Studies of Incorporation of Radioactivity into Lipids by Human Blood

II. Pattern of Incorporation of Radioactivity into Fatty Acids by Platelets from Normal Subjects and Patients in Diabetic Acidosis

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SUMMARY

Platelets from diabetics in acidosis incorporate significantly less C-14 into fatty acids than do platelets from control subjects. This decrease in incorporation of C-14 into fatty acids is not uniform. Incorporation of C-14 into myristic and palmitic acids, fatty acids presumably made by de novo synthesis, is suppressed to a much greater extent than is incorporation into other fatty acids.

Since some of these abnormalities of incorporation of C-14 by platelets from comatose diabetics could represent isotope dilution effects, we have been particularly interested in the analysis of these data in terms of percentage of total fatty acid C-14 incorporated into the various groups of fatty acids. Platelets from diabetics in acidosis have a pattern of incorporation of C-14 into platelet fatty acids which is much different from that of normal platelets. There is a great decrease in percentage of C-14 in myristic and palmitic acid with a relative increase in percentage of C-14 in fatty acids with retention time corresponding to arachidic acid (20:0) or greater. A significant increase in relative percentage of C-14 in fatty acids containing ten carbons or less is also seen. These findings presumably mean that the cytoplasmic or malonyl CoA pathway, which is involved primarily in synthesis of palmitic and myristic acids, is suppressed greatly in diabetic acidosis, while the mitochondrial or chain-lengthening pathway is suppressed to a much smaller degree, or is actually stimulated. It is, therefore, important to consider qualitative as well as quantitative alterations in fatty acid synthesis in diabetes.

Formation of lipids by human blood was studied extensively by James, Lovelock, and Webb. These authors concluded that blood was capable of incorporating C-14 from C-14 acetate into a wide variety of fatty acids.[1] In 1960, Marks, Gellhorn, and Kidson demonstrated that leukocyte lipid formation contributed most of the radioactivity to fatty acids following incubation of blood with C-14 acetate. However, they also showed that platelets were capable of significant incorporation of C-14 into lipids.[2]

We have been particularly interested in the use of human blood cells as a tool for studying alterations in lipid metabolism in diabetes, both because blood cells are the only human cells readily available for study of lipid formation and because we are intrigued with the possibility that abnormal factors in plasma or cells may contribute to lipid abnormalities in diabetes.

In a previous study, we reported that whole blood from diabetics in coma incorporated significantly less radioactivity into fatty acids than did blood from controls, a finding similar to that found in liver or adipose tissue of alloxan diabetic animals. Of greater interest to us, however, was a striking shift in pattern of incorporation of C-14 into discrete fatty acids. Blood from subjects in diabetic acidosis showed a highly significant decrease in percentage of total C-14 in myristic and palmitic acids, with an equally marked increase in percentage of fatty acid C-14 in stearic and oleic acids.[3]

Because of the possible role of platelets and platelet lipids in thrombosis and atherosclerosis, we have been particularly interested in formation of lipids by platelets from diabetics.

The present study demonstrates that platelets from diabetics in coma show a strikingly different pattern of incorporation of C-14 into fatty acids when compared to controls. This pattern, however, differs in some aspects from that previously described for whole blood.

PROCEDURE

Five milliliters of platelet-rich plasma containing 0-200 leukocytes per cubic millimeter was prepared by centrifugation of heparinized whole blood. Siliconized glassware was used throughout the procedure. Platelet

Presented at the Twenty-third Annual Meeting of the American Diabetes Association in Atlantic City on June 16, 1963.

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counts were done by the method of Brecher and Cronkite.[4] The platelet-rich plasma was incubated with five microcuries of 1-C-14 acetate for four hours at 37° C. under 95 per cent oxygen and 5 per cent CO_2. At the end of the four-hour incubation period, total lipids were extracted by the method of Folch et al.[5] except that the crude lipid extracts were washed with an equal volume of 0.73 per cent saline solution to remove any traces of unutilized acetate. The lipids were saponified and extracted according to the method of Bjorntorp.[6] The fatty acids were methylated using the method of Metcalf and Schmitz.[7] The methyl esters were then separated by gas liquid chromatography using a twenty-foot 20 per cent Diethylene glycol succinate column. The fatty acid methyl esters in each peak and any material between the recognizable peaks were trapped by inserting melting point tubes containing a defatted cotton plug and silicone oil into the column exit port. The radioactive fatty acids were washed into counting vials and radioactivity was determined in a liquid scintillation spectrometer. Radioactive peaks have been tentatively identified by their retention time relative to palmitic acid and by use of known standard fatty acids.

