)*TI/(2*S+K(9)*(S+SO)*TI)
06.36 S S=S*(1-CS)
06.50 S X(2)=X(2)+(A*CA+S*CS)*R; S B=X(1)+X(2); S U=U+K(1)*XI+K(10)*YI
06.52 S ST=B+X(3)+A+S+U; R
06.55 S B=X(2)*(1+R(1)); S U=U+K(1)*R(1)*YI+K(10)*YI
06.60 S ST=X(2)*(1+R(1)+R(2))+X(1)+X(3)+U


08.02 I (-TY+.2) 8.04,8.2,8.2
08.04 T %3.02,T(J),%5.04
08.06 I (II-2) 8.1,8.15,8.15
08.10 T X(1)," ",B,%4.03,BX(J)," ",X(3),A,S; G 8.17
08.15 T R(1)*X(2)," ",B,%4.03,BX(J)," ",R(2)*X(2),X(1),X(3)
08.17 T " ",U,%3.02,UX(J)," %BAL",100-ST,!
08.20 I (BX(J)-50) 8.3,8.3,8.4
08.30 S SS=SS+(FLOG(B/BX(J)))↑2; G 8.5
08.40 S SS=SS+(FLOG((100-B)/(100-BX(J))))↑2
08.50 I (UX(J)-50) 8.6,8.6,8.7
08.60 S SS=SS+(FLOG(U/UX(J)))↑2; R
08.70 S SS=SS+(FLOG((100-U)/(100-UX(J))))↑2
```

```
10.10  S  TY=1; D 3; D 5; S SF=SQ; S TY=0
10.15  S  Z=1+.2*SF; S RE=FABS(Z-1)/100
10.20  T  !"Z",%4.03,Z,!,"K:"; F I=1,7; T K(I)
10.21  T  %6.05; F I=8,10; T K(I)
10.23  T  !; F JJ=1,PN; D 12
10.30  S  JJ=1; D 12; S JJ=10; D 12
10.50  S  Z=1+.5*FABS(Z-1)+.1*SF/FSQT(2*NP); S RE=(Z-1)/100
10.60  I  (SF-.05) 10.7,10.7,10.8
10.70  T  !; D 5; D 10.2; D 9; Q
10.80  S  MM=MM+1; T !
10.85  I  (MM-3) 10.92,10.9,10.9
10.90  S  MM=0;S TY=1;D 10.2;D 10.21;T !!;D 10.1;D 5.87;S QQ=FABS(QQ-SF)
10.91  I  (-QQ+.001) 10.92,10.92; T !"G 10.2 OR 10.15 FOR >Z?"; Q
10.92  S  QQ=SF; T !"L SD ",SF,"  "; D 3; D 5; S SF=SQ; G 10.2


12.10  S  K(JJ)=Z*K(JJ); D 5; S K(JJ)=K(JJ)/Z
12.20  I  (SQ-SF-RE) 12.3,12.6,12.6
12.30  I  (SQ-SF) 12.4; R
12.40  I  (SQ-SF+RE) 12.5,12.7,12.7
12.50  D  12.7; D 12.1; G 12.3
12.60  S  Z=1/Z; D 12.1; G 12.3
12.70  S  K(JJ)=K(JJ)*Z; S SF=SQ


14.10  S  R=1/(1+R(1)+R(2))
14.30  S  PP(1)=K1+K2+K5+R*(K3+K4+K6)
14.35  S  PP(2)=K5*(K1+K2)+R*(K1*K3+(K1+K2)*(K4+K6)+K5*(K3+K6))
14.40  S  PP(3)=R*K5*(K1*K6+K2*K6+K1*K3)
14.45  S  AA(1)=PP(2)-PP(1)↑2/3; S AA(2)=2*PP(1)↑3/27-PP(1)*PP(2)/3+PP(3)
14.50  I  (AA(2)) 14.51,14.52,14.53
14.51  S  ZZ=1; G 14.6
14.52  S  AA(3)=1.5707965; G 14.8
14.53  S  ZZ=-1
14.60  S  AA(3)=-6.75*AA(2)↑2/AA(1)↑3
14.75  S  AA(3)=FATN(FSQT(1/AA(3)-1))
14.80  S  A(3)=ZZ*2*FSQT(-AA(1)/3);S A(1)=A(3)*FCOS(AA(3)/3)
14.85  S  A(2)=A(3)*FCOS(AA(3)/3+2.094395);S A(3)=A(3)*FCOS(AA(3)/3+4.1889
14.90  S  A(1)=A(1)-PP(1)/3; S A(2)=A(2)-PP(1)/3; S A(3)=A(3)-PP(1)/3
14.95  I  (-A(2)) 14.96; R
14.96  S  A(2)=FSQT(PP(1)↑2/4-PP(2)); S A(1)=-PP(1)/2-A(2)
14.97  S  A(3)=-PP(1)/2+A(2); S A(2)=0
```

