EFFECT OF ANTIBODY AND COMPLEMENT ON PERMEABILITY CONTROL IN ASCITES TUMOR CELLS AND ERYTHROCYTES*

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Krebs ascites tumor cells exposed to the action of rabbit antibodies and complement (C′), promptly undergo a series of structural and chemical changes in their cytoplasm (1, 2). The first change observed by microscopy is cytoplasmic swelling, appearing first as focal pouching, then spreading to involve the entire cytoplasm. With the electron microscope this swelling can be seen to involve the subcellular particles (mitochondria, smooth and rough surfaced endoplasmic reticulum) and the cytoplasmic matrix. Chemical analysis shows a rapid loss of most of the intracellular K^+, amino acids, and ribonucleotides, and a less rapid loss of a good deal of the cell protein and ribonucleic acid. These chemical constituents are lost through a cell membrane which appears to be intact when viewed by phase and electron microscopy.

The present report concerns the mechanism of production of these effects and attempts to place them in causal sequence. It will be shown that the direct effects of antibody and complement may be best explained by the production of "holes" in the cell membrane large enough to permit rapid exchange of inorganic cations and small molecules, but not of macromolecules. The latter become able to pass the cell membrane only after secondary swelling of the cell, resulting from disordered osmotic regulation. The release of hemoglobin in immune lysis of erythrocytes is similarly not the direct consequence of the action of antibody and C′ on the cells, and does not occur if the disorder in osmotic regulation is prevented.

Methods

Most of the experimental methods used have been previously described (1, 2). Krebs-2 ascites tumor cells were maintained by successive passages through the peritoneal cavity of mice. All incubations were performed in a bicarbonate buffered balanced salt solution (BSS, (3)) containing glucose, 1.0 mg./ml., and equilibrated with 5 per cent CO_2 and 95 per cent oxygen, in a shaker–water bath at 37°. Ascites tumor cells and heparinized mouse blood were freshly collected in cold BSS and the cells were washed at least once before use.

The antibody was salt-fractionated gamma globulin obtained from the serum of rabbits immunized by successive injections of the mouse tumor cells. The source of C′ was fresh

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normal rabbit serum stored at −80°C. until just before use. It was found to contain an average of 12 hemolytic units per ml. (4), and was added in excess in all experiments. Inactivated C′ was prepared by heating normal rabbit serum at 56° for 30 minutes. Bovine plasma albumin, the crystalline product of Armour Laboratories (Kankakee, Illinois), was prepared as a solution in BSS. All centrifugations were carried out at 0°. When it was necessary to centrifuge washed cell suspensions, albumin was first added to a concentration of about 0.1 per cent to prevent the cells from sticking to the walls of the centrifuge tubes. K^{42} and Na^{24} were counted in a gamma ray, well-type scintillation counter.

EXPERIMENTAL RESULTS

I. The Leak Rate for K^+

It had previously been noted (2) that the earliest chemical change following the addition of antibody and C′ was the loss of small molecules from

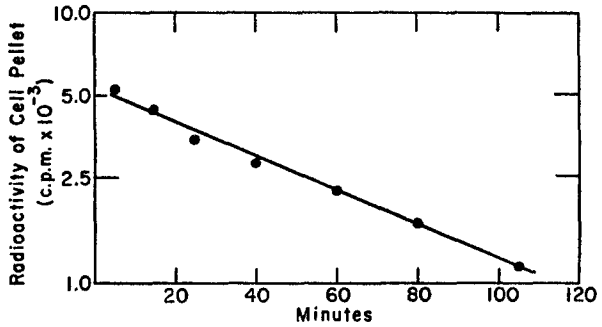

FIG. 1. *The normal leak rate for K^+.* To 20 ml. of washed suspension of acites tumor cells (1.45×10^7 cells/ml.), 0.15 ml. of BSS containing tracer quantities of K^{42} was added, and the mixture incubated for an hour. The suspension was chilled to 0°, and centrifuged at 1000 R.P.M (250 *g*) for 10 minutes. The cell pellet was resuspended in 210 ml. of BSS in a 500 ml. flask and incubation resumed (final cell concentration 1.4×10^6/ml.). 20.0 ml. samples were taken at intervals into chilled tubes and immediately centrifuged at 22,000 *g* for 7 minutes. The supernatants were aspirated, the pellet allowed to drain and the interior of the tube above the pellet was wiped with cotton. The samples were then counted in the γ-ray counter.

the cells; potassium ions in particular were lost extremely rapidly, for 5 minutes' exposure at 37° left only about 10 per cent of the total K^+ still within the cells.

