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MINIREVIEW

Colloidal Gold: A Pluripotent Receptor Probe (41697)

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Abstract. Colloidal gold is an electron-dense, lyophobic colloid that readily forms a stable electrostatic interaction with a variety of macromolecules. Monodispersed colloids ranging from 3–150 nm in diameter can be produced to provide the researcher with flexibility in selecting the optimally sized probe. Gold labeling of antibodies and lectins has been extensively used to study surface antigens and cell components. Recently, the use of gold labeling has been extended to study receptor–ligand binding, enzyme–substrate reactions, and transcellular pathways. Published applications include gold labeling of metabolites (low-density lipoproteins), enzymes (DNAase and RNAase, RNA polymerase, thrombin, collagenase, elastase), hormones (insulin, epidermal growth factor, glucagon), circulating plasma proteins (asialoglycoprotein, α_2-macroglobulin, factor VIII–von Willebrand factor), and endotoxins (tetanus toxin, cholera toxin). This broad spectrum of applications emphasizes the versatility and usefulness of colloidal gold as a probe in areas of cell biology related to receptors, endocytosis, transport, and functions of proteins.

Inexpensive and routine application hardly seem to be appropriate descriptions for the use of gold, in any form. Yet, to the cell biologist, the use of colloidal gold as an ultrastructural tracer meets these and many other criteria. Since its introduction by Palade in 1961 as an ultrastructural tracer to examine capillary endothelial permeability (1), colloidal gold has become increasingly popular in a variety of receptor and tracer applications. Colloidal gold will electrostatically adsorb glycoproteins, lectins, and antibodies to its surface to form a relatively stable monolayer of the macromolecule. Colloidal gold can be visualized by darkfield microscopy and transmission and scanning electron microscopy. Different sizes of gold have been used in multiple-labeling experiments (2–5), and in combination with ferritin (6) or peroxidase (6,7). Colloidal gold is nontoxic and well-tolerated by cells and animals (2,8). While colloidal gold has been extensively used as a label for immunoglobulins and lectins, this review will focus on recent applications related to enzyme labeling, receptor–ligand interactions, and transcellular pathways.

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I. Preparation and Properties.

Colloidal gold is most often prepared by the reduction of chloroauric acid ($HAuCl_4$) by white phosphorus (9, 10), sodium ascorbate (11), or trisodium citrate (12). Other reductants include formaldehyde (13), ethanol (14, 15), or tannin (16). The colloidal suspensions obtained from the first three reductants are simple to prepare and have mean particle diameters of 5.2 nm, 13.0 nm, and possible range of 14–150 nm, respectively. The number of particles per volume can be calculated from the quantity of $HAuCl_4$ added and the average particle size as chloroauric acid is completely reduced to elemental gold during colloid formation (17, 18). The homogeneity of the colloid particle diameter in the final suspension is governed by the rate of icosahedral nuclei formation compared with shell condensation (14). Sucrose-gradient centrifugation (19) and sedimentation field flow fraction (20) may be used to improve further the homogeneity of the diameter by size subfractionation. Particles as small as 0.82 nm composed of 11 gold atoms have been prepared by sodium borohydride reduction of triaryiphosphine gold complexes (21, 22). These gold particles are spherical and consist of elementary crystallites (1–2 nm) with 0.5 to 0.8-nm lattice images of metallic gold (23).

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All gold colloids are negatively charged, electron-dense, and stable for months regardless of the reductant used (14). Colloids in the 5- to 18-nm range exhibit an orange–red color and an absorption peak (λ_{max}) of 520–540 nm (17) that is somewhat influenced by the mean particle size (2, 17). The optical density at 530 nm can be used to determine the amount of the gold colloid remaining in a supernatant after cellular interaction. Since gold colloids are strong emitters of secondary electrons they can be visualized by scanning electron microscopy and X-ray microanalysis (2, 14, 24).

