AGE DIFFERENCES IN THE INTRAVENOUS GLUCOSE TOLERANCE TESTS AND THE RESPONSE TO INSULIN

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In addition to providing information with regard to the status of carbohydrate metabolism, the time course of the disappearance of injected glucose from the blood stream offers information concerning the overall effectiveness of a variety of physiological mechanisms involved in maintaining homeostasis. In a number of investigations, reduction in glucose tolerance, *i.e.,* a slower rate of return to fasting levels of blood sugar following the oral (1–17) or intravenous (18–20) administration of glucose, has been reported in older people. The diminished glucose tolerance in the older individual might be due to: (a) inadequate release of insulin from the pancreas, or greater inactivation of endogenously released insulin, (b) the loss of functioning protoplasm with increasing age so that less metabolizing tissue is removing glucose from the blood, (c) a diminution in the effectiveness of the metabolic processes involved in the removal of sugar from the blood stream, (d) alterations in the rate of release of glucose from the liver, or (e) a reduction in the volume in which the glucose is originally distributed in the aged. By comparing the glucose and glucose-insulin tolerance tests in the same individual, an estimate of the effect of insulin may be obtained (21–27). In the experiments to be reported, standard amounts of insulin were administered along with glucose to both old and young subjects with the aim of investigating age differences in the response to the insulin.

EXPERIMENTAL METHODS

Subject selection. Thirty-five male subjects, age 23 to 86 years, were selected on the basis of a detailed history, physical examination and a series of laboratory tests. The presence of any of the following served to exclude a subject from the study: (a) history or known evidence of diabetes or glycosuria, (b) severe alcoholism, hepatomegaly, cirrhosis or other liver disease, (c) cardiac decompensation or edema, (d) infections, temperature elevation or acute or chronic trauma (including surgical) within one week of test, or (e) the taking of steroid drugs (other medication such as aspirin was omitted for 12 hours preceding the tests). All were ambulatory in-patients on a routine full hospital diet for at least one week. Fasting blood sugars were within normal limits (27, 28) as shown in Table I.

Experimental procedure. The intravenous glucose tolerance test (GTT) and the glucose-insulin tolerance test (GITT) were performed in each subject under basal conditions and separated by an interval of not less than one week. In 8 of the subjects, each of the tests (GTT and GITT) was carried out twice in order to evaluate reliability.

Twenty minutes before either test was begun, a modified Lindeman needle was placed in an antecubital vein and left in place for the duration of the test. The needle was kept patent by heparinization of the stylus and was used subsequently only for withdrawing blood specimens without a tourniquet (29). A vein in the opposite arm was used for the injection of 50 ml. of 50 per cent glucose in water over a period of two minutes. For the GITT, 5 units of hyperglycemic factor-free insulin[3] (Lilly), per square meter of body surface area, were rapidly injected, followed immediately by the standard amount of glucose. The fasting blood specimen was obtained through the Lindeman needle a few minutes before zero time, which was recorded as the beginning of the injection of glucose.

Blood samples were collected at 5-minute intervals for the first hour and at 20-minute intervals during the second hour, and were placed immediately in tubes containing a dried heparin and sodium fluoride mixture. All analyses were completed the day of the test, using the Nelson-Somogyi method (30). Determinations were in duplicate and were read on a Model DU Beckman Spectrophotometer.

Data analysis. For each tolerance test, the observations obtained between 10 and 60 minutes of the experiment were fitted to the equation $\log_e y = \log_e A - kt$ ($y = Ae^{-kt}$) where y is the blood glucose concentration in

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TABLE I
Subject characteristics

