

Effect of Normal Sera of Several Related Rodents on 6C3HED Lymphoma *in vivo*.^{*} (28391)

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The *in vivo* inhibitory effect of normal guinea pig serum on the 6C3HED mouse lymphoma observed by Kidd(1) was not present in any detectable degree in the sera of the horse and rabbit, and was only slight in the serum of a single human being. In an attempt to find another animal which might possess this factor which inhibits the growth of the 6C3HED lymphoma, an agouti and a paca, natives of South America and closely related to the guinea pig, were obtained. These rodents are members of the same superfamily, Caviioidea, as the guinea pig, but have been designated as members of the family Dasyproctidae, whereas the guinea pig is a member of the family Caviidae(2). Another rodent called the coypu or nutria, native of South America and common in Louisiana, was also investigated. This rodent is a member of a different superfamily, the Octodontoidea. The sera of the agouti and the paca were equally effective as guinea pig serum in inhibiting the growth of the 6C3HED lymphoma and also contained a relatively high level of L-asparaginase activity which Broome(3) has suggested to be the tumor inhibitory factor in guinea pig serum.

Materials and methods. Animals and tumor. The 6C3HED lymphoma was obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine and was transplanted in these experiments into untreated young female C3H mice obtained from the Cumberland View Farms, Clinton, Tenn. The tumor was transferred aseptically into C3H mice every 10 days and on repeated trials was found to be completely susceptible to normal guinea pig serum.

The guinea pigs were purchased locally and the agouti and paca were imported from Iquitos, Peru. The nutrias were obtained from the Louisiana Wildlife Commission. Se-

rum was collected from these animals by cardiac puncture and was quick-frozen in alcohol, cooled by dry ice, and kept frozen in a deep freeze until used.

Enzyme determinations. Levo-asparaginase determinations were performed on the normal guinea pig, agouti, paca, nutria, and human sera by the technique described by Meister(4). Five-tenths ml of the serum was incubated for one hour with 0.5 ml of 0.04 M L-asparagine monohydrate and 0.01 M sodium borate buffer at pH 8.5. The mixture was then treated with trichloroacetic acid, and after centrifugation the ammonia present was determined colorimetrically by reaction with Nessler's reagent.

In vivo tests. The 6C3HED mouse lymphoma was tested for its susceptibility to the various sera in the following manner: The tumor was removed aseptically from a mouse and was pressed through a fine meshed sieve into 10 ml of Ringer's solution buffered to pH 7.4 with a monobasic-dibasic phosphate mixture to which glucose was added. The cell suspension was placed in a 100 ml graduated cylinder for 5 minutes to allow clumps of tissue to settle. The concentration of cells in the supernatant was determined by use of a blood counting chamber; this supernatant was used as the tumor cell suspension and was diluted to one million cells per 0.5 ml with buffered Ringer's solution plus glucose. Five-tenths ml of this suspension was injected into both groins of 4 to 8 mice for each serum to be tested. In each group, 2 to 4 of the mice were untreated, and 2 to 4 mice were given 2 ml of the test serum on the days of injection of the tumor cell suspension and on the first and second days after injection. Beginning with the seventh day following injection of the tumor, the animals were examined daily to detect the appearance of the tumor and to follow the course of its growth until the animal died or until 50 days elapsed, whichever came

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TABLE I. *In vivo* Serum Effect on 6C3HED Lymphoma and Corresponding L-Asparaginase Levels of Sera.

Serum	Trials	No. of mice injected with tumor	Survival	L-asparaginase activity gamma NH ₃ nitrogen/hr/ml of serum
Guinea pig	6	20	20 free of tumor after 50 days	472-608
Agouti	3	10	10 free of tumor after 50 days	520-640
Paca	2	6	6 free of tumor by 50 days	416-520
Nutria	1	4	4 died of tumor by 22 days	none detectable
Human	2	6	6 died of tumor by 23 days	" "
None	6	20	20 died of tumor by 21 days	—

sooner. As indicated in Table I, the experiments with the agouti and paca sera were repeated several times.

Results. As demonstrated in Table I, the animals injected with 6C3HED lymphoma cells and treated with guinea pig, agouti, and paca sera failed to develop tumors; whereas those injected with the same number of tumor cells and with the human and nutria sera developed tumors by the seventh or eighth day and died with tumors by the end of the third week as did the untreated animals.

It was also found that the guinea pig, agouti, and paca sera, which were effective in inhibiting the 6C3HED lymphoma, had a significant level of L-asparaginase activity, but the nutria and human sera did not have a measurable amount of L-asparaginase activity.

Discussion. The 6C3HED mouse lymphoma grows readily in C3H mice, and the host usually dies within 3 weeks after injection of the tumor. Since Kidd observed that normal guinea pig serum would effectively inhibit the growth of the 6C3HED lymphoma, little has been learned about how this inhibition occurs. The testing of sera of a variety of unrelated animals such as the rabbit, horse, and human have indicated that these sera have no significant inhibitory effect on the 6C3HED mouse lymphoma *in vivo*. The experiments reported here indicate that the agouti and paca, rodents very closely related to the guinea pig, do possess serum which can exert an *in vivo* inhibitory effect on the 6C3HED lymphoma equal to that of guinea pig serum. These rodents are in the same superfamily as the guinea pig.

The fact that the serum of the nutria, a rodent in a different superfamily from the

guinea pig, agouti, and paca, does not exert an inhibitory action on this lymphoma is an interesting biochemical difference in these rodents which have been placed into different superfamilies on the basis of gross morphologic characteristics.

Broome(3) has recently indicated that the high level of L-asparaginase in guinea pig serum is responsible for its tumor inhibitory action. This view is supported by the observation reported here that no asparaginase activity was found in the sera which failed to inhibit the tumor whereas a high level of asparaginase activity was present in the agouti and paca sera which did inhibit the tumor.

Summary. Serum from the agouti and paca, rodents closely related to the guinea pig, exerted an inhibitory effect *in vivo* on the 6C3HED mouse lymphoma equal to that of guinea pig serum. Agouti, paca, and guinea pig sera have a measurable level of L-asparaginase activity; whereas human and nutria sera, which are inactive against the 6C3HED lymphoma, do not. The results support the hypothesis that this enzyme is the active principle responsible for inhibition of this tumor.

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