II. Labeling Procedures and Technical Considerations. Colloid stability is maintained by electrostatic repulsion of negative charges which are distributed around the colloid particle in the form of an ionic double layer. The presence of electrolytes compresses the ionic double layer, reduces the electrostatic repulsive forces between particles, and destabilizes the colloid. As a result, flocculation and cohesion will occur, indicated by an immediate color change from red–orange to blue (24) followed by precipitation (2, 24, 25). A surface layer of absorbed protein (or any macromolecule) will protect against electrolyte flocculation and stabilize the gold particles in a dispersed suspension (2). The electrolyte flocculation test provides a convenient and accurate method to establish the minimal amount of protein needed to stabilize a given volume of gold colloid (24), thus obviating the need to remove excess unbound protein. The amount of protein needed to stabilize a colloidal gold suspension is proportional to the total colloidal surface area and the number of colloidal particles per unit volume (18, 24). However, some proteins do not exhibit a saturation behavior, suggesting the formation of multiple protein layers on each colloid particle (2, 25, 26).

Proteins are rapidly adsorbed (24, 25) to the surface of the negatively charged gold colloid by noncovalent electrostatic interactions via their amino groups (14, 24, 26). Available evidence indicates that the adsorbed proteins are tightly bound to the gold particle surface (2, 24, 27), and little desorption is seen after several weeks (9) to years of storage (2, 28) or during fixation and embedding procedures (2). However, it is advisable that the gold-labeled protein be purified by centrifugation to remove any protein that has dissociated from the gold surface (29). Optimal protein adsorption occurs at a pH equivalent to the isoelectric point (pK_I) of the protein (30, 31). The pH of the gold colloid should be adjusted to this pK_I (with either potassium carbonate or acetic acid) before labeling. Since electrolytes reduce the adsorptive capacity of the colloidal particle, proteins should be dissolved in water or very dilute buffers (27). Once the optimal labeling conditions (protein concentration and pH) have been determined, they may be applied to similar labeling studies involving the same protein. In some cases, the absorbed protein can be observed by negative staining of the labeled gold as an electron-lucid halo around the electron-dense gold particle (5, 12, 32, 33).

Adsorbed macromolecules generally retain their biological activity after binding to the gold surface as no chemical changes transpire in the ligand during the labeling procedure (29). However, molecules such as enzymes may express reduced activity. Factors that reduce enzymatic activity include steric hinderance of the active site, loss of mobility due to adsorption, and limited access of substrate to the adsorbed protein.

III. Receptor Applications. *A. Immunoglobulins.* Gold labeling of immunoglobulins represents the earliest application of colloidal gold as an electron-dense probe (10, 34). Preparation of gold labeled to antibodies (30, 32, 34, 35) or to staphylococcal protein-A (36–40) has been shown to be a convenient and reproducible procedure (2, 38, 39). The protein-A gold technique is an indirect postembedding method reported to be superior to direct enzyme- or ferritin-labeled antibodies (41). It is based on the specific interaction of protein-A with the Fc fragment of the immunoglobulin (42). Gold labeled to antibodies or protein-A have been used to localize antigen structures on frozen tissue sections (41, 43), ultrathin resin sections (39, 41, 44–47), glutaraldehyde-fixed cells (48, 49), paraffin sections (37), and double labeling of two (50, 51) or more (3, 52) antigenic sites. While initial gold labeling experiments were directed toward red cell antigen profiles (17, 35) and immunoglobulin agglutination (53), recent applications of gold labeled to antibodies or protein-A as surface probes include: the intracellular transport of rat IgG in the yolk sack endoderm (54), distribution of yeast chi-

tin (55) and mannan (17, 55), identification of human B lymphocytes by detection of surface immunoglobulins (56), detection of leukocyte surface antigens (57), surface distribution of fibronectin (38, 58), and mapping of receptor-bound low-density lipoproteins in cultured fibroblasts (49). Subcellular applications include organization of microtubules during interphase (59, 60), effects of antimicrotubule agents on microtubule function (61), detection of actin in myofilaments in smooth muscle cells (62), localization of the vitamin D-dependent calcium-binding protein of the chick duodenum (63) and kidney (64), localization of secretory proteins in the rat pancreas (39, 45, 50, 51, 65, 66), and detection of intracellular antigens of Hela cells infected with measles virus (48). Other studies have identified subcellular distribution of mitochondrial proteins in the rat liver (47, 67, 68), insulin distribution in the rat pancreas (47), granular localization of enkephalins in catecholamine-containing cells (46), the intracellular hormone ACTH in porcine adenohypophyseal cells (44), localization of DNA or RNA sequences (69), the endocytotic pathways of the asialoglycoprotein receptor (70), the zymogen granule membrane GP-2 glycoprotein of the rabbit pancreas (43), and compartmentalization of Golgi enzymes (71).

