phils' oxidative metabolism capacity. Neutrophils of CGD patients, which seem to be defective in the activating system of NADPH oxidase, did not consume oxygen after FMLP preincubation, but neutrophils of CGD carriers showed an enhancement after FMLP-preincubation similar to that found in normal neutrophils. These data suggest that FMLP preincubation enhances not NADPH oxidase activity itself but an activating system of oxygen consumption. Kitagawa[11] reported that FMP-preincubated neutrophils increased concanavalin A (Con A)-induced O_2^- production and this enhancement effect was almost completely abolished by the washing out of FMP. They speculated that FMP-receptor complexes and Con A-receptor complexes may interact directly on the surface membrane to result in the marked enhancement of O_2^- production, or FMP-receptor complexes may affect the redistribution of Con A-receptor complexes through intracellular events. Beswick[12] reported that FMLP enhanced OPZ-induced O_2^- production, but oxygen consumption was not enhanced by FMLP. They speculated that the increased number of complement receptors by FMLP lead to a greater receptor/particle interaction resulting in an enhanced O_2^- production[13]. In regard to oxygen consumption, our results conflict with those reported by Beswick. This discrepancy may be related to the dose of OPZ which they used, i.e., 2 mg and the method of calculation of oxygen consumption activity. In this study the enhancement effect of FMLP preincubation was abolished by washing, indicating that FMLP and its receptors may interact to form a reversible complex with a resultant enhancement of oxygen consumption. In vivo, neutrophils during chemotaxis are exposed to a gradient of a chemotactic factor. It may be important in the host defense system that an interaction of the neutrophil membrane and a chemotactic factor modulates responsiveness of neutrophils to stimuli of oxidative activation.

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2 Goldstein, I.M., Brai, M., Osler, A.G., and Weissmann, G., J. Immun. *111* (1973) 33.
3 Hatch, G.E., Gardner, D.E., and Menzel, D.B., J. exp. Med. *147* (1978) 182.
4 Goldstein, I.M., Roos, D., Kaplan, H.B., and Weissmann, G.J. clin. Invest. *56* (1975) 1155.
5 Goetzl, E.J., and Austen, K.F., J. clin. Invest. *53* (1974) 591.
6 Van Epps, D.E., and Garcia, M.L., J. clin. Invest. *66* (1980) 167.
7 English, D., Roloff, J.S., and Lukens, J.N., Blood *58* (1981) 129.
8 Issekutz, A.C., Lee, K.Y., and Biggar, W.G., Infect. Immun. *24* (1979) 295.
9 Babior, B.M., New Engl. J. Med. *298* (1978) 659.
10 Nakamura, M., Baxter, C.R., and Master, B.S.S., Biochem. biophys. Res. Commun. *98* (1981) 734.
11 Kitagawa, S., Takaku, F., and Sakamoto, S., J. Immun. *125* (1980) 359.
12 Beswick, P.H., and Kay, A.B., Clin. exp. Immun. *43* (1981) 399.
13 Kay, A.B., Glass, E.J., and Salter, D.M., Clin. exp. Immun. *38* (1979) 294.

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Inhibitory action of serum from a Laron dwarf on normal cellular function[1]

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Summary. Glucose uptake and O_2 consumption of confluent glial cells grown in culture were measured in the presence of serum-free buffer and compared with those measured in the presence of serum from a normal volunteer, from an hGH-deficient dwarf and from a Laron dwarf. Cellular glucose uptake and respiration in the absence or presence of insulin or hGH are inhibited by Laron serum.

