stick chemistry, and complete microscopic examination—or should only specific parts of the examination be done? Although these questions may seem redundant and mundane, the paper of Christenson et al. correctly addresses an issue that clinicians encounter on a daily basis.

A complete urinalysis is performed in my office on all patients presenting with urinary tract complaints and in those who have recently undergone surgery of the urinary tract. The character of the urine is noted (turbid or clear) and qualitative dipstick sugar and protein determinations are made. An aliquot of the urine is routinely centrifuged and the sediment examined microscopically by the physician. Staining of the sediment is not routinely performed. My experience with dipstick tests (blood/hemoglobin, leukocyte esterase, nitrite) is similar to that of the authors. False-positive and false-negative rates are significantly higher as compared with the microscopic examination. Often, tests for blood and hemoglobin are positive and the microscopic examination shows no or only an occasional erythrocyte. Similarly, tests for infection (leukocyte esterase or nitrite) also have a high false-positive and false-negative rate, and for this reason cultures are done only if more than five leukocytes are seen or if bacteria are evident on the microscopic examination. In my experience, this is the most reliable basis for deciding when a urine culture should be performed. It is interesting that the authors identified 46 patients who had demonstrable bacteria microscopically, and this is equal in number to those patients having positive cultures (their Table 2).

Another important point is that microscopic examination often permits determination of contaminated specimens. The presence of many epithelial cells in a voided urine specimen from a female generally indicates contamination, and if bacteria or leukocytes are seen in association with epithelial cells, a "clean-catch" or catheterized specimen will often reveal a normal urine. Additionally, trichomonas-infected specimens are occasionally seen in both men and women with symptoms of lower urinary tract irritability. As the authors emphasize, these organisms can only be detected by microscopic examinations, but they can be pathogenic, because their detection is essential in establishing the correct diagnosis so that appropriate therapy can be instituted.

In summary, the microscopic aspect of the urinalysis is an essential part of the study that should not be omitted in daily clinical practice. When properly performed, more reliance can be placed on the microscopic observation than on dipstick chemical studies.

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An L-Lactate Sensor with Immobilized Enzyme for Use in in Vivo Studies with an Endocrine Artificial Pancreas

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We report the features of a sensor for determining L-lactate. An oxygen sensor, coupled to a nylon net with chemically bound L-lactate oxidase (EC 1.1.3.2), is inserted into an artificial pancreas (Biostator, Miles) "downstream" from the glucose sensor. We used the sensor to continuously monitor the L-lactate concentration in blood after a "glucose clamp" experiment with a diabetic patient. L-Lactate determinations in blood drawn from the patient every 15 min agreed well with results obtained by use of the L-lactate sensor.

Additional Keyphrases: *glucose sensor · L-lactate oxidase · diabetes*

The importance of L-lactate measurement in blood has increased because of its relation to specific pathological states such as shock, respiratory insufficiencies, and heart disease and because of its involvement in glucose metabolism.

The recently developed artificial pancreas, the "Biostator" (Ames Division, Miles Laboratories), is a glucose-controlled insulin infusion system for diabetic patients (*1–4*). However, the infusion of insulin provokes changes in the concentrations of certain other metabolites, including L-lactate, pyruvate, α-alanine, and ketone bodies (*5–7*). Information on the concentration of these metabolites might be useful for establishing the metabolic pattern in diabetic patients and eventually for deriving a more precise algorithm for the insulin infusion.

Here we report the development of an L-lactate sensor in which an oxygen probe is coupled with L-lactate oxidase (EC 1.1.3.2) chemically bound to nylon net. We placed this sensor in the flow stream of a Biostator unit so we could monitor the L-lactate concentration continuously during infusion of insulin into diabetics.

