The Pinocytosis of ^{125}I -Labelled Poly(vinylpyrrolidone), $[^{14}\text{C}]$ Sucrose and Colloidal $[^{198}\text{Au}]$ Gold by Rat Yolk Sac Cultured *in vitro*

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The rates of uptake of ^{125}I -labelled poly(vinylpyrrolidone), $[^{14}\text{C}]$ sucrose and colloidal $[^{198}\text{Au}]$ gold by 17.5-day rat yolk sac cultured *in vitro* were studied. Over a 6.5 h period each substrate was accumulated at a constant and reproducible rate of approx. $2\mu\text{l/h}$ per mg of protein. After accumulation *in vitro*, the three substances were released from the tissue into substrate-free medium at low rates. Sucrose present in the medium at concentrations up to 10mg/ml was without effect on the accumulation of either $[^{14}\text{C}]$ sucrose or ^{125}I -labelled poly(vinylpyrrolidone), but at higher concentrations inhibited the uptake of both substrates. Some batches of colloidal $[^{198}\text{Au}]$ gold had a significantly higher Endocytic Index (up to $5\mu\text{l/h}$ per mg of protein). The Endocytic Index of such a batch decreased with increasing substrate concentration, but colloidal gold did not decrease the Endocytic Index of ^{125}I -labelled poly(vinylpyrrolidone). It is concluded that the three substrates enter the yolk sac by pinocytosis in the liquid phase. Those batches of colloidal $[^{198}\text{Au}]$ gold with higher Endocytic Indices are considered to enter also by adsorption on membrane binding-sites.

Jacques (1975) has proposed two mechanisms by which a substrate can enter a cell by pinocytosis: it can enter in the liquid phase, through engulfment of microscopic droplets of extracellular fluid in which it is present, or it can adsorb on the external surface of the plasma membrane which subsequently invaginates to form the limiting membrane of a pinocytic vesicle. The most general model of the pinocytic process would envisage both routes of entry being available to a substrate.

Recently, certain features of Jacques's (1975) model have been examined experimentally with the rat yolk sac, a tissue whose pinocytic activity has been extensively studied. The rates of ingestion of ^{125}I -labelled poly(vinylpyrrolidone) and of different preparations of ^{125}I -labelled bovine serum albumin by 17.5-day yolk sac incubated *in vitro* indicated that the major route of entry of albumin, particularly of those preparations in which the native conformation had been modified by chemical treatments, is by adsorption on the plasma membrane (Williams *et al.*, 1975a,b; Moore *et al.*, 1977). ^{125}I -labelled poly(vinylpyrrolidone) was ingested at a far lower rate, from which it was possible to calculate a minimum value for the amount of ^{125}I -labelled protein that must enter by the adsorptive route. The precise amount entering by this route can only be determined if the rate of pinocytic ingestion of extracellular fluid can be established.

If substrates could be identified that were known not to adsorb on the plasma membrane and hence

could enter cells pinocytically only in the liquid phase, then each should exhibit an identical rate of uptake when expressed in the form of an Endocytic Index [defined by Williams *et al.* (1975a) as the volume of culture medium (μl) whose contained substrate is captured/h by unit quantity of yolk-sac tissue]. In the present study three chemically diverse non-protein substances [^{125}I -labelled poly(vinylpyrrolidone), $[^{14}\text{C}]$ sucrose and colloidal $[^{198}\text{Au}]$ gold] were used as substrates in the yolk-sac system and their Endocytic Indices determined under a number of experimental conditions.

Materials and Methods

^{125}I -labelled poly(vinylpyrrolidone) (mean mol.wt. 30000–40000, preparation IM.33P, 20–60 $\mu\text{Ci/mg}$), $[^{14}\text{C}]$ sucrose (preparation CFB.146, > 350 mCi/mmol), colloidal $[^{198}\text{Au}]$ gold (preparation GCS.1P, 4–12 mCi/mg), and non-radioactive colloidal gold (batch H41) were all from The Radiochemical Centre (Amersham, Bucks., U.K.). Toluene (scintillation grade) was obtained from Hopkin and Williams (Chadwell Heath, Essex, U.K.), butyl-PBD [5-(bi-phenyl-4-yl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole] from Koch–Light Laboratories (Colnbrook, Bucks., U.K.) and Triton X-100 from Lennig Chemicals (Croydon, Sussex, U.K.). Tissue-culture medium 199 and inactivated calf serum (preparations TC 20 and CS 07 respectively) were obtained from Wellcome Reagents (Beckenham, Kent, U.K.). Bovine serum albumin (fraction V) was a product of Sigma

(London) Chemical Co. (London S.W.6, U.K.). All other chemicals were of AnalaR grade.