Variable		Age group: Young	Middle	Old	Total
N		12	11	12	35
Age (*yrs.*)	Mean	31.3	49.1	78.7	53
	Range	23	42	65	23
		37	58	87	87
Height (*cm.*)	Mean	175.3	174.4	162.6	170.8
	σ_{Mean}	2.0	2.7	2.3	2.2
	Range	165.7	165.1	143.5	143.5
		185.4	186.7	171.4	186.7
Weight (*Kg.*)	Mean	71.0	64.6	66.8	67.5
	σ_{Mean}	2.4	3.5	3.9	3.2
	Range	57.3	52.0	45.1	45.1
		85.0	92.7	88.2	92.7
Surface area ($M.^2$)	Mean	1.83	1.77	1.70	1.77
	σ_{Mean}	.033	.047	.060	.040
	Range	1.67	1.58	1.39	1.39
		2.00	2.08	2.00	2.08
Fasting blood glucose*	Mean	68.0	78.5	85.0	77.1
	σ_{Mean}	1.9	2.0	2.3	2.1
	Range	60	64	70	60
		77	90	97	97
Glucose administered (*Gm./Kg. body wt.*)	Mean	.356	.397	.389	.381
	σ_{Mean}	.012	.018	.024	.020
	Range	.294	.270	.283	.270
		.436	.454	.554	.554

* Mean of control observations on glucose and glucose-insulin tests.

milligrams per 100 ml. and t is time in minutes, following the injection of the glucose load. The method of least squares (31) was utilized for the computation of A and k for each experiment. The value of k was taken as the index of tolerance for this study. The difference between the k for the glucose (k_G) and the glucose-insulin test (k_{GI}) is called Δ k and served as the index of the response to insulin in each subject. Visual fits to plots of log of the glucose level against time were also made and compared with the least squares fitting.

Age changes in the data were evaluated by determining the regression of the derived measures on age and also by comparing mean values for three groups: young (12 subjects, age 20 to 39 years), middle (11 subjects, age 40 to 59 years), and old (12 subjects, age 60 to 90 years).

RESULTS

Characterization of the subjects

No significant ($P = > 0.10$) differences were found among the three age groups with respect to body weight, surface area or dose of glucose per Kg. of body weight (Table I). A small, but statistically significant, increase in fasting venous blood sugar levels with age was observed in this sample.

Reliability of methods

The standard deviation of repeated glucose determinations on a single filtrate was ± 1.1 mg. per cent (N = 58). Comparing two filtrates, prepared from the same blood sample, the standard deviation was 2.1 mg. per cent (N = 53).

There was no systematic difference between fasting blood sugar levels determined on the same individual on different days. The standard error of estimate between measurements made on the first and second days was ± 6.5 mg. per 100 ml.[4]

In the 8 subjects (4 old and 4 middle-aged) who had duplicate glucose tolerance and glucose-insulin tolerance tests, no significant differences occurred between the results of the first and second tests, with respect to k_G or k_{GI}. The dupli-

[4] Mean values are reported with standard errors of the mean.

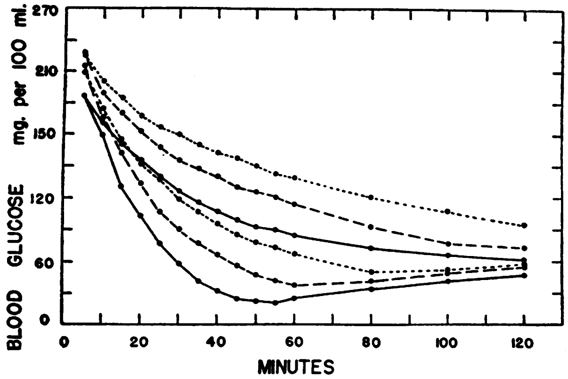

FIG. 1. LINEAR PLOTS OF AVERAGE BLOOD SUGAR VALUES (MG. PER 100 ML.) AT EACH TIME INTERVAL (MIN.) FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 25 GM. GLUCOSE FOR EACH OF THREE AGE GROUPS

Glucose tolerance (GTT) open circles; glucose plus 5 units insulin per sq. M. surface area (GITT) closed circles; old subjects (65 to 87 years old) dotted lines; middle-aged subjects (42 to 58 years old) dashed lines, and young subjects (23 to 37 years old) solid lines.

cate tests were not performed in any specific order; the lapse of time between tests varied from one week to three months. In contrast to results reported by Hlad, Elrick, and Witten (32) values for k_G or k_{GI} were repeatable and characteristic for the individual.

