Diabetologia 4, 188–194 (1968)

The Metabolism of [1-^{14}C]-, [2-^{14}C]-, [3,4-^{14}C]-, and [6-^{14}C]- Glucose in Normal and Diabetic Polymorphonuclear Leukocytes and during Phagocytosis

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Received: November 16, 1967

Summary. 1. Polymorphonuclear leucocytes from normal and diabetic subjects and from normal and alloxan diabetic rats were incubated with ^{14}C-glucose, and allowed to phagocytize. — 2. The major $^{14}CO_2$ production originated from the pentose cycle. Approx. one third of $^{14}CO_2$ was formed by decarboxylation of pyruvate, whereas Krebs cycle activity was minimal. — 3. The pentose cycle metabolized 0.1—0.5% of the phosphorylated glucose, and phagocytosis increased this fraction 2—3 fold. No difference was found between normal and diabetic cells. — 4. The major endproduct of glucose metabolism was lactic acid. The randomization of ^{14}C of glucose was not consistent with the sole operation of the oxidative or non-oxidative parts of the pentose cycle, but indicated the participation of a symmetrical C_3 intermediate, i.e. dihydroxy-acetone, in the metabolism of part of the glucose carbons. — 5. Very small amounts of ^{14}C from glucose were found in aminoacids, metabolic acids not extractable with ether, and lipids. Appreciable amounts of ^{14}C were, however, found in a compound (A), part of which precipitated as glyceroltribenzoate, and a compound (B), which presumably was of protein nature. The incorporation of ^{14}C in compound A from normal leucocytes decreased during phagocytosis, and was insignificant in human diabetic cells. The incorporation of ^{14}C from [1-^{14}C]- and [2-^{14}C]-, but not from [6-^{14}C]-glucose, into compound B was greatly stimulated in normal cells during phagocytosis, but not so in diabetic leucocytes.

Le métabolisme du glucose [1-^{14}C]-, [2-^{14}C]- [3, 4-^{14}C]- et [6-^{14}C]- dans des leucocytes normaux et diabétiques polynucléaires et au cours de la phagocytose

Résumé. 1. Des leucocytes polynucléaires de sujets normaux et diabétiques et de rats normaux et rendus diabétiques par l'alloxane ont été incubés avec du ^{14}C-glucose et mis à phagocyter. — 2. La majeure production de $^{14}CO_2$ venait du cycle des pentoses. Approximativement un tiers du $^{14}CO_2$ était formé par la décarboxylation du pyruvate, tandis que l'activité du cycle de Krebs était minime. — 3. Le cycle des pentoses métabolisait 0.1—0.5% du glucose phosphorylé, et la phagocytose augmentait 2—3 fois cette fraction. On n'a trouvé aucune différence entre les cellules normales et les cellules de diabétiques. — 4. Le produit final principal du métabolisme du glucose était l'acide lactique. La dispersion du ^{14}C du glucose n'était pas compatible avec le seul fonctionnement des parties oxydatives et non-oxydatives du cycle des pentoses, mais indiquait la participation d'un intermédiaire symétrique en C_3, c-à-d la dihydroxyacétone, dans le métabolisme d'une partie des carbones du glucose. — 5. De très petites quantités de ^{14}C du glucose ont été trouvées dans les acides aminés, les acides métaboliques non-extractibles par l'éther et dans les lipides. Cependant on a trouvé des quantités appréciables de ^{14}C dans un composé (A) dont une partie précipitait comme tribenzoate de glycérol, et un composé (B) qui était probablement de nature protéique. L'incorporation de ^{14}C dans le composé A de leucocytes normaux diminuait au cours de la phatocytose et était insignifiante dans les cellules de diabétiques humains. — L'incorporation de ^{14}C du glucose [1-^{14}C]- et [2-^{14}C]-, mais non du glucose [6-^{14}C] dans le composé B était fortement stimulée dans les cellules normales au cours de la phagocytose, mais pas autant dans les leucocytes de diabétiques.