Incubation of 17.5-day rat yolk sacs with radioactively labelled substrates

In each experiment yolk sacs (8–12), taken at 17.5 days of gestation from the same pregnant Wistar rat, were incubated each in a separate flask containing medium 199 (9.0ml), calf serum (1.0ml) and labelled substrate by the method of Williams *et al.* (1975a) with minor modifications. Unless otherwise stated, the concentrations of radioactive tracer in the medium were 2–5 $\mu\text{g/ml}$ for ^{125}I -labelled poly(vinylpyrrolidone), 1 $\mu\text{g/ml}$ for colloidal [^{198}Au]-gold and 0.1 $\mu\text{g/ml}$ for [$\text{U-}^{14}\text{C}$]sucrose.

Measurement of radioactivity in the culture medium

In experiments with either ^{125}I -labelled poly(vinylpyrrolidone) or colloidal [^{198}Au]-gold as substrate, the amount of radioactivity in the medium was determined by counting duplicate 1.0ml portions in 3ml disposable tubes on a Selektroic gamma spectrometer (Packard Instrument, Caversham, Berks., U.K.) with a standard counting geometry.

Media from experiments where [$\text{U-}^{14}\text{C}$]sucrose was used as substrate were assayed in duplicate, by adding 1.0ml samples from each flask to 10.0ml of scintillation 'cocktail' [butyl-PBD (6g/l) in toluene/Triton X-100 (2:1, v/v)] and counting samples for radioactivity in a Packard 2425 liquid-scintillation spectrometer with a sample-chamber temperature of 13°C. Samples remained homogeneous in appearance and no drift in the observed count was detected over periods up to 5 days. No suitable hydrophilic internal standard containing ^{14}C was commercially available and, since both Phenol Red (present as an indicator in the culture medium) and [$\text{U-}^{14}\text{C}$]sucrose are likely to be found in the aqueous phase of the emulsion, relative counting efficiencies, rather than absolute efficiencies based on a hydrophobic internal standard, were used. The relative counting efficiency of a sample was defined as the increase in the observed c.p.m., on addition of a given quantity of [$\text{U-}^{14}\text{C}$]sucrose to the sample, divided by the observed c.p.m. when an identical addition was made to 1.0ml of water in 10ml of scintillation 'cocktail'. After the initial count of each sample of medium, a second count was made after addition of 25 μl of a [$\text{U-}^{14}\text{C}$]sucrose stock solution (equivalent to approx. 10^5 d.p.m. of radioactivity) to each of the vials containing medium and to four vials each containing 1.0ml of water and 10.0ml of scintillation 'cocktail'.

Measurement of radioactivity in yolk sac

With either ^{125}I -labelled poly(vinylpyrrolidone) or colloidal [^{198}Au]-gold as substrate, each yolk sac was

solubilized by incubation in 5.0ml of 1M-NaOH at 37°C for 1h and duplicate 1.0ml portions of the resulting solution were assayed by using the method for the associated media (see above). Because of the short half-life (2.69 days) of ^{198}Au , it was essential to count solutions of yolk sac for radioactivity after their corresponding media to minimize the differential effects of radioactive decay on the observed values.

When determining the amount of radioactivity associated with the yolk sac in experiments with [$\text{U-}^{14}\text{C}$]sucrose as substrate, a milder method of tissue solubilization was developed to decrease the possible adverse effects of either high pH or salt concentration on the stability of the emulsion and on the counting efficiency. Preliminary experiments with different amounts of NaOH and differing regimes of incubation and/or sonication showed that the tissue could be fully solubilized, in a reproducible manner, by sonicating each yolk sac for 2h at 37°C in 2.0ml of 0.25M-NaOH. Such solutions were then neutralized by adding 2.0ml of 0.25M- HNO_3 , and the volume was made up to 5.0ml with water before duplicate 1.0ml portions were assayed by using the method described for the samples of associated medium. The counting emulsion was stable for at least 5 days and the observed count was insensitive to slight fluctuations in the pH of the sample. The relative counting efficiencies of solutions of yolk sac were shown to decrease progressively in an almost linear manner with respect to time as the quantity of tissue protein in a sample was increased; a quench-correction curve was constructed and used to determine the relative counting efficiency of individual yolk-sac solutions after the protein content had been determined (see below).

