Assembled and Unassembled Pools of Clathrin: A Quantitative Study Using an Enzyme Immunoassay

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ABSTRACT Using polyclonal antibodies raised against clathrin, we have developed an enzyme-linked immunoassay that can specifically measure the quantity of clathrin in crude cell extracts. We found that the quantity (weight percent of total protein) of clathrin was similar in cell types that exhibit large differences in their levels of endocytosis and exocytosis (lymphoid cells, 0.11%; liver cells, 0.07%, fibroblasts, 0.18%; myeloma cells, 0.16%). However, the quantity of clathrin was found to be significantly higher in brain cortex (0.75%).

Cellular clathrin was separated by high-speed centrifugation into two fractions: an unassembled form present in high-speed supernatants and an assembled form (clathrin coats) present in the pellets. We show that the fraction of clathrin in the unassembled state varies considerably depending on the cell type studied (14% in brain cortex to 70% in lymphocytes).

Our data support the view that the amount of clathrin (relative to total cell protein) in eucaryotic cells is not related to the extent of receptor-mediated endocytosis and intracellular membrane traffic. However, the fraction of assembled clathrin seems to be higher in endocytically and/or exocytically active cells.

Clathrin is the main constituent of the polyhedral protein lattice that forms the coat of coated pits and coated vesicles (1). Considerable data has shown that coated pits and coated vesicles are involved in many cellular processes, including receptor-mediated endocytosis (for a review, see reference 2), exocytosis of newly synthesized proteins (3), and plasma membrane recycling (for a review, see reference 4). However, an important question still unresolved concerns the molecular mechanism(s) underlying the fission and fusion events that occur during the vesicular transport.

Many arguments support the view that the fission and fusion events are related to a cycle of assembly-disassembly of clathrin coats. In vitro, clathrin coats dissociate reversibly into triskelions, flexible, three-armed structures comprising three clathrin heavy chains (180,000 mol wt) associated with three light chains (30,000–40,000 mol wt depending on the tissue and species) (5, 6). Triskelions are able to bind to stripped coated vesicles reforming clathrin cages (7). In vivo, coated vesicles arising from coated pits during receptor-mediated endocytosis appear to shed their clathrin coats rapidly before fusing with endosomes (8, 9). It is likely that the coats are removed enzymatically from the coated vesicles (10) and that the disassembled components return to the plasma membrane to be reassembled.

However, the question of whether a pool of free triskelions exists is still being debated (11, 12). Immunocytochemical studies that use polyclonal anticlathrin antibodies have revealed clathrin associated with coated pits or coated vesicles with very little background staining in the nonmembranous regions of the cytoplasm. This has been interpreted to indicate the absence of clathrin within the cytoplasm (13–16). On the other hand, Louvard et al. (16) have recently produced a monoclonal antibody that recognizes an epitope present on the clathrin heavy chain. This monoclonal antibody gives diffuse staining throughout the cytoplasm, which suggests the presence of a "soluble" pool of clathrin.

In an effort to determine whether or not an intracellular pool of unassembled clathrin exists, we developed an enzyme immunoassay in which crude cell lysates (containing clathrin) inhibit the binding of anticlathrin antibodies to immobilized clathrin. We separated clathrin pools into their assembled and unassembled forms by centrifugation. Our results indicate that although the amount of clathrin is relatively constant from one cell type to another (0.1–0.2% of cellular proteins), with the notable exception of brain cortex (0.7%), the ratio of assembled to unassembled clathrin varies greatly. A positive
correlation exists between the size of the clathrin assembled pool and the extent of cellular activities mediated by coated structures.

MATERIALS AND METHODS

**Animals:** Male 5-8-mo-old rats of the Fischer 344 strain were used. They were raised in specific pathogen-free conditions in the breeding center of the Pasteur Institute (Dr. J.-L. Guénét).