```
15.05 S XD(1)=-(K1+K2)*X(1)+K3*X(2); S XD(3)=K4*X(2)-K5*X(3)
15.10 S XD(2)=R*(K2*X(1)-(K3+K4+K6)*X(2)+K5*X(3))
15.15 S XS(1)=-(K1+K2)*XD(1)+K3*XD(2); S XS(3)=K4*XD(2)-K5*XD(3)
15.20 S XS(2)=R*(K2*XD(1)-(K3+K4+K6)*XD(2)+K5*XD(3))
15.25 S DN=(A(1)-A(2))*(A(3)-A(1)); S DM=(A(1)-A(2))*(A(2)-A(3))
15.28 F I=1,3; D 15.85; D 15.9
15.30 F I=1,3; S PP(I)=FEXP(A(I)*TI)
15.35 I (-A(1)*TI-100) 15.45,15.45,15.36
15.36 S PP(1)=0
15.45 S XI=-A(2)*TI
15.46 I (XI-.001) 15.47,15.47,15.48
15.47 S XI=AB(1)*TI; S YI=AB(2)*TI; G 15.5
15.48 S XI=AB(1)*(PP(2)-1)/A(2); S YI=AB(2)*(PP(2)-1)/A(2)
15.50 S XI=XI+AA(1)*(PP(1)-1)/A(1); S YI=YI+AA(2)*(PP(1)-1)/A(1)
15.55 S XI=XI+(X(1)-AA(1)-AB(1))*(PP(3)-1)/A(3)
15.56 S YI=YI+(X(2)-AA(2)-AB(2))*(PP(3)-1)/A(3)
15.60 F I=1,3; S X(I)=AA(I)*PP(1)+AB(I)*PP(2)+(X(I)-AA(I)-AB(I))*PP(3)
15.66 R; T %5.04, T(J),X(1),X(2),X(3),A,S,U,!
15.67 T "XI",XI," YI",YI,!; R
15.85 S AA(I)=(XD(I)*(A(2)+A(3))-A(2)*A(3)*X(I)-XS(I))/DN
15.90 S AB(I)=(XD(I)*(A(1)+A(3))-A(1)*A(3)*X(I)-XS(I))/DM

19.10 I (TA-T(I)) 19.2; R
19.20 S P1=I; S I=NP+1; R

21.01 T "NORM #3",!
21.10 S T(1)=.004;S T(2)=.045;S T(3)=.156;S T(4)=.96;S T(5)=4
21.11 S T(6)=10;S T(7)=14;S T(8)=21;S T(9)=29;S T(10)=35;S T(11)=49
21.12 S K(1)=3.9173;S K(2)=246.6;S K(3)=13.06;S K(4)=135.6;S K(5)=344.07
21.13 S K(6)=.7675;S K(7)=.305;S K(8)=.1707; S K(9)=.0014;S K(10)=.09505
21.20 S BX(1)=86.9;S BX(2)=69.9;S BX(3)=59.2;S BX(4)=29.8;S BX(5)=14
21.30 S BX(6)=6.8;S BX(7)=4.5;S BX(8)=2.46;S BX(9)=1.36;S BX(10)=.74
21.40 S BX(11)=.23
21.50 S UX(1)=.9;S UX(2)=1.8;S UX(3)=7.5;S UX(4)=23.1;S UX(5)=36.3
21.60 S UX(6)=44.7;S UX(7)=48.6;S UX(8)=53.4;S UX(9)=55.6;S UX(10)=58.4
21.70 S UX(11)=61.6
21.90 S NP=13; S PN=10
21.95 S T(12)=63; S T(13)=78;S  BX(12)=.21;S BX(13)=.2;
21.96 S UX(12)=65.2;S UX(13)=67.5;
*

*
```

```
*3
MMC5C2: MAMM 5 CMPTS; LA1<<LA2..<<LA5
NORM #3

Z 0.000
K: 4.000 238.0 12.18 126.5 303.6 0.716 0.305 0.17070 0.00156 0.08805

T1,T2,T3 0.01240 0.02163 0.00988 TA 0.07319  P1 3.00000
   0.01 39.255    88.544 86.90    10.31 0.038 0.321    1.243 0.90  %BAL 0.00
   0.05 3.2740    68.090 69.90    27.07 1.966 0.472    2.399 1.30  %BAL 0.00
   0.16 2.9985    62.399 59.20    24.31 6.749 1.648    4.396 7.50  %BAL 0.00
   0.96 1.6992    35.360 29.80    14.06 27.93 7.822    14.86 23.1  %BAL 0.00
   4.00 0.5786    12.040 14.00    4.737 34.95 17.23    30.99 36.3  %BAL 0.00
   10.0 0.3041    6.3289 6.800    2.516 20.41 25.45    45.32 44.7  %BAL 0.00
   14.0 0.2099    4.3668 4.500    1.736 14.05 28.72    51.13 48.6  %BAL 0.00
   21.0 0.1109    2.3074 2.460    0.917 7.361 32.35    57.36 53.4  %BAL 0.00
   29.0 0.0551    1.1463 1.360    0.456 3.593 33.79    61.02 55.6  %BAL 0.00
   35.0 0.0338    0.7029 0.740    0.280 2.154 34.35    62.51 58.4  %BAL 0.00
   49.0 0.0133    0.2774 0.230    0.110 0.775 34.61    64.23 61.6  %BAL 0.00
   63.0 0.0079    0.1636 0.210    0.065 0.407 34.33    65.04 65.2  %BAL 0.00
   78.0 0.0063    0.1313 0.200    0.052 0.304 33.88    65.63 67.5  %BAL 0.00
    0   L SD 0.1768