Determination of protein

The method of Lowry *et al.* (1951) was used to determine the protein content of yolk sacs, with bovine serum albumin as reference protein.

Expression of rates of uptake

The rate of uptake of each labelled substrate was expressed in the form of an Endocytic Index, a form of expression that has the merit of rendering the results independent of the effects of variation in the specific radioactivity of the substrate, radioactive decay of the labelled substrate and variation in the size of individual yolk sacs; it also permits a direct comparison of the rates of ingestion of different substrates.

Determination of the rate of release of labelled substrate from yolk sacs

To measure the rate of release of a substrate that had been accumulated *in vitro*, individual yolk sacs were incubated for 3h, as above, but with five times

the normal concentration of labelled substrate in the medium. After rinsing at 37°C for 2min periods in 3×10ml of fresh medium gassed with O₂/CO₂ (19:1), the individual yolk sacs were reincubated in 10.0ml of substrate-free medium for a further 4h. The pattern of release with time was determined as described by Williams *et al.* (1975a).

Effect of prior exposure of yolk sacs to non-radioactive sucrose on their capacity to accumulate ¹²⁵I-labelled poly(vinylpyrrolidone)

To determine whether exposure to high concentrations of sucrose permanently modified the pinocytic capacity of the tissue, through cytotoxic or other effects, yolk sacs were first incubated for 2.5 h in the presence of various concentrations of sucrose, then incubated for 2min periods in 3×10ml of fresh gassed substrate-free medium at 37°C, to remove extracellular sucrose, before reincubation for 2.5 h in medium (10.0ml) containing the usual concentration of ¹²⁵I-labelled poly(vinylpyrrolidone) but no sucrose.

Results

Fig. 1 shows the results of three representative experiments with ¹²⁵I-labelled poly(vinylpyrrolidone), [U-¹⁴C]sucrose and colloidal [¹⁹⁸Au]gold respectively as substrate. Uptake of all three substrates was linear with time over a 6.5 h period. The Endocytic Index of ¹²⁵I-labelled poly(vinylpyrrolidone) was 1.57±0.35 (s.d.) μl/h per mg of protein in 46 experiments and that of [U-¹⁴C]sucrose was 2.04±0.21 (s.d.) μl/h per mg of protein in five experiments; no inter-batch variation was seen in the Endocytic

Table 1. Inter-batch variation in the Endocytic Index of colloidal [¹⁹⁸Au]gold

Each value of the Endocytic Index was derived from the results from a separate experiment in which yolk sacs (eight to ten) from a single 17.5-day pregnant rat were incubated in the presence of radioactively labelled substrate for up to 6.5h. Uptake (calculated as described in the text) was plotted against time; the Endocytic Index is equal to the gradient of the regression line through these points. Values are given as means±s.d. where appropriate.

Batch of colloidal [¹⁹⁸ Au]gold	No. of experiments	Endocytic Index (μl/h per mg of yolk-sac protein)
I	2	1.45 (1.73, 1.27)
II	3	2.75 ± 1.25
III	2	1.79 (1.64, 1.94)
IV	2	1.87 (2.40, 1.33)
V	3	4.83 ± 0.43
VI	5	4.79 ± 1.50
VII	2	3.22 (3.53, 2.91)

Fig. 1. Accumulation of radioactivity by rat yolk sacs incubated in the presence of tracer amounts of labelled substrates

The results of three separate representative experiments are shown. Substrates were: ▲, colloidal [¹⁹⁸Au]gold (1 μg/ml); ○, [U-¹⁴C]sucrose (0.1 μg/ml); ●, ¹²⁵I-labelled poly(vinylpyrrolidone) (2–5 μg/ml). The ordinate axis shows the volume of culture medium whose contained radioactivity has been accumulated within a given time interval; values are normalized on the basis of the protein content of the tissue.

Index of ¹²⁵I-labelled poly(vinylpyrrolidone) for the six batches examined. Table 1 shows that the rate of uptake of colloidal [¹⁹⁸Au]gold was both less reproducible for a given batch and exhibited inter-batch variation. Certain batches showed a mean Endocytic Index substantially greater than those of ¹²⁵I-labelled poly(vinylpyrrolidone) or [U-¹⁴C]-sucrose.