**Cells and Tissues:** Macrophage-depleted lymphoid cells were prepared from rat cervical and mesenteric lymph nodes as previously described (17). Mouse myeloma IgG-secreting cell line X63Ag8 (18) was grown in Dulbecco modified Eagle's medium (Boehringer GmbH, Mannheim, Federal Republic of Germany [FRG]) supplemented with 1 mM sodium pyruvate (Flow Laboratories, Irvine, U. K.), 50 IU/ml penicillin and 50 μg/ml streptomycin (Flow Laboratories), and 10% fetal calf serum (Boehringer) in an atmosphere of 90% air and 10% CO2. Cells were harvested during exponential growth. Vero cells (monkey kidney fibroblasts) were grown in monolayer in Dulbecco's modified Eagle's medium (Seromed, Munich, FRG), supplemented with 25 mM glucose (Sigma Chemical Co., St. Louis, MO), penicillin-streptomycin, and 10% fetal calf serum (Seromed) in an atmosphere of 90% air and 10% CO2. Cells were seeded 24 h before the experiments and were just confluent when they were removed from the petri dishes. Brains and livers were taken from freshly killed rats. Sheep erythrocytes were purchased from Institut Pasteur Production (Paris, France). They were kept at 4°C in Abeer.

**Preparation and Fractionation of Cell Lysates and Tissue Homogenates:** Before lysis, lymphoid cells and myeloma cells were washed four times in PBS (10 mM potassium phosphate buffer, pH 7.4, 0.15 M NaCl). Fibroblasts were removed from petri dishes with 0.05% trypsin, 0.2% Triton X-100. Homogenates were centrifuged for 10 min at 1,500 g to remove cell debris.

Preparation and fractionation of cell lysates and tissue homogenates

| Step | Description |
|------|-------------|
| 1. | Cells and tissue were homogenized in buffer A (pH 6.5) containing 0.5 mM MgCl2, 0.02% NaN3 (wt/vol), 0.005% phenylmethylsulfonyl fluoride, and Veto cells (3-6 x 10^7 cells) were lysed in 2-3 ml of a buffer (Pearse's buffer). |
| 2. | For lymphoid cells and myeloma cells, cell lysates were prepared as described above. For some experiments, fibroblasts were removed from petri dishes with 0.05% trypsin, 0.2% Triton X-100. Homogenates were centrifuged for 10 min at 1,500 g to remove cell debris. |
| 3. | After 30-45 min at 4°C, these fractions were centrifuged for 1 h at 100,000 g. The supernatants were collected, the pH was raised to pH 9.5 with 1 M Na2CO3, neutralized with 1 M phosphate buffer, pH 7.4, and recentrifuged for 1 h at 100,000 g. The supernatants were then collected, and neutralized with KH2PO4 as described above. |
| 4. | Clathrin present in cell or tissue extracts was quantitated using an enzyme-linked immunoadsorbent assay. To perform this assay, we have used a crude rabbit immune serum raised against empty clathrin cages prepared from pig brain coated vesicles (16). In control experiments, a pool of normal rabbit sera was used instead of the immune serum. Polyethylene flat-bottom microtiter plates (Nunc, Denmark) were coated with purified clathrin. Coating was carried out for 2 h at 37°C and overnight at 4°C in 1 M sodium carbonate buffer, pH 9.5 (1.5 μg/ml clathrin, 50 μl/well). The cells were then washed five times with PBS supplemented with 0.1% Tween 20 (Merck) and free-binding sites were saturated in the same buffer containing 0.4% BSA (fraction V, Industrie Biologique Francaise, Villeneuve-la-Garenne, France) (PBS-Tween-BSA buffer) for 30 min at 4°C. Cell lysates or tissue homogenates (1-20 mg/ml) were serially diluted in PBS-Tween-BSA (dilutions: 1/5 up to 1/640, preincubated for 1 h at 37°C, and overnight at 4°C, with rabbit anti-clathrin immune serum (final dilution: 1/3, 200) and then added onto the plates for 3 h at 4°C (60 μl/well). A standard curve was raised with purified clathrin diluted in buffer A containing 0.2% Triton X-100 and 0.4% BSA was carried out simultaneously for each experiment. A stock solution of clathrin was raised to pH 9.5 with 1 M Na2CO3, neutralized with 1 M phosphate buffer, pH 7.4, or 1 M K2HPO4 diluted in PBS-Tween-BSA, and preincubated with rabbit anti-clathrin immune serum under the same conditions as cell lysates. To determine 100% antibody binding, we added immune serum to dilutions of buffer A containing 0.2% Triton X-100 and 0.4% BSA (previously raised to pH 9.5 and neutralized) in PBS-Tween-BSA. To measure nonspecific binding, we performed control experiments using a pool of normal rabbit sera (final dilution: 1/3,200) instead of the immune serum. The microtiter plates were washed six times with PBS-Tween, the wells were filled with β-galactosidase-linked affinity-purified horseradish peroxidase IgG (a gift from Dr. L. Leclercq, Institut Pasteur), diluted in PBS-Tween-BSA, and incubated for 2 h at 4°C. After six additional washings with PBS-Tween, the enzymatic reaction was allowed to take place at 37°C in 0.1 M phosphate buffer, pH 7.0, containing 10 mM MgSO4, 2 x 10^-4 M MnSO4, 2 x 10^-5 M magnesium triflate (Merck), and 0.4% BSA. The enzyme activity was determined as described above. The absorbance at 490 nm was read. |