The normal maintenance of intracellular potassium in erythrocytes (5) and ascites tumor cells (6) depends on a balance of two processes: (*a*) the normal rate of leakage of K^+ from inside the cell to the outside in the direction of the electrochemical potential gradient and (*b*) the transfer of K^+ into the cell against the gradient by an energy requiring process involving hypothetical pumps. It is therefore possible that the loss of K^+ from cells treated with antibody and complement could result from either an accentuation of (*a*) or interference with (*b*).

An experiment was performed to measure the normal leak rate of intracellular K^+ independently of the rate of uptake of K^+ from the medium. After a preliminary incubation of ascites tumor cells with K^{42}, the labelled cells were suspended in isotope-free BSS. The loss of K^{42} from the cells was followed, and Fig. 1 shows their isotope content plotted on a logarithmic scale against time. After temperature equilibration the rate of decline of K^{42} content was logarithmic with time, and the half-life of the cell K^{42} was estimated to be about 48 minutes. In this experiment, the cell concentration was low enough so that K^{42} released into the medium was diluted to low specific activity, and reincorporation of medium K^{42} by the cell pumps was minimized (the specific activity of the medium K^{42} after one half-life was approximately 8 per cent of that of the cells). It is evident that the turnover rate of intracellular K^{42} of ascites tumor cells at 37° is normally considerably faster than that of erythrocytes (half-life = 39 hours (7) or 35.5 hours (8)).

The effect of the addition of antibody and C′ on the leak rate for K^+ was next determined. To labelled cell suspensions prepared as before, antibody + C′, and antibody + inactivated C′ were added and the content of K^{42} was followed with time. Fig. 2 (curve A) shows that in the presence of antibody + inactivated C′, the cells lost their K^{42} content at a logarithmic rate with a half-life of 65 minutes, somewhat longer than in the previous experiment.[1] However, the addition of C′ to the suspension already containing antibody resulted in much more rapid loss of K^{42} (curve B). This rate of loss, evidently much greater than the leak rate, cannot be explained by inactivation of the active transport system for K^+. It seems necessary to suppose that the leak rate had actually been increased, such as would occur if "holes" were made in the cell membrane.

II. Rate of Influx of Na^+

If the effect of antibody and complement is to produce "holes" through which cellular K^+ diffuses rapidly into the medium, Na^+, which is in high concentration in the medium as compared to the cells (6), should pass rapidly into the cells during the same period. An experiment was performed to test this point. To a washed cell suspension were added tracer quantities of Na^{24}, antibody, and then C′ at time = 5 minutes (Fig. 3). Control cells were incubated either in the presence of antibody + inactivated C′, or simply in BSS. In either case, the control cell pellets contained the same small quantity of radioactivity, which remained constant throughout the incu-

[1] In order to conserve antibody by maintaining a small final volume, this experiment was performed at a higher cell concentration than the previous experiment. Dilution of released K^{42} being not as great, significant recycling probably occurred, resulting in a slightly slower apparent leak rate. The presence of antibody does not influence the leak rate if C′ is absent.

bation period. This radioactivity is equal to that contained by 0.02 ml. of medium, and can therefore be partly accounted for by the fluid trapped between the packed cells (total packed cell volume of each aliquot $\approx$ 0.11 ml.) on the assumption that 21 per cent of the packed cell volume is extracel-