General description of results

Average values of blood sugar concentration at each time interval, following the administration of glucose, are plotted for each of the three age groups in Figure 1. The rate of fall in blood glucose level was greater for the young than for the old subjects under both experimental conditions. When tests of significance of age differences were applied to specific time points along the glucose or glucose-insulin tolerance curves, true differences ($P < 0.001$) were found between young and old subjects at 15, 30, and 60 minutes after injection of the glucose. Differences over shorter age spans, *i.e.*, between young and middle, and middle and old subjects, were usually significant at $P < 0.01$ or $P < 0.05$.

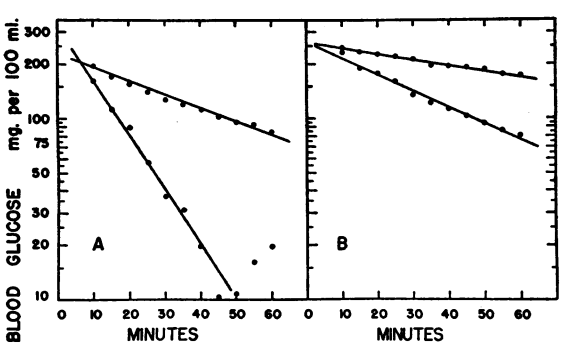

FIG. 2. ESTIMATION OF K_G AND K_{GI} IN INDIVIDUAL EXPERIMENTS

Log glucose concentration in blood (mg. per 100 ml.) plotted against time (min.). A. Young subject (26-year-old). B. Old subject (86-year-old). Upper lines (open circles) represent data from GTT; lower lines (solid circles) represent data from GITT.

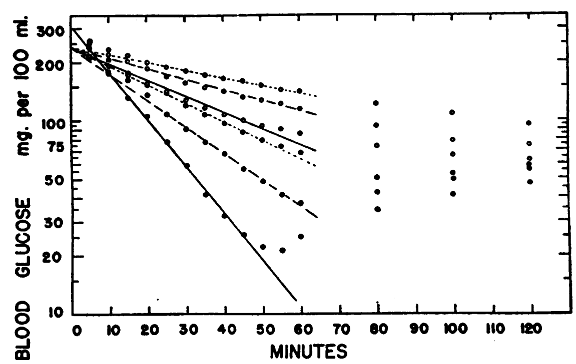

FIG. 3. AGE DIFFERENCE IN K_G AND K_{GI}

Plots of log glucose concentration in the blood (mg. per 100 ml.) against time (min.) following intravenous administration of 25 Gm. glucose. Glucose tolerance (GTT) open circles; glucose plus 5 units insulin per M.[2] surface area (GITT) solid circles; old subjects (65 to 87 years old) dotted lines; middle-aged subjects (42 to 58 years old) dashed lines, and young subjects (23 to 37 years old) solid lines. (Lines are fitted to points for 10 to 60 min. inclusive.)

Age differences in rate of disappearance of glucose from the blood

When the log glucose concentration was plotted against time, a linear relationship was obtained for the points between ten and 50 to 60 minutes. Sample plots for a young (26-year-old) and an old (86-year-old) subject are shown in Figure 2A and 2B. Figure 3 shows the log of the mean glucose values for the three groups of 8 subjects, plotted against time after glucose administration. Deviations from linearity are apparent in all curves beyond 50 or 60 minutes. Therefore, the expression $\log_e y = \log_e A - kt$ fails to describe the total process, but may be used to derive an index of the rate of disappearance of glucose from the

TABLE II

Blood glucose levels at specified times following intravenous administration of 25 grams glucose (G) and intravenous administration of 25 grams glucose plus 5 units per M.² of insulin (GI) in young males