New immunocolloid approaches include radioactive gold colloids (^{195}Au or ^{198}Au) that permit quantitation by radioanalysis in parallel with examination at the ultrastructural level by electron microscopy (72). Other advances include simultaneous labeling of protein-A with fluorescein isothiocyanate and colloidal gold (14, 73), brightfield (37, 56) and darkfield (57) microscopy of localized gold, and replica immunocytochemistry using colloidal gold to study the distribution of surface proteins (48). In the gold-labeled antigen detection (GLAD) technique, specific antibodies are reacted in slight excess with plastic tissue sections. This results in a free antigen binding site of the antibody that is then able to react with the same antigen previously labeled with colloidal gold (3).

B. Lectins. Labeling of lectins with colloidal gold to visualize surface binding has been reviewed in detail elsewhere (2, 14). Examples of the use of gold-lectin probes include surface labeling of red blood cells (5, 18, 74, 75), rat hepatocytes (76, 77), rat liver epitheloid cells (6, 7), yeast (78, 79), protein bodies in the embryo axis of soybean (80), glycoproteins in milk fat globules (81), and distribution of the polysaccharide galactan in the myxomycete *Physarum polycephalum* (82). Dual labeling of lectins with rhodamine and gold was used to observe cell wall mannan on yeast by light and electron microscopy (83). A new procedure for demonstration of lectin binding sites at the light microscopic level employs lectin–gold probes reacted with paraffin sections (84). In multiple labeling studies, the accessibility

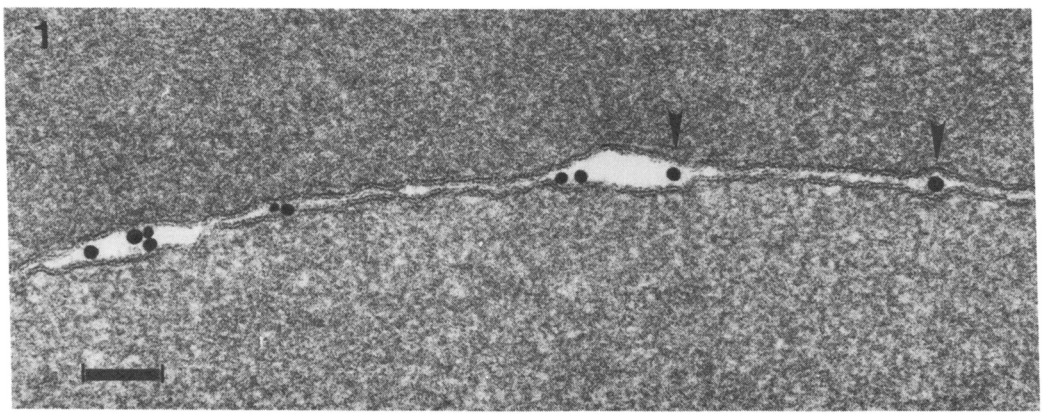
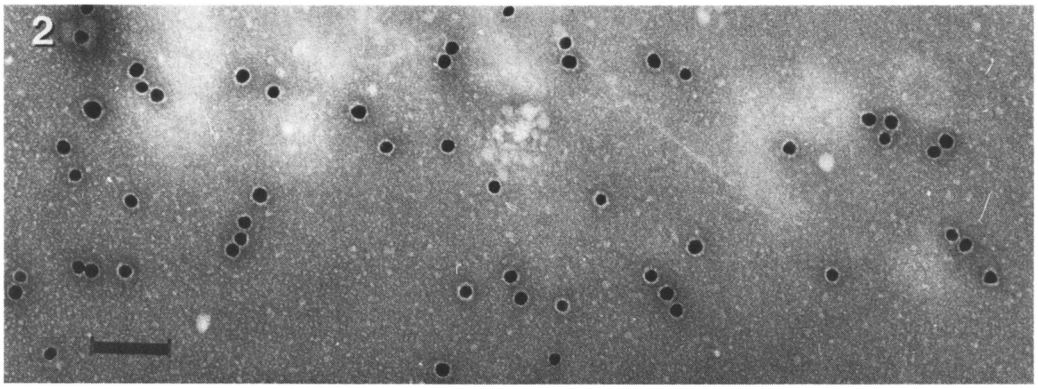
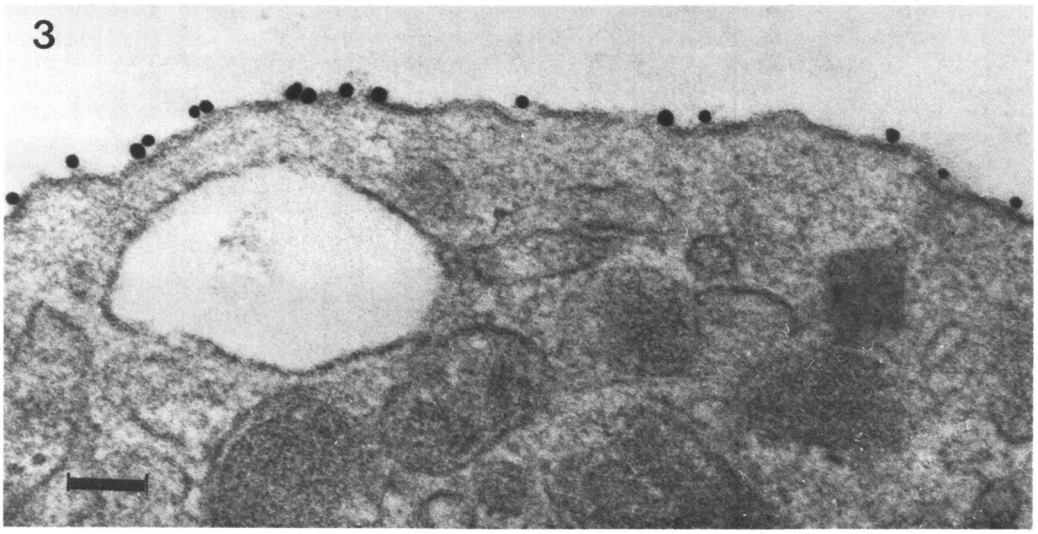


