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X-RAY SPECTRAL ANALYSIS OF CHEMICAL ELEMENTS IN THE SERUM OF HERITABLE XANTHOMATOSIS

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X-RAY SPECTRAL ANALYSIS OF CHEMICAL ELEMENTS IN THE SERUM OF HERITABLE XANTHOMATOSIS

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(Ph. D. Thesis)

May 1963

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ABSTRACT

An exploration of possible derangements in metabolism of the chemical elements in two inborn errors of lipoprotein metabolism was attempted by analyzing 66 chemical elements in the serum of these patients with heritable xanthomatosis -- xanthoma tuberosum and xanthoma tendinosum -- with x-ray emission spectroscopy. X-ray spectrochemical methodology and experimental procedure are described.

Significance tests done at the level of 5% show some differences in the level of certain chemical elements: phosphorus, sulfur, chlorine and zinc. The experiment does not show any possible deficiency or excess in certain elements in the serum of the above heritable hyperlipoproteinemia patients: vanadium, arsenic, iron, chromium, cobalt, copper, cerium, selenium, iodine, and manganese which have been considered to have some relationship with lipid metabolism. The relationship between chemical elements and lipid metabolism are briefly reviewed.

99% confidence limits were calculated for 66 chemical elements in the difference of mean values between (a) xanthoma tendinosum and xanthoma tuberosum (b) reference group and xanthoma tuberosum (c) reference group and xanthoma tendinosum.

Some implications of these findings for the nature of biochemical defects in hyperlipoproteinemia are briefly discussed along with lipoprotein metabolism, and suggest some possible future research aspects in this "lipid-protein" disorder.

INTRODUCTION

The heritable nature of xanthomatosis has been recognized since the last century (1). It has been considered a disorder of lipid metabolism but only comparatively recently carefully studied and classified (2, 3).

The two inborn errors of lipid metabolism to be studied in this thesis, xanthoma tuberosum and xanthoma tendinosum, present certain biochemical and clinical differences (2, 3, 4). From the clinical point of view these lie in their frequent association with overt coronary artery disease.

Gofman et al have (2, 3) distinguished between xanthoma tendinosum and xanthoma tuberosum on the basis of lipoprotein patterns. In the former he reported in "Hyperlipoproteinemia," that the greatest increase was in the standard Sf^{o} 0-12 class lipoproteins, the next greatest in the Sf^{o} 12-20 class, and least in the Sf^{o} 20-100 class. The Sf^{o} 100-400 lipoprotein level is slightly, but significantly lower in xanthoma tendinosum than in matched controls.

In xanthoma tuberosum, the Sf^{o} 20-100 and Sf^{o} 100-400 lipoprotein showed the largest increase; the Sf^{o} 12-200 lipoproteins were elevated but to a much lesser degree; the Sf^{o} 0-12 fraction was, if anything, lower than normal. (See Fig (1, 2) and Appendix I, II)

In spite of the fact that this work leads to a critical and most precise insight to the problem, this was often mistreated (5, 6).

This misunderstanding has brought nothing but more confusion on the above issue.

This study was undertaken in an attempt to find the possible basic clue to correlate the lipid and lipoprotein metabolism with chemical elements (trace elements and non-trace elements).

In spite of vast literature directed to the study of lipid metabolism and lipid metabolic abnormality, the role of enzymatic system remains a subject more of conjecture than one of understanding (7).

Claude Bernard, in his early age, suggested the possiblity of finding metal enzyme complex or metalloenzyme molecule by trace elemental study (8).

Various known substances, several of them chemical elements, are capable of inducing serum lipid derangement (9, 10). Co, V -- are well known in this connection. Therefore it is reasonable to consider the derangement in metabolism of some common or uncommon chemical elements might underlie some function of lipid metabolism.

Animals with deficiency or excess storage of one or another element, copper, Co, Zn, iron, magnesium, molybdenum, etc. of these elements which are known to be essential for metabolic cycle, show specific metabolic derrangement (11, 12).

At an initial step, serum out of all tissues is of special interest because it is the probable medium of transport of chemical substances among tissues and also it is a fundamental medium in the homeostasis of "milieu interne" (13). Furthermore, it is very common to have an aberration of the level of biochemical substances in the blood in association with the changes of the levels in other tissues. Xanthoma formation in various tissues in the xanthomatosis is a good illustration of this.

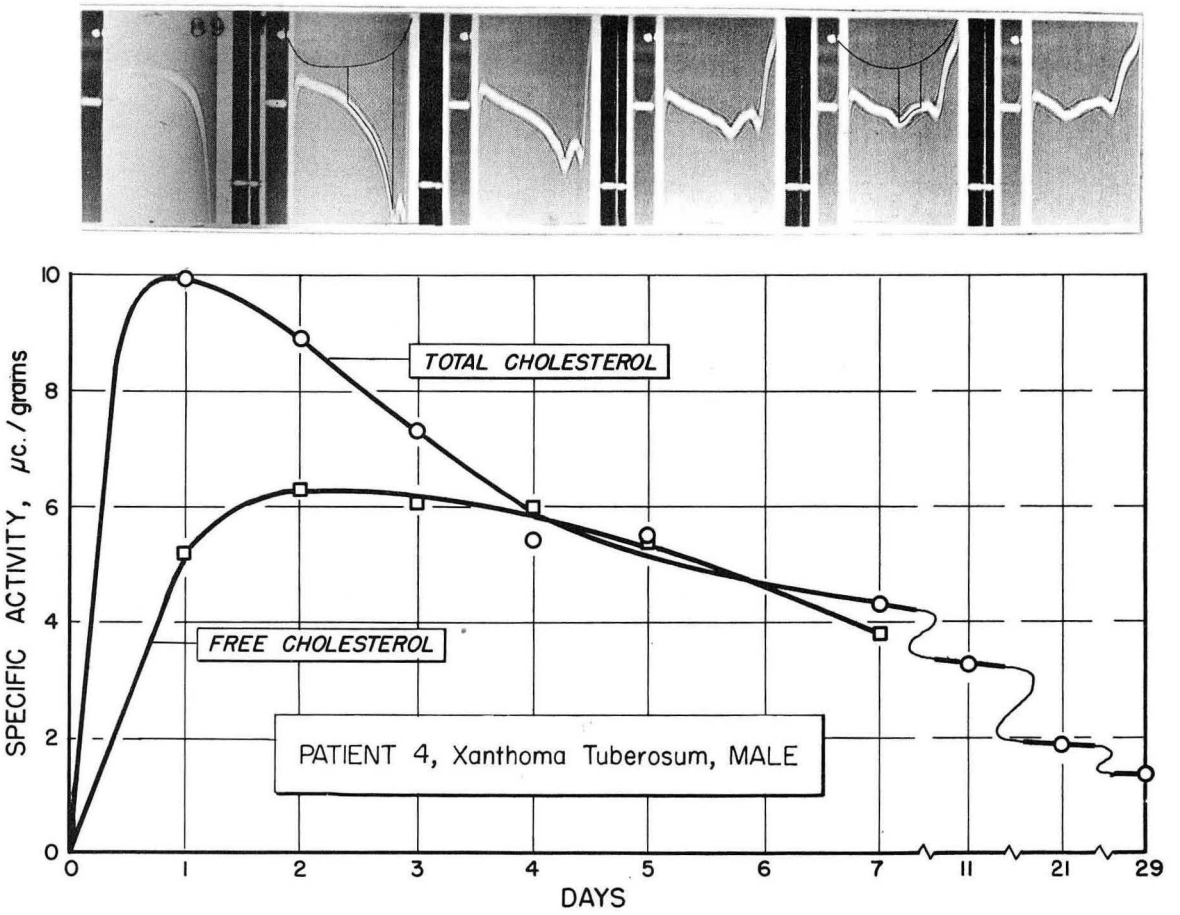

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Fig. 1. Changes in serum cholesterol specific activities as a function of time following a single feeding of 0.72g of cholesterol $-H^3$ (sp. act. = 1.07 c/mg) in a fatty meal. The lipoprotein spectrum shows: Sf 0–12 = 124 mg%, Sf 12–400 = 537 mg%. Serum cholesterol values: free = 73 mg%, total = 259 %.

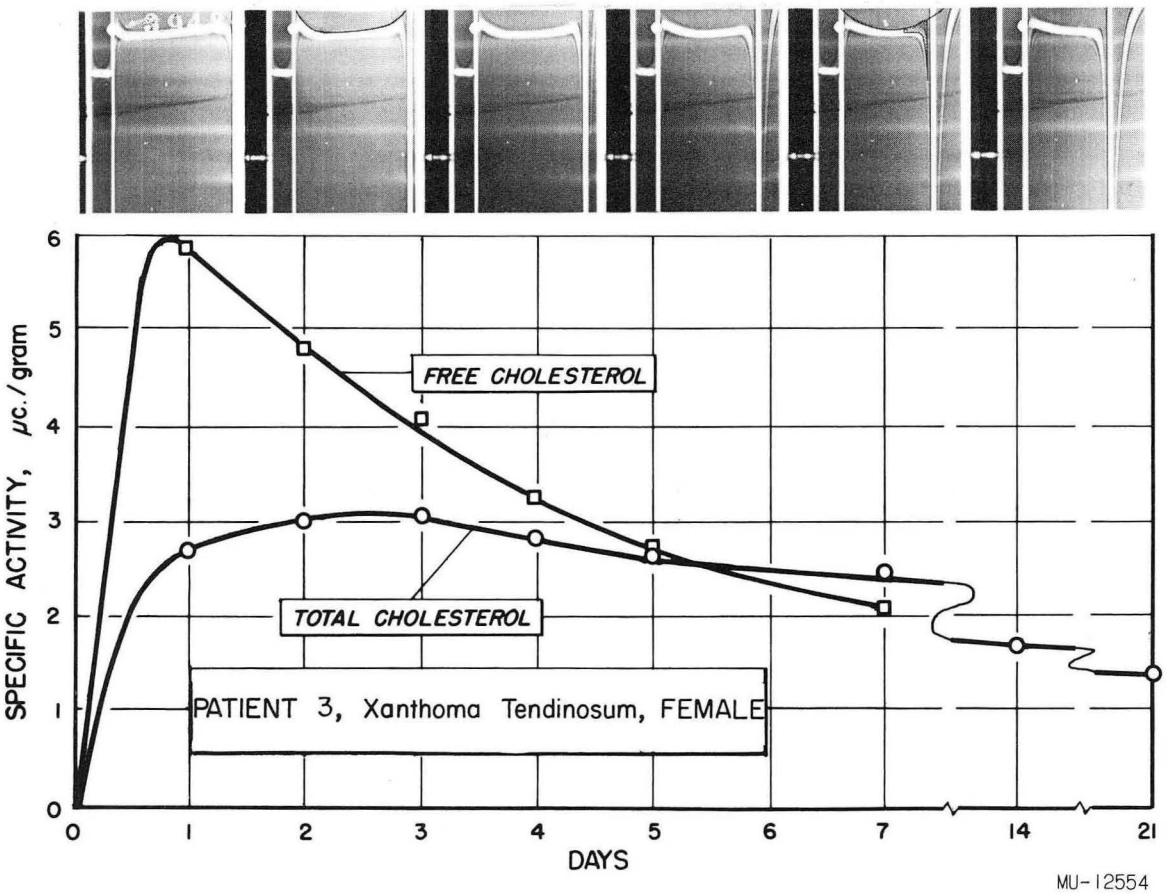

Fig. 2. Changes in serum cholesterol specific activities as a function of time following a single feeding of 0.60g of cholesterol -H^3 (sp. act. = 1.07 c/mg) in a fatty meal. The lipoprotein spectrum shows: Sf 0–12 = 974 mg%, Sf 12–400 = 193 mg%. Serum cholesterol values: free = 143 mg%, total = 519 %.

Is it less important to study the steady concentration of biochemical substances in the blood than dynamic studies of influx and efflux rate in the blood? Since steady state concentration measurements reveal abnormality that provides the basis for more penetrating study of dynamic aspects, it is obvious that both approaches are essential. In case of possible derangements of function of a particular enzyme system, the actual concentration of a specific element or ionic group in blood may be involved directly because serum as a primary part of the extra cellular fluid compartment, may in large measure define the chemical environment in which that enzyme system functions (13).

Then what element is important to measure in the blood? Do some particular elements have special merit to be studied while others not?

There is no priori reason to single out any particular element or group of elements in this study.

X-ray emission spectroscopy (initiated by Gofman et al at Donner Laboratory) was applied in this chemical analysis (14, 15, 16, 17).

X-ray spectrochemical analysis is outstanding among analytical methods for the following reasons. First, the x-ray emission spectroscopy technique has proved applicable to every element over the region from magnesium through uranium. In this region encompassing some 80 elements a single physical principle underlies analysis for all the elements, namely, excitation of either of the relatively simple K or L spectral x-ray lines. The excitation varies in a regular and predictable manner from element to element based upon Moseley's Law relating atomic

number to the x-ray spectra (13).

Second, the processing of samples of blood to render them usable for x-ray emission spectroscopy can be made minimal, with a single processed sample being suitable over the entire region from atomic number 12 to atomic number 92. Also the method is non-destructive to the sample (13).

Third, the minimum goal of sensitivity, namely 0.1 ppm or less appeared to be realistically possible of achievement with minimum time and effort per analysis by means of this technique (13).

MATERIAL AND METHODS

A. X-ray spectroscopy

1. Introduction

It was in 1895 when Röntgen experimented with "highly evacuated" tubes, the glass walls of which suffered pulsating electron bombardment in the course of a systematic attempt to see if any radiation could be produced which would traverse matter opaque to ordinary light (18). For his work with x-rays Röntgen in 1901 received the first Nobel prize in physics, also the first of six to be awarded in the field of x-rays by 1927 (19).

He studied the properties of the new rays so well that he laid the foundation not only for important methods of x-ray detection but for the application of x-ray absorption to analytical chemistry as well (19).

In 1911 Von Laue's suggestion that a crystal would diffract x-rays was confirmed by Friedrich and Knipping. Soon afterwards, Bragg let a beam of x-rays fall on the natural cleavage face of a mica crystal and proved the beam was reflected when the condition known as Bragg's Law

$$n\lambda = 2d \sin \theta \text{ is fulfilled.}$$

In 1913-14 Mosely discovered the regularity of spacing of x-ray emission lines and the simple law governing the relative displacements of the lines of different elements

$$\frac{1}{\lambda} = \text{const } (z - 1)^2$$

The spectrochemical analysis by means of x-rays is based on this simple relation.

The methodology soon played a vital role in the discovery of several elements -- that of hafnium by Coster and Von Hevesy (20) as an example.

Hadding seems to have been the first to use x-ray emission specifically for analysis, and it is appropriate that his first published data should have been for a metal because x-ray spectroscopy is particularly suited to the examintion of metals (19).

2. Atomic structure and x-ray spectra

The origin of spectral lines is easily understood by simple Bohr theory. Its essential feature is the assumption that the atom may exist in any one of a series of discrete states and that while in such a state no radiation is emitted, however when the atom changes from one state to another of less energy, the energy lost is radiated in a single quantum unit (18).

$$h\nu = E_k - E_i$$

The phenomenon takes place as follows: when an atom is supplied with sufficient energy, an electron, e. g., from the "K" shell can be expelled from the atom (21, 22).

Thereupon, the gap in the "K" shell is at once filled by an electron from one of the more outlying shells. When "jumping" from an outer to an inner shell, the electron radiates excess energy in the form of an x-ray quantum. The energy of a quantum jump from the L shell to the K shell is given by

$$E_{12} = \frac{2\pi^2 e^4 (Z-1)^2 m}{h^2} \left(1 - \frac{1}{2^2}\right)$$

where $e = 4.8029 \cdot 10^{-10}$ esu = electronic charge

$m = 9.1085 \cdot 10^{-28}$ g = electron mass

$c = 2.9979 \cdot 10^{10}$ cm/sec = velocity of light

$h = 6.6252 \cdot 10^{-27}$ erg sec = Planck's constant

z = atomic number

If this same quantum jump is observed in a series of different atoms we shall therefore expect to find that a graph of $\sqrt{\nu}$ against Z will be a straight line (22).

The multiplicity of energy levels is determined by the total angular momentum vector j, defined by $j = \ell + s$ where $S = \frac{1}{2}$ always (23). Thus the K state is single as $n = 1$, $\ell = 0$ and $j = \frac{1}{2}$. Higher n states are degenerate. For the L shell, $n = 2$, $\ell = 1, 0$ and $j = \frac{1}{2}, 3/2$. The selection rules for transitions are as follows $\Delta \ell = \pm 1$, $\Delta j = \pm 1, 0$. Thus $\nu_k = \frac{E_k - E_j}{h}$ gives rise to the K series of lines where E_j = energy level satisfying selection rules. If we express the quantum energy in electron volts, we have for the K_α lines

$E \approx 10(Z - 1)^2$ ev (a convenient "rule of thumb") (22).

The corresponding wavelength is given by

$$\lambda = \frac{hc}{E} \approx \frac{120}{(Z - 1)^2} \quad A. \ (1A = 10^{-8} \text{ cm})$$

Thus, for example, for Cu(Z = 29), we should have E = 7.8 Kev, = 1.5A. (22)

The intensity of lines increases with voltage across the tube as short wavelength x-rays generated by bramsstrahlung processes are effective when $V > V_0$ is the minimum voltage required to excite the characteristic line. The relative intensities of the lines are determined by the probabilities of transitions. For the K and L series (19) the following relationships hold:

K_{α_1} :K_{α_2} :K_{β_1} = 100:50:21, and L_{α_1} :L_{α_2} :L_{β_1} :L_{β_2} =100:11:50:20. The values of the relative intensities vary from elements but the ranking of the important analytical lines remains the same (19). The subscripts refer to the transition from various initial to final quantum levels.

The relation between energy E(Kev) and wave length λ(Å) of an x-ray quantum is given by the following equation:

$$E = \frac{12.39}{\lambda}$$

For the elements P to U e.g. the quantum energy varies from 6.15Å to 0.12Å.

The wave lengths of x-ray spectra are independent of the chemical combination of the atom as well as of physical states of the specimen.

Production of the Spectra: The energy required for the excitation of x-ray spectra can be supplied to the atoms by absorption of x-ray quanta (or by electron, or particles, etc). Obviously, the excitation energy must be greater than the energy liberated by the electrons jumping from an outer to an inner shell, since the former must expel the electron entirely from the atomic union. In the case of elements P to U, x-ray quanta of minimum energy of 2.14 to 115.6 Kev for an electron of the "K" shell are required, while quanta of at least 6 to 21.7 Kev are necessitated for an electron of the "L" shell of element Ba(56) to U(92)(21).

Hence analyzing a compound of elements of different atomic numbers, an exciting radiation of adequately high energy must be chosen. Hence it follows that tube voltage of at least 116 Kev would be required for exciting the "K" spectrum of uranium. For technical

reasons, however, the excitation voltage were limited to 60 Kv. For elements atomic number above 60, their "L" spectrum is used.

3. Analysis of the spectra

The wave lengths of the emitted x-ray quanta are determined with the aid of the x-ray diffraction produced by the atoms of a crystal lattice. The diffraction angles are found by Bragg's Law

$$N \lambda = 2d \sin \theta$$

λ : the wave length

d : the grating space (distance between atomic layers parallel to the reflecting face of the crystal

θ : angle between the beam and the reflecting surface

It is obvious that a diffraction maximum is observed at angle 2 θ but with the same optical grating, the reflection may repeat in several orders, n = 1, 2, 3, etc.

The angle between the incident beam and reflecting lattice plane family of the analyzer crystal is equal to that between this lattice -- plane family and diffracted beam. The incident and reflected beams form together an angle of 2 θ. The measurement of 2 θ enables a clear inference to be drawn as to the wave lengths of the emitted spectrum.

4. Spectroscope arrangement

An x-ray spectrometer consists basically of an x-ray tube which excites the fluorescence spectrum, a crystal analyzer to disperse the spectrum, and a detector.

Figure (3) shows schematically the simplest arrangement of components for x-ray spectroscopy.

X-ray equipment, Kristalloflex 4, designed for direct voltage of

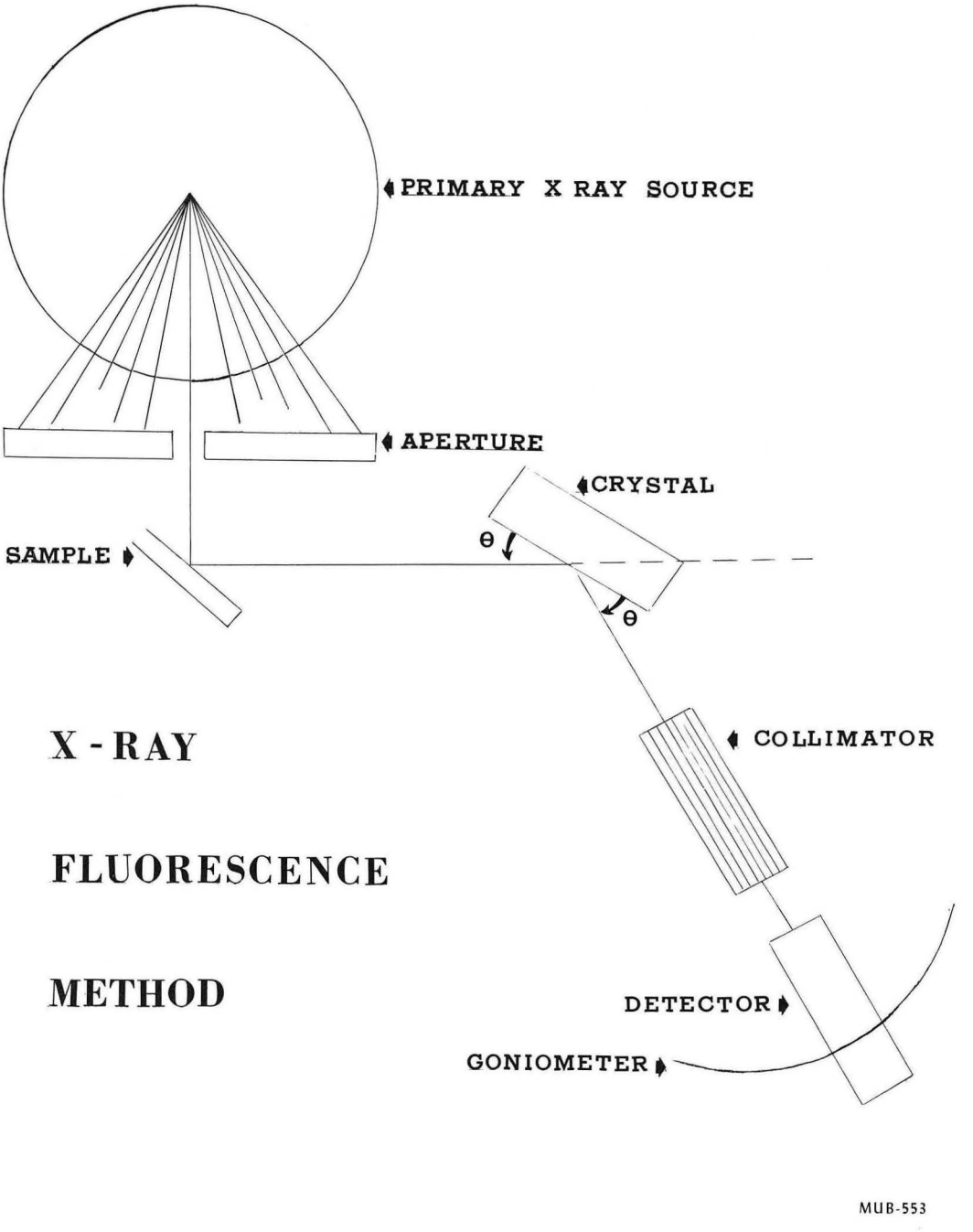

Fig. 3. The x-ray fluorescence method, with flat crystal monochromatization. (X-ray fluorescence, x-ray spectro-chemistry and x-ray emission spectroscopy have all been used as names for this same methodology.)

