bow with prealbumin mobility(15), so that the sum of the amount of alpha-1-lipoprotein and lipo-prealbumin remains constant. The complex of alpha-1-lipoprotein and radio-thyroxine or radio-triiodothyronine on auto-radiograph may therefore show some variability as a function of storing and possibly, by chemically induced splitting by addition of the labelled hormone. Also the lipoprotein with alpha-2-mobility seems to have a somewhat variable position depending on amount of lipo- and normal serum proteins(15). Thus on the basis of immuno-electrophoretical tracings within the lipoprotein system and our investigation of binding properties, the confusing results which have been obtained concerning thyroxine binding proteins in human serum are understandable. The reason some workers have found a very high thyroxine binding capacity to the tryptophan rich prealbumin of Schultze(16), is being investigated, as our antisera have a very low titer against this protein.

Summary. Immuno-electrophoresis of normal human serum incubated with radio-thyroxin and radio-triiodothyronine combined with auto-radiography and special staining for lipoproteins with Oil red O showed that the albumin (identical with lipalbumin), alpha-1-lipoprotein and alpha-2-lipoprotein have the capacity to bind thyroxin and triiodothyronine.

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Effect of *Pseudomonas* on Lactate Production by Mouse Monocytes.* (25721)

R. S. BERK AND E. L. NELSON (Introduced by A. J. Salle)
Dept. of Bacteriology, University of California, Los Angeles

Recent data obtained in this laboratory indicate that succinoxidase activity of cells obtained from mouse peritoneal exudates, as well as of homogenates of liver and spleen, was inhibited in presence of heat-killed *Pseudomonas aeruginosa*(1). However, since metabolism of mouse monocytes appears to be mainly glycolytic, this study was undertaken to determine in what way, if any, *P. aeruginosa* might affect lactate production by these cells.

Method. Warm mineral oil (0.5 ml/mouse) was used to induce exudates in 10 week old female mice (Webster strain), 18 hours before harvesting of cells from peritoneal cavities with chilled 0.9% sodium chloride containing 0.1% heparin. Pooled monocytes were washed once with saline by centrifuging for 10 minutes at 500 x G. In experiments with

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infected monocytes, washed *Pseudomonas* (10^8) were administered intraperitoneally one hour before autopsy. Extracellular organisms were killed by incorporating polymyxin B (500 units/ml) in heparinized saline. Where indicated, exudates were obtained from mice immunized with suspension of dead *Pseudomonas* as previously reported(1). Most experiments employed intact cells, but where disrupted cells were required, homogenates of equivalent numbers of cells were prepared by sonication for 15 minutes in 9 KC Raytheon sonic oscillator. Counts with a Spencer hemacytometer indicated that 1 ml contained approximately 5 x 10^8 packed cells. All glycolysis experiments were run aerobically in heparinized phosphate buffer (0.1 M), pH 7.4, for 90 minutes. Lactic acid was determined by method of Barker and Summerson (2) and reported as amount/ml obtained from 20 μmoles of sugar/2 ml of a 5 x 10^7 cell suspension. Manometric experiments were run in conventional manner(3). Liver and spleen homogenates were prepared by homogenizing tissues in phosphate buffer, pH 7.4, for 60 seconds in Waring blendor.

Results. A survey of monocyte glycolysis, using lactate production as indicator, showed that only glucose and fructose were metabolized (Table I). Glucosamine, glucuronate, gluconate, methyl glucose, galactose, maltose, sucrose, fructose diphosphate, lactate, ribose, sorbose, l-xylose, mannose, arabinose, d-xylose, l-fucose, galacturonate, starch, dextrin, salicin, chondroitin sulfate, trehalose, raffinose, cellobiose, melezitose, melibiose, l-rhamnose, ascorbate, and glycogen were not utilized.

Since permeability may be a factor in preventing some sugars from being converted to lactate, monocytes were disrupted by sonic methods, and substrates related to glucose were tested with cell-free extracts. Nevertheless, substrates such as methyl glucose, glucosamine, glucuronate, galactose, maltose, and galacturonate were either not metabolized or were converted to substances other than lactate. Lactate production from glucose and fructose was not adversely affected by sonic treatment of the monocytes since cell-free preparations yielded 600 μg of lactate per ml with either sugar. This value compares well with 690 μg/ml obtained with intact cells. It should be pointed out that lactate production from glucose and fructose was almost always identical.