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calculated the absolute amount of clathrin in the different fractions of cell lysates or tissue homogenates at 50% inhibition by referring to the concentration of purified clathrin giving the same inhibition. Knowing the total protein content, we calculated the absolute amount of clathrin in the different fractions of cell lysates or tissue homogenates. The percentage of unassembled clathrin was calculated by the formula:

\[
\text{percent unassembled} = \frac{\text{amount of clathrin in high-speed supernatants}}{\text{amount of clathrin in high-speed supernatants} + \text{amount of clathrin in corresponding pellets}} \times 100.
\]

To estimate the clathrin recovery during the fractionation procedure, we calculated the amount of clathrin in the total clathrin fraction (with the protein content of this fraction taken as 100%). The yield of the fractionation was given by the formula:

\[
\text{percent recovery} = \frac{\text{amount of clathrin recovered in high-speed supernatants} + \text{pellets}}{\text{amount of total clathrin}} \times 100.
\]

RESULTS

The polyclonal rabbit anticlathrin immune serum used in these experiments has been shown to bind to clathrin coats in situ and to clathrin heavy chains and its associated light chains blotted on nitrocellulose (16). Binding to immobilized native triskelions is now reported.

**Standard Curve with Purified Clathrin**

In preliminary experiments, we were able to show that binding of the rabbit anticlathrin immune serum to clathrin immobilized on microtiter plates was efficiently inhibited by the addition of serial dilutions of purified clathrin. This observation allowed us to develop a quantitative immunoassay (see Materials and Methods). The purified clathrin, under the conditions of our assay, should be present in solution as triskelions (6, 25) and should not reassociate into baskets (see section below). Fig. 2A shows a typical curve of inhibition with purified clathrin. 50% binding (or 50% inhibition) was usually obtained by preincubating a dilution of immune serum (1/3, 200) with 0.3–0.5 μg/ml of clathrin.

In control experiments, the binding of a pool of normal rabbit sera was found to be <10% of the binding of anticlathrin immune serum at the same dilution. We also tested the ability of unrelated antigen to interfere with the specific binding. Concentrations up to 0.5 mg/ml of mouse actin, mouse myosin, pig and mouse tubulin, rabbit IgG, or pig thyroglobulin produced no significant inhibition. Finally, when a sheep erythrocyte lysate was used, no inhibition was observed. This observation is consistent with the hypothesis that this cell type lacks clathrin (<0.01% of proteins in cell lysate). It is known that mature erythrocytes do not perform either endocytosis or intracellular membrane transport (26, 27), two cellular mechanisms thought to require the participation of clathrin.

**Inhibition Curves with Cell and Tissue Extracts**

The binding of anticlathrin immune serum was inhibited by extracts obtained from either tissue culture cells or organs. The cell extracts were prepared in a buffer (buffer A) known to stabilize clathrin coats in vitro. Clathrin coats were then efficiently disassembled into triskelions (see Table II) by raising the pH of the extracts to 9.5, centrifuging them at 100,000 g, and neutralizing the supernatent to pH 7.4. Next, the immune serum was added to serial dilutions of these supernatants. Fig. 2B shows a typical curve of inhibition obtained with a rat liver extract. The slope of this curve was parallel to that obtained with purified clathrin, a result consistent with the inhibition of binding being due to clathrin in the extract.