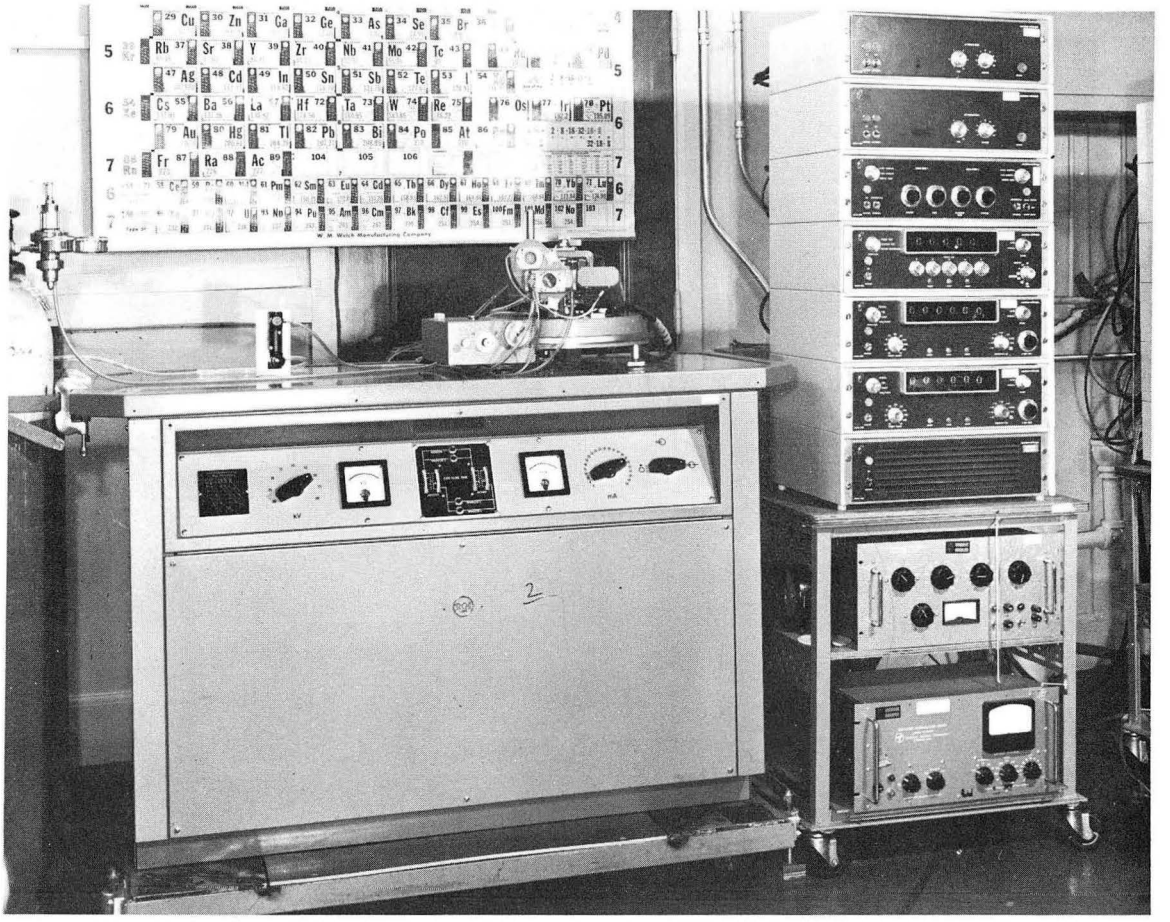

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Fig. 4. X-ray spectrophotoscopy and counting device.

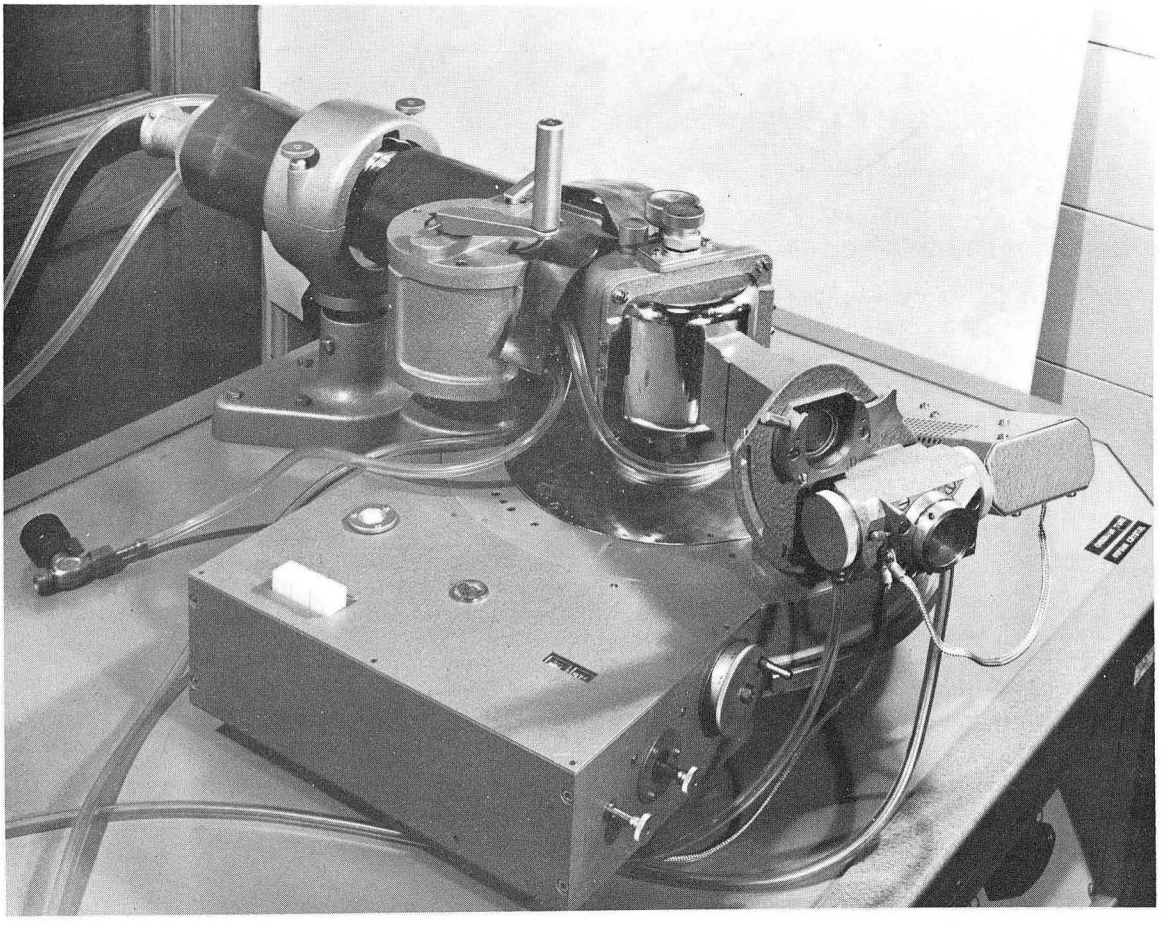

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Fig. 5. X-ray spectroscope
1) Tube
2) Sample chamber
3) Goniometer
4) Crystal chamber
5) Collimator
6) Prop. counter

up to 60 Kv and a current load of up to 50 ma. (Fig 4, 5)

For quantitative analysis, a very constant intensity of x-rays is pre-requisite. The intensity, however, is proportional to the current flowing through the x-ray tube and to the square of the tube voltage.

Our machine is stabilized to an accuracy of 0.1% fluctuation of mean voltage.

The x-ray tube is fastened horizontally in the tube holder of the equipment (21). It radiates vertically downwards on the specimen, which is inclined towards the horizontal plane at an angle of 45 degrees. The specimen is accommodated in a radiation-proof changing device. The specimen changer is mounted to the goniometer separated from the x-ray equipment and the tube. The analyzer crystal is fitted in the center of rotation of the goniometer behind a screen protecting against radiation. The counter tube holder with its collimator is pivoted on a bracket, facilitating accurate adjustment (21).

The intensity of the fluorescent spectra excited increases rapidly as the operating voltage V , exceeds the minimum voltage V_o, and

$$I \propto (V - V_o)^2 \quad \text{up } V \approx 2 \sim 3 \text{ time } V_o$$

$$I \propto V - V_o \quad \text{for } V > 3V_o \qquad (24).$$

These tubes have beryllium windows and these will preferentially absorb the softer radiation. Therefore at low operating voltages only a very small portion of the radiation gets through the window, which substantially reduces the excitation efficiency.

The best approach appears to have the vacuum tube with specimen inside. But it brings another difficult problem.

5. Primary x-ray source

The ideal x-ray souce should radiate all its power in nearly monochromatic emission at a wave length equal to the critical excitation potential of the element to be excited.

However there is always continuous white radiation.

The absorption energy expressed in terms of wave length as λ_{Kabs} or λ_{Labs} for removal of a K or L electron varies with the atomic number.

The minimum potential V_o required to excite the K series of the target element is then calculated by substituting the λ_{Kabs} for that element in place of the λ min in Duane-Hunt Law:

$$\lambda = \frac{12350}{V}$$

For each element λ_{Kabs} is just shorter in wave length than the corresponding K emission line and λ_{Labs} is just shorter than L emission line (24).

The minimum potential V_o for three common target materials we used is given in table (24).

Table I

Target	Minimum Potential V_o	X-ray Line
W	12.1 Kev	L
Mo	20.0 Kev	K
Cr	6.0 Kev	K

Since the absorption coefficient of x-rays is proportional to $\rho\lambda^u Z^v$, (in most of formula U has a value of about 3 and V a value of about 4, but in extremely soft roentgen region these values are changed)

(26), the long wave x-ray spectra of elements are strongly absorbed by air. Therefore a vacuum spectrometer was used with gas flow proportional counter for the soft radiation with the stream of gas mixture consisting of 90% argon and 10% methane passing through the counter tube at atmospheric pressure with the flow rate of approximately two liters per hour. In the last stage of this work, we replaced the vacuum system with helium flow because we had encountered some difficulties with gypsum crystal dehydration in the vacuum system.

6. Analyzer crystals and collimator

The heart of the x-ray spectroscopy might be the analyzing crystal because it does distinguish the various wavelengths and also intensity and resolution depend on the properties of crystal (24). According to the Bragg Law, a crystal cannot reflect a wavelength larger than twice the lattice spacing. The shortest wave length reflected is governed by the reduction in the projected crystal surface seen in the direction of the incident radiation at small angles. (25)

The dispersion of the spectra can be calculated by the formula derived from differentiating Bragg's Law (24, 25).

$$\frac{d\theta}{d\lambda} = \frac{n}{2d\cos\theta.}$$

Gypsum crystal and LiF crystal were used. A blade type collimator was used with different kinds of blades (tungsten, stainless steel, etc.). The purpose of the collimator (this collimator located between a sample and a crystal does not appear in fig. 3)

is to allow only a parallel bundle of the polychromatic radiation from the specimen to strike the crystal. Resolution is limited by the divergence allowed by the collimator and by the rocking curve of the crystal (24).

B. Experimental

1. Subjects

a. Xanthomatosis patients

Two groups of heritable xanthomatosis are under investigation. One group is represented by 16 subjects with xanthoma tendinosum and the second group is represented by 4 subjects with xanthoma tuberosum and 4 additional untypical type of later form in the case of certain elements.

The individual clinical data for the series of patients are presented in Table II.

A variety of metabolic states in which lipid metabolic alterations was known to exist or suspected to exist has been investigated (2, 3, 27, 29, 30, 31, 32, 33). Such study has revealed that there is a considerable diversity in the types of lipoprotein transport derangement present in certain metabolic disturbances, whereas for a particular metabolic disorder the lipoprotein transport pattern is reasonably specific. One important corollary finding of such studies is that a classification of metabolic states by the older chemical determination of serum cholesteral is wholly inadequate for satisfactory description of the lipid metabolic disturbance present. Thus hypercholesterolemia must be regarded as simply a conglomerate of several

disorders, unrelated, not only becuase of etiologic basis, but also unrelated in terms of the actual lipoprotein transport aberration present (4).

Some of the patients with xanthoma tendinosum reported their lesions had been present since childhood (4).

Characteristic sites of the tuberosum lesion in this group were (1) extensive surface of the elbow, (2) buttocks, (3) exterior aspects of the knees, (4) the hands, especially the volar surfaces, (5) over the ankle malleoli, especially laterally (3, 4).

In contrast to the patients with xanthoma tendinosum, none of the patients with xanthoma tuberosum reported the presence of lesions in childhood (3, 4).

b. Control

In most of elemental studies, series of 39 ostensibly healthy adult males have been analyzed using the pooling procedures (14).

The adult males range in age from 21 to 53 years with a mean of 35.3 years. All subjects in the group were free of major disease discoverable by a complete physical examination (14).

All were residents of the Northern California Bay Area, and included urban and rural dwellers. Common allergies and minor dermatosis did not constitute basis for exclusion of cases. Acute febrile illnesses did constitute a basis for exclusion, because certain elements as copper and Zn, are altered during infection.

110 normal male and female individual blood samples were used for the comparison in the Fe studies, 22 randomly selected (male and

Clinical Data

run no.	name	sex	age**	Sf_{0-12}	Sf_{12-20}	Sf_{20-100}	$Sf_{100-400}$	A. I.***	Diagnosis	Treatment (drug)
				(Lipoprotein Data)**						
23	J. S.	M	43	207	115	621	684	269	H., X.Tbs.	Heparin
24	J. U.	F	57	500	45	190	110	110	H., X.Tnd.&A.	Thyroid,Premarin
25	M. J.	F	39	1334	-----303****			186	H., X.Tnd.	Heparin,Premarin
27	H. L.	F	48	125	92	368	82	107	H., X.Tbs.	Thyroid
28	R. S.	F	40	257	170	374	89	136	H., X.Tbs.	None
29	J. C.	F	48	777	107	57	10	108	H., X.Tnd.	Mer. Nicotinic acid,th-roid.
30	L. J.	M	31	771	134	171	56	140	H., X.Tnd	TThyroid
31	J. M.	M	68	196	139	1042	487	311	H., X.Tbs.&B.	None
32	R. R.	M	51	750	30	24	5	85	H., X.Tnd.&C.	Nicotinic acid
199	A. C.	F	57	1035	45	0	0	112	H., X.Tnd.&D.	Thyroid
200	P. B.	F.	39	806	128	91	33	125	H., X.Tnd.	Thyroid,MER
201	B. B.	F	12	498	51	58	16	72	H., X.Tndd type	Thyroid
202	C. G.	F	12	527	51	58	5	73	no lesion Same as above	Thyroid
374	P. S.	F	75	394	87	181	188	119	H., X. Tnd.	Digitalis,Urinase
409	H. C.	M	57	554	29	8	0	62	H., X. Tnd.	Thyroid
421	J. O.	M	45	584	32	43	3	72	H., X. Tnd.	Thyroid
584*	H. S.	F	56	947	167	177	21	159	H., X. Tnd.	Diuril,KCL,Digitalis
733*	G. S.	M	53	250	59	583	374	203	H.,& F	Thyroid,Nitroglycerin
735*	E. V.	M	49	223	35	371	302	146	H.,Hyperemic type&G	None
753*	G. G.	M	33	500	88	262	201	146	H.,Untypical type&H	Thyroid
758*	E. U.	F	52	472	-----960****			215	H.	Thyroid, Formatrix
759*	M. M.	F	53	965	70	72	11	123	H., X. Tnd.	Premarin
812*	S. R.	F	35	573	64	206	67	116	H., X. Tnd.	Thyroid, Premarin
963*	H. H.	F	59	255	259	465	99	170	H., X. Tbs.	Thyroid

* These samples were run on certain elements only.
** Age at the time of sampling.
*** The latest available data.
**** Sf_{12-400}
A : ASHD with posterior wall infarction
B : Diabetes Mellitus,diffuse lipomatosis, history of epileptic seizure and peptic ulcer
C : ASDH with anterior wall infarct,Dupuytran's contracture
E : ASDH with old myocardiac infarction, left ventricular hypertrophy, interventricular conduction defect by E.C.G.
D : ASDH with anginal syndrome
G : Essential Hypertension
H : Obesity
H., : Hyperlipoproteinemia
X.Tnd. : Xanthoma Tendinosum
X.Tbs. : Xanthoma Tuberosum

female)blood samples with the same age distribution were used for the Zn study.

Normal subjects for Zn and Fe had the same basis for exclusion of cases as the other normal group.

2. Sampling procedure

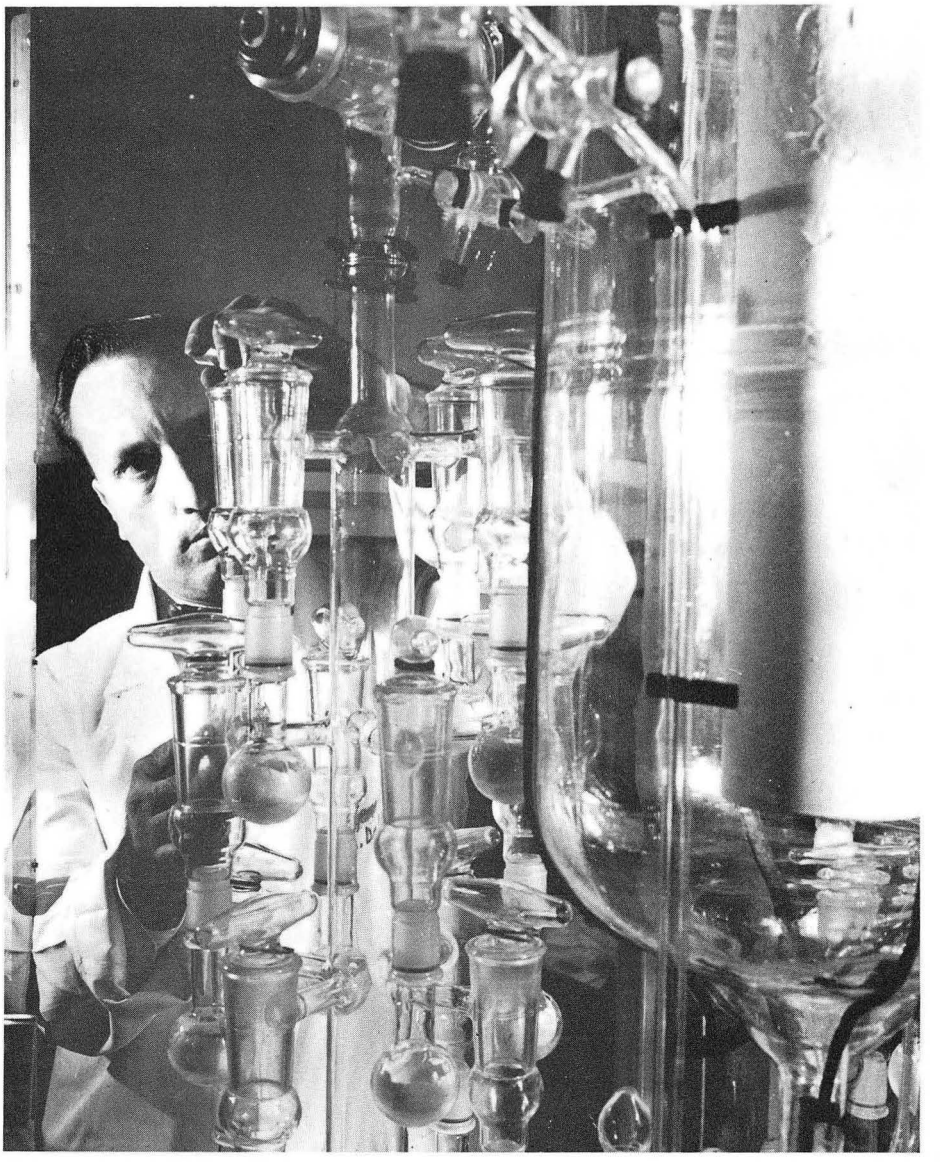
Collection of blood samples. Blood samples were collected by means of the sampling kit. It includes a collecting tube which is evacuated and sterile bleeding set, which has a 20 gauge stainless steel needle at each end of a plastic tube. The use of such a system allows for complete control over contamination of the sample by some foreign material. In most cases of xanthomatosis 60 cc of blood was drawn to make more than just one sample for each individual to allow for sample breakage.

After successful venipuncture, the blood will gradually and continuously flow toward the other end of the set which is inserted through the diaphragm of the rubber stopper in the collecting tube. The first tube is just for flushing off the needle. The second and third tube together make 60 cc of the blood.

Extraction of serum. The blood samples are left at room temperature for at least 2 hours to allow for sufficient clot retraction.

Serum is then transferred to a centrifuge tube for centrifugation at around 2500 rpm for 15 to 20 minutes. After the first centrifugation the serum is transferred to another centrifuge tube for centrifugation at the same rpm and period.

Parafilm cut to size is used to top the serum sample before



ZN-3878

Fig. 6. All glass lyophillizer apparatus designed by de Lalla.

the lyophillization.