Z 1.035
K: 4.000 238.0 12.18 126.5 303.6 0.716 0.305 0.17070 0.00156 0.08805
    1   L SD 0.1778
    1   L SD 0.1788
    2   L SD 0.1786
    2   L SD 0.1777
    3   L SD. 0.1767
    4   L SD 0.1766
    5   L SD 0.1762
    5   L SD 0.1760
    6   L SD 0.1750
    6   L SD 0.1753
    7   L SD 0.1760
    7   L SD 0.1758
    8   L SD 0.1760
    8   L SD 0.1754
    9   L SD 0.1754
    9   L SD 0.1747
   10   L SD 0.1749
    1   L SD 0.1771
    1   L SD 0.1753
   10   L SD 0.1749


L SD  0.1747  T1,T2,T3 0.0124 0.0209 0.0092 TA 0.0709  P1 3.0000
   10   L SD 0.1747
```

Z 1.021
K: 4.000 238.0 12.61 131.0 325.5 0.741 0.305 0.17070 0.00151 0.08805
```
   1   L SD 0.1758
   1   L SD 0.1747
   2   L SD 0.1747
   3   L SD 0.1742
   3   L SD 0.1745
   4   L SD 0.1742
   5   L SD 0.1740
   5   L SD 0.1738
   6   L SD 0.1736
   7   L SD 0.1737
   8   L SD 0.1739
   8   L SD 0.1738
   9   L SD 0.1739
   9   L SD 0.1734
   9   L SD 0.1732
   9   L SD 0.1730
  10   L SD 0.1729
   1   L SD 0.1741
   1   L SD 0.1729
  10   L SD 0.1726
  10   L SD 0.1725
```

L SD 0.1725   T1,T2,T3 0.0122 0.0205 0.0089 TA 0.0691 P1 3.0000
```
  10   L SD 0.1725
```

Z 1.014
K: 3.917 243.0 12.88 133.7 339.3 0.757 0.305 0.17070 0.00142 0.09375
```
   1   L SD 0.1730
   1   L SD 0.1726
   2   L SD 0.1725
   3   L SD 0.1723
   3   L SD 0.1724
   4   L SD 0.1723
   5   L SD 0.1721
   6   L SD 0.1720
   7   L SD 0.1720
   8   L SD 0.1722
   8   L SD 0.1721
   9   L SD 0.1722
   9   L SD 0.1719
  10   L SD 0.1718
   1   L SD 0.1722
   1   L SD 0.1719
  10   L SD 0.1718
```

