durch einen elektrischen Funken gezündet. Während der Verbrennung wird über das Ventil (13) in das Absorptionsgefäss (12) eine vorgegebene Menge Absorptionslösung (z.B. Monoäthanolamin in Methanol) eingefüllt. Ebenfalls während der Verbrennungszeit wird die durch die Fülleinrichtung (2) mit Szintillatorlösung (z.B. Dioxanszintillator[9]) gefüllte Messflasche unter den Ausstoss (3) des Absorptionsgefässes gefahren. Nach einer der kurzen Verbrennungsdauer angemessenen Verzögerung wird durch eine am Ende des Rohres (10) befindliche Sprühdüse (11) eine bestimmte Menge destillierten Wassers eingeblasen. Sofort nach der Spülung veranlasst die Steuervorrichtung (17) das langsame Absenken des gasdichten Kolbens (8) im Verbrennungsraum, währenddessen die Steuervorrichtung das Einwegventil (7) geöffnet hat und durch das Sprührohr (10) Stickstoff eingeleitet wird. Auf diese Weise werden Verbrennungsprodukte der Probe zusammen mit dem Spülwasser in die Absorptionslösung eingebracht. Wenige Sekunden nach dem Aufsetzen des Kolbens (8) auf dem Boden des Verbrennungsgefässes (5) öffnet die Steuervorrichtung (17) das Mehrwegventil (15) und die im Absorptionsgefäss (12) befindliche Lösung läuft über den Auslass (3) in das bereitstehende mit Szintillator-Lösung versehene Messfläschchen. Das Transportband (18) setzt sich in Bewegung und bringt diese Fläschchen unter den Mechanismus (4), der das Fläschchen verschliesst. Während dieses Vorgangs wird über das Ventil (13) das Absorptionsgefäss (12) gespült. Dann wird durch den Schaft (19) der Kolben (8) wieder in die Lochscheibe zurückbefördert, und diese dreht sich um eine Stelle weiter, so dass der nächste Kegelstumpf mit der Probe einfahrbereit ist. Die Steuervorrichtung (17) wird durch die Zeitvorwahl des Flüssigszintillationsspektrometers getriggert. Werden 5 min Zählzeiten vorgewählt, so rückt die Lochscheibe alle 5 min um einen Platz weiter. Bei Verwendung einer 25plätzigen Lochscheibe kann die Apparatur also 2 h unbeaufsichtigt vollautomatisch laufen. Wird die Lochscheibe für 50 Proben ausgelegt oder die Zählzeit verkürzt, so kann die Apparatur dementsprechend länger unbeaufsichtigt funktionieren.

Summary. To avoid quench corrections in liquid scintillation counting, an automatic sample-combustion device has been developed. Triggered by the preset time of whatever liquid scintillation counter is available and connected to it by an extended surveyor belt, the designed machine burns each sample, dissolves the combustion products, CO_2 and H_2O, in the scintillator solution and introduces the vial in the liquid scintillation counter.

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[9] Dioxanszintillator = in 1000 ml Dioxan befinden sich: 4 g DPO + 60 g Naphthalin + 20 ml Äthylenklykol + 100 ml Methanol.

A Simple Method for the Study of the Uptake of Radioactive ^{32}P Phosphate by Red Blood Cells

Generally, uptake of phosphate labelled with ^{32}P by red blood cells is studied either by determining the decrease in radioactivity of the medium[1–7] or by determining the increase of the radioactivity of the cells after centrifuging and washing with medium of 0 °C[8–10]. The former method is less satisfying for the study of initial rates of uptake, because the decrease of radioactivity will be small after short incubation periods. The second method, on the other hand, is rather elaborate because of the washing procedure. This washing is avoided in the following method which consists essentially in one centrifugation and determining the radioactivities of equal volumes of cell sediment and corresponding supernatant. The ratio of the activities is a measure for the uptake of the isotope. We have determined phosphate uptake according to this method and the results are compared with those of the other two procedures.

