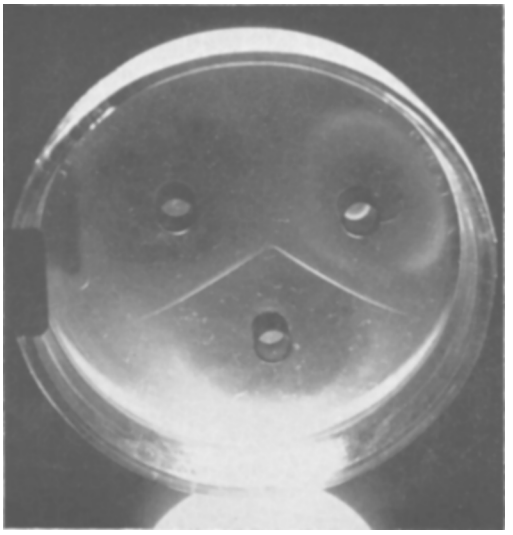

FIG. 1. Gel diffusion plates. *Left cup*: Normal human plasma. *Right cup*: Atherosclerotic plasma. *Center cup*: Chicken anti-human lipoprotein serum. The smooth blending of the lines of individual precipitates indicates the reaction of identity.

saturation with ammonium sulfate. 3) Chicken anti-human sera reacts with purified S_f6 lipoproteins. The reaction of identity (Ouchterlony) was given between purified lipoprotein and those present in atherosclerotic plasma. 4) A high degree of positive correlation existed between the total lipoproteins as measured by the ultracentrifuge and the quantity of precipitate produced by chicken antisera.

Summary. The injection of a lipoprotein fraction from the sera of cholesterol-fed rabbits or human atherosclerotic patients into chickens brought about the formation of antibodies specific for either rabbit or human lipoproteins. A positive correlation existed between the amount of precipitate produced by mixing antisera with sera containing lipoproteins and the lipoprotein content measured ultracentrifugally.

The authors wish to acknowledge the aid given by Dr. James A. L. Mathers of Columbia University in providing blood samples from diagnosed patients.

1. Baker, S. P., Ogden, E., and Riddle, J. W., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 119.

2. Kabat, E. A., and Meyer, M. M., *Exp. Immunochemistry,* C. C. Thomas, Springfield, Ill., 1948.

3. Gofman, J. W., Lindgren, F., Elliott, H., Mantz, W., Hewitt, J., Strisower, B., and Herring, V., *Science,* 1950, v11, 166.

4. Ouchterlony, O., *Arkiv. Kemi. Mineral Geol.,* 1949, v26B, 1.

5. ———, *ibid.,* 1950, v1, 43.

6. ———, *ibid.,* 1950, v1, 55.

Received July 6, 1954. P.S.E.B.M., 1954, v86.

Inhibitory Effects of Normal Guinea Pig Serum and Immune Rabbit Serum on Transplanted AKR Lymphomas.* (21232)

JOHN G. KIDD AND JEAN ELIZABETH TODD.

From the Department of Pathology, The New York Hospital—Cornell Medical Center.

When normal guinea pig serum is given intraperitoneally in relatively large amounts to C3H mice carrying the Gardner 6C3HED lymphosarcoma or to A mice carrying Lorenz's Lymphoma II, the growths regularly regress, as a recent study has shown(1); furthermore, the potency of the normal guinea pig serum against 6C3HED lymphoma cells *in vivo* is considerably enhanced when a small quantity of an immune serum, made by injecting the lymphoma cells into rabbits, is given together with it(1). In the work now to be reported normal guinea pig serum and immune rabbit serum, separately and in combination, have been tested for inhibitory effects *in vivo* against the cells of 8 transplanted lymphomas of AKR mice. As will be shown, the findings with the AKR lymphomas differ in at least one respect from those obtained with the C3H

* These investigations were supported by grants from Mr. John W. O'Boyle, The Committee on Growth Acting for The American Cancer Society, and The National Cancer Institute, and by gift from Mr. Salvatore Scarzafava.

and A lymphomas; this difference may have significance in light of the fact that lymphomas occur spontaneously in a high proportion of untreated AKR mice while being rare in animals of the C3H and A breeds.