```
Z 1.010
K: 3.917 246.4 13.06 135.6 344.1 0.768 0.305 0.17070 0.00140 0.09505

T1,T2,T3 0.01199 0.02018 0.00872 TA 0.06814  P1 3.00000
  0.01 37.897    88.148 86.90    10.73 0.096 0.021    1.009 0.90   %BAL 0.00
  0.05 3.4367    69.097 69.90    25.94 2.137 0.479    2.350 1.80   %BAL 0.00
  0.16 3.1299    62.951 59.20    23.63 7.316 1.666    4.438 7.50   %BAL 0.00
  0.96 1.7118    34.430 29.80    12.92 29.74 7.757    15.15 23.1   %BAL 0.00
  4.00 0.5744    11.553 14.00    4.336 36.23 16.78    31.10 36.3   %BAL 0.00
  10.0 0.3083    6.2007 6.800    2.327 21.24 24.77    45.46 44.7   %BAL 0.00
  14.0 0.2139    4.3028 4.500    1.615 14.71 27.99    51.38 48.6   %BAL 0.00
  21.0 0.1140    2.2920 2.460    0.860 7.781 31.32    57.75 53.4   %BAL 0.00
  29.0 0.0569    1.1439 1.360    0.429 3.826 33.10    61.50 55.6   %BAL 0.30
  35.0 0.0348    0.7000 0.740    0.263 2.298 33.69    63.05 58.4   %BAL 0.00
  49.0 0.0133    0.2679 0.230    0.101 0.810 34.03    64.80 61.6   %BAL 0.00
  63.0 0.0075    0.1499 0.210    0.056 0.405 33.81    65.58 65.2   %BAL 0.00
  78.0 0.0058    0.1158 0.200    0.044 0.289 33.42    66.13 67.5   %BAL 0.00
  10   L SD 0.1718
```

N#1

0.02	6.5962	67.376	69.50	29.49	0.639	0.139	2.356	2.10	%BAL	0.00
0.03	3.7637	60.789	60.50	34.85	1.298	0.283	2.784	2.40	%BAL	0.00
0.05	3.5315	59.072	57.80	34.83	2.267	0.496	3.332	3.50	%BAL	0.00
0.10	3.3618	56.311	52.70	33.21	4.719	1.042	4.723	4.00	%BAL	0.00
0.32	2.7602	46.209	30.00	27.26	13.52	3.076	9.929	18.0	%BAL	0.00
0.92	1.7087	23.605	23.00	16.88	27.81	6.924	19.78	31.5	%BAL	0.00
3.92	0.4758	7.9647	10.60	4.699	34.09	14.34	38.91	43.3	%BAL	0.00
14.0	0.1625	2.7197	3.380	1.635	12.99	22.33	60.36	56.4	%BAL	0.00
28.0	0.0445	0.7450	0.970	0.440	3.445	25.38	69.99	63.0	%BAL	0.00
36.0	0.0230	0.3848	0.390	0.227	1.705	25.72	71.96	66.0	%BAL	0.00
49.0	0.0171	0.1681	0.140	0.099	0.660	25.61	73.46	70.0	%BAL	0.00
80.0	0.0052	0.0862	0.200	0.051	0.271	24.58	75.01	74.3	%BAL	0.00
135	0.0045	0.0759	0.160	0.045	0.233	22.63	77.01	80.0	%BAL	0.00

K: 5.295 247.3 16.00 77.55 124.5 0.871 0.269 0.18942 0.00210 0.14317

1 L SD 0.2144

N#2

K: 5.206 172.0 8.118 44.28 145.0 0.900 0.356 0.14000 0.00297 0.08452

T1,T2,T3 0.01693 0.05725 0.02069 TA 0.15812 PI 4.00000

0.02	5.0984	75.696	77.60	19.77	1.153	0.180	3.203	2.64	%BAL	0.00
0.05	3.2066	72.129	71.40	21.13	2.582	0.405	3.777	3.60	%BAL	0.00
0.09	3.0297	68.851	65.30	20.22	5.304	0.838	4.782	4.70	%BAL	0.00
0.17	2.8119	64.408	55.00	18.77	9.388	1.503	6.324	8.00	%BAL	0.01
0.36	2.3511	53.513	45.00	15.69	18.02	2.991	9.768	15.4	%BAL	0.01
0.94	1.4547	33.114	30.00	9.710	33.55	6.264	17.36	27.0	%BAL	0.01
1.96	0.8215	18.700	21.60	5.484	41.10	9.595	25.12	34.3	%BAL	0.01
7.00	0.3758	8.5530	10.90	2.508	28.23	17.30	43.41	44.9	%BAL	0.01
15.0	0.1775	4.0394	5.200	1.185	13.26	23.24	58.27	53.4	%BAL	0.01
28.0	0.0563	1.2388	1.700	0.376	4.089	26.40	67.84	62.0	%BAL	0.01
42.0	0.0205	0.4656	0.390	0.137	1.334	26.77	71.24	65.3	%BAL	0.01
84.0	0.0077	0.1743	0.300	0.051	0.429	24.91	74.43	72.1	%BAL	0.01
112	0.0071	0.1634	0.200	0.047	0.391	23.53	75.87	75.4	%BAL	0.01

10 L SD 0.1993

H#1
T1,T2,T3 0.0094 0.0224 0.0563 TA 0.1466 PI 3.0000
```
0.01 13.450    63.407 57.00    28.33 0.214 0.081    7.972  9.00   %BAL 0.00
0.09 0.8704    26.194 30.80    59.20 2.163 0.822    11.62 11.0    %BAL 0.00
0.20 0.8146    24.513 21.20    55.40 4.137 1.603    14.29 22.5    %BAL 0.00
1.92 0.2932    8.8241 7.400    19.94 21.34 9.273    43.63 50.2    %BAL 0.00
2.84 0.1842    5.5453 5.400    12.53 23.70 11.13    47.39 53.0    %BAL 0.00
8.00 0.0491    1.4762 2.350    3.336 19.50 14.91    63.79 61.0    %BAL 0.00
20.0 0.0199    0.5981 0.700    1.351 8.237 17.58    72.18 69.0    %BAL 0.00
27.0 0.0125    0.3769 0.380    0.852 5.069 18.15    75.56 72.0    %BAL 0.00
34.0 0.0081    0.2446 0.270    0.553 3.148 18.35    77.71 76.1    %BAL 0.00
48.0 0.0039    0.1177 0.110    0.266 1.312 18.19    83.12 76.8    %BAL 0.00
69.0 0.0020    0.0607 0.060    0.137 0.502 17.44    81.86 79.0    %BAL 0.00
94.0 0.0015    0.0454 0.040    0.103 0.304 16.40    83.15 82.6    %BAL 0.00
227  0.0010    0.0306 0.010    0.069 0.191 11.64    83.87 89.3    %BAL 0.00
  1   K SD 0.1667
```

z 1.033
K: 28.50 292.0 11.00 123.0 53.34 3.807 0.127 0.33584 0.00336 0.06302

H#2
T1,T2,T3 0.02893 0.06545 0.07073 TA 0.27513 PI 5.00000
```
0.02 21.483    67.645 61.10    16.51 1.059 0.103    14.63  7.20   %BAL 0.00
0.04 4.1177    45.831 49.40    32.23 2.977 0.303    18.61 12.1    %BAL 0.00
0.09 1.4741    36.523 37.50    35.32 6.811 0.700    20.65 23.4    %BAL 0.00
0.18 1.2579    31.874 28.00    30.92 12.77 1.336    23.19 31.6    %BAL 0.01
0.38 0.9375    23.971 18.00    22.97 22.80 2.437    27.75 46.3    %BAL 0.03
0.91 0.4988    12.755 11.20    12.22 35.51 4.360    35.12 57.4    %BAL 0.03
1.92 0.2512    6.4237 6.500    6.156 39.07 6.136    42.19 63.9    %BAL 0.03