Material and methods. The new method was carried out as follows: uptake of phosphate is stopped at appropriate times by pipetting 2 ml of red blood cell suspension into centrifuge tubes chilled at 0 °C. Five capillary tubes are filled for about 80% with the chilled suspension, and are centrifuged in a hematocrit centrifuge after sealing with a small gas flame. The capillaries are then placed in a copper plate provided with 5 parallel holes in which they just fit. The slide is covered by a 1 mm copper shield with on top 2 mm of lead. An opening is left in this shield in the middle and perpendicular to the capillaries (Figure 1). A Geiger-Müller tube is placed just above this opening. In this way we are able to determine the radioactivity of a 5 mm part of the capillaries filled with trapped cells or medium. The mean internal diameter of the capillaries used is 1 mm. The concentration of the absorbed phosphate is calculated by means of equation (1).

$$C_{c,t} = \frac{(A_{c,t} - \mathrm{ECF}\, A_{s,t})\, C_{s,o}}{(1 - \mathrm{ECF})\left(A_{s,t} + \frac{(A_{c,t} - \mathrm{ECF}\, A_{s,t})\, H\,(1 - \mathrm{ECF})}{100 - H\,(1 - \mathrm{ECF})}\right)} \quad (1)$$

$C_{c,t}$ is the concentration of the absorbed phosphate in mmoles/l of cells. $A_{c,t}$ and $A_{s,t}$ are the activities of a 5 mm piece of the capillaries filled with sedimented cells and with supernatant respectively. ECF is the fraction of extracellular fluid in the cell sediment. $C_{s,o}$ is the concentration of added phosphate expressed in mmoles/l of medium (concentration in the supernatant at zero time). H is the hematocrit value in percents. The values of ECF are determined with ^{131}I labelled iodinated serum albumin after a modification of the procedure of BEILIN et al.[11] by using capillaries instead of 11 mm centrifuging tubes and avoiding in this way the necessity of freezing the tubes first before cutting them into pieces.

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The uptake of labelled phosphate determined by measuring the decrease in medium activity (the supernatant method) is calculated according to equation (2).

$$C_{c,t} = (A_{s,o} - A_{s,t}) \\ (100 - H\,(1 - ECF))\, C_{s,o}/A_{s,o}\, H\,(1 - ECF) \qquad (2)$$

The symbols are the same as in formula (1), $A_{s,o}$ is the activity of the medium at the start of the experiment, and is related to the same volume as $A_{s,t}$.

The absorption of phosphate determined by measuring the radioactivity of the hemolysate after washing the cells 3 times with 2 volumes of medium and hemolyzing them with one volume ice-cooled 5% w/v trichloroacetic acid (washing method) is calculated according to equation (3).

$$C_{c,t} = A_{c,t}\, C_{s,o}/A_{s,o} \qquad (3)$$

$A_{c,t}$ and $A_{s,o}$ are measured in equal volumes of red cells (H = 100%) and medium respectively.

Results and discussion. It is seen from Figure 2 that the initial rates of phosphate uptake can be evaluated far more accurately from the data obtained with the capillary method than by measuring the decrease in activity of the supernatant. The mean standard error of the values by the capillary method amounted to 2.32% with a minimum value of 0.002 mmoles/l of cells at an initial concentration of 1.59 mmoles/l of medium. The average standard error for the supernatant method was 0.015 mmoles/l of cells. For the washing method we found a mean standard error of 1.5%. With this method the standard error is smaller than with the capillary method, but there is a loss of activity as can be seen in Figure 2. Approximately 10% of the original activity is found in the precipitate obtained after addition of trichloroacetic acid, and about 20% of the activity is lost during washing the cells.