Der Stoffwechsel von [1-^{14}C]-, [2-^{14}C]-, [3-^{14}C]- und [6-^{14}C]-Glucose in normalen und diabetischen polymorphkernigen Leukozyten und während der Phagozytose

Zusammenfassung. 1. Polymorphkernige Leukozyten von Normalpersonen und Diabetikern und von normalen und alloxandiabetischen Ratten wurden mit ^{14}C-Glucose inkubiert und zur Phagozytose angeregt. — 2. Der überwiegende Teil der $^{14}CO_2$ Produktion stammte aus dem Pentosezyklus. Etwa ein Drittel des $^{14}CO_2$ wurde bei der Decarboxylierung von Pyruvat gebildet, während die Aktivität des Krebs-Zyklus nur sehr gering war. — 3. Den Pentose-Phosphat-Zyklus durchliefen 0.1 bis 0.5% der phosphorylierten Glucose. Phagozytose erhöhte diesen Anteil auf das 2- bis 3-fache. Zwischen normalen und diabetischen Zellen fanden sich dabei keine Unterschiede. — 4. Milchsäure stellte das Hauptendprodukt des Glucosestoffwechsels dar. Die Verteilung des ^{14}C aus der Glucose spiegelte nicht nur die Einwirkungen des oxydativen und des nicht-oxydativen Anteils des Pentose-Phosphat-Zyklus wider, sondern deutete auch auf die Beteiligung eines symmetrischen C_3 Zwischenproduktes, nämlich Dihydroxyaceton, beim Stoffwechsel eines Teils des Glucose-Kohlenstoffs hin. — 5. Nur sehr kleine Mengen des ^{14}C aus Glucose fanden sich in Aminosäuren, nicht mit Äther extrahierbaren Stoffwechsel-Säuren und Lipiden. Hingegen ließen sich beträchtliche Anteile des ^{14}C in einem Stoffgemisch (A) nachweisen, von dem ein Teil als Glycerin-Tribenzoat präzipitierte, und in einem Gemisch (B), das wahrscheinlich Eiweißcharakter besitzt. Die Einlagerung von ^{14}C in das Gemisch A durch normale Leukozyten nahm während der Phagozytose ab und war unbedeutend bei diabetischen Zellen. Der Einbau von ^{14}C aus [1-^{14}C]- und [2-^{14}C]-, nicht aber aus [6-^{14}C]-Glucose in das Gemisch B war bei normalen Zellen während der Phagozytose erheblich gesteigert, jedoch nicht bei diabetischen Zellen.

Key-words: Polymorphonuclear leucocytes, glucose metabolism, diabetes, phagocytosis, pentose cycle, Embden Meyerhof pathway, Krebs cycle activity, dihydroxyacetone, glycerol.

Previous experiments with polymorphonuclear (PMN) leucocytes from human peripheral blood and rabbit peritoneal exudates have shown, that the aerobic catabolism of different hexoses occurs mainly by the Embden-Meyerhof(EM)[1] pathway, whereas a lesser amount of glucose, for human cells 10—15%, is converted to glycogen by the Leloir pathway [6, 10, 24]. The EM pathway is irreversible in leucocytes due to the abscence of fructose-1,6-diphosphatase [18]. The randomization pattern observed in glycogen upon incubation of leucocytes with C_2, C_3, and C_5 compounds indicates the presence of transketolase, transaldolase, and transaldolase-exchange activity [19, 23, 25]. The extent of the pentose cycle(PC) has been intensively investigated, especially in relation to phagocytosis [1, 2, 9, 17, 21].

