thus producing an orange-coloured precipitate which is probably a lead uranyl complex together with a surplus of lead acetate.

Method. Germinating seeds of *Phalaris canariensis* were used, the root-tips being fixed in permanganate of potash at 2% in distilled water for 2 h at room temperature. The fixed material was then dehydrated in the acetone series up to that of 70%. At this stage the roots usually remain all night in a saturated solution of uranyl acetate in acetone at 70%, but we substituted lead uranyl for this solution and then continued the process of dehydration and inclusion in Durcupan ACM by the usual method.

Results and discussion. The pictures thus obtained (Figure) are both quantitatively and qualitatively on a par with those obtained by the use of REYNOLD's stain in point of contrast and definition. Since the impregnation is done without prolonging the normal process of dehydration and inclusion, and the sections can be observed as soon as they have been obtained, our method enables us to avoid a certain amount of handling and saves time.

One of the drawbacks of all lead solutions is that they age so quickly, with the consequent increased risk of contamination. In the present case we were able to observe that the lead uranyl solution presented no signs of alteration after 3 months, and examination of the sections from roots contrasted by this procedure showed no contamination of any kind. We think the absence of contamination is due to the fact that the piece itself acts as an ultrafilter, retaining any particles the solution may contain on its surface, and the contrast is effected by uranium and lead atoms forming a deposit upon the structures under study and avoiding such aggregations as give rise to the sort of particles that cause contamination.

Resumen. Mediante el empleo de una solución saturada de acetato de uranilo-plomo en acetona de 70%, durante el proceso convencional de deshidratación, se ha conseguido incrementar marcadamente el contraste electrónico en ápices radicales de *Phalaris canariensis*, sin riesgo alguno de contaminación o deformación de las estructuras.

G. GIMÉNEZ-MARTÍN, M. C. RISUEÑO and J. F. LÓPEZ-SÁEZ

Instituto de Biología Celular, C.S.I.C., Madrid-6 (Spain), 7th October 1966.

A Simple Method for Reduction of Non-Specific Fluorescence

The advantages afforded by the sensitivity and rapidity of immunofluorescence may be nullified by non-specific dye deposition[1,2]. This problem is particularly acute with exudate specimens of viral antigens where debris tends to intensify background fluorescence[3]. The empirically derived procedure presented here, utilizes methods similar to those reported from other laboratories but combines them in a specific sequence[4-7].

The following is a summary of 1 of 3 experiments utilizing vaccinia infected chorioallantoic membranes (CAM) and HeLa monolayers which demonstrated the necessary sequence of adsorption and gel filtration. Fluorescein isothiocyanate labeled vaccinia antibody was dialyzed and 1.0 ml aliquots stored at − 70 °C[7]. Immediately before use, 1.0 ml of the conjugate was thawed, adsorbed once with 100 mg of buffered-saline saturated rhesus monkey liver powder and centrifuged at 23,000 *g* for 30 min at 4 °C. The supernatant was then transferred to a Sephadex-25 (medium) column which measured about 0.6 · 10.0 cm. Collection of 1.5 ml of eluate was carried out with 0.02 *M* phosphate buffer, pH 7.6. Other aliquots were treated as follows: (1) 2 adsorptions, (2) gel filtration alone, and (3) gel filtration followed by adsorption. All 4 aliquots were utilized simultaneously using standard washing and mounting procedures[7].

The Table presents staining intensities obtained with conjugates processed by different combinations of liver powder adsorption and gel filtration. Adsorption followed by gel filtration gave maximum staining intensity with infected cells where conjugate-antigen reaction was unblocked by negative serum. In addition the elimination of background staining resulted in the desired absence of fluorescence in uninfected cells or infected cell preparations pre-treated with vaccinia serum. The striking differences in staining are demonstrated by Figures (a), (b), and (c). All 3 photomicrographs are of vaccinia infected HeLa cells. Cells in (b) and (c) received a vaccinia serum

Immunofluorescence intensity with vaccinia infected CAM and HeLa cells

Conjugate treatment	Infected cells		Uninfected cells	
	Vaccinia serum	Negative serum	Vaccinia serum	Negative serum
I Adsorption and gel filtration	0	++++	0	0
II Gel filtration and adsorption	++	++++	++	++
III Gel filtration	++++	++++	++++	++++
IV 2 adsorptions	++	+++	++	++

(1) Method: direct staining. (2) Pre-conjugate serums: 1:4 dilutions of vaccinia and negative rabbit serums. (3) Conjugate: vaccinia rabbit globulin-isothiocyanate.

1 A. H. COONS, Rev. Microbiol. *8*, 333 (1954).
2 E. BEUTNER, Bact. Rev. *25*, 49 (1961).
3 M. SCHAEFFER, E. V. ORSI and D. WIDELOCK, Bact. Rev. *28*, 402 (1964).
4 W. GEORGE and K. W. WALTON, Nature *192*, 1188 (1961).
5 M. A. GORDON, M. R. EDWARDS and V. N. TOMPKINS, Proc. Soc. exp. Biol. Med. *109*, 96 (1962).
6 D. KIRSH and R. KISSLING, Bull. Wld Hlth Org. *29*, 126 (1963).
7 W. B. CHERRY, M. GOLDMAN, T. R. CARSKI and M. D. MOODY, Publ. Hlth Serv. Publs, Wash. *729*, 1 (1960).