3.92 0.1703    4.3539 5.400    4.173 32.96 8.144    50.35 68.9    %BAL 0.03
9.10 0.0994    2.5424 2.580    2.437 19.33 11.43    64.24 74.3    %BAL 0.03
23.0 0.0255    0.6577 0.840    0.624 4.323 14.38    79.51 79.1    %BAL 0.03
51.0 0.0040    0.1229 0.110    0.099 0.634 13.81    85.33 83.5    %BAL 0.03
86.0 0.0025    0.0641 0.005    0.062 0.357 12.34    87.45 86.6    %BAL 0.03
149  0.0019    0.0491 0.004    0.047 0.272 9.331    90.27 89.7    %BAL 0.03
 10   L SD 0.2842
```
  K: 18.84 84.38 4.243 41.60 42.41 2.097 0.363 0.21232 0.00512 0.06514

H#3

Z 1.009
K: 6.772 243.2 8.810 133.4 399.9 2.774 0.237 0.14972 0.00227 0.07311

T1,T2,T3 0.01200 0.02109 0.00750 TA 0.06766   P1 3.00000
     0.00 50.381   92.275 91.00   6.297 2.053 0.010   1.365 1.00   %BAL 0.00
     0.05 2.4183   70.783 72.00   22.86 2.317 0.451   3.583 3.80   %BAL 0.00
     0.11 2.2924   67.123 65.00   21.63 5.328 1.046   4.826 6.60   %BAL 0.00
     0.17 2.1632   63.341 55.00   20.46 8.412 1.663   6.124 9.70   %BAL 0.00
     0.88 1.2306   36.033 31.00   11.64 29.49 6.533   16.31 25.2   %BAL 0.00
     2.04 0.6386   13.732 22.80   6.039 39.51 10.70   25.05 38.7   %BAL 0.00
     7.04 0.2692   7.8363 11.40   2.547 29.36 13.51   41.72 46.7   %BAL 2.00
    14.0 0.1469   4.3019 5.100   1.389 16.00 24.14   54.17 49.5   %BAL 0.00
    21.0 0.0815   2.3369 2.720   0.771 3.799 27.01   61.03 55.9   %BAL 0.00
    28.0 0.0463   1.3553 1.380   0.433 4.922 28.41   64.37 60.0   %BAL 3.00
    36.0 0.0255   0.7453 0.810   0.241 2.627 29.06   67.32 61.2   %BAL 0.00
    49.0 0.0116   0.3336 0.350   0.109 1.100 29.15   69.02 64.1   %BAL 0.00
    64.0 0.0068   0.1979 0.180   0.064 0.575 23.71   70.45 67.6   %BAL 0.00
    10   L SD 0.2170
R 0.9762 1.0720 1.1155

L SD 0.2170   T1,T2,T3 0.0120 0.0211 0.0075 TA 0.0677   P1 3.0000
    10   L SD 0.2170

Z 1.009
K: 6.772 243.2 8.810 133.4 399.9 0.774 0.237 0.14972 0.00227 0.07311

All SD in this calculation has to be multiplied by 1.08.

INPUT DATA

N#1
```
21.10 S T(1)=.014; S T(2)=.027; S T(3)=.047; S T(4)=.1; S T(5)=.324
21.12 S K(1)=5.5; S K(2)=252;S K(3)=16.2; S K(4)=65;S K(5)=125;
21.13 S K(6)=.85; S K(7)=.23;S K(8)=.18; S K(9)=.004;S K(10)=.17;
21.20 S T(6)=.917; S T(7)=3.92; S T(8)=14; S T(9)=28; S T(10)=36
21.30 S T(11)=49
21.40 S BX(1)=69.5; S BX(2)=60.5; S BX(3)=57.8; S BX(4)=52.7; S BX(5)=32
21.45 S BX(6)=23; S BX(7)=10.6; S BX(8)=3.38; S BX(9)=.97; S BX(10)=.39
21.46 S BX(11)=.14
21.50 S UX(1)=2.1; S UX(2)=2.4; S UX(3)=3.5; S UX(4)=4; S UX(5)=18
21.55 S UX(6)=31.5; S UX(7)=43; S UX(8)=56.4; S UX(9)=63; S UX(10)=66
21.56 S UX(11)=70
21.90 S NP=13; S PN=10
21.95 S T(12)=80; S T(13)=135;S BX(12)=.2;S BX(13)=.16
21.96 S UX(12)=74.3;S UX(13)=80;
```

N#2
```
21.10 S T(1)=.022;S T(2)=.045;S T(3)=.091;S T(4)=.166;S T(5)=.356
21.11 S T(6)=.938; S T(7)=1.96; S T(8)=7; S T(9)=15; S T(10)=28
21.12 S K(1)=5;S K(2)=172;S K(3)=8.8;S K(4)=45;S K(5)=145;
21.13 S K(6)=.9;S K(7)=.35;S K(8)=.14;S K(9)=.0039;S K(10)=.088;
21.20 S BX(1)=77.6; S BX(2)=71.4; S BX(3)=65.3; S BX(4)=55; S BX(5)=45
21.30 S BX(6)=30; S BX(7)=21.6; S BX(8)=10.9; S BX(9)=5.2; S BX(10)=1.7
21.40 S BX(11)=.23
21.50 S UX(1)=2.64; S UX(2)=3.6; S UX(3)=4.7; S UX(4)=8; S UX(5)=15.4
21.60 S UX(6)=27;S UX(7)=34.3;S UX(8)=44.9;S UX(9)=53.4;S UX(10)=62
21.70 S T(11)=42; S BX(11)=.39; S UX(11)=65.8
21.90 S NP=13;S PN=10;
21.95 S T(12)=84;S T(13)=112;S  BX(12)=.3;S BX(13)=.2;
21.96 S UX(12)=72.1;S UX(13)=75.4;
```

N#3
```
21.10 S T(1)=.004;S T(2)=.045;S T(3)=.156;S T(4)=.96;S T(5)=4
21.11 S T(6)=10;S T(7)=14;S T(8)=21;S T(9)=29;S T(10)=35;S T(11)=49
21.12 S K(1)=4;S K(2)=238;S K(3)=12.18;S K(4)=126.5;S K(5)=303.6
21.13 S K(6)=.716;S K(7)=.305;S K(8)=.1707; S K(9)=.00156;S K(10)=.08805
21.20 S BX(1)=86.9;S BX(2)=69.9;S BX(3)=59.2;S BX(4)=29.8;S BX(5)=14
21.30 S BX(6)=6.8;S BX(7)=4.5;S BX(8)=2.46;S BX(9)=1.36;S BX(10)=.74
21.40 S BX(11)=.23
21.50 S UX(1)=.9;S UX(2)=1.8;S UX(3)=7.5;S UX(4)=23.1;S UX(5)=36.3
21.60 S UX(6)=44.7;S UX(7)=48.6;S UX(8)=53.4;S UX(9)=55.6;S UX(10)=58.4
21.70 S UX(11)=61.6
21.90 S NP=13; S PN=10
21.95 S T(12)=63; S T(13)=78;S  BX(12)=.21;S BX(13)=.2;
21.96 S UX(12)=65.2;S UX(13)=67.5;
```

П#1
```
21.10 S T(1)=.0066;S T(2)=.093;S T(3)=.1975;S T(4)=1.92;S T(5)=2.834;
21.11 S T(6)=8;S T(7)=20;S T(8)=27;S T(9)=34;S T(10)=48;
21.12 S K(1)=29;S K(2)=292;S K(3)=11;S K(4)=104;S K(5)=50;
21.13 S K(6)=.95;S K(7)=.1;S K(8)=.27;S K(9)=.003;S K(10)=.1;
21.20 S BX(1)=57;S BX(2)=30.8;S BX(3)=21.2;S BX(4)=7.4;S BX(5)=5.4
21.30 S BX(6)=2.35;S BX(7)=.7;S BX(8)=.38;S BX(9)=.27;S BX(10)=.11
21.40 S BX(11)=.06;S BX(12)=.04;S BX(13)=.01;
21.50 S UX(1)=9;S UX(2)=11;S UX(3)=22.5;S UX(4)=50.2;S UX(5)=53;
21.60 S UX(6)=61;S UX(7)=69;S UX(8)=72;S UX(9)=76.1;S UX(10)=76.8;
21.65 S UX(11)=79;S UX(12)=82.6;S UX(13)=89.3;
21.70 S T(11)=69;S T(12)=94;S T(13)=227;
21.90 S NP=13;S PN=10
```

