Some reports suggested that the release of these fatty acids does not take place[12] or is very weak[11] during phagocytosis of inert material such as latex beads. On the contrary, phagocytosis of inflammatory particles parallels the synthesis and release of prostaglandins. In a recent publication, Hsueh et al.[21] showed that the phagocytosis of red blood cells induces the release of prostaglandins from rabbit alveolar macrophages. Further experiments are necessary to throw light on this process and determine whether the action of asbestos on prostaglandin release is related to phagocytosis or simply a toxic effect on the membrane.

Our results with endotoxin and indomethacin confirmed previous reports on the synthetic potential of macrophages for fatty acids[11,13,20,22,23] and showed that our perifusion system is adequate for short term culture of the cells. Cumulated amounts of prostaglandins released are comparable to those reported using classic techniques[11,20,24,25]. In our system, unstimulated cells release detectable levels of the mediators and endogenous substrate appears to be sufficient for maintenance of a very satisfactory level of synthesis.

As described before[10,25,26], zymosan and asbestos are powerful stimulants of phagocytosis and prostaglandin release. These particulate substances produced only a transient stimulation which could probably correlate with active phagocytosis. However with asbestos fibers the increase in the liberation of LDH corresponds the decrease in viability of the cells observed at the end of the experiments and confirms the cytotoxic effect of this particulate pollutant.

In conclusion, the results presented extend previous observations on the action of various pharmacological and particulate substances on the release of prostaglandins by free airway cells (mainly macrophages). Our technique represents an application of perifusion to the study of the progression of inflammatory reactions in the macrophage. The utility of this technique for the evaluation of the toxicity of various types of asbestos and other inhaled particles is warranted by the clear advantages which it offers over the standard culture methods: continuously renewed medium and sequential event analysis.

Because of the ever increasing feasibility of bronchoalveolar lavages in animals and man we believe that this approach will contribute to the clarification of the early biochemical events in the host response to various environmental toxic substances.

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The preparation of colloidal gold particles using tannic acid as an additional reducing agent

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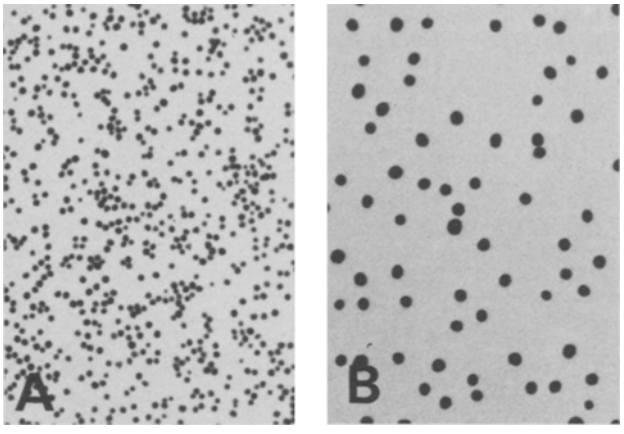
Summary. A description of a simple procedure using a tannic acid/citrate reducing agent for the preparation of gold particles having an average diameter of 5.7 nm is given.

Colloidal gold particles, being electron dense and non-cytotoxic markers, are increasingly used in cytochemistry[2]. Various reducing agents have been employed in the preparation of colloidal gold[3]. Up until now, the smallest particles have been obtained with white phosphorus[4]. However, after having investigated the reduction of gold solutions with the aid of a combination of tannic acid and sodium citrate, we now describe a simpler alternative to the white phosphorus method of preparing stable gold particles.

A stock solution of 0.1% (w/v) $HAuCl_4$ (Riedel de Haen) is prepared in water distilled twice in glass. This solution can be stored for months in well-stoppered brown glass bottles. This stock solution is diluted with distilled water to provide a fresh working solution of 0.01% (w/v). 100 ml of a 0.01% (w/v) freshly prepared chloroauric acid solution is trans-

ferred to a 500-ml Duran-glass Erlenmeyer flask. The solution is stirred vigorously, and brought to the boiling point in exactly 6.5 min. While the solution boils, the reducing agent, prepared from 2 ml of a 1% (w/v) sodium citrate, dihydrate solution (Merck) and 0.45 ml of a freshly prepared 1% (w/v) tannic acid solution (Merck), is poured rapidly into the boiling solution. The solution immediately turns dark violet, and with continued heating, the color changes to a clear wine red within 5 sec. The boiling of the solution is continued for another 5 min (with no further change in color, however). The solution is cooled under running tap water and then is transferred to a polypropylene bottle for storage at 4 °C. It is a well-known fact that small quantities of contaminants stemming from the glassware used, and alterations in the preparation technique can influence the size of the gold particles which are obtained[5]. Several modifications of the standard procedure described above were carried out, and it was found that larger gold particles are produced when a) the volume of the gold solution is increased up to 400 ml; b) a 250-ml instead of a 500-ml Erlenmeyer flask is used, or when a 800-ml glass beaker is used; c) the tannic acid concentration is increased or reduced; d) the tannic acid/citrate solution is added slowly, drop by drop; e) the tannic/citrate solution has been boiled before being added to the gold solution. The average diameter varied between 8.4 nm and 15.0 nm, depending on the conditions.

Using the standard procedure, we were able to obtain gold particles with an average diameter of 5.7 nm. We were unable to produce similarly small particles when using either tannic acid or sodium citrate alone. Particle size in a suspension is determined by the number of nuclei over which the available gold is divided[6]. It is assumed that tannic acid has a reducing as well as a protective effect during the production of colloidal gold[3]. Particles produced with phosphorus have an average diameter of 5.9 nm and a coefficient of variation of 25%[4]. No significant differences appear to exist between the results reported by Slot and Geuze[4] and the results reported here. Thus, the tannic acid/citrate method, due to its simplicity, represents a good alternative to use of white phosphorous in the preparation of gold particles.



Electron micrographs of colloidal gold particles (× 100,000). 2.5 µl of the respective gold sol were dropped on a formvar coated grid, dried on filter paper, photographed and 100 distributed gold particles were measured at random with a micro comparator. *A* Tannic acid/citrate method: average particle diameter is 5.7 nm (95% range 3.0–8.4 nm). *B* Sodium citrate method[6]: average particle diameter is 13.8 nm (95% range 10.0–17.6 nm). Both methods were tested for significant differences. The t-test gave $p < 0.001$ and the F-test demonstrated a highly significant, smaller variance ($p < 0.01$).

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