was only 86±7 mm Hg. However, as can be seen in the upper panel, the blood loss necessary to lower the MAPs to 60 and 30 mm Hg were significantly higher in the pentobarbital group (6.8±0.9 ml/kg and 12.6±1.4 ml/kg) than in the chloralose-urethane group (2.3±0.4 ml/kg and 9.2±0.8 ml/kg). In addition, the maximum shed volume during compensation which occurred during shaded area around 105 min was also significantly greater in the pentobarbital group (23.1±0.8 ml/kg) compared to the chloralose-urethane group (17.6±0.8 ml/kg). Also of significance was the fact that in each of the 10 sets of experiments the pentobarbital series decompensated to 20% utpake while the chloralose-urethane rats failed to exhibit this type of cardiovascular decompensation. Upon restoration of normal blood volume, the MAPs of both groups returned toward control; however, after 2 h only the pentobarbital group was able to maintain this pressure.

Conclusions. The fact that the prehemorrhage control MAP was higher in rats anesthetized with pentobarbital than chloralose-urethane suggests that choralose-urethane has less of an effect on blood pressure control than pentobarbital. However, these rats were able to lose significantly more blood at 60 and 30 mm Hg than the chloralose-urethane group suggesting that they are better able to compensate for acute blood loss. This compensatory effort noted in the pentobarbital group progresses into cardiovascular decompensation as seen by the uptake of blood between 105 and 190 min but not in the chloralose-urethane group. The fact that the chloralose-urethane animals were unable to maintain their blood pressure 2 h postreinfusion suggests that these animals were more severely compromised during the hemorrhagic hypotensive phase of the experiment even though they bled less and did not show cardiovascular decompensation, (i.e. take back blood from the buret) during hypovolemic hypotension (180 to 205 min in the fig.).

1 Acknowledgments. The author wishes to thank Tim Cronin and Mary Alice Secola for their technical assistance in the conduction of these studies. This study was supported by Oral Roberts University Research Funds.

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0014-4754/83/060602-03$1.50+0.20/0
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Enhancement of human neutrophil oxygen consumption by chemotactic factors[1]

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Summary. Human neutrophils preincubated with chemotactic factors showed enhanced OPZ-induced oxygen consumption. Maximum capacity of neutrophils to consume oxygen was found to be limited for both FMLP-treated cells and control cells. But with lower doses of OPZ, FMLP-treated cells consumed more oxygen than control cells.

Chemotaxis of neutrophils is an important part of the host defense system. Chemotactic factors induce not only chemotaxis but also neutrophil activation, which includes degranulation[2], chemiluminescence[3], superoxide anion production[4] and activation of the hexose monophosphate shunt[5]. Furthermore, preincubation of neutrophils with chemotactic factors enhances the stimulation of superoxide production[6,7], hexose monophosphate shunt activity[8],

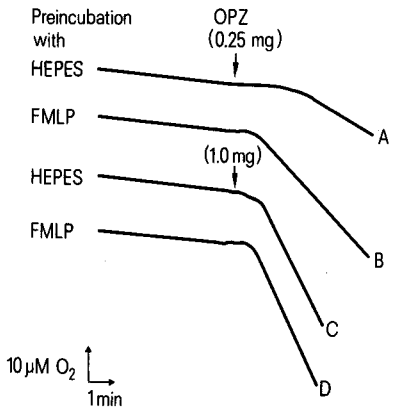

Figure 1. Enhancement of neutrophil oxygen consumption by FMLP preincubation. Tracings illustrate changes of oxygen concentrations in 1.0 ml HEPES-buffer containing 2×10^6 cells at 37 °C. The cell suspension was preincubated for 30 min with either FMLP (10^{-7} M) or HEPES-buffer prior to the addition of OPZ.