Fig. 2 shows the pattern of release of [U-¹⁴C]-sucrose and colloidal [¹⁹⁸Au]gold from yolk sacs after accumulation *in vitro*. During the 4.0h re-incubation period, all three substrates showed a variable, rapid, release in the initial 30–60 min, then a slower, reproducible, constant rate of release over the remaining period. In each experiment the rate of

Fig. 2. Release of radioactivity into culture medium on reincubating yolk sacs that had accumulated a labelled substrate *in vitro*

The radioactivity released into the culture medium is expressed as a percentage of the radioactivity associated with the tissue at the time of its transfer to fresh medium containing no labelled substrate. The rate of release in the slow phase is given by the gradient of the regression line fitted to the points in the period 1–4 h. The intercept on the ordinate, obtained by extrapolation of this line, gives an estimate of the radioactivity freed in the initial rapid phase of release. The results of two representative experiments are shown, one with colloidal [^{198}Au]-gold as substrate (▲) and one with [^{14}C]sucrose (○).

release in the linear region was determined from the gradient of the regression line fitted to those points between 1 and 4 h of reincubation. The mean rates of release of colloidal [^{198}Au]gold and [^{14}C]sucrose were determined from six such experiments with each substrate and found to be 0.88 ± 0.29 and $3.36 \pm 0.69\%/h$ respectively. A value of $1.43 \pm 0.86\%/h$ was reported by Roberts *et al.* (1976) for the rate of release of ^{125}I -labelled poly(vinylpyrrolidone) in equivalent experiments.

Table 2 shows the effect of non-radioactive sucrose in the medium on the rate of uptake of [^{14}C]-

Table 2. Effect of sucrose on the Endocytic Index of [^{14}C]sucrose by 17.5-day rat yolk sacs

Variable amounts of non-radioactive sucrose, dissolved in medium 199, were added to culture media containing a constant amount of [^{14}C]sucrose ($0.1\text{ }\mu\text{g/ml}$). Each value of the Endocytic Index was derived from a separate experiment, as described in Table 1. Results are given as means $\pm$ S.D.

Concentration of non-radioactive sucrose in medium (mg/ml)	No. of experiments	Endocytic Index ($\mu\text{l/h}$ per mg of yolk-sac protein)
0	5	2.04 ± 0.21
0.1	3	2.27 ± 1.21
1.0	3	1.99 ± 0.29
10.0	4	2.00 ± 0.79
25.0	3	0.93 ± 0.20
50.0	4	0.38 ± 0.04
100.0	4	0.36 ± 0.09

Table 3. Effects of sucrose and colloidal-gold concentration on the Endocytic Index of ^{125}I -labelled poly(vinylpyrrolidone) by 17.5-day rat yolk sacs

Variable amounts of the non-radioactive colloidal-gold preparation or sucrose, dissolved in medium 199, were added to the culture medium containing a tracer amount of ^{125}I -labelled poly(vinylpyrrolidone) ($1\text{--}10\text{ }\mu\text{g/ml}$). Each value of the Endocytic Index was derived from a separate experiment, as described in Table 1. Results are given as means $\pm$ S.D.

Concentration of non-radioactive substance in medium	No. of experiments	Endocytic Index ($\mu\text{l/h}$ per mg of yolk-sac protein)
Sucrose		
1 mg/ml	4	1.49 ± 0.27
10 mg/ml	4	1.17 ± 0.22
100 mg/ml	8	0.11 ± 0.08
Colloidal gold		
25 $\mu\text{g/ml}$	3	1.26 ± 0.15
40 $\mu\text{g/ml}$	3	1.87 ± 0.34
100 $\mu\text{g/ml}$	3	1.52 ± 0.11

sucrose. Concentrations from $100\text{ }\mu\text{g/ml}$ to 10 mg/ml had no detectable effect, but a sharp and significant decrease in uptake was noted at concentrations above 25 mg/ml . Table 3 shows these same high concentrations of sucrose to have a similar effect on the rate of ingestion of ^{125}I -labelled poly(vinylpyrrolidone).

Table 4 shows the effect of increasing concentrations of colloidal gold on the rate of ingestion of one of the batches of colloidal [^{198}Au]gold that exhibited a high Endocytic Index when present at $1\text{ }\mu\text{g/ml}$. Increasing the concentration of non-

Table 4. *Effect of colloidal-gold concentration on the Endocytic Index of colloidal [^{198}Au]gold by 17.5-day yolk sacs*
Variable amounts of a preparation of non-radioactive colloidal gold, dissolved in medium 199, were added to the culture medium containing batch-VII colloidal [^{198}Au]gold (0.01–3.0 $\mu\text{g}/\text{ml}$). Each value of the Endocytic Index was derived from a separate experiment, as described in Table 1. Results are given as means $\pm$ s.d. where appropriate.

