EFFECTS OF VARIOUS IMMUNE RABBIT SERUMS ON THE CELLS OF SEVERAL TRANSPLANTED MOUSE LYMPHOMAS IN VITRO AND IN VIVO*

With Special Reference to Inhibition by an Antibody That Reacts with Neoplastic and Non-Neoplastic Lymphocytic Cells of Mice

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In the experiments now to be described, we have found that immune serums prepared in rabbits with cells of various mouse lymphomas as antigens will not only kill several types of lymphoma cells rapidly *in vitro* in the presence of complement but will act upon them *in vivo* as well, the effects being to a considerable extent specific and dependent upon the presence of an antibody that reacts with neoplastic and non-neoplastic lymphocytic cells of mice. Furthermore, the anti-lymphoma serums acted more powerfully upon the lymphoma cells *in vivo* than did such chemotherapeutic agents as amethopterin, azaguanine, ethionine, azaserine, and 6-mercaptopurine, given singly or in various combinations in maximal tolerated amounts, though their effects were not so powerful as those exerted by normal guinea pig serum on lymphoma cells of two types that are susceptible to its effects *in vivo* (1).

Although by no means extensive, the older work of several investigators provides little reason for supposing that heterologous immune serums—that is to say, antiserums made by injecting cancer cells into animals of alien species—have the capacity to act powerfully or specifically against cancer cells *in vivo*. For example, Borrel in 1907 failed to find antibodies that were effective *in vivo* against the cells of a mouse carcinoma in immune serums procured from sheep and fowls that had been given large quantities of the cancer as antigen (2), and Tyzzer in 1916 found that an antiserum produced in rabbits, with mouse sarcoma cells as antigen, did not retard growth of the sarcomas even when the antiserum was injected several times directly into the subcutaneous tumors, while subsequently Rohdenburg noted that an antiserum prepared in guinea pigs with the Flexner-Joblin rat carcinoma as antigen was devoid of effect on the tumor *in vivo* (3). Furthermore, Lambert in 1914 found that guinea pigs injected with rat blood or rat embryo skin provided immune serums that acted quite as powerfully against rat sarcoma cells cultured *in vitro* as did immune serums made with the sarcoma cells as antigens (4), and Lumsden, in an extensive study begun in 1925, found

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that immune serums prepared in rabbits with cells from the Jensen rat sarcoma as antigen, although toxic for the neoplastic cells when brought into contact with them *in vitro*, were devoid of noteworthy effect *in vivo* (5).

Under suitable conditions, however, heterologous immune serums will inhibit the cells of two transplanted lymphomas to some extent *in vivo*. For Nettleship in 1945 showed that immune rabbit serums made with chemically separated fractions of Murphy-Sturm lymphoma cells as antigens, when given in large amounts to rats implanted with the lymphoma cells 4 to 5 days previously, brought about regression of the growths in 57 per cent of 41 treated animals and slowed the growth of the lymphomas in most of the remainder (6). Nettleship's results are obviously open to the objection, recognized by him, that his animals were resistant to the transplanted cells employed; even so his findings seem significant, since he set up his controls carefully and found that regression occurred in approximately 10 per cent of 83 control animals. In further studies, Kidd showed that an anti-Lymphoma 6C3HED immune rabbit serum enhanced the inhibitory effect of normal guinea pig serum on the cells of Lymphoma 6C3HED *in vivo* (1); indeed, the latter effect was discovered incidentally in experiments designed to test for inhibition by the immune serum, the guinea pig serum being added with a view to enhancing its effect. More recently, Nungester and Fisher have reported that immune serums, prepared in rabbits with sedimented materials separated from Lymphoma 6C3HED cells as antigen, inhibited growth of these cells to a considerable extent when injected intraperitoneally into the host animals within 48 hours following implantation of the cells, but the serums had little or no effect when given after 96 to 144 hours (7).

Lumsden (8), Phelps (9), and Gorer (10) have shown furthermore, that isoantibodies often appear in the blood of animals manifesting resistance to cancer cells implanted into hosts of alien breeds, while Kidd (11) and Burmester (12) have found that humoral antibodies are effective *in vitro* and *in vivo* against cells of the Brown-Pearce rabbit carcinomas and those of fowl lymphomatosis, respectively. The observations of Michison (13) and those of Algire, Prehn, and Weaver (14) have made it plain, however, that cells of resistant and immune hosts are more influential than are humoral isoantibodies in bringing about regression of transplanted cancer cells of several types.

Materials and Methods

In brief, 43 lots of pooled immune rabbit serums (hereinafter IRS) were made by injecting Freund's adjuvants plus suspensions of various normal and neoplastic mouse tissues (normal kidney, liver, brain, thymus, lymph nodes, spleen, erythrocytes; transplanted malignant lymphomas of seven types which had originated in C3H, AKR, and A mice, and a transplanted mammary carcinoma of C3H mice) into large domestic rabbits. The immune serums thus obtained were then tested, together with serums from normal rabbits, for capacity to act upon the cells of several transplanted mouse lymphomas *in vitro* and *in vivo*.

The immune rabbit serums were prepared in a standardized way. Mouse tissues of three general sorts were used as antigens: (*a*) normal mouse organs that were presumably devoid of large numbers of lymphocytic cells—brain, kidney, liver, muscle, whole embryos, erythrocytes; (*b*) non-neoplastic lymphatic organs, such as thymus, lymph nodes, and spleen procured from C3H or AKR mice 2 to 3 months old; and (*c*) the cells of transplanted lymphomas of seven different types which had originated in either AKR, C3H, or A mice and been carried in host animals of the strain of origin. For further comparison, an immune serum was also made with tissue from a transplanted mammary carcinoma which had recently originated in a C3H mouse in this laboratory. The tissue in each instance, usually 50 gm. or more, was procured with an aseptic technique and pressed with a pestle through a 40 mesh monel metal sieve into 50 cc. of Ringer's solution; the heavy suspension of cells was then mixed in a commercial Waring blendor or by means of a hand syringe with Freund's adjuvants (50 cc. falba,

50 cc. bayol, and 330 mgm. dried *Mycobacterium butyricum*). The antigen was injected intramuscularly in six sites in 4 to 10 adult hybrid rabbits (2.0 cc. to 4.0 cc. in each site, 2.0 to 10.0 gm. of tissue being injected into each animal); large granulomas regularly appeared where the antigen had been deposited. The animals were bled from the heart on the 21st, 28th, and 35th days, approximately 60 cc. being removed at each of the first two bleedings, and as much blood as possible at the final bleeding. After the final bleedings the serum from all rabbits of a given immunization was pooled, cultured in broth and on agar, and stored in the deep freeze at −16°C.