Earlier experiments have shown an overall decrease of glucose uptake, lactic acid production, and glycogen synthesis in human diabetic leucocytes [6], which were not correctable by insulin *in vitro*, thus excluding a defective transport mechanism for glucose in these cells [7]. It was therefore of interest to examine the extent of different pathways in glucose catabolism, especially during the strain of phagocytosis when the PC normally increases. The present communication demonstrates, that the relative contribution of the EM, PC, and Krebs cycle in diabetic leucocytes is not different from that of normal cells. As a side path it was observed, however, that diabetic leucocytes responded otherwise than normal cells with respect to the incorporation of radioactivity into two as yet unidentified compounds. The decreased glycogen synthesis in diabetic leucocytes has been dealt with in the preceding communication, where a glycogen transferase activity was described with 77% of the activity obtained in insulin-corrected diabetes [9].

Experimental

Material

Leucocytes. Male rats of the HOLZMANN strain weighing 150—200 grams were injected intraperitoneally with 12—15 ml 9% sterile casein solution. 16 h later the leucocytes were recovered from the fasted rats by washing the peritoneal cavity with a Krebs-Ringer-bicarbonate buffer containing 0.2% sodium-acetate, 1% gelatine (Merck), and 0.04 mg/ml of heparin. The exudates were collected in siliconized tubes and centrifuged at 150 × *g* for 2 min, followed by resuspension in buffer without heparin and renewed centrifugation. The final suspension was made in buffer and supplemented with glucose.

Leucocytes from human blood were isolated as previously described [5].

[1] EM designates the Embden-Meyerhof pathway, and PC the pentose cycle. C_2, C_3, or C_5 is a two-, three,- or five-carbon compound. C-1, C-2, etc. refer to the position of the carbon atom in a compound.

Diabetes was produced in rats fasted 48 h, by intraperitoneal injection of 30 mg alloxan (British Drug Houses). The rats were deprived of insulin before use, and were severely diabetic with 100—125 ml diuresis per day and ketonuria. The human diabetics were middle aged subjects with blood sugar 250—300 mg/100 ml and ketonuria.

Although enough material for several incubations was obtainable from a single human blood sample, it was necessary to pool leucocytes from 3—6 rats. The leucocyte concentration in experiments with normal cells was 10—15 × 10^6/ml, whereas the concentration chosen for experiments with diabetic cells was somewhat higher [6]. The final suspensions of leucocytes from peritoneal exudates contained 93—97% polymorphonuclear leucocytes, the remainder being lymphocytes, monocytes, and endothelial cells. The suspensions of human leucocytes contained approx. 85% PMN leucocytes.

Radioactive substrates. [1—^{14}C]-, [2—^{14}C]- and [6—^{14}C]-glucose were obtained from Volk Radiochemical Co., and [3, 4—^{14}C]-glucose from New England Nuclear Corp. The position of the label was verified by control degradations.

Methods

Incubations. The leucocytes were incubated for 30 min at 37° in 10 ml buffer (pH 7.43) in siliconized, conical flasks equipped with a centre well. Before incubation 0.167 mmoles glucose and 0.12—5 μC of labelled substrates were added. The vessels were closed with serum caps and equilibrated for 2 min with 95% O_2 + 5% CO_2. Phagocytosis was induced at the start of the incubation by injecting 0.2 ml polystyrene particles (Bacto latex 0.81) through the serum caps. Samples of leucocytes were withdrawn at 10 and 20 min for examination. At 10 min, 2—10 particles were found ingested per cell, which is less than seen in PMN leucocytes from guinea pig exudates [21]. No further phagocytosis was seen at 20 min. The incubation was terminated by injection of 1 ml 3 M HCl in methanol into the cell suspension. CO_2 was absorbed in 2 ml 3 M NaOH injected into the centre well.

