STUDIES ON PROTEIN UPTAKE BY ISOLATED TUMOR CELLS

II. Quantitative Data on the Adsorption and Uptake of I^{131}-Serum Albumin by Ehrlich Ascites Tumor Cells

H. RYSER, M.D., J. C. AUB, M.D., and J. B. CAULFIELD, M.D.

From the Department of Pathology and the John C. Warren Laboratory of the Huntington Memorial Hospital at the Massachusetts General Hospital, Harvard Medical School, Boston. Dr. Ryser's present address is Department of Pharmacology, Harvard Medical School, Boston

ABSTRACT

Surface adsorption is studied in some detail because it is believed to be a major artifact in measurements of protein uptake by mammalian cells. Adsorption increases linearly with the I^{131}-albumin concentration between 0.001 and 300 mg/ml. After short exposure to 300 mg/ml and two cell washings, the adsorption amounts to 38 mg albumin per gm cell proteins. Further washings remove 80 per cent of this value, leaving a small irreversibly bound residue. At equilibrium, adsorbed albumin can be labeled by a simple albumin exchange. This labeling reaches a steady state within seconds and stays at constant level over 30 minutes. Significant increases above this initial level are measured over periods of 2 hours. In our experimental conditions these increases can be considered due to albumin uptake. This uptake rises linearly with the albumin concentration between 0.5 and 50.0 mg/ml, and reaches 0.2 mg/gm cell protein or 4×10^5 molecules per cell. Compared to the incorporation of free amino acids in similar conditions, this value does not appear to contribute significantly to the N-metabolism of the tumor cells. Adsorption was generally greater than uptake. Both processes are linear functions of the same variable over the whole range of concentration tested. It is suggested that albumin is taken up by pinocytosis.

INTRODUCTION

Evidence has been reported in a preceding study that large protein molecules such as ferritin can be transported into the cytoplasm of morphologically intact tumor cells (19). The main question to be considered in this paper is whether such an uptake is readily measurable. As discussed previously, ferritin molecules are taken up by means of pinocytotic membrane movements (19). Little is known about the dynamics and regulations of this transport process. Information on this subject will largely depend on methods of quantitative evaluation. Any efforts in this direction will, therefore, be of essential value.

Several requirements must be met to measure a protein uptake by mammalian cells, using radioactive tracer techniques. The procedure must exclude a reincorporation of small molecular label liberated in the medium during a possible protein breakdown. Secondly, it must allow a clear distinction between true penetration and surface adsorption. Furthermore, the incubation conditions must be optimal so as to prevent pathological

changes of cellular permeability and adsorption to injured cells.

None of the quantitative data on protein uptake currently available in the literature have been obtained in a way to satisfy all these criteria (2–5, 17, 22). In addition, publications on this subject have led to contradictory results. In a recent study on ascites tumor cells, Thomason *et al.* (22) did not find any measurable uptake of smaller than the number of ferritin molecules estimated to enter a tumor cell during 2 hours of incubation (19).

A series of experiments has been performed under experimental conditions satisfying the requirements mentioned above, and the present paper summarizes the data obtained for the adsorption and uptake of albumin by ascites tumor cells during a 2-hour incubation.

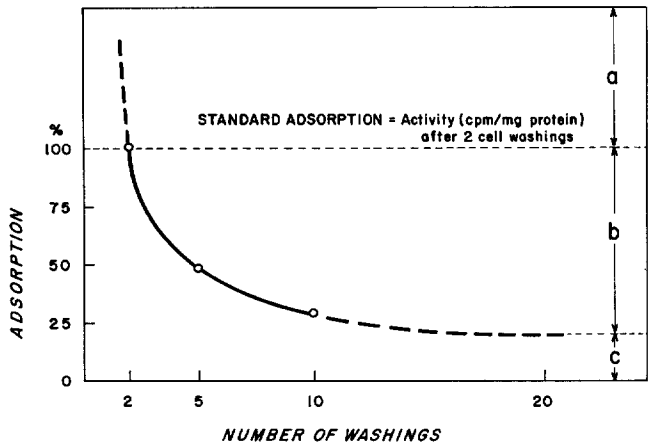


FIGURE 1

Decrease of albumin adsorption upon successive washings. The sample activity after 2 washings in Tyrode is set as 100 per cent (standard value). Extrapolation to an infinite number of washings makes it possible to distinguish between an irreversible (*c*) and a reversible (*a* + *b*) adsorption. Fraction (*a*) corresponds to the albumin discarded during the first 2 washings. The points are averages of 5 samples exposed for 15 minutes at 25°C to I^{131}-albumin. The percentage of desorption is identical in various albumin concentrations.

I^{131}-albumin, whereas Pileri *et al.*, using the same cell type and comparable incubation techniques, described a considerable uptake of ribonuclease (17).

From a calculation based on the number of ferritin particles seen in some of our electron microscopic sections of ascites tumor cells (19), it appears likely that an uptake of labeled protein would reach a measurable order of magnitude, provided that the measurements are done on a large enough cell sample, with protein of high specific radioactivity and over several hours of incubation.