We recently described (*5*) the features of a L-lactate sensor obtained by immobilizing on an oxygen probe the commercially available L-lactate oxidase from *Mycobacterium smegmatis* (*8*). Although that sensor in aqueous solutions and in serum samples of controlled composition gave reliable results, it failed with the Biostator unit, because of the low (10-fold) dilution of blood and the high concentration of chemicals (phosphate buffer, sodium chloride, and preserva-

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tive) added to the heparinized blood. Therefore, we assembled a new sensor, based on L-lactate oxidase from *Pediococcus* sp., the features of which have been described (*9, 10*) and which is now commercially available.

We put the L-lactate sensor in the flow stream of the Biostator unit, after the glucose sensor, so as to measure the actual in vivo concentration of L-lactate in diabetic patients. Results of these studies have led to several new and exciting speculations on the role and control of this metabolite.

Materials and Methods

Materials

L-Lactate oxidase (activity 18 kU/g) from *Pediococcus* sp. was obtained from Toyo Yozo Co., Shizuoka, Japan; commercial lyophilized human-serum controls were from Boehringer Mannheim GmbH and Merz + Dade AG, Dudingen, Switzerland. Human fresh serum samples were from a local hospital and from diabetic patients during treatment with the Biostator artificial pancreas. L-Lactate (lithium salt; Sigma Chemical Co.) was used as the standard. All other reagents and buffers were AR grade. The nylon net used (A. Bozzone, Appiano Gentile, Italy) has a mesh of 120/cm^2, is 100 μm thick, and has 35% of its area as free surface.

The oxygen probe was a p_{O_2} electrode (Instrumentation Laboratory, Milano, Italy) consisting of a 0.1 mol/L KCl internal solution, a 12 μm-thick Teflon membrane, a platinum cathode, and a silver anode. To measure the current, we used a Model 213 ammeter (Instrumentation Laboratory) with an Omniscribe recorder (Houston Instruments).

Procedures

We immobilized the enzyme on nylon net as described previously (*8*), then secured the nylon net to the oxygen probe with the Teflon membrane and fixed the probe in a flow-through cell with an estimated volume of 40 μL.

Before determining L-lactate in serum samples, we diluted them 10- to 20-fold with the commercially available buffer used in the Biostator (an isotonic solution of phosphate buffer, sodium chloride, and preservative). These are the same conditions used with the Biostator artificial pancreas; in a series of preliminary experiments, we had checked these conditions by using standard solutions, reconstituted sera, human sera obtained from the hospital, and whole blood.

Before and after each sample, the Biostator buffer was pumped at the same flow rate, 1 mL/min.

In the experiment with the Biostator we placed a dialysis membrane over the nylon net to obviate microbial action on the immobilized enzyme.

Results and Discussion

Characteristics of the Sensor

The features of this L-lactate sensor are very similar—in terms of activity of the immobilized enzyme, the range of the calibration curve, and useful lifetime—to results previously published for a similar probe obtained by immobilizing L-lactate oxidase obtained from *Mycobacterium smegmatis* (*8*). The major difference is in the buffer and in pH effect. The results (Figure 1) clearly show the lack of the inhibitory effect that was seen for the previous enzyme (*8*).

Application to serum samples was therefore straightforward, and a correlation of 0.985 was found for nine samples of reconstituted commercially available sera as analyzed spectrophotometrically and with the sensor. Results for another analysis with human sera, performed with a commercial analysis kit for L-lactate (Sigma Chemical Co.) and the electrode probe, did not substantially differ from the results previously published, except that the same results could be obtained whatever buffer was used.