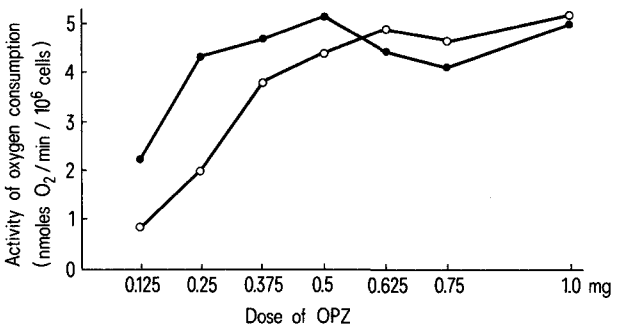

Figure 2. Relationship between the dose of OPZ and oxygen consumption. Neutrophils were preincubated for 30 min at 37 °C with FMLP (—●—) or with HEPES-buffer (—○—). The ordinate indicates activity of oxygen consumption. Representative data are shown from 1 subject.

chemiluminescence[6] and bactericidal activity[8]. In the present study, we demonstrate that preincubation of human neutrophils with chemotactic factors enhances their oxygen consumption activity.

Material and methods. Zymosan A and N-formyl-methionyl-phenylalanine (FMP) were purchased from Sigma Chemical Co. N-formyl-methionyl-leucyl-phenylalanine (FMLP) was obtained from the Protein Research Foundation, and both FMLP and FMP were dissolved in dimethylsulfoxide (DMSO) and diluted with HEPES-buffer (17 mM N-hydroxyethyl-piperazine-N′-ethanesulfonic acid, pH 7.4, 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$ and 5 mM glucose) prior to use. Zymosan A was incubated with fresh human AB serum at 25 mg/ml for 30 min at 37 °C. After centrifugation, zymosan-activated serum (ZAS) was incubated for 30 min at 56 °C. Opsonized zymosan (OPZ) was washed and resuspended in saline at 25 mg/ml.

Human neutrophils from normal adults were isolated with a Ficoll-diatrizoate sodium gradient, 3% dextran sedimentation and hypotonic hemolysis. Neutrophils were suspended in HEPES-buffer. Oxygen consumption was measured with a Clark type oxygen electrode (Yellow Springs Instrument). The rate of oxygen consumption was calculated from the difference between the linear segment of decrease in oxygen concentration before and after the addition of OPZ during stirring at 37 °C. The total reaction volume was 1.0 ml, which contained 2×10^6 neutrophils and chemotactic factors or control substances (HEPES-buffer or heat-inactivated serum) in appropriate concentrations. The preincubation time was 30 min, which included 20 min of equilibration with air, 5 min of stabilizing time after an electrode attachment and 5 min of recording of prestimulated oxygen concentration. The reaction was started by the addition of an appropriate dose of OPZ.

Results. When the cells preincubated with FMLP were stimulated with enough OPZ (1.0 mg) to give the maximum response (5.16 nmoles O_2/min/10^6 cells) by itself (fig. 1,C), no enhancing effect of the chemotactic factor was demonstrated (fig. 1,D). With a reduced dose of OPZ, i.e., 0.25 mg, control cells showed a 39% decrease in oxygen consumption (fig. 1,A). Figure 1,B, indicates the change of oxygen consumption capacity of FMLP-treated cells stimulated with 0.25 mg OPZ, which almost reached the maximum response obtained with 1.0 mg of OPZ alone. Maximum enhancement was noted after 30 min preincubation with 10^{-7} M FMLP (data not shown). No further increase of oxygen consumption was demonstrated with other combinations of the concentrations of OPZ and FMLP, i.e., lower OPZ and higher FMLP and vice versa. In neutrophils preincubated with FMLP for 30 min, the relationship between the dose of OPZ and oxygen consumption is shown in figure 2. Neutrophils preincubated with FMLP showed a larger oxygen consumption than the control cells at lower doses of OPZ, but the maximum activity of oxygen consumption was similar between FMLP-treated cells and control cells with doses of OPZ higher than 0.5 mg.