FIG. 1. Human red blood cells agglutinated with colloidal gold (16.5 nm diameter, prepared according to (12)) labeled with the lectin from *Helix pomatia* (74, 75). In the area of cell–cell agglutination, opposing cell bilayers show a curvature profile around the gold probe (arrowheads), suggesting membrane deformation. Marker bar 0.1 μm.

of the gold probes may be limited due to steric hindrance at the membrane surface (5), thus compromising absolute quantitation (2, 76). Furthermore, at areas of cell-to-cell contact, membrane deformation in the form of a curvature around the probe is seen, suggesting steric interference (Fig. 1).

C. Enzymes. Colloidal gold has been conjugated to enzymes to examine substrate interactions. Collagenase, elastase, and amylase have been labeled to gold and used to localize the corresponding collagen, elastin, and glycogen substrates (47, 66). DNAase I and RNAase A labeled with gold are highly suitable for ultrastructural localization of nucleic acids (heterochromatin, euchromatin), as evaluated in rat acinar cell organelles (85–87). Binding sites of DNA-dependent RNA polymerase to chick oviduct DNA have been examined by complexing the polymerase to colloidal gold (88). Careful consideration of fixation and embedding artifacts are important when assessing enzyme specificity (87). Thrombin labeled with colloidal gold has been used as a probe to investigate enzyme cleavage of fibrinogen to form fibrin strands (89) and to visualize receptor distribution on human platelets (90). The adsorbed thrombin can be





FIGS. 2 AND 3. Human α-thrombin labeled to colloidal gold (89, 90). The α-thrombin is seen by negative staining with phosphotungstate as an electron-lucid halo around the gold particle (Fig. 2). Receptor labeling to human platelets (prepared from platelet-rich plasma and fixed for 10 min at 37°C with 0.5% paraformaldehyde in 0.15 *M* Na-cacodylate buffer) shows uniform surface distribution (Fig. 3). Probes are closely opposed to the membrane, suggesting direct contact with a surface receptor. Marker bar represent 0.1 μm.

visualized by negative staining (Fig. 2), and the thrombin–gold probe is observed randomly distributed on the platelet membrane (Fig. 3).

D. Hormones. Receptor binding and endocytosis of hormones represents a fundamental mechanism for stimulation and regulation of cellular activities. Insulin labeled with colloidal gold has been used to visualize receptor binding and internalization by coated vesicles in human monocytes and neutrophils (91). Colloidal gold labeled to glucagon–bovine serum albumin reagent has been used to visualize glucagon binding sites on the surface of human peripheral leukocytes (92). Avidin-labeled gold probes have been used to identify the receptor-mediated pathway of biotinylated epidermal growth factor in culture granulosa cells (38, 93). This technique relies on the strong interaction between avidin and biotin.