Subject	Condition	Age (yrs.)	Wgt. (Kg.)	Ht. (cm.)	Blood sugar levels (mg./100 ml. at specified time—min.) 0	10	15	20	25	30	35	40	45	50	55	60	80	100	120	k (%/min.)
J. B.	G	32	69.1	180.3	73	152	146	126	119	105	93	80	78	77	72	67	68	65	67	1.73
	GI				81	153	123	87	58	37	19	24	22	23	28	35				8.26
F. B.	G	33	80.0	182.2	74	215	161	165	139	115	90	70	63	61	57	56	60	63	73	3.13
	GI				74	156	128	97	80	55	39	34	27	19	20	22	39	42	51	5.30
E. C.	G	36	71.4	175.3	61	190	168	154	140	129	120	112	101	96	89	85	66	55	52	1.60
	GI				65	162	97	77	54	42	14	13	6	4	7	25				9.36
L. C.	G	35	71.8	165.7	73	212	182	165	157	149	137	130	120	117	109	98	83	75	67	1.39
	GI				72	181	155	129	97	84	70	46	47	41	30	27	31	55	60	3.92
R. H.	G	32	66.0	165.7	57	205	189	171	155	135	131	116	112	112	98	95	68	51	41	1.55
	GI				67	194	142	112	83	55	40	27	13	7	2	6	27	33	40	9.30
J. J.	G	27	60.4	172.7	67	216	190	170	158	152	147	139	132	115	116	108	84	70	60	1.36
	GI				69	256	159	139	110	88	67	49	42	37	27	30	43	46	57	4.74
D. L.	G	37	71.0	176.5	60	176	166	128	107	88	68	73	71	60	68	59	56	68	59	2.81
	GI				73	142	113	82	59	38	17	17	17	23	29	29				7.80
M. N.	G	37	83.4	175.3	74	193	176	167	152	141	135	126	119	112	109	102	83	72	65	1.26
	GI				68	160	117	94	74	54	42	37	22	23	23	31	37	42	49	5.07
T. R.	G	23	57.3	171.4	65	191	169	156	135	128	119	113	102	93	85	80	58	52	49	1.70
	GI				55	153	120	94	74	52	30	25	12	12	16	18	22	28	35	6.88
B. T.	G	28	72.2	185.4	66	195	172	155	139	127	120	112	102	94	92	84	66	58	53	1.63
	GI				60	161	112	89	57	37	31	19	9	10	17	19	23	27	27	7.78
C. W.	G	25	64.8	168.9	68	202	185	186	172	161	149	137	124	120	118	110	91	73	69	1.27
	GI				73	221	162	142	105	91	76	51	48	39	35	33	50	51	59	3.85
L. Y.	G	31	85.0	184.2	73	201	185	183	176	170	165	155	147	142	139	136	123	109	97	0.78
	GI				73	175	136	93	74	55	45	37	35	29	22	21	33			4.44
Mn	G	31	71.0	175.3	67	191	171	156	141	127	116	107	99	93	90	85	73	66	62	1.68
	GI				69	179	131	103	77	58	41	32	25	22	21	25	34	41	47	6.39
SE_{Mn}	G		2.4	2.0	2.1		5.8			9.7			10.7			8.9				.19
	GI				2.0		6.1			5.8			4.3			2.3				.60

TABLE III

Blood glucose levels at specified times following intravenous administration of 25 grams glucose (G) and intravenous administration of 25 grams glucose plus 5 units per M.² of insulin (GI) in middle-aged males