As already mentioned, *seven transplanted mouse lymphomas were employed.* Two of these (Lymphomas 6C3HED and E9514) had originated in C3H mice, three (Lymphomas AKRL1, AKR17, and AKR13) in AKR mice, and two (Lymphomas A1 and A2) in Strong A mice. The growths were transferred as routine in young adult mice of the three respective breeds, all procured from the Jackson Memorial Laboratory, Bar Harbor. They grew equally well in male and female mice, and progressively until death of the host except in the case of Lymphoma 6C3HED, which regressed after a period of transitory growth in approximately 5 per cent of the animals. Suspensions of the lymphoma cells, for use in routine transfers and in the *in vitro* and *in vivo* tests to be described further on, were prepared as in previous studies (1), briefly as follows: Subcutaneous growths, usually 1.5 to 3.0 cm. across, which had resulted from implantations made 8 to 10 days previously, were procured with aseptic precautions and pressed through the 40 mesh monel metal screen into a buffered-glucose-Ringer's solution, in which the pH was adjusted to 7.4 with 0.01M phosphate buffer and to which glucose was added in a concentration of 200 mg. per cent—hereinafter BGR. The suspension was allowed to stand a few minutes in a cylinder to allow the tissue debris and clumps of cells to settle out. From the center of the column the required material was then carefully pipetted off. Counts of the cells were made microscopically with an ordinary hemocytometer, and the volume of the suspension was so adjusted that 10 thousand to 10 million or more cells were present in each milliliter. Cultures of the final suspensions in broth and on agar regularly proved sterile. For routine transfers, 1 million or more cells in 0.5 ml. of BGR were implanted by means of a 1.0 ml. syringe and 25 gauge needle into the subcutaneous tissue of each groin. Palpable lymphomas regularly developed at the implanted sites, usually within 5 to 7 days, and these enlarged progressively as a rule until death of the host animals with generalized lymphomatosis. Microscopic studies furthermore showed that the lymphoma cells spread rapidly following the subcutaneous implantations; for example, within 3 to 5 days following implantation into the subcutaneous tissues of 3 to 6 week old AKR mice, Lymphoma AKRL1 cells were often more numerous in lymph nodes throughout the body and in the spleen, liver, and other viscera, than at the implanted sites.

For the in vitro studies, the immune serums, and normal rabbit serum that had been procured and stored in the same way, were mixed in suitable dilutions in test tubes with suspensions of the lymphoma cells prepared as described above. As a rule, the normal and immune rabbit serums were heated at 65°C. for 20 minutes to inactivate complement and natural antibodies; such heating has no deleterious effect on induced antibodies, as much experience has shown (15). Normal guinea pig serum, pooled from a dozen or more adult, market-bought guinea pigs and tested for hemolytic activity in conjunction with rabbit amboceptor, was usually added to each of the *in vitro* mixtures in a final concentration of 1:10, to provide a uniform quantity of complement. The mixtures were then held 1 hour or longer at 37°C., centrifuged at 600 R.P.M. for 5 minutes, and the deposited cells resuspended in an equal volume of BGR—this to limit contact between serums and cells. To test for viability, the cells of each mixture were then implanted subcutaneously in two or four susceptible test mice, with careful charting afterwards of all resulting growths.

For the in vivo tests, 5,000 to 300,000 or more lymphoma cells were first implanted in each groin of susceptible, healthy adult mice. After an interval of 1 hour to 6 days or more, the test serums were injected intraperitoneally; usually the intraperitoneal injections were

repeated several times, as will be noted in the accounts of the various experiments. The mice were carefully observed afterwards, repeated chartings of all growths being made and scrutiny of the animals for toxic effects of the immune serums as well.

Tests for Viability of Mouse Lymphoma Cells Exposed In Vitro in the Presence of Complement to Various Immune Serums

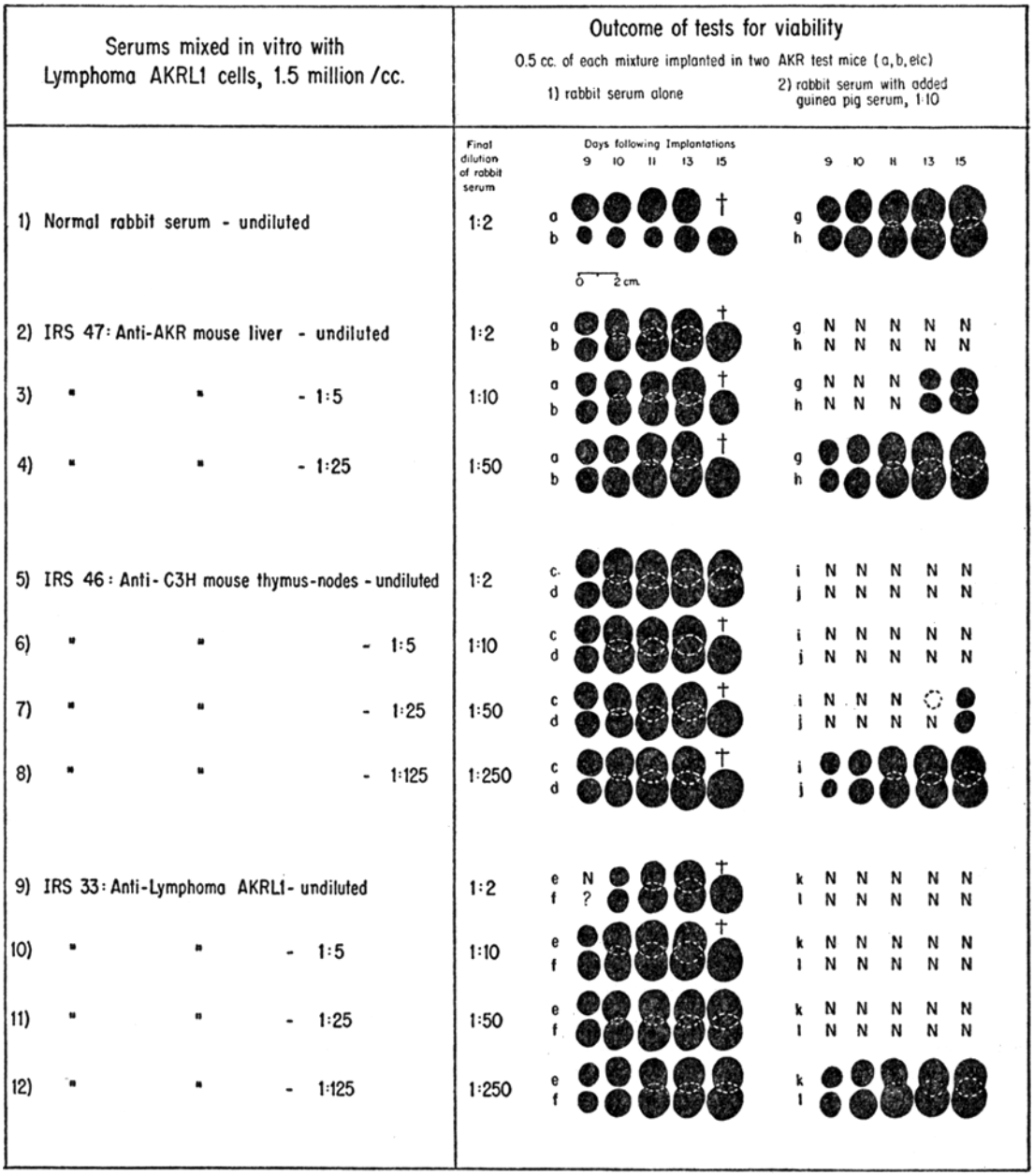
In an initial experiment viability tests were made with Lymphoma AKRL1 cells that had been held in contact during one hour at 37°C. with (*a*) normal rabbit serum and (*b*) three pooled immune rabbit serums. One of the immune rabbit serums (IRS 47) had been prepared by injecting a suspension of liver cells from normal C3H mice, together with Freund's adjuvants, into rabbits; another (IRS 46) with a suspension of cells from thymuses and lymph nodes from normal C3H mice; and the third (IRS 33) with Lymphoma AKRL1 cells. The serums were first heated at 65° for 20 minutes, to inactivate complement and natural antibodies (15). In each instance the serum specimens were tested in several dilutions as such and with unheated guinea pig serum added in a final concentration of 1:10 to provide complement.

