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Effect of Specific Anti-Sera on C^{14} Amino Acid Incorporation into Cellular Protein of Mouse Ascites Tumor Cells.* (28626)

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Goldberg *et al.* have described the effect of specific anti-sera on Erhlich ascites tumor cells(1). When observed by phase microscopy, numerous small clear pouches immediately form upon exposure of the cells to specific anti-serum and after one-half hour incubation at 37.5°C the cells become markedly enlarged. The nucleus, along with the intracellular particles, is restricted to one side of the cell and the remaining area of the cell (congealed pouches) is clear and apparently contained by a membrane. Green *et al.*(2) have reported that these cells lose a significant amount of total cell protein, amino acids, potassium and nucleotides. It has also been reported(3,4,5) that these cells have lost the capacity to oxidize exogenous glucose, but maintain the ability to oxidize succinate.

We have found that the uptake of amino acid by cells and subsequent incorporation of labeled amino acid into the gross protein is inhibited by anti-serum and that percent of inhibition correlates well with percent of cell rupture. This observation has yielded a rapid assay method for anti-sera and this method and results are reported below.

Materials and methods. L-valine-1-C^{14} (5.73 μc/μmole) was obtained from the New England Nuclear Corp. and made up in saline so that 0.1 ml contained 0.25 μc. The solution was frozen in small aliquots and stored at —20°C until required. NCTC-109 was obtained from Microbiological Associates. Male white rats between 180-200 g were obtained from Holtzman Co. The S-180 ascites tumor and lymphosarcoma 6C3HED was provided by Dr. T. McCoy, Noble Foundation, Ardmore, Okla., and maintained in Swiss mice and C3H mice, respectively. The C3H mice were purchased from the Texas Inbred Co., Houston, Texas, and the Swiss mice from A. R. Schmidt Co., Madison, Wis.

Preparation of antigen. All steps were carried out under sterile conditions. Tumor cells were removed from mice injected intraperitoneally with tumor one week previously, transferred to a graduated centrifuge tube and the tube sealed with a serum cap. The tube was centrifuged at 800 $\times$ *g* for 5 minutes and the supernatant fluid removed from the cell pellet by aspiration through the serum cap. Nine volumes of saline were injected into the tube with enough force to break the cell pellet. The tube was again centrifuged, the saline wash was removed by aspiration and the cells resuspended in 9 volumes of saline.

This preparation always contained varying amounts of red cells (1-5% of total cell volume). Rats weighing approximately 200 g

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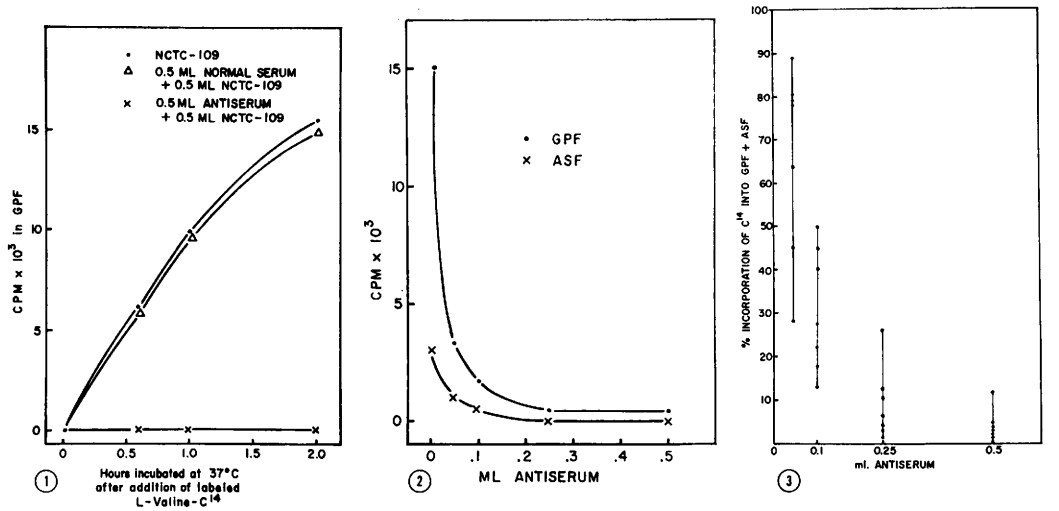

FIG. 1. Incorporation of labeled amino-acid into gross protein fraction of S-180 cells incubated in normal serum and in specific anti-serum.

FIG. 2. Effect of titrated anti-serum on incorporation of labeled amino-acid into gross protein fraction and acid soluble fraction of S-180 cells.

FIG. 3. Effect of titrated anti-sera from seven rats on incorporation of labeled amino-acid into combined gross protein fraction and acid soluble fraction.

were given 0.1 ml of this suspension intraperitoneally for 3 consecutive days. Cell suspensions were prepared each day one-half hour before use. Phase-contrast microscopy was used to ascertain the number of intact cells. These preparations contained about 5% dead cells.