Lyophillization. The serum is transferred to a proper flask for lyophillization. Dry ice and acetone are used to freeze the sample. The flask is turned continuously while kept in the acetone-dry ice mixture so that all the serum is in evenly distributed serum film inside the flask. Then it is transferred to the de Lalla Apparatus for drying (Fig. 6).

This lyophillization is the compromise we choose as the simplest concentration process with assurance of minimal loss of any particular element present in the sample. Lyophillization of serum gives about a twelve-fold concentration of all elements present.

Samples for analysis have been prepared by accurate weighing of 400 milligrams of dry powder which is pressed into a wafer using a press lined with teflon carefully cleaned before each sampling procedure (34).

Standardization is performed by preparing sample with known additions of a soluable salt of the element in question, lyophillizing to dry powder in a manner identical with that for the study sample and pressing 400-mg disk for use in the x-ray emission spectrometer.

Since the powder yield per unit serum is not identical from sample to sample, each dry sample is weighed immediately after lyophillization, so that appropriate calculations can be made for the true volume of serum presented in a 400-mg powder sample used in the x-ray spectrometer.

The linearity of counting rate versus quantity of element present

in such a 400-mg disk for the elements analyzed was tested.

Some representive studies for manganese, chromium, and iron are presented in Figures 7a, 7b, 7c.

The final dried powder disks have been mounted in fixed-dimension permanent lucite holders for insertion into the x-ray spectroscope.

The Geometry was very carefully considered and handled for the sample in the sample drawer.

The x-ray beams used have been provided with limiting aperture so that the beam shines only upon the serum disk and not upon the lucite holder, except for multiple scattered radiation which unavoidably irradiates the holders.

Our experience has been that the dried serum powder disk tolerates the x-irradiation very well with respect to preservation of structural features. Such samples have been exposed to the full intensity of the x-ray beam for periods of 30 to 40 hours in the course of multiple analysis without any evident sample deterioration. Many plastic materials are grossly altered structurally with even less irradiation.

3. Analytical procedure

A standard Siemens Krystalloflex was used. The x-ray instrumentation including tube, generator, goniometer, analyzing crystal collimator, and detector was described in a previous section. 2θ angle: Since the goniometer provides 2 : 1 tracking between the crystal holder and the detector it was necessary to allign the detector at the 2θ angle (Bragg's Law).

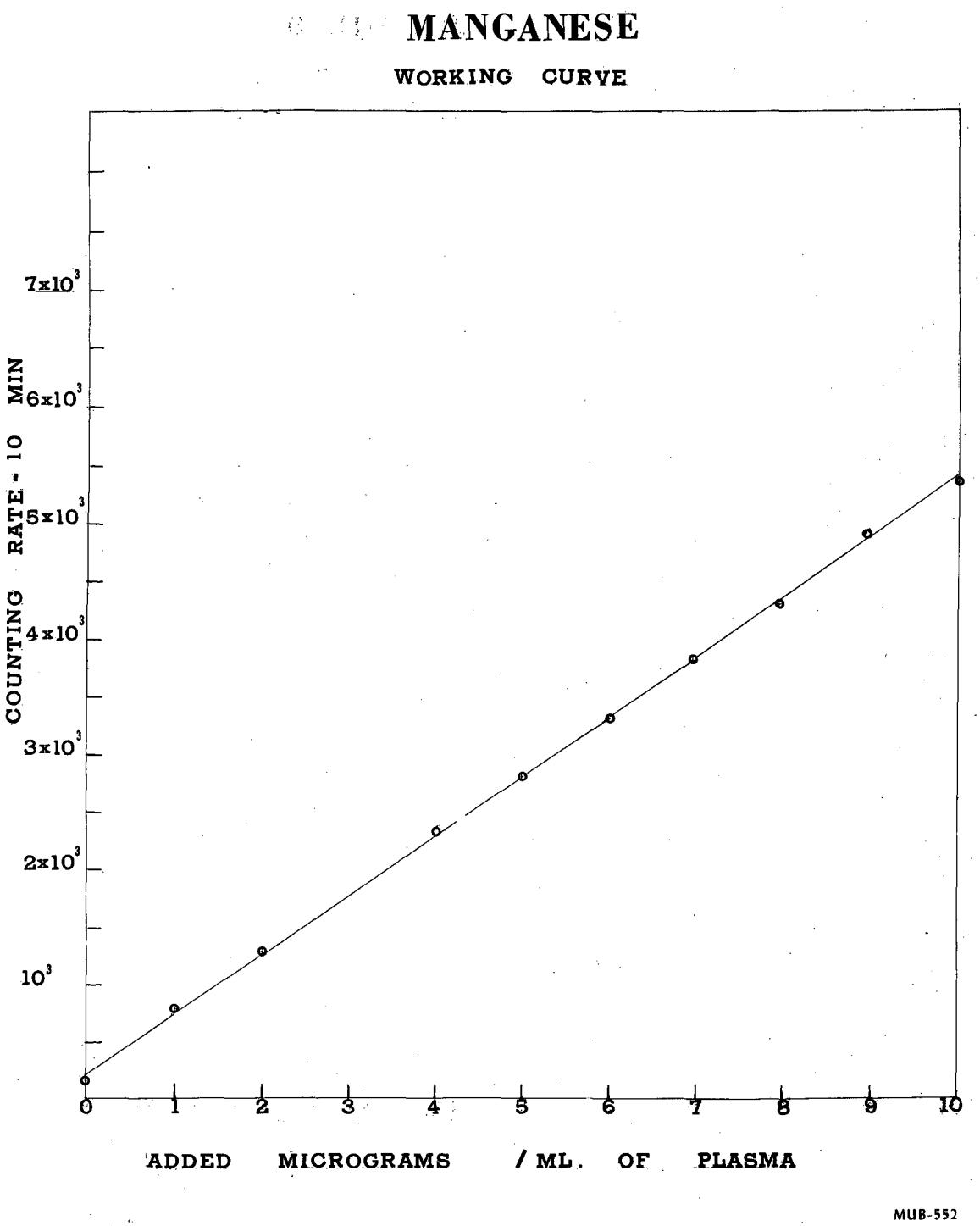


Fig. 7a. Study of linearity of counting rate (at diffraction maximum for the specific characteristic radiation for the element studied) versus concentration of element in the serum (range 0–10 ppm).
(a) manganese (b) chromium (c) iron

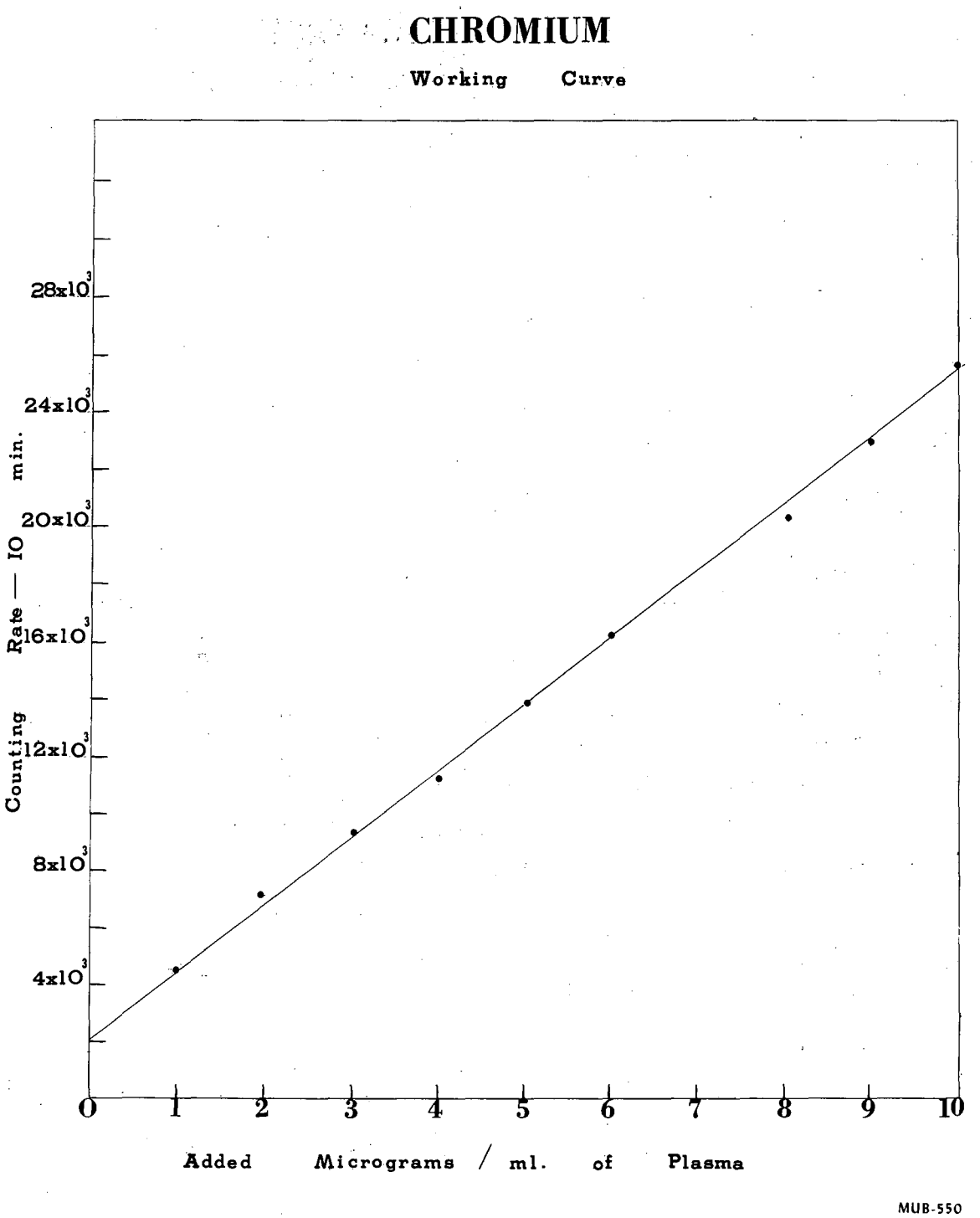

CHROMIUM
Working Curve
Counting Rate — 10 min.
28x10^3
24x10^3
20x10^3
16x10^3
12x10^3
8x10^3
4x10^3
0
1
2
3
4
5
6
7
8
9
10
Added Micrograms / ml. of Plasma
MUB-550

Fig. 7b.

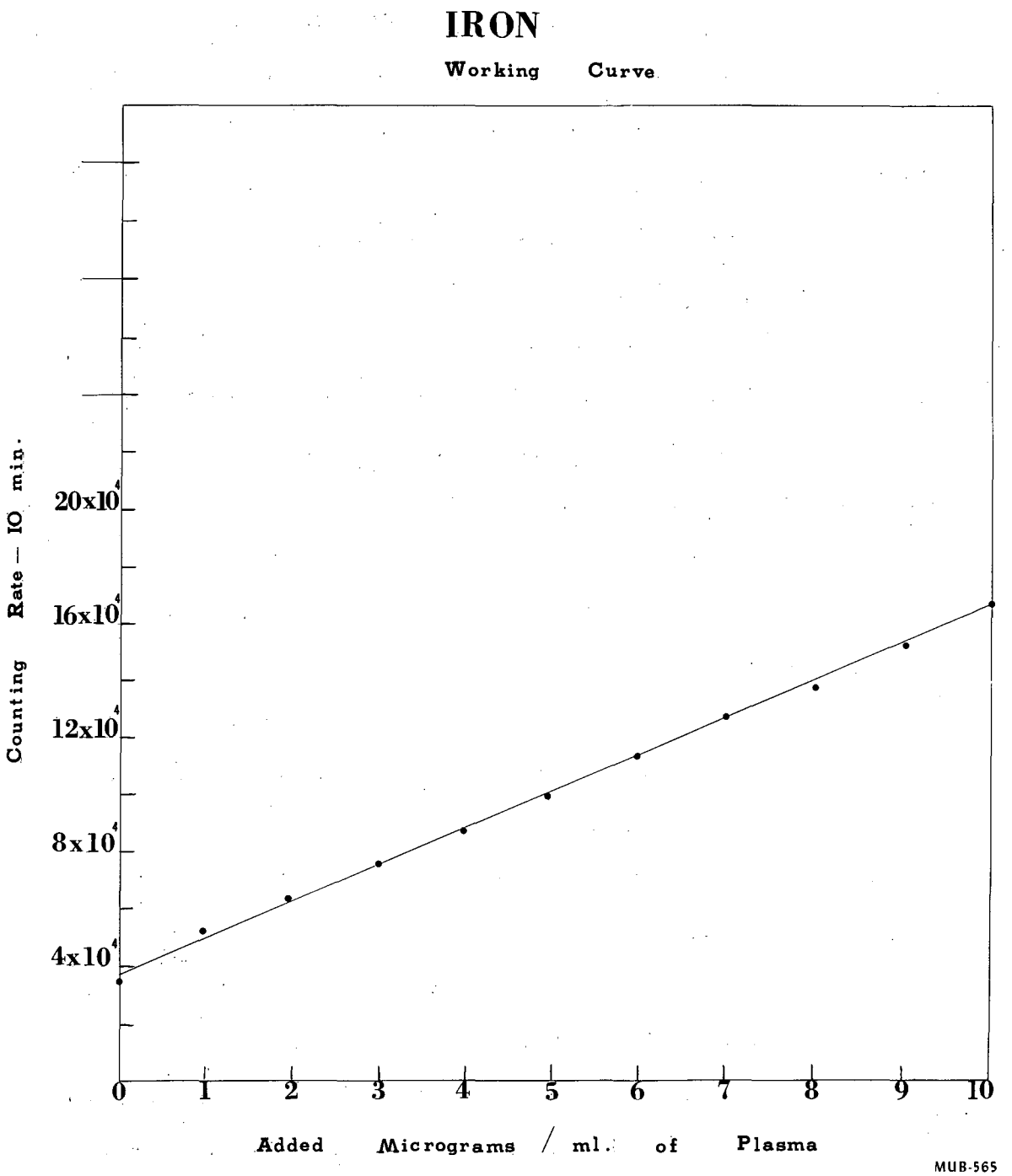

IRON
Working Curve
Counting Rate — 10 min.
20x10⁴
16x10⁴
12x10⁴
8x10⁴
4x10⁴
0
1
2
3
4
5
6
7
8
9
10
Added Micrograms / ml. of Plasma
MUB-565

Fig. 7c.

The system is aligned by inserting an epon plastic sample prepared by the adding of a known element in the sample drawer, setting the detector at the 2 θ value calculated from Bragg's equation for the particular crystal, and spectral line, and measuring the x-ray intensity in the detector.

The fine alignments were done by rotating the crystal and detector until maximum peak intensity of the radiation (e.g.) Zn K_α is found at appropriate angle. Flat LiF (2d = 4.0269) was used for most of the elements and gypsum crystal (2d = 15.1980) was used for soft radiation. The high vacuum we employed brought some destructive changes on the gypsum crystal after use for some period.

The "off angle" was carefully selected. (See section on Quantitation).

Voltage

The operating voltage applied to the detector was carefully optimized to give proper peak to background ratio. Background arising from a) higher order reflection of x-rays being emitted from elements in the sample more energetic than the one being analyzed, b) first order reflection plus higher order reflections at the wave length being measured of either the continuous white spectra or x-ray characteristic lines, c) diffuse coherent and incoherent scattering radiation from x-ray tube is the most crucial problem in the x-ray analysis. The optimization of the voltage applied to the detector is one of the most essential procedures for above P/B ratio.

The differential method was used. Different operating conditions are set up for each element to be studied. An electronic window of 10

to 20 volts in width was utilized with proper base line value. With the above setting, the counter voltage is progressively raised until it reaches a peak value. If the voltage is raised further, then the intensity of the characterisitc radiation is too large for the window setting.

The detector voltage at which intensity of K or L radiation for the particular element to be analyzed was a maximum used the the measurement. By utilizing the above procedure we can improve P/B ratio.

However, there are two processes, namely "edge effects" and "escape peak" phenomena which could not be reduced by the above procedure. The incident x-ray quanta give up their energy by a complicated mechanism when their energy exceeds the critical x-ray absorption edge of the atoms in the gas or crystal of the detector. In those cases where the fluorescent x-ray quantum is reabsorbed and the entire energy of the incident x-ray quantum is thus dissipated by absorption processes in the active volume of counter, one average pulse amplitude A_1 is produced which is proportional to the energy E_1 of the primary x-ray quantum

$$A_1 = k\ E_1$$

A certain fraction of the x-ray quanta of energy E_2 will not be absorbed but instead will escape from active volume of the detector. Therefore, if pulse height analyzer is set to reject it, quantum counting efficiency will be correspondingly reduced (35).

If the quantum produces an electron in the detector by photoelectric absorption, the full pulse is obtained provided the electron loses <u>all</u> of its energy in the detector. If the electron carries some of its energy out of the detector volume, the pulse produced is correspondingly smaller. As a result the pulse produced can be of a size

that will get into the electronic window set for a much lower energy x-ray quantum ("edge" effects) (17).

The differential procedure does decrease background considerably; however, it requires stability of detector voltage and also a stable electronic system in the pulse height analyzer and these requirements gave serious problems at the beginning of the study.

The operating voltage and current of the x-ray tube and target of the tube were properly selected according to the particular energy to be analyzed.

A chromium target tube was usually used with gas flow counter to measure soft radiation (below Ti), and W and Mo tubes were also carefully chosen for particular element under analysis. The operating condition of the tube, current, and voltage was determined by studying the intensity of peak versus background ratio.

A useful rough value of voltage on the x-ray tube is 5 to 10 times the absorption edge voltage for particular elements.

For the element from Z = 45 to 55, it would require between 115 KV and 180 KV on x-ray tube (23.2 KV - 35.9 KV absorption edge voltage) even to provide 5 times the absorption edge voltage. Since our power supply and x-ray tube are limited to 60 KV, sensitivity has been a serious problem for these elements.

Various thicknesses of Al or Cu foils were used to filter the primary x-ray beam for the particular element being analyzed.

This was again for good P/B ratio (e.g. Al filter for Cu K_α measurement -- which will decrease the Cu K_α radiation from the tube itself, since Al absorption edge lies below the Cu K_α).

4. Quantitation

The interrelation between the intensity of the spectra lines and the concentration of the element in the specimen is a pre-requisite to quantitative analysis.

To eliminate the background previously described from the quantitation and to establish the baseline we adopted the "on" and "off" angle method with a proper plastic scatterer.

After the characteristic radiation was measured at the proper 2 θ angle with proper optimal voltage, another measurement was made approximately one degree away from the position of diffracted maximum which does not interfere with other characteristic radiation from some other element (especially from Mo, W, Cu, etc.).

If the element being measured in the sample is absent or present below the limit of detection, the counting rates at the on and off angle show essentially a straight line or smooth curve on the counting rates versus 2 θ angle graph. Otherwise, it should show a peak at 2 θ value of the diffracted maximum for the characteristic line of the element being measured. This consists of two main components namely (a) characteristic radiation from the element under analysis, (b) background due to the previously described, namely scattering radiation of this wave length from the element which is present in the x-ray tube and from the sample drawer or its scattered radiation by the blood sample.

To differentiate quantitatively the radiation (a) from (b) it was necessary to study the same substance which does not contain the element under analysis but otherwise has identical scattering power

as the blood sample at the same angular position with same geometry. The substance used for this base line study was called "scatterer," which is made of plastic. However, whether the element in question is present or not at the level of a few parts per million is not an easy problem.

The problem was attacked by preparing a set of plastic scatterers of the same dimensions as the sample that scatter same extent as the blood sample, measuring at 2 θ on and off angle value, and plotting the counting rate versus 2 θ in the graph.

If there is no peak present with the scatterer at the angle and so the plot is a smooth curve one obtains all the information needed to determine the amount of an element whose fluorescence line is at that angle.

If <u>all</u> of plastics show some peak at the 2 θ diffraction maximum and quantitatively just as much as blood sample, it is very unlikely to be due to the presence of the particular element present to precisely the same content in all the plastic and blood. Most likely the source of radiation is from tube or drawer constituents. Effort has been made to eliminate the problem by inserting foil or changing the material in the drawer.

The slope of plastic scatterer was used for the correction of the background radiation. If the correction was overestimated, then absolute value of the elements in all blood samples would be high by a fixed amount.

This may bring a serious systematic error if the purpose of the study is to determine absolute levels. However the absolute value

was not very crucial problem here where the purpose was to measure the difference between normal series versus xanthomatosis series. But still it is desirable to measure the absolute value itself.

Normal blood and xanthomatesis blood was measured at on and off angles. The absolute value of normal which is necessary in the calculation is from Gofman's data. To eliminate possible mistake arising from not having constant output of x-ray from the tube, it was measured at on and off angle 5 minutes alternately for 6 times.

To convert net counting rates into parts per million or any other concentration measure a previously described standard sample was measured.

From the difference in the counting rate ($9\mu g$ and $0\mu g$ standard sample) and also known amount of the element to the standard sample, net counting rate per part per million of the element in the blood was obtained (counting yield). Here we obtained all the necessary data. It is straightforward to derive the following formula from the experimental procedure.

$$\text{(I)}\quad (ppm)_u = \left\{ \frac{(ppm)_k}{p.\,y_k} + \frac{\text{ratio(u.)} - \text{ratio (k.)}}{p.y_{st}} \quad \frac{(off)_u}{ct.y} \right\} p.y_u$$

$(ppm)_u$ = part per million of unknown sample

$(ppm)_k$ = part per million of known sample

$p.y_{st}$ = powder yield of standard sample

$p.y_k$ = powder yield of known sample

$p.y_u$ = powder yield of unknown sample

$ct.y$ = counting yield of standard sample

$(off)_u$ = off angle value of unknown sample

$$\text{ratio (u.)} = \frac{\text{on angle value}}{\text{off angle value}} \quad \text{ratio of unknown sample}$$

$$\text{ratio (k.)} = \frac{\text{on angle value}}{\text{off angle value}} \quad \text{ratio of known sample}$$

(II) $(ppm)_k$ is calculated from open study with same basic formula

(III) If off angle values are much different from each other it is advisable to use formula III where no assumption on off angle values of known and unknown is made.

$$\text{III} \qquad (ppm)_u = \left\{ \frac{(ppm)_k}{p.y_k} + \frac{b - a + d + c}{p.y_{st} \quad ct._y} \right\} p.y_u$$

b = on angle value of unknown

d = on angle value of known

a = off angle value of unknown

c = off angle value of known

RESULTS

The experimental findings for the analysis of 66 chemical elements are presented in the following tables:

(1) Individual values of xanthoma tendinosum and tuberosum

(2) Mean level of element in normal control

(3) Normal versus xanthoma tuberosum

(4) Normal versus xanthoma tendinosum

(5) Xanthoma tendinosum versus xanthoma tuberosum

The tables contain individual values, mean values, differences in mean, standard deviations of mean, standard errors of mean, standard errors of difference of mean, significance at 5% level and confidence limits upon the difference in the mean value. Normal statistics was applied for the above calculation. Significance test was based on the t-test.