Subject	Condition	Age (yrs.)	Wgt. (Kg.)	Ht. (cm.)	Blood sugar levels (mg./100 ml. at specified time—min.) 0	10	15	20	25	30	35	40	45	50	55	60	80	100	120	k (%/min.)
S. C.	G	42	65.8	165.1	78	231	209	199	184	179	164	155	144	140	131	124	103	86	73	1.21
	GI				81	216	198	171	144	126	111	96	87	77	68	65	51	51	59	2.54
C. C.	G	53	66.9	182.9	73	199	157	126	107	86	73	71	59	65	69	62	56	58	69	3.41
	GI				78	159	121	96	71	57	46	36	35	28	20	21				4.39
W. D.	G	43	61.8	186.7	92	231	215	189	170	148	149	139	130	121	115	106	73	73	76	1.49
	GI				87	213	167	136	105	93	71	63	50	46	40	37	44	50	56	3.56
J. D.	G	42	72.5	166.4	77	216	200	187	172	164	149	143	135	131	126	117	100	89	75	1.20
	GI				73	205	167	133	102	86	71	59	46	40	29	31	41	39	50	4.21
A. E.	G	58	60.9	185.4	76	224	198	185	174	167	159	158	145	138	136	128	112	96	81	1.02
	GI				85	214	192	173	146	126	107	96	81	69	58	53	39	52	45	2.91
L. G.	G	50	55.7	167.6	66	218	197	173	162	145	134	127	114	103	96	94	62	58	47	1.72
	GI				62	169	138	116	96	75	59	45	35	25	26	15	26	41	49	4.71
E. H.	G	54	55.7	170.2	70	231	226	212	208	198	187	181	170	168	157	150	129	111	86	0.87
	GI				85	199	170	128	106	98	81	71	61	46	40	40	43	51	54	3.48
D. J.	G	58	52.0	166.4	80	248	216	190	174	158	152	148	144	138	128	121	97	80	75	1.29
	GI				80	206	180	157	139	113	109	100	92	80	75	69	44	55	59	2.17
J. J.	G	44	92.7	175.3	71	193	175	167	150	139	137	128	119	115	110	106	89	80	74	1.19
	GI				86	180	142	121	91	69	74	60	48	42	32	32	59	67	72	3.49
J. R.	G	53	71.1	185.4	87	220	186	175	163	149	141	135	124	123	116	109	93	88	83	1.29
	GI					193	153	119	93	77	63	56	47	44	34	33	32	45	57	3.55
R. W.	G	43	55.1	167.6	80	249	227	214	193	188	181	166	162	152	146	138	106	87	79	1.13
	GI				70	195	147	118	77	70	55	49	37	28	25	23	39	43	49	4.67
Mn	G	49	64.6	174.4	77	223	201	183	169	156	148	141	131	127	121	114	93	78	74	1.44
	GI				79	196	162	134	107	90	77	67	56	48	40	37	42	49	55	3.61
SE_{Mn}	G		3.5	2.7	2.3		6.5			9.0			8.9			6.9			3.1	.21
	GI				2.6		8.1			7.2			6.4			5.8			2.4	.25

TABLE IV

Blood glucose levels at specified times following intravenous administration of 25 grams glucose (G) and intravenous administration of 25 grams glucose plus 5 units per M.² of insulin (GI) in aged males

Subject	Condition	Age (yrs.)	Wgt. (Kg.)	Ht. (cm.)	Blood sugar levels (mg./100 ml. at specified time—min.) 0	10	15	20	25	30	35	40	45	50	55	60	80	100	120	k (%/min.)
F. B.	G	72	68.2	143.5	80	223	195	181	162	150	144	132	127	121	116	109	94	86	77	1.36
	GI				88	193	162	143	132	120	105	99	89	77	74	68	46	51	58	2.04
T. B.	G	86	56.1	160.0	90	243	219	204	195	178	168	158	150	139	133	130	105	95	79	1.26
	GI				74	182	151	142	117	107	100	84	78	64	57	59	39	41	42	3.58
J. H.	G	73	53.5	161.3	75	211	194	180	176	171	162	155	152	148	146	158	108	108	95	0.79
	GI				65	188	150	134	120	94	83	76	65	56	51	49	39	37	42	2.74
E. K.	G	76	55.6	156.8	83	238	238	217	205	204	197	181	166	165	157	152	136	120	105	0.96
	GI				80	232	208	184	165	150	138	124	118	112	99	85	67	54	57	1.88
J. He.	G	85	70.1	162.6	95	255	223	190	184	186	181	178	174	165	155	141	142	131	114	0.91
	GI				83	202	170	153	135	121	98	86	71	69	66	60	54	63	71	2.52
W. L.	G	87	83.4	168.9	92	220	204	184	177	167	156	155	144	141	134	130	113	102	88	0.82
	GI				96	192	152	128	111	95	74	76	62	59	64	51	45	53	71	2.95
D. M.	G	84	45.1	156.2	82	278	258	236	211	206	188	182	174	163	141	141	122	100	88	1.35
	GI				73	223	193	160	142	119	108	89	75	65	54	50	36	33	39	3.06
J. M.	G	65	82.4	171.4	96	220	216	211	205	194	189	185	179	176	165	155	147	122	104	0.67
	GI				94	212	197	180	168	148	141	130	118	106	103	93	69	63	66	1.66
G. P.	G	79	60.7	171.4	86	206	194	179	176	171	166	162	158	151	149	143	127	113	101	0.67
	GI				86	186	177	154	143	131	121	109	101	94	86	80	58	49	58	1.73
D. R.	G	82	61.8	161.3	78	211	193	174	164	149	135	132	124	113	111	105	92	80	71	1.36
	GI				78	210	171	140	118	97	67	64	54	51	38	31				3.74
A. T.	G	73	77.0	167.6	101	247	232	228	209	212	198	191	192	186	176	174	149	136	123	0.68
	GI				92	231	190	168	159	135	122	113	103	94	86	81	66	66	74	2.04
L. W.	G	82	88.2	170.2	83	215	204	186	183	173	165	162	150	144	138	138	119	104	99	0.91
	GI				82	201	174	144	140	128	121	101	94	89	97	89	73	68	64	1.99
Mn	G	78	66.8	162.6	87	231	214	198	187	180	171	164	158	151	143	140	121	108	95	0.98
	GI				83	204	175	152	138	119	107	96	86	78	73	67	50	53	58	2.49
SE_{Mn}	G		3.9	2.3	2.3		6.0			6.0			5.9			5.7				.08
	GI				2.7		5.6			5.4			6.2			5.2				.02