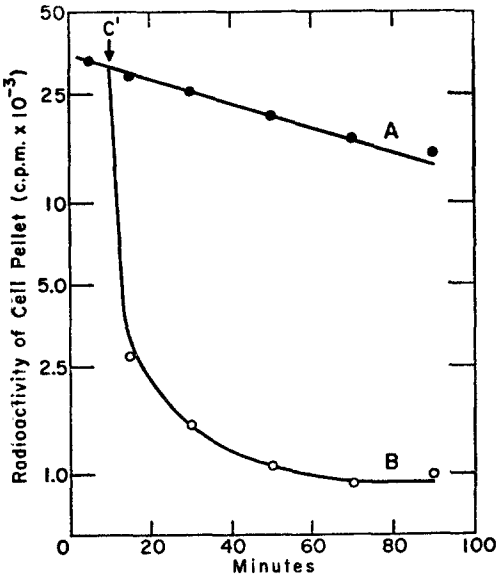

FIG. 2. *Effect of antibody + C′ on the leak rate of intracellular K^{42}.* To 40 ml. washed cell suspension containing 2.0 × 10^7 cells per ml., 2.0 ml. of BSS containing tracer quantities of K^{42} was added, and incubation continued for an hour. The cells were then chilled to 0° and the suspension centrifuged for 10 minutes at 1000 R.P.M. (250 *g*). The supernatant was aspirated and the cells suspended in 130 ml. of BSS. Into each of 2 flasks containing 63 ml. of this suspension the following additions were made: Flask A, 7.5 ml. of rabbit immune globulin (10.0 mg./ml.), 4.0 ml. inactivated C′. Flask B, 7.5 ml. rabbit immune globulin (10.0 mg./ml.), 4.0 ml. C′. The arrow indicates time of C′ addition. Final cell concentration, 5.2 × 10^6/ml. Final protein concentration, approximately 4.4 mg./ml. 3.0 ml. samples were taken at intervals into chilled tubes, and centrifuged at 22,000 *g* for 7 minutes. The pellets were counted as usual.

lular fluid (9). When, however, both antibody and C′ were added to the cells, a very sharp increase in cell Na^{24} occurred which was of the order of magnitude to be expected if the whole cell space became freely permeable to Na^+. The sample taken 5 minutes after the addition of C′ (t = 10 minutes, Fig. 3) contained considerably more Na^{24} than the same cells allowed to incubate longer. This is explained by the fact that the packed cell volume of this sample was considerably larger than that of the samples taken at later intervals. This is due to the initial cytoplasmic swelling resulting from an-

tibody and C′ action. Upon incubation for longer than 5 minutes in the presence of C′ the cells began to lose significant quantities of cytoplasmic protein and nucleic acid. Centrifugation of these aliquots produced smaller packed cell volumes by squeezing out some of the intracellular fluid containing Na^{24}.

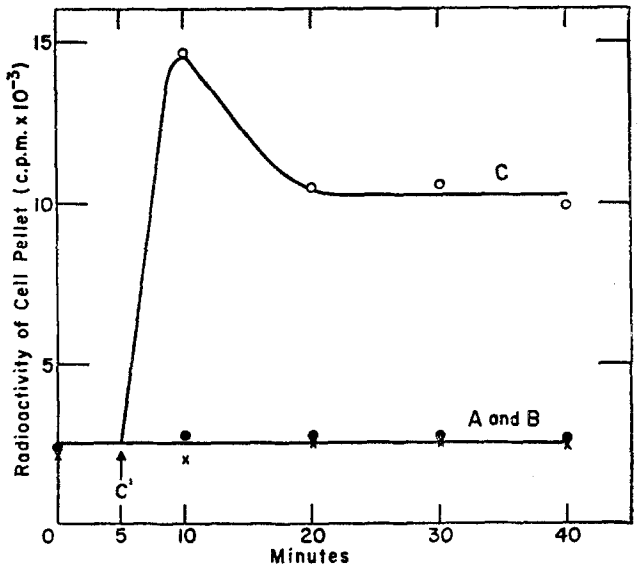

FIG. 3. *Effect of antibody + C′ on exclusion of Na^{24} by the tumor cells.* ×, cells incubated in BSS; ●, cells incubated in presence of antibody + inactivated C′; ○, cells incubated in presence of antibody + C′. To three flasks containing 15.0 ml. of washed cell suspension the following additions were made: Flask A (control): 3.0 ml. BSS, 0.050 ml. Na^{24} in BSS. Flask B (control): 2.0 ml. immune globulin (10 mg./ml.), 0.050 ml. Na^{24} in BSS, and 1.0 ml. inactivated C′. Flask C: 2.0 ml. immune globulin (10 mg./ml.), 0.050 ml. Na^{24} in BSS, and 1.0 ml. C′. The tracer quantities of Na^{24} were added at $t = 0$, and C′ at $t = 5$ minutes. The medium in all flasks contained 120,000 C.P.M./ml. throughout the experiment. Final cell concentration, 1.6×10^7/ml. Final protein concentration, approximately 4 mg./ml. 3.0 ml. aliquots of suspension were taken at intervals and prepared for Na^{24} counting in the usual manner.

III. Osmotic Regulation by the Cell—the Mechanism of Cell Swelling

It has long been recognized that mammalian cells contain a much higher macromolecular concentration than the extracellular fluid in which they are bathed. In order, therefore, not to develop an osmotic pressure gradient across the cell membrane, the cell is obliged to maintain its concentration of inorganic ions at a level slightly lower than that of the outside fluid. This regulation is now thought to be effected by means of $Na^+ - K^+$ pumps (10). As pointed out by Jacobs (11) and Davson (12), any situation which permits the free exchange of these ions between the inside and outside of the cell will

lead to an increase in the osmotic pressure of the cytoplasm, and water must flow into the cell. Exactly this condition occurs in cells exposed to antibody + C′.