Chart 1 shows the results. It can be seen that the heated normal rabbit serum, in a final concentration of 1:2, had no effect on the lymphoma cells either alone or with added complement. The three heated immune serums likewise proved innocuous for the lymphoma cells in those mixtures that were devoid of guinea pig serum. In the presence of guinea pig serum 1:10, however, all three of the immune serums rendered the lymphoma cells non-viable, IRS 47:Anti-liver being completely effective in a final concentration of 1:2 and nearly so at 1:10 but devoid of effect at 1:50, while IRS 46:Anti-thymus-node was considerably more potent, and IRS 33:Anti-Lymphoma AKRL1 was still more so, no tumors resulting from the injection of the mixture containing lymphoma cells and this serum in final concentrations of 1:2, 1:10, and 1:50.

In several additional experiments heated specimens of various immune rabbit serums that had been prepared with various normal mouse tissues and with mouse lymphoma cells as antigens, regularly killed lymphoma cells of three types (6C3HED, E9514, AKR17) when the serums, diluted 1:40 to 1:160, were held in contact with the lymphoma cells for 1 or 2 hours *in vitro* in the presence of added guinea pig complement; but all failed to do so in final concentrations of 1:2 in the absence of complement. Hence unheated guinea pig serum was employed as routine in the subsequent *in vitro* tests.

Table I shows the outcome of more extensive tests. 15 immune serums—9 prepared with various normal mouse tissues as antigens, and 6 with lymphoma cells of several types as antigens—were tested for ability to kill lymphoma cells of three kinds during exposure *in vitro* in the presence of complement. All the immune serums except one exhibited some ability to kill the lymphoma cells *in vitro* in the presence of complement, as the table shows. Furthermore the three types of lymphoma cells differed in susceptibility, 6C3HED cells being most suceptible to the effects of the various serums, E9514 cells slightly less so, and AKRL1 cells comparatively insusceptible. Serums that were most potent against 6C3HED cells (IRS 58 and IRS 35) likewise acted powerfully against E9514 and AKRL1 cells, while serums that were least potent against 6C3HED cells (IRS 54 and 66) had comparatively little ability to kill the E9514 and AKRL1

CHART 1. Tests for viability of Lymphoma AKRL1 cells exposed *in vitro* to specimens of heated immune rabbit serums as such and with added guinea pig serum (complement).



IRS = immune rabbit serum. So, too, in the charts that follow.

The rabbit serums were heated in the undiluted state at 65°C. for 20 minutes immediately before use to inactivate complement and natural antibodies.

The test mixtures were heated one hour at 37°C prior to implantation.

TABLE I

Tests for Viability of Lymphoma Cells of Three Types Exposed in Vitro in the Presence of Complement to Various Immune Rabbit Serums

In vitro mixtures	Outcome of tests for viability											
	6C3HED cells				E9514 cells				AKRL1 cells			
Lymphoma cells (6 million/cc.) and guinea pig serum (complement) 1:5, plus an equal part of:—	Final dilutions of serums											
	1:5	1:20	1:80	1:320	1:5	1:20	1:80	1:320	1:5	1:20	1:80	1:3
(1) Normal rabbit serum	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++
(2) IRS 53:anti-AKR muscle	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++
(3) IRS 54:anti-C3H embryo	0	±	+++	++++	0	+	+++	++++	++++	++++	++++	+++
(4) IRS 66:anti-C3H RBC	0	±	++++	++++	±	+	++++	++++	+++	++++	++++	+++
(5) IRS 47:anti-AKR liver	0	0	±	+++	0	+	+++	+++	+	++++	++++	+++
(6) IRS 50:anti-AKR kidney	0	0	+++	++++	0	0	+++	++++	0	+	++++	+++
(7) IRS 52:anti-AKR brain	0	0	0	+++	0	++	++++	++++	0	+	++++	+++
(8) IRS 57:anti-C3H thy+n+s	0	0	0	+++	0	0	+	+++	0	0	++++	+++
(9) IRS 45:anti-C3H spleen	0	0	0	+++	0	0	++++	++++	0	+	++++	+++
(10) IRS 46:anti-C3H thy + n	0	0	0	+++	+	+	++++	++++	0	0	++++	+++
(11) IRS 58:anti-Lym AKRL1	0	0	0	+	0	0	0	++	0	0	+	+++
(12) IRS 35:anti-Lym AKR17	0	0	0	++	0	0	0	++	0	0	++	++
(13) IRS 63:anti-Lym A2	0	0	0	+++	0	0	0	++	0	±	++	+++
(14) IRS 60:anti-Lym A1	0	0	0	+++	0	0	+	++	0	++	++++	+++
(15) IRS 49:anti-Lym 6C3HED	0	0	0	+++	0	0	+	+++	0	++	++++	+++
(16) IRS 62:anti-Lym E9514	0	0	+++	++++	0	0	+	++++	0	+++	++++	+++

IRS = immune rabbit serum. The test serums were first heated at 65° for 20 minutes, then diluted 1:2.5 to 1:160 with BGR, and added to thr tubes containing respectively the three types of lymphoma cells and complement. The mixtures were next incubated 2 hours at 37°. The mixtur containing 6C3HED and AKRL1 cells were centrifuged at 600 R.P.M. for 5 minutes with resuspension of the sedimented cells in BGR immediate prior to implantation—this to limit contact between the cells and serums. The resuspended cells from each mixture were implanted in two susce tible mice (C3H mice for 6C3HED and E9514 cells and AKR mice for AKRL1 cells). Each mixture containing E9514 cells was implanted direc into two susceptible C3H mice.

thy + n + s = thymus plus node plus spleen.

Growth of the 6C3HED cells was recorded according to the following scale:—

++++, palpable growths approximately 1.0 cm. in diameter appearing in both test mice by the 10th day, enlarging progressively thereaft

+++, growths approximately 1.0 cm. in diameter appearing in both test mice by the 12th day.

++, growths approximately 1.0 cm. in diameter appearing in both test mice by the 14th day.

+, growths approximately 1.0 cm. in diameter appearing in both test mice by the 16th day.

±, growth approximately 1.0 cm. in diameter appearing in one mouse by the 16th day.

0, no growth during observation period (16 days in this experiment).