The confidence limit upon the difference in mean values was calculated because there is a great interest in estimating the extent to which differences in means might differ at a reasonable level of probability even if significance test is "no".

This is obtained by adding 2.65 times the standard errors of the differencesto the differences in means and by subtracting those same numbers from the differences in means.

By doing this, we can predict how large a difference may ever be discovered with good confidence. The confidence limit shows a very narrow range for some elements and appreciably larger for others. The reason is that it is the direct result of the difference in the sensitivity

(or error) of x-ray spectrochemical analysis and biological variation for some elements as compared with others.

The meaning of the "negative result" could be easily understood from a previous section (see analytical procedure).

Of course, it is impossible to have a negative value for the concentration of certain elements inthe human blood. This was resulting from the problem of baseline determination.

However, it is not proper to record all negative results as zero, because it is still true that blood of -0.05 ppm has less concentration than the blood sample of 0.00 ppm.

Even if incorrect on an absolute scale, the importance of the relative differences is of obvious significance.

DISCUSSION

I

History

An inheritable tendency toward increased concentrations of plasma lipids frequently associated with xanthoma tendinosum and tuberosum has been recognized, and among the earliest descriptions of tendinous and tuberous xanthoma appear to be those of Rayer (36) and Addison and Gull (37) and Fox (38). Fagge (39) observed the association of xanthomatosis and cardiovascular disease. In 1920 Chauffaud, Laroche and Grigaut and Burns demonstrated that there is a defective cholesteral metabolism -- increased amount of total serum cholesterol -- in patients with this syndrome. In 1929, Wile, Eckstein and Curtis stated that the formation of xanthoma could not be explained solely by the theory of hypercholesteremia and that a defect in fat metabolism, in which cholesterol undoubtedly played a part, was apparently responsible (44). In 1893 Török (1) recognized the familial tendency of tuberous xanthoma and made an extensive study of the literature concerning the various aspects of xanthomas.

Definition and Classification

Lipidoses is a general term applied to disorders characterized by abnormal concentrations of lipids in tissues or in extracellular fluid. Xanthomatosis often is employed in the same sense, although it more specifically refers to certain morphologic changes in tissues associated with lipid accumulation. Subclassification of nomenclature seems very chaotic, many types of classification are available at the

present time. According to the classification by Fredrickson, et al., primary hyperlipidemia can be subdivided by (6):

a) Essential familial hypercholesteremia

b) Essential familial hyperlipemia.

The former represents a heritable disorder having hypercholesterolemia even though plasma phopholipids (hyperphospholipidemia) are always, and triglycerides occasionally, elevated as well. Significant hyperglyceridemia, producing lactescence in plasma (hyperlipemia), is less common. The latter essential hyperlipidemia has high triglycerides with proportionately much less associated increase in other lipids. Biochemical tests are lacking for clear separation of the hyperlipidemic syndromes into above subclassification, even though it is frequently used (41).

Gofman, et al., proposed the most accurate biophysical methodology to clarify the complex phenomena of lipid metabolism in mammals. One author described it as "very interesting from a heuristic point of view for the investigation of multiplicity of serum protein aggregates, but it seems not justified, or at least premature, to attribute to the various Svedberg units diagnostic significance in clinical medicine" (5), and also (5) "The determination of so-called lipoproteins by electrophoresis or ultra-centrifugation does not offer a better or a different classification for differential diagnosis of xanthomatous disorders."

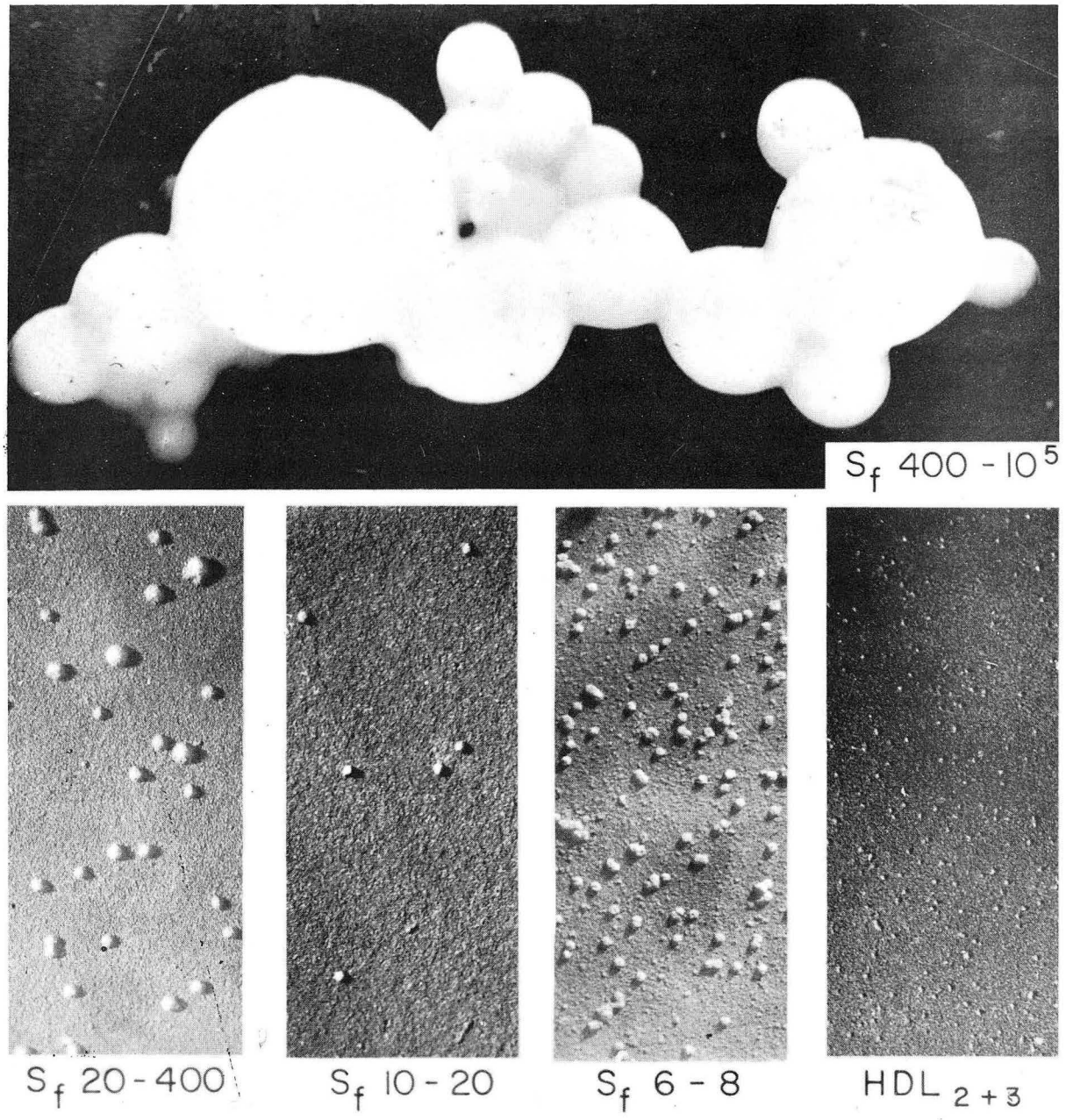
Another author stated "Sound arguments could be advanced for the term Essential Hyperlipoproteinemia, since concentrations of some

lipoproteins can be elevated without recognizable hyperlipidemia (3) but too few physicians have the facilities to measure lipoproteins, etc." (6) Even though it requires an ultracentrifugal study of patient's blood to have a proper diagnosis, it is justifiable to do this rather than using a confusing "generic name". Otherwise it will bring only confusion on the above issue.

The "hyperlipoproteinemia" is a term based on a sound basis, whereas "hyperlipidemia" is just a "generic" term. In a recently discovered rare inheritable disorder, a - beta - lipoproteinemia, hypocholesterolemia is associated with the absence of one major lipoprotein groups. According to others, this should be called again as "hypocholesterolemia".

Lipoprotein

It is certain that the lipids in plasma and other extracellular fluids, except for a few unesterified fatty acids, are present as a complex of lipid linking with protein. The first discovery of a lipid-protein complex in plasma is credited to Machebeuf in 1929 (42) who manipulated both salt concentrations and PH to fractionate horse serum. Organic solvent fractionation was applied for lipoprotein isolation by using alcohol or other solvents at a low ionic strength together with PH manipulation (43). Similarly, electrophoresis was attempted for purification also with failure (44). The first application of the analytical ultracentrifuge to blood lipoprotein was done with undiluted whole sera soon after the introduction of the ultracentrifugal pattern (45, 46). These phenomena, resulting from accumulations of low density lipoproteins in the region of the albumin boundary was analyzed and



ZN-2326

Fig. 8. Electron micrographs of the major classes of serum lipoproteins. These lipoproteins were isolated ultracentrifugally fixed in 1% OsO_4, shadowed with Pt–Pd–Au alloy, and viewed in RCA EMU 2-E electron microscope (magnification 36,000 x).

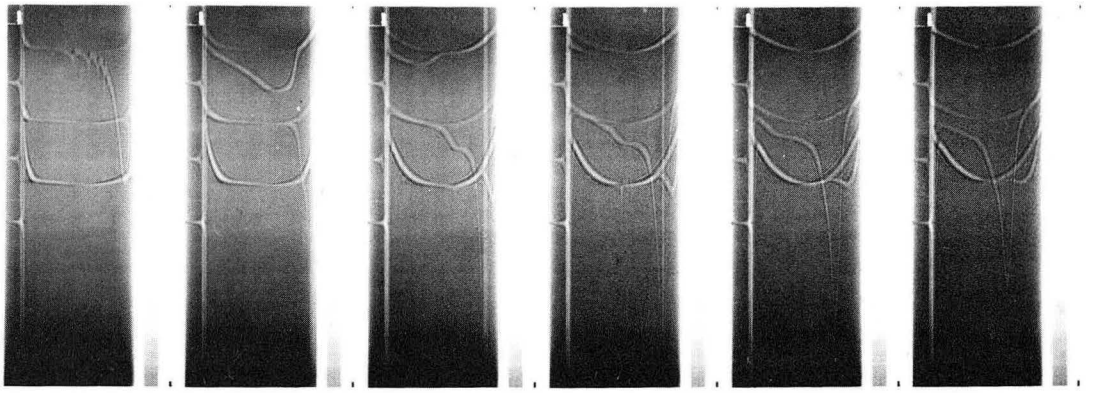


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Fig. 9. Representative schlieren flotation patterns obtained in the ultracentrifuge for the three major lipoprotein classes of human sera. The upper pattern is Sf 20–400 class floated in a medium of 1.063 g/ml. The middle pattern is Sf 0–20 class floated in a medium of 1.063 g/ml. The lower pattern is the HDL-1, 2, 3 class floated in a medium of 1.21 gm/ml. Time of each exposure after ultracentrifuge obtained speed of 52,640 rpm, is, from left to right, 0, 6, 22, 30, 48, and 64 minutes respectively. This run was made three double-sector-type ultracentrifuge cells in a spinco series ANC-1 four-cell rotor.

explained by Gofman, et al., by utilizing the theory of Johnston and Ogston which treated the problem of a mixture of proteins of different densities undergoing sedimentation in an ultracentrifuge (47). Gofman, et al., noticed that since the concentrations of lipoprotein in sera are usually less than that of other serum proteins, therefore, it was necessary to isolate and concentrate the lipoprotein from the other serum protein before characterization in the analytical ultracentrifugal flotation procedure. (48). Thus, the first purified lipoprotein could be obtained from human serum by this ultracentrifugal flotation procedure.

In the past, the chemistry (molecular weight, chemical composition and transport of human serum lipid and serum lipoprotein) has been described by Gofman and his colleagues (49-52). They also proposed the nomenclature for the lipoprotein and the lipoprotein metabolic abnormalities on the basis of ultracentrifuge. According to them, floatation rates of lipoproteins of density less than 1.063 are expressed in Sf units (Svedberg floatation unit in 10^{-13} cm/sec/dyne/gm). (3, 4, 48, 50, 51)

Ultracentrifugal pattern, average distribution, chemical composition, etc. are well shown in the Figures 8-18.

It will be self-explanatory that even though there are several types of lipoprotein measurement, ultracentrifugal lipoprotein flotation methodology provides better information. (Fig. 10) Therefore it is desirable to use ultracentrifugal nomenclature applied to lipoproteins, rather than the other nomenclatures. Separation of the diseases in the xanthomatosis group of hyperlipidemia has been more

difficult because of the great variations in cholesterol and phospholipid levels. Clinical terms, such as "tuberous" and "tendinous" which describe localization or appearance rather than their specific chemical abnormalities have been used (27). The introduction of the ultracentrifuge has made it possible to define the specific defect in lipid transport in these syndromes. In intensive studies of the hyperlipoproteinemia associated with xanthoma tendinosum, Gofman found, in a group of 18 patients, that the low density serum lipoproteins standard Sf^{o} 0-12 standard Sf^{o} 12-20, and standard Sf^{o} 20-100 were significantly elevated and standard Sf^{o} 100-400 significantly decreased in concentration compared with the lipoprotein values observed in a series of controls matched by age and sex (3, 4). The high density lipoprotein HDL-1 was significantly elevated in concentration compared with the matched control group, and the HDL-2 and HDL-3 high density lipoproteins were significantly decreased (3, 4). In xanthoma tuberosum the increase in lipoprotein levels is greatest in the standard Sf 20-100 lipoproteins and in the standard Sf 100-400 lipoproteins. The standard Sf 12-200 lipoprotein level is significantly increased, but to a much lesser extent than the standard Sf 20-100 or standard Sf 100-400 lipoprotein classes. However, the standard Sf 0-12 lipoprotein level is significantly lower than in the matched controls (3, 4). The HDL_1 levels cannot be proven to be significantly altered in xanthoma tuberosum within the framework of his available data. The HDL_2 levels are significantly lower than for matched controls, but the extent of lowering is less than that observed in the metabolic disturbance which characterizes xanthoma tendinosum. The HDL_3 level cannot be proven

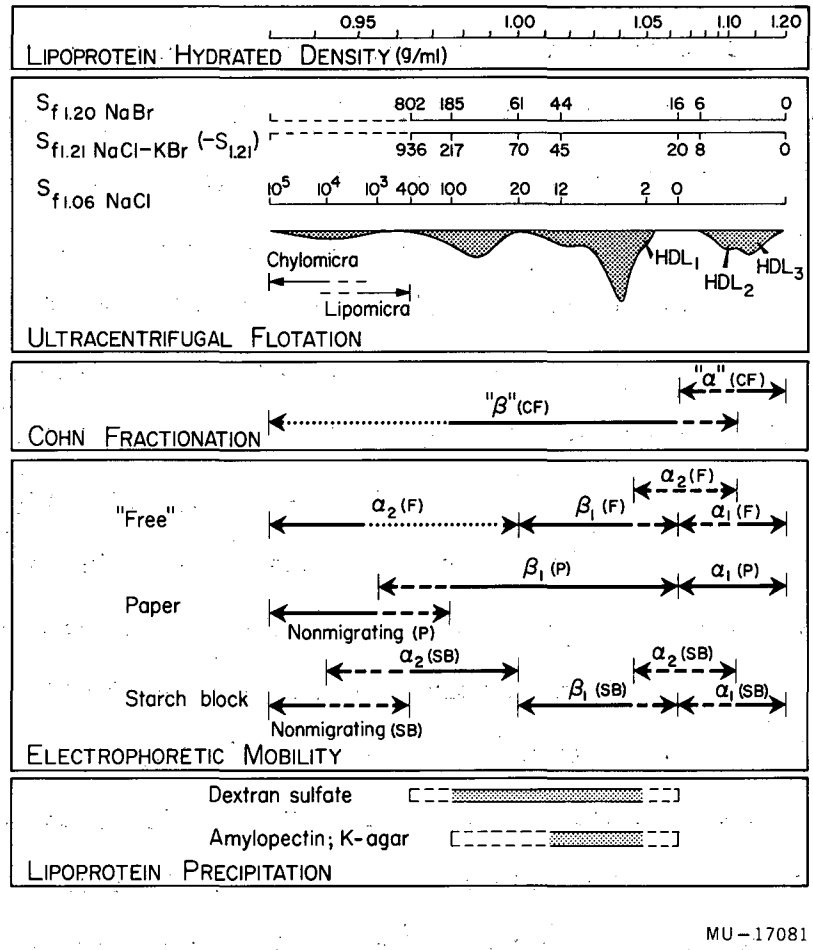

Fig. 10. Interrelationships among lipoprotein measurements and lipoprotein hydrated density. Lipoprotein flotation characteristics are for Sf 1.20 (0.95 molal NaCl; 2.771 molal NaBr), minus S or Sf 1.21 (1.748 molal NaCl; 2.051 molal KBr) and Sf 1.063 (1.748 molal NaCl) measured at a temperature of 26°C. The values of 936 and 217 are calculated from the experimentally obtained values of 802 and 185 by correcting for the solution (exclusive of lipoproteins) viscosity and density differences between the two media. For lipoproteins obtained by the Colin procedure, overlapping and certain hydrated densities are indicated by dashes and dotted lines, respectively. Similarly, for serum lipoprotein electrophoretic characterization, the dashed lines indicate regions of overlapping mobilities and dotted line indicates a region of uncertainty (or technical difficulties in determining mobilities). Subscript CF, F, and P, and SB refer to Cohn fraction, free, paper, and starch block electrophoresis, respectively. Regions of potentially effective measurement by the lipoprotein precipitants are indicated by the shaded rectangles.

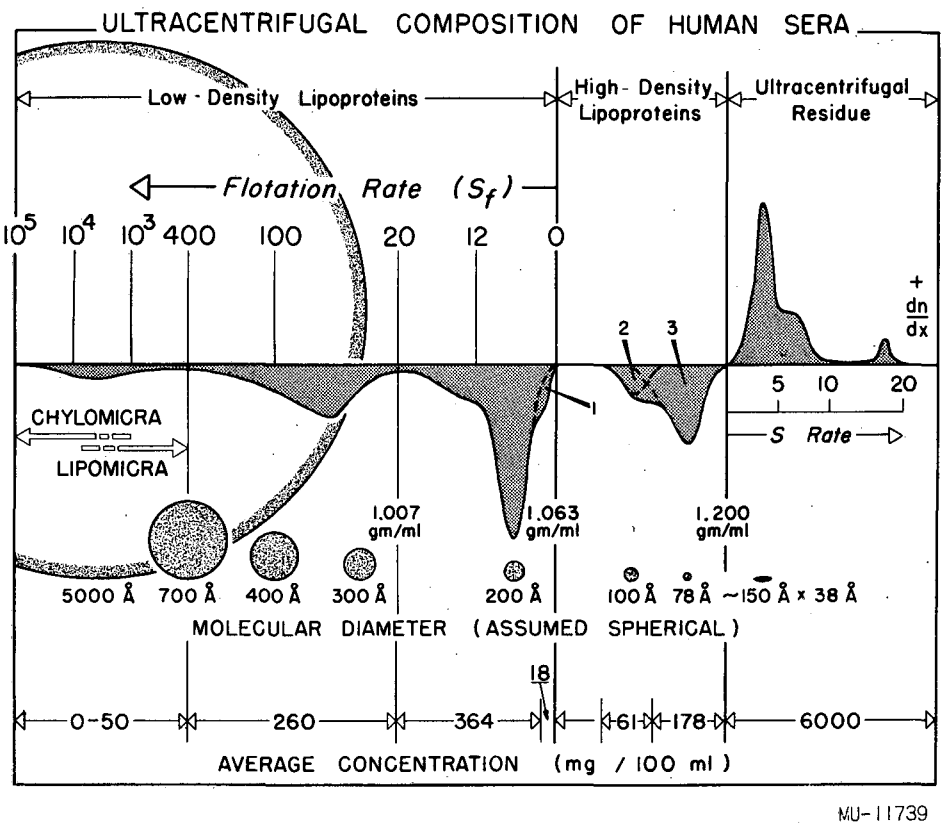

Fig. 11. The ultracentrifugal composition of human serum showing relative molecular size as well as average serum concentration in 45-year-old males. The non-lipoprotein residue is plotted on a thirty-fold-reduced dn/dx scale.

significantly lower than that for matched controls (3, 4).

Essential hyperlipemia represents a vaguely defined clinical state usually discovered by the incidental finding of creamy serum in a fasting blood specimen. Manifestly, the criteria for such a diagnosis can hardly be regarded as quantitative or definitive (3, 4). Some authors refer to xanthoma tuberosum as essential hyperlipemia in those patients with the typical cutaneous tuberous lesions in whom the fasting serum is creamy. (3, 4) In his considerations of essential hyperlipemia, reference is made only to those patients referred for study because of creamy serum, but who do not show any visible xanthomatosis lesion (3, 4).

In essential hyperlipemia, the standard Sf^{o}100-400 and Sf^{o} 20-100 lipoproteins show the greatest elevation in level as compared with matched controls. The standard Sf^{o} 12-20 lipoprotein class is not significantly different in level from that of the matched control group. The standard Sf^{o} 0-12 lipoprotein level is significantly and markedly lower than that in matched control groups (3, 4).

No mechanism of biochemical defect in essential hyperlipoproteinemia is understood, even though there are some suggestive hypothesis (6). Possible defects in the homeostasis of influx and efflux of lipid substances in the blood were largely attacked.

Bergström and Borgström (53), Frazer (54) Bloom, et al., (55) reported on the mechanism of digestion of fat and transfer phenomena in the intestinal mucosa which are far from complete understanding.

The nature of triglyceride rich chylomicron removal from the

blood is only partially solved. Exponential rate of removal and decreased fractional turnover rate by higher initial concentration of chylomicron (56), and tissues (57, 58, 60) involved in the removal of chylomicron were reported.

There are two schools of though concerning the site of hydrolysis and participation of other lipoprotein in the process of removal of chylomicron (6). Lindgren, Nichols, Freeman, Gofman (32, 33, 59) and Pierce (60) had suggested the degradation of chylomicron takes place primarily intravascularly. This involves the hydrolysis of glycerides, with removal of the product fatty acid bound to albumin, leaving higher density lipoprotein which may be degraded sequentially (Fig. 18).

