absorb these stains are considered viable. Damaged and dead cells take up the stain readily.

The pH of the Hanks' B.S.S. in which the cells were rotated was 7.4. At the end of the experiment the pH of the Hanks' had dropped to 7.2.

It is possible to measure changes in optical density by connecting an automatic pen recorder to the galvanometer. This is useful for recording changes over a short period as when platelet clumping occurs in a matter of minutes in the presence of A.D.P.[12]. When cell aggregation is a gradual process, there is no problem in accurately recording the correlated decrease in optical density by taking readings at appropriate times[13].

Résumé. Une technique a été établie pour estimer quantitativement l'aggrégation des cellules des tissus dissociés par une méthode turbidimétrique. Des fibroblastes de l'embryon de poulet ont été mis en rotation sur un absorbtiomètre. Pendant l'aggrégation des cellules vivantes, on observe une diminution de la densité optique de la suspension mésurée par un galvanomètre.

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[12] W. F. J. CUTHBERTSON and D. C. B. MILLS, J. Physiol. *168*, 29P (1963).

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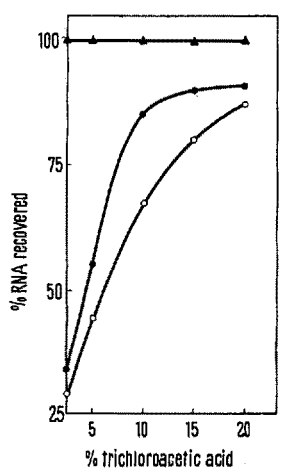
On the Prevention of Loss of RNA into Lipid Solvents

In the current methods for delipidation of acid-precipitated liver homogenates, in which ethanol or acetone is used as the first of the lipid solvents, considerable amounts of RNA are lost into the solvent phase[1-3]. Such losses have been shown to be minimal if 96% ethanol containing 10% potassium acetate is used instead of aqueous ethanol[4]. It will be shown in the present note that 100% recoveries of RNA could be obtained from the acid precipitates if dioxane is used as the first of the lipid solvents.

Rat liver cytoplasmic fraction, prepared according to the method of PALADE and SIEKEVITZ[5], was used in these studies, since maximum losses of RNA into the lipid solvents have been shown to occur from this fraction[2]. Duplicate samples were treated with increasing concentrations of cold trichloroacetic acid and after 10 min centrifuged in the cold at 2500 rpm, for 5–10 min. The precipitates were extracted thrice by centrifuging with cold trichloroacetic acid of the same concentrations, and were then treated individually either with 96% ethanol or acetone or dioxane. In other experiments, mixed solvents (ethanol-ether, 2:3; ether-dioxane, 2:3; acetone-dioxane, 2:3; or acetone-ether, 2:3) were used for the first extraction. Precipitates obtained with low concentrations of trichloroacetic acid dissolve in small amounts of dioxane. However, complete reprecipitation occurs when the dioxane added is about 8-fold excess of the amount of acid in the precipitates. All operations were carried out at 2–4 °C except treatment with dioxane, which was done at 5–8 °C in order to avoid freezing. The sediments were then successively extracted twice with ethanol-ether (3:1) and twice with ether. RNA in the final precipitates was extracted with hot 5% trichloroacetic acid and determined by the orcinol reaction as described by SLATER[6].

RNA recovered from samples precipitated with different concentrations of acid, and subsequently extracted with cold ethanol or acetone or dioxane, is shown in the Figure. Treatment with cold ethanol or acetone results in an appreciable loss of RNA into the solvent phase. The losses are more marked in samples precipitated with low concentrations of trichloroacetic acid. Thus after precipitation with 2.5 or 10% acid, the RNA recoveries after ethanol treatment are 30% and 87% and after acetone treatment, 35% and 90% respectively. However, RNA equivalent to that present in the samples not subjected to any lipid solvent treatment, could be



Recovery of RNA from trichloroacetic acid precipitated cytoplasmic fraction after extraction with 96% ethanol (o—o) or acetone (●—●) or dioxane (▲—▲). Amounts are expressed as % of RNA present in samples precipitated with 2.5 or 5% trichloroacetic acid, which were not extracted with lipid solvents.