RESULTS

Platelets from five comatose diabetic subjects showed a significant decrease in incorporation of C-14 into saponifiable lipids as compared to control subjects. Platelets from comatose diabetics incorporated 10,093 ± 7,800 dpm./10^9 platelets, while platelets from controls incorporated 32,306 ± 12,248 dpm./10^9 platelets (table 1).

To simplify analysis, we have analyzed the data in terms of certain major fatty acids, or groups of fatty acids, which account for approximately 95 per cent of total fatty acid radioactivity. It should be borne in mind that these different fatty acids presumably have different sites of origin within the cell if fatty acid synthesis in the platelet is like that in the liver. Myristic and palmitic acids (14:0 and 16:0) are thought to be made primarily in the cytoplasmic or malonyl CoA pathway by de novo synthesis from acetyl CoA and malonyl CoA. Stearic and oleic (18:0 and 18:1), as well as arachidic (20:0) and other 20 carbon unsaturated fatty acids, are thought to be made by the mitochondrial or chain-lengthening pathway.[8] The exact origin of 22 carbon fatty acids and fatty acids with longer retention time is not known with certainty. Both de novo synthesis and chain lengthening may be involved.[9] Fatty acids with retention time corresponding to behenic acid (22:0) or greater could not be separated from each other completely because of overlapping of the radioactive peaks; nor could arachidonic acid (20:4) always be separated with certainty from 22:0, and is therefore included with this group of fatty acids, when complete separation was not possible. It can be seen from table 1 that suppression of incorporation of radioactivity is most striking in myristic and palmitic acids, controls incorporating 15,098 ± 5,392 dpm./10^9 platelets as compared to 1,916 ± 1,688 in platelets from comatose diabetics ($p < .001$). Less striking, but significant, decreases in absolute radioactivity are seen in fatty acids with retention time corresponding to palmitoleic (16:1), stearic (18:0), and oleic (18:1). There are suggestive, but not significant, decreases in radioactivity in fatty acids with retention time corresponding to arachidic acid (20:0) or greater. On the other hand, a significant increase in the small amount of radioactivity present in fatty acids with retention time less than capric (10:0) was found in fatty acids from platelets of comatose diabetic patients.

Thus, actual incorporation of C-14 into fatty acids is decreased to by far the greatest extent in myristic and palmitic acids (14:0 and 16:0), fatty acids presumably made by de novo synthesis.

Since some of these effects on incorporation of C-14 into fatty acids could be due to isotope dilution with acetate, ketones, or fatty acids in plasma and cells of the comatose diabetics, we have been primarily interested in analysis of these data in terms of percentage of recovered fatty acid C-14 in the various groups of fatty acids. When the data are examined in this way, controls are found to have a very consistent pattern of incorporation of C-14 into fatty acids (table 2). Distribution of radioactivity in platelet fatty acids from diabetics in acidosis differs significantly from this pattern in showing a striking decrease in percentage of total counts in myristic and palmitic acids, with an almost equally striking increase in percentage of fatty acid C-14 in fatty acids of chain length corresponding to arachidic acid (20:0) or greater. In addition, a highly significant increase in percentage of total radioactivity is seen in fatty acids of chain length corresponding to capric acid (10:0) (table 2). Significant increases are also seen in the percentage of radioactivity recovered in fatty acids with retention times less than that of capric acid (10:0) and with retention time corresponding to linoleic acid (18:2).

The degree of abnormality corresponds reasonably well with the severity of acidosis as judged by plasma CO_2 in mEq. (table 2).