E. Plasma macromolecules. The cellular pathway of internalization of low-density lipoproteins (LDL) labeled with colloidal gold has been examined in perfused livers of ethinyl estradiol treated rats (33, 94), in normal cultured fibroblasts (95, 96) and in 3T3 cells (97). In the steady-state condition, all phases of 3T3 cell endocytosis of the gold–LDL conjugate can be demonstrated, including binding to coated pits and lysosomal delivery (Fig. 4). Treatment of 3T3 cells with the lysomotrophic amine chloroquine to prevent catabolism of LDL results in internal deposition of the conjugate against the inner aspect of the lysosome membrane (Figs. 5A, B), instead of the normally observed random dispersion (98). The internalized conjugate retains a structure similar to the initial negative stained image (Fig. 5C), suggesting the absence of lysosomal catabolism.

Studies of receptor-mediated endocytosis of gold labeled to the asialoglycoprotein asialofetuin showed involvement of a coated pit–coated vesicle pathway in Kupffer cells (99), results which demonstrated that cells other than rat hepatocytes express this receptor. Binding and endocytosis of asialoceruloplasmin labeled with colloidal gold has been similarly examined in rat hepatocytes (4). Labeling of the tetrameric protein α_2-macroglobulin with colloidal gold has permitted the identification of a receptor-mediated endocytotic pathway in fibroblasts (100) and hamster ovary cells (101) involving a coated pit–coated vesicle pathway. Gold colloids labeled to Factor VIII were used to examine the distribution of

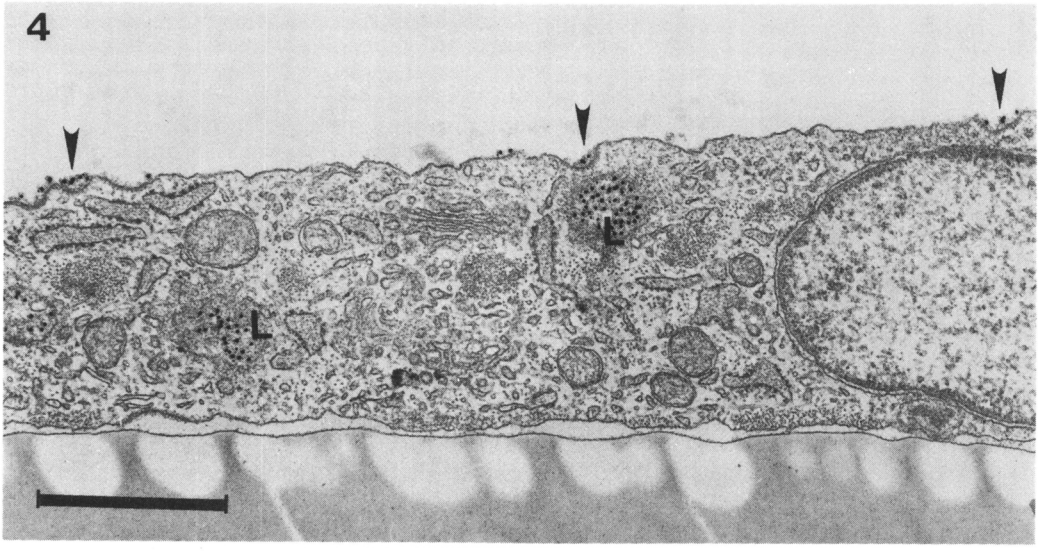


FIG. 4. Human low-density lipoproteins (LDL) labeled to colloidal gold (97) reacted with 3T3 cells grown in lipoprotein deficient serum. The gold–LDL was reacted with the cells for 2 hr at 4°C, followed by 20 min at 37°C. Receptor binding at coated pit regions (arrowheads) and lysosomal accumulation (L) are evident. Cell were fixed with glutaraldehyde, osmicated and treated with tannin as a counter stain. Marker bar 1.0 μm.