With a sample of 5×10^7 cells, a total sample activity of 100 CPM could be expected if each cell took up 400 albumin molecules from a medium containing 50 μc/mg albumin, or 20,000 molecules from a medium containing 1 μc/mg albumin. These required figures of uptake per cell are

METHODS

Tumor Cells

A hyperdiploid strain of Ehrlich ascites tumor cells maintained in A strain mice was used throughout. The ascites tumor was harvested 7 to 8 days after intraperitoneal implantation of 0.1 ml of ascites fluid. The differential count of the tumor cells (at that time) was comparable to that described by Klein (13, 14). The ascites fluid of 2 to 3 mice was pooled in a chilled test tube and centrifuged for 5 minutes at 4°C and 170*g*. The supernatant was discarded and the pellet was washed twice in 30 ml of cold Tyrode bicarbonate solution. The final suspension was adjusted to contain 5×10^7 cells/ml. The cells were tested at this point according to the method of Schrek (20), by shaking a cell sample of 0.1 ml for 3 minutes in Tyrode solution containing 0.05 per cent eosin Y and counting the percentage of stained cells. The count of dye-permeable cells

varied between 0.2 and 4.0 per cent. Suspensions showing higher counts were discarded. For experiments carried out in high albumin concentrations, the cells were resuspended, after their last washing, in the final glucose-Tyrode solution containing the desired inert albumin concentration.

Incubation

Two different procedures were used. (*a*) In the short time experiments on albumin adsorption, the cells were pipetted into 10 ml beakers placed in a Dubnoff-precision metabolic shaking incubator, run at 110 cycles per minute, at 25°C, in an atmosphere of 95 per cent O_2 and 5 per cent CO_2. The beakers contained 1.0 ml of a Tyrode bicarbonate cell suspension counting 5×10^7 cells/ml; 0.1 ml of 0.1 M glucose; 0.05 to 0.4 ml of an I^{131}-albumin-Tyrode solution of suitable concentration. The total volume of 1.5 ml was made up with Tyrode. At the end of incubation the samples were diluted in 2 volumes of Tyrode solution, at 25°C, centrifuged for 5 minutes at room temperature and 170*g* and washed twice with Tyrode.

(*b*) In experiments on the time course of albumin fixation, the cells were incubated in a medium of similar composition, but in a single volume of 25 to 40 ml (5×10^7 cells/ml) placed in a double wall glass container, thermoregulated to 37°C and agitated with a magnetic stirrer. Oxygenation was achieved by aerating the incubation medium with the O_2-CO_2 gas mixture. The electrodes of a Beckman pH-meter were kept in the suspension during incubation for frequent control measurements, and the pH was adjusted to pH 7.4 ± 0.05 by adding 0.1 N NaOH or HCl from a microburette. In experiments run over a period of 2 hours, the medium was supplemented with streptomycin 30 μg/ml and penicillin 40 μg/ml. Frequent tests for bacterial growth in the incubating medium were performed and found negative. In several experiments the incubation medium was fortified with embryo extract (5 per cent final concentration) and folic acid (5×10^{-5} M). These additions have been shown to create optimal conditions for cell division and nucleic acid synthesis (7). The cells were preincubated under these conditions for 15 minutes, in a medium of known total albumin concentration. A small aliquot of labeled albumin adjusted to the pH, temperature, and total albumin concentration of the incubation was added at time zero. Cell samples of 1.5 ml were withdrawn, after time intervals of ½, 5, 15, 30, 60, and 120 minutes, from the large suspension. The incubation was terminated by diluting the samples with 2 volumes of chilled Tyrode solution, centrifuging for 5 minutes at 4°C and 170*g*, and washing the cells twice with 3.0 ml of cold Tyrode.

Protein Purification and Counting Procedure

The cell pellet resulting from the last washing was lysed by suspension in 10 ml of distilled water. The cell proteins were then precipitated with 0.9 ml of 60 per cent trichloroacetic acid and extracted (23). The purified protein was plated on filter papers and counted twice in a low background gas flow counter (Nuclear Chicago, C115) and corrected for self-absorption. The activities of the samples were consistently higher than twice the background count and a total of 2000 counts was collected for each sample. Contamination with small molecular label resulting from an albumin degradation *in vitro* was precluded by the procedure used for the protein extraction.

TABLE I

Comparison of the Albumin Adsorption to Tumor Cells Incubated in Identical, but Washed in Different, Conditions

Incubation: in Tyrode containing 0.5 mg/ml I^{131}-albumin, at 25°C. Washing: twice in Tyrode containing zero, 0.5, and 5.0 mg inert albumin per ml. Raising the concentration of inert albumin from 0.5 to 5.0 mg/ml decreases the efficiency of the washing. Increasing the incubation time from ½ to 15 minutes does not modify the level of adsorption.

Incubation time *min.*	Number of samples	Adsorption (CPM/mg cell protein) remaining after washing the cells twice in Tyrode containing inert albumin		
		0 mg/ml	0.5 mg/ml	5.0 mg/ml
½	8	28.5	26.4	42.5
15	8	24.4	28.0	43.9

I^{131}-Serum Albumin

Iodinated albumin was considered to be a well suited tracer for these measurements, because the amino acids carrying the label are not reutilized for protein biosynthesis (8, 9, 18). This excluded any artifact due to a breakdown of albumin in the medium, followed by a reincorporation of the labeled amino acids into cellular proteins.