Since two obvious abnormalities which occur in dia-

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TABLE 1

Incorporation of C-14 from 1-C-14 acetate into fatty acids by platelets from controls and comatose diabetics (DPM per 10^9 platelets)

Subjects: Condition	Case	Platelets (10^3 per cmm.)	DPM per 5 ml. platelets in plasma	DPM per 10^9 platelets (total)	<10:0	10:0	12:0	14:0	16:0	16:1
Controls	KA	336.0	26,000	15,476	28	42	408	3,106	5,039	54
	RM	247.5	41,337	33,404	17	49	856	6,250	9,213	214
	FF	228.7	57,728	50,483	0	282	1,752	7,971	12,949	146
	JJ	243.0	18,023	14,834	0	34	360	2,572	3,608	45
	EL	395.0	78,886	39,929	8	152	1,254	8,483	12,091	156
	KH	665.0	135,408	40,724	0	53	994	7,143	8,857	200
	WC	475.0	74,320	31,293	0	72	836	8,238	10,172	422
	Mean	370.0	61,672	32,306	8	98	922	6,252	8,847	177
	S.D.			±12,248	±10	±84	±446	±2,263	±3,200	±117
Comatose diabetics	JK	485.0	5,907	2,436	32	28	32	61	33	62
	WZ	200.0	11,944	11,944	27	98	436	1,340	1,390	36
	DB	278.0	33,669	24,222	27	337	1,284	2,631	2,100	63
	AW	490.0	9,548	3,897	14	31	84	190	212	16
	VH	380.0	15,132	7,964	45	100	194	927	698	55
	Mean	366.6	15,240	10,093	29	119	406	1,030	887	46
	S.D.			±7,800	±10	±113	±460	±929	±767	±18
	P Value			.005 —.01	.01 —.005	NS*	.10 —.20	.001 —.005	<.001	.025 —.05

*NS—Not significant
†ND—Not determined separately
‡>22:0 fatty acids with retention time greater than that of behenic acid (22:0).
§>20:0 fatty acids with retention time greater than that of arachidic acid (20:0).

TABLE 2

Percentage of total fatty acid C-14 incorporated into various fatty acids by platelets from control and comatose diabetic subjects

Subjects: Condition	Case	Sex	Age	Plasma glucose (mg./d.)	CO_2 content (mEq./L.)	<10:0	10:0	12:0	14:0	16:0	16:1	14:0 +16:0	18:0
Controls	KA	M	32	97	Not determined	0.18	0.27	2.64	20.07	32.56	0.35	52.63	10.69
	RM	M	31	83		0.05	0.15	2.56	18.71	27.58	0.64	46.29	7.52
	FF	M	41	84		0	0.56	3.47	15.79	25.65	0.29	41.44	10.29
	JJ	M	20	86		0	0.23	2.42	17.33	24.31	0.30	41.64	7.43
	EL	M	25	104		0.02	0.38	3.14	21.23	30.28	0.39	51.51	4.48
	KH	M	26	88		0	0.13	2.44	17.54	21.75	0.49	39.29	8.35
	WC	M	24	67		0	0.23	2.67	26.32	32.50	1.35	58.82	4.42
	Mean					0.04	0.28	2.76	19.57	27.80	0.54	47.39	7.60
	S.D.					±0.06	±0.14	±0.36	±3.20	±3.86	±0.11	±6.66	±2.30
Comatose diabetics	JK	M	62	1,125	2.7	1.30	1.13	1.29	2.49	1.37	2.53	3.86	8.30
	WZ	F	75	1,125	5.8	0.23	0.82	3.65	11.22	11.64	0.30	22.86	7.30
	DB	M	35	875	7.1	0.11	1.39	5.30	10.86	8.67	0.26	19.53	6.15
	AW	F	50	720	3.6	0.36	0.79	2.16	4.88	5.43	0.41	10.31	7.13
	VH	F	46	1,000	5.8	0.57	1.26	2.44	11.64	8.77	0.69	20.41	8.44
	Mean					0.51	1.08	2.97	8.22	7.18	0.84	15.39	7.46
	S.D.					±0.42	±0.24	±1.39	±3.78	±3.60	±0.86	±7.16	±0.84
	P Value					.02	<.001	NS*	<.001	<.001	NS	<.001	NS

*NS—Not significant
†ND—Not determined separately

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TABLE 1 (*continued*)