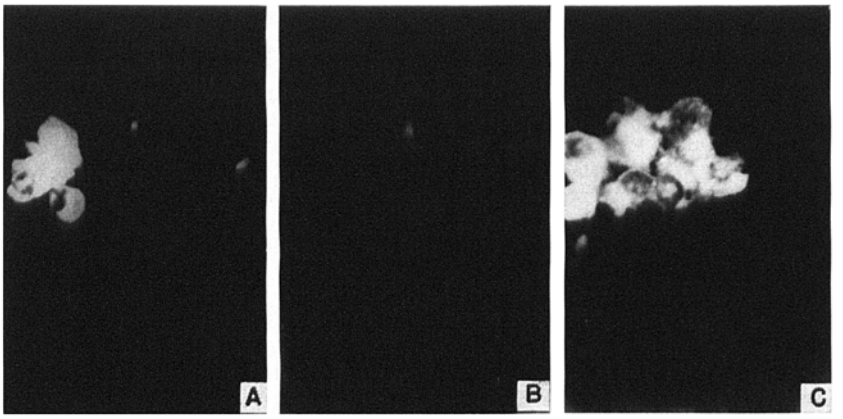

Fig. A. Vaccinia infected cells, acetone fixed, pre-treated with vaccinia negative serum for 1 h at 37 °C and for $^1/_2$ h at 37 °C with vaccinia conjugate which had been adsorbed and gel filtered.

Fig. B. Vaccinia infected cells subjected to the same procedure as in (A) but pre-treated with vaccinia serum.

Fig. C. Vaccinia infected cells subjected to the same procedure as in (B) except for substitution of vaccinia conjugate which had been gel filtered and adsorbed. × 250.

overlay but vaccinia negative serum was employed with cells in (a). Anti-vaccinia conjugate processed by adsorption-gel filtration was used with cells in Figures (a) and (b) in contrast to the reverse method of gel filtration followed by adsorption utilized for cells in Figure (c). It is noteworthy that the expected negative staining in Figure (c) which should have resembled the blocked reaction in Figure (b) actually appeared similar to the positive reaction seen in Figure (a).

The mechanisms underlying the effectiveness of adsorbing followed by filtering and the failure of the reverse procedure have still to be elucidated. For the present this method has been a conveniently simple and reliable method for enhancing immunofluorescence specificity in the diagnostic and teaching laboratory[8].

Résumé. Cette technique pour la réduction de la fluorescence non-spécifique utilise diverses méthodes communes en une série spéciale. Premièrement, les anticorps fluorescents sont adsorbés avec la poudre du foie. Suivant la centrifugation, une colonne de Séphadex-25 est utilisée pour séparer la fluorescéine libre. La méthode contraire donne une fluorescence non-spécifique qui ressemble à celle obtenue avec une seule adsorption ou Séphadex filtration.

E. V. ORSI[9], S. J. MILLIAN and R. FRANZELL

Bureau of Laboratories, New York City Department of Health, New York (New York, USA),
4th November 1966.

[8] We are grateful to Miss THELMA BURT and Mr. E. CARDONE for technical assistance.
[9] Present address: Department of Biology, Seton Hall University, South Orange (New Jersey 07079, USA).

A Micro-Technique for Performing Reactions with Volatile Reagents

Chemical reactions of very small amounts of material (micrograms or less) are performed by adaptation of macro-preparative procedures. For reasons of convenience, this involves the use of a very large excess of reagents and solvents, any minor impurities of which may lead to a major contamination of the reaction product and hence interfere with its detection and measurement. When, as often in analytical work, a large number of samples is to be submitted to the same chemical treatment, the customary procedures become tedious and time-consuming. We report a simple micro-technique for reacting small quantities of material with volatile reagents. It consists in depositing the material on a suitable support and exposing it to the vapour of the reagent. The procedure is illustrated by the following examples of the esterification of steroidal alcohols and acids.

Compounds deposited on paper. 3β-acetoxyandrost-5-en-17β-ol (150 μg) was deposited on a small piece of filter paper and suspended over a mixture of acetic anhydride and pyridine (a few drops of each) in a glass-stoppered test tube. After 16 h at room temperature, the paper was removed, dried in vacuo for 10 min and the product then eluted with ethanol. The crude product gave an IR-spectrum identical with that of authentic androst-5-ene-3β,17β-diol diacetate. Identical treatment of cortisol (3 μg), followed by paper chromatography, gave only one detectable chromatographic fraction with the mobility of cortisol acetate.

[1,2-^{3}H]testosterone (2 μg, specific activity 5.6 $\mu C/\mu M$) was deposited on paper and suspended over a mixture of [^{14}C]acetic anhydride (specific activity 0.56 $\mu C/\mu M$) in benzene (20%, w/v; 30 μl) and pyridine (50 μl). After 38 h at 37 °C, the paper was dried in vacuo and the product eluted. The ^{3}H/^{14}C ratio of the crude product was 20.2, after purification by paper chromatography it was 23.6. When acetylation was performed in the conventional manner (in solution), the corresponding values were 4.7 and 21.3 respectively. After identical treatment of [6,7-^{3}H]oestrone (2 μg, specific activity 4.7 $\mu C/\mu M$) the corresponding values were 19.2 and 17.8 respectively when the reaction was performed by the present technique