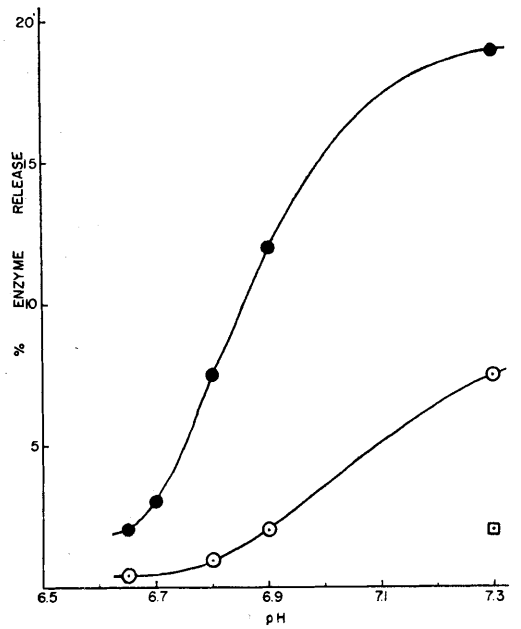

FIG. 1. Effect of pH and adenosine on enzyme release from platelets. Points represent average release in per cent of 3 enzymes. Each point represents 2-4 experiments. ● , no adenosine; ⊙ , 10^{-6}M adenosine; square with dot in it, + 10^{-4}M adenosine.

Summary. 1. Nucleoside diphosphokinase, 3-phosphoglycerate kinase and enolase are released from human platelets during the preparation of platelet concentrates. 2. Enzyme release is reduced by lowering the pH or by addition of adenosine. 3. Enzyme release is greater when the platelets in the concentrates are clumped.

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Measurement of Low Rates of Oxygen Consumption With a Horizontal Capillary-Differential Syringe Manometer.* (32169)

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The simplicity and accuracy of measurement obtainable with differential respirometers, especially where low rates of gas consumption are concerned, are decided advantages when this type of instrument is compared with conventional open limb manometers. Differential manometry is especially useful in systems where it is necessary to distinguish oxygen consumption by cells from a high rate of oxygen uptake by the suspending medium (sperm cells in seminal plasma, for example). Other applications and advantages of the differential techniques have been well described by Umbreit(1). This report describes a small differential syringe manometer with a 30 mm horizontal capillary which offers important advantages over

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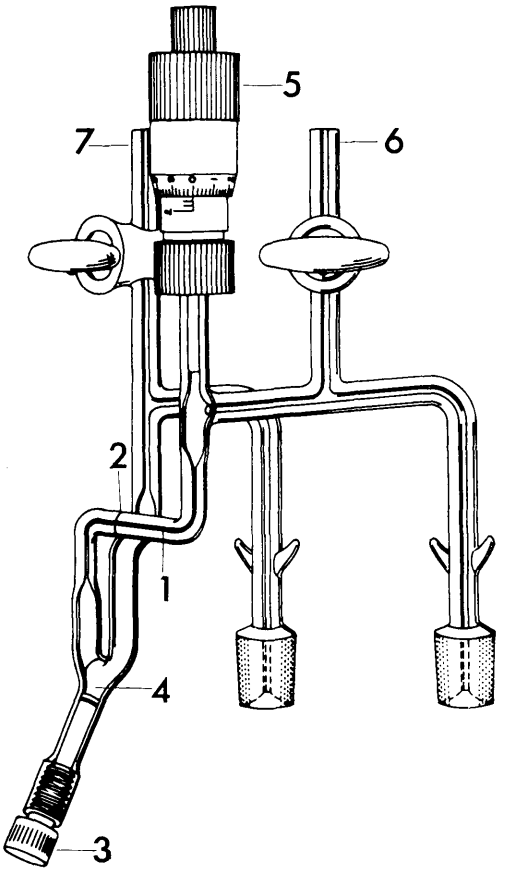

FIG. 1. Differential Syringe Manometer: (1) Horizontal capillary (30mm), (2) Reference line, (3) Adjustment screw, (4) Manometric fluid reservoir, (5) Micrometer syringe, (6) Venting tube for reaction flask, (7) Venting tube for reference flask.

the conventional vertical column differential manometer both in ease of handling and in simplicity and accuracy of measurement and which is capable of reproducibly measuring oxygen consumption by cells at rates as low as 1 μliter per hour.