Materials and Methods. Seven of the 8 lymphomas here employed were originally transplanted in this laboratory. One of the growths (Lymphoma L-1) was transferred initially from the enlarged nodes of an AKR mouse supplied by Dr. Lloyd Law of the National Cancer Institute, and 6 others from AKR/JAX mice with autochthonus lymphomas procured from the Jackson Memorial Laboratory. The provenance of the 8th growth (Lymphoma E-1) will be given further on. All the growths were carried in AKR/JAX mice, suspensions containing known numbers of cells being implanted in the groins of each host animal according to a standardized technique(1). As a rule palpable and visible tumors appeared some 6-15 days following implantation of the lymphoma cells; the growths regularly enlarged progressively thereafter until death of the animals, usually within 18 to 30 days. The normal guinea pig serum was procured by bleeding 12 to 36 normal, adult, market-bought guinea pigs without anesthesia, and allowing the blood to clot in paraffin-lined tubes. The serum specimens were harvested the same day, pooled, and stored in the deep freeze at approximately −22°C until used, usually within 6 weeks. The immune rabbit serum was prepared as follows: approximately 6 g of tumor tissue from Lymphoma L-1 was pressed through a monel metal sieve and suspended in 25 cc of 0.9% NaCl; the suspension was mixed with 25 cc Falba, 25 cc Bayol F, and 165 mg dried *Mycobacterium butyricum,* the mixture then being emulsified in a Waring blender. 2 cc of the creamy emulsion was injected intramuscularly into 6 sites in each of 6 large, market-bought, hybrid rabbits. The rabbits, all with large granulomas at the injection sites, were bled from the heart (approximately 50 cc) on the 21st day, and again 10 and 20 days later, the serum specimens being pooled and stored in the deep freeze.

The tests for *inhibition in vivo* were made as follows: One to 6 million lymphoma cells, suspended in 0.5 cc of buffered Ringer's solution, were injected into the subcutaneous tissues of the groins of 8 to 24 or more AKR/JAX mice weighing 18-22 g each. The mice were then divided into groups, 3 or 4 animals in each. One or more of the groups was kept untreated as controls. The mice of 2 or 3 or more groups were given various amounts of normal guinea pig serum intraperitoneally, those of one group always receiving 2.0 cc of whole serum approximately 1 hour after implantation with the tumor cells; often the animals of another group were given 2.0 cc of the normal guinea pig serum 1 hour after the implantations and again on the 2nd and 3rd days of the experiment, though sometimes a single injection of a smaller amount of serum was given, or repeated injections of 2.0 cc of 3:1 "fan-concentrated" guinea pig serum(1). The rabbit immune serum was usually given to at least 2 groups of the implanted mice, usually in doses of 0.5 cc and 0.1 cc, respectively, sufficient 0.9% NaCl being added to make the volume of each injection up to 2 cc. Mixtures containing 1.5 cc normal guinea pig serum plus 0.5 cc immune rabbit serum, or 1.9 cc of normal guinea pig serum plus 0.1 cc immune rabbit serum, were injected into each of the animals in additional groups, again approximately 1 hour after the implantations. The immune rabbit serum was usually used unheated; in several experiments, however, its effectiveness was found to remain undiminished when it had been diluted 1:2 and heated at 65°C for 30 minutes. For control purposes, normal rabbit serum was used in a number of experiments; it had no effect on the AKR lymphomas when given alone or in combination with normal guinea pig serum. Except for some transitory sluggishness and tachypnoea, the injections as a rule did not bring about any signs of discomfort, the mice remaining lively throughout the periods of observation. Palpable tumors usually appeared at the implanted sites within 7 to 10 days in the control animals. Beginning at this time records were made every day or every other day of the state of each animal, with chartings of the tumors or a note that growths were absent.

Results. In several comparative tests done

TABLE I. Inhibitory Effects *in Vivo* of Normal Guinea Pig Serum and Immune Rabbit Serum, Separately and in Combination, on the Cells of Eight Transplanted Lymphomas of AKR Mice.

8 lymphomas employed	Serial transfers tested	Summary of results of tests for inhibition *in vivo*		
		With normal guinea pig serum	With immune rabbit serum	With normal guinea pig serum plus immune rabbit serum
1–3	2nd, 3rd	0	?	?
1–6	1st	0	nt	?
2–2	1st, 2nd	0	++	++
1–4	2nd, 3rd	0	++	+++
1–7	2nd, 3rd	?	+++	++++
1–5	2nd, 3rd	?	+++	++++
L-1	1st, 2nd, 6th, 7th	?	+++	++++
E-1*	26th, 30th	++	nt	++++

See text for details of materials and methods and for results of concurrent tests with Gardner lymphosarcoma in C3H mice.

0 = No inhibition—growths in 3 or 4 test mice comparable in time of appearance, size, and course to those in untreated control mice.

? = Dubious inhibition; sometimes definite but slight inhibition in one experiment with little or no inhibition in a subsequent test.

++ = Moderate inhibition—growths appeared 2 to 5 days later than in controls, remained smaller throughout period of observation.

+++ = Marked inhibition—growths appeared 4 to 8 days later than in controls, remained smaller throughout period of observation.