We have already shown that as incubation of antibody + C′-treated cells proceeds, and the swelling process continues, the cells begin to lose larger molecules—proteins and nucleic acids (2). The question is therefore of interest, does the action of antibody + C′ produce holes in the cell membrane sufficiently large to allow the passage of the largest of these macromolecules independently of the swelling process, or is the swelling of the cell and concomitant stretching of the cell membrane required to permit these substances to escape? In order to answer this question it was necessary to find a means of preventing the influx of water following the antibody + C′-induced equilibration of cations. This could be accomplished by placing in the medium a non-penetrating substance in sufficient concentration to balance the colloid osmotic pressure of the intracytoplasmic macromolecules.

The major macromolecular component of the ascites tumor cell is protein; the nucleic acid concentration is of the order of $\frac{1}{6}$ as much (13) and contributes an even smaller fraction of the total colloid osmotic activity. The concentration of cell protein should therefore give an approximate index of colloid osmotic activity. Direct determinations of total cell protein were performed on known numbers of cells either by Kjeldahl nitrogen determination of cells whose nucleic acids had been extracted with hot 5 per cent trichloracetic acid or by the copper-Folin reaction on unextracted cells (14). The values obtained were adjusted to 1 ml. of packed cells (4.0×10^8 cells = 1.0 ml.), and corrected upward for extracellular fluid trapped in the pellet by a factor of 21 per cent. The final value for average protein concentration in the cell was about 150 mg./ml. This ignores any question of inequality of distribution between nucleus and cytoplasm.

A means of estimating the protein concentration of the cytoplasm alone is to compare the refractive index of the cytoplasm with that of media containing different known protein concentrations, a phase difference between the cell cytoplasm and the medium presumably indicating a difference of protein concentration between the cytoplasm and the medium. The cytoplasm is most transparent and difficult to see when the extracellular protein concentration approaches that of the cytoplasm, though heterogeneity of the cytoplasm prevents all of its contents from being completely invisible at any given extracellular protein concentration.

The results of an experiment of this kind are shown in Table I. Cells were observed when suspended in different concentrations of bovine serum albumin in BSS. It is evident that at an albumin concentration in the region of 170 mg./ml. the phase difference between cytoplasm and medium is at a minimum. This protein concentration is not very different from that determined

analytically for the cells. At an albumin concentration of 170 mg./ml., the mean density of the cells is also close to that of the medium, centrifugation dividing the cell population into cells heavier and cells lighter than the medium.

Cells can be maintained in albumin concentrations of up to 200 mg./ml. for a few hours with no obvious change in their morphology; if the suspension is diluted into BSS the cells regain an appearance typical of cells in a low protein medium. Albumin concentrations appreciably higher than 200 mg./ml. appear to be toxic to the cells, for the normal cell morphology is not long retained, and impure albumin, such as that generally used for serological tests, is toxic at even lower concentrations.

TABLE I

Phase Appearance and Sedimentation Behavior of Ascites Tumor Cells in Albumin Solutions of Different Concentration

Albumin concentration	Cytoplasm	Sedimentation direction
mg./ml.		
93	Dark	Down
124	Dark	Down
155	Mostly transparent	Down
185	Mostly transparent	Mostly down
216	Bright	Up
248	Bright	Up

Both the molecular weight and charge are involved in the osmotic activity of macromolecules at any fixed concentration. If, however, these factors are not too greatly different for serum albumin and the cytoplasmic proteins, it should be possible to balance the colloid osmotic pressure of the cytoplasm with the same concentration of albumin in the medium, and prevent the swelling of the cell which follows the action of antibody + C′.

IV. Effect of High Serum Albumin Concentrations on the Altered Permeability Induced by Antibody + C′

(*a*) *The Swelling Process.*—When antibody is added to a cell suspension in a serum albumin concentration of 150 to 200 mg./ml., the antibody interacts normally with the cells, as can be seen by the characteristic agglutination. But if C′ is added, the morphological effects are very different from those observed in media of low protein concentration.[2] There soon begins,

[2] Low protein medium will henceforth refer to a medium to which no serum albumin has been added. Immune globulin and C′ constitute the only proteins in such a medium and the final protein concentration is always less than 5 mg./ml.