Incorporation of C-14 from 1-C-14 acetate into fatty acids by platelets from controls and comatose diabetics (DPM per 10^9 platelets)

Subjects Condition	Case	14:0 +16:0	18:0	18:1	18:2	18:0 +18:1	20:0 to 20:3	20:4	20:4 +22:0 +>22:0‡	20:0 +>20:0§
Controls	KA	8,148	1,654	145	28	1,800	3,016	ND†	1,746	4,764
	RM	15,460	2,512	528	284	3,040	5,214	ND	7,379	12,600
	FF	20,918	5,195	581	273	5,774	11,525	ND	7,956	19,480
	JJ	6,180	1,102	156	111	1,258	3,114	ND	3,357	6,471
	EL	20,574	1,789	255	196	2,044	5,981	124	8,768	14,750
	KH	16,000	3,400	407	265	3,808	8,898	204	9,521	18,418
	WC	18,410	1,383	426	100	1,810	4,018	ND	4,359	8,380
	Mean	15,098	2,434	357	180	2,790	5,967	164	6,155	12,123
	S.D.	±5,392	±1,314	±161	±93	±1,478	±2,944	±40	±2,762	±5,362
Comatose diabetics	JK	94	202	79	41	281	632	ND	985	1,618
	WZ	2,730	872	204	125	1,076	2,860	ND	4,324	7,182
	DB	4,730	1,490	203	165	1,692	7,446	ND	7,720	15,164
	AW	402	278	78	48	356	1,842	ND	1,012	2,856
	VH	1,624	672	115	70	786	2,628	57	2,254	4,880
	Mean	1,916	703	136	90	838	3,082	57	3,259	6,340
	S.D.	±1,688	±1,039	±127	±48	±516	±2,317		±2,558	±4,798
	P	<.001	.025	.02	.10	.025	.10		.05	.10
	Value		—.05	—.025	—.20	—.05	—.20		—.10	—.20

*NS—Not significant
†ND—Not determined separately
‡>22:0 fatty acids with retention time greater than that of behenic acid (22:0).
§>20:0 fatty acids with retention time greater than that of arachidic acid (20:0).

TABLE 2 (*continued*)

Percentage of total fatty acid C-14 incorporated into various fatty acids by platelets from control and comatose diabetic subjects

Subjects Condition	Case	Sex	Age	Plasma glucose (mg./d.)	CO_2 content (mEq./L.)	18:1	18:2	18:0 +18:1	20:0 to 20:3	20:4	20:4 +22:0 +>22:0	20:0 +>20:0
Controls	KA	M	32	97	Not determined	0.94	0.18	11.63	19.49	ND†	11.28	30.77
	RM	M	31	83		1.58	0.85	9.10	15.61	ND	22.09	37.70
	FF	M	41	84		1.15	0.54	11.44	22.83	ND	15.76	38.59
	JJ	M	20	86		1.05	0.75	8.48	20.99	ND	22.63	43.62
	EL	M	25	104		0.64	0.49	5.12	14.98	0.31	21.96	36.94
	KH	M	26	88		1.00	0.65	9.35	21.85	0.50	23.38	45.23
	WC	M	24	67		1.36	0.32	5.78	12.84	ND	13.93	26.77
	Mean					1.10	0.54	8.70	18.37	0.41	18.72	37.08
	S.D.					±0.28	±0.28	±2.33	±3.58	±0.10	±4.57	±4.63
Comatose diabetics	JK	M	62	1,125	2.7	3.25	1.68	11.55	25.95	ND	40.40	66.35
	WZ	F	75	1,125	5.8	1.71	1.05	9.01	23.95	ND	36.20	60.15
	DB	M	35	875	7.1	0.84	0.68	6.99	30.74	ND	31.87	62.61
	AW	F	50	720	3.6	2.00	1.22	9.13	47.26	ND	25.97	73.23
	VH	F	46	1,000	5.8	1.44	0.88	9.88	33.00	0.71	28.30	61.30
	Mean					1.85	1.10	9.31	32.18	0.71	32.55	64.73
	S.D.					±0.80	±0.34	±1.47	±8.21		±5.23	±4.74
	P					.05	.005	NS	.001		.001	<.001
	Value					—.10	—.01		—.005		—.005	