Isolation and degradation of intermediates. The incubation mixture was centrifuged free of cells, and the acidified supernatant fluid subjected to 48 h continuous ether extraction after addition of 0.1 mmoles of each of the following carriers: lactic acid, sodiumacetate, sodiumpropionate, sodiumfumarate, succinic acid, methylmalonic acid, and malic acid. The ether-extractable acids were separated by chromatography on a Dowex-1 (formate) column [3]. The activity of the eluates from the columns was determined on a Packard Tri-Carb liquid scintillation counter. Insignificant amounts of activity were found in the peaks corresponding to propionic acid, fumaric acid, succinic acid, methylmalonic acid, and malic acid, and these compounds were not subjected to further isolation and degradation. The neutralized acetic and lactic acids

were purified by chromatography on acid-Celite [28], and degraded carbon by carbon [19]. The determination of radioactivity of the $^{14}CO_2$ from the incubations and degradations was carried out by gas-phase counting in the proportional range.

The neutralized, nonether-extractable fraction of the supernatant of incubation was passed through Dowex-50 (H^+), and the aminoacids recovered with 1.5 N NH_4OH. The radioactivity which was not retained on the Dowex-50 column, was next passed through a column of Duolite A-4 (OH^-), and the retained "metabolic" acids displaced by formic acid. The further identification of amino acids and metabolic acids was not attempted, as the radioactivity residing in these fractions was low.

The radioactivity incorporated into lipids was investigated in a few separate experiments, where the cells were killed with 0.2 N H_2SO_4 and extracted thrice with methanol-ethanol (3 : 1).

Results

$^{14}CO_2$-production

With normal human cells equal amounts of $^{14}CO_2$ were released from [1-^{14}C]- and [2-^{14}C]-glucose, but with other cells 5—7 times more $^{14}CO_2$ was formed from [1-^{14}C]-glucose (Table 1). The yield of $^{14}CO_2$ from [6-^{14}C]-glucose was just detectable, whereas the $^{14}CO_2$ yield from [3, 4-^{14}C]-glucose, presumably by decarboxylation of pyruvate, was approx. one third the yield of $^{14}CO_2$ from [1-^{14}C]- and [2-^{14}C]-glucose.

Table 1. *$^{14}CO_2$ production from labelled glucose in leucocytes*
Leucocytes from rat peritoneal exudates or human peripheral blood were incubated in a Krebs-Ringer-bicarbonate buffer with added sodium acetate (0.2%), gelatine (1%), and glucose (16.7 mM) under 95% O_2 + 5% CO_2 for 30 min. Phagocytosis were induced by addition of 0.2 ml polystyrene particles (latex 0.81). Labelled substrates were as indicated. The results are expressed as per cent incorporation/10^8 leucocytes of added activity (0.12—5 μC)

Exp.	Source of leucocytes	Substrate	$^{14}CO_2$ formation −latex	+latex
			%	%
1	Normal rat	[1-^{14}C]-glucose	0.045	0.065
1	,,	[2-^{14}C]-	0.009	0.008
1	,,	[6-^{14}C]-	0.001	0.001
2	,,	[3,4-^{14}C]-	0.020	0.024
3	Diabetic rat	[1-^{14}C]-	0.35	0.61
3	,,	[2-^{14}C]-	0.048	0.105
4	,,	[6-^{14}C]-	0.001	0.001
5	,,	[3,4-^{14}C]-	0.111	0.071
6	Normal human	[1-^{14}C]-	0.069	0.212
6	,,	[2-^{14}C]-	0.067	0.164
6	,,	[6-^{14}C]-	0.002	0.011
7	Diabetic human	[1-^{14}C]-	0.032	0.100
7	,,	[2-^{14}C]-	0.007	0.025
8	,,	[6-^{14}C]-	0.001	0.001