```
H#2
21.10 S T(1)=.0154;S T(2)=.036;S T(3)=.086;S T(4)=.178;S T(5)=.383;
21.11 S T(6)=.91;S T(7)=1.92;S T(8)=3.92;S T(9)=9.1;S T(10)=23;
21.12 S K(1)=23;S K(2)=90;S K(3)=4;S K(4)=31;S K(5)=40;
21.13 S K(6)=1.8;S K(7)=.327;S K(8)=.22;S K(9)=.005;S K(10)=.06;
21.20 S BX(1)=61.1;S BX(2)=49.4;S BX(3)=37.5;S BX(4)=28;S BX(5)=18;
21.25 S T(1)=.0154;S T(2)=.036;S T(3)=.086;S T(4)=.178;S T(5)=.383;
21.30 S BX(6)=11.2;S BX(7)=6.5;S BX(8)=5.4;S BX(9)=2.58;S BX(10)=.84;
21.40 S BX(11)=.11;S BX(12)=.005;S BX(13)=.0038;
21.50 S UX(1)=7.2;S UX(2)=12.1;S UX(3)=23.4;S UX(4)=31.6;S UX(5)=46.3;
21.60 S UX(6)=57.4;S UX(7)=63.9;S UX(8)=68.9;S UX(9)=74.8;S UX(10)=79.1;
21.65 S UX(11)=83.5;S UX(12)=86.6;S UX(13)=89.7;
21.70 S T(11)=51;S T(12)=86;S T(13)=149;
21.90 S NP=13;S PN=10;