in at least some cells, a sign of incipient swelling in the cytoplasm near the cell membrane, as indicated by a decrease in refractive index and some tendency toward pouching of the membrane. However, this change never progresses to the markedly swollen cell resulting from Ab + C′ action in low protein media. On the contrary, the few pouches which are formed tend to become smaller with time and are probably due to local high concentrations of protein in a heterogeneous cytoplasm. After 1 to 2 hours the cells look substantially normal except for the following: (*a*) evidence in some cells of aborted pouch formation, (*b*) a slight fuzziness of the cell membrane of most of the cells, and (*c*) irregularity of shape of many of the cells. Though the average diameter of these cells is not significantly changed from that of the controls incubated in albumin alone, the change of shape from the spherical suggests an over-all decrease in cell volume, an interpretation supported by subsequent observations. Evidently the addition to the medium of serum albumin in a concentration approximately equal to the cytoplasmic protein concentration largely prevents the swelling process which follows the action of antibody and complement. However, if cells mixed with antibody and C′ in the presence of high albumin concentration are diluted into 10 volumes of BSS, the cell swelling develops in a manner typical of that which occurs when the reactants are mixed in a low protein medium.

(*b*) *Loss of* K^+.—The morphological changes characteristic of the action of antibody + C′ in low protein media were seen to be largely aborted in the presence of high albumin concentrations. Chemical components of the cell were therefore examined to see if their characteristic loss from the cell was affected by the presence of the albumin. A cell suspension was incubated in the presence of K^{42} for 1 hour, then centrifuged, and the cells resuspended in a solution of concentrated albumin in BSS. Antibody + complement was added and K^{42} content of the cells followed in the usual manner. The loss of K^{42} from the cells followed a course indistinguishable from that of cells treated with antibody + C′ in the absence of albumin; that is, over 90 per cent of the cell K^{42} was lost within the first 5 minutes. The control cells (with antibody + inactivated C′) in the same final protein concentration (167 mg./ml.) preserved a normal leak rate for K^{42}. Evidently the loss of cell K^+ in the presence of antibody + C′ was completely independent of the swelling process, which was nearly completely inhibited in this experiment. The same experiment was performed at an even higher protein concentration (218 mg./ml.), and the same result was obtained.

(*c*) *Entrance of* Na^+.—The experiment described in section II on the effect of antibody + C′ on the normal exclusion of Na^+ from the cells was repeated in the presence of high albumin concentrations. Fig. 4 (curve B) shows that the action of antibody + C′ resulted in entrance of Na^{24} into the cells as abruptly as occurred in the absence of added albumin. Small differences,

however, are noted between the two conditions. The initial peak in Na^{24} content of cells treated with antibody + C′ in low protein medium did not occur in the presence of a high albumin concentration because the cell swelling was prevented. In fact there was some reduction in the size of the antibody + C′–treated packed cell pellets, consistent with the reduction in individual cell volumes noted previously (see section IV (*a*)). Perhaps for this reason the radioactivity values were not quite as high as expected. The

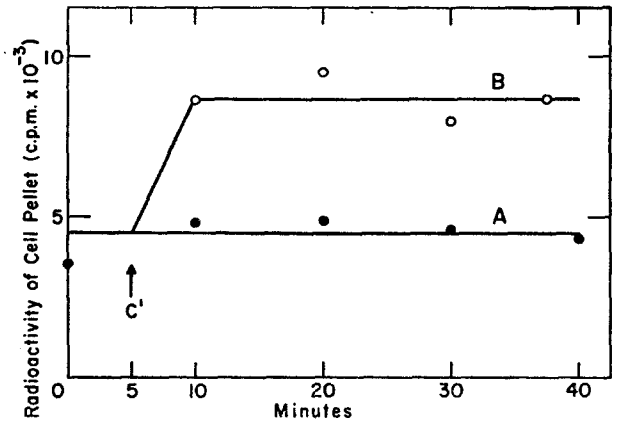

FIG. 4. *Effect of antibody + C′ on entrance of Na^{24} into cells in the presence of a high albumin concentration.* ●, control cells incubated in presence of albumin, antibody, and inactivated C′; ○, cells incubated in presence of albumin, antibody + C′. To 2 flasks containing 4.0 ml. of concentrated washed cell suspension the following additions were made: Flask A (control): 13.0 ml. albumin solution (224 mg./ml.), 1.0 ml. rabbit immune globulin (55.5 mg./ml.), 0.20 ml. Na^{24} in BSS, and 4.5 ml. inactivated C′. Flask B: 13.0 ml. albumin solution (224 mg./ml.), 1.0 ml. rabbit immune globulin (55.5 mg./ml), 0.20 ml. Na^{24} in BSS, and 4.5 ml. C′. Time of C′ addition indicated by arrow. The medium in both flasks contained 100,000 C.P.M./ml. throughout the experiment. Final protein concentration in both flasks, 151 mg./ml.

radioactivity of the control cells (curve A) was on the other hand somewhat higher than expected, probably owing to poorer packing of the cells by centrifugation in high albumin concentration, and trapping of a larger quantity of extracellular water. In spite of these differences in detail, a comparison of Figs. 3 and 4 leads to the conclusion that in high protein medium, as in low protein medium, antibody + C′ induces permeability of the cells to Na^+ from the medium.