TABLE V

Rate of disappearance of glucose from the blood with (k_{GI}) and without insulin (k_G) (k expressed as per cent per minute)

		Venous blood						Arterial blood*		
		Glucose (k_G)		Glucose-insulin (k_{GI})		Diff. (Δk)			Glucose (k_G)	
Age group	N	Mn.	$\sigma_{Mn.}$	Mn.	$\sigma_{Mn.}$	Mn.	$\sigma_{Mn.}$	N	Mn.	$\sigma_{Mn.}$
Young	12	1.68	.19	6.39	.60	4.12	.59	13	1.94	.20
Middle	11	1.44	.21	3.61	.25	2.17	.26	13	1.61	.09
Old	12	0.98	.08	2.49	.02	1.52	.17	38	1.28	.06
Total	35	1.37	.11	4.15	.11	2.62	.09	64	1.48	.07

* Data from Smith and Shock (19) recalculated.

blood during the first 50 to 60 minutes of the experiment. Examination of Figure 3 shows that: (a) the early rate of disappearance of excess of glucose from the blood was more rapid in the young than the old, (b) the rate of disappearance was increased in all age groups by the simultaneous administration of insulin, and (c) the influence of insulin was greater in the young than the old subjects.

Computations by the method of least squares of the slope for each individual test provided data for estimates of the variability of k within each age group. Tables II, III, and IV present the values of k for both the GTT and the GITT with increasing age. Average values of k, expressed as per cent per minute, for the young and old, respectively, were as follows: k_G, 1.68 and 0.98; k_{GI}, 6.39 and 2.49, and Δ k ($k_{GI} - k_G$), 4.12 and 1.52. Values of P were less than 0.01 for these age differences. Regressions of k on age were significant at the 0.01 level for k_G and at less than 0.001 for k_{GI} and Δ k. The effect of insulin in the average adult male was to increase the rate of fall in blood glucose level from 1.37 per cent per minute to 4.15 per cent per minute.

DISCUSSION

Subject selection—Activity and diet

Since age differences in glucose tolerance are relatively small, it is necessary to give careful consideration to the selection of subjects. In order to minimize the effects of prior diet, which has been shown to have an influence on the glucose tolerance (33–36), only subjects who had been on a standard hospital diet for at least one week were tested in the present series.

Since reduced activity has been shown to reduce glucose tolerance (36, 37), only ambulatory patients were studied. The young subjects were also patients drawn from an ambulatory in-hospital population. Thus, the level of activity was probably more uniform between the different age groups than would have been the case had staff members been used for the younger age groups.

