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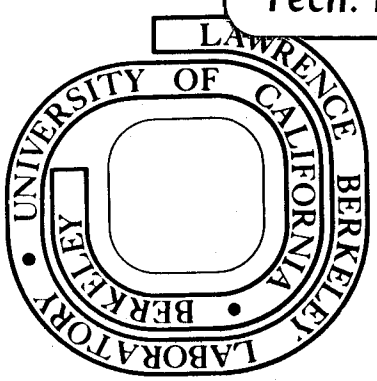

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REDUCED CHROMIUM RETENTION IN PATIENTS WITH HEMOCHROMATOSIS – THE BASIS OF HEMOCHROMATOTIC DIABETES?

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INTRODUCTION

Chromium has been recognized as an essential element since 1959, when it was found to be necessary for maintenance of normal glucose tolerance in rats (1). A severe degree of chromium deficiency in rats leads to a syndrome indistinguishable from diabetes mellitus (2). Chromium occurs in many tissues and appears to be required for the action of insulin in controlling glucose metabolism. It also occurs in microsomes and the nucleus, although its functions in these organelles is unknown. The biologically active form is considered to be the trivalent metal incorporated into a low molecular weight compound known as "glucose tolerance factor" (GTF), recently reported to be a ligand in which the chromium is bound by nicotinic acid, glutamic acid, glycine and possibly cysteine (3). Chromium function and metabolism have recently been reviewed by Hambidge (4), who discusses the possibility that at least some forms of diabetes, particularly adult-onset, may be associated with chromium deficiency or some aberration of chromium metabolism. An earlier review by Mertz (5) considers the occurrence and biological function of chromium.

Studies of the body distribution and turnover of chromium (III) in animals using ^{51}Cr have been reviewed most recently by Onkelinx (6), who studied the body distribution in rats for the relatively short period of 11 days and derived a three-compartment model for chromium kinetics. The only report of human studies with ^{51}Cr contained a limited amount of data (7). We report here the first long term study of ^{51}Cr whole-body retention and blood clearance in human subjects, comparing normal subjects to patients with hemochromatosis. Hemochromatosis is an iron storage disease characterized by hepatomegaly, bronze skin coloration, diabetes, and various other symptoms, some or all of which may be present in any one patient. The symptoms result

from excessive stores of iron in body tissues, and in the idiopathic or primary form this has been shown to result from abnormally increased absorption of iron from the diet (8). The most characteristic sign of hemochromatosis is a high saturation of the plasma iron concentration.

Trivalent chromium is carried in the blood bound to transferrin, the betaglobulin in plasma which also binds and transports iron. This binding has been found to be competitive, saturation with either Fe^{+++} or Cr^{+++} causing diminished binding of the other (9). Recently it has been reported that the two binding sites A and B of the transferrin molecule have different affinities for iron in vitro, depending on the pH. Of particular interest here was the finding that Cr (III) is bound only at the B site, while iron at lower levels of saturation is bound preferentially at the A site (10). Thus, when saturation of transferrin by iron exceeds 50% as in hemochromatosis, one would anticipate that the binding, and thus presumably also the transport, of chromium would begin to be affected.

Consideration of these three factors, vis., (1) chromium deficiency is associated with a diabetic state in animals; (2) diabetes occurs frequently in hemochromatosis, an iron storage disease in which the plasma iron binding capacity is highly saturated with iron; (3) chromium and iron are bound competitively by transferrin, led us to the following hypothesis. The diabetes of hemochromatosis is due not to destruction of the islets of Langerhan by excessive iron in the pancreas, but by a functional chromium deficiency caused by saturation of binding capacity by iron to the exclusion of chromium. This exclusion could occur at the level of plasma transferrin or at binding sites in the liver, in tissue or in all of these.

We have tested this hypothesis by administering $^{51}CrCl_3$ to patients with endogenous hemochromatosis who had highly saturated plasma iron binding capacity, to treated patients with normal or below-normal saturation, and to normal subjects. We measured the fraction of ^{51}Cr retained in the body with a whole-body counter for periods up to six months and the plasma clearance up to 40 days, and have shown that the iron-loaded hemochromatotic patients retain significantly less chromium than iron-depleted patients or normal subjects.

MATERIALS AND METHODS

Radioisotope. Chromium-51 ($T_{1/2}$ = 27.8 days, Amersham-Searle) was used as a sterile pyrogen-free saline solution at pH 3-4 at a specific activity of 100-200 mCi/mg. In some subjects the dose was injected directly into an antecubital vein, in others it was incubated for 20 min. with 10 cc of the subject's own (homologous) plasma, and in two patients it was incubated with donor plasma from a normal subject (heterologous) before intravenous injection. The method of administration of the dose is shown for each subject in Table I.

Counting methods. All counting was done in an Argonne-type whole-body counter, a steel room with 15 cm thick steel walls containing a NaI (Tl) crystal 24 cm diameter and 10 cm thick (11). The crystal can be positioned at the center of curvature of a 1-meter arc couch (arc geometry) or 40 cm from the back and bottom of a tilted chair (chair geometry). The crystal may also be positioned to count urine, stool and blood samples at distances from 1 meter to directly on top of the crystal, and samples in this study were counted in this manner with appropriate standards to relate the counts obtained to the injected dose. Utilizing a 100 channel pulse height analyzer, the counts from the .325 MeV gamma ray in the photopeak from 0.27 to 0.39 MeV were used, and corrected for the 27.8 day half-life of ^{51}Cr.