Commercially available human I^{131}-serum albumin (Risa, Abbott Laboratories, Oak Ridge) was used throughout. The original solution containing 7.0 to 10.0 mg albumin/ml and 0.4 to 0.6 mc/ml was diluted 10 times with distilled water. After dialysis overnight at 4°C against distilled water, it was supplemented with streptomycin 30 μg/ml and penicillin

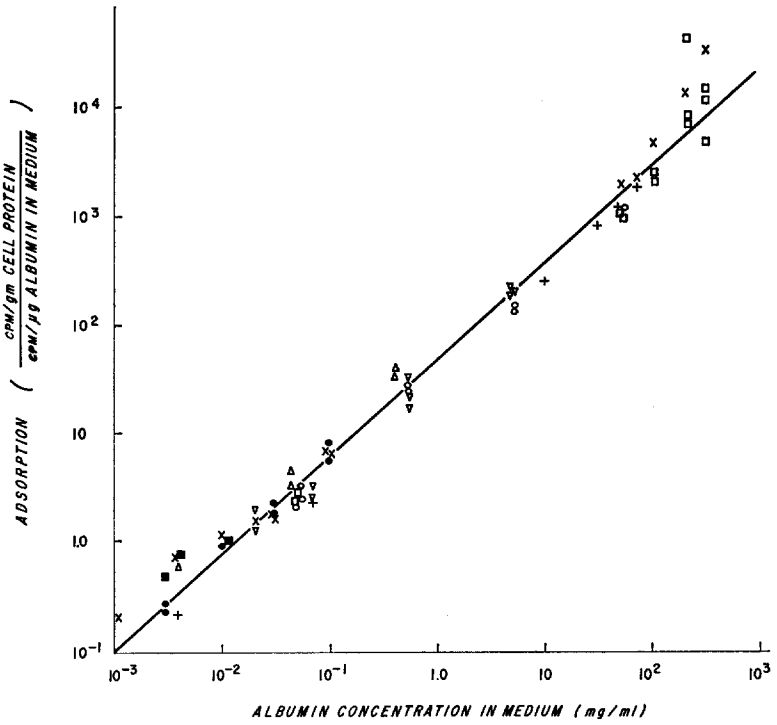


FIGURE 2

In increasing albumin concentrations, the albumin adsorption assumes the form of a linear isotherm over a range of 6 logarithmic units (abscissa). No signs of saturation are apparent at 300 mg/ml. The different symbols correspond to values from 8 experiments, corrected for isotopic dilution and decay. The ordinate corresponds to the radioactivity of purified cell protein, divided by the specific activity of the albumin in the medium. Incubation: 15 minutes at 25°C followed by 2 washings in Tyrode.

40 μg/ml, stored at 4°C and used over a period of 20 days.

Albumin solutions of desired activity were prepared for each experiment by adding aliquots of this labeled solution to Tyrode bicarbonate solutions of known inert albumin concentrations. The average specific activities of the albumin in the medium were 5.3×10^5, 4.7×10^4, and 6.7×10^3 CPM/mg for the 3 series of experiments performed in 0.5, 5.0, and 50 mg/ml total albumin concentrations.[1]

Tests of Cellular Integrity

The percentage of cells stained by eosin or nigrosin was determined at regular time intervals during the incubation according to Schrek (20) and Kaltenbach *et al.* (12). Furthermore, a number of samples were fixed with buffered osmium tetroxide (16) for electron microscopic examination, after ½, 5, 60, and 120 minutes of incubation.

RESULTS

Macromolecules in solution tend to be adsorbed on the surface of isolated cells in suspension. Measuring the uptake of macromolecules requires therefore subtracting the adsorption from the total fixation. The first series of experiments was devised to determine the importance and the characteristics of albumin adsorption by Ehrlich ascites tumor cells.

Data on Albumin Adsorption

When the samples exposed for a few minutes to I^{131}-albumin were washed twice in order to remove the radioactive albumin trapped between the cells, the activity remaining on the cell after this step was still considerable. Thus, this double-

[1] The inert human serum albumin was a gift from the Protein Foundation Laboratories, Boston.

washing procedure was used even in the experiments studying albumin adsorption. The decrease of adsorption upon increasing the number of cell washings is illustrated by Fig. 1. It is seen that 75 per cent of the activity measured after 2 washings is removed by raising the number from 2 to 10. Extrapolation suggests that 20 per cent of the "standard value" is irreversibly fixed by the cell.

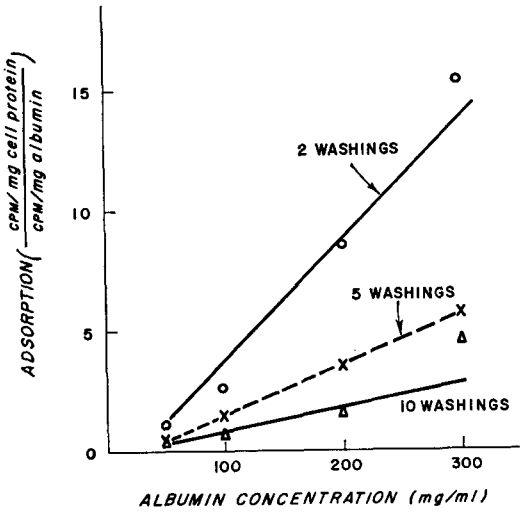


FIGURE 3

Three adsorption isotherms obtained after different washing procedures. Adsorption decreases with successive washings but its relation to the concentration remains linear. Incubation as in Figs. 1 and 2.

The same per cent value of residual adsorption was found in samples incubated for ½ and for 15 minutes and in samples exposed to various albumin concentrations. When inert albumin was added to the washing saline, the adsorption of I^{131}-albumin was modified as indicated in Table I. Whereas an albumin concentration equal to that of the incubation medium did not change the results obtained with Tyrode alone, a 10 times higher concentration of inert albumin increased the amount of I^{131}-albumin left on the cell surface.

The I^{131}-albumin adsorption was studied as a function of the total albumin concentration in 15-minute incubations at 25°C. The concentrations tested ranged from 10^{-3} to 3×10^{2} mg/ml. Each experiment demonstrated a linear relationship between albumin adsorption and concentration. The results of 8 experiments corrected for decay and isotopic dilution are condensed in Fig. 2 on a double logarithmic scale. No leveling off was noticed at the highest concentration. Increasing the number of washings decreased the albumin adsorption without altering the linearity of the isotherm (Fig. 3).