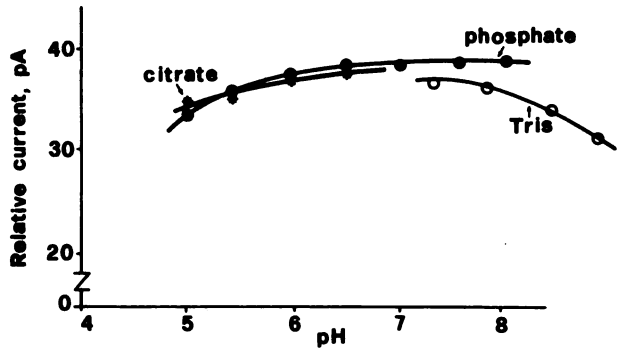

Fig. 1. pH and buffer effect on the new L-lactate sensor
On adding L-lactate to give a final concentration of 1 × 10^{-4} mol/L the oxygen current decreased; the variation is reported on the *y*-axis as relative current (percentage of saturation value)

Experiments with the Biostator

Figure 2 reports the results of an "in vivo" determination of L-lactate in heparinized blood from a diabetic being treated with the artificial pancreas (the Biostator) in a "glucose-clamped" experiment. Blood was taken from the patient via a double-lumen catheter, heparinized, diluted

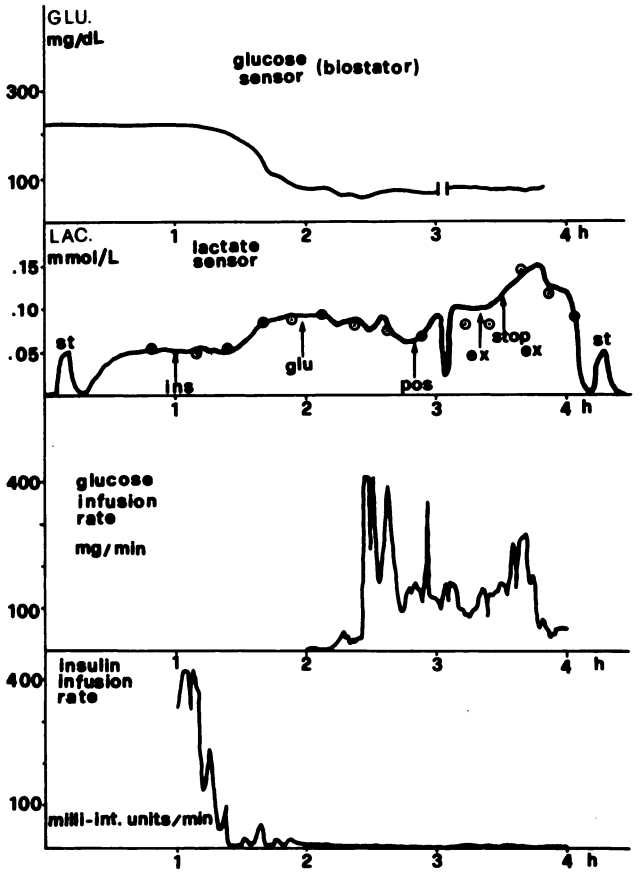

Fig. 2. Glucose and L-lactate concentration during a "glucose clamp" experiment with the Biostator
The *top panel* shows the glucose recording obtained by the Biostator, the *second panel* shows the L-lactate concentration as measured with the L-lactate sensor, and the *third* and *fourth panels* show the glucose and insulin infusion rate realized by the algorithm in the computer system of the Biostator for a glucose clamp experiment and guided by the glucose response. The meaning of the *arrows* is explained in the text

with buffer, and pumped through the glucose sensor, then through the L-lactate sensor that had been placed in series with it. The value for glucose read by the computer drives, via a complex algorithm, an infusion pump for insulin and a second pump for glucose.

We calibrated the L-lactate sensor by using the device used for glucose calibration in the Biostator system, substituting for the glucose standard provided with the Biostator an L-lactate standard prepared in the same buffer.

During the first hour we recorded the glucose and the L-lactate concentration and calibrated the L-lactate sensor.

At the time indicated by "ins" in Figure 2, insulin was infused; the glucose concentration soon returned to normal, but the L-lactate concentration suddenly increased.

At the second hour, at "glu" in Figure 2, glucose was introduced into the bloodstream to "clamp" the glucose concentration at a predetermined value. To this we ascribe the changes in concentration recorded for the L-lactate as a result of the glycolytic pathway.