The subjects were counted within a few minutes after injection and this count was taken as 100%; counts were made at increasing intervals up to 6 months thereafter. In the arc geometry, the counting rate has greater independence from isotope location in the body than the chair geometry, but total counting efficiency is lower. Hence the subjects were counted initially on the arc, then as the required counting time increased due to decay and excretion of the isotope both geometries were used for each scheduled count. Within 4 weeks after injection, the ratio of arc to chair counts became constant. When the counting time in the arc required for 1% statistical accuracy became greater than 20 minutes, the chair geometry only was employed and the previously determined ratio was used to convert the counts to equivalent counts on the arc. In this manner it was possible to count subjects for as long as 8 months with a dose of 100 μCi of ^{51}Cr, which resulted in a total body radiation dose calculated to be 18 mRad. This radiation dose may be compared to the dose received from the average yearly background radiation of 50-150 mRad. The protocol was approved by the Lawrence Berkeley Laboratory Committee for Safeguards in Human Research and informed consent was obtained from all subjects.

Blood sampling. Blood samples were obtained at suitable intervals and counted as whole blood. Separation of plasma from red cells confirmed reports of other workers that ^{51}Cr activity was localized almost entirely in the plasma, and subsequent counts were made without separation; decay-corrected ^{51}Cr counts are expressed as the fraction of the injected dose per ml of whole blood. In the untreated hemochromatotic patients, therapeutic weekly phlebotomies of 500 ml were initiated approximately 4-6 weeks after injection of ^{51}Cr, providing ample blood for accurate counting. At the time phlebotimies were begun, the fraction of ^{51}Cr in the plasma was sufficiently low that its removal had no

significant effect on the whole-body counts. The entire urine and stool samples from subjects A and P were collected for the first 6 days of the study, counted and related to the injected dose by appropriate standards.

Subjects and clinical data. Table I summarizes clinical and laboratory information on the patients and volunteer normal subjects. The normal volunteers A-E were all males with ages from 23 to 50 years, with no known pathology; SMA-12 panels on subjects C, D and E were all within normal limits. One subject, C, had a plasma iron concentration at the upper limit of normal (172 μgm%); his father and an uncle have adult diabetes. A subsequent measurement four months later showed normal plasma iron levels. Subject D also has an uncle with diabetes, but the other subjects had no known familial history of this disease. The six patients with hemochromatosis, K-P, were studied immediately upon referral to the Donner Laboratory Clinic and confirmation of diagnosis. They had received no prior phlebotomies and the saturation of plasma iron binding capacity ranged from 75 to 93%. Liver function tests (SGOT, BSP BUN) were generally mildly elevated, skin pigmentation ranged from none to very dark, hepatomegaly was present in five out of six patients, heavy use of alcohol occurred in only one out of six, clinical diabetes in one out of six, chemical diabetes (elevated blood glucose at two hours after glucose challenge) in an additional three out of six, some degree of arthritis in four out of six. Liver biopsies in all cases confirmed heavy deposits of iron, and cirrhosis in five out of six patients. Subsequent to our study, phlebotomies were continued until the patient became clearly iron deficient, as evidenced by a hypochromic microcytic anemia and a below normal plasma iron. The total amount of iron so removed was calculated from the hemoglobin concentration and the amount of blood removed at each phlebotomy; this value is shown in Table I as grams iron removed by phlebotomy. Subjects I and J were brother and sister whose father

TABLE I

Clinical information and data on normal volunteer subjects and patients. Except where noted, all values were obtained prior to the ^{51}Cr study and prior to beginning phlebotomy therapy in the patients. The indicated grams of iron were removed by phlebotomy therapy after the beginning of the ^{51}Cr study, except in patients 6 and 7 who were studied after phlebotomy therapy had depleted their iron stores. The amount of iron was calculated from: ml blood removed X Hgb(gm/ml) X 0.003466(gm Fe/gm Hgb).

Patient	Diagnosis	Sex	Age	Arthritis [1]	Clinical diabetes [1]	Skin pigmentation [1]	Alcohol intake [1]	Hepatomegaly (cm below costal margin)	Liver biopsy: Iron	Liver biopsy: Cirrhosis	SGOT (% at 45 min; normal 10-40)	BSP	BUN	Glucose tolerance test: Fasting	Glucose tolerance test: ½ hr	Glucose tolerance test: 1 hr	Glucose tolerance test: 2 hr	Glucose tolerance test: 3 hr	Plasma iron (ugm/100 ml)	Plasma iron binding capacity (ugm/100 ml)	% Saturation of iron binding capacity	Gm iron removed by phlebotomy
A	Normal	M	50	0	0	0	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
B	Normal	M	23	0	0	0	+	-	-	-	-	-	-	-	-	-	-	-	71	298	24	0
C	Normal [2]	M	38	0	0	0	++	-	-	-	38	-	-	94	156	123	95	65	95 [4]	282 [4]	33 [4]	0
D	Normal [3]	M	28	0	0	0	+	-	-	-	20	-	-	91	120	112	120	85	96	-	-	0
E	Normal	M	41	0	0	0	+	-	-	-	23	-	-	80	167	172	122	63	107	-	-	0
F	Hemochrom. depleted	M	52	0	0	+	++	4	yes	yes	-	-	11.8	-	-	-	-	-	65	301	22	21.5
G	Hemochrom. depleted	M	38	0	0	0	+	4	yes	yes	18	18	14	80	-	-	-	135	42	230	18	20.5
H	Hemochrom. borderline	M	38	0	0	0	+	0	no	ne	19	1.3	-	71	139	129	65	56	173	288	60	6.4
I	Hemochrom. father	F	31	0	0	0	0	0	-	-	22	-	-	90	107	72	90	93	236	315	75	.8
J	Hemochrom. father	M	23	0	0	0	+	0	-	-	48	-	-	86	112	110	96	74	240	279	86	6.7
K	Hemochrom.	M	44	0	0	0	+++	6	yes	yes	37	2	10.1	-	-	-	-	-	249	274	91	15.2
L	Hemochrom.	F	52	+++	0	+	+	0	yes	no	176	-	9.5	94	218	193	191	149	208	264	79	12.4
M	Hemochrom.	M	55	++	0	+++	+	7	yes	yes	56	-	18	98	192	137	72	64	240	296	81	28.2
N	Hemochrom.	M	60	0	0	+	+	9	yes	yes	59	12.5	-	89	144	153	158	-	229	307	75	10.2
O	Hemochrom.	F	46	+	0	+	+	8	yes	yes	82	2.1	-	84	160	228	234	202	178	192	93	21.0
P	Hemochrom. [5]	M	58	+	+	+	+	8	yes	yes	75	2.3	-	111	226	262	195	88 [6]	252	304	83	19.1

