or in Figs. 1c and d (condition B), the actual salting-out curve and differential curve will be the sum of the individual curves.

The modified procedure (condition A) has been applied to a study of the proteins in saline extracts of normal and pathological human thyroid glands[5].

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Wellcome Laboratory of the Unit of Clinical Investigation, P. G. STANLEY
The Royal North Shore Hospital of Sydney,
Crow's Nest, N.S.W. (Australia)

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A device for rapid collection of cells from radioactive medium

Rapid exchange of metabolites between cells and the medium may make accurate determinations of exchange rates difficult, because of the time required to wash the cells free of radioactive medium. A device, called a cell washer, has been designed which permits fairly accurate, rapid sampling of cells from radioactive medium (Fig. 1). Cells suspended in radioactive medium are separated from the latter as the cell washer is centrifuged and the cells pass through the denser, non-radioactive rinse solution and collect at the tip of the column.

Routinely, 12-cm sections of polyvinyl tubing (Transflex No. 18, Minnesota Mining and Manufacturing Company, Minneapolis, Minn.) are held horizontally and filled with the desired rinse solution by means of a syringe. One end of the tubing is carefully doubled over for a distance of about 1 cm and fastened with a few turns of thin copper wire. Into the other end of the polyvinyl tubing is inserted the tip of an empty, funnel-shaped polyethylene sample holder having an over-all length of 5.5 cm. The sample holders are made by drawing out one end of a piece of polyethylene tubing, inside diameter 0.45 cm, to a tip fine enough so when cut off short it fits tightly into the polyvinyl tubing. The completed cell washer is supported by inserting the polyvinyl tubing into a glass sleeve, 11 cm long, inside diameter 0.635 cm, with the shoulder of the sample holder resting on one end of the tubing. The glass sleeve, in turn, is contained in an 18 × 150 mm test tube. Radioactive cell suspensions up to 3.0 ml are placed in the sample holder and centrifuged horizontally for 3 min at 840 × *g*. The cells, quantitatively collected at the tip of the cell washer, are recovered by slicing off the tip with a blade, removing the wire, and rinsing out the cells with a fine stream of water. Sample chambers are washed and used again while the tubing is discarded. In practice, a number of cell washers are assembled in advance of use.

In order to test the efficacy of this device, human cells of two strains, HeLa[1] and conjunctival[2], grown *in vitro*, were incubated in EAGLE's medium[3] with ^{45}Ca under the conditions specified in each experiment. Table I shows that the recovery of counts at the tip of the cell washer, in the 11th cm of tubing, depends upon the presence

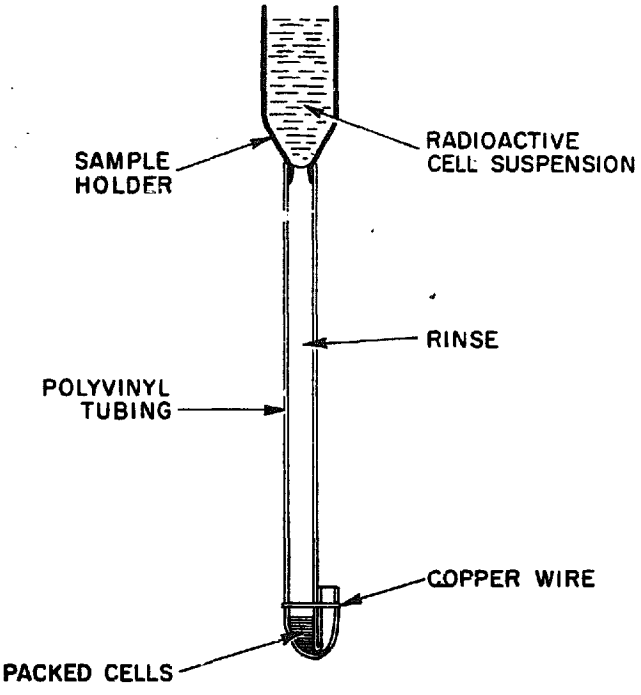

Fig. 1. The cell washer. Radioactive solution overlies a denser non-radioactive rinse solution. The interface between the two solutions is less than 0.92 mm². Cells pass through rinse and collect at tip of washer, upon centrifugation. This device permits rapid collection of cells from radioactive medium. Inside diameter of polyvinyl tubing 0.042 in.