Growths from implantation of the control mixtures containing E9514 cells appeared approximately 4 days later than those from correspondi mixtures containing 6 C3HED cells, while the AKRL1 cells in the control mixtures gave rise to tumors 1.0 cm. across on the 8th day. Arbitra scale adjusted accordingly. In all instances the tumors enlarged progressively throughout the observation period.

cells, while the other serums fell more or less regularly in between these extremes. In general, it will be noted, the immune serums prepared with the several types of lymphoma cells as antigens, and those made with cells from the lymphatic organs of mice, were more powerful than the serums prepared with antigens composed of mouse embryo (IRS 54), erythrocytes (IRS 66), liver (IRS 47), kidney (IRS 50), and brain (IRS 52) respectively, while that prepared with muscle as antigen (IRS 53) was devoid of effect. More will be said further on about the specificity of the antibodies responsible for the *in vitro* effects and about the susceptibility of lymphoma cells of different types.

The findings given in Chart 1 and Table I are representative of our experience as a whole. All told, 36 pooled immune serums—somewhat more than half prepared with antigens made from mouse lymphoma cells and the rest with normal mouse tissues as antigens—were tested for ability to kill lymphoma cells of several sorts *in vitro*. Regularly the immune serums prepared with lymphoma cells as antigens, and those made with cells from non-neoplastic lymphatic organs of mice (thymus plus lymph nodes, occasionally spleens), proved potent; and in general they were more potent than immune serums prepared with antigens made from mouse cells of other sorts, notably mouse erythrocytes or suspensions of cells from mouse brain, kidney, liver, or whole embryos. The findings bear out and extend observations previously made by Dulaney and Arnesen (16) and by Werder, Kirschbaum, MacDowell, and Syverton (17) on the effects of rabbit antiserums on leukemic cells *in vitro*. Furthermore, the fact that the immune serums of the present work were prepared in a standardized way, as already described, gives added weight to the possibility that the differences in potency might be meaningful. Absorption tests, now to be described, provide further evidence on this point.

Evidence That Antibodies of More Than One Sort Are Responsible for the in Vitro Effects: Results of Tests with Absorbed Sera

In the experiment of Chart 2, an unabsorbed specimen of IRS 37:Anti-mouse liver, in final dilutions of 1:40, 1:80, and 1:160, rendered non-viable all the AKRL1 and AKR17 lymphoma cells which were brought into contact with it in the presence of complement. When absorbed with mouse liver, however, the serum had no effect on either type of lymphoma cells, and the same proved true of a specimen of this serum that had been absorbed with Lymphoma AKRL1 cells. Two anti-lymphoma immune serums—IRS 36 and IRS 35, prepared respectively with AKRL1 and AKR17 lymphoma cells as antigens—when used in the unabsorbed state and tested in dilutions 1:40, 1:80, and 1:160, likewise killed all the AKRL1 and AKR17 cells in the test mixtures. Absorption of these two serums with the preparation of mouse liver cells did not alter their ability to kill the two types of lymphoma cells, though absorption with AKRL1 cells rendered the serums innocuous, as the chart shows.

The results of more extensive tests of similar sort are shown in Tables II, III, and IV.

Considered with the findings of the preceding section, the results of the absorption tests make it plain that antibodies of at least two sorts are responsible for the *in vitro* effects: (*a*) antibodies that appear in the blood of rabbits injected with cells from any one of a variety of non-neoplastic mouse tissues (*e.g.*, liver, kidney, brain, embryo, erythrocytes), which are readily absorbed from the immune serums by mouse liver

CHART 2. Tests for viability of Lymphoma AKRL1 cells exposed *in vitro* in the presence of complement to unabsorbed and absorbed specimens of various immune serums.

In vitro mixtures	Outcome of tests for viability 0.5 cc. of test mixtures implanted in each of two AKR test mice (a, b, etc.) Final dilutions of rabbit serums 1:40 / 1:80 / 1:160
Lymphoma AKRL1 cells (3 million/cc.) and guinea pig serum (1:5) plus an equal part of	Days following Implantations: 8 10 12 / 8 10 12 / 8 10 12
1) Normal rabbit serum, 1:20	a) b) / e) f) / k) N, l)
	0 2 cm.
2) IRS 37: Anti-AKR mouse liver, 1:20 - unabsorbed	a) N N N, b) N N N / e) N N N, f) N N N / k) N N N, l) N N N
3) " " " - absorbed with AKR mouse liver cells	a) b) / g) h) / k) l)
4) " " " - absorbed with AKRL1 lymphoma cells	a) b) / g) h) / k) l)
5) IRS 36: Anti-Lymphoma AKRL1, 1:20 - unabsorbed	c) N N N, d) N N N / g) N N N, h) N N N / m) N N N, n) N N N
6) " " " - absorbed with AKR mouse liver cells	c) N N N, d) N N N / g) N N N, h) N N N / m) N N N, n) N N N
7) " " " - absorbed with AKRL1 lymphoma cells	c) d) / i) j) / m) n)
8) IRS 35: Anti-Lymphoma AKR17, 1:20 - unabsorbed	c) N N N, d) N N N / i) N N N, j) N N N / m) N N N, n) N N N
9) " " " - absorbed with AKR mouse liver cells	e) N N N, f) N N N / i) N N N, j) N N N / o) N N N, p) N N N
10) " " " - absorbed with AKRL1 lymphoma cells	e) f) / i) j) / o) p)

The rabbit serums were heated in the undiluted state at 65°C. for 20 minutes immediately before use.

For absorption, approximately 25 million lymphoma cells, twice-washed in Ringer's solution, and approximately twice as many liver cells prepared in the same way from 3 months old AKR mice were used per milliliter of serum 1:20. The absorption mixtures were incubated at 37°C. for 1 hour, spun at 4,000 R.P.M. for 20 minutes, and the supernatant liquids diluted as shown with BGR.

The test mixtures were held one hour at 37°C. prior to implantation.

Concurrent tests with the same unabsorbed and absorbed serum specimens in mixture with Lymphoma AKR17 cells yielded identical results.

TABLE II

Tests for Viability of Lymphoma AKRL1 Cells Exposed in Vitro in the Presence of Complement to Unabsorbed and Absorbed Specimens of Various Immune Serums