[1] 0 = none or absent + = mild or present to a small degree ++ = moderately large +++ = severe or heavy

[2] Subject 3's father and uncle had diabetes.

[3] Subject 4's brother had chemical diabetes.

[4] Subject 3's plasma iron values shown here were done 4 months after the beginning of the study. The plasma iron done at the time of the study was 172.

[5] Patient 16 was taken 1/2 tab Diabinase daily for his diabetes.

[6] This glucose tolerance test was done after completion of phlebotomy and ^{51}Cr study.

died of hemochromatosis. They were completely asymptomatic when first seen in our clinic, but showed highly saturated plasma iron levels, and subject J had an elevated SGOT. Their glucose tolerance curves did not show a very marked increase in blood glucose levels at 30 minutes and might be considered "flat." Neither had hepatomegaly or arthritis, and liver biopsies were not considered justified. Both consented to phlebotomies, which in addition to being prophylactic, would also provide a measure of iron stores. Both are still undergoing phlebotomy with amounts removed to date shown. Subject I requested temporary suspension of phlebotomies for personal reasons, so the total amount of iron in stores is not known at this time.

Patients F and G were studied after having their iron stores depleted by phlebotomy, with the amounts removed shown in Table I. Patient F was referred after treatment elsewhere and the clinical data is accordingly less. Both were iron deficient or nearly so at the time of study, as evidenced by their plasma iron concentrations.

Patient H has a plasma iron concentration which at 173 µgm/100 ml was just above the upper limit of normal (170 µgm/100 ml). His percent saturation, due to a relatively low total iron binding capacity, was 60%, above the normal limit of 50%. It had risen from 50% four years previously, at which time a liver biopsy appeared normal. His brother had died of diabetes and heart disease scondary to hemochromatosis, and his mother had an elevated plasma iron and percent saturation. He agreed to a trial series of phlebotomies in lieu of another biopsy, so that the actual amount of iron stores could be determined. After removal of only 0.8 grams of iron, an entirely normal amount of stores, he became mildly iron deficient and the plasma iron concentration fell to 81 µgm/100 ml with saturation of 25%. This patient thus apparently had only a mildly penetrant form of hemochromatosis with the only sign being an increased

plasma iron concentration but normal iron stores. By the time this was determined the ^{51}Cr study had been initiated, so it was continued to determine the effects of this borderline form of hemachromatosis on chromium metabolism.

Some of the patients and volunteers were also studied with a whole-body scanner interfaced to a computer to obtain information as to organ concentration within the body (12). These results and details of distribution kinetics will be reported in full elsewhere (13).

Data analysis. The data from the whole-body counting and plasma samples on a semilogarithmic plot yielded curves which were fit to a multiple exponential function of the form

$$f(t) = \sum_{i,j} A_i \, e^{-r_j t} \, . \qquad (1)$$

The data were submitted to a computer minimization program to determine the rate constants r_j and coefficients A_i of the exponential components.

The choice of the number of exponential components to which the data were fit was determined as follows. The data were fit to 2, 3, 4, and 5 components, and the sum of the squares of the differences between the data points and the predicted points obtained. This sum was then plotted vs. the number of components, and the point at which the sum of squares leveled off, that is, at which additional components did not improve the fit, was chosen as the proper number of exponential components. For the whole body counting the resulting number was 3 components, and for the ^{51}Cr blood retention, 4 components.