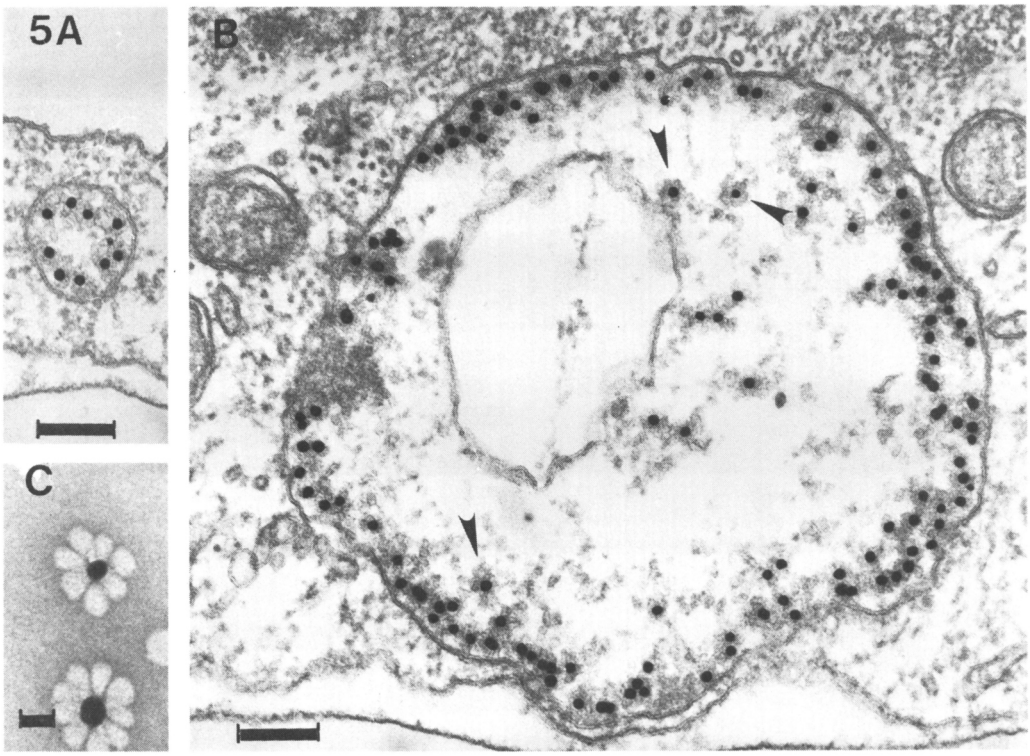


FIG. 5. A–C. Gold–LDL reacted with 3T3 cells (97) treated with chloroquine (98) to abolish lysosome catabolism. After 10 min at 37°C, initial lysosomal delivery of the probe is apparent as internal deposition at the inner membrane surface (A). After 60 min, extensive accumulation is still marked by peripheral clustering against the inner part of the lysosome membrane instead of random distribution within the lysosome (B). The conjugate appears intact (arrowheads), with a structure equivalent to the initial structure of the conjugate seen by negative staining (C). Marker bars equal 0.1, 0.1, and 0.03 μm, in A, B, and C, respectively.

platelet surface receptors (102) and the optimal multimer size of the Factor VIII for expression of platelet aggregation effects (103).

IV. Transport Applications. Colloidal gold labeled with neutral proteins or polysaccharides is a highly suitable tracer to investigate cellular transport and permeability. The electron opacity permits accurate identification against the cell background and use of a specific diameter gold probe is of value for investigations of selective cellular transport.

A. Absorptive pinocytosis. Endothelial pinocytosis, a transcellular movement of macromolecules mediated by vesicles, was examined by Palade using colloidal gold (1). Recently, colloidal gold formed with ^{198}Au and then coated with gelatin results in a probe suitable for radioisotopic autoradiography and electron microscopic quantitation of transport (104). The permeability of the vascular endothelium of the rabbit thoracic aorta has been examined using radioactive colloidal gold (^{198}Au, 14–40 nm) (105). Uptake of the colloidal particles was influenced by perfusion time and hydrostatic pressure but independent of particle size (105). Several studies have shown that the transport rate of nonfenestrated endothelium is closely related to particle diameter (106). Thus, the independence of the rate of endothelial uptake on particle diameter may reflect the contribution of the fenestrated endothelium of the vasa vasorum. The use of ^{198}Au-colloidal gold to quantitate pinocytosis has been investigated in rat (107, 108) and mouse (109) peritoneal macrophages. Uptake was linear with time and dependent upon temperature and concentration of the label (108, 109). The limited amount of nonspecific

surface adsorption and the small size of the gold particles make it suitable for examination of fluid phase pinocytosis related to quantitative rate constants (108). In isolated dog carotid artery, transmural pressure has been shown to alter endothelial pinocytosis examined using different-sized gold colloids coated with polyethylene glycol (110). Protein-A gold and antibodies specific for albumin have been used as an indirect probe to study capillary permeability of albumin (111, 112). Colloidal gold coated with polyvinylpyrrolidone has been used as a tracer to examine passage and subcellular distribution of macromolecules within the ameba *Chaos chaos* (113).