*NS—Not significant
†ND—Not determined separately

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betic acidosis are ketosis and an increase of hydrogen ion concentration, we have investigated the effects of ketones and *p*H on lipid formation by blood. These studies have shown that a number of compounds containing a carbonyl group inhibit formation of fatty acids and can reproduce a pattern similar to that seen in diabetic acidosis.[11] Table 3 shows the effect of several ketones and beta-hydroxybutyric acid on formation of lipids by platelets. Ethyl acetoacetate and alpha-ketobutyric acid at a concentration equivalent to 150 mg. per 100 ml. of acetoacetate produce a definite decrease in incorporation of C-14 into saponifiable lipid, with a disproportionately great decrease in incorporation of C-14 into myristic and palmitic acids. Acetone caused a less noticeable decrease in incorporation of C-14 into 14:0 and 16:0. On the other hand, beta-hydroxybutyric acid at the same concentration produced no change in pattern of formation of fatty acids, although there was a slight decrease in incorporation of C-14 into saponifiable lipid. This latter finding could be due simply to isotope dilution.

When the *p*H of the platelet suspension was decreased to as low as 6.6 with phosphate buffer, there was a moderate decrease in percentage of fatty acid C-14 in 14:0 and 16:0. Even at *p*H 6.6, however, the changes were very slight compared to the findings in diabetic acidosis (table 4). Whole blood at a similar *p*H showed no change in fatty acid pattern.[3]

To determine if plasma factors, including ketones or increased hydrogen ion concentration were responsible for the changes in fatty acid formation seen in diabetic acidosis, platelets from three patients in acidosis were removed from plasma by centrifugation and resuspended

TABLE 3

Effect of ketone bodies* on the incorporation of C-14 into platelet fatty acids

Subject	Condition	Percentage of total CPM recovered							
		<10:0	10:0	12:0	14:0	16:0	16:1	14:0 +16:0	18:0
WC	Control	0	0.23	2.67	26.32	32.50	1.35	58.82	4.42
	+ ethylacetoacetate	0.19	0.07	4.21	20.78	25.82	0.36	46.60	10.78
	+ beta-hydroxybutyric acid	0.12	0.20	1.65	16.32	40.93	0.60	57.25	6.22
	+ acetone	0.01	0	1.96	18.80	30.56	1.11	49.36	6.17
	+ alpha-ketobutyric acid	0	0.06	4.95	17.67	23.30	1.06	40.97	5.76

*Each compound was added at a concentration of 73.5 μM per 5 ml. plasma.

TABLE 4

Effect of decreasing *p*H of plasma on the incorporation of C-14 into platelet fatty acids

Subject	Condition	Percentage of total CPM recovered							
		<10:0	10:0	12:0	14:0	16:0	16:1	14:0 +16:0	18:0
WC	Control	0	0.23	2.67	26.32	32.50	1.35	58.82	4.42
	*p*H 7.4	0.02	0.08	3.32	26.19	33.00	1.46	59.19	5.10
	*p*H 7.0	0.16	0.35	4.29	20.33	22.69	1.30	43.02	5.47
	*p*H 6.6	0	0.01	3.20	18.58	22.66	1.15	41.24	5.91

TABLE 5

Effect of suspending platelets in Tyrode's solution

Subjects Case	Sex	Age	Plasma glucose (mg./dl.)	CO_2 content (mEq./L.)	Media of platelets	Percentage of total fatty acid C-14 incorporated into various fatty acids by platelets						
						<10:0	10:0	12:0	14:0	16:0	16:1	14:0 +16:0
VH	F	46	1,000	5.8	Own plasma	0.57	1.26	2.44	11.64	8.77	0.69	20.41
					Tyrode's solution	0.26	0.17	0.40	10.21	13.24	0.26	23.45
EC	M	54	1,240	5.8	Own plasma	Not determined						
					Tyrode's solution	1.69	0.37	0.81	2.93	10.33	0.07	13.26
BA	F	17	690	6.9	Own plasma	0	0.20	2.96	10.75	12.68	0.15	23.43
					Tyrode's solution	0.02	0.02	1.66	14.24	18.43	0.25	32.67