This interconversion process has been reported in human beings after ingestion of fat (61). Even though one author claims that passive exchange of labeled protein and lipid between chylomicrons and other lipoprotein may mimic actual metabolic "conversion" (6), it is quite reasonable to believe there is a defect in conversion of chylomicrons in essential hyperlipoproteinemia. Also, the "interconversion" defect was proposed as the origin of hyperlipemia in the nephrotic syndrome (62). The report by Biggs and Gofman on the tritium labeled cholesterol study is consistent with the above idea (63).

It is also suggested chylomicron triglyceride does not pass through other low-density lipoprotein in plasma during its removal; or it may pass with rapid turnover rate. The possibility of intact chylomicron removal was suggested (64, 65). In any event, it is quite reasonable to believe that two processes are competing with

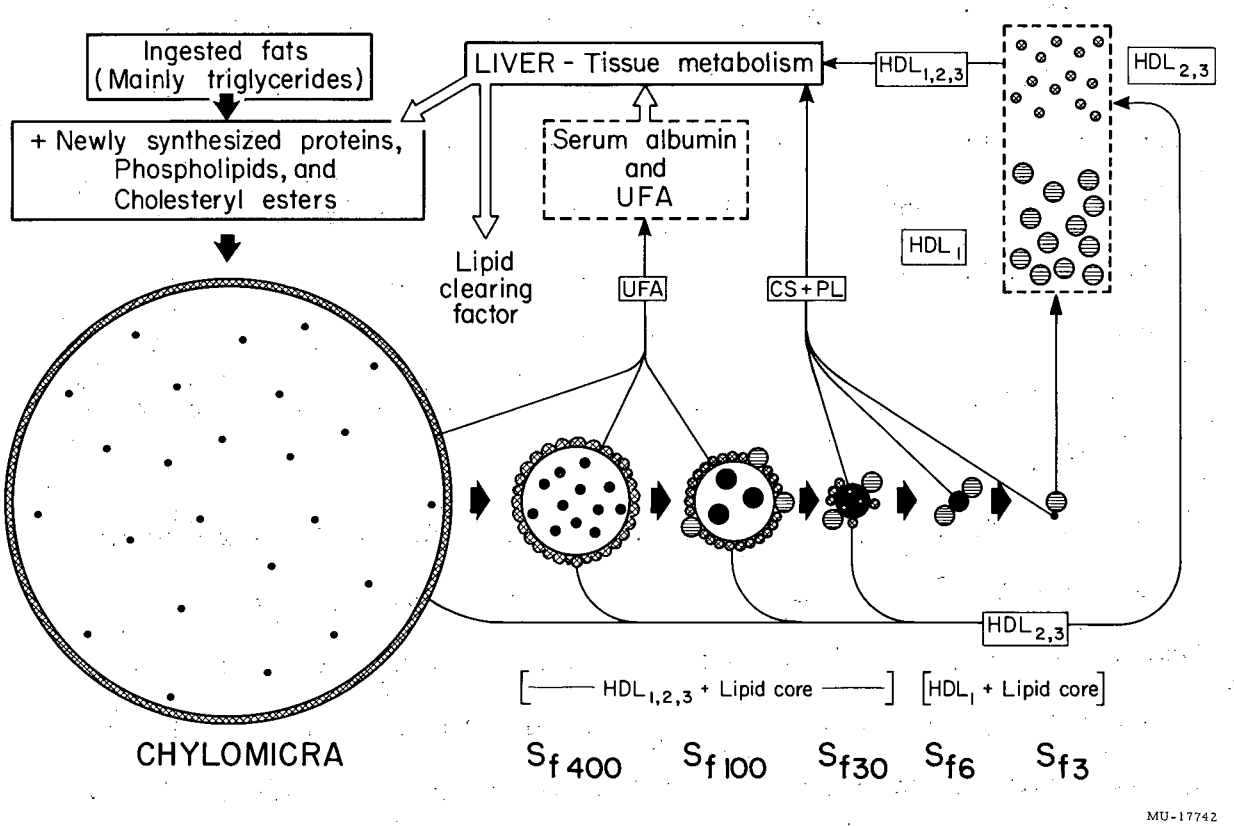

Fig. 12. Model of serum lipoprotein transformations between the various lipoprotein classes found in human sera, presenting schematically the pathway for the transport of ingested fatty acids.

each other in human chylomicron metabolism.

Chaikoff, et al., showed some difference in the disappearance curves for labeled cholesterol and labeled triglyceride in the rats (66). Whether an abnormally slow rate of removal or rapid influx of lipid is the cause of the defect is not yet clearly understood. Thanhauser (67), Crofford (68), Lever (69) have reported slower clearance from blood and Crofford (70) reported an accelerated influx of lipid into the blood stream.

The interrelationship between the lipoprotein lipase and hyperlipoproteinemia is not yet completely established, even though numerous works have been done on this hydrolytic enzyme (71, 72, 73, 74, 75, 76, 77).

Lipoprotein lipase deficiency was reported in some cases (78, 79) but another did not detect it (70). Schettler suggested that chylomicron may have abnormal composition, but Fredrickson reached the opposite conclusion. (6)

The abnormal nature of lipoprotein is not yet clear, but what is clear is the existence of an abnormal lipoprotein transport pattern (3, 4, 49, 80).

The possible association of hyperlipoproteinemia with carbohydrate metabolism was reported (5, 28, 68, 81), but the nature is not clear in the hyperlipoproteinemia. Net synthesis of triglycerides involves the intermediate formation of phosphatidic acid and, 1, 2-diglyceride (82). ATP and Acyl CoA are essential for these steps (82), and it is logical to assume that a major share of the latter comes from glucose degradation (6). Langdon (83), reported in those lines.

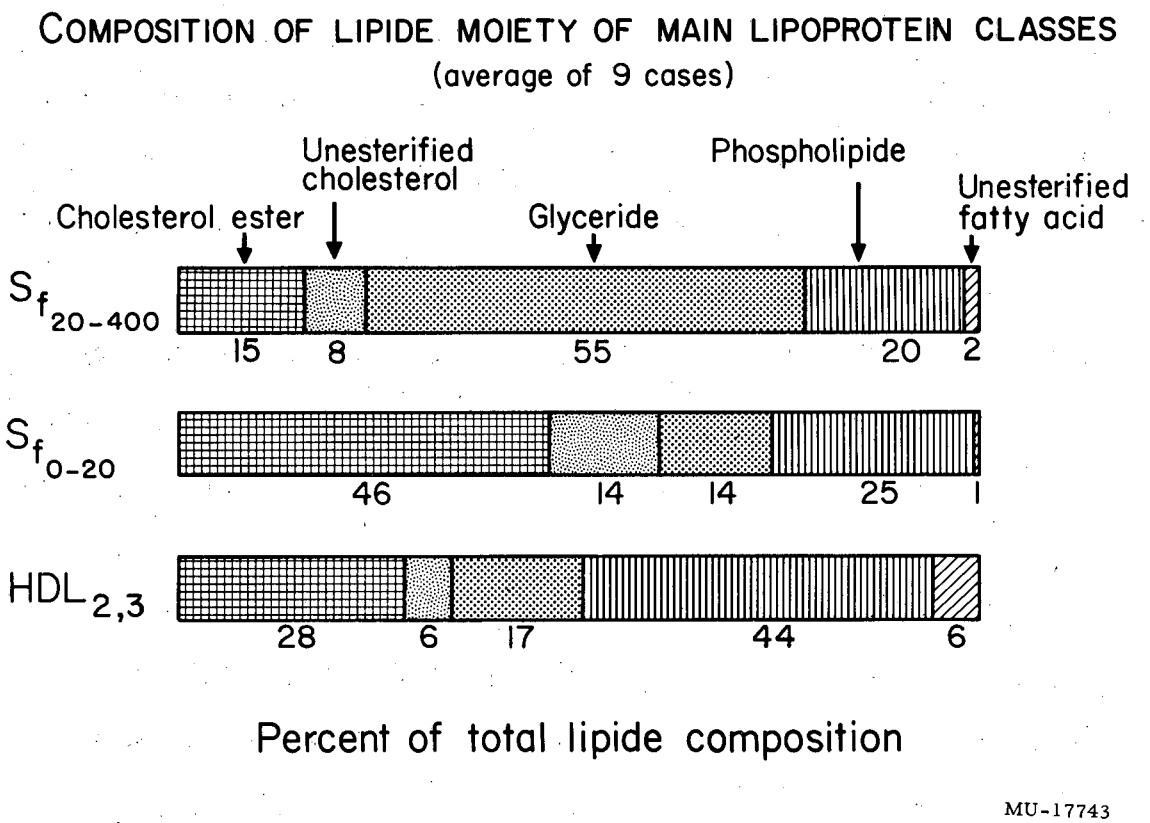

Fig. 13. The average lipide composition of nine individuals is given with respect to the three major lipoprotein classes. The phospholipides are presented as a single fraction.

The cholesterol aspect is also incompletely understood. The liver is reported to be the organ responsible for its homeostasis (84, 85, 86, 87).

Hellman, et al., have shown no difference in the rate of acetate incorporation as determined by appearance of radioactivity in the plasma in hypercholesterolemia (88). Since diabetes is often associated with hypersholesteremia, it is interesting that whereas Wilkins (89) found no abnormality in the glucose tolerance test Waddell (90) reported abnormal glucose and insulin tests in the hypercholesterolemia or hyperlipemia because increased hexose monophophate shunt and increased production of $TPNH_2$ would lead to increased cholesterol synthesis (91). The catabolism of cholesterol into bile acid was studied (92, 93, 94, 95, 96). Biggs and Gofman have made an interesting study with tritium labeled cholesterol on the dynamic aspect of lipid transport in health and disease (63). These authors reported a difference of specific activity of free and esterified cholesterol level between the blood of normal, xanthoma tuberosum and xanthoma tendinosum (Fig. 1, 2, 17, 18). Also they showed this specific activity absorption curve is a function of the $Sf^{o}0$-12 versus Sf^{o} 12-400 lipoprotein molecule in the serum and concluded that it is consistent with their idea of migration (63).

The endocrine and vitamin aspects were reported (97, 98, 99, 100, 101, 102, 103, 104).

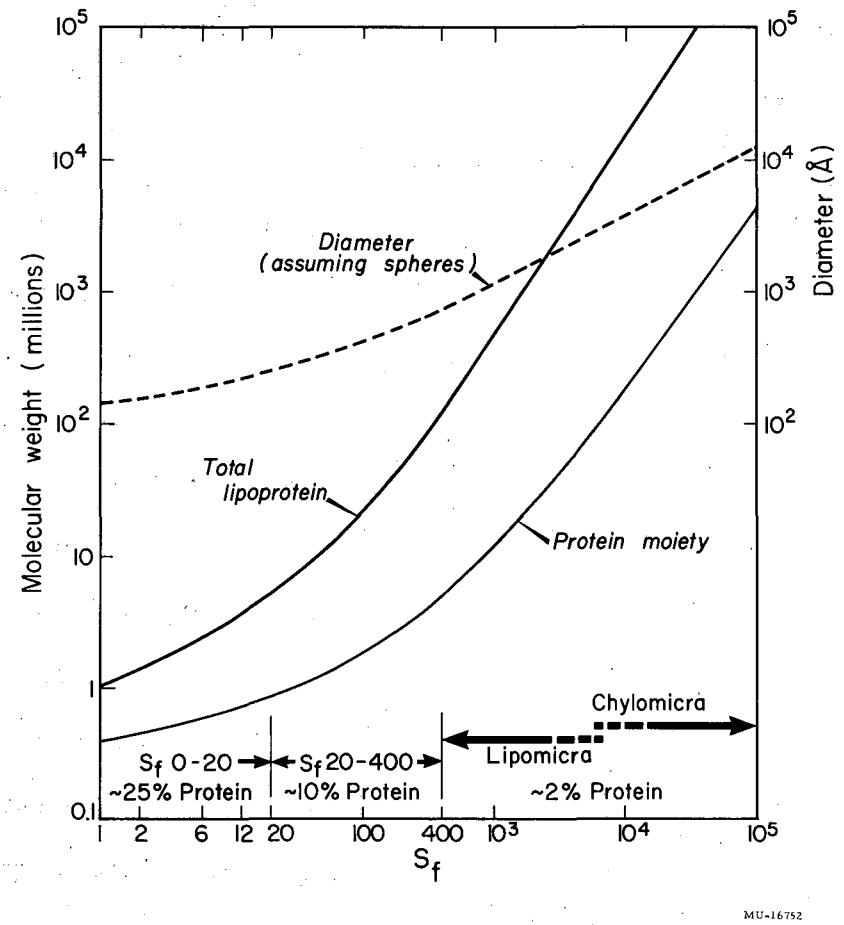

Fig. 14. Total molecular weights of the low density lipoproteins of Sf^o 1 to 10^5 as calculated for spheres (also indicated is the estimated molecular weight of the protein moiety). Molecular diameter (for spheres) is indicated by the dash line.

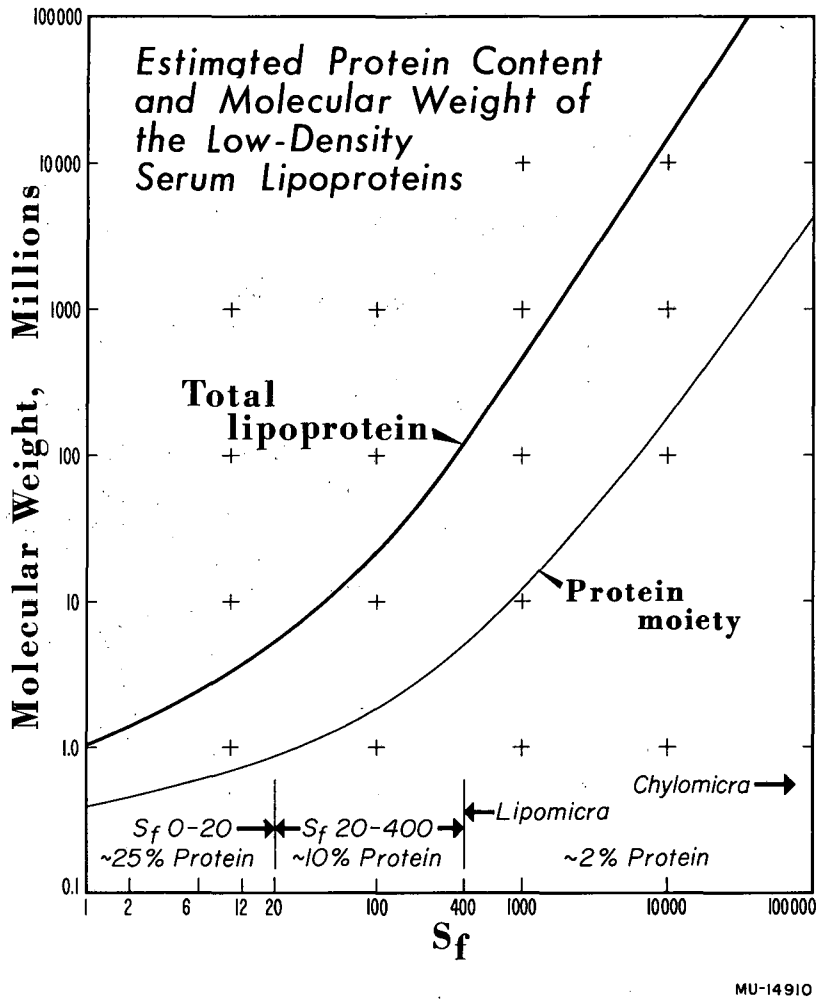

Fig. 15. Total molecular weight of the low density lipoproteins of Sf° 1–10 as calculated for spheres (also indicated is the estimated molecular weight of the protein moiety).

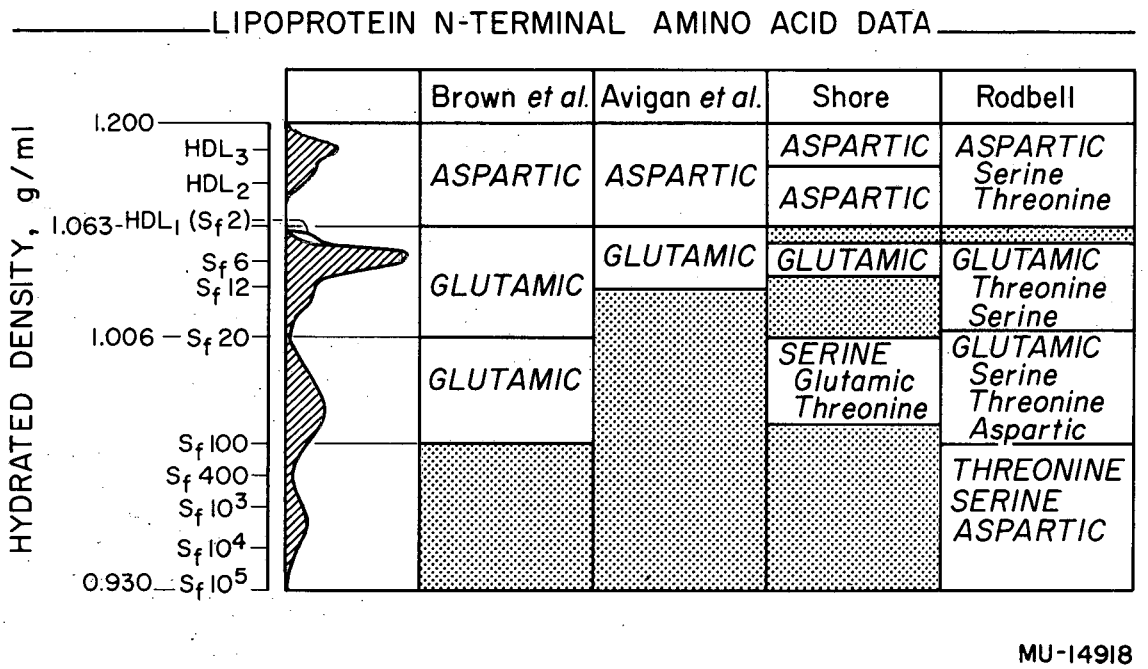

Fig. 16. Major N-terminal amino acid composition of the serum lipoproteins. The rectangles are placed to indicate which lipoprotein bands within the total lipoprotein distribution were studied. Dominant components are shown in the longer print. Trace componiment are not shown.

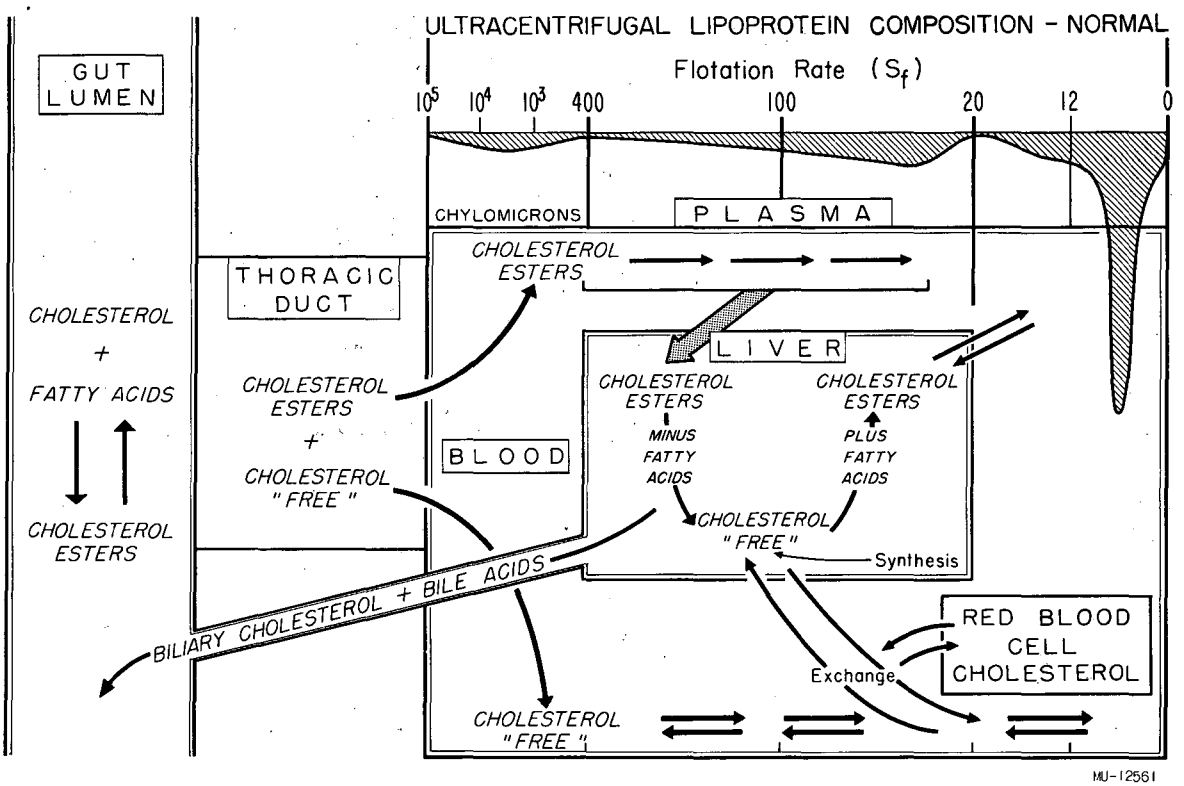

Fig. 17. Schematic representation of exogenous cholesterol metabolism in the normal patient. The Sf^o 20–400 lipoprotein pool is small and hepatic uptake is rapid.

