The infusion of 16 µg DA in 0.9% NaCl–5% glucose resulted in a significant ($P < 0.01$) decrease in plasma prolactin (figure). A continued decrease in renal plasma prolactin was observed for 5 min after infusion was terminated. At the end of this time a rebound in prolactin secretion was observed reaching the initial value at the end of 10 min. A significant decrease of lower magnitude ($p < 0.05$) in renal plasma prolactin levels was also observed when 16 µg DA in 0.9% saline was infused (figure). The decrease in renal plasma prolactin using DA saline-glucose solution was significantly greater ($p < 0.05$) than that observed for the DA saline solution.

Discussion. It has recently been reported that there was no difference in plasma prolactin levels between the renal and jugular veins for rats with a single pituitary transplanted to the kidney capsule[9]. We have observed a similar finding when only one pituitary was grafted, however, when 3 or more pituitaries were transplanted to the kidney capsule the concentration of plasma prolactin for the renal plasma blood was significantly greater than that for aortic blood (Gala, unpublished observations). We have found a similar result in this study with 3 grafted pituitaries.

The infusion of either saline or saline-glucose without DA resulted in some decrease in renal plasma prolactin (approx. 14%). The reason for the decrease is not known but could reflect some alteration in renal blood flow as a result of infusion of the solutions. In order to visualize the effect of DA on suppressing prolactin release, the percent decrease due to saline or saline-glucose was subtracted from that of DA for the respective carrier solution. It is believed that the suppression of prolactin secretion by DA infusion was due to an action directly on the transplanted pituitary because of the magnitude of the decrease and because the transplanted pituitary contributed the major portion of the prolactin in circulation. It can not be discounted, however, that some of the effect observed may be on the in situ pituitary since the amount of DA administered has been reported in long term infusion studies to suppress prolactin release by the in situ pituitary[10]. The addition of glucose to the carrier solution amplified the inhibitory effects of DA on prolactin release and confirms the observation of others who infused DA into the hypophysial portal vessels[11]. It was suggested that the glucose retards the autooxidation of DA in solution[11].

Our purpose in examining the rebound of prolactin release after DA suppression was to see how rapidly it would occur. It appears that within 1 min after termination of DA infusion (saline-glucose group) there was a slight increase in renal plasma prolactin which was followed by a subseqent decrease over a 5 min period. From this point of maximum inhibition (approx. 50%), 10 min was required for prolactin to reach preinfusion levels. The strongest in vivo evidence for a releasing factor comes from the rapid 2–3 min increase in plasma prolactin induced by ether anesthesia[12,13]. We believe that the initial 1 min increase in prolactin release reflects an increase in vascular flow due to the removal of the catheter from the renal artery. It cannot be completely discounted that the rapid effects of ether on prolactin release may be due to alterations in vascular blood flow to the pituitary. It was demonstrated a number of years ago that ether may induce an initial ($2^1/_2$ min) 40% increase in blood flow[14]. Thus, it may be possible to explain the increase in prolactin to ether without the necessity of a releasing factor. Further, the increase in prolactin induced by ether is about 15–25 ng/ml[12,13], which is comparable to the 17.2 ng/ml change which we have observed here. Once maximum suppression of prolactin is observed, however, approximately 10 min is required for prolactin levels to return to normal, a time compatible with most observations of prolactin release by physiologic stimuli or drug administration. Thus, it may be possible to explain both the rapid increase in prolactin release due to ether and the slower increase due to suckling and drugs by only a hypothalamic inhibitory factor.

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PRO EXPERIMENTIS

A simple technique for scintillation counting of cell preparations on coverslips[1]

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Summary. A simple, rapid and versatile technique for scintillation counting of cells on coverslips is described. The cells are conserved for subsequent staining and autoradiography so that other data can be collected from the same specimen.

A simple scintillation counting technique which avoids the destruction of a specimen of cultured cells and allows further data to be obtained from precisely the same cell population has evident benefits of accuracy and versatility. Errors due to collating results from parallel or analogous cultures are removed, and extensive data can be obtained from relatively little material. There follows a description of such a technique which we are employing in our laboratories, involving scintillation counting of tritium labelled cells grown on coverslips. The cells are conserved for subsequent staining for morphological examination, accurate cell counts, microspectrophotometry and autoradiography.

Materials and methods. Cells are plated out in replicates at a density of 10^5/ml in Leighton tubes containing 11 × 22 mm coverslips and are fed routinely with Eagle's MEN supplemented with 20% foetal bovine serum. When the cultures are semi-confluent and in the log phase of growth (2–4 days), labelled precursor (tritiated thymidine – ^{3}H-TdR) is added to the growth medium for the exposure period, before the cells are harvested by rinsing with saline to remove residual medium and fixing in 95% ethanol. Alternatively, the coverslips may be removed to fresh tubes containing labelled precursor, leaving approximately half of the culture growing on the glass of the Leighton tube, which permits serial sampling

of the same material. The coverslips are transferred to scintillation vials, covered with 4 ml Aquasol (N.E.N), a xylene-based scintillation cocktail and counted in a Packard Tri-Carb liquid scintillation spectrometer. After counting, the scintillant may be removed by thoroughly washing the coverslips in xylene. The cells may then be stained for microscopic examination or may be mounted, cells uppermost, on immersion oil for autoradiographic processing[2].

Both Kodak AR 10 stripping film and Ilford K2 autoradiographic emulsion have been used with good results on specimens prestained with Feulgen or lactic-acetic orcein, and cells have also been poststained with Giemsa. Bone marrow and cultured blood specimens prepared on a cytocentrifuge (Shandon Elliot Cytospin model, 5 min spin at $27 \times g$) have been counted using this technique and later autoradiographed and stained through the film with May-Grünwald and Giemsa or with Leishman so that labelling and mitotic indices as well as differential counts could be made.

