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Studies on Glycogen Metabolism in Human Leukemic Cells. I. Glycogen Content, Glycogen Synthetase Forms and Their Metabolic Interconversion *

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A decrease in glycogen content and glycogen-synthetase activity was observed in leukemic leucocytes compared to those of normal humans, but the decrease was lesser in chronic myeloid leukemic leucocytes than in those of acute leukemic patients.

Incubation of crude leucocyte homogenates at 30° C before assay of the enzyme activity, revealed a conversion of the glycogen-synthetase from the D to the I form. It took place if the enzyme source were enzyme extracts from acute myeloblastic, acute lymphoblastic, chronic lymphocytic, acute menoblastic and undifferenciate leucocytes. The conversion did not occur in crude homogenates from leucocytes of chronic myeloid leukemic patients.

ATP-Mg caused a conversion of the glycogen-synthetase I form to the D form in acute myeloblastic, chronic lymphocytic and undifferenciate leukemic leucocytes. This conversion was not observed in chronic myelocytic leukemic leucocytes.

The presence of glycogen-synthetase activity (transferase of synthase; UDPG: α-1, 4-glucan α-4-glucosyltransferase E.C. 2.4.1.11) in leucocytes from healthy human subjects has been demonstrated by several authors (4, 5, 7, 12, 15). It is known that human lymphocytes possess the systems for the interconversion between the glycogen-synthetase dependent on glucose-6-P for activity (D form) and the glycogen-synthetase independent form (I form) (4). It was not possible to demonstrate the presence of these systems in normal polymorphonuclear leucocytes.

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using freshly prepared homogenates (12). This behavior has been discussed also by others (8). These systems have been found in polymorphonuclears from diabetic patients on insulin therapy (2). Interconversion from glycogen-synthetase D into I form obtained from normal leucocytes has recently been demonstrated by incubation of a cell suspension with glucose, but only when the cells had previously been starved by incubation without substrate for 2 hours (17).

The presence of glycogen and the enzymes involved in its biosynthetic pathway were demonstrated by VANDERWENDE (15), LUGANOVA *et al.* (6) and NAKA *et al.* (9) in leucocytes from leukemic humans. Glycogen levels and glycogen-synthetase activity in leukemic leucocytes have been the subject of reports displaying controversial results.

This report presents an investigation of the levels of glycogen, the glycogen-synthetase activity and the possibility of interconversion between its two forms in different types of leukemic cells. The incubation of a leucocyte homogenate at 30° C results in a time-dependent conversion of glycogen-synthetase from a form which is inactive in the absence of glucose-6-P to one almost completely independent of this ligand (I form). This conversion has been observed in leucocyte homogenates from acute myeloblastic, acute lymphoblastic, chronic lymphocytic, acute monoblastic and undifferenciated leukemic cells, but it has not been possible to detect this conversion in leucocyte homogenates from chronic myelocytic leukemic subjects.

Glycogen levels have been measured in different types of leukemic leucocytes and the results are compared with those from leucocytes of normal humans.

Materials and Methods

Blood samples. To obtain leucocytes, 15-30 ml of heparinized blood was withdrawn from patients affected by chronic myelocytic, acute myeloblastic, chronic lymphocytic, acute lymphoblastic, acute monoblastic and undifferenciated leukemias. These leukemias were diagnosed by morphological and cytochemical methods.

Leucocytes were separated from red blood cells by differential sedimentation and from platelets by differential centrifugation at $670 \times g$. Relatively pure suspensions were obtained. About 97 % of leucocytes were polimorphonuclears in chronic myelocytic leukemias, 98 % were lymphocytes in chronic lymphocytic leukemias and 70 % to 90 % were blast cells in acute and undifferenciated leukemias, as shown by differential counting in an haemocytometer.

The cells were washed twice in saline and suspended in 0.05 M Tris-0.005 M EDTA, pH 7.8 to a final concentration of about $2.5\text{-}5 \times 10^8$ cells/ml depending on the type of leukemic leucocytes.

These suspended cells were completely destroyed by means of a teflon pestle homogenizer manually operated. Afterwards they were frozen and thawed once and finally centrifuged at $14{,}000 \times g$ during 15 minutes in a Sorvall refrigerated centrifuge. The transferase activity was assayed on the supernatant (crude extract) about two hours after withdrawal of the blood from the patients.