21.01 T "H #3",!
21.10 S T(1)=.03278;S T(2)=.04653;S T(3)=.10625;S T(4)=.1722;S T(5)=.375
21.11 S T(6)=2;S T(7)=7;S T(8)=14;S T(9)=21;S T(10)=23;S T(11)=36
21.12 S K(1)=5;S K(2)=225;S K(3)=12;S K(4)=125;S K(5)=200;
21.13 S K(6)=.7;S K(7)=.3;S K(8)=.17;S K(9)=.002;S K(10)=.1;
21.20 S BX(1)=91;S BX(2)=72;S BX(3)=65;S BX(4)=55;S BX(5)=31
21.30 S BX(6)=22.8;S BX(7)=11.4;S BX(8)=5.1;S BX(9)=2.7;S BX(10)=1.39
21.40 S BX(11)=.91
21.50 S UX(1)=.5;S UX(2)=3.8;S UX(3)=6.6;S UX(4)=9.7;S UX(5)=25.2
21.60 S UX(6)=38.7;S UX(7)=46.7;S UX(8)=49.5;S UX(9)=55.9;S UX(10)=60
21.70 S UX(11)=61.2
21.90 S NP=13; S PN=10
21.95 S T(12)=49; S T(13)=64;S   BX(12)=.35; S BX(13)=.13
21.96 S UX(12)=64.1;S UX(13)=67.6
*
```

*G -- SENSITIVITY TEST OF K(I)
MMC5C2: MAMM 5 CMPTS; LA1<<LA2..<<LA5

Z 0.000
K: 28.50 292.0 11.00 123.3 53.34 0.807 0.127 0.30504 0.00336 0.06302

T1,T2,T3 0.00936 0.02234 0.05624 TA 0.14657 P1 3.00000
   0 L SD 0.1667

Z 0.167
K: 34.20 292.0 11.00 123.3 53.34 0.807 0.127 0.30504 0.00336 0.06302
   1 L SD 0.2535

Z 0.167
K: 28.50 350.4 11.00 123.3 53.34 0.807 0.127 0.30504 0.00336 0.06302
   2 L SD 0.2165

Z 0.167
K: 28.50 292.0 13.20 123.3 53.34 0.807 0.127 0.30504 0.00336 0.06302
   3 L SD 0.2568

Z 0.167
K: 28.50 292.0 11.00 148.0 53.34 0.807 0.127 0.30504 0.00336 0.06302
   4 L SD 0.1684

Z 0.167
K: 28.50 292.0 11.00 123.3 64.01 0.807 0.127 0.30504 0.00336 0.06302
   5 L SD 0.1711

Z 0.167
K: 28.50 292.0 11.00 123.3 53.34 0.969 0.127 0.30504 0.00336 0.06302
   6 L SD 0.1667

Z 0.167
K: 28.50 292.0 11.00 123.3 53.34 0.807 0.152 0.30504 0.00336 0.06302
   7 L SD 0.1803

Z 0.167
K: 28.50 292.0 11.00 123.3 53.34 0.807 0.127 0.36605 0.00336 0.06302
   8 L SD 0.1787

Z 0.167
K: 28.50 292.0 11.00 123.3 53.34 0.807 0.127 0.30504 0.00403 0.06302
   9 L SD 0.1701

Z 0.167
K: 28.50 292.0 11.00 123.3 53.34 0.807 0.127 0.30504 0.00336 0.07563
   10 L SD 0.1694

C-PS/8 FOCAL, 1971

```
01.01 T !"LEAST SQ; ORGAN CR51, N #2",!
01.09 E
01.20 D 21
01.30 D 22; D 2
01.40 D 23; D 2
01.50 D 24; D 2
01.90 Q

02.10 S RR=0;S MM=0;S SS=0;S RM=0;S RS=0; S MS=0; S RL=0; S ML=0; S SL=0
02.20 F I=1,10; D 3
02.30 D 4; D 5
02.40 S B=NB/DN; S C=NC/DN; S D=ND/DN
02.50 T !"B,C,D",%6.04,B,C,D,!!; T %5.03
02.60 S SQ=0; S LD=0; F I=1,10; D 7
02.65 S SD=FSQT(SQ/10)
02.70 T !"L SD",SD,!
02.80 T "AV DQ",LD/10,!!!

03.10 S RR=RR+R(I)↑2
03.20 S MM=MM+M(I)↑2
03.30 S SS=SS+S(I)↑2
03.40 S RM=RM+R(I)*M(I)
03.50 S RS=RS+R(I)*S(I)
03.60 S MS=MS+M(I)*S(I)
03.70 S RL=RL+R(I)*L(I)
03.80 S ML=ML+M(I)*L(I)
03.90 S SL=SL+S(I)*L(I)

04.10 S DN=RR*MM*SS+RM*MS*RS+RS*MS*RM
04.30 S NB=RL*MM*SS+RM*MS*SL+RS*MS*ML
04.50 S NC=RR*ML*SS+RL*MS*RS+RS*SL*RM
04.60 S ND=RR*MM*SL+RM*ML*RS+RL*MS*RM

05.20 S DN=DN-RS*MM*RS-MS*MS*RR-SS*RM*RM
05.40 S NB=NB-SL*MM*RS-MS*MS*RL-SS*RM*ML
05.60 S NC=NC-RS*ML*RS-SL*MS*RR-SS*RL*RM
05.80 S ND=ND-RS*MM*RL-MS*ML*RR-SL*RM*RM

07.05 S LC(I)=B*R(I)+C*M(I)+D*S(I)
07.30 S DQ=FLO G(L(I)/LC(I)); S SQ=SQ+DQ*DQ
07.50 T R(I),M(I),S(I)," ",L(I),LC(I)," ",DQ,!
07.60 S LD=LD+DQ
```