Labeled amino uptake. Tumor cells were prepared as described above and the washed cell pellet was resuspended in 19 volumes of NCTC-109. One-half ml aliquots of this suspension were added to 15 ml screw-top test tubes. Antisera, or other solutions to be tested were then added, and final volume adjusted to 1.0 ml with NCTC-109. The tubes were capped and incubated for one-half hour at 37.5°C. The tubes were then removed, 0.1 ml of the labeled amino acid solution added, and the tubes again incubated at 37.5°C for the length of time described in each experiment. The tubes were then centrifuged at 800 $\times$ *g* for 5 minutes, the supernatant discarded and 5 ml of saline added to each tube. The cell pellet was dispersed by gently tapping and the tubes again centrifuged. The supernatant was discarded and the above wash repeated once. Five ml of 5% TCA was added to each tube, the pellet dispersed again by tapping, and after 15 minutes the tubes were centrifuged at 800 $\times$ *g* and the supernatant decanted into clean test tubes. This supernatant will be described as acid soluble fraction (ASF). Preparation of gross protein fraction (GPF) has been described(6).

Cell extracts were assayed for radioactivity with a Packard Tricarb liquid scintillation counter.

Results. Fig. 1 illustrates the uptake of amino acid into GPF of S-180 cells incubated in NCTC-109, normal serum of 18 rats and antibody serum of 32 rats. Cells incubated in normal rat serum or NCTC-109 incorporated labeled amino acid into protein at a linear rate for a period of almost 2 hours. Cells incubated in anti-serum did not incorporate labeled amino acid over the 2-hour incubation period. Phase-contrast microscopy revealed rupture of 98-99% of the cells exposed to anti-serum. Inhibition of anti-serum was found to be complement linked (Table I). Heating anti-serum for one-half hour at 57° C destroyed its inhibitory property and addition of complement (0.10 ml of normal rat serum) reactivated the heated anti-serum. Failure to completely destroy inhibitory property of anti-serum by heat treatment may be explained on the basis that heating normal rat serum under the same conditions produces

TABLE I. Requirement of Complement of Inhibition of C^{14}-Amino Acid Incorporation into Protein of S-180 Cells by Specific Anti-serum.*

Exp No.	Additions to suspension of S-180 cells	CPM in GPF
1	.5 ml normal rat serum	10,000
2	.5 ml anti-serum	150
3	.5 ml heated† anti-serum	8,800
4	.4 ml heated† anti-serum and .1 ml normal rat serum (complement)	200
5	.5 ml heated† normal rat serum	9,800

* See text for experimental details.
† Heated 1 hr at 60°C.

an inhibitory substance which interferes with uptake of amino acid.

In Fig. 2, the effect of titrating anti-serum of 33 rats against a constant number of S-180 cells may be observed. The radioactivity of both protein and the amino acid soluble fraction of the cells is plotted. One-half ml of anti-serum completely inhibits amino acid uptake into ASF and also of amino acid into GPF. Significant inhibition was observed at concentrations as low as 0.05 ml of serum, however, at 0.01 ml no significant inhibition of amino acid uptake into ASF or GPF was noted. It was found that this amount of anti-serum did not contain enough complement to rupture the cells. Phase-microscopy studies demonstrated that cell rupture closely parallels uptake of amino acid at all concentrations of anti-serum.

Since the uptake of amino acid into both ASF and GPF was inhibited by anti-serum it was possible to simplify the assay in the following manner. The cells were washed 3 times with saline and dissolved in 5 ml of 0.1 N NaOH. One-tenth ml of the solution was then assayed for radioactivity and the results for 7 rats are shown in Fig. 3. The results are plotted as percent inhibition *vs* ml of anti-serum added per tube.

Similar results were obtained when 6C3HED cells were used instead of S-180 cells. When 6C3HED cells were incubated in S-180 anti-serum, cell rupture did not occur and uptake of labeled amino acid was the same as observed when the cells were incubated in normal rat serum. Anti-serum produced against blood of non-tumor bearing Swiss mice did not rupture S-180 cells or inhibit C^{14}-amino acid uptake by these cells.

Discussion. Although Goldberg *et al.* noted minor alterations in the membrane of ascites tumor cells exposed to specific anti-serum in absence of complement, it would not appear that uptake of labeled amino acid by the cell or incorporation of labeled amino acid into protein is affected. The inhibition of amino acid uptake by the cell and incorporation into cellular protein occurs only when the cell is ruptured; it is the result of interaction of both antibody and complement. In some experiments 0.01 ml of anti-serum caused clumping of the tumor cells but did not rupture a significant number of cells. In these experiments uptake of amino acid was similar to that found in the controls. Apparently agglutination of cells does not affect amino acid uptake.

Summary. Mouse ascites tumor cells exposed to specific anti-sera in the presence of complement failed to concentrate and incorporate labeled amino acid into gross cellular protein. Inhibition of amino acid uptake by specific anti-sera closely parallels cell rupture by anti-sera (in the presence of complement) and this appears to be a rapid and sensitive biochemical assay for antibody produced against mouse ascites tumor cells by the rat.

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