Analytical. Glycogen-synthetase (E.C. 2.4.1.11) was determined by the filter paper method of THOMAS *et al.* (14) measuring the transfer of (^{14}C) glucose from uniformly labeled UDP-(^{14}C) glucose into glycogen in the presence and absence of 10 mM glucose-6-P (I and total activities respectively).

Glycogen was determined by the anthrone method according to VAN HANDEL (16).

Glucose-6-P was determined with an enzymatic glucose-6-P dehydrogenase method, measuring the variation of optical

density at 340 mμ in a Beckman DV spectrophotometer.

Materials. UDP-glucose and rabbit liver glycogen were purchased from Sigma Chemical Co. UDP-(^{14}C) glucose was obtained from the Radiochemical Centre (Amersham, England), Hydroxymethylaminometane (Tris), EDTA and anthrone were obtained from Merck. Glucose-6-phosphate dehydrogenase and NADP were supplied by Boheringer Mannheim.

Results

Levels of glycogen and glycogen-synthetase activity. Table I shows the average glycogen content (mean ± S.E.) and glycogen-synthetase activity in different types of leukemic leucocytes. Glycogen synthetase activity was determined by incubation of the supernatant at 30° C with and without glucose-6-P. Under these conditions the detection of transferase activity in all types of leukemic leucocytes was almost entirely dependent on the addition of glucose-6-P.

Transformation from the D form to the I form of glycogen-synthetase. Several enzyme extracts from different types of leukemic leucocytes were preincubated at 30° C, with 50 mM mercaptoethanol and 10 mM Cl_2Mg during 1 hour before assay of the enzyme activity. The experiment revealed generally a steady conversion of the enzyme from the D to the I form. However figure 1 shows the effect of preincubation on two different enzyme crude extracts from two types of leukemias. Figure 1A depicts the behavior of the crude extract from leukocytes of an acute myeloblastic leukemia. Preincubation of this leucocyte preparation resulted in an increase in the I activity. Figure 1B represents the preincubation of a supernatant (crude extract) from chronic myelocytic leucocytes in the same conditions. No rise in activity measured in the absence of added glucose-6-P could be detected during the time of preincubation. Glucose-6-P levels were controlled during the experiments because it could be argued that if enough hexose phosphate was originated in the assay, the aparent glycogen-synthetase I activity would be increased. However glucose-6-P levels remained constant or changed slightly. Therefore the interconversion was real and could not be attributed to the increase of glu-

Table I. *Glycogen content and glycogen-synthetase activities in leukemic leucocytes.*

Diagnosis	Glycogen-synthetase activity nmols/hour/10^3 cells		Glycogen mg/10^{10} cells
	I form	Total	
Chronic myelocytic leukemia	0.21±0.25 (11)*	2.40±1.42 (11)	12.73±7.45 (7)
Acute myeloblastic leukemia	0.11±0.12 (17)	0.99±0.64 (17)	5.89±2.55 (8)
Chronic lymphocytic leukemia	0.05±0.02 (11)	0.25±0.12 (11)	3.22±2.22 (8)
Acute lymphoblastic leukemia	0.07±0.07 (6)	0.30±0.18 (6)	5.51±2.91 (5)
Acute monoblastic leukemia	0.15±0.13 (3)	0.78±0.40 (3)	10.00 (1)
Undifferenciate leukemia	0.01±0.01 (2)	0.25±0.26 (2)	1.54±1.05 (2)
Normal polymorphonuclears		3.10-7.04 (Rosell-Pérez and Esmann)	140 (Rosell-Pérez and Esmann)
Normal lymphocytes		1.80-3.21 (Hedeskov *et al.*)	31±6 (Hedeskov *et at.*)

* The numbers in parentheses correspond to the number of experiments.