TABLE I. Lactic Acid Production by Monocytes from Normal and Immunized Mice.

Substrate	μg lactate/ml N*	I*
-(Endogenous)	160	158
Glucose	690	690
Fructose	680	685

* Monocytes from normal (N) and immunized (I) mice.

TABLE II. Lactic Acid Production by Infected* and Non-Infected Cells.

Substrate	μg lactate/ml Non-infected	Infected*
-(Endogenous)	71	150
Glucose	250	605
Net lactate	179	455

* Living *Pseudomonas* inj. intraper. into mice 90 min. prior to cell harvest.

Measurements of lactate production with infected and non-infected monocytes indicated greater acid production by infected cells. The results are seen in Table II. Infected cells yielded at least twice as much lactate as did uninfected cells whether supplemented with glucose or not. Net lactate production from glucose was 455 μg/ml for infected cells *vs.* 179 μg/ml for uninfected cells. Manometric studies suggested that the lactate produced with infected monocytes was solely monocytic in origin, since no intracellular microbial oxidation of either glucose or gluconate could be demonstrated. This assumption was supported by the fact that no net oxygen consumption could be noted using uninfected monocytes supplemented with glucose or gluconate.

Since previous experiments indicated that the succinoxidase activity of monocytes was susceptible to inhibition by bacteria and bacterial fractions, several different preparations of *P. aeruginosa* were added to monocytes to determine their effect on glycolysis. Heat-killed *Pseudomonas,* cell-free sonic extracts, and particles obtained by centrifuging sonic extracts of *Pseudomonas* were added to uninfected monocytes *in vitro.* Examination of

TABLE III. Lactic Acid Production by Monocytes Treated *In Vitro* with Intact and Fractionated *P. aeruginosa*.

Substrate	μg lactate/ml	*Pseudomonas**		
	—	Intact bacteria	Supernatant	Particles
-(Endogenous)	40	39	48	43
Glucose	590	600	599	690
Fructose	585	590	585	595

* Heat-killed intact organisms, supernatant of heat-inactivated sonicated *Pseudomonas*, or unheated particles from sonicated bacteria were added to give a concentration of 2.5 mg N/ml.

Table III indicates that 2.5 mg N/ml of each microbial preparation had no inhibitory effect on lactate production by monocytes. Similar results were obtained when microbial extracts were added to either spleen or liver homogenates.

Discussion. The data indicate that mouse monocytes exhibit a highly selective substrate specificity. Such substrates as methyl glucose, glucosamine, and maltose are closely related to glucose and yet were not metabolized to lactate. Permeability apparently is not a factor in substrate specificity. It has been established that polymorphonuclear leukocytes store sugars in the form of glycogen and that lactate production proceeds endogenously from this substance(4). However, when glycogen was added as substrate to monocytes, no lactate was detected. Previous immunization of donor mice with *Pseudomonas* did not affect lactic acid production by monocytes.

Kun and Miller(5) injected Salmonella endotoxin intravenously in rabbits. Blood lactic acid increased, but succinic dehydrogenase system of muscle and liver was inhibited. Although their experiments differed from ours, they obtained similar results. Sbarra and Karnovsky(6) have also noted increased lactate production by polymorphonuclear leukocytes when phagocytosis of polystyrene particles was allowed to proceed *in vitro* in presence of serum. However, in our *in vitro* studies when bacteria were added to monocytes in absence of serum no change in lactic acid production occurred.

Since similar studies had indicated that the succinoxidase activity of monocytes and of spleen and liver homogenates was inhibited by small concentrations of both intact and disrupted *Pseudomonas*, it was interesting to note that these preparations had no effect on lactate production. The results suggest that glycolysis is more refractory to microbial inhibition than is succinoxidase activity.

Summary. Mouse monocytes, induced by intraperitoneal injection of mineral oil, produced lactate from only glucose and fructose. No net lactate production was noted from 29 other sugars. *In vivo* infection of monocytes resulted in at least a 2-fold increase in lactate production. However, no effect was noted when either heat-killed *Pseudomonas aeruginosa* or sonic fractions thereof were added to monocytes *in vitro*. No significant differences in lactate production were noted between cells obtained from immunized and non-immunized animals.

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