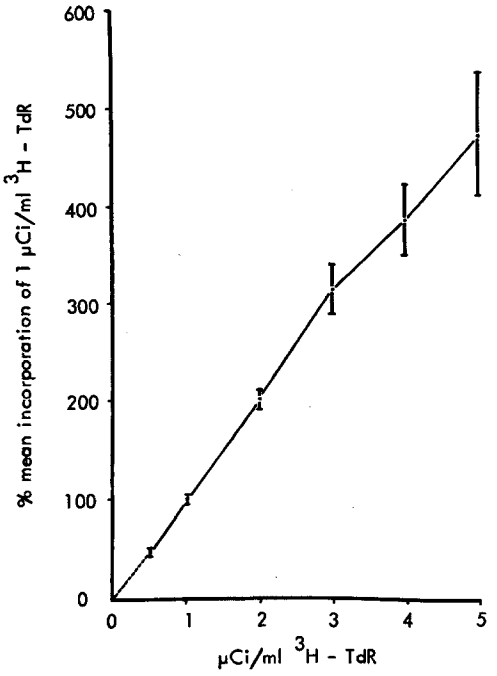

Fig. 1. Incorporation of ^{3}H-TdR in human fibroblasts in 1 h. Data are presented as means of 6 duplicate assays; bars represent SEM.

Results. Figure 1 shows data obtained using this technique in 6 series of duplicate assays of the incorporation of ^{3}H-TdR (spec. act. 46 Ci/mmole) at concentrations between 0.5 and 5.0 µCi/ml in the growth medium of human skin fibroblasts for 1 h. The curve is linear up to a concentration of 3 µCi/ml ^{3}H-TdR, but then falls off a little and the error increases, presumably because these relatively low numbers of cells are saturated with thymidine at higher concentrations. Also, since cultures must be assayed soon after plating out to avoid contact inhibition causing depression of DNA synthesis, some problems with cell synchrony may be encountered. Reproducibility is good. The overall varience (s^2) of all the duplicates represented on the graph was $\pm$ 36.5%. 10 repeat counts of 2 samples chosen at random gave mean $\pm$ SEM counts of 18.038 $\pm$ 0.011 and 17.596 $\pm$ 0.014 kcpm. 2 series of 11 and 9 replicate cultures of fibroblasts gave mean $\pm$ SEM values of 20.929 $\pm$ 0.403 and 22.570 $\pm$ 0.371 kcpm. Figure 2 illustrates that scintillation counting using this technique causes no adverse effect on different cell preparations subsequently stained and autoradiographed in a variety of ways, except that if traces of the scintillation cocktail remain because of inadequate xylene washing, chemography[3] can occur. This simple, rapid and versatile technique is adaptable and may prove useful for in vitro investigations in cell biology, cell kinetics, pharmacology and chemotherapy.

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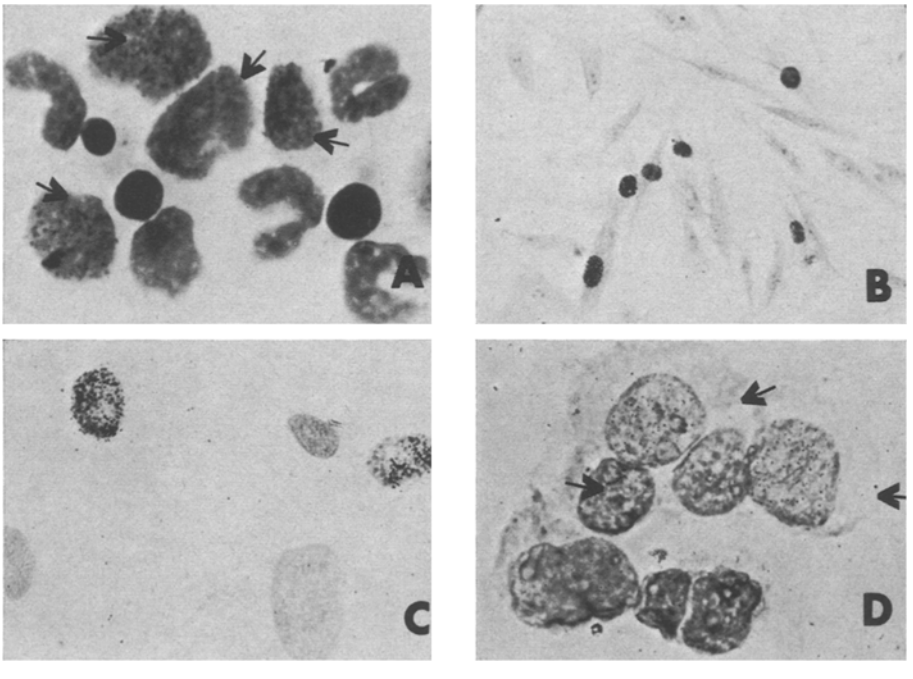

Fig. 2. Autoradiographs of coverslip preparations after scintillation counting by the technique described Cells were exposed to 3 µCi/ml ^{3}H-TdR for 1 h. *A* Bone marrow, cytocentrifuge preparation after dextran sedimentation; Kodak AR 10 stripping film, poststained with May-Grünwald and Giemsa, labelled cells are arrowed. ×3750. *B* Fibroblasts, grown on coverslip; Kodak AR 10 stripping film, poststained with Giemsa. ×2400. *C* Fibroblasts, grown on coverslips; Ilford K2 autoradiographic emulsion, prestained with Feulgen. ×3250. *D* Ovarian carcinoma cells, grown on coverslips; Kodak AR 10 stripping film, prestained with lacto-acetic orcein, labelled cells are arrowed. ×3750.