RESULTS

The whole-body retention of ^{51}Cr in subjects A-H and patients I-P is shown in Fig. 1. The final slope is linear for a period of time at least equal to the final half-time in all cases with the exception of patients

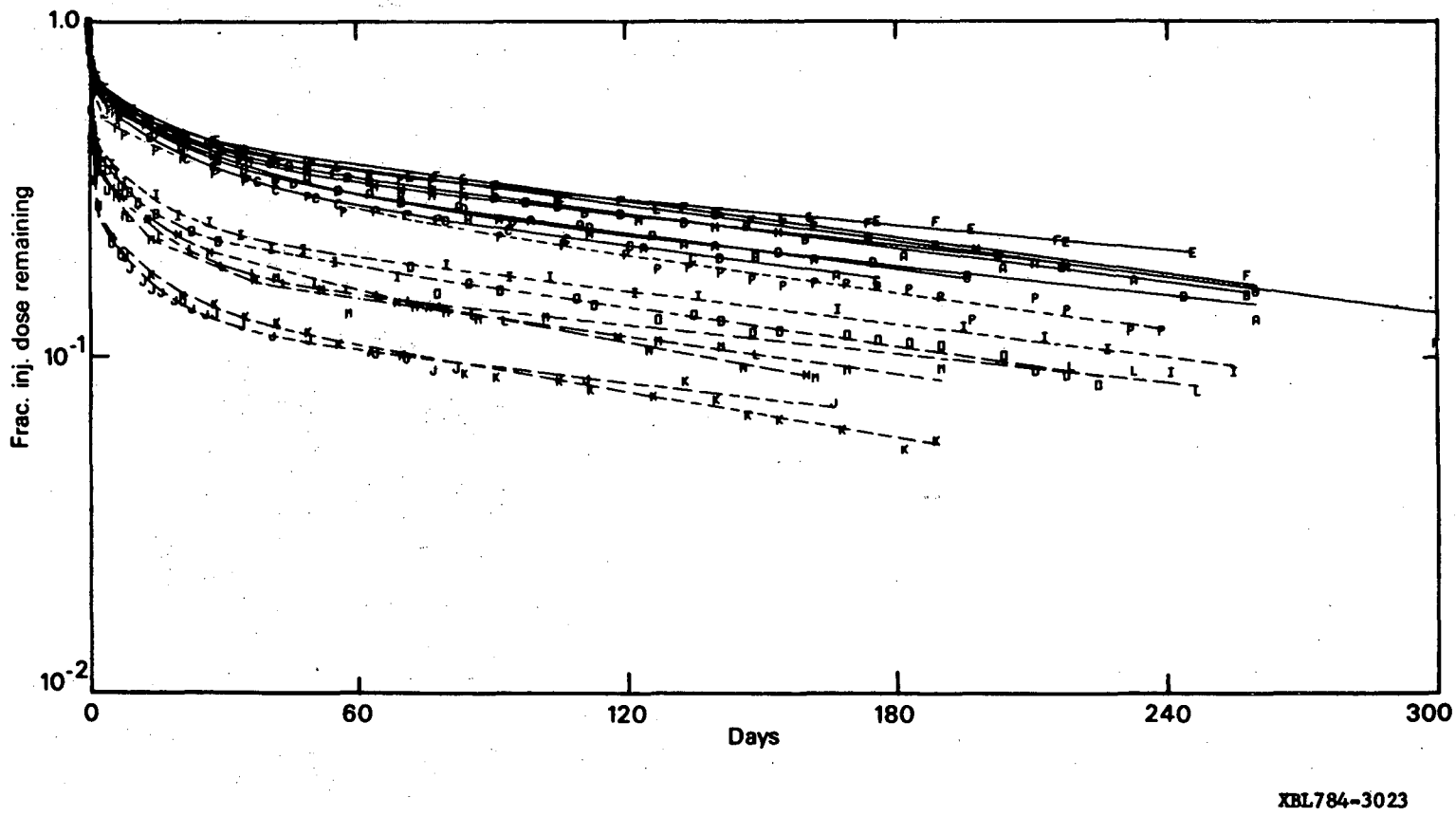

Fig. 1. Whole-body retention of ^{51}Cr as a function of time after injection. Normal subjects A-E and patients with normal iron stores F, G and H are shown by solid lines; iron-loaded patients with hemochromatosis K-P are shown by broken lines.

J, K, M and N, who were counted for somewhat shorter periods. This provides reasonable assurance that there is not a still longer half-time that would be observed by using a larger dose of ^{51}Cr and a longer period of counting. The mean half-times corresponding to the three component slopes r_1, r_2 and r_3 for the normal subjects were 0.56 days, 12.7 days and 192 days. The values of r_1, r_2 and r_3, and the coefficients A_1, A_2 and A_3 for equation (1) obtained by the least-squares fitting program are shown for all subjects in Table II.

The clearance of ^{51}Cr from whole blood is shown for subjects A-H in Fig. 2a and patients I-P in Fig. 2b. The mean half times of the four components for the normal subjects were 13 min., 6.3 hr, 1.9 d and 8.3 d. The linear portions of the final slope extend over 2 to 3 half times, again evidence that there is not a yet longer component with a significant effect on the final slope. The values for the r_4-r_7 and A_4-A_7 of equation (1) obtained by the fitting program are given in Table II. The half-time corresponding to the rate constant is given by $T_{1/2} = \ln 2/r$.

Counting of the urine and stool specimens for subjects A and P showed that in the first 4 days after injection only 0.5% of the injected dose appeared in the feces. The balance of the activity was excreted in the urine, the loss by this route agreeing with that determined by whole body counting within 2%.