Concentration of colloidal gold ($\mu\text{g}/\text{ml}$)			No. of experiments	Endocytic Index ($\mu\text{l}/\text{h}$ per mg of yolk-sac protein)
Radioactive	Non-radioactive	Total		
0.01	—	0.01	3	4.88 ± 1.62
0.1	—	0.1	3	4.80 ± 0.61
0.01	0.09	0.1	3	4.34 ± 0.66
1.0	—	1.0	2	$3.22 (3.53, 2.91)$
0.1	0.9	1.0	2	$2.54 (2.05, 3.02)$
1.0	9.0	10.0	3	1.58 ± 0.27
1.0	11.0	12.0	3	1.95 ± 0.47
1.0	24.0	25.0	3	2.33 ± 0.48
3.0	47.0	50.0	3	2.10 ± 0.34
1.0	69.0	70.0	3	1.13 ± 0.78
1.0	99.0	100.0	3	1.13 ± 0.01

Table 5. *Effect of preincubation with sucrose on the subsequent ingestion of ^{125}I -labelled poly(vinylpyrrolidone)*

In each experiment six yolk sacs from a single 17.5-day pregnant rat were incubated in the presence of a given concentration of non-radioactive sucrose for 2.5 h, incubated for 3×2 min in fresh medium to remove extracellular sucrose, then finally reincubated for 2.5 h in sucrose-free medium containing ^{125}I -labelled poly(vinylpyrrolidone) (2.0 $\mu\text{g}/\text{ml}$ of medium). Results are given as means $\pm$ s.d.

Concentration of sucrose in medium in initial 2.5 h period (mg/ml)	No. of experiments	Endocytic Index of ^{125}I -labelled poly(vinylpyrrolidone) ($\mu\text{l}/\text{h}$ per mg of yolk-sac protein)
0	3	1.80 ± 0.36
10	4	1.57 ± 0.55
50	4	1.00 ± 0.19
100	3	0.19 ± 0.15

radioactive colloidal gold over the range 0–99 $\mu\text{g}/\text{ml}$ of medium caused the rate of ingestion of colloidal [^{198}Au]gold (batch VII) to fall progressively from 4.88 ± 1.62 to 1.13 ± 0.01 $\mu\text{l}/\text{h}$ per mg of yolk-sac protein. Table 3 shows that these concentrations of the non-radioactive colloidal gold were without detectable effect on the rate of uptake of ^{125}I -labelled poly(vinylpyrrolidone).

Table 5 summarizes the results of a number of experiments in which the pinocytic ability of yolk sacs, after preincubation with various concentrations of sucrose for 2.5 h, was determined on reincubating the tissue in the presence of ^{125}I -labelled poly(vinylpyrrolidone) but in the absence of extracellular sucrose. The preincubation and washing procedures did not decrease the Endocytic Index of ^{125}I -labelled

poly(vinylpyrrolidone) on subsequent reincubation, nor did inclusion of 10 mg of sucrose/ml of medium during the preincubation. However, initial exposure of the tissue to either 50 or 100 mg of sucrose/ml of medium grossly impaired its subsequent ability to accumulate ^{125}I -labelled poly(vinylpyrrolidone).

Discussion

The low rates of substrate release observed on reincubating yolk sacs in tracer-free medium, after accumulation *in vitro* of [$\text{U-}^{14}\text{C}$]sucrose, colloidal [^{198}Au]gold or ^{125}I -labelled poly(vinylpyrrolidone), demonstrated that all three substrates had become similarly associated with the tissue and indicated that pinocytic ingestion rather than reversible surface adsorption was responsible for the observed progressive accumulation. Most probably, the initial rapid phase of release arises almost entirely from a small quantity of bulk culture medium occluded in the crypts between the villi on the tissue surface, that is not removed in the 3×2 min wash before reincubation. This explanation is compatible with the observed poor inter-experimental reproducibility of this phase of release and the absence of any correlation with the associated Endocytic Index. (Such a correlation would be expected if the initial phase represented a slow dissociation of tracer from binding sites on the plasma membrane.) The slower, more reproducible, linear phase of release represents the combined effects of cell death, exocytosis and slow release of any residual extracellular tracer. Thus exocytosis of all three substrates most probably occurs at such a low rate that the rates of accumulation by the tissue are to a good approximation equal to the true rates of pinocytic ingestion.