The albumin fixation by the cell was examined in the half-hour following the addition of the label. The time curve revealed an extremely rapid adsorption process, reaching equilibrium within seconds. In order to study an albumin exchange between medium and cell surface, cells were preincubated in a medium containing the final albumin concentration, and I^{131}-albumin was then

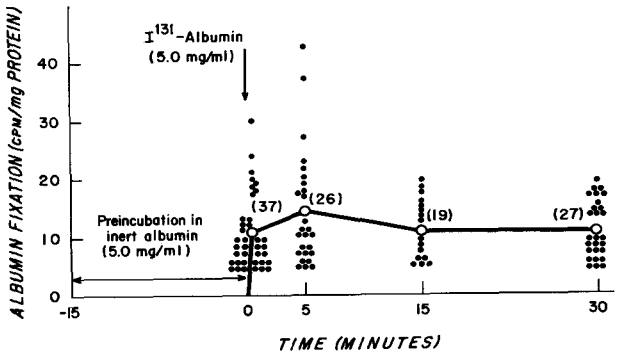


FIGURE 4

I^{131}-albumin fixation by tumor cells preincubated in inert albumin. Vertical arrow indicates the time of label addition. As the albumin concentration of both added label and incubation medium are the same (5.0 mg/ml), the steady state of adsorption is not modified at time zero, and the labeling of the cells occurs by albumin exchange between medium and cell surface. The curve goes through the means. The figures in parentheses indicate the number of samples for each time.

added as an aliquot of identical albumin concentration. Although the addition of the label did not change the total albumin concentration, it produced an immediate rise of the cellular radioactivity (Fig. 4). Moreover, the means of the activities at ½, 2, 5, 15, and 30 minutes were essentially the same. This result indicates an extremely rapid exchange of unlabeled albumin adsorbed on the cell surface with labeled albumin present in the medium, a process which reaches equilibrium in ½ minute and remains at a constant level for 30 minutes. This procedure of labeling adsorbed albumin was used in our subsequent experiments. The constant level of activity in the first half-hour allowed the choice of any time between ½ and 30 minutes as a representative initial value of the albumin adsorption. The smallest scattering of individual results was observed after 15 minutes; therefore this time was preferred as "initial value" in uptake measurements extended over a longer period of time.

Although the curve joining the average values of Fig. 4 is flat, the individual time curves of various experiments showed differences in their profiles. The 30-minute values were occasionally the highest. More often, the maximum of activity was seen after 5 minutes, explaining the larger scattering of single results found at this time (Fig. 4). In single experiments the dispersion among samples taken at different times was usually, but not always, greater than the dispersion among the duplicates taken at the same time. Efforts to control these variations by modifying the incubation medium (albumin concentration, pH and nature of the buffer, salt composition, addition of embryo extract) did not prove successful, nor did modifications of the washing procedure after incubation (increasing the number of washings; adding mild detergents to the washing saline; changing its pH, albumin content, or NaCl concentration). These experimental variations, therefore, remain unexplained, and could reflect changes in the membrane properties of the cell population.

Data on Albumin Uptake

In spite of the scattering of the results observed during short incubations, an attempt was made to measure an increase in activity over a period of 2 hours. The cells were incubated as a single suspension in a medium subjected to constant pH adjustment. Both light and electron microscopic surveys of cells were repeatedly carried out during the incubation. Neither showed any change in the gross morphology or in the cellular ultrastructure (Figs. 5 *a* and *b*). In particular, when tested for non-specific changes in cell permeability by exposure to dyes such as nigrosin and eosin, the cells did not reveal any signs of membrane damage, after 2 hours *in vitro*.