To confirm the specificity of the immunoassay, Vero or X63Ag8 cell extracts were depleted of their clathrin content using an immunoprecipitation procedure. For this purpose, an aliquot of the cell extracts was incubated with an excess of affinity-purified rabbit anticlathrin antibodies. The immunocomplexes were subsequently precipitated using sheep antirabbit IgG. The removal of the excess of anticlathrin antibodies was monitored by incubating the cell extracts on microtiter plates coated with clathrin. Using a cell lysate depleted in clathrin by this procedure, we observed no inhibition of binding to immobilized clathrin (data not shown).

In other sets of experiments, we added purified clathrin to the extracts before performing the immunoassay. In the experiment shown in Fig. 2B, clathrin was added to a liver extract to a final concentration of 10 μg/ml. The resultant clathrin level was found almost equal to the sum of endogenous and exogenous (added) clathrin. This indicates that in our experimental conditions, the amount of measurable clathrin...
rin was not affected by other cellular proteins. It is worth mentioning that we have been able to measure clathrin in cell or tissue extracts that contained up to 4-5 mg/ml of total cellular protein. Therefore, given that 0.3–0.5 μg/ml of purified clathrin produced 50% inhibition of the binding of immune serum (Fig. 2A), it should be possible to detect clathrin in an extract when it makes up only 0.01% of the total protein. The experiment shown in Fig. 2B also indicates that clathrin is stable during the whole experimental procedure. We cannot rule out that some proteolytic degradation occurred; however, it did not quantitatively affect the antigen-antibody reaction.

**Quantification of Total, Assembled, and Unassembled Clathrin in Cell and Tissue Extracts**

Total, assembled, and unassembled clathrin was quantitated in rat lymphoid cells, Vero cells, X63Ag8 myeloma cells, rat liver, and rat brain cortex. Figs. 3 and 4 give typical titration curves that we obtained with rat brain cortex and rat lymphocytes. We then measured the ratio of clathrin in an assembled and unassembled state using the centrifugation procedure. We found that most of the clathrin was in an assembled form in rat brain cortex extracts (86%), whereas only 30% was in the assembled form in lymphocytes (Table 1). In these experiments, the yield of clathrin was 110% (brain cortex) and 87% (rat lymphocytes). Similar experiments were performed with Vero cells, myeloma cells, and rat livers. We summarize in Table 1 the results we obtained. We observed that the relative abundance of clathrin with respect to total cellular protein is similar in Vero cells (0.18%), myeloma cells (0.16%), rat liver cells (0.07%), and lymphocytes (0.11%). The amount of total clathrin was found to be significantly higher (0.75%) in brain cortex. From these values, we also calculated the average number of triskelions per cell, assuming a molecular weight of 640,000 for a clathrin triskelion. This number ranged from $2.8 \times 10^4$ in lymphoid cells to $4.8 \times 10^5$ in myeloma cells.

**Additional Control Experiments**

The following experiments were performed to further validate our experimental procedure.

**Effectiveness of the Fractionation Procedure**

Clathrin pools were separated by centrifugation (Fig. 1). We assumed that unassembled clathrin would be recovered in the supernatants whereas clathrin coats would be recovered in the corresponding pellets. As the clathrin quantification assay requires that clathrin triskelions be free in solution, the pellet that contained clathrin coats, once isolated, was exposed to a pH of 9.5 to dissociate clathrin coats into triskelions. Triskelions were subsequently separated from the insoluble residue by centrifugation, and the super-

| Table 1 | Clathrin Content and Distribution of Clathrin Pools in Various Cell Types |
|---------|-------------------------------------------------|
| Cell extract | Clathrin (% of cellular proteins) | Triskelions per cell | Unassembled clathrin | Assembled/unassembled |
| Rat brain cortex | 0.75 ± 0.1* | ND | 14 ± 8 | 6.0 |
| Rat livers (perfused) | 0.07 ± 0.0 | ND | 35 ± 3* | 1.8 |
| X63Ag8 | 0.16 ± 0.07 | $3.8 \times 10^3 \pm 1.25 \times 10^3$ | 45 ± 5 | 1.2 |
| Vero cells | 0.18 ± 0.02 | $4.8 \times 10^5 \pm 10^5$ | 59 ± 5 | 0.7 |
| Rat lymphoid cells | 0.11 ± 0.03 | $2.8 \times 10^4 \pm 0.9 \times 10^4$ | 70 ± 2 | 0.42 |
| Sheep erythrocytes | Undetectable | ND | ND | ND |