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in Tyrode's solution containing 100 mg. per 100 ml. glucose at *p*H 7.4-7.2. This allowed study of platelet lipid formation in the absence of any exogenous insulin. Removal of these cells from plasma and resuspension in Tyrode's solution was not associated with return of pattern to normal in two experiments. However, these findings could be due to prolonged exposure of the cells to plasma factors or retention of plasma factors by the cells. On the other hand, the pattern of incorporation of C-14 into fatty acids by platelets from one subject (BA) returned almost to normal when they were incubated in Tyrode's solution, suggesting that plasma factors were of great importance in this instance (table 5).

DISCUSSION

No work has been reported on the enzymatic pathways involved in fatty acid formation by the platelet. However, if fatty acid formation by the platelet is like that in the mammalian liver, at least two pathways may be involved. According to Wakil and co-workers, palmitic and myristic acids are made in the cytoplasmic or malonyl CoA pathway by de novo synthesis from malonyl CoA and acetyl CoA.[8] Eighteen, and perhaps twenty, carbon fatty acids are made primarily by the mitochondrial or chain-lengthening pathway.[8] In the chain-lengthening pathway, preformed 16 or 18 carbon fatty acids are converted to 18 or 20 carbon fatty acids by addition of acetyl CoA. The energy requirements and requirement for hydrogen as TPNH are approximately seven times greater per mole of fatty acid formed via the malonyl CoA pathway, as they are in the chain-lengthening pathway. Further, the distribution of radioactivity within the fatty acid after incubation with C-14 acetate is much

TABLE 3 (*continued*)

Effect of ketone bodies* on the incorporation of C-14 into platelet fatty acids

Subject	Condition	Percentage of total CPM recovered 18:1	18:2	18:0 +18:1	20:0 to 20:3	20:4+22:0 +>22:0	20:0 +>20:0	DPM per 10^9 platelets
WC	Control	1.36	0.32	5.78	12.84	13.93	26.77	31,293
	+ ethylacetoacetate	1.21	0.62	11.99	7.49	26.84	34.33	12,043
	+ beta-hydroxybutyric acid	1.35	0.39	7.57	16.18	13.11	29.29	24,554
	+ acetone	0.92	0.36	7.09	17.36	20.14	37.50	29,662
	+ alpha-ketobutyric acid	1.18	0.45	6.94	20.42	22.26	42.68	18,800

*Each compound was added at a concentration of 73.5 μM per 5 ml. plasma.

TABLE 4 (*continued*)

Effect of decreasing *p*H of plasma on the incorporation of C-14 into platelet fatty acids

Subject	Condition	Percentage of total CPM recovered 18:1	18:2	18:0 +18:1	20:0 to 20:3	20:4+22:0 +>22:0	20:0 +>20:0	DPM per 10^9 platelets
WC	Control	1.36	0.32	5.78	12.84	13.93	26.77	31,293
	*p*H 7.4	1.39	0.50	6.49	7.83	16.21	24.04	20,504
	*p*H 7.0	1.37	0.47	6.84	14.10	24.40	38.50	30,745
	*p*H 6.6	2.34	0.21	8.25	16.71	21.79	38.50	31,209

TABLE 5 (*continued*)

Effect of suspending platelets in Tyrode's solution

Subjects Case	Sex	Age	Plasma glucose (mg./dl.)	CO_2 content (mEq./L.)	Media of platelets	Percentage of total fatty acid C-14 incorporated into various fatty acids by platelets 18:0	18:1	18:2	18:0 +18:1	20:0 to 20:3	20:4+22:0 +>22:0	20:0 +>20:0
VH	F	46	1,000	5.8	Own plasma	8.44	1.44	0.88	9.88	33.00	28.30	61.30
					Tyrode's solution	9.45	1.40	0.78	10.85	32.29	29.15	61.44
EC	M	54	1,240	5.8	Own plasma	Not determined						
					Tyrode's solution	8.17	2.49	0.07	10.66	28.82	43.72	72.54
BA	F	17	690	6.9	Own plasma	8.76	1.51	0.78	10.27	34.61	26.63	61.24
					Tyrode's solution	10.34	1.23	0.65	11.57	27.65	23.83	51.48