(*d*) *Loss of Other Cellular Constituents.*—The cell pellets obtained from the experiments described in sections I, II, and IV (*c*) were analyzed for cell amino acids, proteins, ribonucleotides and RNA by methods already described (2). The results obtained from cells incubated in the presence and absence of added albumin are shown in Figs. 5 and 6. In order to permit

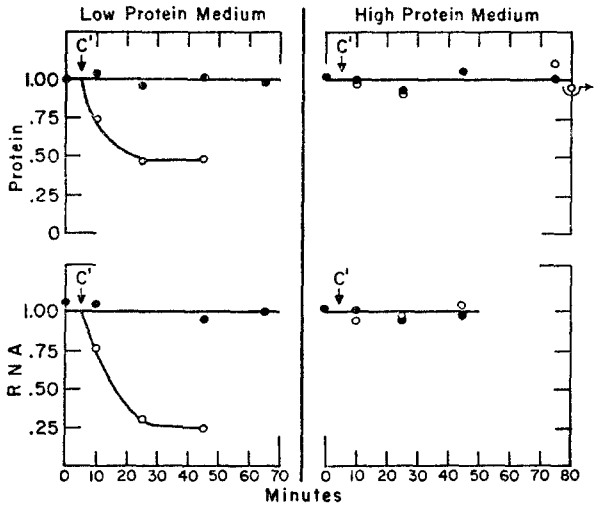

FIG. 5. *Loss of protein and RNA from cells treated with antibody + C′ in high and low protein media.* ●, control cells incubated in presence of antibody and inactivated C′; ○, cells incubated in presence of antibody and C′. Left, in absence of added albumin; right, in presence of added albumin. Ordinates represent fractions of cell protein and RNA remaining in pellet (see text). Time of C′ addition indicated by arrows. Final protein concentrations: low protein medium, 4.4 mg./ml.; high protein medium, 167 mg./ml.

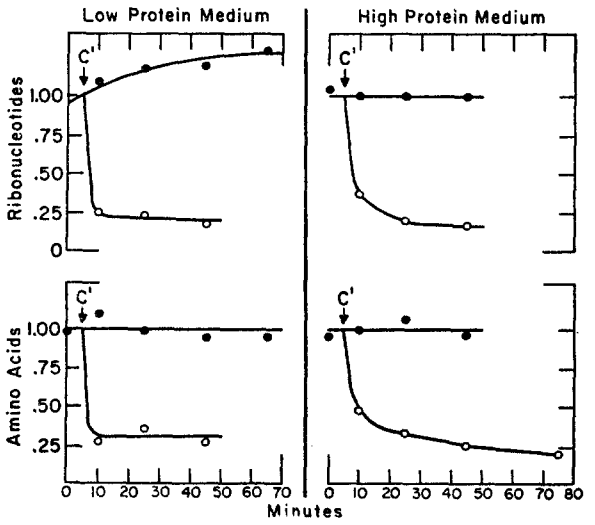

FIG. 6. *Loss of ribonucleotides and amino acids from cells treated with antibody + C′ in high and low protein media.* ●, control cells incubated in presence of antibody and inactivated C′; ○, cells incubated in presence of antibody and C′. Left, in absence of added albumin; right, in presence of added albumin. Ordinates represent fractions of cell ribonucleotides and amino acids remaining in pellet (see text). Time of C′ addition indicated by arrows. Final protein concentrations: low protein medium, 4.4 mg./ml.; high protein medium, 167 mg./ml.

direct comparison, all values are expressed as their respective fraction of the mean control value through the incubation period, with the exception of the ribonucleotide values, which are expressed as a fraction of the level at the time of C′ addition.[3]

The first result to be noted is that whereas, in agreement with previously reported experiments, cells treated with antibody + C′ in low protein medium lose about half their protein and three-quarters of their RNA, the presence of a high concentration of albumin in the medium completely prevents the loss of both cell protein and RNA (Fig. 5). In the case of the cell ribonucleotides and amino acids, the cells in high albumin concentrations lost about as much as those without added albumin (Fig. 6). However, the rate of loss in the presence of high albumin was consistently somewhat slower; it was not complete within 5 minutes after the addition of C′, as was the case in low protein medium.

It appears, therefore, that if swelling of the cells is prevented by high protein concentrations, the loss of macromolecules is also prevented, the rate of loss of amino acids and nucleotides is only slightly reduced and the rates of K^+ efflux and Na^+ influx are unaffected.