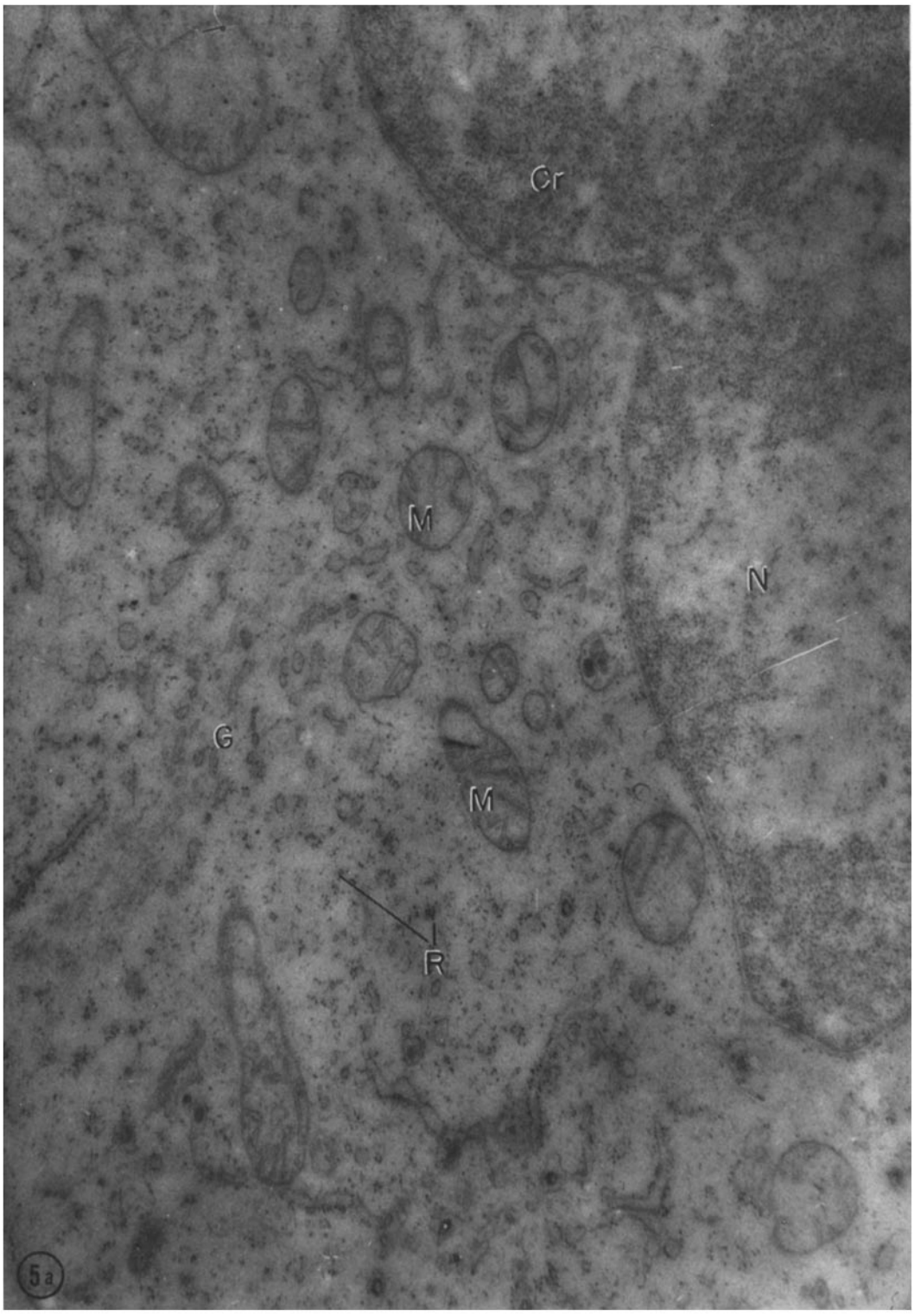
The results of a series of experiments performed in 3 different concentrations of albumin are represented in Figs. 6 *a* to *c*. An increase in activity with time is recognized in all 3 series. Incubations carried out in a medium supplemented with embryo extract and folic acid, or followed by more than 2 washings, yielded similar results. The differences in activity between 15 and 120 minutes are statistically significant in the 3 groups.

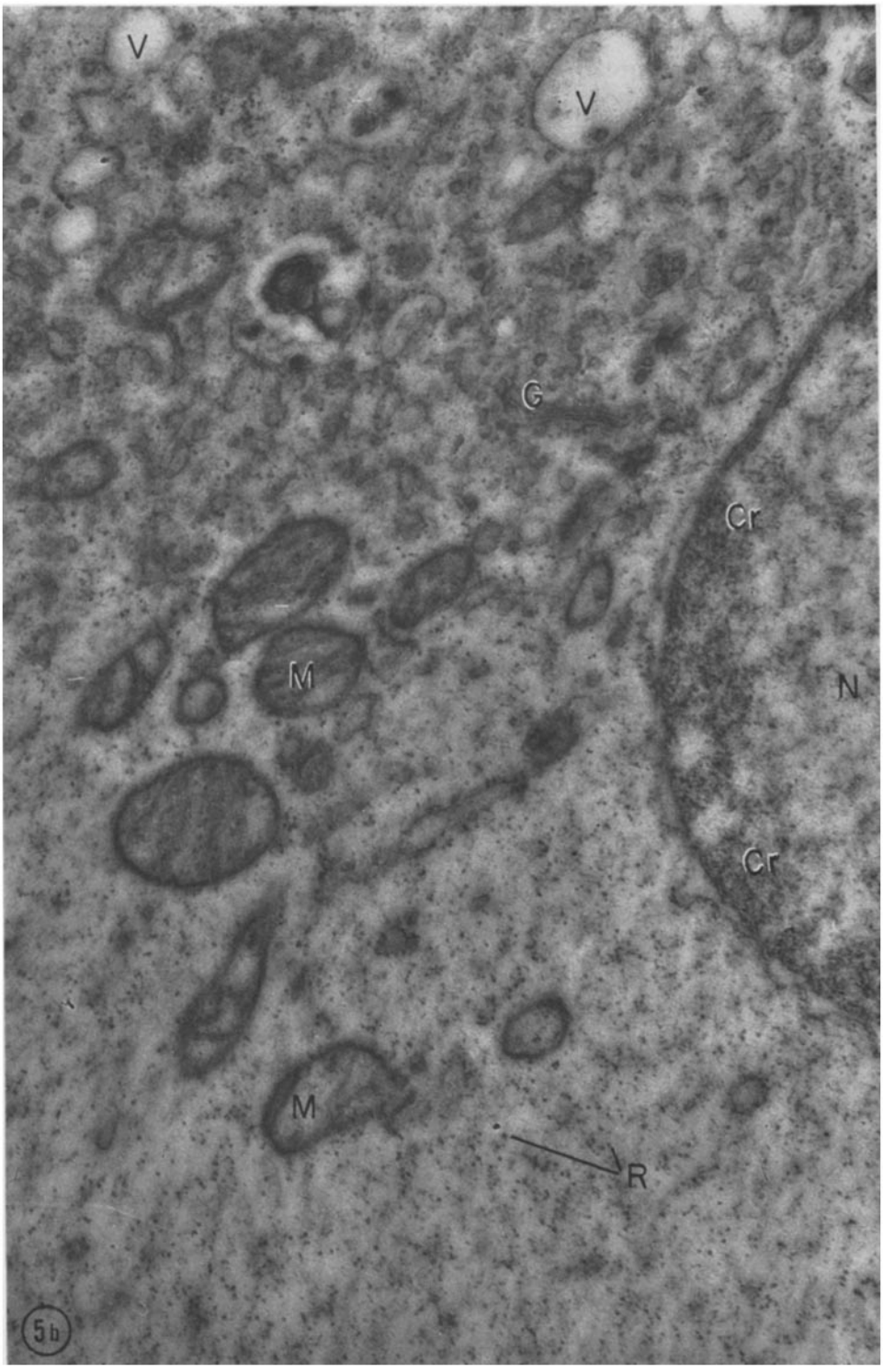
Abbreviations

Cr, Chromatin	*G*, Golgi body	*M*, Mitochondria
N, Nucleus	*R*, Ribosome	*V*, Vesicle