* Means ± 1 SD of three or four experiments.
* Unperfused livers were used for the measurements of assembled and unassembled clathrin to avoid a possible redistribution of clathrin pools induced by perfusion.
natant was recovered and adjusted to pH 7.4. To quantify unassembled clathrin in the same experimental conditions, we also adjusted the pH of the first supernatant to pH 9.5 and then neutralized it.

The following experiments were designed to test the validity of this procedure. First, we centrifuged at 100,000 g a solution of purified clathrin (10 μg/ml) in buffer A containing 0.2% Triton X-100 and 4 mg/ml BSA (previously adjusted to pH 9.5 and neutralized). Under these conditions, no significant amount of triskelions was found in the pellet. Then we centrifuged fresh prepared coated vesicles and centrifuged them at 100,000 g in buffer A containing 0.2% Triton X-100 and 4 mg/ml BSA (Fig. 5). Two concentrations of coated vesicles were tested (20 and 2 μg/ml), corresponding to the range of concentrations of assembled pools of clathrin calculated to be in extracts from brain cortex and lymphocytes, respectively. As shown in Fig. 5, >90% of clathrin from coated vesicles could be recovered as a pellet. The above results demonstrate the stability of clathrin coats during our experimental procedures. Moreover, these results indicate the efficiency of a centrifugation to separate quantitatively unassembled from assembled clathrin.

**Efficiency of Clathrin Disassembly by High pH**

It is known that high pH leads to a nearly complete dissociation of clathrin coats into triskelions (20). To confirm this, we analyzed the pellet resulting from centrifugation at pH 9.5 of a fibroblast extract. We treated this pellet at pH 9.5 with Na2CO3 and centrifuged for 1 h at 100,000 g. The total amount of clathrin was estimated in the first (column 1) and second (column 2) supernatants. The residual pellet was also analyzed for clathrin content (column 3). The results are presented in Table II. These results have also been confirmed by immunoblotting of the residual pellet (data not shown).

**Effect of Cell Lysis Procedure on the Recovery of Unassembled Clathrin**

In order to lyse the cells efficiently, we routinely added 0.2% Triton X-100 to buffer A. Triton X-100 up to a concentration of 1% does not dissociate clathrin from coated vesicles (28). Under these cell lysis conditions, we might be isolating assembled clathrin in the form of complete cages (derived from coated vesicles) and partial cages (derived from coated pits) (29). Pearse has reported that some of the coated particles isolated in the presence of Triton X-100 from human placenta are possibly derived from both coated pits and coated vesicles (21). To determine whether the recovery of unassembled clathrin is preferable, we either broke X63Ag8 myeloma cells in the absence of Triton X-100 with a Dounce homogenizer or homogenized liver with a Waring blender, and fractionated this cell extract. As shown in Table III, the amount of re-extracted clathrin represented 50% of the total clathrin, whereas in the control

![Figure 5](image-url)

**Figure 5** Stability of purified coated vesicles. Purified coated vesicles (20 μg/ml [△, ●] and 2 μg/ml [○, ●]) in 1 ml of stabilizing buffer (0.2% Triton X-100 containing 4 mg/ml BSA) were centrifuged for 1 h at 100,000 g. The supernatant was recovered and adjusted to pH 7.4. To quantify unassembled clathrin in the same experimental conditions, we also adjusted the pH of the first supernatant to pH 9.5 and then neutralized it.

**TABLE II**

| Cell extract          | First supernatant | Second supernatant | Pellet |
|-----------------------|-------------------|--------------------|--------|
| Vero cells*           | 26.5 μg           | 0.4 μg             | ND     |
| Rat brain cortex†     | 39 μg             | 5.1 μg             | 1 μg   |

*ND, not detectable.