Dose of glucose and insulin

Although different amounts of glucose have been used by previous investigators, recent studies have shown that adjustment of the dose of glucose to body size is unnecessary (18, 28, 38). Consequently, a standard dose of 25 Gm. of glucose was administered at a uniform rate (18, 38) to all subjects in the present study. The glucose load varied from 0.57 Gm. per Kg. to 0.27 Gm. per Kg. in different subjects, but there were no systematic differences between age groups (Table I). The dose of insulin was set at 5 units per $M.^2$, which is approximately 0.1 unit per Kg. body weight. Experimental studies (22–24, 27, 39–41) indicate that insulin gives a maximum effect on rate response at dose levels of 0.05 unit per Kg. and up to 0.3 unit per Kg. The effect of differences in endogenous insulin production would be obliterated at this dose range of injected insulin.

Comparison of venous and arterial blood samples

Nelson's modification of the Somogyi method (30) was used for sugar determination in this study in order to minimize the effects of nonfermentable reducing substances (42, 43). The use of venous blood samples in conjunction with an indwelling needle has the advantages of good patient acceptability and a minimum of patient trauma (44–46). Although the differences between arterial and venous blood glucose levels are small under fasting conditions (arterial bloods average 9 mg. per 100 ml. higher than venous), there is a wide range of individual variation (1 to 17 mg. per 100 ml. in normal subjects) and the difference increases markedly (average 30 to 43 mg. per 100 ml.) following the administration of glucose (43, 47). Since Blotner (37) has found that glucose tolerance determined on venous blood samples was influenced less by physical activity in both children and adults than were estimates derived from capillary blood samples, it follows that the use of venous blood might be a more adequate test of age differences in glucose tolerance.

Since previous studies of glucose tolerance from this laboratory were based on arterial blood samples (19), the data were fitted to the tolerance equation. A total of 64 subjects, divided into three age groups, were tested under conditions closely approximating the present study, except that blood samples were drawn from the femoral artery (19). Insulin was not given. Table V gives the k values based on arterial blood samples.[5] As was true for venous blood samples, there was a significant decrement in k_G with increasing age. However, the trend toward increasing fasting blood sugar levels with age, found in the present study on venous blood and in the report on capillary blood by Schneeberg and Finestone (20), was not apparent in arterial blood.

The tolerance equation

One simple expression which can serve to express the rate of disappearance of glucose from the blood, as a single number, is the equation $\log_e y = \log_e A - kt$ (48–51). If one assumes that no distinction is made between the glucose added to the blood from an external source and the glucose added by the liver or other cells of the body, the estimates of the slope of the curve must be made on the total glucose content at successive time intervals. This assumption may be applied safely only to the early parts of the curve, since it is obvious that alterations in the glucose concentration will be introduced by other processes in the body which tend to add glucose to the circulation, particularly when blood sugar levels fall to low values, as in the case when insulin is administered. Greville (50) as well as Hlad, Elrick, and Witten (32) found that subtraction of a calculated asymptotic value of blood sugar level resulted in a somewhat better fit to glucose tolerance data beyond 90 minutes. In this study where curves were limited to the first 60 minutes, the fit was very good; the introduction of an asymptote had only a small insignificant effect on goodness of fit. Furthermore, the use of an asymptote gave rise to difficulties in comparing the GTT and the GITT, since subtraction of a calculated asymptote for the glucose-insulin curves often resulted in values less than zero. Although the value of k is related to the level of the asymptote, both parameters are determined by the same set of experimental points.

In agreement with other studies (49–51), the blood glucose level at 5 minutes, following the injection, was found to be higher than predicted from the exponential curve, suggesting an interaction with the early extra-cellular mixing phase. By 10 minutes after the glucose injection, the mixing phase is indistinguishable from the body of the curve. Conard, Franckson, Bastenie, Kestens, and Kovacs (52) injected glucose and thiocyanate simultaneously and found a thiocyanate space of 14.31 L. and a glucose space of 14.01 L. In our experiments, the average glucose space was 14.1 L. or 22 per cent of the body weight. This value is only slightly lower than the glucose space (23.3 per cent of body weight) reported by Hlad, Elrick, and Witten (32) on the basis of continuous infusions of glucose. Since there are no significant changes with age in the thiocyanate space (53), it appears that the values of k are determined by the distribution of glucose in