In vitro mixtures	Outcome of tests for viability			
Lymphoma AKRL1 Cells (3 million/cc.) and guinea pig serum (1:5) plus an equal volume of:	Final dilutions of serums 1:20	1:40	1:80	1:160
(1) Normal rabbit serum, 1:10	++++	++++	++++	++++
(2) IRS 37:anti-AKR mouse liver, 1:10—unabsorbed	0	0	0	+++
(3) " " " " " " " —absorbed with AKR kidney cells	++++	++++	++++	++++
(4) " " " " " " " —absorbed with AKR liver cells	++++	++++	++++	++++
(5) " " " " " " " —absorbed with C3H spleen cells	++++	++++	++++	++++
(6) " " " " " " " —absorbed with C3H thymus-node cells	++++	++++	++++	++++
(7) " " " " " " " —absorbed with AKRL1 cells	++++	++++	++++	++++
(8) IRS 42:anti-C3H mouse spleen, 1:10—unabsorbed	0	0	0	++
(9) " " " " " " " —absorbed with AKR kidney cells	++++	++++	++++	++++
(10) " " " " " " " —absorbed with AKR liver cells	+++	+++	++++	++++
(11) " " " " " " " —absorbed with C3H spleen cells	++++	++++	++++	++++
(12) " " " " " " " —absorbed with C3H thymus-node cells	++++	++++	++++	++++
(13) " " " " " " " —absorbed with AKRL1 cells	++++	++++	++++	++++
(14) IRS 38:anti-AKR mouse spleen-thymus-node, 1:10—unabsorbed	0	0	0	0
(15) " " " " " " " " " —absorbed with AKR kidney cells	0	0	0	+
(16) " " " " " " " " " —absorbed with AKR liver cells	0	0	+	+++
(17) " " " " " " " " " —absorbed with C3H spleen cells	++	++	++	++++
(18) " " " " " " " " " —absorbed with C3H thymus-node cells	+	+	+++	++++
(19) IRS 38: " " " " " " " —absorbed with AKRL1 cells	++++	++++	++++	++++
(20) IRS 31:anti-Lymphoma 6C3HED, 1:10—unabsorbed	0	0	0	0
(21) " " " " " " —absorbed with AKR kidney cells	0	0	±	+
(22) " " " " " " —absorbed with AKR liver cells	0	0	0	+
(23) " " " " " " —absorbed with C3H spleen cells	++	++	+++	++++
(24) " " " " " " —absorbed with C3H thymus-node cells	++++	++++	++++	++++
(25) " " " " " " —absorbed with AKRL1 cells	++++	++++	++++	++++
(26) IRS 34:anti-Lymphoma AKRL1, 1:10—unabsorbed	0	0	0	++
(27) " " " " " " —absorbed with AKR kidney cells	0	0	0	++
(28) " " " " " " —absorbed with AKR liver cells	0	0	0	++
(29) " " " " " " —absorbed with C3H spleen cells	+	+	+++	++++
(30) " " " " " " —absorbed with C3H thymus-node cells	++++	++++	++++	++++
(31) " " " " " " —absorbed with AKRL1 cells	++++	++++	++++	++++
(32) IRS 43:anti-AKR mouse thymus-node, 1:10—unabsorbed	0	0	0	0
(33) " " " " " " " " —absorbed with AKR kidney cells	0	0	0	0
(34) " " " " " " " " —absorbed with AKR liver cells	0	0	0	0
(35) " " " " " " " " —absorbed with C3H spleen cells	0	0	+	++
(36) " " " " " " " " —absorbed with C3H thymus-node cells	+++	+++	++++	++++
(37) " " " " " " " " —absorbed with AKRL1 cells	++++	++++	++++	++++

Rabbit sera heated at 65°C. for 20 minutes immediately before use. The absorption mixtures contained respectively 30 million lymphoma cells, 25 million thymus-node cells, and approximately twice as many cells from the other organs per cubic centimeter. They were incubated at 37°C. for 1 hour, spun at 4000 R.P.M. for 20 minutes, and the supernatant liquids diluted as shown. For the viability studies, the test mixtures were held 1 hour at 37°C. prior to implantation of 0.5 cc. of each mixture into two test mice.

Outcome recorded as in scale of Table I.

TABLE III

Tests for Viability of Lymphoma AKRL1 and 6C3HED Cells Exposed in Vitro in the Presence of Complement to Unabsorbed and Absorbed Specimens of Various Immune Serums

In vitro mixtures	Outcome of tests for viability			
	AKRL1 cells		6C3HED cells	
Lymphoma cells (3 million/cc.) and guinea pig serum (1:5) plus an equal part of:	Final dilutions of serums 1:40	1:160	1:40	1:160
(1) Normal rabbit serum, 1:20	++++	++++	++++	++++
(2) IRS 38:anti-AKR mouse thymus-node-spleen, 1:20—unabsorbed	0	0	0	0
(3) " " " " " " " " " —absorbed with AKR spleen cells	+	+++	±	++
(4) " " " " " " " " " —absorbed with AKR thymus-node cells	++++	++++	++++	++++
(5) IRS 38: " " " " " " " " —absorbed with AKRL1 cells	++++	++++	++++	++++
(6) " " " " " " " " " —absorbed with 6C3HED cells	++++	++++	++++	++++
(7) IRS 27:anti-Lymphoma AKR 17, 1:20—unabsorbed	0	0	0	0
(8) " " " " " " " " —absorbed with AKR spleen cells	+	+	0	0
(9) " " " " " " " " —absorbed with AKR thymus-node cells	++++	++++	++++	++++
(10) " " " " " " " " —absorbed with AKRL1 cells	++++	++++	++++	++++
(11) " " " " " " " " —absorbed with 6C3HED cells	++++	++++	++++	++++
(12) IRS 31:anti-Lymphoma 6C3HED, 1:20—unabsorbed	0	0	0	0
(13) " " " " " " " —absorbed with AKR spleen cells	0	++	0	0
(14) " " " " " " " —absorbed with AKR thymus-node cells	++++	++++	++++	++++
(15) " " " " " " " —absorbed with AKRL1 cells	++++	++++	++++	++++
(16) " " " " " " " —absorbed with 6C3HED cells	++++	++++	++++	++++
(17) IRS 30:anti-Lymphoma AKR 13, 1:20—unabsorbed	0	0	0	+
(18) " " " " " " " —absorbed with AKR spleen cells	++++	++++	0	++
(19) " " " " " " " —absorbed with AKR thymus-node cells	++++	++++	++++	++++
(20) " " " " " " " —absorbed with AKRL1 cells	++++	++++	++++	++++
(21) " " " " " " " —absorbed with 6C3HED cells	++++	++++	++++	++++
(22) IRS 34:anti-Lymphoma AKRL1, 1:20—unabsorbed	0	0	0	++
(23) " " " " " " —absorbed with AKR spleen cells	++++	++++	++++	++++
(24) " " " " " " —absorbed with AKR thymus-node cells	++++	++++	++++	++++
(25) " " " " " " —absorbed with AKRL1 cells	++++	++++	++++	++++
(26) " " " " " " —absorbed with 6C3HED cells	++++	++++	++++	++++

Rabbit sera heated at 65°C. for 20 minutes immediately before use. The absorption mixtures contained respectively 50 million spleen cells, 100 million thymus-node cells, and 25 million lymphoma cells per cc. They were incubated 1 hour at 37 C., then centrifuged. For the viability studies the mixtures were held 1 hour at 37°C., then implanted as such, 0.5 cc. into each of two susceptible mice (AKR and C3H, respectively).

Outcome recorded as in scale of Table I.