TABLE I

ASSOCIATION OF ^{45}Ca ACTIVITY WITH CELLULAR UPTAKE

Trypsinized HeLa cell suspensions were incubated with ^{45}Ca and then centrifuged in cell washers. The polyvinyl tubings containing the rinse and the cells were then detached from the sample holders, and 1-cm segments of tubing, at various distances from the interface, cut off. Their contents were rinsed out with water, dried and assayed for radioactivity. The 11th cm of tubing contained packed cells or any sediment which might have resulted from centrifugation of the radioactive medium. Medium A: EAGLE's medium[3] containing 10% dialysed horse serum and 1 mM Ca^{2+}. Medium B: solution of 55 mM EDTA, 3.2 μM Ca^{2+}, pH 7.6–8.0. Expt. 1: 152 500 counts/min/ml; Expt. 2: 218 000 counts/min/ml. Rinse solutions were similar in composition to the respective incubation media, but contained 20% (w/v) dextrose. Values are given as means ± standard error of the mean.

Expt.	*Millions of cells per sample*	*Incubation medium and time (min)*	*No. of observations*	*^{45}Ca activity (counts/min/cm)*			
				Distance from interface (cm)			
				4	*7*	*9*	*11*
1	0	A (10)	2	6 ± 5	0 ± 0.5	0 ± 0	0 ± 0
	1.97	A (10)	2	2 ± 0.5	0 ± 0.5	0 ± 0.5	154 ± 10
2	0.80	A (20)	4	—	2 ± 2	4 ± 1	145 ± 6
	0.80	B (20)	4	—	14 ± 14	4 ± 3	11 ± 1

TABLE II

^{45}Ca ACTIVITY ASSOCIATED WITH DIFFERENT NUMBERS OF CELLS

Different volumes of a single cell suspension were centrifuged in cell washers. Aliquots were also taken for cell enumeration in each experiment. Cells were incubated for 15 min in medium A containing ^{45}Ca. Expt. 1: 321 000 counts/min/ml; Expt. 2: 302 600 counts/min/ml. The rinse solution was medium A containing 20% (w/v) dextrose. Where two observations were made, mean value is given.

Expt.	*Millions of cells per planchet*	*No. of observations*	*^{45}Ca activity*	
			counts/min/planchet	*counts/min/million cells*
1	0.04	2	70	1750
	0.08	1	160	2000
	0.12	1	219	1825
	0.16	2	250	1560
2	0.28	2	101	375
	0.84	1	276	329
	1.12	1	415	371

of cells. Incubation of cells with EDTA diminishes the amount of radioactivity recovered in association with the cells, indicating that the radioactivity present at the tip of the column can be largely attributed to cellular uptake of ^{45}Ca, rather than contamination of the cells with radioactive medium. The amount of radioactivity recovered at the tip of the washer is proportional to the number of cells in the sample (Table II).

The Ca^{2+} concentration of the rinse has no significant effect upon the amount of ^{45}Ca recovered, indicating that the passage of cells through the non-radioactive rinse is so rapid as to prevent a significant amount of interaction between the cells and the rinse solution. Addition of EDTA to the rinse solution had no effect upon ^{45}Ca recovery[4]. The efficacy of the rinsing procedure depends upon the relative densities of the sample medium and the rinse solution. The presence of 20% (w/v) glucose in the latter prevented the downward movement of extraneous ^{45}Ca.

The usefulness of this device is shown by the fact that ^{45}Ca-labelled cells collected by means of the cell washer lose approx. 25% of the original radioactivity in 15 min of incubation with non-radioactive Ca (ref. 4). This rate of exchange would probably have given rise to less accurate determinations of ^{45}Ca activity, if the method of collection had been done by conventional, more time-consuming methods.

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Harvard School of Public Health, Department of Nutrition, Boston, Mass. (U.S.A.)

HSIANG JU LIN
ROBERT P. GEYER

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