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different when fatty acids are formed by these two pathways. In the malonyl CoA pathway, each alternate carbon should be labeled, resulting in a fatty acid with approximately one eighth of the total radioactivity in the carboxyl carbon of palmitate after incubation with 1-C-14 acetate. Similarly, approximately one seventh of the total fatty acid radioactivity should be present in the carboxyl carbon of myristic acid (14:0) after incubation with 1-C-14 acetate. In the case of 18 and 20 carbon fatty acids, most of the radioactivity should be present in the carboxyl carbon, provided little mixing of radioactive 16 carbon fatty acids occurs during the time of the experiment. Degradation studies of fatty acids formed by normal platelets are at present under way in our laboratory, and data are quite compatible with the existence of both of these pathways in the normal platelet. Approximately one eighth of the total radioactivity of palmitic acid, and slightly more than one seventh of the total radioactivity in myristic acid, is present in CO_2 liberated from the carboxyl carbon. In contrast, 18 and 20 carbon fatty acids contain 90 per cent or more of their total radioactivity in the carboxyl carbon; capric (10:0) and lauric (12:0) acids also contain approximately 90 per cent of their total radioactivity in the carboxyl carbon, suggesting that these, too, are primarily made by chain lengthening.[10]

It appears likely from these findings that the malonyl CoA pathway is suppressed to a much greater degree in the platelet in diabetic acidosis than in the mitochondrial or chain-lengthening pathway. This results in an apparent qualitative difference in type of fatty acids synthesized by the platelet. It is of interest that whole blood from subjects in diabetic acidosis shows a decrease in percentage of C-14 in myristic and palmitic acids, but an increase in percentage of C-14 in 18 carbon fatty acids, rather than an increase in percentage of C-14 in 20 carbon fatty acids.[3] The findings in whole blood presumably reflect primarily leukocyte lipid synthesis, rather than platelet lipid synthesis, since leukocytes appear to be responsible for approximately 70 per cent of the C-14 incorporated into fatty acids by whole blood.[2]

Neither whole blood nor platelets respond to insulin in vitro with an increase in incorporation of C-14 into fatty acids. Recovery from diabetic acidosis is associated with return of pattern of whole blood radioactivity to normal.[3] However, the pattern of platelet fatty acid radioactivity returns toward, but not completely to, normal following recovery from acidosis (figure 1). It appears then that cells which are themselves not sensitive to insulin, nevertheless, exhibit abnormalities in metabolism when diabetes is poorly controlled. We have been unable to reproduce these changes by lowering *p*H of normal whole blood to as low as 6.6. Decreasing *p*H of normal platelet suspensions has produced changes qualitatively similar to but much less marked than those seen in diabetic acidosis. Compounds containing a carbonyl group, including alpha ketobutyric acid, lithium acetoacetate, ethyl acetoacetate and even ascorbic acid, when incubated with normal whole blood or platelets have rather consistently produced changes like those seen in diabetic acidosis. However, beta-hydroxybutyric acid at the same concentration has been completely ineffective.[11] It is possible that these compounds utilize available hydrogen in their reduction, thus making hydrogen unavailable for fatty acid synthesis. It is of interest that the pattern of incorporation of C-14 into fatty acids by platelets from one acidotic diabetic returned almost to normal when the platelets were removed from plasma and resuspended in Tyrode's solution. This finding clearly shows that factors other than insulin deficiency were largely responsible for the abnormalities in this instance. It is unlikely that hyperketonemia can be the sole cause for this abnormality since similar, but less striking, changes can be consistently demonstrated in platelets and at times whole blood from apparently well controlled, nonketotic, insulin-dependent diabetics.[12] Thus, abnormal plasma or cellular factors may be in-