TABLE IV

Tests for Viability of Lymphoma 6C3HED and AKRL1 Cells Exposed in Vitro in the Presence of Complement to Unabsorbed and Absorbed Specimens of Various Immune Serums

In vitro mixtures	Outcome of tests for viability			
	Final dilutions of serums			
	1:20	1:40	1:80	1:160
Lymphoma 6C3HED cells (3 million/cc.) and guinea pig serum (1:5) plus an equal part of:				
(1) Normal rabbit serum, 1:10—unabsorbed	++++	++++	++++	++++
(2) " " " " —absorbed with Swiss RBC	++++	++++	++++	++++
(3) " " " " —absorbed with Swiss liver cells	++++	++++	++++	++++
(4) " " " " —absorbed with Swiss kidney cells	++++	++++	++++	++++
(5) " " " " —absorbed with Swiss muscle cells	++++	++++	++++	++++
(6) IRS 48:anti-AKR-RBC, 1:10—unabsorbed	0	0	+	++++
(7) " " " " " " —absorbed with Swiss RBC	++++	++++	++++	++++
(8) " " " " " " —absorbed with Swiss liver cells	++++	++++	++++	++++
(9) " " " " " " —absorbed with Swiss kidney cells	++++	++++	++++	++++
(10) " " " " " " —absorbed with Swiss muscle cells	0	++	+++	++++
(11) IRS 58:anti-Lymphoma AKRL1, 1:10—unabsorbed	0	0	±	++++
(12) " " " " " " —absorbed with Swiss RBC	0	0	+++	++++
(13) " " " " " " —absorbed with Swiss liver cells	0	0	+++	++++
(14) " " " " " " —absorbed with Swiss kidney cells	0	0	+	++++
(15) " " " " " " —absorbed with Swiss muscle cells	0	0	+	++++
Lymphoma AKRL1 cells (3 million/cc.) and guinea pig serum (1:5) plus an equal part of:				
(16) Normal rabbit serum, 1:10	++++	++++	++++	++++
(17) IRS 59:anti-AKR kidney, 1:10—unabsorbed	0	0	0	++++
(18) " " " " " " —absorbed with AKR RBC	++++	++++	++++	++++
(19) " " " " " " —absorbed with sheep RBC	0	0	0	++++
(20) IRS 47:anti-AKR liver, 1:10—unabsorbed	0	0	++++	++++
(21) " " " " " " —absorbed with AKR RBC	++++	++++	++++	++++
(22) IRS 48:anti-AKR RBC, 1:10—unabsorbed	0	0	0	+
(23) " " " " " " —absorbed with AKR RBC	++++	++++	++++	++++
(24) IRS 43:anti-AKR thymus-lymph nodes, 1:10—unabsorbed	0	0	0	0
(25) " " " " " " " " —absorbed with AKR RBC	0	0	0	0
(26) " " " " " " " " —absorbed with sheep RBC	0	0	0	0
(27) IRS 55:anti-Lymphoma AKRL1, 1:10—unabsorbed	0	0	0	0
(28) " " " " " " —absorbed with AKR RBC	0	0	0	0
(29) " " " " " " —absorbed with AKR kidney cells	0	0	0	0
(30) " " " " " " —absorbed with sheep RBC	0	0	0	0

The rabbit sera were heated in the undiluted state at 65°C. for 20 minutes immediately before use.

In the tests with Lymphoma 6C3HED cells, the absorption mixtures contained approximately 0.6 cc. of packed cells per cubic centimeter of serum 1:10. They were incubated at 37°C. for 1 hour, spun at 3,000 R.P.M. for 20 minutes, and the supernatant liquids diluted as shown. For the viability studies the test mixtures were held 2 hours at 37°C. and spun at 600 R.P.M. for 8 minutes, the supernatant liquids being discarded and the cells resuspended in BGR prior to implantation into two susceptible C3H mice.

In the tests with AKRL1 cells the absorption mixtures contained approximately 0.2 of packed mouse erythrocytes or 0.4 cc. of packed sheep erythrocytes, or 0.4 cc. of packed kidney cells per cc. of serum, 1:10. Rest of procedure as above except that susceptible AKR mice were used in the tests for viability.

Outcome recorded as in scale of Table I.

or kidney cells or mouse erythrocytes; and (*b*) antibodies that appear in the blood of rabbits injected with neoplastic and non-neoplastic lymphoid tissues of mice (*e.g.* lymphomas, normal thymus + normal lymph nodes), which are not absorbed from the serums by mouse liver cells, mouse kidney cells, or mouse erythrocytes but are readily absorbed by mouse lymphoma cells and to some extent also by cells procured from the thymuses and lymph nodes of normal mice. The distinction has importance for an understanding of the *in vivo* results, which will be given next.

Tests for Effects of the Immune Serums on Mouse Lymphoma Cells in Vivo

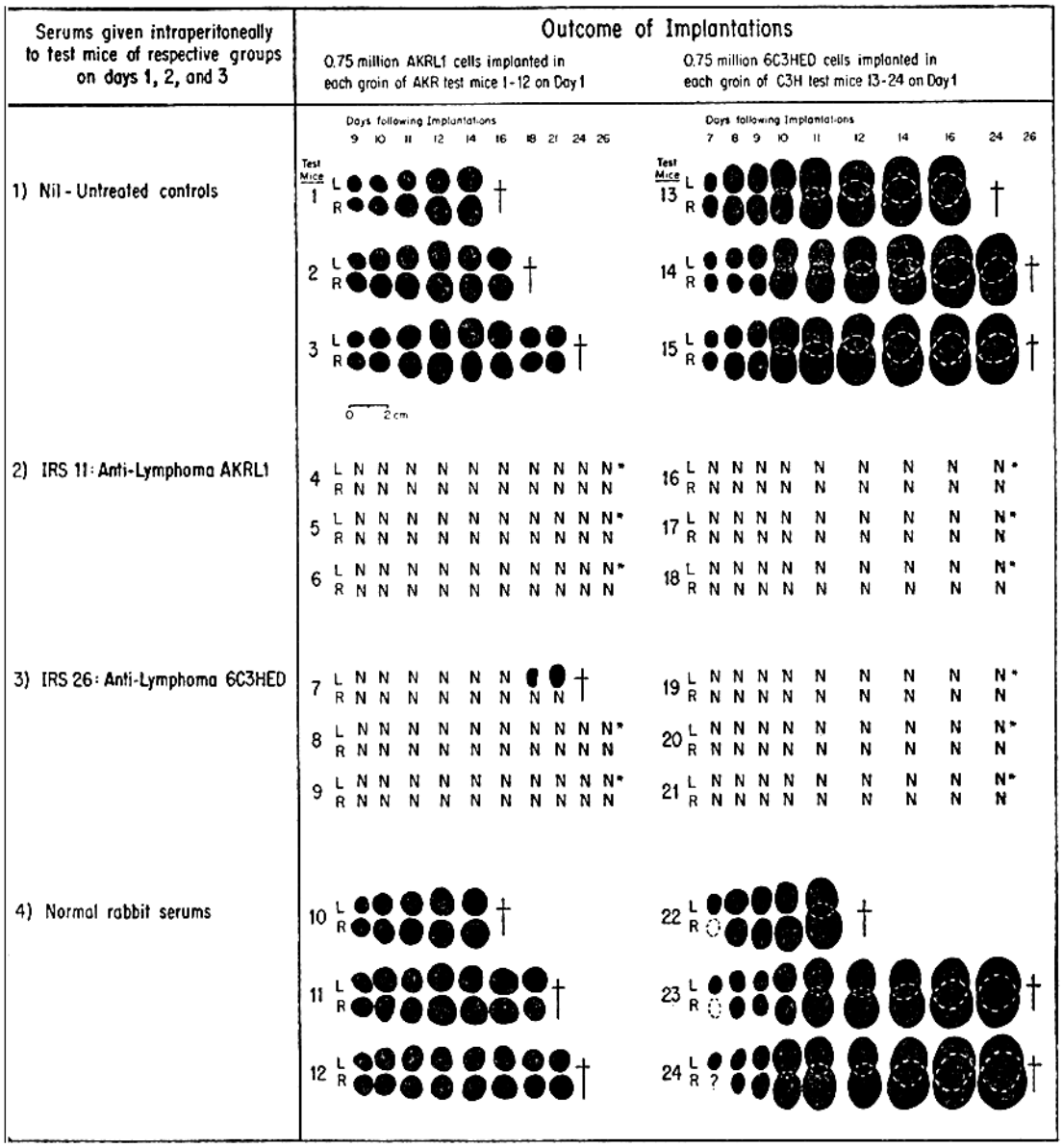
In the experiment of Chart 3 two anti-lymphoma serums and a specimen of normal rabbit serum were injected intraperitoneally into groups of AKR and C3H mice that had been implanted subcutaneously with AKRL1 and 6C3HED lymphoma cells respectively. The two immune serums inhibited growth of the lymphoma cells of both types either completely or almost completely, while the normal rabbit serum had no effect on them.