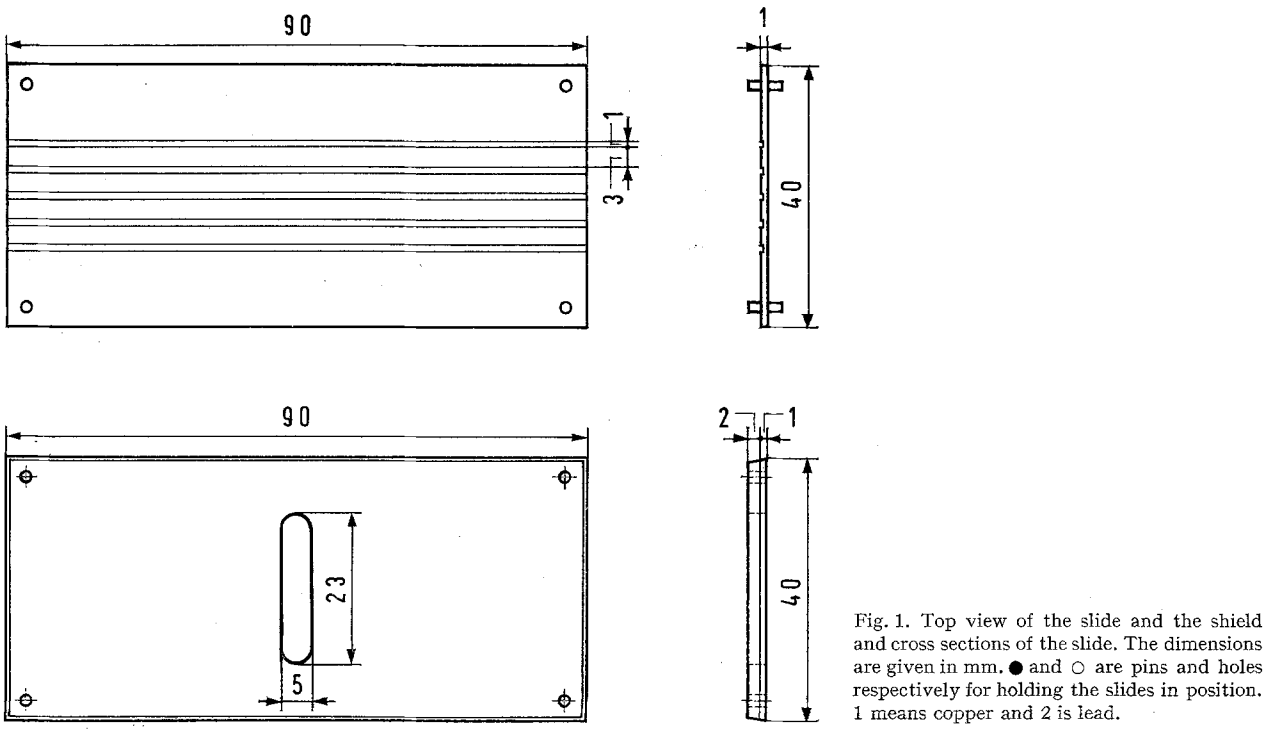

Fig. 1. Top view of the slide and the shield and cross sections of the slide. The dimensions are given in mm. ● and ○ are pins and holes respectively for holding the slides in position. 1 means copper and 2 is lead.

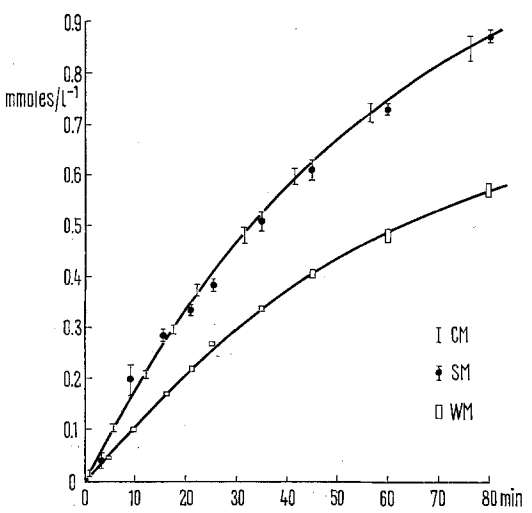

Fig. 2. Uptake of radioactive phosphate by human erythrocytes at pH 7.4 and 37 °C, expressed in mmoles phosphate/l of cells. Initial orthophosphate concentration is 1.59 mmole/l medium. The heights of the signs is equal to the standard error. CM, capillary method; SM, supernatant method; WM, washing method. The value of trapped volume (ECF) used for the calculation of $C_{c,t}$ (see equation (1)) was 0.023 with a standard error of 0.0014. H was 37%.

Zusammenfassung. Die P^{32}-Aufnahme in Erythrocyten wird durch Aktivitätsmessungen über Sediment und Überstand in einer Hämatokrit-Kapillare bestimmt.

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