[5] Arterial k values were based on a visual fit. A comparison between the derivations of k by least squares and graphic estimates, made from a visually fitted line using the venous data, gave mean values of 137 ($k \times 10^4$) for both methods for the GTT ($r = 0.96$). For the GITT, the mean values of k by the least squares' method was 415 as compared to 439 by the visual method ($r = 0.89$). Age did not influence the correlation between methods. Thus, a visual fit to the data yields substantially the same results as analysis by least squares. However, the latter permits a quantitative statement of the "goodness of fit" of the equation. This is not possible when the visual method above is used. Age did not influence the goodness of fit.

the intra-cellular fluids and metabolic pathways, and are influenced very little by extra-cellular mixing and not in a manner which is age biased.

Age differences

Although there is an overall reduction in the rate of removal of glucose from the blood and a reduced response to insulin with increasing age, it cannot be assumed that this reduction is necessarily associated with altered cellular metabolism. A similar overall effect could result from a reduction in the number of metabolizing units. Other studies from this laboratory have indicated a gradual loss of metabolizing tissue with increasing age (54). This conclusion is based on the observed decrease in intra-cellular water (antipyrine space minus thiocyanate space) with age. The intra-cellular space, calculated in this fashion, averages 10.5 L. in young and 8.6 L. in aged subjects; a reduction of 18 per cent. Over the same age span, k shows a reduction of 42 per cent for the glucose tolerance data and 61 per cent for the glucose-insulin tests. Although the data do not represent observations made on the same subjects, and the dimensions are incongruous, it seems difficult to account for all of the changes observed on the basis of a loss of functioning protoplasm alone.

It is conceivable that the age differences in the rate of removal of glucose from the blood might be due to differences in blood flow and delivery of glucose to the tissues. Clearly, a reduction in the total amount of blood delivered, per unit of time, would influence k if all the glucose were removed in a single passage through a vascular bed. However this is not the case. If there were a substantial reduction in blood flow to tissues in the older subject, an increase in the A-V difference should appear. Since we do not have simultaneous arterial and venous glucose levels on the same subjects, no final decision can be reached on this question, but it does not seem likely that differences in blood flow can account for the age differences observed.

It is recognized that the concentration of glucose in the blood at any given time represents an equilibrium between the rate of removal and the rate of release of glucose from the liver. The differential effect of insulin in the three age groups makes it improbable that the results obtained can be ascribed to differences in the rate of release of glucose from the liver in the old and young subjects. Recent studies indicate that the early effect of insulin action is that of increasing peripheral uptake and metabolism of glucose. The liver response is minimal and delayed (55).

Inactivation of insulin by a plasma constituent may be a factor in some phases of diabetes, particularly in regard to the mechanism of clinical insulin resistance. Welsh, Henley, Williams, and Cox (56) studied the plasma binding of insulin I^{131} in 118 subjects, 43 of which were non-diabetic ranging from 14 years to 90 years of age. Analyzing their data with reservation for the inclusion of patients with other active disease in their group of non-diabetic controls, no trend is discernible between the age of the subject and the potential insulin inactivation by plasma binding.

Although proof cannot be offered, it seems reasonable to assume that at least part of the age differences can be ascribed to alterations in the metabolic effectiveness and response of the functioning cells in the aged male.

SUMMARY

Intravenous glucose tolerance and glucose-insulin tolerance tests were performed on 35 normal male subjects under standardized conditions using venous blood samples. The subjects ranged in age from 23 to 86 years. Blood samples drawn at 5-minute intervals, between 5 and 60 minutes after administration of 25 Gm. glucose, were analyzed for glucose by the Nelson method. The rate of fall of the blood sugar level between 10 and 60 minutes was determined by fitting the experimental points to the equation $\log_e y = \log_e A - kt$. A significant decrease in k with age was observed in both the glucose and the glucose-insulin tolerance curves. The administration of insulin had a greater effect on the rate of disappearance of glucose from the blood in the young than the old subjects. It is proposed that the age difference may result from both a reduction in the amount of functioning protoplasm and an alteration in intra-cellular glucose metabolism.

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