†2.5 ml (4.1 mg/ml) of a Vero cells extract was adjusted to pH 9.5 with Na2CO3 and centrifuged for 1 h at 100,000 g. The pellet was resuspended in 0.5 ml of buffer A, 0.2% Triton X-100 (pH 6.5) and clathrin was re-extracted at pH 9.5. The total amount of clathrin was estimated in the first (column 1) and second (column 2) supernatants. The residual pellet was also analyzed for clathrin content (column 3).

‡0.5 ml of a rat brain cortex “pellet” fraction (Fig. 1) was centrifuged for 1 h at 100,000 g. We then re-extracted clathrin from the pellet as described above and estimated clathrin content in the first and second supernatants as well as in residual pellet.

§Numbers in parentheses indicate the percentage of the first supernatant.

**TABLE III**

| Lysis procedure       | Cell extract       | % of cellular proteins | % of unassembled clathrin |
|-----------------------|--------------------|------------------------|---------------------------|
| Mechanical breaking   | Myeloma cells      | 0.20                   | 45                        |
| alone                 | Liver              | 0.10                   | 42                        |
| In presence of Triton | Myeloma cells      | 0.22                   | 51                        |
| X-100                 | Liver              | 0.07                   | 37                        |

X63Ag8 myeloma cells were lyzed in buffer A containing Triton X-100 (0.2%) or in the same buffer without Triton X-100 with a Dounce homogenizer (100 strokes, on ice). Livers were homogenized with a Waring blender in buffer A containing Triton X-100 (0.2%) or without detergent. Cell lysates were obtained and each subcellular fraction was analyzed for clathrin content as usual.
experiment only 16% of clathrin was found in an unassembled form. We reasoned that most of the added clathrin was
recovered in the high-speed supernatant (unassembled form), suggesting that no reassocation of added triskelions had oc-
curred during the experiment.

**Clathrin Heavy Chains and Associated Light Chains are Both Present in Assembled and Unassembled Clathrin Pools:** One could argue that clathrin heavy chains and associated light chains may not be stoichiometrically represented in the assembled and unassem-
bled pools of clathrin. Since our antiserum recognizes both, we tested this possibility by immunoblotting analysis using
affinity-purified rabbit anticlathrin antibodies (as previously
described in reference 16). These experiments were carried
out using total assembled and unassembled fractions from
brain and lymphocyte extracts. In all cases, heavy and light
chains were easily identified. Furthermore, the ratio of heavy
to light chains appeared to be similar (data not shown).
Therefore, we conclude that each clathrin pool contains both
polypeptides.

**Discussion**

The aim of this study was to determine the clathrin content
and the distribution of assembled and unassembled clathrin
pools within various cell types. For this purpose, we have
developed a competitive solid-phase enzyme immunoassay
that can measure clathrin in cell lysates. A fractionation
procedure was also designed to separate the pools of assembled
and unassembled clathrin.

As illustrated in Fig. 2B, cell lysates inhibited the binding
of the rabbit anticlathrin immune serum to immobilized
clathrin in a specific way. The inhibition of binding was found
to be nearly total, which indicates that similar antigenic
determinants are recognized on clathrin molecules present in
cell lysates and on immobilized clathrin. In all cell types so
far tested, these antigenic determinants were present on both
clathrin heavy chains and associated light chains as demon-
strated by immunoblotting (reference 16 and unpublished
results). In addition, the curves of inhibition were found to
be parallel, which suggests that the anticlathrin antibodies
have the same affinity for clathrin present in all cell extracts
tested. These antibodies were found to cross-react efficiently
with clathrin from chicken, rodents, and other mammalian
cells including dog, bovine, and human cell lines (16). There-
fore, the assay we have developed could be applied to a wide
variety of cells or tissues from various origins.