The syndrome of Laron dwarfism is a familial disorder characterized by high levels of circulating immunoreactive growth hormone, which is considered biochemically normal[2-4]. Patients with this disease are known to have very low levels of circulating somatomedins[5] and to be unresponsive to exogenously administered human growth hormone[6]. The tentative hypothesis of cellular unresponsiveness has recently been substantiated and extended by Golde and collaborators[7]. They were able to show that in large erythroid colonies grown in culture from normal adult circulating peripheral blood erythropoietic stem cells human growth hormone (hGH) had growth-promoting effects in nanogram concentrations, whereas in cultures of the cells from Laron dwarfs either no or minimal effects were observed. It is well known that somatomedins have insulin-like actions in addition to their mitogenic and growth-promoting effects[8]. The insulin-like activity of somatomedins includes stimulation of transmembranal transport of sugars[9] as well as intracellular glucose oxidation[10]. In view of the somatomedin deficiency in Laron serum the present study was undertaken to investigate whether other hormones with insulin-like activity could substitute for somatomedins in such serum. Thus, the effect of serum from a Laron dwarf was tested on cellular respiration and glucose uptake of normal cells in vitro and

Table 1. Glucose consumption of glial cells (NN) grown in culture*

Incubation medium	mg glucose consumed/mg protein/h $\times 10^{-2} \pm$ SEM**		
	O	Insulin (220 µU/ml)	hGH (200 ng/ml)
Serum-free buffer	4.08 ± 0.52 (4)	11.72 ± 0.91 (4)	9.61 ± 1.02 (4)
Normal serum	8.43 ± 0.70 (15)	14.77 ± 1.39 (7)	13.34 ± 1.10 (4)
hGH-deficient serum	5.82 (2)	-	10.19 (2)
Laron serum	3.79 ± 0.77 (10)	3.58 ± 2.07 (7)	-

*Cells were between 54th and 62nd passage and were always harvested on the 7th days after passage. ** All values based on 2-h incubation period.

Table 2. Respiratory rates (µl O_2/mg protein) of glial cells (NN) grown in culture*

Time interval (min)	Serum-free buffer No addition	Normal serum No addition	Laron serum No addition	Glucose	Glucose + insulin	Glucose + hGH
0- 30	2.9	2.7	2.4	2.3	2.6	2.3
30- 60	2.3	2.6	2.2	1.9	1.8	1.7
60- 90	1.6	2.6	1.2	1.2	1.0	1.2
90-120	1.7	3.2	0.8	0.7	0.6	0.4
120-150	1.5	1.8	1.0	1.1	0.8	0.8
150-180	1.5	1.2	1.0	1.1	1.2	1.0
Cumulative rates over 3 h ± SEM	11.5 ± 1.4	14.1 ± 1.2	8.6 ± 0.9	8.3 ± 1.0	8.0 ± 0.9	7.4 ± 0.9

*Cells were between 54th and 62nd passage and were always harvested on the 7th day after passage. All values represent means of 5-7 determinations, the SEM when not given was found to be consistently less than 10% of each respective mean value. Additions: glucose, 1 mg/ml; insulin, 200 µU/ml; hGH, 200 mg/ml.

compared to that from a hGH-deficient dwarf and a normal adult. In addition, the response to both insulin and hGH was examined. Confluent glial cells grown in culture were chosen as test cells since they are considered good target cells for glucose metabolism[11] and growth-promoting substances[12].

Materials and methods. The case history of the Laron dwarf used in this study has been described extensively[13]. A normal healthy adult and a classic hyposomatotropic dwarf with growth hormone deficiency were used as control subjects for this study with informed consent from all subjects. Peripheral blood from each subject was obtained, following an overnight fast, in heparin-coated tubes and plasma was obtained by centrifugation (refrigerated). All experiments were repeated 3 times with different plasma samples. Blood glucose was found to vary between 0.85 and 0.90 g/l for the healthy control, to be 0.79 g/l for the hGH-deficient dwarf, and between 0.47 and 0.60 g/l for the Laron patient.

Glial cells (clone NN), initially isolated and subsequently characterized by Shein and collaborators[14], were obtained from North American Biologicals Inc. and cultivated under conditions identical to those described[11]. Prior to the incubations the cells were rinsed twice with warm saline and then harvested into 6 ml of the respective incubation medium (buffer or sera) with the aid of a rubber spatula. Glucose uptake was measured according to previously described methods[11]; the rates of oxygen consumption were measured in a standard Warburg apparatus with each flask containing 6.5 ± 0.71 mg (SEM) protein as estimated by Lowry's method. Glucose, when added, amounted to 1 mg/ml, insulin to 200 µU/ml and human growth hormone to 200 ng/ml.