Table 2 shows the results of the calculation of the PC. In the present experiments the calculation of the PC from the specific yields of $^{14}CO_2$ according to KATZ and WOOD [16] is of special advantage, as this method is sensitive to low values of the PC. The presence of an extensive transaldolase exchange activity [25] does not invalidate the calculation unless significant amounts of C-6 of glucose are drained into pentoses, and a carbon balance leaves only a few per cent for this purpose. The extremely small production of $^{14}CO_2$ from [6-^{14}C]-glucose excludes significant Krebs cycle and uronic acid pathways. A calculation of the PC from the randomization pattern in the hexose-6-P pool (i.e. glucose of glycogen) gives unreliable results with PMN leucocytes, since the extent of the randomization is low, and the pattern is mixed with that imposed by the transaldolase-transketolase activity of the cells. It is seen from Table 2, that very small amounts of the metabolized glucose traverse the PC both in normal and diabetic tissue, and that the effect of phagocytosis is to increase the PC 2—3 fold irrespective of the presence of diabetes.

Table 2. *Pentose cycle in leucocytes*
The pentose cycle was calculated according to Katz and Wood[16] from the specific yields of $^{14}CO_2$ from [1-^{14}C]- and [6-^{14}C]-glucose and is expressed in per cent of metabolized glucose

	Pentose cycle −latex	+latex
	%	%
Normal rat	0.10	0.30
Diabetic rat	0.10	0.30
Normal human	0.55	1.25
Diabetic human	0.50	1.40

Table 3. *Distribution of ^{14}C from labelled glucose in lactate*
Lactate was isolated from incubations of polymorphonuclear leucocytes with different labelled substrates and degraded carbon by carbon. The percentage distribution of radioactivity in the different carbon atoms is given in the table, together with the percentage recovery of counts during the degradation procedure

	n	CH_3 (C-3)	-CHOH (C-2)	-COOH (C-1)	Recovery
		%	%	%	%
[1-^{14}C]-glucose	8	93	1	6	103
[2-^{14}C]-	8	4	89	7	97
[3,4-^{14}C]-	4	12	6	82	104
[6-^{14}C]-	6	99	0	1	99

Lactate production

The incorporation of ^{14}C from glucose into lactate was between 0.3% and 3.5% of the initial activity. No major difference was found between rat and human, or between normal and diabetic cells. Phagocytosis

increased lactic acid production to some extent in human leucocytes, whereas no effect was observed with rat leucocytes.

The randomization of ^{14}C of glucose in lactate is shown in Table 3. The data from experiments with the same substrate are pooled, for the randomization patterns were essentially the same irrespective of the nature of the leucocytes or the presence of phagocytosis or diabetes; i.e. the values obtained in the single experiments did not differ by more than 1% from the value stated in the table, except in the experiments with [3, 4-^{14}C]-glucose, where the percentage activity in the methyl group of lactate was 8-11-13-16% in experiments with normal and diabetic rat cells and the activity in C-1 correspondingly altered. It is observed that C-1 of glucose randomized with 6% to lactate-COOH. C-2 of glucose labelled both C-3 and C-1 of lactate, but with twice as much activity in the carboxyl group. C-3, 4 of glucose preferentially labelled the methyl group of lactate. The randomization of [6-^{14}C]-glucose to C-1 of lactate was small but consistent.

Table 4. *Distribution of ^{14}C from labelled glucose in acetate*[a]

Substrate	Source of leucocytes	n	Normal CH_3	Normal -COOH	n	Diabetic CH_3	Diabetic -COOH
			%	%		%	%
[1-^{14}C]-glucose	rat	2	99	1	2	99	1
[1-^{14}C]-	human	2	97	3	[a]	—	—
[2-^{14}C]-	rat	2	55	45	2	6	94
[2-^{14}C]-	human	2	32	68	[b]	—	—
[6-^{14}C]-	rat	[b]	—	—	2	100	0
[6-^{14}C]-	human	2	89	11	—	—	—

[a] cf. legend to Table 3.
[b] Too few counts for degradation.

activity was retained at the original position, but that acetate formed in normal leucocytes from [2-^{14}C]-glucose was heavily randomized. No effect of phagocytosis on the randomization of ^{14}C was observed, and these data have therefore been pooled.

