# Effect of Antilymphocyte Serum on Parameters of Tumor Growth in a Syngeneic Tumor–Host System* (33793)

BERNARD FISHER, OSAMA SOLIMAN, AND EDWIN R. FISHER

*Departments of Surgery and Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213*

Evidence indicates that experimental tumors induced by carcinogens (1, 2) and viruses (3, 4) possess tumor-specific transplantation antigens (TSTA). Such antigens are capable of eliciting immune responses which inhibit the growth of a subsequent syngeneic tumor challenge. Exposure of nonimmunized animals to drugs, X-irradiation and other immunosuppressive modalities prior to tumor inoculation increases "takes" of such tumor transplants. The presence of TSTA in "spontaneous" tumors has been less firmly established although mammary tumors of C3H mice recently have been shown to possess TSTA (5). The importance of immune mechanisms on "take," growth, and metastases of such tumors was revealed in this study by observing an augmentation of these parameters of tumor growth following the administration of antilymphocyte serum (ALS), a widely employed immunosuppressant in clinical and experimental organ and tissue transplantation.

*Procedure and Results.* Antimouse lymphocyte serum was prepared in New Zealand white rabbits utilizing lymph nodes and thymus from C3H mice according to previously described methods (6). Leuko-agglutination titers of all batches of ALS that were utilized were greater than 1:64. Total leukocyte and lymphocyte counts were performed at intervals and a consistent reduction in the latter was demonstrated in treated animals (Fig. 1). Also, lymph nodes of ALS-treated animals, when compared with those from controls, demonstrated a depletion of cortical lymphocytes.

In the first experiment C3HeB/FeJ mice were inoculated intraportally with a suspension of 100,000 cells derived from C3H tumors maintained by serial transplantation.

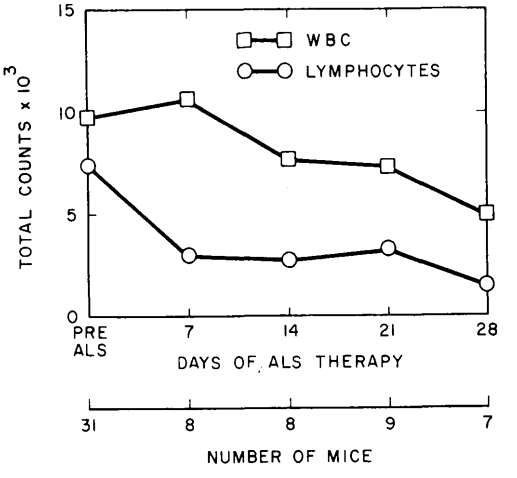


FIG. 1. Effect of antilymphocyte serum on white blood and lymphocyte counts of C3H mice.

Random groups received daily intraperitoneal injections of 0.25 ml of either ALS, normal rabbit serum (NRS) or saline from the day of tumor cell injection until the time of sacrifice 14 or 21 days later. The experiment was carried out four times and the results consistently demonstrated that liver "metastases" occurred in ALS-treated mice, but not in control animals (Table I).

In a second experiment, the effect of ALS was evaluated on the growth and metastases of the first transplant generation of C3H mammary tumors. Six uniform plugs were prepared from a spontaneous tumor and each was implanted subcutaneously by trocar into the thigh of a C3HeB/FeJ mouse. Three mice were injected daily with ALS and three with NRS until sacrifice 30 days after implantation. This experiment was performed six times—each time with a different spontaneous tumor. Six of the ALS-treated animals died at various times prior to 30 days and were not included in the results. Tumor was detected in all surviving ALS-treated animals, but in only 50% of controls (Table

* Supported by USPHS Grants CA-05716, CA-03731, AI-08206, and CA-05949.

TABLE I. Effect of Antilymphocyte Serum on Development of Liver Metastases in C3H Mice by C3H Mammary Tumor Cells.

| Expt. | Treatment | Sacrifice time (days) | No. of mice | No. + | % + |
|---|---|---|---|---|---|
| A | ALS[a] | 14 | 6 | 1 | 17 |
| | NRS[a] | | 10 | 0 | 0 |
| | Saline | | 10 | 0 | 0 |
| B | ALS | 21 | 3 | 2 | 67 |
| | NRS | | 8 | 0 | 0 |
| C | ALS | 14 | 4 | 2 | 50 |
| | NRS | | 4 | 0 | 0 |
| D | ALS | 14 | 6 | 5 | 83 |
| | NRS | | 9 | 0 | 0 |
| | Saline | | 10 | 0 | 0 |
| Total | ALS | | 19 | 10 | 53 |
| | NRS | | 31 | 0 | 0 |
| | Saline | | 20 | 0 | 0 |

[a] ALS = antilymphocyte serum; NRS = normal rabbit serum.

TABLE II. Effect of Antilymphocyte Serum on Growth and Metastases of First Transplantation Generation of C3H Mammary Tumors.

Tumor growth by 30 days
- Control 9 of 18 (50%)
- Treated 12 of 12 (100%)

Day of tumor appearance (av)
- Control 22.2
- Treated 17.6

No. of tumors reaching 10 mm by 30 days
- Control 3 of 9 (33%)
- Treated 9 of 12 (75%)

Size at sacrifice (30 days) (mm)
- Control 3.8
- Treated 11.4

Metastases (no. of animals)
- Control 0 of 18
- Treated 4 of 12 (2 to liver, 1 to lung, 1 to lung and liver)

II). Tumors also appeared earlier, grew larger, and occasionally metastasized to liver and lungs in ALS-treated mice. Spontaneous metastases were not observed in control animals.