Differences in retention are apparent from inspection of Figs. 1 and 2, but a more accurate and more easily interpretable comparison is that of the parameters derived by fitting the curves to equation (1). The subjects may be divided into a variety of different groups, first according to degree or type of illness and second according to the method of injection of the ^{51}Cr. The groupings for which comparisons have been made in Table III are as follows: "normal", normal subjects A, B, C, D, and E; "depleted", the patients with hemochromatosis who had been treated by phlebotomy until their iron stores were

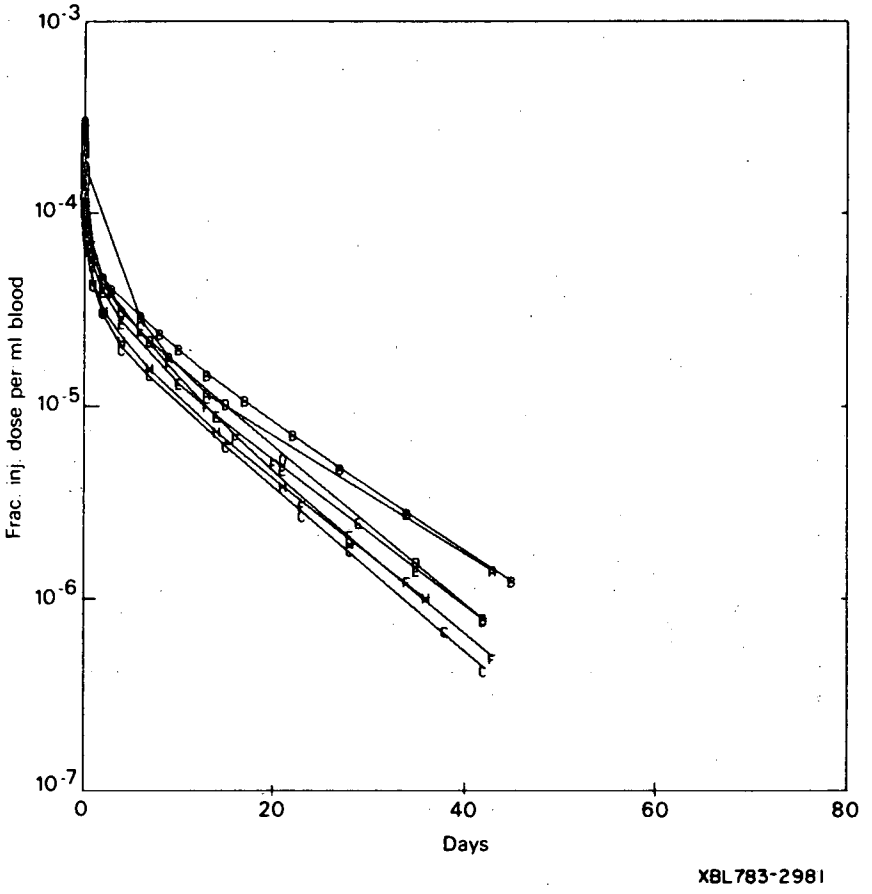

Fig. 2a. Blood levels of ^{51}Cr in normal subjects and patients F,G and H with normal iron stores.

Fig. 2b. Blood levels of ^{51}Cr in patients K-P, who had hemochromatosis with excessive iron stores.

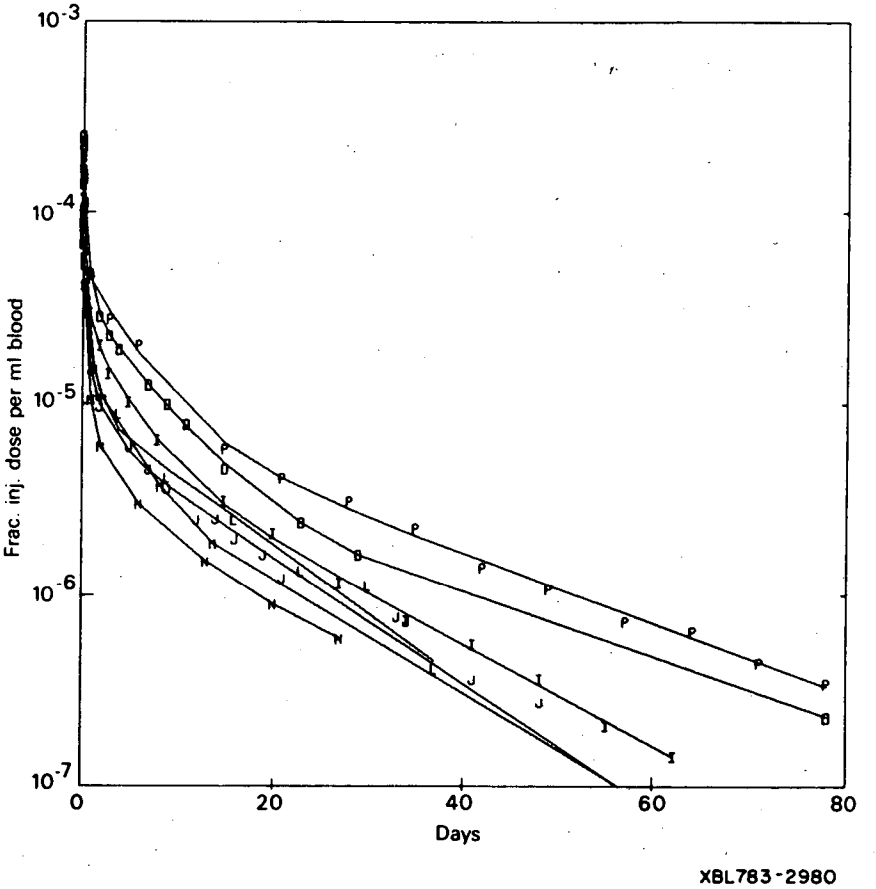

TABLE II

Multi-exponential fitting parameters A_i and r_i for the best fit of equation (1) to whole-body retention curves. The fitting for the whole-body retention data was performed independently of the blood data. The half-times $T_{1/2}$, calculated from $T_{1/2} = \ln 2/r$, are given for the mean value of each r; for the cases where there is only a single value, the $T_{1/2}$ is for that single value.