```
21.10 S R(1)=20;S R(2)=21;S R(3)=20;S R(4)=19;S R(5)=16;S R(6)=9.7
21.20 S R(7)=5.5; S R(8)=2.5; S R(9)=.38;S R(10)=.05
21.30 S M(1)=1.2;S M(2)=2.6;S M(3)=5.3;S M(4)=9.4;S M(5)=18
21.40 S M(6)=34;S M(7)=41; S M(8)=28;S M(9)=4.1;S M(10)=.43
21.50 S S(1)=.18;S S(2)=.41;S S(3)=.84;S S(4)=1.5;S S(5)=3.0
21.60 S S(6)=6.3;S S(7)=9.6;S S(8)=17;S S(9)=26;S S(10)=25

22.05 T !"LIVER",!
22.10 S L(1)=.21;S L(2)=.12;S L(3)=.105;S L(4)=.14;S L(5)=.3
22.20 S L(6)=. 3;S L(7)=.31;S L(8)=.33;S L(9)=.31;S L(10)=.24

23.05 T !"SLPEEN",!
23.10 S L(1)=.32; S L(2)=.315; S L(3)=.3; S L(4)=.31; S L(5)=.315
23.20 S L(6)=.4; S L(7)=.4; S L(8)=.35; S L(9)=.3; S L(10)=.25

24.05 T !"THIGH",!
24.10 S L(1)=.23; S L(2)=.24; S L(3)=.25; S L(4)=.26; S L(5)=.32
24.20 S L(6)=.30; S L(7)=.28; S L(8)=.25; S L(9)=.22; S L(10)=.12
*0000000000000000
*


      G

LEAST SQ; ORGAN CR51, N #2

LIVER

B,C,D  0.0064   0.0049   0.0103
```

20.000	1.200	0.180	0.210	0.136	0.433
21.000	2.600	0.410	0.120	0.152	- 0.235
20.000	5.300	0.840	0.105	0.163	- 0.440
19.000	9.400	1.500	0.140	0.184	- 0.271
16.000	18.000	3.000	0.300	0.222	0.302
9.700	34.000	6.300	0.300	0.294	0.021
5.500	41.000	9.600	0.310	0.335	- 0.078
2.500	28.000	17.000	0.330	0.328	0.006
0.380	4.100	26.000	0.310	0.290	0.068
0.050	0.430	25.000	0.240	0.259	- 0.077

```
L SD  0.249
AV DQ- 0.027
```

SLPEEN

B, C, D 0.0138 0.0054 0.0102

20.000	1.200	0.180	0.320	0.284	0.121
21.000	2.600	0.410	0.315	0.307	0.025
20.000	5.300	0.840	0.300	0.312	- 0.041
19.000	9.400	1.500	0.310	0.327	- 0.055
16.000	18.000	3.000	0.315	0.348	- 0.098
9.700	34.000	6.300	0.400	0.380	0.050
5.500	41.000	9.600	0.400	0.394	0.016
2.500	28.000	17.000	0.350	0.358	- 0.021
0.380	4.100	26.000	0.300	0.291	0.030
0.050	0.430	25.000	0.250	0.257	- 0.026

L SD 0.059
AV DQ 0.000

THIGH

B, C, D 0.0114 0.0043 0.0061

20.000	1.200	0.180	0.230	0.234	- 0.018
21.000	2.600	0.410	0.240	0.253	- 0.053
20.000	5.300	0.840	0.250	0.256	- 0.024
19.000	9.400	1.500	0.260	0.266	- 0.024
16.000	18.000	3.000	0.320	0.279	0.139
9.700	34.000	6.300	0.300	0.296	0.012
5.500	41.000	9.600	0.280	0.299	- 0.066
2.500	28.000	17.000	0.250	0.254	- 0.015
0.380	4.100	26.000	0.220	0.181	0.194
0.050	0.430	25.000	0.120	0.156	- 0.259

L SD 0.115
AV DQ- 0.011

APPENDIX B

CLINICAL LABORATORY TEST DATA

SUBJ. SEX-AGE	DIAG.	S.I.	% SAT.	SGOT [mU/ml]	GLUCOSE TOLERANCE/INSULIN TESTS [mg/100ml] (uU/ml)					GTT**
					FAST	30 min.	60 min.	120 min.	180 min.	
N#1 M-38	Norm.	95	34	38	94/16.5	156/73.2	123/113	95/62.4	65/18.5	Norm.
N#2 M-27	Norm.	96	32	20	91/10.8	120/55.3	112/81.1	120/56.1	85/22.5	Norm.
N#3 M-41	Norm.	118	31	23	80/22.2	167/160.7	172/161.3	151/142.7	122/20.9	Pre-Diab.
H#1 F-31	Hemo-chr.	236	75	22	90/18.9	107/163.5	72/88.7	90/152	93/81.4	Norm.
H#2 F-52	Hemo-chr.	208	79	176	94/8.9	218/62.1	193/57.9	191/86.5	149/43.6	Adult Diab.
H#3 M-38	Early Hemo-chr.	173	60	20	71/6.7	139/69.4	129/97.7	65/19.3	56/78	Norm.

* S.I.: Serum Iron [µg/100ml]

** This comment is based on FAJAN and CONN criteria. Syllabus Twenty-eighth Annual Postgraduate Assembly of the Endocrine Society, held in Seattle, Wash., Oct. 1976, pg. 25.