Incorporation of activity into other compounds

It has generally been assumed, that most glucose in PMN leucocytes is metabolized to CO_2, lactic acid, and glycogen; and this was also found to be so in the present experiments, where only small amounts of radioactivity were recovered in the fractions of amino acids (0.01—0.1%), nonether-extractable acids (0.01—0.05%) and lipids (< 0.01%). It was found, however, that appreciable amounts of radioactivity resided in two, as yet not fully identified, compounds (Table 5).

Compound A was obtained from the water eluate of the Dowex-1 (formate) columns (cf. Methods). The activity found in this neutral or weakly acid compound was of the same order of magnitude as the incorporation of ^{14}C into lactate of normal rat and human leucocytes. With normal leucocytes the effect of phagocytosis was consistently to lower the incorporation of ^{14}C. With diabetic leucocytes no activity was found in compound A with human leucocytes, whereas rat leucocytes showed some activity in "A". The effect of phagocytosis in rat diabetic leucocytes was equivocal.

The nature of compound A has been the object of intense investigations by various methods including co-chromatography, and crystallization with carriers. Glucuronic, galacturonic, mucic, and gluconic acids, as well as glyceraldehyde, dihydroxyacetone, 1, 2-propandiol, propionaldehyde, and polymerization products of lactic and pyruvic acids have been ruled out. 20—30% of the radioactivity was volatile at room temperature. Part of the activity was, however, recovered after several recrystallizations with carrier as the glycerol-

Table 5. *Effect of phagocytosis on incorporation of ^{14}C from labelled glucose in compounds A and B from normal and diabetic leucocytes*

Percentage of radioactivity/10^8 leucocytes per hour recovered in two operationally defined compounds (cf. text) appearing during incubation of leucocytes (cf. legend to Table 1)

Source of leucocytes	Substrate	Compound A Normal —latex	Compound A Normal +latex	Compound A Diabetic —latex	Compound A Diabetic +latex	Compound B Normal —latex	Compound B Normal +latex	Compound B Diabetic —latex	Compound B Diabetic +latex
		%	%	%	%	%	%	%	%
Rat	[1-^{14}C]-glucose	11	6	0.7	3.4	0.05	0.79	0	0.03
—	[2-^{14}C]-	1.6	0.16	0.9	1.2	0.30	3.60	0.13	0.41
—	[6-^{14}C]-	2.2	0.17	0.7	0.4	0.07	0.03	—	—
Human	[1-^{14}C]-	2.1	1.1	0	0	0.06	0.24	0.58	1.00
—	[2-^{14}C]-	1.5	0.6	0.1	0.1	0.03	0.68	0.42	1.57
—	[6-^{14}C]-	1.6	1.0	—	—	0.11	0.10	—	—

Acetate production

The incorporation of ^{14}C from glucose into acetate was very small (0.02—0.05%), and in some of the experiments there were not sufficient activity for purification and degradation. The randomization pattern observed (Table 4) shows that in most cases the

tribenzoate.[2] It should also be mentioned, that compound A was formed, when leucocytes were incubated with labelled glycerol and glyceraldehyde [7], but not

[2] The recovery of part of the radioactivity in compound A as glyceroltribenzoate was done by Dr. STJERNHOLM.

when ^{14}C-labelled formate, [2-^{14}C]-acetate, [2-^{14}C]-propionate or [2-^{14}C]-lactate were the substrates. Whether part of compound A may be a glycerol derivative or polymer must await methods for the production of sufficient quantities for purification and chemical analysis.

The compound B was found in the nonether extractable fraction of the supernatant of incubation. The radioactivity was not retained by subsequent passage through Dowex-50 (H^+) and Duolite-A-4 (OH^-). The resulting neutral eluate containing the activity from non-metabolized glucose was subjected to prolonged dialysis against water in the cold. An appreciable amount of activity was found to reside inside the dialysis bag, and it is seen (Table 5), that this activity increased appreciably during phagocytosis, when the labelled substrate was [1-^{14}C]- and [2-^{14}C]-glucose, but not when [6-^{14}C]-glucose was used. With diabetic leucocytes the increase in activity during phagocytosis seems to be much less than with normal cells.

