Therefore we have devised a technique based on the previous oxidizing treatment of the intestinal mucosa in order to avoid the cell conglutination through the mucous. The technique combines this new procedure with the usual air-dried preparation.

Technique. (1) Kill the animal (possibly after treatment with colchicine in order to obtain a great number of metaphases) and rapidly remove the intestinal tract. (2) Wash the intestines in saline, open longitudinally and cut into small pieces about 1 cm long. (3) Place the pieces in hypotonic solution (sodium citrate 1% in distilled water) and add H_2O_2 3% (10 drops of H_2O_2/5 cm^3 of hypotonic solution). Shake rapidly for 2–3 min. The mucous coating is thus removed and the solution becomes turbid. This solution is discarded and the pieces are put into a test-tube containing hypotonic solution. (4) Shake again until the solution becomes homogeneally turbid. Then transfer the pieces to a new test-tube containing hypotonic solution and shake again. At this stage the pieces are thin; their histological examination shows a complete exfoliation of the cells. The pieces are discarded and the latter two tubes remain at room temperature for 30–45 min. (5) Then centrifuge and remove the supernatant. Add Carnoy's fixative (alcohol–acetic acid 3:1) and resuspend by shaking. Leave for 10 min and repeat this procedure. (6) Centrifuge and remove all the supernatant, add some drops of fixative and resuspend the cells. (7) Put droplets of the cell suspension with a Pasteur pipette on clean slides and leave to dry thoroughly. (8) Stain with diluted Giemsa.

In the sediment of the 2nd and 3rd tubes, there are normally several mitoses (Figures 1 and 2). This technique has been tried not only with mice, to which colchicine was given (Figures 3 and 4), but also with animals which have been poisoned with different antimitotic drugs (Cyclophosphamide, Methotrexate) (Figures 5 and 6).

An attempt to identify the type of cell in mitosis has been performed. For the demonstration of the enterochromaffin cells, we have previously treated with formalin (hypotonic solution with 10% formalin) the cell suspension[16]. No mitoses were found in the enterochromaffin cells, while some appeared in goblet cells and the greatest number in the epithelial cells.[17]

Zusammenfassung. Es wird eine Technik zur cytogenetischen Untersuchung von Dünndarmzellen beschrieben. Die Arbeit vergleicht Ergebnisse bei der normalen Maus mit solchen bei Tieren, die mittelst verschiedener Cytostatika vergiftet worden sind.

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[17] We should like to mention that a technique of intestinal cell dispersal in which oxidised ascorbic acid is used, has recently been applied for other purposes by W. K. Cowan, J. Path. Bact. *84*, 439 (1962).

A Simple Method for Separation and Determination of Aldosterone, Hydrocortisone and Corticosterone

Several methods for determination of aldosterone have been described. Most of them use paper chromatography[1-4] or column chromatography combined with paper chromatography[5,6] for separation of the steroids after extraction from biological fluids. The method to be described here makes use of thin-layer chromatography. This makes it possible to separate aldosterone, hydrocortisone, cortisone, corticosterone, oestrone, oestradiol and oestriol in a very short time.

After elution of the aldosterone spot directly with concentrated sulphuric acid, this steroid can be measured quantitatively by its fluorescence[7].

Material and Methods. The thin layer of silica gel is prepared as described by Stahl[8]. The silica gel is mixed with about 3% of a fluorescent drug[9]. Standard solutions of pure steroids in ethanol are made in a concentration of 1 mg per ml. The solution is applied to the silica gel with a capillary pipette. As mobile phase we used 8% ethanol (96%) in chloroform (Merck, p.a.). The T.L. is placed in a closed chamber. The chromatogram is developed at 38.5°C for 1 h (ascending technique). The spots of the corticosteroids are visible in UV-light (Figure), oestron and oestradiol are visible in UV-light after about 24 h. After marking the steroids, the aldosterone spot is scraped off and transferred to a centrifuge tube. Aldosterone is eluated directly from the silica gel by means of 1 ml concentrated sulphuric acid. The silica gel is well mixed with

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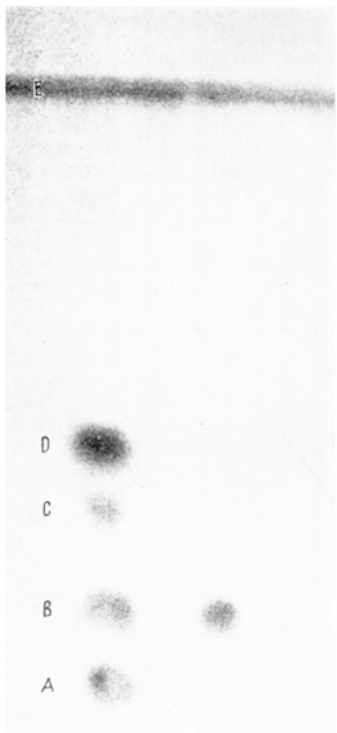
the sulphuric acid and is left at room temperature for 1 h. The silica gel is centrifuged off and the tube is placed in an oil bath at 100°C for 1 h. The tube is cooled in ice water and the fluorescence of aldosterone is measured as already described[7].

The hydrocortisone and corticosterone can be eluated with a mixture of ethanol-sulphuric acid. Measurements of these steroids are described by several authors[10,11].

Results and Discussion. The method described has several advantages over the known methods. (1) Simple and rapid determination of aldosterone; 12 determinations can be done by one person in one day. (2) Quantities of 0.5–4 μg can be estimated in this manner. Recovery 95 ± 3.4% (mean ± S.E.). (3) With this solvent the following steroids may be separated: aldosterone, corticosterone cortisone, hydrocortisone, oestrone, oestradiol and oestriol (Table). Therefore it is not necessary to remove the oestrogens from the extract with alkali as they are well separated from aldosterone in the chromatogram. (4) It is possible to determine also hydrocortisone and corticosterone from the same sample in the same chromatogram. The spots of these steroids can be eluated with a mixture of ethanol-sulphuric acid. The small difference in Rf value between hydrocortisone and oestriol does not disturb the determination of hydrocortisone[12]. (5) The reagents need no further purification[13].

Steroid	Rf × 100
Oestriol	21
Hydrocortisone	25
Aldosterone	37
Cortisone	50
Corticosterone	62
Oestradiol	70
Oestrone	93



Separation of corticosteroids after 1 h. (A) hydrocortisone, (B) aldosterone, (C) cortisone, (D) corticosterone, (F) front. Photographed in UV-light.

Zusammenfassung. Eine Mischung von Corticosteroiden (Corticosteron, Cortison, Hydrocortison und Aldosteron) und Oestrogenen (Oestron, Oestradiol und Oestriol) wurde mit Hilfe der Dünnschichtchromatographie getrennt. Die quantitative Bestimmung von Aldosteron geschah mit Hilfe der Fluoreszenz in konzentrierter Schwefelsäure.

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CORRIGENDUM

N. DASTOOR und H. SCHMID: *Über die Alkaloide von Aspidosperma discolor* A. DC., Exper. vol. XIX, fasc. 6, p. 297 (1963). In der Fussnote 19 muss es, wie aus dem Text der Arbeit hervorgeht, anstelle von 11-Methoxy-dihydrocorynantheol **10**-Methoxy-dihydrocorynantheol heissen.