One important question, still debated, concerns the exist-
ence or the nonexistence of unassembled clathrin inside the
cells (11, 12). The results we report here strongly suggest that
in fact a measurable fraction of clathrin is present in an
unassembled form (Table I). These data provide further in-
dependent evidence for the presence of unassembled clathrin
in the cytoplasm, as we previously suggested using a mono-
clonal antibody reacting with an epitope unavailable in clath-
rin coats but accessible in triskelions (16). It is unlikely that
this cytosolic pool could be generated during our experimental
procedures, since in using purified coated vesicles, as a model
system, we found that only a small amount of clathrin disso-
ciated during the whole procedure (Fig. 3). It also seems
unlikely that the unassembled pool of clathrin could result
from the dissociation of incomplete cages (for example, from
coated pits) after the solubilization of associated membranes
with Triton X-100 because we found a similar amount of
clathrin in the high-speed supernatants after mechanical
breaking of the myeloma cells or livers prepared in the same
stabilizing buffer (Table III), a treatment that probably pre-
serves the association of coated structures with membranes
(30).

We have been able to estimate the clathrin content in
various cell types and tissues. As summarized in Table I, it
appears that the percentage of clathrin in eucaryotic cells is
relatively constant. Our data indicate that clathrin represents
~0.1–0.2% of total cellular proteins. The slightly lower value
obtained for perfused liver may be due to the fact that
hepatocytes contain a significant amount of protein destined
for secretion, in addition to their cellular proteins. A remark-
able exception, however, is the brain cortex, in which clathrin
represents 0.7% of total cellular proteins. The significance of
this result is currently unknown.

The cell types we have chosen in these experiments exhibit
large differences in the cellular activities that are thought to
require the participation of clathrin. In brain cells, coated
vesicles play a major role in the retrieval of excess cell surface
membrane in presynaptic neurons (31). In fibroblasts and
liver cells, clathrin, organized as coated pits and coated vesi-
cles, has been shown to be involved in receptor-mediated
endocytosis of various ligands (for a review, see reference 2).
In myeloma cells, a major function of coated vesicles could
be to mediate plasma membrane retrieval following the secre-
tory process of immunoglobulins (17, 32). In this context,
that cells contain a constant level of clathrin confirms that
the degree of expression of clathrin is not directly related to a
particular cellular function as first suggested by Pearse (33).
Perhaps the most striking data concern normal lymphoid
cells, because these cells display low secretory and endocytic
activities yet contain as much total clathrin as other cells. For
example, we have calculated that normal rat lymphoid cells
internalize ~0.3 nl/h per 10⁶ cells of fluid medium (34). This
represents 106 times less than mouse L fibroblasts (35) or 364
times less than rat hepatocytes (36).

It has been suggested (8, 9) that coated pits and vesicles
form in vivo by a cycle of assembly-disassembly of clathrin
coats that has been shown to occur in vitro (5–7, 10, 20, 25,
28, 37). Furthermore, whereas the concentration of clathrin
(relative to other proteins) is similar in most of the cell types
we studied, the ratio of assembled to unassembled clathrin
varies greatly (Table I). It is striking that brain contains the
highest level of assembled clathrin and lymphoid cells the
lowest. This raises the possibility that a positive correlation
may exist between the size of the assembled pool and the
intracellular membrane transport activity of the cell. As a
consequence, an activated lymphocyte could recruit clathrin
from its unassembled pool to form coated pits and/or coated
vesicles when the membrane activity increases, for instance,
during differentiation. Salisbury et al. have indeed observed a
recruitment of clathrin coats during the capping of surface
IgG induced by multivalent anti-Ig antibodies on lympho-
blastoid cells (38). Note also that in the secreting myeloma
cells there is only 45% of unassembled clathrin compared
with 70% in the lymphoid cells (Table I). In cultured fibro-
blasts, Lubinski and Huet (39) have recently shown that
various ligands can induce accumulation of coated vesicles,
individually from coated pits, and Larkin et al. (40) reported
that in fibroblasts depleted in potassium, there is a dramatic
inhibition of low-density lipoprotein uptake, associated with
a disappearance of immunoreactive clathrin coats at the cell surface. In each of these examples, it is likely that the size of the unassembled clathrin will be decreased with increased membrane transport and, conversely, will increase when membrane transport is arrested.

In conclusion, the results of experiments we report in this paper favor the existence of at least two intracellular pools of clathrin. The enzyme immunoassay that we have developed to quantify these two pools of clathrin should provide a useful tool to analyze the state of clathrin in response to changes in cellular demands for intracellular membrane transport.

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