Williams *et al.* (1975a) showed that the Endocytic

Index of ^{125}I -labelled poly(vinylpyrrolidone) in 17.5-day rat yolk sac cultured under defined conditions was approx. $2\mu\text{l/h}$ per mg of protein and remained constant over a wide range of substrate concentrations ($0.15\mu\text{g/ml}$ to 1mg/ml). The Endocytic Index of undenatured ^{125}I -labelled bovine serum albumin was approx. $5\mu\text{l/h}$ per mg of protein, but values up to $80\mu\text{l/h}$ per mg of protein were seen with preparations that had been exposed to chemical treatments (Williams *et al.*, 1975b; Moore *et al.*, 1977). It was concluded that the rapid pinocytosis of denatured albumin was due to adsorption of substrate on binding sites on the invaginating plasma membrane. These results were consistent with a model in which the rate of ingestion of liquid by the tissue was $2\mu\text{l/h}$ per mg of protein and in which uptake of ^{125}I -labelled poly(vinylpyrrolidone) was entirely in the liquid phase. This model would predict that, unless the rate of pinosome formation was altered by a substrate, all substrates able to penetrate freely into the pinocytic invaginations would have the same Endocytic Index ($2\mu\text{l/h}$ per mg of protein) if they entered solely in the liquid phase and a higher value if they entered also by adsorption on membrane.

In contrast with the rat peritoneal macrophage, in which colloidal ^{198}Au gold has been shown to be ingested at a far higher rate than either ^{14}C sucrose or ^{125}I -labelled poly(vinylpyrrolidone) (Pratten *et al.*, 1977), the yolk sac ingests these three substrates at approximately the same rate. This implies that either all three enter yolk sacs solely in the liquid phase or all adsorb to the same extent on plasma-membrane binding sites. Since the three substances differ widely in chemical properties and molecular size, the former explanation is much more plausible. It is therefore concluded that the rate of pinocytosis of liquid by the yolk sac in culture is approx. $2\mu\text{l/h}$ per mg of protein.

The Endocytic Index of $[\text{U-}^{14}\text{C}]$ sucrose was independent of the sucrose concentration within the range $0.1\mu\text{g/ml}$ to 10mg/ml . This result too is consistent with $[\text{U-}^{14}\text{C}]$ sucrose entering only in the liquid phase, a suggestion made by Dingle *et al.* (1969) from studies on chick limb-bone rudiments. At higher concentrations, the Endocytic Index was much decreased. This could have indicated competition between normal and labelled species for sucrose-binding sites on the plasma membrane, but since these high sucrose concentrations similarly decrease the Endocytic Index of ^{125}I -labelled poly(vinylpyrrolidone) it is more likely that such concentrations of sucrose inhibit the pinocytic uptake of liquid. The latter explanation is confirmed by the observation that preincubation of yolk sacs in these concentrations of sucrose greatly decreased the capacity of the tissue to capture ^{125}I -labelled poly(vinylpyrrolidone) even when reincubated in the absence of extracellular

sucrose. Wagner *et al.* (1971) showed that accumulation of sucrose by Chang-strain human liver cells resulted in inhibition of pinocytosis.

The Endocytic Indices of three batches of colloidal ^{198}Au gold were significantly higher than $2\mu\text{l/h}$ per mg of protein. When the effect of altering the substrate concentration on the rate of uptake of one of these batches was examined, it was found that the Endocytic Index decreased as the concentration of colloidal gold increased. Inhibition of pinocytosis by increasing concentrations of colloidal gold is compatible with such concentrations of the colloid being toxic to the tissue, but this can be excluded by the observed failure of colloidal gold to decrease the Endocytic Index of ^{125}I -labelled poly(vinylpyrrolidone). It is concluded that batch-VII colloidal gold is ingested partly in the liquid phase and partly by an adsorptive mechanism of low capacity that becomes saturated at fairly low substrate concentrations. Differing degrees of adsorption on the plasma membrane are thus the probable explanation of the varying Endocytic Indices of different batches of colloidal ^{198}Au gold and could result from differing amounts or preparations of gelatin being used as stabilizer in individual preparations. Caro *et al.* (1970) have reported that the rates of clearance of colloidal gold *in vivo* differ markedly when stabilized with gelatin rather than with poly(vinylpyrrolidone). Also, Normann & Benditt (1965) have shown that the rate of clearance of colloidal carbon *in vivo* is strongly dependent on the amount of gelatin used as stabilizer.

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