Results. In table 1 are summarized the amounts of glucose taken up per mg cellular protein either in the presence of serum-free buffer, in the presence of serum from a healthy volunteer or an hGH-deficient dwarf, and in the presence of Laron serum. The highest rate of glucose consumption was found in the presence of normal serum. In the presence of serum-free buffer or Laron serum, glucose uptake was approximately half of that measured in the normal serum ($p < 0.01$, Student's t-test), with that in the presence of serum from the hGH-deficient dwarf being intermediary. The addition of 200 µU insulin/ml resulted in significant increases in the rate of glucose consumption, both in the buffer and in the presence of normal serum, but had no effect on cellular glucose accumulation in the presence of Laron serum. Although the stimulatory effect of insulin (percent increase) was greatest in the presence of serum-free buffer (170% vs 85% in normal serum), the absolute amount of glucose consumed was significantly higher ($p < 0.05$) in the presence of normal serum. When human growth hormone at a concentration of 200 ng/ml was added to the incubation, cellular glucose uptake was increased in the presence of buffer, normal serum and serum from the hGH-deficient dwarf. There was no significant difference between the effect of human growth hormone and that of insulin on glucose consumption.

The rates of O_2 consumption of glial cells in the presence of serum-free buffer, normal serum, and serum from the Laron dwarf were compared in the absence of any exogenously added substrate, thus reflecting endogenous respiration, and in the case of the serum from the Laron dwarf, in the presence of glucose, glucose plus insulin, and glucose plus human growth hormone (table 2). As can be seen, initial endogenous respiratory rates appear to be identical in the presence of the 2 sera, but with time, O_2 uptake in the presence of Laron serum is progressively reduced. At the end of the incubation period total O_2 consumption in the presence of Laron serum amounts to only 60% of that in the presence of normal serum.

Furthermore, when glucose was added as substrate, either alone or in the presence of insulin or hGH, no change in O_2 uptake could be detected (table 2). Under all conditions glial cell respiration in the presence of Laron serum remained below the endogenous respiratory rate of glial cells in the presence of buffer or normal serum.

Discussion. The present results indicate that Laron serum exerts inhibitory effects on normal cellular glucose consumption. This probably represents a decrease in glucose uptake. The reduction in glucose consumption is reflected in a decrease in cellular oxygen consumption. These 2 observations could be related to the fact that Laron serum is hypoglycemic and low in somatomedins, known to have insulin-like activity. However, in that case, exogenous insulin or hGH should exhibit at least some facilitation of glucose uptake. This was not observed, despite the use of supramaximal concentrations of both hormones. These concentrations were adequate to elicit the expected facilitation of cellular glucose consumption when added to serum-free buffer, normal serum and serum from an hGH-deficient dwarf. Thus, it could be concluded that a putative inhibitor of glucose uptake and utilization is present in Laron serum. Such an inhibitor could affect reduced cellular metabolism, which might be related to some of the growth-inhibitory characteristics of the Laron syndrome.

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2 Elders, M.J., Gerland, J.T., Daughaday, A., Fischer, D.A., Whitney, J.E., and Hughes, E.R., J. Pediatr. *83* (1973) 253.
3 Pierson, M., Malaprade, D., Fortier, G., Belleville, F., Lasbennes, A., and Wuilbercq, L., Archs fr. Pediatr. *35* (1978) 151.
4 Jacobs, L.S., Sneid, D.S., Garland, G.T., Laron, Z., and Daughaday, W.H., J. clin. Endocr. Metab. *42* (1976) 403.