B. Phagocytosis and lysosomal uptake. Endocytosis and lysosomal accumulation of colloidal gold has been used to examine alterations of lysosomal composition associated with aging in mouse (114) and rat liver hepatocytes (115). As colloidal gold is nondegradable, accumulation has been observed in residual bodies and quantitated by X-ray microanalysis (108). Internalization of the macromolecules tetanus toxin, cholera toxin, and nerve growth factor labeled with colloidal gold has been examined in the preterminal axons of the anterior chamber of the rat eye (27). Subsequent retrograde transport of these labeled components to the cervical ganglion suggests the presence of a pathway related to neuron innervation (27).

V. Future Directions. The many examples cited in this review serve to emphasize the versatility of colloidal gold as a discrete electron-dense probe that can be easily and reproducibly labeled to a large variety of macromolecules. A recent development in gold labeling is the monomeric or dimeric surface tagging technique which has the advantage of reducing the steric hindrance contributed by the gold. In this method, 1–3 gold particles of less than 6 nm size are adsorbed to each protein molecule. An example of this approach is shown in Fig. 6, where rat intermediate-density lipoproteins (35.0 nm) were reacted with a controlled volume of 5.2 nm colloidal gold, followed by albumin to quench the tagging reaction. Surface tagging with small (0.82 nm) undecagold gold probes has been used to examine avidin–biotin interactions (116). Resolution in the order of 1.5 nm is possible by this technique (116), permitting for visualization of functional sites and protein subunits related to biological activity. These newer tagging techniques will undoubtedly find applications in many investigations of receptors

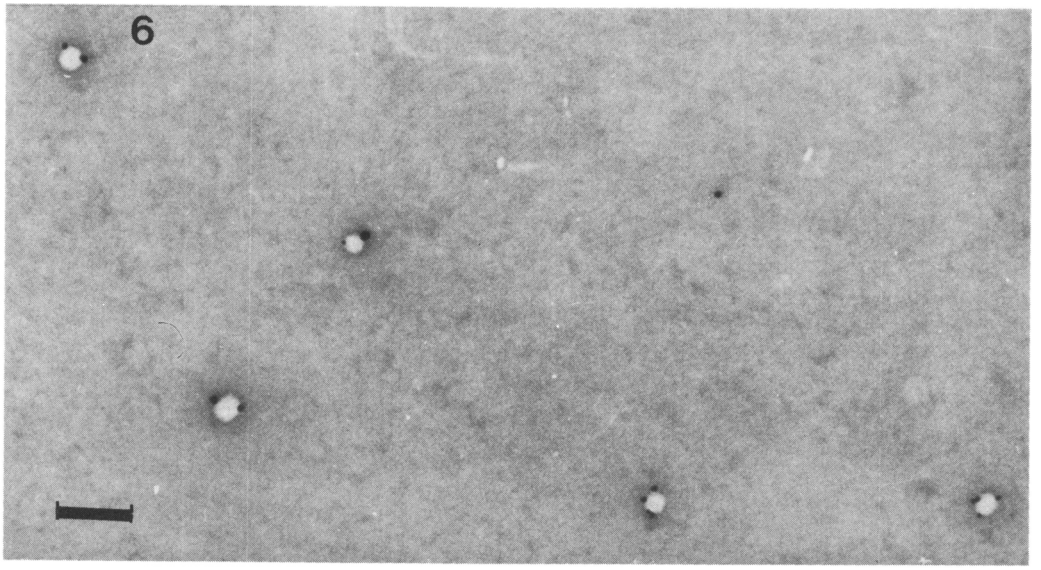


FIG. 6. Rat intermediate density lipoproteins (35 nm diameter) reacted with colloidal gold (5.2 nm, prepared as described in (9) to yield dimeric surface tagging. Labeling by this approach minimizes the steric hindrance and interference by the gold particle to receptor binding. Marker Bar 0.1 μm.

and transport using gold-labeled macromolecules.

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