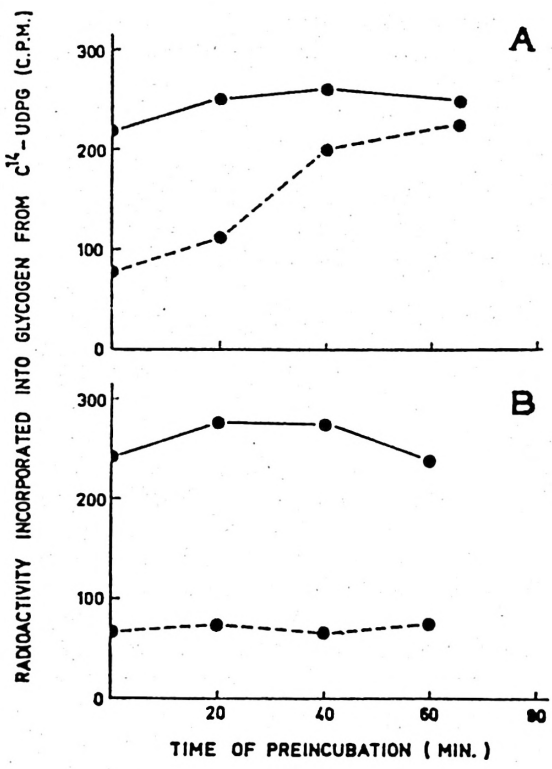

Fig. 1. *Effect of preincubation time on the D to I form conversion.*
Enzyme activities were assayed at different time intervals after preincubation with 50 mM mercaptoethanol and 10 mM $MgCl_2$. Dotted lines mean enzyme activity measured without glucose-6-P, and fine lines glycogen-synthetase activity measured with 10 mM glucose-6-P. A) Increase of glycogen-synthetase I activity in leucocytes from an acute myeloblastic leukemia. B) Effect of preincubation on the enzyme obtained from leucocytes of a chronic myelocytic leukemia patient.

cose-6-P as has been demonstrated in platelets (1).

In order to verify that this transformation was similar to the transformation catalized by a synthetase phosphatase as has been shown in other tissues (3, 11) the enzyme was incubated with some activators and inhibitors. In figure 2 the results of such an experiment are plotted.

During the 60 minutes of preincubation the crude extract with no additions presented a lag period and at the 40 minutes slow activation could be seen. When the enzyme was incubated with 50 mM mercaptoethanol the lag period disappeared and the I activity increased markedly. The total activity measured with glucose-6-P did not change significantly. This indicated that an almost complete transformation of the D form to the I form had occurred. Pi and especially Mg^{2+}

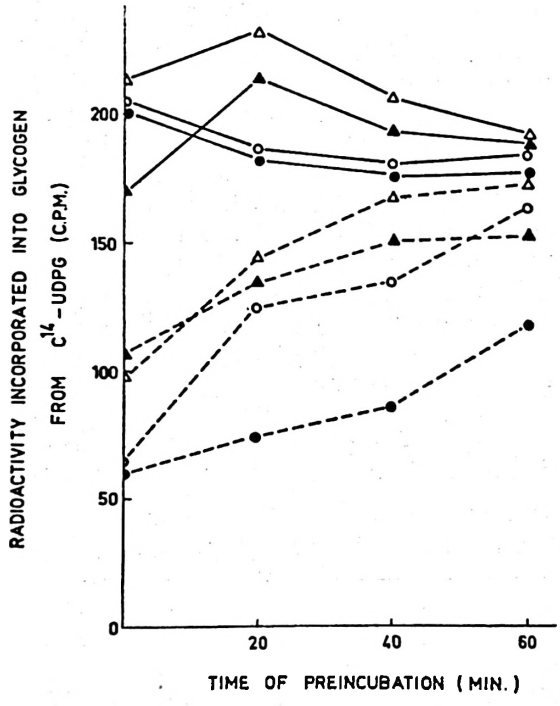

Fig. 2. *The stimulation of glycogen-synthetase interconversion by different conditions during preincubation.*
The enzyme source was a leucocyte homogenate. It was assayed at different time intervals after preincubation alone (●) with 50 mM mercaptoethanol (○), with 8 mM P_i (▲) and 10 mM $MgCl_2$ (△). Dotted lines represent the enzyme activity measured without glucose-6-P and fine lines represent the enzyme activity measured with 10 mM glucose-6-P. The homogenate was from a myeloblastic leukemia.

Table II. *Glycogen-synthetase D-phosphatase and glycogen-synthetase I-kinase activity in different types of leukemic cells.*

Diagnosis	Glycogen-synthetase D-phosphatase % of I activity before and after 60′ of preincubation		Glycogen-synthetase I-kinase % of I activity 20′ after the addition of ATP-Mg compared with controls	
	—Preincubation	+Preincubation	—ATP-Mg	+ATP-Mg
Chronic myelocytic leukemia	10.0± 5.0 (10)*	11.0± 7.2 (10)	14.1± 7.2 (7)	15.0± 6.1 (7)
Acute myeloblastic leukemia	22.9±15.5 (11)	74.1±15.2 (11)	68.5±26.1 (4)	21.1±16.2 (4)
Chronic lymphocytic leukemia	25.2±20.4 (5)	76.4±20.6 (5)	85.5± 4.9 (2)	51.0±11.4 (2)
Acute lymphoblastic leukemia	22.7± 5.5 (4)	74.7±28.0 (4)		
Acute monocytic leukemia	16.1± 7.0 (2)	93.5± 9.1 (2)		
Undifferenciated leukemia	14.1± 0.9 (2)	74.5±14.8 (2)	91.00 (1)	27.00 (1)

* The numbers in parentheses correspond to the number of experiments.

stimulated the transformation. The effect of sodium fluoride was to prevent the D to I conversion (not shown).