*Discussion.* Although ALS treatment has been noted by us to abrogate the skin allograft reaction, it has not been as consistent in this regard as it has been in augmenting tumor growth in the species studied. This dichotomy may be related to antigen differences of skin and tumor and consequently the degree of immunity requiring suppression by ALS.

The mechanism responsible for the effect of ALS on the enhancement of allogeneic transplant survival has not been satisfactorily elucidated. It has been attributed to the lymphocytopenic effect of the serum, possible "blindfolding" of lymphocytes or so-called "sterile activation" (7). Since a number of experiments have demonstrated the role of a cell-mediated immune response in tumor growth and transplantation, the marked reduction of circulating lymphocytes observed in this study may be responsible for our findings. Similar effects on tumor growth have been observed following neonatal thymectomy in this species. However, the size and histologic appearance of the thymus appeared unaltered in the mice receiving ALS, and thymic localization of such serum is minimal in this species (8). The possibility that ALS may have a direct effect upon the tumor cells cannot be discounted at this time and is now under investigation.

These findings represent experimental evidence coincident with clinical observations of the growth of tumors inadvertently transferred with allografts to patients receiving immunosuppressive therapy (9).

*Summary.* Administration of antilymphocyte serum (ALS) resulted in augmentation of "takes," growth, and metastases of transplanted mouse mammary tumors in their syngeneic C3HeB/FeJ hosts.

1. Foley, E. J., Cancer Res. **13**, 835 (1953).
2. Prehn, R. T. and Main, J. M., J. Natl. Cancer Inst. **18**, 769 (1957).
3. Sjögren, H. D., Virology **15**, 214 (1961).
4. Hable, K., Proc. Soc. Exptl. Biol. Med. **106**, 722 (1961).
5. Silobrocic, V. and Suit, H. D., J. Natl. Cancer Inst. **39**, 1113 (1967).
6. Gray, J. G., Monaco, A. P., Wood, M. L., and Russell, P. S., J. Immunol. **96**, 217 (1966).
7. Levey, R. H. and Medawar, P. B., Proc. Natl. Acad. Sci. U. S. **56**, 1130 (1966).
8. Denman, A. M. and Frenkel, E. P., Immunology **14**, 107 (1968).

9. Wilson, R. E., Hager, E. B., Hampers, C. L., Corson, J. M., Merrill, J. P., and Murray, J. E., New Engl. J. Med. 278, 479 (1968).

Received Oct. 6, 1968. P.S.E.B.M., 1969, Vol. 130.

# Plasma Corticosterone and Contractility of Glycerinated White Muscle Fibers in the Rat* (33794)

A. W. SEXTON
(Introduced by J. W. Gersten)
*Department of Physical Medicine and Rehabilitation, University of Colorado School of Medicine, Denver, Colorado 80220*

Insufficient or excessive amounts of circulating adrenal steroid hormones can cause skeletal muscle weakness (1). Excessive glucocorticoids increase gluconeogenesis and the resulting depletion of muscle protein has been considered to be the cause of muscle weakness in hyperfunction of the adrenal cortex (2, 3). The precise mechanism of skeletal muscle weakness in hypofunction of the adrenal cortex is not known, but it is associated with sodium depletion (4) and may represent changes in microsomal activity of the muscle cells (5). This laboratory has previously demonstrated that the contractility of glycerinated muscle fibers from adrenalectomized rats is significantly decreased (4). No information is available, however, relating increased adrenal cortical activity to glycerinated muscle contractility. This report attempts to provide such information. Procedures were adopted which subjected rats to stress and thus achieved adrenal hyperfunction as measured by plasma corticosterone levels. The data relating glycerinated muscle contractility to adrenal hypofunction have been previously published (4). They are included in the present report so that a comparison may be made of the contractility of glycerinated skeletal muscle during adrenal hyperfunction and hypofunction.

*Materials and Methods.* Sixty-eight male Holtzman rats weighing 150–250 g were divided into four groups, housed in pairs, and maintained on Purina lab chow and water *ad libitum*. The first group was anesthetized with ether, subjected to bilateral adrenalectomy, maintained without replacement therapy for 6 days, and sacrificed. The control group received a sham-operation with bilateral penetration into the peritoneum and was maintained for 6 days before being sacrificed. The third group was subjected to ether anesthesia stress to elevate the naturally occurring plasma corticosterone levels. Light ether anesthesia was administered for 0.5 hr daily for 6 consecutive days before sacrifice. The final group was subjected to a severe stress to drive corticosterone levels as high as possible. A horizontal wheel 4 ft in diameter was constructed with four narrow tubular wire cages placed equidistant around the periphery. An electric motor provided power to rotate the wheel. By varying the angular velocity of the wheel and the distance of the cages from the axis of rotation, a controlled angular *g* force could be administered. These animals were subjected to 10*g* for 0.5 hr daily for 6 consecutive days before sacrifice.

Samples of tail blood were drawn for determination of plasma corticosterone 0.5 hr after stress administration on the third and the sixth or terminal day. Sampling was carried out between 1:30 and 2:30 p.m. to minimize diurnal variations of corticosterone levels. Corticosterone was determined fluorometrically by the method of Glick *et al.* (6) using a Turner fluorometer model 110 with a 47-B primary and a 2A-12 secondary filter. Immediately after drawing the final blood sample the rats were killed with ether and the su-

* Supported in part by a Research and Training Center Grant (RT-10) from the Social and Rehabilitation Service, Department of Health, Education, and Welfare, Washington, D. C.