Table V shows the results of a larger experiment in which 14 immune serums were tested for ability to inhibit growth of Lymphoma AKRL1 cells *in vivo*. The four anti-lymphoma immune serums were all markedly inhibitory, and two of the three immune serums made with non-neoplastic lymphoid tissues as antigens were also effective, though less so than the anti-lymphoma serums, while the third was toxic and brought about death before the 5th day of all three of the mice into which it was injected. None of the 6 immune serums prepared with antigens made from normal mouse organs —brain, kidney, liver, embryo—inhibited growth of the lymphoma cells, however, and the same was true of an immune serum (IRS 59) for which cells from a mammary carcinoma of C3H mice had been employed as antigen.

In a number of additional experiments immune serums prepared in rabbits with antigens composed of cells from various non-lymphoid organs and tissues of mice all regularly failed to inhibit lymphoma cells of four types (6C3HED, E9514, AKRL1, AKR17) *in vivo*, while immune serums made with antigens containing cells of normal lymphoid organs of mice—notably thymus and lymph nodes—usually exhibited some inhibitory effect. In contrast, 21 pools of anti-lymphoma immune rabbit serums, made with mouse lymphoma cells of one or another of the seven types as antigens, all proved notably inhibitory for the 4 types of lymphoma cells *in vivo* when given within a few days following implantation. The anti-lymphoma serums varied considerably in potency, however, and they often proved more effective against lymphoma cells of the type employed as antigen than against other types of lymphoma cells.

The inhibition *in vivo* was influenced by other factors as well. For example, when relatively small numbers of lymphoma cells (*e.g.* 5,000 to 50,000) were implanted in each groin of susceptible mice, and 1.0 cc. of any one of the various anti-lymphoma immune serums was given intraperitoneally on the same day, the lymphoma cells nearly always failed to grow; but when larger numbers of cells were implanted (*e.g.*, 300,000 or more), or if the serum injections were delayed 3 days or more, inhibition was often incomplete even when larger injections of immune serum were given repeatedly. Furthermore, the lymphoma cells varied in susceptibility, those of Lymphoma 6C3HED and E9514 being more or less readily inhibited *in vivo* by a variety of anti-lymphoma immune serums, while those of AKRL1 were influenced much less readily, as will be brought out further on.

CHART 3. Tests for effects *in vivo* on Lymphoma AKRL1 and 6C3HED cells of immune serums prepared in rabbits with lymphoma cells of the two types as antigens.



L, R, = left and right groins, respectively.

The C3H mice were given 2.0 cc. of the respective serums and the AKR mice 1.0 cc. of the respective serums plus 1.0 cc. of Ringer's solution on days 1, 2, and 3.

The serums were heated at 65°C. for 20 minutes immediately before use.

* Remained lively and devoid of tumors throughout observation period (42 days).

TABLE V

Tests for Effects in Vivo of Immune Serums Prepared in Rabbits with Lymphoma Cells and Normal Mouse Tissues as Antigens

Serums given intraperitoneally to mice of respective groups on days 1, 2, and 3	Outcome of implantations: 300,000 AKRL1 cells implanted on day 1 in left and right groins of 3 AKR test mice (*a*), (*b*) (*c*) in each of 16 groups		
	Test mice (*a*)	(*b*)	(*c*)
(1) Nil-untreated controls	++++	++++	++++
(2) Normal rabbit serum	++++	++++	++++
(3) IRS 58:anti-Lymphoma AKRL1	0	d5	d15
(4) IRS 55:anti-Lymphoma AKRL1	0	++	++
(5) IRS 63:anti-Lymphoma A2	0	0	++
(6) IRS 60:anti-Lymphoma A1	0	0	++++
(7) IRS 57:anti-C3H thymus + node + spleen	d2	d4	d5
(8) IRS 45:anti-C3H spleen	0	++	++
(9) IRS 43:anti-AKR thymus + node	0	++	++++
(10) IRS 53:anti-AKR brain	++++	++++	++++
(11) IRS 50:anti-AKR kidney	++++	++++	++++
(12) IRS 47:anti-AKR liver	++++	++++	++++
(13) IRS 51:anti-AKR liver	++++	++++	++++
(14) IRS 44:anti-C3H kidney	++++	++++	++++
(15) IRS 54:anti-C3H embryo	++++	++++	++++
(16) IRS 59:anti-C3H mammary carcinoma	++++	++++	d10

The serums were heated at 65°C. for 20 minutes immediately before use. Serums IRS 55 and IRS 50 were given in amounts of 0.25 cc. to each test mouse on the 3 successive days. The rest were given in amounts of 0.5 cc., the total volume in each instance being made up to 2.0 cc. with Ringer's solution.

++++, died with large tumors in groins and signs of visceral lymphomatosis before day 20.

++ , developed lymphoma in only one groin; survived 20 days.

0 , groins devoid of palpable tumors, no sign of visceral lymphomatosis during observation period (20 days).

d2, d5, etc. = died on day indicated by numeral. So, too, in the tables that follow.