Description of the apparatus. The microgasometer to be described is a modification of a commercially available instrument.‡ An isometric drawing of the modified instrument is shown in Fig. 1. The length of the horizontal capillary has been increased to 30 mm so that the meniscus of the manometric fluid may be kept in the horizontal portion of the capillary throughout an experiment. This overcomes a serious shortcoming in the original design since it eliminates the necessity for frequently adjusting the fluid mensicus in order to keep it within the horizontal portion of the capillary (and, therefore, retain the desired degree of sensitivity). Furthermore, the use of a 30 mm horizontal capillary permits the sensitivity and accuracy§ to be significantly increased by reducing its bore. In addition, the reference mark has been placed in vertical alignment with the large leg of the manometer. This alignment minimizes any error which can occur if the manometer is not mounted precisely in the same angular position each time.

In order to achieve the desired degree of sensitivity, the bore of the capillary was reduced to approximately 0.6 mm. Because of the fine capillary it was necessary to keep the glassware very clean and to employ a suitable manometric fluid (distilled water containing approximately 0.2% of a non-ionic, non-foaming surfactant, such as "Liqui-Nox" was found adequate). The small size and compactness of the apparatus made cleaning and handling relatively simple operations, as compared to the cleaning and handling difficulties encountered with the larger conventional vertical column differential manometers.

To facilitate mounting on the Warburg Bath, an aluminum channel 1″ wide and ½″ deep (outside dimensions) by ⅛″ wall thickness was used. Two thumbscrews were used to hold 2 aluminum strips against the vertical legs of the manometer. Thin rubber strips between the glass and metal protected the glass against breakage.

Volume changes due to gas absorption or

‡ Manufactured by Roger Gilmont Instruments, Inc., 161 Great Neck Road, Great Neck, N. Y., Catalog No. W-4200.

§ Assuming the ideal gas law to apply, it has been shown(3) that the sensitivity of a horizontal capillary approaches infinity as the capillary bore appoaches zero. The sensitivity of a vertical capillary, however, approaches a finite value which is determined by the ratio of the total pressure to the total volume of the system. For this particular apparatus, further reduction in the bore of a vertical capillary type results in no significant increase in sensitivity. Any further increase in sensitivity may be achieved only with a horizontal capillary of finer bore.

TABLE I. Accuracy and Reproducibility of Measurements of the Release of Known Volumes of Nitrogen.

	Volumes in μliters						
Theoretical	6.097	12.19	24.38	60.97	121.95	63.68*	
Experimental (mean of 4 determinations)	6.500	12.73	22.91	55.87	118.99	65.94	
Systematic error†	+.403	+.54	—1.47	—5.10	—2.96	+2.26	
Random error ($2\bar{\sigma}$)‡	.31	.88	1.21	3.07	5.07	5.80	
							Avg‖
% Systematic error§	+6.6	+4.4	—6.0	—8.4	—2.4	+3.6	—0.4
% Random error§	5.1	7.2	5.0	5.0	4.2	9.1	5.9

* Measurements made at 37.0°C, all other measurements at 24.8°C.

† Experimental (mean) — theoretical.

‡ $\bar{\sigma}$ = standard deviation of mean = $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$. d = exp (individual) — exp (mean); n = No. of determinations.

$2\bar{\sigma}$ corresponds to 95% confidence limits from the mean.

§ % error = $\frac{\text{error}}{\text{theoretical}} \times 100$.

‖ Algebraic average.

Measurements were made with 6 ml Warburg Flasks. The center compartment of the reaction flasks contained 0.4 ml of a standard solution of potassium ferricyanide, 0.4 ml 4N NaOH and 0.2 ml distilled water. The side arms contained 0.1 ml saturated hydrazine sulfate solution. The control flasks were prepared in the same manner except that 0.4 ml distilled water was added in place of potassium ferricyanide. After a 10 min equilibration period the contents of the side arm were tipped in and measurements taken. The reaction was complete within 10 minutes. Mean values were taken from 4 replicates.

release were measured directly from the changes in the settings of the micrometer syringe necessary to restore the manometric fluid meniscus to the reference point. In order to achieve an equilibrium fluid meniscus and consequently a reproducible reading, the final reading was always taken by approaching the reference point from the syringe side of the capillary; that is, by turning the micrometer in a clockwise direction. The readings were consistently reproducible to ± 0.3 μliter.