* = insufficient data early in the curve to determine these parameters accurately, – = no blood obtained on this patient; dir = direct injection of ^{51}Cr; hom = injection of ^{51}Cr after incubation with patient's own plasma; het = injection of ^{51}Cr after incubation with donor plasma.

Patient	Diagnosis	Method of Injection	Whole Body Retention Parameters						Blood Retention Parameters							
			A_1	r_1	A_2	r_2	A_3	r_3	A_4	r_4	A_5	r_5	A_6	r_6	A_7	r_7
A	Normal	hom	.367	1.12	.257	.0376	.342	.00334	9.60	248.	13.5	4.11	7.50	.313	2.42	.0663
B	Normal	hom	.314	1.43	.263	.0678	.427	.00390	3.42	34.4	8.56	2.21	2.22	.212	3.53	.0746
C	Normal	dir	.377	1.14	.263	.0534	.345	.00391	6.06	6.53	3.99	.947	.308	.241	2.65	.0977
D	Normal	dir	.345	1.42	.267	.0596	.381	.00405	3.82	37.9	6.65	3.01	3.47	.669	4.12	.0945
E	Normal	dir	.311	1.12	.269	.0552	.415	.00287	2.39	55.0	6.83	2.81	3.27	.424	2.96	.0865
	Mean		.343	1.25	.264	.0547	.382	.00361	5.06	76.4	7.91	2.62	3.35	.372	3.14	.0839
	$T_{1/2}$			.56 d		12.7 d		192 d		13 min		6.3 hr		1.9 d		8.3 d
F	Hemochromatosis depleted	hom	.307	1.45	.237	.114	.464	.00410	3.07	97.1	11.5	2.03	3.23	.228	2.74	.0932
G	Hemochromatosis depleted	dir	*	*	*	*	.480	.00413	–	–	–	–	–	–	–	–
	Mean						.472	.00412								
	$T_{1/2}$			.48 d		6.08 d		168 d		10.3 min		8.19 hr		3.04 d		7.43 d
H	Hemochromatosis borderline	dir	.366	1.25	.240	.0554	.396	.00337	2.35	129.	6.06	2.72	1.96	.334	2.52	.0900
	$T_{1/2}$			.55 d		12.5 d		206 d		7.7 min		6.12 hr		2.07 d		7.7 d
I	Hemochromatosis relative	hom	.572	2.42	.200	.0836	.245	.00373	6.13	40.6	6.91	2.66	2.09	.266	.650	.0616
J	Hemochromatosis relative	hom	.739	2.23	.145	.0882	.129	.00357	10.2	83.2	6.43	3.60	.945	.517	.728	.0764
	Mean		.655	2.33	.173	.0859	.187	.00365	9.16	61.9	6.67	3.13	1.52	.391	.689	.0690
	$T_{1/2}$			.30 d		8.07 d		190 d		16.1 min		5.31 hr		1.77 d		10.0 d
K	Hemochromatosis	hom	*	*	*	*	.147	.00517	*	*	*	*	*	*	.550	.0898
L	Hemochromatosis	dir	.616	3.42	.193	.0257	.183	.00322	5.45	145.	5.65	11.0	2.89	1.10	.966	.0826
M	Hemochromatosis	dir	.605	2.10	.216	.119	.191	.00423	6.45	33.7	3.14	1.98	1.28	.243	.338	.0633
N	Hemochromatosis	dir	.643	2.49	.158	.0774	.215	.00555	6.85	29.7	2.28	1.56	.437	.201	.233	.0518
O	Hemochromatosis	het	.573	2.42	.196	.106	.238	.00440	17.9	12.8	6.49	1.55	2.99	.164	.395	.0364
P	Hemochromatosis	het	.473	1.46	.205	.0388	.324	.00410	7.76	126.	12.6	4.81	4.29	.223	.896	.0423
	Mean		.582	2.38	.194	.0834	.216	.00444	8.88	69.4	6.03	4.18	2.38	.386	.563	.0610
	$T_{1/2}$			.29 d		8.3 d		156 d		14.4 min		3.98 hr		1.80 d		11.4 d

normal, subjects F and G; "relatives", the two siblings whose father had died of hemochromatosis, who had highly saturated plasma iron; "hemochrom" the patients with hemochromatosis who had not been phlebotomized at the start of the study. A final grouping was made of all subjects considered to have normal iron stores, "nor stores", which included the normal subjects A, B, C, D, and E, the depleted hemochromatosis F and G, and the borderline patient H, compared to "ex stores", the relatives I and J and hemochromatotics K, L, M. N, O, and P considered to have excessive iron stores. For comparison of the injection method, subgroups were chosen from the above groups in those cases where two or more subjects were available. The three methods of injection were: "dir", direct injection of $^{51}CrCl_2$; "hom" for homologous injection, in which a sample of the subject's own plasma was incubated with $^{51}CrCl_2$ and then injected; "het" for heterologous injection, in which plasma from a heterologous donor was incubated with $^{51}CrCl_2$ prior to injection. Table III lists the results of comparison of the significance of difference between the means according to the "t" test, of a number of different pairs of these groups, for each of the derived parameters.