5 Phillips, L.S., and Vassilopoulou-Sellin, R., New Engl. J. Med. *302* (1980) 438.
6 Laron, Z., Pertzelan, A., and Mannheimer, S., Israel J. med. Sci. *2* (1966) 152.
7 Golde, D.W., Noelle Bersch, B.A., Kaplan, S.A., Rimoin, D.L., and Li, C.H., New Engl. J. Med. *303* (1980) 1156.
8 Phillips, L.S., and Vassilopoulou-Sellin, R., New Engl. J. Med. *302* (1980) 371.
9 Uthne, K., Reagan, C.R., Gimpel, L.P., and Kostyo, J.L., J. clin. Endocr. Metab. *39* (1974) 548.
10 Clemmons, D.R., Hintz, R.L., Underwood, L.E., and Van Wyk, J.J., Israel J. med. Sci. *10* (1974) 1254.
11 Roth-Schechter, B.F., and Mandel, P., Biochem. Pharm. *25* (1976) 563.
12 Westermark, B., and Wasteson, A., Adv. Metab. Disorders *8* (1975) 85.
13 Flori, J., Nanisme Type Laron: Etude d'ensemble à propos d'une observation personnelle, thesis No. 235, Université Louis Pasteur, Fac. Médecine, Strasbourg 1978.
14 Shein, H.M., Britva, A., Hess, H.H., and Selko, D.J., Brain Res. *19* (1970) 497.

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Karyological studies on established mosquito cell lines[1]

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Summary. Chromosome frequency distribution and cellular DNA estimations in different established mosquito cell lines were studied. These cell lines exhibited a wide range of cell types with a diploid stem-line comprising 50–55% and a haploid substem-line comprising 12–30% of the population. Estimation of cellular DNA contents by impulse cytoflowmetry and by Feulgen cytophotometry supported these observations. Because of their low diploid counts, these cell lines cannot be classified as diploid.

Since the first successful establishment of a mosquito cell line on hemolymph-free medium by Singh[3], several cell lines from different species of *Aedes* mosquitoes have been developed[4–6] and used for virological studies[7–15]. However, studies on characterization of these cell lines were only limited[6,16,17]. In this paper, we report the chromosome constitutions of 4 cell lines and cellular DNA contents of 5 cell lines from different species of *Aedes*.

Material and methods. Established mosquito cell lines of *Aedes aegypti* (ATC-10)[3], *A. albopictus* (ATC-15)[3], *A. vittatus* (ATC-121)[4], *A. w-albus* (ATC-136)[5] and *A. novalbopictus* (ATC-173)[6] were maintained at 28 °C on Mitsuhashi-Maramorosch medium[18] supplemented with 10% foetal calf serum and antibiotics (penicillin-streptomycin). Chromosome studies were carried out on ATC-10, ATC-15, ATC-136 and ATC-173 cell lines; DNA estimations by impulse cytoflowmetry were made on all the 5 cell lines while ATC-10 and ATC-136 cell lines were used for Feulgen cytophotometry.

2–3-day-old cultures were treated with colcemid (0.5 µg/ml) for 4–6 h. Metaphase cells were removed by shaking, treated with 0.5% KCl hypotonic solution and fixed in chilled acetic acid-methanol (1:3) fixative. Chromosomes were spread by air-drying and stained with dilute Giemsa's stain. About 400 metaphases from each cell line were screened. Chromosome preparations were also made from coverslip cultures without hypotonic pretreatment to corroborate the results obtained by the above method.

Full-grown cultures were trypsinized to obtain single-cell suspensions and were fixed in 5% buffered glutaraldehyde. These were washed 3 times with Tris buffer (0.1 M, pH 7.4), resuspended in ethidium bromide (EB) in Tris buffer (10 µg/ml) and stained in the dark for 60 min. Fluorescence intensities of these cells were recorded with Phywe ICP

a) ATC-10 (passage 210):

Chromosomes	2	3	4	5	6	7	8	9	10	11	21
% metaphases	2	30	4	1	55	1	1	1	1	2	2

b) ATC-15 (passage 110):

Chromosomes	2	3	4	5	6	7	8	9	10	12	13	14	15	17	18	24
% metaphases	2	12	2	3	55	2	3	2	1	7	2	2	2	1	2	2

c) ATC-136 (passage 180):

Chromosomes	2	3	4	5	6	7	8	9	11	12	15	21	24
% metaphases	1	20	3	1	50	3	2	2	2	11	2	2	1

d) ATC-173 (passage 105):

Chromosomes	2	3	4	5	6	7	9	10	11	12	13	14	15	16	17	19	24
% metaphases	2	15	2	3	52	2	1	2	0.5	2	15	0.5	0.5	0.5	0.5	0.5	1