V. The Effect of High Albumin Concentrations on Immune Hemolysis of Erythrocytes

Since more detailed work has been performed on the action of antibodies and C′ on erythrocytes than on any other cell type (15), it was of interest to know whether the effects observed in ascites tumor cells occur also in erythrocytes. The same immune globulin preparations used in the mouse ascites tumor cell experiments contain antibodies to mouse red blood cells; they agglutinate suspensions of the erythrocytes and in the presence of C′ produce hemolysis. The effect of high albumin concentrations on the hemolysis was examined, and the results are shown in Fig. 7. At final protein concentrations of albumin up to about 50 mg./ml. hemolysis was nearly complete under the conditions described; at higher protein concentrations hemolysis was inhibited and at protein concentrations over 200 mg./ml., it was completely prevented. It should be noted that hemolysis was inhibited at albumin concentrations far lower than the normal hemoglobin concentration within the red blood cell (340 mg./ml.). This is probably due to the ability of the disc-shaped erythrocytes to swell without stretching of the cell membrane, and thus reduce their hemoglobin concentration without losing any to the medium. That swelling had occurred, was confirmed by examination of the pellets obtained from the cell suspensions treated with antibody and C′, particularly in the middle of the protein concentration range.

Evidence that antibody and C′ have acted upon the cell membrane even

[3] The concentration of free nucleotides rises during the course of incubation in low protein medium as was noted previously (2). This does not occur in the high protein medium.

in high albumin concentrations is provided by the constant loss of 90 per cent of the cell K^+ over the whole protein concentration range (Fig. 7, upper curve). The loss of hemoglobin therefore does not follow loss of K^+ unless the cell is permitted to swell freely, and protein in the medium prevents the swelling to a degree dependent on its concentration. At protein concentra-

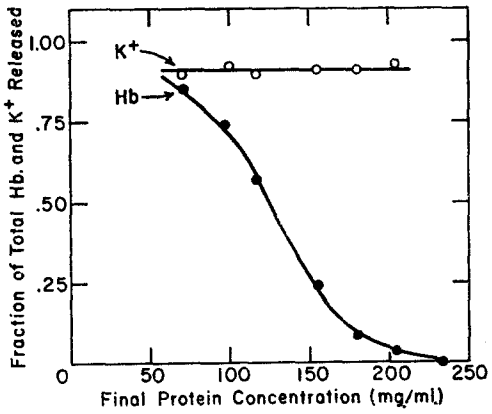

FIG. 7. *Uncoupling of immune hemolysis from K^+ loss in high protein medium.* 1.25 ml. of heparinized mouse blood was washed once in a large volume of BSS, and the cells taken up in a total volume of 1.0 ml. Into each of a duplicate series of tubes were placed 1.0 ml. of albumin solutions of varying concentration, 0.050 ml. immune globulin (55.5 mg. protein/ml.) and 0.050 ml. of cell suspension. To each tube of one series was added 0.20 ml. C′, to the other series (control) the same volume of inactivated C′. The suspensions were incubated at 37° for 15 minutes. The tubes were then centrifuged at 1500 R.P.M. (500 *g*) for 30 minutes (0°) and the supernatants aspirated. 1.0 ml. of each supernatant was diluted with 1.0 ml. of BSS, and the optical density read at 541 mμ. No hemolysis occurred in any of the series incubated in the presence of inactivated C′, but because of the faint pink color of the concentrated albumin solution, each supernatant from a C′ tube was read with the corresponding inactivated C′ supernatant as the blank.

The cell pellets containing either whole red cells, ghosts, or a mixture of both were allowed to drain, and the tubes wiped. Distilled water was added to complete lysis, the lysates were transferred to 10 ml. volumetric flasks containing 0.100 ml. of 2.0 N Li^+ and the volumes made up with water. K^+ was determined in the flame photometer using the Li^+ as an internal standard. Each measured K^+ value was subtracted from the corresponding control value to give the quantity of K^+ released during incubation. This was divided by the control value to give the fraction of the total K^+ released. The final medium protein concentration represents chiefly albumin but there is also a small contribution from the immune globulin and the C′.

tions of the order of magnitude existing in blood plasma (70 mg./ml.) the protection would be definite but slight.