++++ = Complete inhibition—no growths in test mice during 44-60 days; growths palpable in control mice after 7 to 10 days—these enlarged steadily, bringing about death with widespread lymphomatosis within 18-30 days.

nt = not tested.

* Lymphoma E-1 was transplanted for 25 generations during 2 years in laboratory-bred AKR mice of the line in which it had originated; it was then transferred to AKR/JAX mice for the tests here reported.

along with the experiments of the present work, inhibition of the Gardner 6C3HED lymphoma cells was regularly marked (+++) or complete (++++) when 2 cc of normal guinea pig serum was given intraperitoneally 1 hour after the subcutaneous implantation of 4 to 6 million 6C3HED lymphoma cells in C3H mice, and it was always complete (++++) when 2 cc was given on the day of implantation and on the 2 following days as well. Table I provides a summary of the results of the tests for inhibition of the 8 AKR lymphomas *in vivo*. The findings as a whole (Table I) show that normal guinea pig serum was far less effective against the cells of transplanted AKR lymphomas *in vivo* than it was against the cells of the C3H and A lymphomas previously tested(1) and confirmed in the case of the 6C3HED lymphoma in the present work. Indeed under the conditions here employed normal guinea pig serum did not notably inhibit the cells of any of the 7 recently transplanted AKR lymphomas against which it was tested, this being generally true when 2.0 cc of the serum was given 1 hour after implantation with the lymphoma cells and again on each of the following 2 days. Additional tests showed that 2 cc of 3:1 "fan-concentrated" guinea pig serum(1), given to test mice on the day of implantation and again on each of the 2 succeeding days, had no effect on the later transfers of the cells of Lymphomas 1-7 and L-1. Furthermore, the normal guinea pig serum had only limited inhibitory effects *in vivo* on the cells of an AKR lymphoma (E-1) that had recently been transferred to a new line of AKR mice after 25 serial transplantations during approximately 2 years in the laboratory-bred AKR mice in which it had originated. It is possible that intrinsic differences in the neoplastic cells of the AKR and C3H lymphomas might account for the diverse results obtained in the inhibition tests with the AKR and C3H lymphomas. It is also possible that the mice of the 2 breeds

might react in significantly different ways following the injection of normal guinea pig serum intraperitoneally. And since the 6C3HED cells are killed *in vivo* when guinea pig serum is given to mice carrying them, but not *in vitro* when the cells are held in contact with whole guinea pig serum during several hours at 37°C—the effect *in vivo* obviously depending upon some reaction in which the animal host and the guinea pig serum both participate(1)—, the latter possibility has added interest. A third possible explanation for the findings will receive mention further on, after consideration has been given to the effects of normal guinea pig serum on the AKR lymphoma E-1.

The AKR lymphoma E-1 was moderately inhibited in the test mice given 2 cc of normal guinea pig serum one hour after the implantations, as Table I indicates; in other tests it was markedly but incompletely inhibited (++++) when 2 cc of the serum was given one hour after the implantations and repeated on each of the 2 succeeding days. In additional experiments, subcutaneous E-1 lymphomas measuring up to 1.5 cm across, resulting from implantations made 6-9 days previously, dwindled markedly in size within 24-48 hours following the injection of 2 cc of 3:1 "fan-concentrated" guinea pig serum intraperitoneally into mice carrying them, while gross and microscopic studies of the tissues of several animals of this experiment made it plain that visceral infiltrations of this lymphoma—*e.g.*, in thymus, lungs, liver, spleen, and kidneys—had either failed to develop or regressed following injection of the concentrated serum. This growth had been transplanted longer than any of the other AKR lymphomas, and the fact may have significance for the interpretation of the results, though in this relation it may also be significant that the lymphoma L-1 was not inhibited after 6 and 7 serial transfers (Table I). Lymphoma E-1 differed from the rest of the AKR growths in another way as well: it had been transferred during approximately 2 years in AKR mice of the line in which it had originated (mice bred in the laboratories of Dr. Ralph Engle of this Medical Center) and then was transferred to AKR/JAX mice for the experiments here reported. All the other lymphomas had originated in AKR/JAX mice and were regularly transferred in hosts of that line. These facts deserve consideration in relation to the fact that the 2 lymphomas previously found to be markedly inhibited by normal guinea pig serum *in vivo*—Gardner's lymphoma 6C3HED and Lorenz's lymphoma II (1)—had both been repeatedly transplanted during several years' time and eventually were tested in hosts manifesting some resistance against them. Considered together the findings lend weight to the supposition that isoantibodies might be provided by the hosts which act together with the intraperitoneally injected guinea pig serum in bringing about regression of subcutaneous lymphomas *in vivo*(1).