DISCUSSION

Dymock and Williams have reviewed the relationship of hemochromatosis and diabetes (14). They noted that diabetes is not universal in hemochromatosis, with an incidence ranging from 11 to 87% in different series. Sheldon (15) thought that islet damage was caused by the excessive iron in the pancreas, but Dymock and Williams note that Even in 1932 felt that a pancreatic cause was insufficient and that there was an impaired glycogenic activity in the liver. They describe their own and other authors' observation of insulin resistance in hemochromatotic diabetes; generally insulin requirements were reduced after

phlebotomy therapy had reduced the iron stores. They felt that the mechanism of this improvement in the diabetes was difficult to explain. In glucose tolerance tests they found impaired insulin response in some patients but in others a normal early insulin release with high insulin levels later in the test. Thus present understanding of the cause of diabetes in hemochromatosis is not entirely satisfactory.

For purposes of providing evidence for our basic postulate that chromium retention is reduced in hemochromatosis, the comparison of primary interest is the first line of Table III, comparing normal subjects and the patients with hemochromatosis. Inspection of Fig. 1 indicates that the final slopes of whole-body retention in the normals and hemochromatotics are the same, but that the zero-time intercept of this slope is lower in the patients with hemochromatosis. (The parameters r_i are the slopes and the coefficients A_j are the zero-time intercepts of the corresponding slopes). In Table II the mean of this intercept, A_3, is .382 in normals and .216 in the patients; from Table III, this difference is significant at the .01 level. A_2 is also lower in the patients, significant at .01, while A_1 and r_1 are higher in the patient group. In the blood clearance study there was considerable variability in the early parts of the curve, with less in the last two parameters A_7 and r_7. There was not a significant difference between slopes r_7, but the mean of the intercept A_7 was 3.14 in the normals and .563 in the patients, a factor of 5.6 difference significant at the .01 level.

Clearly there must be three or more physically or physiologically distinct pools or compartments of Cr (III) in the human body. Consideration of a model which describes the interrelationships of these compartments requires additional data on the relative amount of ^{51}Cr in each compartment or organ in the body, and a mathematical solution of this model. Such a solution is beyond the scope

TABLE III

Levels of significance of the difference between the means of the parameters A and r, determined by the "t" test, comparing groups of subjects according to disease status in the upper portion and according to method of injection in the lower portion. * = insufficiant data for determination; - = not significant at $p < .05$; dir = direct injection; hom = injection after homologous incubation; het = injection after heterologous incubation.

Comparison Groups			Whole Body Retention						Blood Retention							
Groups		Subjects	A_1	r_1	A_2	r_2	A_3	r_3	A_4	r_4	A_5	r_5	A_6	r_6	A_7	r_7
Normal Hemochrom		ABCDE KLMNOP	.01	.01	.01	-	.01	-	-	-	-	-	-	-	.01	-
Normal Depleted		ABCDE FG	*	*	*	*	.05	-	*	*	*	*	*	*	*	*
Normal Relatives		ABCDE Hi	.01	.01	.01	.05	.01	-	-	-	-	-	-	-	.01	-
Hemochrom Depleted		KLMNOP FG	*	*	*	*	.91	-	*	*	*	*	*	*	*	*
Hemochrom Relatives		KLMNOP HI	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nor stores		ABCDEFGH IJKLMNOP	.01	.01	.01	-	.01	-	.05	-	-	-	-	-	.01	.05
Normal Hemochrom	dir dir	CDE LMN	.01	.05	.05	-	.01	-	-	-	-	-	-	-	.01	.05
Normal Hemochrom	hom hom	AB IJK	-	.05	-	-	.05	-	-	-	-	-	-	-	.05	-
Normal Normal	dir hom	CDE AB	-	-	-	-	-	-	-	-	-	-	-	-	-	.05
Hemochrom Hemochrom	dir het	LMN OP	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemochrom Hemochrom	dir hom	LMN IJK	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemochrom Hemochrom	hom het	IJK CP	-	-	-	-	-	-	-	-	-	-	-	-	-	-

of this communication but will be the subject of a subsequent report (13). It is nevertheless clear that the amount of ^{51}Cr retained in the hemochromatotic patients is significantly less than that retained by normal subjects, and this applies to both the 192 day component and the 12.7 day components. The shortest component, with a $T_{1/2}$ = .56 d in normals and .29 d in the patients, has a correspondingly higher intercept A_1 in the patients than in the normals. Since both r_1 and A_1 are significantly higher in the patients ($p < .01$), an interpretation that accords with these results is that the high saturation of transferrin prevents binding of the injected ^{51}Cr which is then rapidly excreted at the rate r_1. Hence both the rate r_1 and the intercept A_1 are higher in the patients, while the amount of ^{51}Cr available to the rest of the body is reduced, so that A_2 and A_3 are lower. The rate constants r_2 and r_3 associated with these components are the same however. The rates r_2 for whole body and r_7 for blood are of similar magnitude and may represent turnover of the same physiological compartment. The coefficient A_7 would then correspond to the whole body coefficient A_2, and the reduced value for A_2 for the whole body in the patients is reflected in a significantly reduced value of A_7 for blood. There is presumably a component of the blood curve of $T_{1/2}$ comparable to r_3 in the wholebody, but the amount of ^{51}Cr remaining in blood was too low to measure such a $T_{1/2}$. Thus our principle postulate appears to be confirmed, that highly saturated iron stores results in reduced retention of body chromium (III).