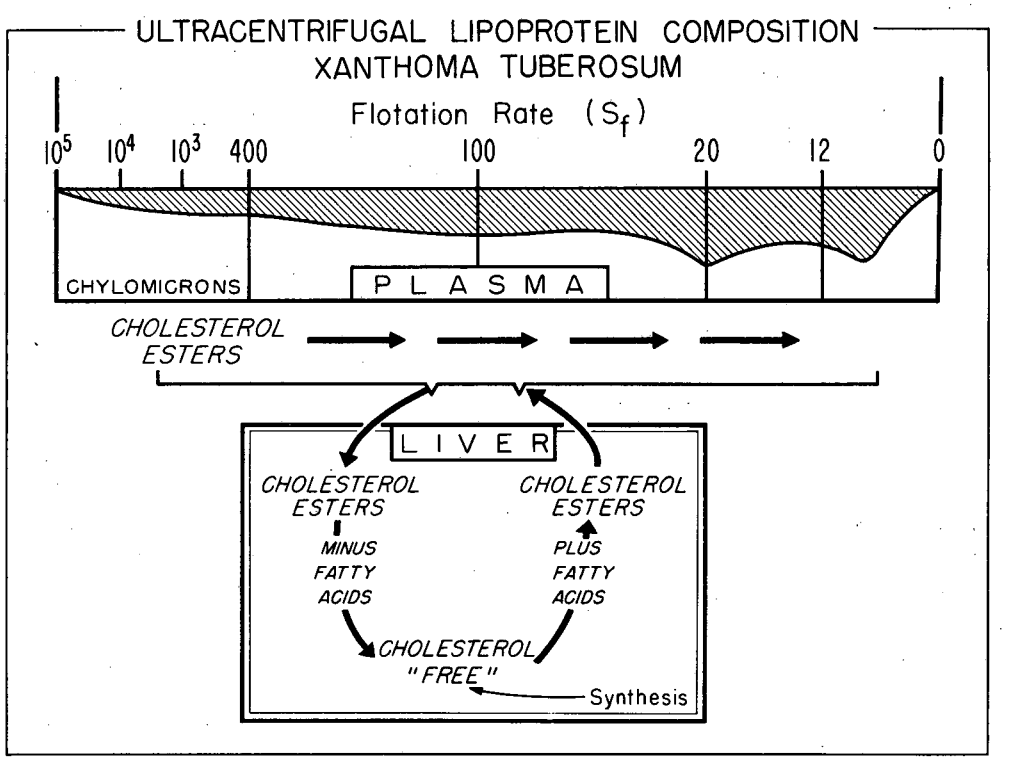

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Fig. 18. Schematic representation of exogenous cholesterol-ester metabolism in xanthoma tuberosum.
The Sf^o 20–400 lipoprotein pool is large, and effective uptake is slow.

II.

The relationship of lipid to mineral metabolism was shown by many authors. Lewis studied the serum lipids of vanadium industry and found a lower mean serum cholesterol level in the worker than the control (105). Curran, et al., also found the serum level of free and total cholesterol inthe young normocholesterolemic subjects lowered by vanadium salt administration. He also noticed reduction of C/P ratio (106), but no significant change in serum phospholipid level. Several authors reported in those lines (107, 108, 109, 110, 111).

Salts of vanadium also have been shown to inhibit utilization of mevalonic acid for cholesterol biosynthesis (107), possibly by competing with manganese or magnesium required for conversion of mevalonic acid to isopentenylpyrophosphate by two successive reactions with ATP (112).

Wright, et al., reported vanadyl inhibition is probably a reflection of the inability of a system to maintain a functional level of ATP (113).

Manganese increases the incorporation of C^{14}-labelled acetate in cholesterol and fatty acid (108). Tietz (114), Wakil, Porter and Gibson (115) reported similar findings with the synthesis of fatty acids in vitro.

Chromium increased hepatic cholesterol and fatty acid (108) synthesis by promoting the reaction between cytochrome c and succinic dehydrogenase (11).

Chromium differs from manganese and vanadium in that it is

mediated through a stimulating effect of chromium on carbohydrate metabolism with resultant production of such cofactors for lipid synthesis as reduced diphosphopyridine nucleotide and triphosphopyridine nucleotide (116). It was also suggested by studying of antagonistic actions of these metals (116).

Iron was reported to possess moderate depressant action on cholesterol synthesis in vitro and a lesser effect on fatty acid synthesis (116).

Many relations were reported on these metal functions on the above issue and (117, 118) suggested the possibility of an etiologic vanadium deficiency needs further investigation (119).

Arsenic was also reported for its inhibition of cholesterol biogenesis (120).

Cadmium and lead were reported to possess a lowering effect in the serum cholesterol of rats (121). Zyl reported elemental iodine caused a decrease of serum lipids in totally thyroidectomized baboons (122).

Perry, Camel have demonstrated that abnormally elevated levels of cholesterol in human plasma diminished following parenteral administration of disodium calcium chelate of EDTA, $CaNa_2$ EDTA and subsequently there is a markedly increased zincuria and increased excretion of Cd. V, Pb and Mn in the urine (117, 123). It was interpreted as indirect action rather than due to the molecule per se by Boyle, Clarke, Mosher and McCann (124). They also found no constant changes in the total cholesterol levels of plasma or in the cholesterol level of the three lipoprotein fraction (Sf^o 20-400, Sf^o 4-20, and HDL)

studied (117). Also they showed one of the two patients with xanthomatosis showed good response with a somewhat smaller daily oral intake of $CaNa_2$ EDTA for a longer period of time (117).

Caplan, Curtis reviewed hyperlipemia and xanthoma of skin produced by cobalt (125).

A slight inhibition of synthesis of triglyceride was suggested by the lowering of radioactivity of triglyceride synthesized by rat liver from C^{14}-labelled acetate in the presence of vanadium (108).

The metal effect on phospholipid metabolism was reported by Bernheim and Bernheim. They found that vanadium increased the oxidation of phospholipids (126, 127). In 1956, Mountain, et al., also showed that dietary vanadium produced a lowering of hepatic phospholipid content (110).

From a P^{32} study Snyder and Carnatzer have interpreted the decreased hepatic phospholipid value after administration of vanadium was due to the inhibition of phospholipid synthesis (128).

The interrelationship between copper deficiency and phospholipid was carefully studied by Gallagher, Judah, and Rees. They demonstrated that the defect in phospholipid synthesis in copper deficient rats was due to an abnormally low capacity to couple acyl coenzyme A with α-glycerophosphate to form phosphatidic acids (129).

Snyder, et al., also noticed abnormal fatty acid oxidation in the fatty liver induced by cerium (130, 131).

Selenium was also reported for its function on lipid metabolism (132, 133).

Thus, chemical elements and the linkage between lipids and

protein in lipoprotein are fascinating riddles and quite possibly the keys to the question of how the plasma lipid is regulated in normal and abnormal lipid metabolism (112).

III

Several chemical elements are shown in this study to be significantly different in the xanthoma tuberosum and xanthoma tendinosum.

Although the differences are significant, they are not large. Listed in tables are the elements showing abnormal differences.

Table

Significant differences expressed in ppm of the mean value of the reference population and also between two xanthomatosis *

Element	Mean Value in Reference Population	Difference in Xanthoma Tuberosum	Difference in Xanthoma Tendinosum	Difference between Two Xanthomatosis
Phosphorus	163	16	6.6	9.5
Sulfur	1201	110	45	64.6
Cholorine	3798	354	143	210.
Zinc	0.80	-0.09	0.04	-0.13

*underlined values showed positive significant test at 5% level.

Serum phosphorous occurs in several forms as does serum sulfur or serum zinc. Since x-ray spectroscopic analysis of chemical elements does not discriminate between the various chemical structures of a particular element, it is sometimes quite useful. Thus, it provides total concentration of all forms, organic plus inorganic.

Serum total phosphorus findings. Total serum phosphorus represents the sum of contributions from all phosphorous-containing entities of serum, organic and inorganic.

Literature tabulations (134) shows 35 ppm (3.5 mg/100 ml) for serum inorganic phosphorus ($H_2PO_4^-$, $HPO_4^=$ $PO_4^{\equiv}$), and some 92 ppm as phospholipid phosphorus, there is left some 36 ppm of phosphorus distributed among all other forms (135, 136). If this observed aberration in serum total phosphorus level in xanthoma tuberosum is due to one form of serum phosphorus, it is a sizable alteration. Why do two diseases show a different value? If one agrees with one author who described it as different manifestations of the same disease (6) he is very unlikely to expect these results. With this data, it is impossible to know which form of phosphorus is high. It is interesting to match this with phospholipid phosphorus since there is some different phospholipid distribution among various members of the lipoportein transport system.

According to Gofman, Freeman, deLalla, Nichols, Lindgren (4, 49) the phospholipid contents of various lipoproteins in xanthoma tuberosum and xanthoma tendinosum shows as Table 3.

Phospholipid content

Lipoprotein Classes	Xanthoma Tuberosum Lipoprotein	Xanthoma Tendinosum Lipoprotein	Fraction of Phospholipid	Fraction of Lipid
HDL_1	25.6	25.8	0.20	0.50
$HDL_{2.3}$	38.5+165.9	14.9+149.8	0.44	0.50
Sf_{0-20}	334	793	0.25	0.75
SF_{20-400}	1266	164	0.20	0.90

The phospholipid phosphorus calculated from the table above shows the following values:

Xanthoma tuberosum:

$$\left\{ (334)(0.25)(0.75) + (25.6)(0.2)(0.5) + (38.5 \pm 165.9)(0.44)(0.5) + (1266)(0.2)(0.9) \right\} 0.4$$

$$= 134 \text{ (ppm)} \quad \text{) (Phospholipid phosphorus)}$$

Xanthoma Tendinosum

$$\left\{ (0.5)(25.8)(0.2) + 149.9 + 149.8)(0.44)(0.5) + (793 + 150)(0.25)(0.75) + (164)(0.2)(0.9) \right\} 0.4$$

$$= 96 \text{ (ppm)} \quad \text{) (phospholipid phosphorus)}$$

These data agree very well with x-ray spectrochemical data.

It is very interesting that 36 ppm component of the phosphorus is very much decreased in xanthoma tuberosum whereas in xanthoma tendinosum there is no effect. If this 36 ppm component is used for some lipoprotein transport system or some other linkage with protein, it is suggestive that the two diseases may have distinctly different structures of lipoprotein system beyond just ultracentrifugal differences.

Chlorine: Chlorine in the serum is probably mostly in the form of choloride as the predominant matching anion for Na.

Chloride ion has a very weak affinity for combination with protein. Evidence of its binding to protein in biological fluids and tissues is sparse and inconclusive (139).

About 2% of serum chloride at the most, binding to protein was described by Cotlove and Hogben on the basis of Manery's Donnan equilibrium experiment, if the result did not stem from analytical error (139). It was suggested that the binding of chloride in plasma is likely to be less because of competition for protein-binding

sites by substances with higher affinities; fatty acids, amino acids, organic acids and other proteins (139).

Evidence for protein binding of Cl was obtained by evaluating ion-protein interaction (140). The binding affinity of Cl^- for serum albumin is about one-fourth that of I^- and binding readily reversible (139).

The average number of moles of anion bound per mole of serum albumin increases at lower pH with increase in net positive change on protein, and increases at higher concentrations of anion. (139).

Several detailed interpretations of binding data have been proposed (141). Also, chloride is reported to have binding with globulin (142).

The activation of enzyme by Cl^- was shown in a few instances (139); among them, Mammalian amylases, polypetidase converting hypertension I to II ("plasma-converting enzyme") (143).

It is of interest to study to what extent the above-deranged chloride of hyperlipoproteinemia is different from the normal functional range. Also, it is desirable to know the distribution of chloride between intra and extra-cellular compartments, and the difference in the Cl^- and lipoprotein interaction in the hyperlipoproteinemia.

None of the subjects could be suspected of having other conditions known to bring about chloride derangement, e.g., diabetic acidosis, gastrointestinal fluid loss and starvation, adrenocortical insufficiency, Renal tubular acidosis, uremic acidoses, or congestive heart failures.

Serum sulfur finding: Total serum sulfur also represents the sum of contributions from a variety of chemical entities, organic and

inorganic.

The sulfur value are very much lower than our x-ray spectrochemical data becuase, as one would expect, it is customary to report serum sulfur as the sum of inorganic plus organic forms without the protein or other macromolecule sulfur which may contribute by its amino acid or bound sulfur (135, 136). A value of 85.5 ppm of serum sulfur was reported (134). Since the mean level of the healthy reference group sample is reported as 1201 ppm, it would follow that approximately 1115 ppm of sulfur must be bound in proteins or other macromolecules (135).

According to Edsall (137) the following data could be obtained for the sulfur content of serum proteins:

Albumins	1.96% Sulfur
α Globulins	1.02% Sulfur
β Globulins	1.32% Sulfur

Calculation done by Gofman (136) based on de Lalla, et al., (138) report on the protein-bound serum sulfur shows:

$4.6 \times 0.0196 + 1.5 \times 0.0102 + 0.3 \times 0.0132 = 0.109$
gms of sulfur per 100 ml serum

(4.6 gm% for albumin, 1.5 gm% for globulin and 0.3% for-
blobulin) (138)

Approximately 7% (some 85 ppm) distributed among inorganic sulfur plus sulfur in various small molecule organic compounds.

Approximately 70% (some 840 ppm) as cystine, cysteine, and methionine in serum albumin.

Approximately 23% (some 275 ppm) as cystine, cysteine, and methionine in various serum globulins (136).

Expressed in ppm, 1095 ppm protein-bound sulfur is calculated (136).

As in the case of phosphorus, it is interesting to know what form of sulfur is changed. The comparison of the sulfur in the two different genetic diseases is also significant. Whether it is the direct result of the lipid part or protein part of the lipoprotein transport system or some other metabolic abnormalities influencing homeostatic mechanism of sulfur remains a challenging unsolved problem. No known sulfur lipid exists in the plasma, although it does in other tissues (e.g. brain tissue). Structural studies of protein moeity of lipoprotein might provide the key information about the above metabolic derangement.

Zinc: This element shows a positive significance test in the difference between the two groups of xanthomatosis, even though the individual groups do not have significant differences with respect to the reference group. It is quite true further study is necessary before making any conclusion. However, this limited data does show the difference between the groups to be in the opposite sense. This should be further explored with a more ideal reference control and a greater number of patients because zinc forms about four different metalloenzymes which are involved dehydrogenation process in TCA cycle and also glycerol part of fatty acid metabolism (alcohol dehydrogenase) (144).

At first glance it is interesting that two diseases show distinct different values for these elements. It is in

quite good agreement with Gofman's xanthoma classification based on ultracentrifugal flotation rate of lipoprotein in the serum of hyperlipoproteinemia. Even though the exact biochemical significance still remains uncertain, two inherited inborn metabolic disorders which show different lipoprotein transport and different homeostasis of chemical elements could be genetically distinctly different disorders.

Other elements such as vanadium, arsenic, iron, chromium, cobalt, copper, cerium, selenium, iodine and manganese which are discussed in relation with lipid metabolism do not show any significant difference between the two types of xanthomatosis at the level where this technique could detect. It might be quite possible that there were some changes at a lower level where our technique cannot reach or some alteration at some organ other than serum. But, nevertheless, it is quite interesting that they do not show any significant difference when other elements do.

Future of hyperlipoproteinemia research: Since all lipid in plasma is transported in association with protein as lipoprotein molecules, abnormalities in the linkage bond or in protein structure could also play an etiologic role along with other deranged lipid metabolism . Ultracentrifugal work achieved by Gofman, et al., on the xanthomatosis was ingenious since it described the lipid metabolic disorder as a "lipid-protein" disorder.

The x-ray spectral data is quite stimulating since it may suggest also disturbances in the protein moiety of lipoprotein macromolecules In short, all of lipoprotein metabolism, normal and abnormal phenomena,

are related to the fine structure of lipoprotein, protein and lipid.

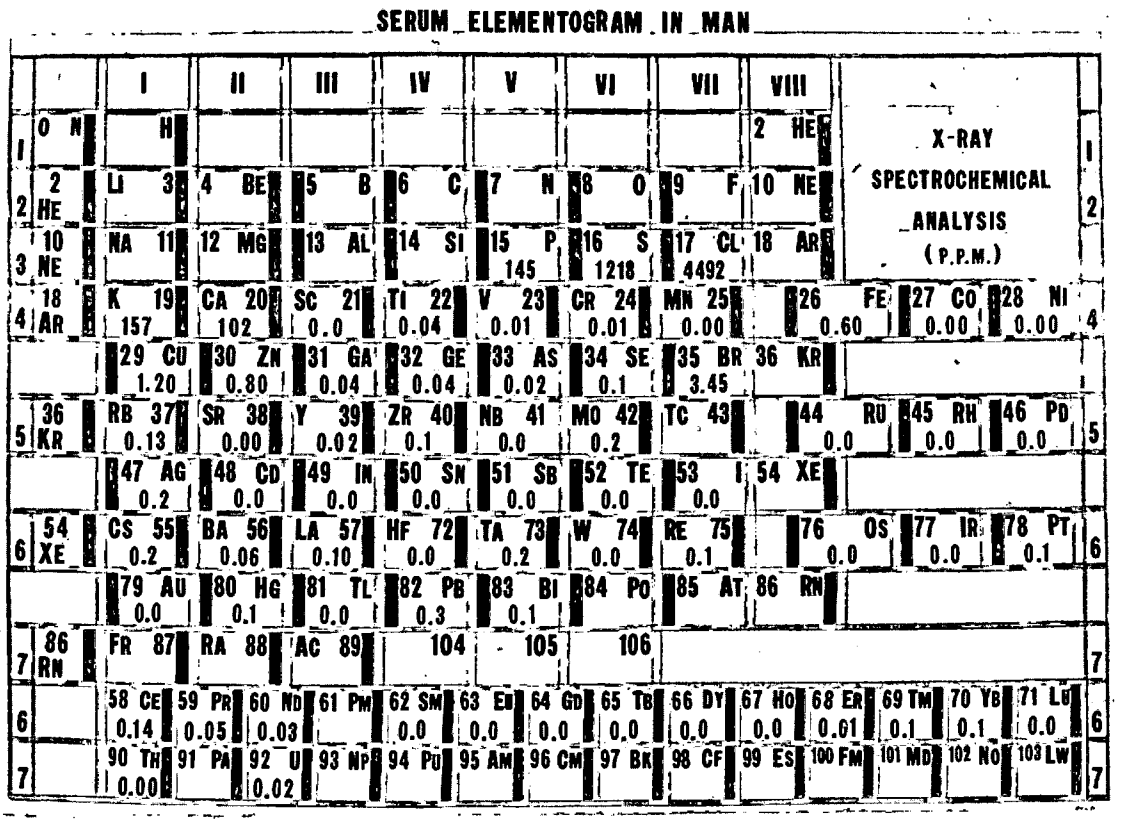
The so-called structure of protein, which consists of amino acid sequence, spatial configuration, the electric charge distribution in the peptide chain at microscopic range is in dynamic equilibrium with lipid component. A subtle linking bond produces a highly specific reaction and thus leads to a specific biochemical transformation or energy transfer (145). The intermolecular forces within biological macromolecules and also the intramolecular forces of such molecules remain unsolved theoretical problems (145).

A more important but rather difficult assignment is the determination of lipoprotein configuration in the solution. It is more important because water is a major constituent of an organism, and lipoprotein is functioning with the mediation of plasma. This subject can be approached from various angles by the various scientific fields and divergent approaches can be united.

Extensive and joint application of various research techniques involving both physical and physio-chemical methods (e.g. Linderstrøm-Lang's deuterium exchange method, Doty's optical rotation dipersion method, electrical conductivity, flourescence spectra, ultracentrifuge, x-ray diffraction, light scattering, infra-red spectroscopy, electron microcopy, flow birefringence, electric bifringence, viscosity, etc.), chemical methods (i.e., paper chromatography, column chromatography, isotopic tracers, terminal analysis, etc.) and also new technique developed E. M. R. for measuring free radical and N.M.R.

for spatial configuration and hydration of protein molecule (145) should be properly applied to understanding this macromolecular genetic disorder at the molecular or submolecular level. Indeed, it is so profound and mysterious that it requires further and further exploration.

Metals, enzymes, lipoprotein, lipids and proteins in hyperlipoproteinemia, other inborn errors of metabolism and living phenomena, these are CHALLENGING PROBLEMS to all physicists, chemists, biologists, biophysicists, molecular biologists, medical scientists, mathematicians and engineers at present and in the future.



ZN-3879

Fig. 19. Serum elementogram in ostensibly normal man arranged by elemental groups and periods (from Z = 15 through Z = 92).
Does not represent the table. (17)

TABLE 1

X-Ray Spectra Analysis of Sixty-Six Chemical Elements in the Serum of Xanthoma Tendinosum

Subject	Diagnosis	Z= 15 Serum Phosphorus	Z= 16 Serum Sulfur	Z= 17 Serum Chloride
* 24	Xanthoma Tendinosum	151	1107	3507
25		169	1238	3921
29		162	1189	3764
30		170	1247	3945
32		163	1201	3797
199		184	1354	4282
200		176	1294	4093
201		168	1237	3908
202		166	1225	3871
394		182	1336	4224
409		156	1150	3638
421		168	1241	3917
ppm mean		170	1246	3942
ppm S. D.		7.9	58.2	183.3
ppm S. E.		2.5	18.4	58.0

*24 is not included in statistical calculation because of its partial hemolysis.