FIGURE 5 *a*

This Ehrlich ascites tumor cell was fixed immediately upon removal from a mouse. Though an occasional degenerate form is present 6 to 7 days after inoculation, most of the tumor cells resemble the one shown. The nuclei of unincubated cells show a definite tendency to have the chromatin material aggregate and these aggregates are generally located near the nuclear membrane. The intervening spaces contain a highly variable amount of fine filaments. The mitochondrial profiles vary greatly and the cristae do not appear to have a constant orientation. The other cytoplasmic components, Golgi body, rough surfaced endoplasmic reticulum, and the various vesicular elements, vary in appearance from area to area and cell to cell sufficiently so that slight changes induced by incubation would be difficult to appreciate. × 26,000.





These increases, measured by subtracting the initial fixation (adsorption) from the final one, are taken to represent an albumin uptake. To allow a better comparison of the data of Figs. 6 *a* to *c*, the values corresponding to adsorption (15 minutes) and uptake (120 minus 15 minutes) were corrected for isotopic dilutions and decay, and were expressed as number of molecules per cell (Table II). It is seen that the adsorption values increase with the albumin concentration, as expected. In addition, Table II indicates that the albumin uptake increases as well. When the values of Table II are plotted against the albumin concentration, they fall on 2 straight lines of different slopes (Fig. 7). In this representation the adsorption increases as in Fig. 2. The linear uptake furthermore suggests a simple relation between adsorption and uptake defined by the slopes of the two linear functions.

Since in our experiments the relative adsorption is measured after 2 cell washings, it can be inferred that the absolute adsorption, prior to washings, would be considerably greater (see Fig. 1). Accordingly, the slope of the isotherm of total adsorption must be steeper than that represented in Fig. 7. It thus appears that, in our system, and over the largest part of the concentration range, the adsorption of albumin is quantitatively more important than the uptake.

DISCUSSION

Our data emphasize the importance of adsorption in the total fixation of albumin by isolated tumor cells.

After a short exposure to albumin 300 mg/ml followed by 2 washings with Tyrode, the amount of albumin remaining on the cells corresponds to 38 mg per gm of cell protein, or to 7.8×10^7 molecules per cell. This is more than would be necessary to cover a sphere of 12 μ in diameter with an albumin monolayer.[2] The adsorption prior to any washing can be expected to be considerably higher.

The observation of a strict linearity between adsorption and concentration up to 300 mg/ml indicates that the adsorption is not limited by saturation phenomena at the cell surface. Our results also indicate that adsorbed albumin is not easily removed since more than 10 washings are required to take off the reversibly bound fraction. Extrapolation to an infinite number of washings suggests that a small amount is irreversibly fixed to the cell.

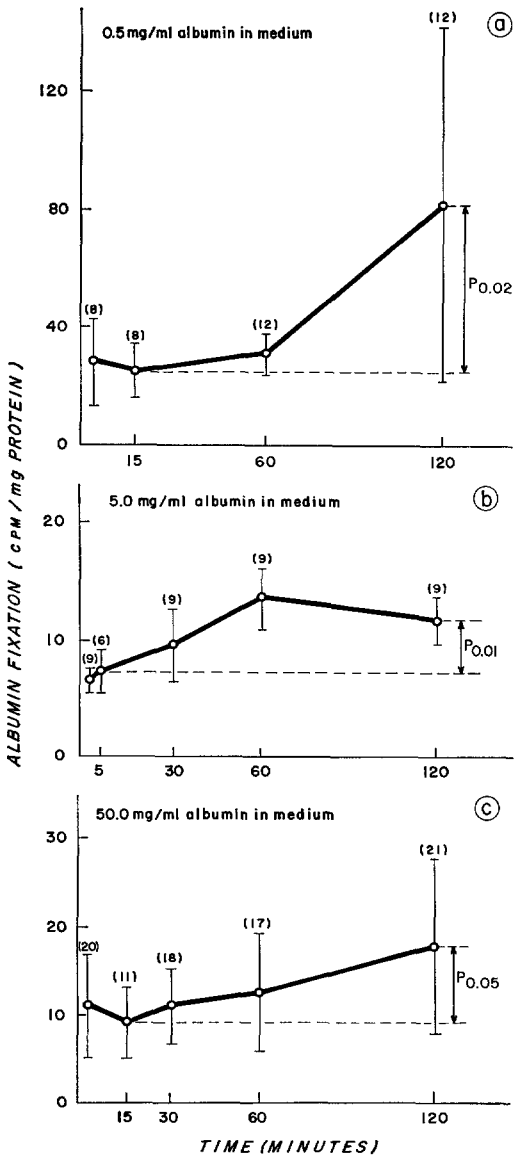
This irreversible fraction is identical after exposures of ½ or 15 minutes. Its nature is not known. A strong binding of albumin to special membrane sites is a conceivable explanation. On the other hand, the complex morphology of the cell membrane with its projections, foldings, and ramifications suggests that small membrane areas could easily be secluded from close contacts with the washing medium. To morphologists a distinction between cytoplasmic structures maintaining or lacking a continuity with the plasma membrane is often impossible. It appears therefore equally hazardous to decide whether this residual adsorption is in fact intra- or extracellular.