An immune rabbit serum—made by injecting the cells of one of the AKR lymphomas (L-1) together with Freund's adjuvants into rabbits, as previously described—, when injected in 0.5 cc amounts intraperitoneally into AKR mice one hour after implantation, proved moderately or markedly inhibitory for the cells of 5 of the 6 recently transplanted AKR lymphomas against which it was tested, its effects in the case of the remaining growth being dubious (Table I). The findings have interest in relation to the observation made some years ago by Nettleship that the Murphy-Sturm lymphosarcoma of rats was to some extent inhibited *in vivo* by large amounts of an immune serum made by injecting extracts of the rat lymphosarcoma cells into rabbits (2), and to the more recent observation of Nungester and Fisher that the 6C3HED lymphoma was likewise inhibited *in vivo* by an immune serum made in rabbits(3). Furthermore, mixtures containing 1.5 cc of normal guinea pig serum and 0.5 cc immune rabbit serum, given intraperitoneally 1 hour after implantation of AKR mice with the various lymphoma cells, proved markedly inhibitory *in vivo* for the cells of 5 of the 8 transplanted growths, the effects of the mixtures in these instances being often complete and usually greater than when either material was injected alone (Table I); in additional tests, mixtures containing 1.9 cc of normal guinea pig serum plus 0.1 cc of the immune serum were often markedly inhibitory, whereas mixtures con-

taining 1.9 cc of Ringer's solution and 0.1 cc of immune serum had only slight inhibitory potency or none at all. The observations may have a relation to a finding already reported—namely that the effectiveness of normal guinea pig serum against 6C3HED lymphoma cells *in vivo* is enhanced upon admixture with an immune serum made in rabbits with the 6C3HED lymphoma cells as antigen(1)—and it may have other implications also. The possibility that one or another of the components of complement may participate in the reactions responsible for these *in vivo* effects has been discussed in a previous paper(1). The question is now being tested whether immune serum alone, or mixtures of normal guinea pig serum and immune rabbit serum, will bring about regression of established AKR lymphomas *in vivo*.

Summary. In the experiments here given, as in those previously reported, normal guinea pig serum, given in amounts of 1 or 2 cc intraperitoneally to C3H mice one hour following the implantation of 2 million 6C3HED lymphoma cells in their subcutaneous tissues, always markedly inhibited growth of the lymphoma cells, and when 2 cc of the serum was given on the day of implantation and again on the 2 succeeding days the inhibition was regularly complete. In striking contrast, normal guinea pig serum, even when given repeatedly in the largest feasible amounts, had little or no inhibitory effect *in vivo* on the cells of 7 AKR lymphomas recently transplanted in AKR mice of the line in which they had originated, and it manifested only limited effectiveness against the cells of a single AKR lymphoma that had been transplanted during about 2 years in laboratory-bred mice of the line in which it had originated with later transfer to AKR mice of another inbred line. An immune serum, prepared by injecting rabbits with the lymphoma cells of one of the AKR growths together with Freund's adjuvants, proved moderately to markedly inhibitory for the AKR lymphoma cells when injected intraperitoneally into mice one hour after implantation of the cells in the subcutaneous tissues, and mixtures of the immune serum with normal guinea pig serum were often completely inhibitory. The significance of the findings is briefly discussed.

1. Kidd, John G., *J. Exp. Med.*, 1953, v98, 565, 583.

2. Nettleship, A., *Am. J. Path.*, 1945, v21, 527.

3. Nungester, W. J., and Fisher, H., *Ca. Research*, 1954, v14, 284.

Received July 8, 1954. P.S.E.B.M., 1954, v86.

Secretion of 17-Hydroxycorticosterone by Adrenal of Hypophysectomized Dog: Effect of ACTH.* (21233)

ELIZABETH W. RAUSCHKOLB,† ROBERT S. ROSNAGLE,† AND GORDON L. FARRELL.
(Introduced by George Sayers.)

From the Department of Physiology, Western Reserve University School of Medicine, Cleveland, O.

The rate of secretion of 17-hydroxycorticosterone by the adrenal of the hypophysectomized dog prior to and following injection of ACTH has been determined to elucidate the time-relationships concerned in the activation of adrenal secretion.

Methods. Male dogs weighing 11.3 to 19.5 kg were anesthetized with sodium pentobarbital and hypophysectomized via the oral approach. The animals were maintained under light sodium pentobarbital anesthesia throughout the remainder of the experiment. At 3.25 to 12.7 hours after the hypophysectomy the left lumboadrenal vein was cannulated

* This investigation was supported by a research grant from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service and by a research grant from the Upjohn Co.

† Experimental work was conducted in partial fulfillment of the Research Project Teaching Program at Western Reserve University School of Medicine.