S. D. = Standard deviation
S. E. = Standard error

[Continued]

TABLE 1

Subject	Z= 19 Serum Potassium	Z = 20 Serum Calcium	Z = 21 Serum Scandium	Z = 22 Serum Titanium	Z = 23 Serum Vandium
24	not available	108	not available	-0.06	0.05
25	183	106	-0.12	-0.10	0.08
29	175	102	not available	-0.08	0.02
30	184	115	-0.09	0.01	-0.04
32	177	109	-0.04	-0.17	0.09
199	200	107	not available	-0.10	0.04
200	191	113	0.31	-0.13	0.06
201	182	115	0.07	-0.15	0.00
202	180	115	-0.07	-0.01	0.10
374	197	118	0.40	-0.09	0.03
409	169	113	-0.20	-0.06	0.04
421	183	121	0.05	-0.03	0.05
ppm mean	184	112	0.00	-0.08	0.04
ppm S. D.	8.6	5.5	0.22	0.05	0.03
ppm S. E.	2.7	1.8	0.07	0.02	0.01
584			-0.42		
759			0.15		

[Continued]

TABLE 1

Subject	Z = 24 Serum Chromium	Z = 25 Serum Manganese	Z = 26 Serum Iron	Z = 27 Serum Cobalt	Z = 28 Serum Nickel
24	0.00	-0.02	not available	-0.01	-0.05
25	-0.03	-0.01	1.17	-0.02	-0.04
29	-0.01	-0.03	0.74	-0.03	-0.04
30	0.00	0.00	0.82	-0.01	-0.08
32	-0.02	0.00	1.14	0.01	-0.06
199	0.00	0.11	0.82	0.00	-0.07
200	0.00	0.02	0.72	0.03	-0.07
201	0.01	0.00	0.96	0.00	-0.06
202	0.03	0.01	0.77	0.00	-0.05
374	-0.02	0.07	1.09	-0.10	-0.06
409	-0.02	0.02	1.16	-0.06	-0.04
421	-0.02	0.04	1.17	-0.05	-0.07
584			0.82		
759			0.22		
ppm mean	0.00	0.02	0.89	-0.02	-0.06
ppm S. D.	0.02	0.04	0.26	0.04	0.01
ppm S. E.	0.00	0.01	0.07	0.01	0.00

[Continued]

TABLE 1

Subject	Z = 29 Serum Copper	Z = 30 Serum Zinc	Z = 31 Serum Gallium	Z = 32 Serum Germanium	Z = 33 Serum Arsenic
24	1.63	1.03	0.06	0.01	-0.01
25	1.36	0.66	0.06	0.08	-0.01
29	1.38	0.65	0.06	0.01	-0.01
30	1.29	0.72	0.07	-0.01	-0.07
32	1.26	0.81	0.05	0.01	-0.02
199	1.62	0.96	0.06	0.03	0.07
200	2.27	0.90	0.05	0.06	0.04
201	1.30	0.97	0.07	-0.01	0.01
202	1.22	0.87	0.03	0.01	-0.01
374	1.29	0.71	0.07	-0.08	0.00
409	1.20	0.83	-0.01	-0.07	-0.01
421	1.12	0.99	-0.04	-0.05	0.03
584	2.30	0.73			
759	1.54	0.82			
812		1.02			
ppm mean	1.48	0.84	0.04	0.00	0.00
ppm S. D.	0.36	0.13	0.03	0.05	0.03
ppm S. E.	0.01	0.03	0.01	0.02	0.01

[Continued]

TABLE 1

Subject	Z = 34 Serum Selenium	Z = 35 Serum Bromine	Z = 37 Serum Rubidium	Z*= 38 Serum Strontium	Z= 39 Serum Yttrium
24	0.09	3.83	-0.10	0.07	-0.01
25	0.04	4.55	0.33	-0.06	0.03
29	0.07	3.12	0.21	-0.03	0.06
30	0.09	3.81	0.23	-0.01	0.10
32	0.12	4.87	0.16	-0.02	0.05
199	0.01	4.85	0.20	-0.07	-0.10
200	0.03	4.92	0.16	-0.08	-0.12
201	0.01	5.41	0.10	0.03	-0.02
202	0.06	3.66	0.08	-0.03	-0.02
374	0.11	3.68	0.14	0.04	0.02
409	0.12	1.87	0.10	-0.05	0.00
421	0.21	2.80	0.09	-0.03	-0.01
ppm mean	0.08	3.96	0.16	-0.02	0.00
ppm S. D.	0.06	1.03	0.07	0.04	0.06
ppm S. E.	0.02	0.32	0.02	0.01	0.02

* 24 was used

[Continued]

TABLE 1

Subject	Z = 40 *Serum Zirconium	Z = 41 Serum Niobium	Z = 42 Serum Molybdenum	Z = 44 Serum Ruthenium	Z = 45 Serum Rhodium
24	-0.08	-0.11	0.28	-0.10	0.20
25	0.13	-0.25	-0.47	-0.15	0.27
29	0.42	-0.05	-0.18	-0.19	0.07
30	-0.33	-1.951	-0.33	0.19	0.00
32	-0.34	-0.02	0.531	0.05	-0.24
199	-0.68	-0.13	0.47	0.15	-0.30
200	-0.27	-0.30	0.31	0.16	-0.26
201	-0.41	-0.13	0.31	0.04	-0.14
202	0.73	-0.35	0.85	0.18	-0.11
374	-0.08	-0.08	0.22	-0.04	-0.51
409	-0.13	0.03	-0.19	0.02	-0.15
421	-0.60	-0.14	0.07	0.05	-0.02
ppm mean	-0.14	0.31	0.15	0.04	-0.11
ppm S. D.	0.24	0.53	0.39	0.12	0.22
ppm S. E.	0.11	0.18	0.12	0.04	0.07

* 24 was used

[Continued]

TABLE 1

Subject	Z = 46 Serum Palladium	Z= 47 Serum Silver	Z = 48 Serum Cadmium	Z= 49 Serum Indium	Z = 50 Serum Tin
24	0.07	-0.16	not available	-1.04	-0.08
25	-0.22	0.11	0.18	-2.77	-1.23
29	-0.38	0.28	0.09	0.25	-0.36
30	0.28	0.19	-0.04	0.09	0.06
32	0.23	0.15	-0.03	-0.77	-1.38
199	0.05	0.25	0.02	-0.22	-1.38
200	-0.09	0.35	-0.02	-0.06	1.04
201	-0.51	0.39	-0.08	-0.18	-0.10
202	0.00	0.36	-0.28	-0.41	0.21
374	0.03	0.22	-0.75	-0.20	-0.37
409	-0.04	-0.24	-0.37	0.05	0.03
421	0.37	0.09	-0.92	0.23	-0.43
ppm mean	-0.03	0.20	-0.20	-0.36	-0.32
ppm S. D.	0.26	0.29	0.33	-0.86	0.71
ppm S. E.	0.08	0.17	0.11	0.27	0.23

[Continued]

TABLE 1

Subject	Z = 51 Serum Antimony	Z= 52 Serum Tellurium	Z = 53 Serum Iodine	Z= 55 Serum Cesium	Z= 56 Serum Barium
24	-2.16	-0.25	-0.78	0.43	0.19
25	-0.88	-0.14	-1.50	0.32	0.37
29	0.61	-2.04	0.89	0.60	0.08
30	-0.54	-1.76	0.23	0.36	0.30
32	-1.16	1.34	-0.17	0.44	0.06
199	-0.30	-0.43	0.50	0.34	0.30
200	-0.76	-0.23	0.40	0.14	0.12
201	-1.19	-0.03	0.27	0.36	0.19
202	0.26	-0.40	1.06	0.31	0.23
374	-0.15	-1.88	-1.68	0.18	0.30
409	-0.06	-1.81	0.72	0.33	0.29
421	0.07	2.76	-0.106	0.35	0.26
ppm mean	m-0.37	-0.42	-0.01	0.34	0.23
ppm S. D.	0.56	1.41	0.84	0.12	0.10
ppm S. E.	0.18	0.45	0.25	0.04	0.03

[Continued]

TABLE 1

Subject	Z = 57 Serum Lanthanum	Z = 58 Serum Cerium	Z = 59 Serum Praseodymium	Z = 60 Serum Neodynium	Z = 62 Serum Samarium
24	0.27	0.16	not available	0.45	-0.06
25	0.32	0.28	0.10	0.09	0.10
29	-0.27	0.04	not available	0.00	-0.06
30	-0.31	-0.03	-0.02	0.02	0.09
32	-0.32	-0.39	-0.05	-0.03	0.23
199	-0.17	-0.25	not available	0.05	0.21
200	-0.22	0.02	-0.05	-0.02	0.18
201	-0.28	-0.07	not available	0.02	0.09
202	-0.22	0.53	0.00	-0.07	0.10
374	-0.20	-0.11	-0.01	0.12	-0.21
409	-0.20	0.14	0.03	0.00	-0.08
421	-0.16	-0.10	-0.03	0.03	-0.26
584			-0.05		
ppm mean	-0.15	0.01	-0.01	0.03	0.04
ppm S. D.	0.18	0.24	0.04	0.05	0.16
ppm S. E.	0.06	0.08	0.02	0.02	0.05

[Continued]

TABLE 1

Subject	Z = 63 Serum Europium	Z = 64 Serum Gadolinium	Z = 65 Serum Terbium	Z = 66 Serum Dysprosium	Z = 67 Serum Holmium
24	0.14	0.02	-0.10	0.52	0.44
25	0.15	-0.15	-0.10	0.08	0.09
29	0.20	-0.17	0.02	-0.04	0.13
30	0.18	-0.03	0.01	-0.07	-0.13
32	0.10	-0.28	-0.07	0.03	-0.09
199	-0.02	-0.19	0.05	0.08	0.18
200	-0.04	-0.21	-0.14	-0.25	-0.11
201	0.06	-0.19	-0.06	0.03	0.04
202	-0.19	-0.07	0.03	0.13	-0.11
374	-0.89	-0.09	-0.04	0.08	-0.14
409	-0.16	0.03	0.02	0.14	0.01
421	-0.13	0.17	0.10	-0.28	-0.04
ppm mean	-0.07	-0.11	-0.02	-0.01	-0.01
ppm S. D.	0.29	0.12	0.07	0.14	0.11
ppm S. E.	0.09	0.04	0.02	0.04	0.03

[Continued]

TABLE 1

Subject	Z = 68 Serum Erbium	Z = 69 Serum Thulium	Z = 70 Serum Ytterbium	Z = 71 Serum Lutetium	Z = 72 Serum Hafnium
24	0.06	0.04	-0.07	-0.30	0.24
25	-0.04	-0.17	0.00	-0.03	-0.31
29	-0.04	-0.05	-0.19	-0.03	-0.18
30	-0.04	0.25	-0.17	0.47	-0.25
32	0.03	-0.01	0.22	0.63	0.47
199	0.04	0.22	0.25	0.54	-0.05
200	0.06	0.16	0.23	-0.02	-0.07
201	0.03	0.27	0.14	-0.31	-0.34
202	0.10	0.18	0.16	0.57	-0.56
374	-0.07	0.22	0.09	0.63	-1.01
409	-0.12	0.06	-0.10	0.46	-0.73
421	-0.02	0.08	-0.02	0.22	-0.85
ppm mean	-0.00	0.11	0.06	0.17	-0.30
ppm S. D.	0.06	0.14	0.15	0.40	0.50
ppm S. E.	0.02	0.04	0.05	0.12	0.16

[Continued]

TABLE 1

Subject	Z= 73 Serum Tantalum	Z= 74 Serum Tungsten	Z= 75 Serum Rhenium	Z= 76 Serum Osmium	Z= 77 Serum Iridium
24	0.36	-0.13	0.00	0.53	-0.02
25	-0.04	-0.15	-0.09	0.39	0.09
29	0.17	-0.22	-0.02	0.10	-0.05
30	0.48	-0.23	0.04	-0.29	0.02
32	0.53	-0.08	-0.03	-0.38	0.08
199	0.34	-0.14	0.01	-0.44	-0.07
200	0.32	-0.26	0.02	0.00	0.00
201	0.31	-0.05	0.00	0.03	-0.07
202	0.14	0.00	-0.18	-0.25	0.01
374	0.26	0.46	-0.05	-0.13	-0.01
409	0.35	0.23	2.07	-0.85	-0.14
421	0.42	0.32	-0.08	-0.19	0.00
ppm mean	0.30	-0.01	0.15	-0.18	-0.01
ppm S. D.	0.16	0.04	0.61	0.3	0.06
ppm S. E.	0.05	0.01	0.19	0.10	0.02

[Continued]

TABLE 1

Subject	Z = 78 Serum Platinum	Z = 79 Serum Gold	Z = 80 Serum Mercury	Z = 81 Serum Thallium	Z = 82 Serum Lead
24	0.40	0.02	0.81	0.20	0.26
25	0.30	0.01	0.51	-0.04	0.40
29	0.10	0.68	0.12	-0.06	0.23
30	0.35	0.10	0.05	-0.09	0.24
32	0.33	0.32	0.21	-0.01	0.36
199	0.42	0.54	0.10	0.00	0.30
200	0.09	0.32	0.15	-0.06	0.33
201	0.30	0.49	0.20	0.08	0.21
202	0.33	0.43	0.20	0.00	0.30
374	0.37	-0.07	1.22	-0.16	0.20
409	0.15	-0.13	0.06	-0.06	0.36
421	0.01	-0.20	0.28	0.01	0.41
ppm mean	0.26	0.23	0.26	-0.04	0.30
ppm S. D.	0.13	0.28	0.31	0.06	0.07
ppm S. E.	0.04	0.09	0.10	0.02	0.02

[Continued]

TABLE 1

Subject	Z= 83 Serum Bismuth	Z= 90 Serum Thorium	Z= 92 Serum Uranium
24	-0.06	0.00	0.20
25	0.09	0.04	0.07
29	0.50	-0.06	0.17
30	0.29	-0.02	0.10
32	0.30	-0.10	0.04
199	0.35	-0.14	-0.02
200	-0.09	-0.07	0.02
201	0.12	0.05	0.04
202	0.28	-0.05	0.05
374	0.08	0.08	0.04
409	0.30	0.12	0.29
421	0.27	0.08	0.23
ppm mean	0.20	-0.01	0.09
ppm S. D.	0.17	0.08	0.08
ppm S. E.	0.05	0.03	0.03

TABLE 2

X-Ray Spectra Analysis of Sixty-six Chemical Elements in the Serum of Xanthoma Tuberosum

Subject	Diagnosis	Z = 15 Serum Phosphorus	Z = 16 Serum Sulfur	Z = 17 Serum Chloride
23	Xanthoma tuberosum	179	1312	4151
27		169	1240	3933
28*		185	1356	4294
31		189	1381	4372
ppm mean		179	1310	4152
ppm S. D.		8.0	57.7	179
ppm S. E.		5.7	40.9	127

* 28 is not included in statistical calculation because of its partial hemolysis.

TABLE 2

Subject	Z = 19 Serum Potassium	Z = 20 Serum Calcium	Z = 21 Serum Scandium	Z = 22 Serum Titanium	Z = 23 Serum Vanadium
23	194	112	-0.03	-0.29	0.08
27	183	107	-0.01	-0.02	0.09
28	not available	143	0.03	-0.03	0.07
31	204	102	-0.03	-0.18	0.01
963			-0.13		
ppm mean	193	107	-0.01	-0.13	0.06
ppm S. D.	8.5	3.9	0.07	0.17	0.03
ppm S. E.	6.0	2.7	0.03	0.10	0.02

TABLE 2

Subject	Z = 24 Serum Chromium	Z = 25 Serum Manganese	Z = 26 Serum Iron	Z = 27 Serum Cobalt	Z = 28 Serum Nickel
23	-0.01	-0.02	0.68	-0.02	-0.06
27	0.00	-0.01	0.80	-0.01	-0.06
28*	-0.03	-0.02	not available	-0.01	-0.10
31	-0.00	-0.01	1.46	0.02	-0.10
647*			0.88		
733			1.08		
735			0.37		
753			0.72		
758			0.67		
ppm mean	0.00	-0.02	0.83	0.00	-0.07
ppm S. D.	0.01	0.01	0.30	0.02	0.02
ppm S. E.	0.00	0.00	0.11	0.01	0.01

* same sample

[Continued]

TABLE 2

Subject	Z= 29 Serum Copper	Z= 30 Serum Zinc	Z= 31 Serum Gallium	Z= 32 Serum Germanium	Z= 33 Serum Arsenic
23	1.45	0.75	0.04	0.03	-0.01
27	1.19	0.75	0.11	0.05	-0.02
28	1.08	0.66	0.09	0.00	-0.02
31	1.30	0.58	0.06	0.06	0.00
733	1.48	0.76			
735	1.42	0.71			
753	1.21	0.68			
758	1.59	0.85			
ppm mean	1.38	0.72	0.07	0.03	-0.01
ppm S. D.	0.14	0.08	0.03	0.02	0.01
ppm S. E.	0.06	0.03	0.02	0.01	0.01

(Continued)

TABLE 2

Subject	Z = 34 Serum Selenium	Z = 35 Serum Bromine	Z = 37 Serum Rubidium	Z= 38 Serum Strontium	Z= 39 Serum Yttrium
23	0.04	4.44	0.34	-0.09	0.03
27	0.04	5.06	0.20	-0.05	0.04
28	0.04	2.93	0.21	0.03	0.06
31	0.09	3.31	0.19	-0.09	0.04
733				0.06	
735				-0.27	
753				0.01	
758				0.06	
ppm mean	0.06	4.27	0.24	-0.04	0.04
ppm S. D.	0.02	0.72	0.07	0.10	0.01
ppm S. E.	0.02	0.51	0.05	0.04	0.01

[Continued]

TABLE 2

Subject	Z= 40 Serum Zirconium	Z= 41 Serum Niobium	Z= 42 Serum Molybdenum	Z= 44 Serum Ruthenium	Z= 45 Serum Rhodium
23	0.25	-0.05	-0.35	-0.17	0.90
27	0.28	-0.13	0.33	-0.22	-0.05
28	0.19	-0.05	0.78	-0.06	-0.05
31	-0.15	-0.05	-0.42	0.03	-0.34
ppm mean	0.13	-0.08	-0.15	-0.11	0.11
ppm S. D.	0.19	0.04	0.33	0.10	0.47
ppm S. E.	0.14	0.03	0.24	0.06	0.27

[Continued]

TABLE 2

Subject	Z = 46 Serum Palladium	Z = 47 Serum Silver	Z = 48 Serum Cadmium	Z = 49 Serum Indium	Z = 50 *Serum Tin
23	-0.05	-0.09	-0.09	0.50	-0.44
27	0.02	0.11	-0.40	0.67	-1.22
28	0.45	0.77	0.06	0.50	0.35
31	-0.17	0.59	-0.11	-0.28	1.10
733			0.25		
735			-0.30		
753			-0.53		
758			-0.27		
ppm mean	-0.07	0.20	-0.20	0.30	-0.04
ppm S. D.	0.08	0.24	0.24	0.41	0.35
ppm S. E.	0.06	0.07	0.09	0.29	0.20

*28 was used

[Continued]

TABLE 2

Subject	Z= 51 Serum Antimony	Z= 52 Serum Tellurium	Z= 53 Serum Iodine	Z= 55 Serum Cesium	Z= 56 Serum Barium
23	-0.17	-1.07	0.70	0.28	-0.21
27	0.39	-0.14	1.65	0.51	0.22
28	-1.93	-0.94	2.15	0.36	0.29
31	-0.53	0.39	0.11	0.35	0.27
ppm mean	-0.10	-0.28	0.82	0.38	0.09
ppm S. D.	0.38	0.60	0.63	0.10	0.22
ppm S. E.	0.27	0.43	0.45	0.07	0.15

[continued]

TABLE 2

Subject	Z= 57 Serum Lanthanum	Z= 58 Serum Cerium	Z= 59 Serum Praseodymium	Z= 60 Serum Neodymium	Z= 62 Serum Samarium
23	0.07	0.243	-0.01	-0.07	-0.03
27	0.11	-0.621	-0.12	0.07	0.05
28	0.07	0.181	0.02	-0.04	0.03
31	0.02	-0.222	-0.03	0.09	0.18
963			-0.01		
ppm mean	0.07	-0.20	-0.04	0.03	0.07
ppm S. D.	0.32	0.35	0.05	0.07	0.09
ppm S. E.	0.18	0.25	0.03	0.05	0.07

[Continued]

TABLE 2

Subject	Z= 63 Serum Europium	Z= 64 Serum Gadolinium	Z= 65 Serum Terbium	Z= 66 Serum Dysprosium	Z= 67 Serum Holmium
23	0.32	0.12	-0.08	-0.28	0.20
27	0.12	0.06	0.04	0.18	0.07
28	0.13	-0.05	-0.01	0.32	0.12
31	-0.11	-0.23	0.05	-0.13	-0.16
ppm mean	0.11	-0.03	0.03	-0.08	0.04
ppm S. D.	0.15	0.17	0.06	0.20	0.15
ppm S. E.	0.09	0.12	0.04	0.14	0.11

[Continued]

TABLE 2

Subject	Z= 68 Serum Erbium	Z= 69 Serum Thulium	Z= 70 Serum Ytterbium	Z= 71 Serum Lutetium	Z= 72 Serum Hafnium
23	-0.01	0.09	0.05	-0.37	0.43
27	0.03	0.03	-0.30	-0.36	0.19
28	-0.05	-0.15	-0.12	-0.09	0.00
31	-0.02	0.02	0.25	0.55	0.05
ppm mean	-0.01	0.05	0.00	-0.06	0.17
ppm S. D.	0.03	0.03	0.22	0.43	0.16
ppm S. E.	0.02	0.02	0.16	0.31	0.09

[Continued]

TABLE 2

Subject	Z= 73 Serum Tantalum	Z= 74 Serum Tungsten	Z = 75 Serum Rhenium	Z = 76 Serum Osmium	Z = 77 Serum Iridium
23	0.29	0.05	2.26	-0.03	-0.15
27	0.31	-0.09	0.23	-0.11	0.03
28	0.22	-0.15	-0.01	0.06	-0.08
31	0.10	0.01	0.04	0.34	-0.01
ppm mean	0.24	-0.01	0.63	0.07	-0.05
ppm S. D.	0.10	0.60	0.95	0.20	0.06
ppm S. E.	0.07	0.35	0.55	0.14	0.04

[Continued]

TABLE 2

Subject	Z = 78 Serum Platinum	Z = 79 Serum Gold	Z =80 Serum Mercury	Z = 81 Serum Thallium	Z = 82 Serum Lead
23	0.33	-0.03	0.08	-0.11	0.38
27	0.30	-0.02	0.18	0.02	0.22
28	0.33	-0.14	0.19	0.00	0.38
31	0.20	0.30	0.11	0.16	0.23
ppm mean	0.29	0.08	0.12	0.01	0.30
ppm S. D.	0.06	0.15	0.04	0.10	0.08
ppm S. E.	0.03	0.11	0.03	0.06	0.04

[Continued]

TABLE 2

Subject	Z= 83 Serum Bismuth	Z= 90 Serum Thorium	Z= 92 Serum Uranium
23	0.20	-0.10	0.13
27	-0.04	-0.13	0.08
28	-0.11	-0.01	0.13
31	0.32	-0.10	0.13
ppm mean	0.16	-0.08	0.11
ppm S. D.	0.15	0.05	0.02
ppm S. E.	0.11	0.03	0.02

TABLE 3

Mean Levels for Sixty-Six Chemical Elements in the Serums of Ostensibly Healthy Adult Male Subjects

Mean Age = 35.3 years 39 Cases

Element	Atomic Number	Mean Level ppm	Standard Error	99% Confidence Limit or Mean Value
Phosphorus	15	163	3.1	155 - 171
Sulfur	16	1201	14.7	1161 - 1241
Chlorine	17	3798	20.0	3744 - 3852
Potassium	19	177	3.1	169 - 185
Calcium	20	115	1.3	111 - 119
Scandium	21	0.0	0.1	0.0 - 0.3
Titanium	22	0.0	0.07	0.00-0.18
Vanadium	23	0.01	0.04	0.00-0.11
Chromium	24	0.01	0.01	0.00-0.04
Manganese	25	0.00	0.02	0.00-0.06
*Iron	26	0.80	0.03	0.72-0.88
Cobalt	27	0.00	0.02	0.00-0.05
Nickel	28	0.00	0.07	0.00-0.18
Copper	29	1.29	0.05	1.16-1.42
**Zinc	30	0.80	0.03	0.72-0.88

*Fe data are from 110 healthy people

**Zn data are from 22 healthy people

[Continued]