The effects of different doses of the 3 chemotactic factors. FMLP, FMP and ZAS, on oxygen consumption of neutrophils were examined. Figure 3 showed that each chemotactic factor enhanced oxygen consumption in a dose-dependent fashion when preincubated with cells at 37 °C for 30 min. Maximum enhancement was obtained at 10^{-7} M FMLP, 10^{-5} M FMP and 1% ZAS, respectively. Neutrophils which had been washed after preincubation with FMLP consumed the same amount of oxygen as control cells (data not shown). Neutrophils of patients with chronic granulomatous disease (CGD) of the X-linked recessive type (n = 3, males) did not consume oxygen when preincubated with or without FMLP. On the other hand, neutrophils of their mothers (n = 3) consumed $1{,}46 \pm 0.1$ nmoles $O_2/min/10^6$ cells at 0.25 mg OPZ, and their cells preincubated with FMLP consumed 2.69 ± 0.18 nmoles O_2/min/10^6 cells. DMSO itself had no effect in this experiment.

Discussion. Oxygen consumption is an initial step in the oxydative metabolism of neutrophils following a pertubation of the membrane by either soluble or insoluble stimulating factors, and consumed oxygen is then converted to biologically active oxygen species (O_2^-, H_2O_2, OH·, $^1O_2$)[9]. Recently it has been shown that chemotactic factors not only cause chemotaxis but also induce or enhance various neutrophil functions, i.e., chemiluminescence, superoxide anion production, activation of the hexose monophosphate shunt and degranulation. The present study demonstrates that chemotactic factors (FMLP, FMP and ZAS) enhance oxygen consumption by neutrophils when stimulated with OPZ; after preincubation the dose of OPZ which was sufficient to induce maximum oxygen consumption was lower than that required to produce a similar effect with OPZ alone.

The oxygen consumption capacity of neutrophils closely reflects NADPH oxidase activity of intact neutrophils[10]. Therefore, its determination may reflect changes of neutro-

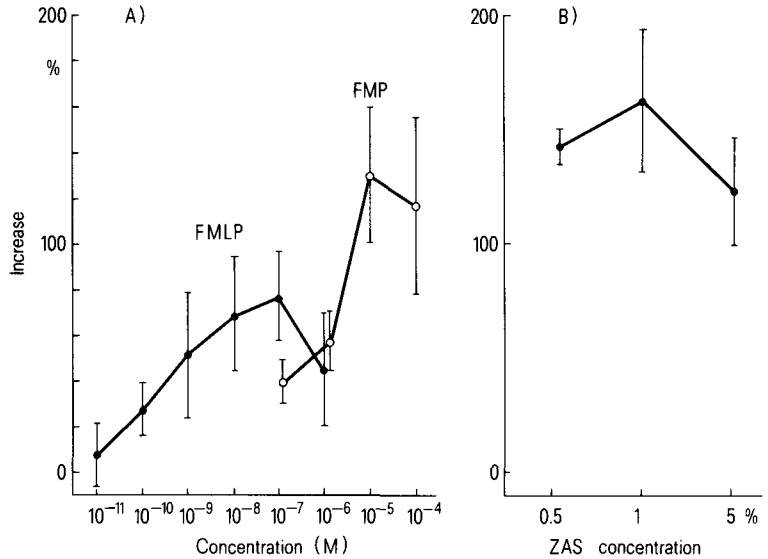

Figure 3. *A* Effect of various concentrations of formyl peptides on neutrophil oxygen consumption. Neutrophils were preincubated (30 min at 37 °C) with various concentrations of FMP (—○—) and FMLP (—●—). Then OPZ (0.25 mg) was added. Points are the mean percent increase (± 1 SD). *B* Effect of various concentrations of ZAS on neutrophil oxygen consumption. Neutrophils were preincubated (30 min at 37 °C) with various concentrations of ZAS and then OPZ was added. Points are the mean percent increase (± 1 SD).

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.

1 This work was supported in part by a Grant-in-Aid for Scientific Research (Project No. 56570363) from the Ministry of Education, Science and Culture of Japan and a Grant-in-Aid from the Japan Medical Research Foundation.

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0014-4754/83/060604-03$1.50+0.20/0
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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.