Conversion of the I to D form of Glycogen-synthetase. Addition of ATP-Mg to those crude extracts whose glycogen-synthetase independent activity had increased after 60 minutes of preincubation, caused a prompt decline of this activity, while total activity remained constant. The net reversal of I to D form means that a comparatively large proportion of the I enzyme was converted to the D enzyme in these experiments (figure 3A).

Addition of ATP-Mg to those homogenates that did not show an increase in glycogen-synthetase independent activity had no or very little effect on the very low independent activity, as shown in 3B, whereas the D activity decreased clearly, the amount of the inactivation depending on the extracts and the ATP-Mg concentration used.

Glycogen-synthetase kinase and glycogen-synthetase phosphatase activities. The time-dependent D to I form transformation produced by preincubation of crude extracts and the I to D form reconversion produced after addition of ATP-Mg were assayed in several enzyme sources from different types of leukemic cells. Table II shows the glycogen-synthetase D phospatase and glycogen-synthetase I kinase activities. Glycogen-synthetase D phosphatase was measured as an increase in the percentage of I form after 60 minutes of preincubation. Glycogen-synthetase I kinase activity was measured as a decrease in the percentage of I activity 20 minutes after the addition of ATP-Mg, compared with a control. It can be concluded that chronic myelocytic leucocytes cannot show synthetase phosphatase and kinase activities *in vitro* and they cannot transform one form of glycogen-synthetase into the other. The other types of leukemic leucocytes present these activities and, as a consequence, have the possibility of transformation.

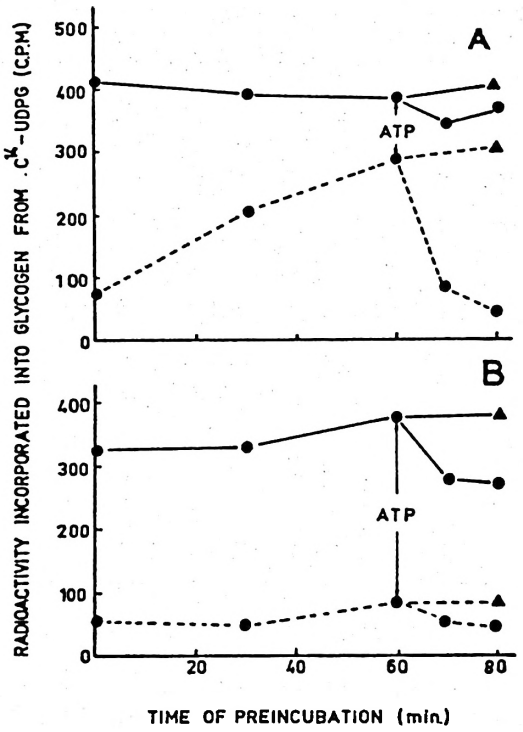

Fig. 3. *Effect of preincubation time and ATP-Mg addition on glycogen synthetase activities.*
After 60 minutes of preincubation Mg-ATP (10 mM, 5 mM) was added to part of the preincubated enzymes. Full lines represent enzyme activities measured with 10 mM glucose-6-P. (▲), control (no Mg-ATP was added). Dotted lines represent the activities measured without glucose-6-P. A) The effect on acute myeloblastic, acute lymphoblastic or chronic lymphocytic leucocytes. B) The effect on chronic myelocytic leucocytes.

Discussion

The glycogen content in different types of leukemic leucocytes was found to be lower than in normal leucocytes: myelocytic leucocytes being those with the highest level compared with the other types of leukemic leucocytes. However the glycogen levels in these cells were still lower than in normal polymorphonuclear leucocytes. Glycogen content was found to be very low in all types of cells from acute leukemias and sometimes these cells were practically void of this storage polysaccharide.