Considering the extremely rapid exchange of albumin between medium and cell surface (Fig. 4) one would expect a rapid rate of desorption upon successive washings, particularly in the presence of added inert albumin. The decreased desorption noticed in washing solutions of high inert albumin

[2] According to data of Hall (6) the average diameter of small globular proteins (mol wt 35,000 to 100,000) is of the order of 40 to 60 A. Taking 40 A as average diameter of serum albumin molecules, the surface of projection of a molecule would be 12.6 mμ^2. This is 3.6×10^7 times smaller than the total surface of a sphere 12 μ in diameter. The sphere would thus be entirely covered by 3.6×10^7 molecules of albumin.

FIGURE 5 *b*

This Ehrlich ascites tumor cell was washed twice with buffer and incubated for 2 hours in the basic Tyrode solution before fixation. Most of the tumor cells after 2 hours' incubation resemble this cell. The concentration of free ribosomes in the cytoplasm is somewhat greater than in the control cell. This degree of difference is within the expected variation among cells and sections. The mitochondria are similar to those of the control cell. The vesicular profiles in the upper portion of the cell are more in evidence after incubation, but again similar structures are present in the unincubated controls. The chromatin aggregates are slightly more diffuse in this cell than in the control, the difference being slight. × 35,000.



FIGURES 6 *a*, *b*, AND *c*

Time curve of albumin fixation between ½ and 120 minutes, in 3 different albumin concentrations (*a*, *b*, and *c*). In all 3 cases the increases in activity between 15 and 120 (as well as between ½ or 30 and 120) minutes are significant in the *t* test. Probability values (*P*) between 0.001 and 0.05.

These differences correspond to an albumin uptake. The increases between ½ or 5 and 60 minutes are significant in Fig. 6 *b* only. The vertical segments indicate the standard deviations; the figures in parentheses correspond to the number of samples. Specific activities of albumin in the media: 53, 4.7, and 0.67 × 10^4 CPM per mg in *a*, *b*, and *c*, respectively.

content (Table I) is therefore an unexpected result. The nature of this inhibition is unknown. It appears reasonable however to assume that desorption is counteracted by a simultaneous greater adsorption of inert albumin, creating steric hindrances to the exchange of the label initially bound to the cell surface. This concept would be consistent with the view that, in 5.0 mg/ml albumin and higher concentrations, albumin is partially adsorbed in plurimolecular layers.

In our experiments, the adsorption value has been generally greater than the values representing uptake. Furthermore, previous observations on

TABLE II

Comparison of Albumin Adsorption and Albumin Uptake in 3 Different Albumin Concentrations

The values are based on the results of Figs. 6 *a* to *c*, corrected for isotopic dilution. The adsorption corresponds to the values of initial albumin fixation (15-minute samples in Fig. 6). The uptake corresponds to the increases in fixation between 15 and 120 minutes. The samples contained 5 × 10^7 cells; mol wt of albumin taken as 50,000.

Albumin in medium	Number of molecules × 10^5 per cell	
	Adsorbed	Ingested
mg/ml		
0.5	0.61	1.0
5.0	2.9	1.7
50.0	32.5	4.4

ferritin fixation by tumor cells have demonstrated that unspecific cell damage opens new sites for protein adsorption, and that membrane remnants of disintegrating cells bind proteins to a greater extent than membrane of normal cells (10, 11, 19). These considerations underline the importance of correcting for adsorption in experiments aimed at measuring a protein uptake. It can be anticipated on this basis that uncorrected values will be abnormally high. This seems to explain to some extent the divergence of our findings from those of others (2–5, 17).

Although our previous electron microscopic study did demonstrate some ferritin penetration after 30 minutes of incubation, our measurements with I^{131}-albumin do not reveal any significant uptake between ½ and 30 minutes (Fig. 4). The

large scattering of individual measurements could well account for the failure to detect a possible, but small, albumin uptake in the first 30 minutes. Even so, this negative result stands in clear opposition to the findings of Pileri *et al.* (17),[3] and confirms those of Thomason and Schofield (22), who found no change in the total I^{131}-albumin fixation by ascites tumor cells during a 20 minute incubation. In our experiments, however, a significant increase was measured when incubations were extended to 2 hours. Whether this increase corresponds to a true uptake depends on the correctness of two assumptions, namely: (*a*) that any I^{131} label getting into the cell in a small molecular form is excluded from the final sample count, and (*b*) that the albumin adsorption remains constant for the duration of the experiment. The validity of the first assumption is documented by the fact, already mentioned, that iodinated amino acids arising from I^{131}-albumin are not reutilized by protein biosynthesis (8, 9, 18) and are eliminated from our samples with the other acid-soluble components during the protein extraction procedure. The second assumption implies a maintenance of the cellular integrity during the experiment, since altered cells would bind more protein (10, 11, 19). Repeated light and electron microscopic examinations of the cells during the incubation, and tests of their staining properties, did not reveal any changes in either gross morphology, ultrastructure, or membrane permeability. In the absence of any sign pointing towards an unspecific albumin fixation, it appears reasonable to assume that the level of adsorption—found constant between ½ and 30 minutes—remains unchanged over 2 hours.[4] The increase in activity recorded between 15 and 120 minutes is therefore considered to correspond to a true albumin uptake. It can be inferred that the intracellular localization of ingested albumin is similar to that of ingested ferritin (see 19) and that in both cases the number of perinuclear protein-containing structures (vacuoles and dense bodies) increases with the incubation time. Protein enclosed in such deep seated structures has been considered "taken up," even though it might remain membrane-bound (19).