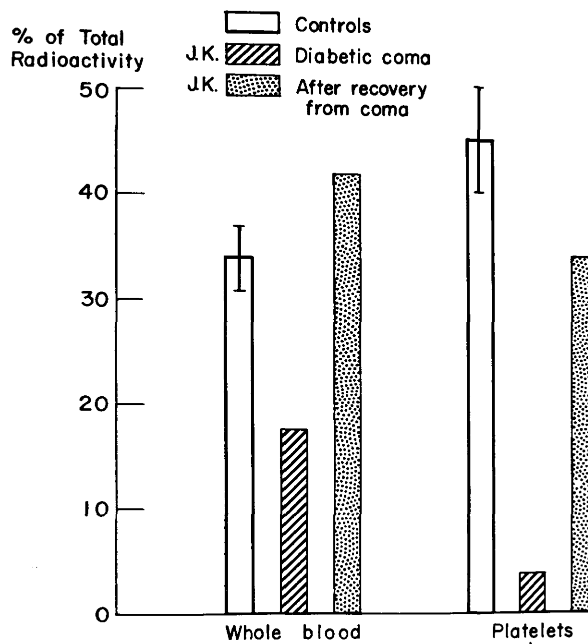

FIGURE 1

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volved in addition to ketones and perhaps *p*H changes.

These observations suggest that lipid abnormalities in diabetes can not be understood solely in terms of an increase or decrease in formation of lipids. The type of fatty acid formed, the enzymatic site at which it is formed, and its function within the cell may all be of importance. Nor can the changes in lipid metabolism in diabetes be thought of as being due to insulin deficiency alone, for cells which do not appear to respond to insulin directly can, nevertheless, show changes in lipid metabolism. Further, changes like those seen in diabetic acidosis can be partially reproduced by addition of certain ketones to plasma. And, in one experiment, the abnormality in fatty acid formation was nearly reversed by simply changing the incubation media, without addition of insulin.

Blood platelets may prove to be a valuable human cell for studying lipid abnormalities in diabetes. They appear to be a particularly important cell to study because of their role in thrombosis and perhaps in atherosclerosis as well.

SUMMARIO IN INTERLINGUA

Studios Concernente le Incorporation de Radioactivitate ad in Lipidos per Sanguines Human.

II. Le Incorporation de Radioactivitate ad in Acidos Grasse per Plachettas ab Subjectos Normal e ab Patientes in Acidosis Diabetic

Plachettas ab diabeticos in acidosis incorpora significativemente minus C-14 ad in acidos grasse que plachettas ab normal subjectos de controlo. Iste declino del incorporation de C-14 ad in acidos grasse non es uniforme. Le incorporation de C-14 ad in acidos myristic e palmitic—le quales es acidos grasse presumitemente fabricate per un synthese de novo—es supprimite a un multo plus alte grado que le incorporation ad in altere acidos grasse.

Viste que certes de iste anormalitates in le incorporation de C-14 per le plachettas de diabeticos comatose poterea representar effectos del dilution del isotopo, nos ha concentrate nostre attention in le analyse de iste datos relative al procentage del total C-14 incorporate in le varie gruppos de acidos grasse. Plachettas ab diabeticos in acidosis ha un distribution de incorporation de C-14 ad in acidos grasse multo differente ab illo trovate in plachettas normal. In le plachettas ab diabetic in acidosis il existe un forte declino in le procentage de C-14 in acido myristic e in acido palmitic, con un relative augmento del procentage de C-14 in acidos grasse le quales ha tempores de retention equal o superior a illo de acido arachidic. Es etiam a notar un augmento significative del procentage relative de C-14 in acidos grasse que contine dece carbones o minus. Iste constatationes pare significar que le circuito cytoplasmic, o le circuito de co-enzyma A, le qual es interessate primarimente in le synthese de acido palmitic e myristic, es extensemente suprimite in acidosis diabetic, durante que le circuito mitochondrian, o le circuito de allongamento de catenas, es supprimite multo minus marcatemente o es, de facto, stimulate. Il es importante, considerar qualitative tanto ben como quantitative alterationes del synthese de acidos grasse in diabete.

ACKNOWLEDGMENT

Dr. Awai is Diabetes Trainee, U.S. Public Health Service (T1 AM 5024-08 and 08S1).

The authors gratefully acknowledge the excellent technical assistance of Mr. Kent Hammarstrand and Mr. John Juntunen.

This investigation was supported by grants from National Institutes of Health (AM 05891-03, T1 AM 5024-08 and 08S1, and AM 07089-01), and Michigan Heart Association (306-0974).

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