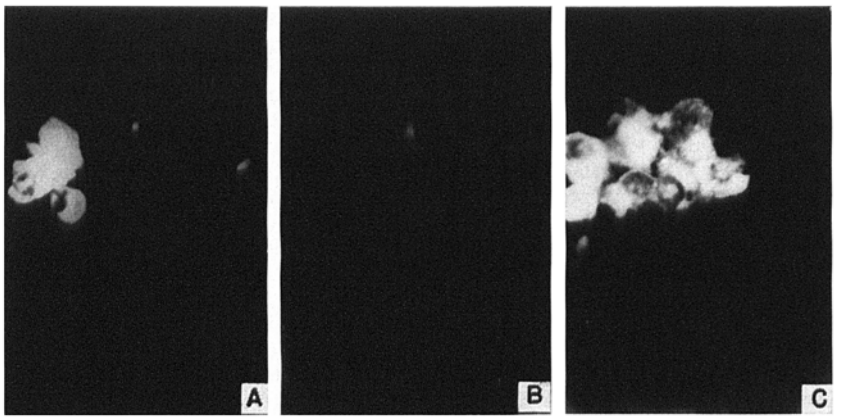

Fig. A. Vaccinia infected cells, acetone fixed, pre-treated with vaccinia negative serum for 1 h at 37 °C and for $^1/_2$ h at 37 °C with vaccinia conjugate which had been adsorbed and gel filtered.

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.

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[8] We are grateful to Miss THELMA BURT and Mr. E. CARDONE for technical assistance.
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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 $^3H/^{14}C$ 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

and 4.9 and 14.0 respectively when it was performed in the conventional manner. (The conditions of acetylation were chosen arbitrarily.)

Deoxycholic acid (170 μg), deposited on paper, was suspended over a mixture of N-methyl-N-nitroso-*p*-tolyl-sulphonamide (a few crystals), ethanol (a few drops) and aqueous 5N-KOH (a few drops). After 16 h at room temperature, the crude product was eluted with chloroform-methanol (1:1, v/v). It gave an IR-spectrum identical with authentic methyl deoxycholate. Paper chromatography gave only one detectable spot with the mobility of the methyl ester. Identical treatment of 11β-hydroxy-3-oxoaetiochol-4-enoic acid (4 μg) gave the expected methyl ester as sole product (paper-chromatographic evidence). The time allowed for reaction (overnight) was convenient but almost certainly unnecessarily long since benzoic acid, deposited on blue litmus paper and exposed to diazomethane as in the preceding examples, was esterified (disappearance of the red spot) within a few minutes.

It was found convenient – particularly when large numbers of esterifications were to be carried out – to roll the paper supports and insert them into holes (5 mm diameter) made in a piece of Teflon sheet which was then suspended from a hook attached to a glass stopper. Care was taken that the papers did not touch the walls of the test tube.

Compounds deposited on stainless steel gauze. Compounds were deposited on cylindrically shaped steel gauzes as previously described[1]. The loaded gauzes were placed in indentations (3 mm diameter, 2 mm deep) of a Teflon disc (18 mm diameter) which was then suspended over a mixture of acetic anhydride and pyridine. After 16 h at room temperature, the gauzes were introduced onto a gas-chromatographic column either manually[1] or automatically[2]. Dehydroepiandrosterone, aetiocholanolone, testosterone, oestrone, pregnanediol – among others – each gave rise to a single gas-chromatographic peak with the retention time of the expected acetate.

The examples cited suffice to illustrate the advantages of the present technique, namely: (1) Large numbers of samples can be reacted in one reaction vessel. (2) Minimum of handling (no 'work-up'). (3) Any need to use freshly distilled reagents is circumvented. (4) Economic use of reagents (important in the case of isotopically labelled reagents). (5) In combination with a solid-injection system the preparation of derivatives for gas chromatography is greatly simplified.

Clearly, the scope of the present technique may be extended to include other volatile reagents and other supporting materials. Of particular interest are supporting materials (e.g. ion-exchange paper) which may act as acid or base catalysts[3].

Zusammenfassung. Es wird eine einfache mikroanalytische Technik zur Ausführung von Reaktionen mit flüchtigen Reagenzien beschrieben.

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[1] E. Menini and J. K. Norymberski, Biochem. J. *95*, 1 (1965).
[2] R. Borth, A. Canossa and J. K. Norymberski, J. Chromat., in press.
[3] We are indebted to F. Hoffmann-La Roche and Co. AG, Basel, for their support of this work and to Miss Maureen Manson for technical assistance.

Sexchromatinbestimmung aus der Haarwurzel

Das Geschlechtschromatin wird heute für klinische Zwecke fast ausschliesslich an Mundschleimhautabstrichen bestimmt. Im folgenden wird eine Methode beschrieben, die als Alternative oder Ergänzung zu dieser herkömmlichen Methode dienen kann. Sie ist für die Person, die das Material beschaffen muss, von unüberbietbarer Einfachheit und liefert Resultate, die der Mundschleimhautabstrichmethode mindestens ebenbürtig sind.

Alles was benötigt wird, ist ein Haar mit Wurzel. Hat man sich versichert, dass das epilierte Haar eine vollständige, lange Wurzelscheide aufweist, so gibt man es entweder direkt an die verarbeitende Stelle weiter oder man wickelt es in ein Stück feuchtes Papier und liefert es innert weniger Stunden zur Präparation ab.

Die weitere Verarbeitung erfordert eine gewisse Übung und Geschicklichkeit. Zuerst wird das Haar auf etwa drei cm verkürzt, dann legt man es auf einen Objektträger und bedeckt die Wurzel mit einem grossen Tropfen von frisch filtriertem, gesättigtem essigsaurem Orcein. Über einer Spiritusflamme wird der Tropfen sorgfältig auf etwa 60 °C erwärmt. Darauf wird das Haar auf einen neuen Objektträger verbracht und die Wurzel mit einem Tropfen 50%iger Essigsäure bedeckt.

Unter der Präparierlupe erkennt man nun die stark rot gefärbte äussere Wurzelscheide, die das Haar distal vom Bulbus schlauchförmig umgibt. Mit einer Lanzette wird diese äussere Wurzelscheide möglichst als Ganzes abgestreift. Bei dieser Manipulation bricht gewöhnlich der Haarbulbus ab. Er wird zusammen mit dem Haarschaft entfernt, und lediglich die Wurzelscheide wird auf dem Objektträger belassen. Mit einem Filterpapier wird die Essigsäure entfernt und das Material mit einem neuen Tropfen Orcein bedeckt.. Sodann wird ein Deckglas aufgelegt und das Präparat nochmals etwa zwei Minuten lang leicht erwärmt. Zwischen zwei Filterpapieren wird das überflüssige Orcein durch sanftes Quetschen mit dem Daumen entfernt und das Material abgeflacht und ausgebreitet. Das Deckglas darf dabei nicht verschoben werden. Mittels Krönig's Deckglaskitt wird das Präparat am Schluss abgedichtet.

Typischerweise findet man nun die mehreren 10000 Zellkerne einer Wurzelscheide in einer einzelligen Schicht ausgebreitet (Figur). Das Chromatin dieser Kerne ist sehr fein verteilt und frei von störenden Chromozentren. Die einzige Chromatinverdichtung wird vom Sexchromatin gebildet. Typische randständige Sexchromatinkörperchen finden sich bei der Frau in rund 30% der Kerne. Wegen der Abwesenheit anderer Chromozentren ist es beim vorliegenden Material durchaus angängig,