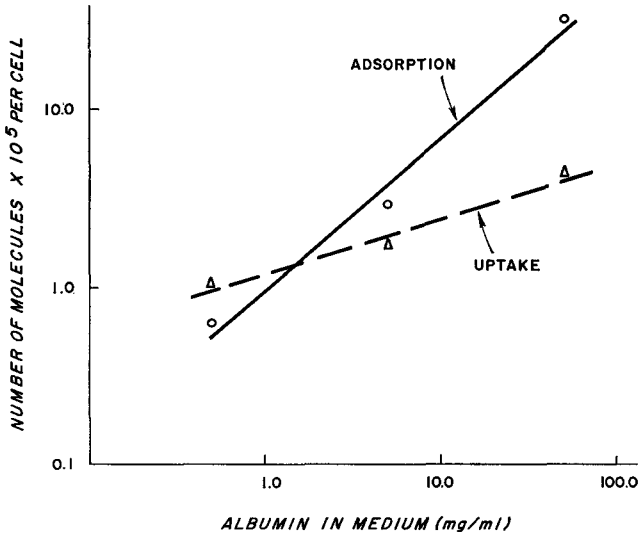


FIGURE 7

Double logarithmic plot of the values of Table II. Both adsorption and uptake show a linear relation to the albumin concentration. As in the previous figures, the adsorption is measured after 2 cell washings. Thus the isotherm of adsorption prior to any washing would be considerably steeper.

The albumin uptake is of the order of 0.06 to 0.2 mg per gm cell protein or 1 to 4 × 10^5 molecules

[3] The finding of a time curve with a sharp maximum of ribonuclease uptake after 5 minutes of incubation has been described as characteristic for the protein ingestion by young cells (17). In our experiments, a similar maximum was occasionally noticed at this time and attributed to variations in the albumin adsorption. A relation to the age of the tumor (number of days after implantation) could not be confirmed.

[4] New data confirming this conclusion have been obtained with an improved technique since this paper went to press. In experiments measuring the adsorption and uptake in parallel samples, the activities resulting from 15 minutes' exposure to I^{131}-albumin were found to be identical when measured at the beginning or at the end of 120 minutes' incubation.

per cell in 2 hours. The same order of magnitude has been arrived at in an estimate of the amount of ferritin taken up by ascites tumor cells in 2 hours (19). The amino acids that can be derived from 4×10^5 molecules of albumin correspond to 0.2 per cent of the amount of free amino acids estimated to be incorporated into the proteins of a cell during the same period of time.[5] Thus, even when a full utilization of the ingested albumin is assumed, this uptake would apparently not contribute significantly to the nitrogen metabolism of the cell.

Attempts to measure the protein uptake by tumor tissue *in vivo* (2–4) have led to estimates which are considerably higher than the figures obtained in our studies on isolated cells. It had been suggested that a direct protein uptake by tumor tissue could account for a 100 per cent increase of the cellular proteins within 24 hours (2). Our highest value extrapolated to a 24-hour period corresponds to only 2.4 per cent of the cell protein. The possibility of greater rates of uptake *in vivo* cannot be excluded, but is not supported by our data on ferritin, which show a similar behavior of ascites tumor cells exposed to proteins *in vivo* or *in vitro* (19). It is conceivable also that certain tumors take up protein more readily than Ehrlich ascites tumor cells. However, pending adequate corrections for the various artifacts mentioned above, the high values of protein uptake proposed for tumor *in situ* (2–4) can be ascribed in part to contaminations in the form of surface adsorption or unspecific fixation by injured cells.

A comparison of our results with the recent data reported by Straus (21) on the *in vivo* uptake of horseradish peroxidase by cells of the kidney cortex strongly suggests that epithelial elements of the kidney display greater rates of protein uptake than tumor cells. Electron microscopic observations describing the protein uptake by both tissues (15, 19) have led to similar conclusions (19). The kinetics of protein uptake by kidney and ascites tumor cells appears to differ in several respects. Whereas the horseradish peroxidase can be measured in kidney cells a short time after injection and reaches saturation values with increasing concentrations, the uptake process of tumor cells is only measurable over longer periods and does not level off at higher albumin concentrations.

The increase of uptake with the albumin concentration (Fig. 7) is in agreement with previous electron microscopic observations, which indicated that media of high albumin content raise the amount of ferritin taken up by tumor cells (19). By analogy to ferritin, it is suggested that albumin penetrates into the cell by pinocytotic membrane movements. The very nature of the pinocytotic process implies that increased adsorption must be connected with increased uptake, since adsorbed molecules are carried into the various formations of the plasma membrane. On this basis alone one would expect a relation between adsorption and uptake similar to that expressed by Fig. 7. However, other mechanisms have been proposed (1) by which adsorption could influence the pinocytotic uptake in a more specific way, namely by triggering membrane movements. The view that protein adsorption might induce pinocytosis is consistent with both our previous electron microscopic data and our present quantitative results. Special attention will be given in future experiments to other factors influencing protein uptake.

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[5] Since the rate of incorporation of single amino acids into tumor cell proteins is known, it can be estimated that in optimal conditions a cell incorporates roughly 7×10^{10} molecules of amino acids in 2 hours (19). On the other hand, 4.5×10^5 molecules of serum albumin contain some 1.6×10^8 amino acid residues, *i.e.*, about 0.2 per cent of the optimal amount incorporated.

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