DISCUSSION

The experiments reported here are best explained as follows: as a result of the action of antibody and C′ on animal cells, functional "holes" are pro-

duced in the cell membrane. These holes permit the free exchange of K^+ and Na^+ between the interior of the cells and the suspending medium. As a result of this equilibration, the osmotic pressure inside the cells rises, water enters the cells, and they swell. The swelling of the cells has no effect on the rate of equilibration of Na^+ and K^+; the "holes" produced by the action of antibody + C′ are already large enough to permit unimpeded passage. That the "unstretched holes" as they are made by antibody + C′ are *not* large enough to permit the passage of macromolecules, is shown by the fact that no loss of these substances occurs in either ascites tumor cells or erythrocytes if the swelling is prevented with albumin. In the absence of a high extracellular protein concentration, about 50 per cent of the protein and 75 per cent of the RNA is lost to the medium in the case of the ascites tumor and over 85 per cent of the hemoglobin in the case of the erythrocyte.

The small molecules investigated—amino acids and ribonucleotides—occupy an intermediate position. These substances are finally lost from the cell to about the same extent whether the antibody + C′ acts in high protein medium or in low protein medium. However, their initial rate of loss is slightly reduced in the high protein medium. This would be consistent with the idea that the "holes" made by antibody + C′ are not large enough to permit the totally unhindered passage of these molecules, and only when the "holes" are stretched by the swelling which occurs in low protein medium, is the passage of these molecules entirely unimpeded.[4]

It appears that the "holes" produced by the action of antibody + C′ are, from the beginning, sufficiently large to allow the passage of substances of the order of molecular size of amino acids and nucleotides, for the swelling of the tumor cells produced by antibody + C′ is not prevented by the presence in the medium of quite high concentrations of sucrose (molecular weight 342), ordinarily a non-penetrating molecule. In a solution of 0.4 M sucrose in BSS, which is quite hypertonic, the swelling changes are at most delayed by a few minutes, probably the time required for the sucrose to equilibrate between the cells and the medium. At lower sucrose concentrations the delays are smaller in magnitude. Since bovine serum albumin, which is able to prevent the swelling, has a molecular weight of 65,000 (16), the "holes" must be of the size of molecules of molecular weight somewhere between 342 and 65,000. This pore size might be susceptible of more precise determination.

These "holes" should be considered to be of a functional character and not necessarily fixed or rigid openings in the cell membrane. The holes might be reversibly formed and sealed, for it is well known that cell membranes

[4] Another possible explanation for this result might be that the high viscosity of the protein medium results in a thicker layer of immobilized water around the cell membrane through which the substances must diffuse in order to reach the free medium. This effect might not be detected in the rate of loss of K^+, for KCl has a diffusion constant approximately fivefold greater than those of the small molecules in question.

have an essentially plastic nature, a property well illustrated in the case of erythrocytes by their ability to undergo so called fragmentation processes (17).

It is possible that these holes represent an enlargement or alteration of the pores postulated to be present normally in erythrocytes (18) and responsible for the leak rates of inorganic ions. Influx of water, produced by placing the cells in hypotonic solutions could produce the same general effect, though here the sequence of events would be inverted: first the influx of water, then the stretching of the pores, then the loss of small ions, followed by the macromolecules.

Davson and Danielli have shown that a variety of weakly lytic agents for erythrocytes, especially dihydric aromatic alcohols can, in concentrations lower than those required to induce loss of hemoglobin, produce a loss of cellular K^+ (19). This problem has also been studied by Ponder (20, 21) with rather similar results. Heavy metals—lead and mercury (22)—and irradiation in the presence of photodynamic dyes (23) also produce liberation of K^+ without equivalent amounts of hemoglobin. The sequence of events in the action of these lysins therefore seems rather like that for antibody + C′.

SUMMARY AND CONCLUSIONS

Rabbit antibody + complement alters the permeability properties of mouse Krebs ascites tumor cells and erythrocytes. When antibody + C′ acts on ascites tumor cells in a low protein medium, intracellular K^+ is lost from the cells at a rate far greater than the normal leak rate. At the same time the cells lose amino acids and ribonucleotides and become fully permeable to the Na^+ of the medium. When antibody + C′ acts in a low protein medium, the cells swell extensively and lose most of their macromolecules to the medium (hemoglobin from erythrocytes, protein and RNA from the ascites tumor cells). If the antibody + C′ acts in a medium containing protein in sufficient concentration to balance the colloid osmotic pressure of the cells, the swelling is prevented; no macromolecules are then lost from the cells, but the loss of K^+ and entrance of Na^+ are not altered, and the loss of amino acids and ribonucleotides is only slightly affected.

It therefore appears that the action of antibody + C′ is to produce functional "holes" in the animal cell membrane which permit the equilibration of cations and small molecules between cell and medium. This leads to an increase in the osmotic pressure of the cell and a rapid influx of water. The cell membrane and its "holes" are thereby stretched, permitting macromolecules to escape from the cell.

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