TABLE 3

Element	Atomic Number	Mean Level ppm	Standard Error	99% Confidence Limit or Mean Value
Gallium	31	0.04	0.02	0.00-0.09
Germanium	32	0.00	0.04	0.00-0.10
Arsenic	33	0.02	0.01	0.00-0.05
Selenium	34	0.1	0.02	0.05-0.15
Bromine	35	4.12	0.16	3.70-4.54
Rubidium	37	0.15	0.05	0.02-0.28
Strontium	38	0.00	0.02	0.00-0.05
Yttrium	39	0.02	0.01	0.00-0.05
Zirconium	40	0.1	0.04	0.00-0.20
Niobium	41	0.0	0.06	0.00-0.16
Molybdenum	42	0.2	0.04	0.10-0.30
Ruthenium	44	0.0	0.09	0.00-0.23
Rhodium	45	0.0	0.06	0.00-0.16
Palladium	46	0.0	0.04	0.00-0.10
Silver	47	0.2	0.07	0.02-0.38
Cadmium	48	0.0	0.09	0.00-0.23
Indium	49	0.0	0.08	0.00-0.21
Tin	50	0.0	0.18	0.00-0.47
Antimony	51	0.0	0.12	0.00-0.31
Tellurium	52	0.0	0.19	0.00-0.49
Iodine	53	0.0	0.22	0.00-0.57

[Continued]

TABLE 3

Element	Atomic Number	Mean Level ppm	Standard Error	99% Confidence Limit or Mean Value
Cesium	55	0.2	0.2	0.00-0.52
Barium	56	0.18	0.1	0.00-0.44
Lanthanum	57	0.0	0.08	0.00-0.21
Cerium	58	0.1	0.17	0.00-0.55
Praseodymium	59	0.0	0.05	0.00-0.13
Neodymium	60	0.0	0.03	0.00-0.08
Samarium	62	0.0	0.07	0.00-0.18
Europium	63	0.0	0.2	0.00-0.52
Gadolinium	64	0.0	0.1	0.00-0.26
Terbium	65	0.0	0.09	0.00-0.23
Dysprosium	66	0.0	0.1	0.00-0.26
Holmium	67	0.0	0.16	0.00-0.41
Erbium	68	0.00	0.03	0.00-0.08
Thulium	69	0.0	0.14	0.00-0.36
Ytterbium	70	0.1	0.08	0.00-0.31
Lutetium	71	0.0	0.05	0.00-0.13
Hafnium	72	0.0	0.16	0.00-0.41
Tantalum	73	0.2	0.08	0.00-0.41
Tungsten	74	0.0	0.07	0.00-0.18
Rhenium	75	0.0	0.06	0.00-0.26

[Continued]

TABLE 3

Element	Atomic Number	Mean Level ppm	Standard Error	99% Confidence Limit or Mean Value
Osmium	76	0.1	0.18	0.00-0.57
Iridium	77	0.0	0.05	0.00-0.13
Platinum	78	0.1	0.14	0.00-0.46
Gold	79	0.0	0.09	0.00-0.23
Mercury	80	0.1	0.03	0.00-0.31
Thallium	81	0.0	0.01	0.00-0.03
Lead	82	0.3	0.09	0.07-0.53
Bismuth	83	0.1	0.04	0.00-0.20
Thorium	90	0.00	0.04	0.00-0.10
Uranium	92	0.02	0.03	0.00-0.10

TABLE 4

Comparison of the Serum Levels of Sixty-Six Chemical Elements in Xanthoma Tuberosum and the Reference Group

Element	Z	Mean Value in Reference Group	Xanthoma Tuberosum Minus Control	Standard Error of Difference of Mean	Significance at 5% Level	99% Confidence Limits upon difference in m Limit A	Limit
Phosphorus	15	163	16	6.5	borderline	16.6	33
Sulfur	16	1201	110	43	borderline	-4	224
Chlorine	17	3798	354	128	borderline	-200	456
Potassium	19	177	16	6.8	no	-2	34
Calcium	20	115	-8	3.0	no	-16	0.
Scandium	21	0.0	-0.01	0.10	no	-0.26	0.26
Titanium	22	0.0	-0.13	-0.12	no	-0.45	-0.19
Vanadium	23	0.01	0.05	0.05	no	-0.08	0.18
Chromium	24	0.01	-0.02	0.01	no	-0.05	0.01
Manganese	25	0.00	-0.02	0.02	no	-0.07	0.03
Iron	26	0.80	0.03	0.13	no	-0.31	0.37
Cobalt	27	0.00	0.00	0.02	no	-0.05	0.05
Nickel	28	0.00	-0.07	0.07	no	-0.26	0.12
Copper	29	1.29	0.09	0.08	no	0.12	0.30
Zinc	30	0.80	-0.09	0.05	no	-0.22	0.04
Gallium	31	0.04	0.03	0.03	no	-0.05	0.11
Germanium	32	0.00	0.03	0.01	no	0.00	0.06
Arsenic	33	0.02	-0.01	0.01	no	-0.04	0.02
Selenium	34	0.1	-0.04	0.03	no	-0.12	0.04

[Continued]

TABLE 4

Element	Z	Mean Value in Reference Group	Xanthoma Tuberosum Minus Control	Standard Error of Difference of Mean	Significance at 5% Level	99 % Confidence Limits Upon Difference in Limit A	Limit B
Bromine	35	4.12	0.15	0.54	no	-1.28	1.58
Rubidium	37	0.15	0.09	0.07	no	-0.10	0.28
Strontium	38	0.00	-0.04	0.04	no	-0.15	0.07
Yttrium	39	0.02	0.02	0.01	no	-0.01	0.05
Zirconium	40	0.1	0.03	0.14	no	0.34	0.40
Niobium	41	0.0	-0.08	0.07	no	-0.27	0.11
Molybdenum	42	0.2	-0.35	0.24	no	-0.99	0.29
Ruthenium	44	0.0	-0.12	0.12	no	-0.43	0.19
Rhodium	45	0.0	-0.29	0.19	no	-0.79	-0.21
Palladium	46	0.0	-0.07	0.07	no	-0.26	0.12
Silver	47	0.2	0.00	0.18	no	-0.48	0.48
Cadmium	48	0.0	-0.19	0.12	no	-0.51	0.13
Indium	49	0.0	0.30	0.30	no	-0.50	1.10
Tin	50	0.0	-1.34	0.70	no	-3.22	0.54
Antimony	51	0.0	-0.10	0.29	no	-0.87	0.67
Tellurium	52	0.0	-0.28	0.47	no	-1.53	0.97
Iodine	53	0.0	0.82	0.50	no	-0.51	2.15
Cesium	55	0.2	0.38	0.21	no	-0.16	0.94
Barium	56	0.18	-0.09	0.18	no	-0.57	0.39
Lanthanum	57	0.0	0.18	0.11	no	-0.11	0.47
Cerium	58	0.1	-0.30	0.30	no	-1.10	0.50
Praseodymium	59	0.0	-0.17	0.07	no	-0.36	0.02

[Continued]

TABLE 4

Element	Z	Mean Value in Reference Group	Xanthoma Tuberosum Minus Control	Standard Error of Difference of Mean	Significance at 5% Level	99 % Confidence Limits Upon Difference in mea Limit A	Limit B
Neodymium	60	0.0	0.03	0.06	no	-0.13	0.19
Samarium	62	0.0	0.07	0.09	no	-0.14	0.31
Europium	63	0.0	0.11	0.24	no	-0.53	0.75
Gadolinium	64	0.0	-0.03	0.16	no	-0.45	0.39
Terbium	65	0.0	0.00	0.10	no	-0.27	0.27
Dysprosium	66	0.0	-0.08	0.17	no	-0.53	0.33
Holmium	67	0.0	0.04	0.19	no	-0.46	0.54
Erbium	68	0.00	0.00	0.03	no	-0.08	0.08
Thulium	69	0.0	0.05	0.14	no	-0.32	0.42
Ytterbium	70	0.1	-0.1	0.18	no	-0.58	0.38
Lutetium	71	0.0	-0.06	0.31	no	0.88	0.76
Hafnium	72	0.17	+0.17	0.16	no	-0.25	0.59
Tantalum	73	0.2	0.04	0.10	no	-0.23	0.31
Tungsten	74	0.0	-0.01	0.38	no	-1.01	0.99
Rhenium	75	0.1	0.74	0.71	no	-1.14	2.62
Osmium	76	0.1	-0.03	0.23	no	-0.64	0.58
Iridium	77	0.0	-0.05	0.06	no	-0.21	0.11
Platinum	78	0.1	0.18	0.15	no	-0.32	0.58
Gold	79	0.0	0.08	0.14	no	-0.29	0.45
Mercury	80	0.1	0.04	0.09	no	-0.20	0.28
Thallium	81	0.0	0.02	0.08	no	-0.19	0.23

[Continued]

TABLE 4

Element	Z	Mean Value in Reference Group	Xanthoma Tuberosum Minus Control	Standard Error of Difference of Mean	Significance at 5% Level	99 % Confidence Limits Upon Difference in mea	
						Limit A	Limit B
Lead	82	0.3	-0.02	0.10	no	-0.29	0.25
Bismuth	83	0.1	0.06	0.11	no	-0.23	0.35
Thorium	90	0.00	-0.08	0.05	no	-0.21	0.05
Uranium	92	0.02	0.09	0.04	no	-0.02	0.20

TABLE 5

Comparison of the Serum Levels of Sixty-Six Chemical Elements in Xanthoma Tendinosum and the Reference Group

Element	Z	Mean Value in Reference Group Control	Xanthoma Tendinisum Minus Reference	Standard Error of Difference of Means	Significance at 5% Level	99 % Confidence Limits Upon Difference in mean Limit A	Limit B
Phosphorus	15	163	6.6	3.9	no	-3.7	16.9
Sulfur	16	1201	45	23.5	no	-17.2	107.2
Chlorine	17	3798	143.8	61	yes	-17.9	305.5
Potassium	19	177	6.7	4.1	no	-4.2	17.6
Calcium	20	115	-2.9	2.2	no	-8.7	2.9
Scandium	21	0.0	0.00	0.12	no	-0.32	0.32
Titanium	22	0.0	-0.08	0.07	no	-0.27	0.11
Vanadium	23	0.01	0.04	0.04	no	-0.07	0.15
Chromium	24	0.01	-0.02	0.01	no	-0.05	0.01
Manganese	25	0.00	0.02	0.02	no	-0.04	0.08
Iron	26	0.80	0.09	0.12	no	-0.23	0.41
Cobalt	27	0.00	-0.02	0.02	no	-0.08	0.04
Nickel	28	0.00	-0.06	0.07	no	-0.25	0.13
Copper	29	1.29	0.19	0.15	no	-0.10	0.48
Zinc	30	0.80	0.04	0.05	no	-0.09	0.17
Gallium	31	0.04	0.00	0.02	no	-0.06	0.06
Germanium	32	0.00	0.00	0.02	no	-0.05	0.05
Arsenic	33	0.02	-0.02	0.01	no	-0.05	0.01
Selenium	34	0.1	-0.02	0.03	no	-0.10	0.06

[continued]

TABLE 5

Element	Z	Mean Value in Reference Group Control	Xanthoma Tendinosum Minus Reference	Standard Error of Difference of Means	Significance at 5% Level	99 % Confidence Limits Upon Difference in mea Limit A	Limit B
Bromine	35	4.12	-0.17	0.34	no	-1.07	0.73
Rubidium	37	0.15	-0.01	0.06	no	-0.18	0.15
Strontium	38	0.00	-0.02	0.02	no	-0.08	0.04
Yttrium	39	0.02	0.00	0.02	no	-0.06	0.06
Zirconium	40	0.1	-0.24	0.11	no	-0.53	0.05
Niobium	41	0.0	-0.31	0.18	no	-0.79	0.17
Molybdenum	42	0.2	0.01	0.14	no	-0.36	0.38
Ruthenium	44	0.0	0.03	0.10	no	-0.24	0.30
Rhodium	45	0.0	-0.11	0.09	no	-0.35	0.13
Palladium	46	0.0	-0.17	0.09	no	-0.41	0.07
Silver	47	0.2	0.00	0.10	no	-0.27	0.27
Cadmium	48	0.0	-0.20	0.14	no	-0.57	0.17
Indium	49	0.0	-0.36	0.28	no	-1.10	0.38
Tin	50	0.0	-0.32	0.29	no	-1.09	0.45
Antimony	51	0.0	-0.52	0.25	no	-1.18	0.14
Tellurium	52	0.0	-0.41	0.45	no	-1.60	0.78
Iodine	53	0.0	0.01	0.25	no	-0.65	0.67
Cesium	55	0.2	0.35	0.20	no	-0.25	0.95
Barium	56	0.18	0.22	0.10	no	-0.05	0.49
Lanthanum	57	0.0	-0.15	0.10	no	-0.42	0.12
Cerium	58	0.1	-0.08	0.18	no	-0.56	0.40

[Continued]

TABLE 5

Element	Z	Mean Value in Reference Group Control	Xanthoma Tendinosum Minus Reference	Standard Error of Difference of Means	Significance at 5% Level	99 % Confidence Limits Upon Difference in mean Limit A	Limit B
Praseodymium	59	0.0	-0.01	0.05	no	-0.14	0.12
Neodymium	60	0.0	0.06	0.05	no	-0.07	0.19
Samarium	62	0.0	0.03	0.08	no	-0.18	0.24
Europium	63	0.0	-0.05	0.22	no	-0.63	0.53
Gadolinum	64	0.0	-0.10	0.11	no	-0.39	0.19
Terbium	65	0.0	-0.02	0.09	no	-0.26	0.22
Dysprosium	66	0.0	0.04	0.12	no	-0.28	0.36
Holmium	67	0.0	0.02	0.17	no	-0.43	0.47
Erbium	68	0.00	0.00	0.04	no	-0.11	0.11
Thulium	69	0.0	0.10	0.15	no	-0.30	0.50
Ytterbium	70	0.1	-0.06	0.09	no	-0.30	0.18
Lutetium	71	0.0	0.13	0.13	no	-0.21	0.47
Hafnium	72	0.0	-0.30	0.23	no	-0.91	0.31
Tantalum	73	0.2	0.10	0.09	no	-0.14	0.34
Tungsten	74	-0.01	-0.02	0.10	no	-0.29	0.25
Rhenium	75	0.1	0.04	0.10	no	-0.23	0.31
Osmium	76	0.1	-0.22	0.21	no	-0.78	0.34
Iridium	77	0.0	0.14	0.05	no	0.01	0.27
Platinum	78	0.1	0.16	0.15	no	0.24	0.56
Gold 79	79	0.0	0.21	0.12	no	-0.11	0.53
Mercury	80	0.1	0.20	0.13	no	-0.14	0.54

[Continued]

TABLE 5

Element	Z	Mean Value in Reference Group Control	Xanthoma Tendinosum Minus Reference	Standard Error of Difference of Means	Significance at 5% Level	99 % Confidence Limits Upon Difference in mean Limit A	Limit B
Thallium	81	0.0	-0.02	0.03	no	-0.10	0.06
Lead	82	0.3	0.00	0.09	no	-0.24	0.24
Bismuth	83	0.1	0.10	0.06	no	-0.06	0.26
Thorium	90	0.00	-0.01	0.05	no	-0.12	0.12
Uranium	92	0.02	0.07	0.04	no	-0.04	0.18

TABLE 6

Comparison of the Serum Levels of Sixty-Six Chemical Elements
Xanthoma Tuberosum versus Xanthoma Tendinosum

Element	Z	Difference in Means	Standard Error of Difference in Means	Significance at 5% Level	99 % Confidence Limits Upon Difference in Mean Limit A	Limit B
Phosphorus	15	9.5	6.2	no	-6.9	25.9
Sulfur	16	64.6	44.7	no	-53.9	183.1
Chlorine	17	210	139	no	-158	578
Potassium	19	9.7	6.6	no	-7.8	27.2
Calcium	20	-5.2	3.2	no	-13.7	3.3
Scandium	21	-0.01	0.08	no	-0.20	0.20
Titanium	22	-0.47	0.12	no	-0.79	0.15
Vanadium	23	0.02	0.03	no	-0.06	0.10
Chromium	24	0.00	0.01	no	-0.03	0.03
Manganese	25	-0.00	0.01	no	-0.03	0.03
Iron	26	-0.06	0.13	no	-0.40	0.28
Cobalt	27	0.02	0.02	no	-0.04	0.08
Nickel	28	-0.01	0.01	no	-0.04	0.02
Copper	29	-0.10	0.12	no	-0.42	0.22
Zinc	30	-0.13	0.05	yes	-0.26	0.00
Gallium	31	0.03	0.03	no	-0.05	0.11
Germanium	32	0.03	0.02	no	-0.03	0.09
Arsenic	33	-0.01	0.01	no	-0.04	0.02
Selenium	34	-0.02	0.03	no	-0.10	0.06
Bromine	35	0.31	0.61	no	-1.31	1.93
Rubidium	37	0.08	0.05	no	-0.05	0.21

[Continued]

TABLE 6

Element	Z	Difference in Means	Standard Error of Difference in Means	Significance at 5% Level	99 % Confidence Limits Upon Difference in Mean Limit A	Limit B
Strontium	38	-0.02	0.04	no	-0.14	0.10
Yttrium	39	0.04	0.02	no	-0.02	0.10
Zirconium	40	0.35	0.21	no	-0.21	0.91
Niobium	41	0.23	0.17	no	0.22	0.68
Molybdenum	42	-0.35	0.28	no	-1.09	0.39
Ruthenium	44	-0.16	0.09	no	-0.40	0.08
Rhodium	45	0.22	0.28	no	-0.52	0.96
Palladium	46	-0.04	0.10	no	-0.31	0.23
Silver	47	0.00	0.18	no	-0.48	0.48
Cadmium	48	0.00	0.14	no	-0.37	0.37
Indium	49	0.69	0.41	no	-0.40	1.78
Tin	50	-0.5	0.30	no	-1.30	0.30
Antimony	51	0.27	0.32	no	-0.58	1.12
Tellurium	52	0.14	0.62	no	-1.50	1.78
Iodine	53	0.25	0.72	no	-1.66	2.16
Cesium	55	0.04	0.08	no	-0.17	0.25
Barium	56	-0.13	0.16	no	-0.55	0.29
Lanthanum	57	0.22	0.22	no	-0.36	0.80
Cerium	58	-0.21	0.26	no	-0.90	0.48
Praseodymium	59	-0.03	0.04	no	-0.13	0.07
Neodymium	60	0.01	0.05	no	-0.12	0.14
Samarium	62	0.03	0.08	no	-0.18	0.24

[Continued]

TABLE 6

Element	Z	Difference in Means	Standard Error of Difference in Means	Significance at 5% Level	99 % Cojfidence Limits Upon Difference in Mean Limit A	Limit B
Europium	63	0.17	0.15	no	-0.23	0.57
Gadolinum	64	0.07	0.13	no	-0.27	0.41
Terbium	65	0.02	0.05	no	-0.11	0.15
Dysprosium	66	-0.07	0.15	no	-0.47	0.33
Holmium	67	0.06	0.11	no	-0.23	0.35
Erbium	68	0.01	0.02	no	-0.05	0.07
Thulium	69	-0.07	0.05	no	-0.20	0.06
Ytterbium	70	-0.06	0.17	no	-0.51	0.39
Lutetium	71	-0.23	0.33	no	-1.10	0.64
Hafnium	72	0.28	0.18	no	-0.20	0.76
Tantalum	73	-0.06	0.08	no	-0.27	0.15
Tungsten	74	0	0.35	no	-0.93	0.93
Rhenium	75	0.69	0.74	no	-1.27	2.65
Osmium	76	0.25	0.17	no	-0.20	0.70
Iridium	77	0.04	0.041	no	-0.07	0.15
Platinum	78	0.03	0.06	no	-0.13	0.19
Gold	79	-0.14	0.14	no	-0.51	0.23
Mercury	80	-0.14	0.10	no	-0.41	0.13
Thallium	81	0.06	0.08	no	-0.15	0.27
Lead	82	-0.03	0.06	no	-0.19	0.13
Bismuth	83	-0.06	0.12	no	-0.38	0.26

[Continued]

TABLE 6

Element	Z	Difference in Means	Standard Error of Difference in Means	Significance at 5% Level	99 % Confidence Limits Upon Difference in mean Limit A	Limit B
Thorium	90	0.07	0.07	no	-0.12	0.26
Uranium	92	0.02	0.03	no	-0.06	0.10

APPENDIX I

Sources of Variation in Population (UCRL) Statistics (49)

Lipoprotein Class	Mean value (mg/100 ml)	Standard deviation of mean (mg/100 ml)	Biological variation (mg/100 ml)	Technical error (mg/100 ml)
Sf^{o} 0-12	352	79	32	15
Sf^{o} 12-20	49	21	11	7
Sf^{o} 20-100	92	51	27	12
Sf^{o} 100-400	53	63	43	12

APPENDIX II

Lipoprotein Levels in Xanthoma Tuberosum (49)

	Low density lipoproteins (n = 23)[a] (mg/100 ml)				HDL lipoproteins (n = 16)[a] (mg/100 ml)		
	Sf^{o} 100-400	Sf^{o} 20-100	Sf^{o} 12-20	Sf^{o} 0-12	HDL_1	HDL_2	HDL_3
Mean lipoprotein levels	650	616	128	206	26	39	166
Matched controls	72	105	74	358	17	76	185

a The symbol n designates the number of cases studied.

APPENDIX III

Lipoprotein Levels in Xanthoma Tendinosum (49)

	Low density lipoproteins (n = 18)[a] (mg/100 ml)				HDL lipoproteins (n = 9)[a] (mg/100 ml)		
	Sf^{o} 100-400	Sf^{o} 20-100	Sf^{o} 12-20	Sf^{o} 0-12	HDL_1	HDL_2	HDL_3
Mean lipoprotein levels	36	128	150	793	26	15	150
Matched controls	56	92	65	336	17	88	193

a The symbol n designates the number of cases studied.

APPENDIX IV

Lipoprotein Levels in "Essential Hyperlipemia" (49)

	Low density lipoproteins (n = 9)[a] (mg/100 ml)				HDL lipoproteins (n = 5)[a] (mg/100 ml)		
	Sf^0 100-400	Sf^0 20-100	Sf^0 12-20	Sf^0 0-20	HDL_1	HDL_2	HDL_3
Mean lipoprotein levels	967	450	66	229	80	54	144
Matched controls	83	109	68	364	20	80	196

a The symbol n designates the number of cases studied.

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