Compound B is further characterized by moving slower through Sephadex G-25 and -50 than proteins, and by being made dialyzable by treatment with a protease or by hydrolysis with 0.5 M sulphuric acid for 24 h at 100°. After hydrolysis most of the activity was retained on Dowex-50 (H^+), indicating the presence of aminoacids or aminosugars, but the sugar portion was, however, only weakly labelled. Some activity was found in an acetyl group.[3] Presumably compound B is a protein of relatively low molecular weight.

Discussion

Several interesting points emerged from the present study. The $^{14}CO_2$ data showed that approximately one third of the CO_2 production in leucocytes occurs by decarboxylation of pyruvate, and that the rest is formed in the oxidative part of the pentose cycle. The contribution of the Krebs cycle to CO_2 formation is insignificant, and a low Krebs cycle activity is also indicated by the observed, very low incorporation of radioactivity into Krebs cycle intermediates. These results are consistent with data obtained by SELVERAJ and SBARRA [22] with [1-^{14}C]- and [3-^{14}C]-pyruvate in guinea pig leucocytes, and are similar to results obtained with normal human lymphocytes [12].

The calculation of the PC showed that a very small amount, 0.1—0.5%, of glucose is metabolized by this route, but that phagocytosis increases this fraction 2—3 fold. No difference was found between normal and diabetic leucocytes in this respect, and the ability of diabetic leucocytes to respond with an increased formation of NADPH upon stimulation was thus not impaired. ELSBACH [4] showed with normal rabbit PMN leucocytes, that phagocytosis resulted in an increase in the rate of turnover of lipids, but that lipid content and composition was not affected. A decrease in the nonmitochondrial cytoplasmatic synthesis of fatty acids in diabetic leucocytes has, however, been demonstrated and amply discussed by HENNES and AWAI [13, 14]. The present results seem to exclude the possibility that NADPH formation in the PC is impaired in diabetic leucocytes, but still leave the possibility that the NADPH formed in diabetic cells may be channelled to other reductive steps than fatty acid synthesis.

A much larger increase (tenfold) in the PC during phagocytosis has been reported in guinea pig leucocytes [2, 11, 21], which may be caused by guinea pig leucocytes being more actively phagocytizing than human and rat leucocytes as evaluated from the number of particles ingested. The causal relationship between phagocytosis and an increased PC has, however, lately been doubted in a preliminary report, which indicates that addition of increasing amounts of serum to human leucocytes increases the PC but lowers the phagocytotic index [17]. Consistent with this observation is a previously reported result of 8—10% PC obtained with rabbit PMN leucocytes incubated with [2-^{14}C]-fructose in the presence of 60% serum [10]. However, the higher PC may also be related to the different source of leucocytes, and to the calculation of the PC from the ratio of radioactivity randomized to C-1 and C-3 of a hexose-P derivative, which, as discussed, is open to criticism when applied to a tissue with an active transaldolase-transketolase system.

The total CO_2 production might be estimated from the recovery of $^{14}CO_2$ from the differently labelled glucoses, and calculated in terms of glucose equivalents. With human cells 1—2% of the metabolized glucose might be accounted for in this way, which agrees favourably with previous results [5], where the CO_2 formation was measured by differential manometry. A limited PC producing the major amount of CO_2 would explain the observation [4] that the fraction of glucose oxidized to CO_2 decreases with increasing glucose phosphorylation, and is consistent with the observation of BECK [1], that an increase in the concentration of glucose-6-P would increase the amount of glucose-6-P entering the EM pathway.