This paper has shown that the glycogen synthetase activity in all freshly prepared homogenates, was practically all in the D form. This situation was similar to that found in normal leucocytes (15). In acute myeloblastic and chronic myelocytic leukemic cells the total glycogen synthetase activity was lower than that described by ROSELL-PÉREZ *et al.* (12) in normal and practically homogeneous polymorphonuclear preparations (3.1 to 7.04 μmols/h/10^8). However the differences between both types of leucocytes seem to be important. Glycogen synthetase activity in chronic myelocytic leucocytes was only slightly lower than in normal polymorphonuclears whilst in acute myeloblastic cells it was 5 times lower.

Glycogen synthetase activity in acute lymphoblastic and chronic lymphocytic leucocytes was much lower than that described by HEDESKOV *et al.* (4) for normal practically homogeneous populations of lymphocytes (1.80 to 3.21 μmols/h/10^8 cells).

Glycogen synthetase activity in leucocytes from undifferenciated leukemia was also very low, but this result cannot be confronted with normal monocytes because there are no available data on these cells.

Incubation of enzyme crude extracts at 30° C evidenced the capacity in almost all cells of converting the glycogen synthetase D to the I form which is more active, in the sense that it does not require glucose-6-P for activity. The system that carries on this conversion operates *in vitro* in leucocytes from acute myeloblastic, acute lymphoblastic, chronic lymphocytic, acute monoblastic and undifferenciated leukemia. This conversion is catalized by a synthetase phosphatase (10).

Leucocyte extracts from chronic myelocytic leukemias under the same experi-

mental conditions cannot show the transformation. That means that the glycogen synthetase D phosphatase system is absent in this type of leucocytes, as in normal polymorphonuclear cells, or that there exist some mechanisms preventing the conversion *in vitro*. This could be the result of the action of some of the neutral proteases present in these cells acting specifically on the phosphatase during enzyme preparations. In any case it is an experimental difference in behavior in our conditions.

The presence of glycogen synthetase D phosphatase activity in cell homogenates from acute myeloblastic leukemia and the absence of this activity in enzyme extracts from chronic myelocytic leukemia could be of interest for the diagnosis.

Since the population of white blood cells in acute myeloblastic leukemia are blast cells, inmature cells, precursors of myelocytic and polymorphonuclear cells, it seems possible that glycogen synthetase D phosphatase and glycogen synthetase I kinase activities became absent or inhibited when cells are more differenciated. Glycogen appears in the first stage of granulocyte and lymphocyte development and its content increase with granulocyte development. Glycogen synthetase D phosphatase activity is evidenced *in vitro* in the first stage of cell differentiation, but it has not been possible to show it in neutrophile development in the same conditions. The situation does not seem to be the same in lymphocytes because glycogen-synthetase D phosphatase is active during development and in the mature stage as the present data point out.

Acute myeloblastic, acute lymphoblastic, chronic lymphocytic, acute monoblastic and undifferenciated leukemic leucocytes possesses the system for the conversion of the I into the D form of transferase, this transformation taking place upon addition of ATP-Mg and therefore attributable to a glycogen-synthetase I kinase (14). Leucocytes from chronic myelocytic leukemia do not show kinase activity. However, it was possible to observe in many prepparations of this type of cells, a time dependent inactivation of the D form of glycogen-synthetase after the addition of ATP-Mg (fig. 3B). This behavior has been described also in other cellular systems devoid of the I activity of glycogen-synthetase either naturally or by inhibiting their phosphatase activity (17) and was explained by an extraphosphorylation of the D form that could inactivate this already phosphorylated molecular species (13). The present experiments with chronic myelocytic leukemic cells seem to corroborate that hypothesis.

Resumen

El contenido en glucógeno y la actividad glucógeno sintetasa se hallan disminuidos en los leucocitos leucémicos humanos, si se comparan con los de leucocitos normales. Sin embargo, estos niveles son significativamente más elevados en los procedentes de leucosis mieloides crónicas que en los de leucosis agudas de cualquier tipo.

Por preincubación de los extractos crudos de leucocitos a 30° C se obtuvo conversión de la glucógeno sintetasa D a la forma I. Se observó transformación en los casos en que el extracto enzimático procedía de leucosis de tipo mieloblástico agudo, linfoblástico agudo, linfoide crónico, monoblástico agudo e indiferenciado. No se observó interconversión en los homogenados procedentes de leucocitos de pacientes con leucosis mieloide crónica.

El ATP-Mg cousaba conversión de la glucógeno sintetasa en forma I a la forma D en los extractos de leucocitos de mieloides agudos, linfoides crónicos e indiferenciados. Tampoco se observó transformación en los de leucosis mieloides crónicas.

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