Accuracy and reproducibility of measurement. We have carried out several experiments with this apparatus in order to obtain data on accuracy and reproducibility and to test its applicability to a typical biological system. One estimate of accuracy and reproducibility was obtained by measuring the release of a known volume of nitrogen gas using the ferricyanide-hydrazine method described by Michaelis and Rona(2). Results are shown in Table I. As can be seen, volumes of gas as small as 6.0 μliters could be accurately determined with good reproducibility. The accuracy of measurement can be estimated by the algebraic average of the systematic errors. Since this value is negligibly small (less than 1%), it may be concluded that there is no significant systematic error so that the true value may be approached by taking a relatively small number of measurements. Reproducibility of measurement is shown by the average of the random errors and amounts to 5.9%. The large value of 9.1% at 37°C is typical and is probably caused by the greater difference between ambient and bath temperature. Total immersion might reduce the error at the higher temperature to the same level as at the lower temperature.

A more practical estimate of reproducibility was obtained by selecting a system in which gas absorption occurred over a relatively long period of time and at rates which could be easily varied. The measurement of the succinic acid dehydrogenase activity of an unsupplemented guinea pig liver homogenate was found adequate for these purposes. The results of studies on oxygen consumption using this system are shown in Table II. Uptake was followed over a 3-hour period and changes in micrometer setting were recorded approximately every

TABLE II. Measurements of Oxygen Consumption by an Unsupplemented Guinea Pig Liver Homogenate.

Homogenate concentration, percent (w/v)	Oxygen consumed, μl/hr	
	24.8°	37°
1.0	15.15†± .52‡	28.23 ± 1.88‡
.5	7.54†± 2.23	—
.5 *	5.70 ± 1.50	—
.25*	2.88 ± 1.05	3.34 ± 1.04

* Homogenate frozen and thawed once.

† Mean of 3 determinations, all others of 5 determinations.

‡ Random error ($2\bar{\sigma}$), see Table I.

A homogenate was prepared by blending fresh guinea pig liver in ice cold 8.5% sucrose solution. Reaction flasks contained 0.8 ml pH 7.4 phosphate buffer (0.1 M) and 0.2 ml liver homogenate. The side arms contained 0.15 ml sodium succinate (0.2 M). Control flasks were the same except that distilled water replaced the liver homogenate in the center compartment. Center wells contained filter paper strips soaked in 4N NaOH. After allowing 10 minutes for equilibration, the contents of the side arms were tipped in and measurements started.

20 minutes. The reported rates were determined from 30-minute averages taken over the linear portion of the uptake curve. It can be seen that oxygen uptake is proportional to tissue concentration over the interval studied and that oxygen consumption at rates as low as 3 μliters/hour could be measured with a reproducibility of approximately ± 1 μliter/hour.

Summary. A small differential syringe manometer with a 30 mm horizontal capillary is described. Estimates of accuracy and reproducibility were made by measuring the release of known volumes of nitrogen gas and by measuring the slow respiration rates that occur in unsupplemented liver homogenates. Absorption of oxygen at rates as low as 3 μliters/hour could be measured with a reproducibility of ± 1 μliter. The small size of the apparatus together with the accuracy, reproducibility, and simplicity of measurement recommend its use over conventional vertical column differential manometers.

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Ouabain Effect on Transmural Transport of Potassium by Canine Small Intestine.* (32170)

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The absorption of Na+ by mucosal epithelium of the small intestine has been shown to be an active process, *i.e.*, Na+ can be transported against an electrochemical gradient, and the kinetics of the relationship between Na+ absorption and NaCl concentration of the luminal solution appear to be those of a saturable carrier system(1,2). In contrast to the situation for sodium, previous work has suggested that potassium absorption can be explained purely on the basis of passive equilibration due to existing electrochemical gradients(3). Recently, however, several reports have suggested that potassium interacts with a mucosal membrane carrier. For example, the apparent affinity of the mobile carrier for the transported sugar, arbutin, is depressed by K+(4), and the Km of the carrier for 6-deoxyglucose is increased from 2 mM to 200-300 mM by K+ loading(5). Another line of evidence that suggests a carrier mechanism is the finding that intestinal K+ absorption can be depressed and Na+ absorption enhanced by aldosterone administration in the rat(6). The present report describes studies that support the hypothesis that K+ interacts

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