Further comparisons of the various groups may yield additional information. The depleted group is difficult to compare because of the limited data for patient G. However, as seen in Table II the whole-body intercept A_3 is higher for the depleted patients than the normals ($p < .05$) and correspondingly higher still than for the iron loaded patients ($p < .01$). Comparison of the single values of each of the other parameters for the depleted patient F shows that they

are closer to the normal values than to the iron-loaded patients; in Figs. 1 and 2 the data for the depleted patients are plotted with the normal subjects, and the curves are very similar. Thus is appears that once a patient with hemochromatosis is depleted of his excess iron, his chromium metabolism is normal, except for a tendency to store a greater amount of ^{51}Cr in the long half-time pool, perhaps compensating for years of chromium depletion when it was being excluded by the excess iron stores.

The two relatives with highly saturated iron stores when compared to the normal subjects (Table III) showed the same relationship of parameters as did the hemochromatotic patients. In addition r_2 was significantly different from normals ($p < .05$) and was not significantly different from the patients. When the relatives were compared to the full clinical cases of hemochromatosis, there was no significant difference in any of the parameters. Thus it appears that at the transferrin saturation level (75% and 86%) of these relatives, the chromium (III) metabolism is indistinguishable from that of the clinical cases. On the other hand, for patient H (whom we classified as "borderline" on the basis of 60% transferrin saturation and had normal iron stores on the basis of the amount of iron removed by phlebotomy)all of the parameters with the exception of A_2 were within one standard deviation of the mean of the normal subjects. This patient appears to be the same as the normal subjects in every respect except for an abnormally saturated transferrin level and affected relatives as noted earlier.

Because of the similarity of the parameters and clinical condition of F, G and H to the normal subjects, and the similarity of relatives I and J to the patients with clinical hemochromatosis, all subjects were divided into two groups, "normal stores" and "excess stores", and the parameters compared. The results were the same as comparison of the normals and hemochromatotics, except that A_4 and r_7 were

also found to be different, but only at the $p < .05$ level. This result supports the division of the subjects on this basis in terms of the parameters of ^{51}Cr metabolism.

If the basis of the lower retention of ^{51}Cr in patients with hemochromatosis were only that the transferrin was saturated in the latter, then we might expect that injection of ^{51}Cr bound to plasma from a normal donor into a patient with hemochromatosis would result in normal retention of the ^{51}Cr. This was tested in patients O and P, and as can be seen in Table III, when the parameters were compared with patients L, M and N receiving direct injection and I, J and K receiving homologous injection, there was no significant difference in any of the parameters (relatives I and J were considered for this purpose to be comparable to the clinical hemochromatotics). Since the injected ^{51}Cr bound to normal plasma in hemochromatotic patients showed the same reduced retention pattern, we may conclude that either the iron from the patients' own saturated transferrin rapidly displaced the ^{51}Cr from the heterologus plasma immediately upon injection, or that the saturated binding sites in other tissues also contributed to the exclusion, and hence excretion, of the ^{51}Cr.

In other comparisons of injection methods in Table III, it can be seen that normals and hemochromatotics, injected both directly (CDE vs LMN) and after homologous incubation (AB vs IJK), showed essentially the same differences as the normal and hemochromatotic groups as a whole, although the significance level was lower in some cases and not significant for A_1 and r_2 in the homologous comparison. The normals injected directly (CDE) were not different in any parameters except r_7 at $p < .05$, from normals injected after homologous incubation (AB). The general conclusion from comparison of methods of injection is that the method used does not affect the retention of chromium, and the simplest method, direct injection, is thus the preferred one.

There appears to be no relationship between the value of A_3 in each patient, and the amount of iron in stores as measured by subsequent phlebotomies. Patient J, the son of a patient with hemochromatosis, had one of the lowest values of A_3 but had only 7 grams of iron in stores, while P had the highest value of A_3 and 19.1 grams of iron. Patient P differed from the other patients in that he was the only one with clinical diabetes, and he was taking Diabinase to control blood glucose. Diabinase is believed to act by increasing the number of insulin binding sites on the cell membrane (16), and chromium is believed to potentiate the action of insulin by taking part in the binding of insulin to the cell membrane (17). Conceivably the high value of A_3 in patient P compared to the other patients resulted from an effect of Diabinase on chromium binding to cell membranes.

To our knowledge the whole-body retention of chromium in humans has not been previously reported. Mertz, et al. (18) reported the whole-body turnover of ^{51}Cr in rats; they found a three-component curve with half-times of 83.4 days, 5.9 days and 0.5 days. The final linear portion of their curve extended from 35 to 72 days, less than half of the final half time; a similar half-time could be obtained from our curves of retention in humans over this interval. The final slope of our curves is linear for longer than one half-time, providing reasonable assurance that there is not yet another slope of still longer half-time that could be seen if the radioactive half-life of ^{51}Cr were long enough or if a larger dose were given. We thus feel that our data provide an accurate measure of the exponential components that comprise human chromium excretion.

In conclusion we have obtained evidence to support part of our hypothesis, that patients with hemochromatosis with excessive iron stores retain less chromium (III) than normal subjects. The other part of the hypothesis, that this exclusion is causally associated with hemochromatotic diabetes, is still

unproven and is of great interest with respect to diabetes mellitus. There is ample evidence that diabetes mellitus involves insulin resistance as well as insulin deficiency, and that these conditions may represent different basic disease processes (19). Since there is evidence that chromium is required for the binding of insulin to the cell membrane (17), it is possible that the phenomenon of insulin resistance may be the result of a failure of metabolic handling of chromium. Investigation of chromium metabolism in diabetes mellitus should be able to demonstrate whether such a failure occurs.

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