[1] P. R. VENKATARAMAN and C. U. LOWE, Biochem. J. *72*, 430 (1959).
[2] P. R. VENKATARAMAN, Biochim. biophys. Acta. *39*, 352 (1960).
[3] T. HALLINAN, A. FLECK and H. N. MUNRO, Biochim. biophys. Acta. *68*, 131 (1963).
[4] W. J. STEELE, N. OKAMURA and H. BUSCH, Biochim. biophys. Acta. *87*, 490 (1964).
[5] G. E. PALADE and P. SIEKEVITZ, J. biophys. biochem. Cytol. *2*, 171 (1956).
[6] T. F. SLATER, Biochim. biophys. Acta. *27*, 201 (1958).

obtained when the precipitates from any trichloroacetic acid concentration were treated once with dioxane prior to extraction with the other lipid solvents. RNA recoveries are also inferior when mixed solvents are used directly on the acid precipitates, except with acetone-ether (2:3) which gives almost 100% recovery of RNA from precipitates obtained with trichloroacetic acid of more than 5% concentration.

Precipitates obtained by treating cytoplasmic fraction at 0 °C with cold 2.5–5% trichloroacetic acid, are finer in texture forming rather loose and voluminous sediments apparently containing much acid. Appreciable portions of these precipitates readily go into solution in ethanol, acetone or even water. On the other hand, the precipitates obtained at higher acid concentrations (15–20%) are extensively aggregated forming rather hard pellets on centrifugation. These pellets contain much less water and are fairly insoluble in the lipid solvents or water. Therefore, in addition to the loss, into the lipid solvents, of that fraction of RNA associated with the lipoprotein complex[4,5], greater losses of RNA due to solubilization of nucleoprotein itself could also occur if low concentrations of acid were used for precipitation (low concentrations of acids are usually preferable for precipitation in order to avoid the risk of hydrolysis of RNA by high acid concentrations[3]).

Since solubilities of proteins in organic solvents depend on the dielectric constants produced in the medium[7], the choice of the first solvent for treatment of the acid precipitated proteins should be such as to produce low dielectric constant when added to the nucleoprotein precipitates containing appreciable amounts of acid. Dioxane is very soluble in water and produces much lower dielectric constant than acetone or ethanol. Moreover, its interaction with water causes extensive dehydration of the protein and denaturation of the lipoprotein complexes resulting in a quantitative precipitation of the nucleoprotein. Thus the loss of RNA into the lipid solvents could be completely abolished by treating the dilute acid precipitated samples once with dioxane prior to extraction with the other lipid solvents.

Résumé. La perte de RNA dans les solvants tels que l'éthanol et l'acétone, au cours de la délipidation des nucléoprotéines précipitées par l'acide, peut être évitée en traitant le précipité (obtenu à une concentration quelconque d'acide trichloracétique) par le dioxane avant d'extraire avec les autres solvants délipidants usuels.

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CNRS et Ecole Pratique des Hautes Etudes, Biochimie des Nucléoprotéides, Institut Biologie Physico-chimique, Paris 5e (France), 2nd January 1967.

[7] A. A. GREEN and W. L. HUGHES, *Methods in Enzymology* (Academic Press, New York 1955), vol. 1, p. 67.

CONGRESSUS

Czechoslovakia

Symposium on Experimental Gerontology

in Prague, November 29–December 1, 1967

The European Section of Experimental Gerontology, the Czech. Medical Association J. E. Purkyně and the Physiological Institute of the Czech. Academy of Sciences in Prague are organizing a Symposium with the following topics: Aging of macromolecules, subcellular structure and cells, collagen structure, aging of different tissues and organs, effect of aging on different regulation mechanisms in organs and systems with regard to their metabolic functions, adipose tissue and lipid metabolism in aging.

Further information and preliminary registration by MUDr, Eva Stuchlíková, IVth Medical Clinic, Charles University Hospital, U nemonice 2, Praha 2 (Czechoslovakia).

CORRIGENDUM

L. MUSAJO, P. VISENTINI, F. BACCICHETTI and M. A. RAZZI: *Photoinactivation of Ehrlich Ascites Tumor Cells in vitro Obtained with Skin-Photosensitizing Furocoumarins*, Experientia 23, fasc. 5, p. 335 (1966). On page 336 the name of the third author reads as follows: F. BACCICHETTI.