The randomization pattern of ^{14}C in lactate is not consistent with the pattern predictable from the sole operation of the PC [28]. Thus with [1-^{14}C]-glucose no activity should appear in C-1 of lactate, with [2-^{14}C]-glucose more activity in C-3 than in C-1 would have been expected, and with [3, 4-^{14}C]-glucose more activity in C-2 than in C-3 of lactate should have been observed.

Neither is the labelling pattern of lactate consistent with the randomization imposed upon the hexose-6-P pool by transketolase-transaldolase activity [15]. With [1-^{14}C]-glucose the ratio of C-1 over C-2 in lactate should have been much less than six (max. four), and with [2-^{14}C]-glucose the activity in C-3 should exceed or at most be equal to the activity in C-1. Similarly the

[3] The identification of labelled acetylated aminosugars in compound B was done by Dr. STJERNHOLM.

activity found in C-3 of lactate in the experiments with [3, 4-^{14}C]-glucose was too much to be explained by transaldolase-transketolase activity. Apparently, too heavy a randomization from C-1 to C-3 of lactate and *vice versa* has occurred. To explain this randomization pattern, it is suggested that some of the upper three carbons of glucose pass through a compound possessing optical rotational symmetry i.e. dihydroxyacetone. Dihydroxyacetonephosphate would be immediately susceptible to the action of a triosephosphatephosphatase, whereas glyceraldehyde-3-phosphate, arising from the lower three carbons of glucose, would be much less susceptible when the triosephosphates are not in isotopic equilibrium during the drain of glycolysis. The small but consistent randomization observed with [6-^{14}C]-glucose to C-1 of lactate, is consistent with such a prediction Experiments with [1-^{14}C]- and [3-^{14}C]-glycerol reported elsewhere [8] seem to support the suggestion of the occurrence of dihydroxyacetone as an intermediate in leucocyte metabolism.

The randomization pattern observed in the acetates is consistent with the formation of acetate through decarboxylation of pyruvate, except in the experiments with [2-^{14}C]-glucose and normal leucocytes. No reasonable suggestion to explain the discrepancy is available at the present moment.

The significance of the "compounds" A and B is difficult to evaluate. It appears that the consistent decrease in incorporation of radioactivity into compound A in normal leucocytes upon phagocytosis, and the corresponding increase in compound B when glucose was labelled in positions 1 and 2 but not in position 6, excludes the possibility of artifacts. No real significance should be paid to the absolute amounts of radioactivity appearing in these compounds for they are inhomogeneous, and some activity certainly belongs to volatile compounds. Further attempts at identification may be rewarding, since the compounds may bear a relation to the diabetic state.

The glucose metabolism in diabetic leucocytes can now be characterized as a decrease in glucose uptake, lactic acid production, and glycogen synthesis [5, 6], with a relative participation of the different major metabolic pathways identical to those of normal cells. A defective transport of glucose through the cell membrane has been eliminated as the cells are not sensitive to insulin *in vitro* [7], whereas the observed response to insulin *in vivo* [7] is consistent with a regulation at the enzyme level. The decreased glycogen synthesis in diabetic leucocytes would be a consequence of the diminished glycogen transferase activity as described in the preceding paper [9]. Several of the glycolytic enzymes have been assayed in normal and diabetic cells without the finding of any differences [20]. Experiments on the regulatory role of phosphofructokinase, however, are in progress.

Acknowledgements. Most of the reported experiments were done in the laboratory of Dr. RUNE STJERNHOLM, Dept. of Biochemistry, Western Reserve University, Cleveland, and were supported through Grant AM-6366 of USPHS. The author wishes to thank Dr. STJERNHOLM for valuable advice and help. The author also appreciate informative discussions with Dr. J. KATZ on the randomizations observed in the present experiments.

The author was supported by grants from the NATO Science Fellowship Programme, Nordisk Insulinfond, NOVO's fond, and Landsforeningen for Sukkersyge.

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