At "pos" in Figure 2 the position of the patient was changed such that the catheter briefly dropped from its initial position and no more blood was aspirated. When this happened, the Biostator stopped recording glucose values, an alarm rang, and the printer discarded the fault values (shown in Figure 2 by a break in the top line).

At "ex" in Figure 2 the patient was requested to do a simple physical exercise and at "stopex" he was told to stop.

At the fourth hour the experiment was stopped and the sensor was recalibrated against the same standard. During this period L-lactate was continuously determined without delay for the patient by using the same sample of the glucose monitor without any added reagent or any pretreatment, a measurement in real time on a specimen that ordinarily is wasted.

The comparison of calibration at the beginning and at the end of the experiment shows that the sensitivity of the probe did not vary during the experiment. Moreover, every 15 min blood was sampled from the patient for an independent analysis of L-lactate, done the next day. The dots on the second panel of Figure 2 show the results of these separate L-lactate determinations and illustrate how comparable the results by both methods are.

The third and fourth panels of Figure 2 show the infusion rates for insulin and glucose, obtained by the computer. These illustrate the "delay" between the infusion of insulin and the response in the blood of glucose and of L-lactate or between the glucose infusion and the L-lactate in the blood. The experiment shows also the large variations in L-lactate concentration that take place within a few minutes and how an L-lactate sensor may be useful in further studies.

The realization of the L-lactate sensor opens new possibilities to studies of diabetics. Several interesting speculations arise after study of the rate and the amount of this metabolite as related to the glucose variations, and by measuring the restoration to normal or the alterations in this circulating metabolite, whose fate is determined by the glycolytic pathway.

Use of the lactate sensor should lead to the development of a more nearly accurate algorithm for infusion of insulin or glucose in delicate cases such as surgery of diabetic patients where the glucose clamp is used.

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References

1. Clemens AH, Chang PH, Myers RW. Le developpement d'un systeme automatique d'infusion controlé pour la glycemie, son systeme de dosage du glucose et ses algorithmes de control. *Journ Annu Diabetol Hotel* 269–278 (1976).

2. Clemens AH, Chang PH, Myers RW. The development of BIOSTATOR, a glucose controlled insulin infusion system (GCIIS). *Horm Metab Suppl* 8, 23–33 (1977).

3. Fogt EJ, Dodd LM, Clemens AH. Development and evaluation of a glucose analyzer for a glucose-controlled insulin infusion system (Biostator). *Clin Chem* 24, 1366–1372 (1978).

4. Clemens AH, Hough DL, D'Orazio PA. Development of the Biostator glucose clamping algorithm. *Clin Chem* 28, 1899–1904 (1982).

5. Cohen RD, Wood HF. *Clinical and Biochemical Aspects of Lactic Acidosis,* Blackwell Sci. Pub., London, 1976.

6. Brunetti P, Calabrese G, Massi-Benedetti M, Santeusanio F. Il pancreas artificiale endocrino nella ricerca e nella clinica. *G Ital Diabetol* 2, 53–74 (1982).

7. Noy GA, Buckle ALJ, Alberti KGMM. Continuous monitoring in vivo of blood glucose, L-lactate, alanine and 3-hydroxybutyrate. *Clin Chim Acta* 89, 135–144 (1978).

8. Mascini M, Moscone D, Palleschi P. A L-lactate electrode with L-lactate oxidase immobilized on nylon net for blood serum sample in flow system. *Anal Chim Acta* 157, 45–51 (1984).

9. Matsunaga T, Karube I, Teraoka N, Suzuki S. Determination of cell numbers of lactic acid producing bacteria by L-lactate sensor. *Eur J Appl Microbiol Biotechnol* 16, 157–160 (1982).

10. Mizutani F, Sasaki K, Shimura Y. Sequential determination of L-lactate and lactate dehydrogenase with immobilized enzyme electrode. *Anal Chem* 55, 35–38 (1983).