TABLE VI

Comparative Effects of Unabsorbed and Absorbed Anti-Lymphoma Immune Serums on AKRL1 Cells in Vivo and in Vitro

Test serums	Growth of AKRL1 cells in treated mice. 50,000 AKRL1 cells implanted in each groin of three test mice ((*a*), (*b*), (*c*)) in each experimental group: 1.0 cc. of test serums given intraperitoneally to each mouse on days 1, 2, 3.			Growth of AKRL1 cells exposed *in vitro* to the various immune serums plus normal guinea pig serum 1:10 (complement).			
	Test mice (*a*)	(*b*)	(*c*)	Final dilutions of serums: 1:8	1:16	1:32	1:64
(1) Normal rabbit serum	++++	++++	++++	++++	++++	++++	++++
(2) " " " —absorbed with Swiss RBC	++++	++++	++++		Not tested		
(3) " " " —absorbed with AKRL1 cells	++++	++++	++++		" "		
(4) " " " —absorbed with 6C3HED cells	++++	++++	++++		" "		
(5) IRS 58:anti-AKRL1—unabsorbed	0	0	d2	0	0	0	0
(6) " " " " —absorbed with Swiss RBC	0	d2	d3	0	0	0	0
(7) " " " " —absorbed with AKRL1 cells	+++	++++	++++	0	0	0	+
(8) " " " " —absorbed with 6C3HED cells	++++	d3	d8	0	0	+++	+++
(9) IRS 61:anti-6C3HED—unabsorbed	0	0	++++	0	0	0	0
(10) " " " " —absorbed with Swiss RBC	0	0	++++	0	0	0	0
(11) " " " " —absorbed with AKRL1 cells	0	+++	++++	+++	+++	+++	+++
(12) " " " " —absorbed with 6C3HED cells	+++	++++	++++	+++	+++	+++	+++

The rabbit sera were heated at 65°C. for 20 minutes before use. Approximately 1.5 cc. of packed erythrocytes and 6C3HED cells, and approximately 1 cc. of packed AKRL1 cells were used per milliliter of serum in the absorption procedures, which were carried out in two steps, each 1 hour at 37°C.

For the *in vitro* tests, mixtures containing 5 million AKRL1 cells and 0.1 cc. guinea pig serum in 1 cc. of the respective serum dilutions were incubated *in vitro* for 16 hours at 37°C., following which they were centrifuged, with resuspension of the cells in the same volume of BGR. 0.5 cc. of each suspension was implanted at different sites in two AKR test mice. The findings are recorded as in Table I.

For the *in vivo* tests, the findings are recorded as follows:—

++++, lymphomas in both groins reaching 1.0 cm. across on day 9, enlarging progressively thereafter until death of the host.

+++ , lymphomas in both groins reaching 1.0 cm. across on day 11, enlarging progressively thereafter.

+ , lymphoma in one groin; survived 20 days.

0 , negative throughout observation period (40 days).

Evidence That an Antibody Which Reacts with Neoplastic and Non-Neoplastic Lymphocytic Cells Is Responsible for the Effects of Anti-Lymphoma Serums in Vitro and in Vivo

Are the *in vitro* and *in vivo* effects of anti-lymphoma immune serums referable to specific antibodies which react directly with the lymphoma cells both in the test tube and in the living animal? A number of observations provide evidence on the point. The findings summarized in Table VI, for example, show clearly that absorption of two anti-lymphoma immune serums with 6C3HED lymphoma cells greatly diminished the ability of these two serums to act upon AKRL1 cells *in vivo* and *in vitro*, and similar results were got when a smaller quantity of AKRL1 cells was employed for absorption. Absorption of the serums with erythrocytes from Swiss mice did not alter their capacity to act upon the AKRL1 cells either *in vivo* or *in vitro*.[1]

In an experiment similar to that of Table VI the capacity of IRS 61:Anti-Lymphoma 6C3HED to act upon 6C3HED cells *in vitro* and *in vivo* was diminished following absorption with 6C3HED cells, and in a further experiment the capacity of two anti-AKRL1 immune serums (IRS 55 and IRS 33) to inhibit growth of implanted AKRL1 cells *in vivo* was diminished following absorption with AKRL1 cells but remained undiminished following absorption with mouse liver cells.

Survival of Lymphoma Cells during Many Hours' Exposure in Vitro to Anti-Lymphoma Serums in the Absence of Complement

The experiment summarized in Table I shows clearly that Lymphoma AKRL1 cells remained viable during exposure for 1 hour at 37°C. to immune serums of three types when the hemolytic complement naturally present in the immune serums had been inactivated by means of heat. In the experiment of Table VII, *q.v.*, Lymphoma AKRL1 cells also remained viable following exposure *in vitro* during 16 hours at 37°C. to each of two "heat-inactivated" anti-lymphoma immune serums admixed with heated guinea pig serum. In two similar experiments, not to be given in detail, AKRL1 cells survived 24 hours' exposure to several immune serums admixed with heated normal rabbit serum, and in a further experiment 6C3HED cells survived 5 hours' exposure—the longest period tested—to two anti-lymphoma serums to which heated guinea pig serum had been added. It is interesting indeed that lymphoma cells should have sur-

[1] It is interesting to note in passing that the unabsorbed specimen of IRS 58, and also the specimens absorbed with Swiss erythrocytes and 6C3HED lymphoma cells, proved quite toxic. All the mice receiving these specimens became obviously sick during the injection period, manifesting diarrhea, sluggishness, ruffled fur, and a loss of 2 to 4 gm. in weight, and three of them died. The test mice that received serum absorbed with AKRL1 cells, however, remained sleek and lively during the course of injections and for the week thereafter, becoming sick and dying only after the development of manifest lymphomatosis. The findings suggest that the AKRL1 cells alone absorbed an undefined "toxic" antibody. Further evidence that this "toxic" antibody reacts specifically with some constituent of AKR mouse cells was provided by an observation made repeatedly in other experiments, namely that AKR mice were notably more susceptible to the "toxic" effects of anti-AKR Lymphoma immune serums than were C3H mice. More will be said further on about the toxicity of the various immune serums.

vived under such circumstances and promptly begun to proliferate upon implantation in new hosts. For the results of the absorption experiments already given make it seem probable that the lymphoma cells had absorbed as much or nearly as much antibody as they could possibly absorb during the prolonged contact with the immune serums *in vitro*, though whether they had actually done so was not determined in the experiments cited. Since mouse serum is known to be devoid of one or more of the components of hemolytic complement (18), the animals implanted with the antibody-soaked lymphoma cells could hardly be expected to supply this. The findings suggest, however, that the mouse host somehow provides conditions that are essential for the effectiveness of the anti-lymphoma serums *in vivo* and that these conditions are different from those prevailing in the test tube.

Will Normal Guinea Pig Serum Enhance the Inhibitory Effects of Anti-Lymphoma Serums on Mouse Lymphoma Cells in Vivo?

A number of reasons can be cited for supposing that normal guinea pig serum might enhance the effects of immune serums on lymphoma cells *in vivo*. Firstly, it is a rich source of complement, as is well known, and this must be present if such immune serums are to act powerfully upon lymphoma cells *in vitro*, as we have here shown; so too in the case of the structural changes brought about in Brown-Pearce carcinoma cells by means of a specific antibody *in vitro* (19). Secondly, mouse serum lacks one or more of the known components of complement (18), as has already been mentioned. Thirdly, in a previous study normal guinea pig serum enhanced to some extent the effects of an anti-lymphoma immune serum on several types of AKR lymphoma cells *in vivo* (20). Hence in the next two experiments normal guinea pig serum was given along with various anti-lymphoma serums to learn whether it would enhance their effects on lymphoma cells in the living animal.

Chart 4 shows the results of the first test. The mixture containing IRS 36:Anti-AKRL1 and guinea pig serum completely inhibited growth of the Lymphoma AKRL1 cells, while that containing IRS 43:Anti-thymus-nodes and guinea pig serum had a marked though incomplete inhibitory effect. IRS 39:Anti-AKR kidney and IRS 37: Anti-AKR liver, both in mixture with normal guinea pig serum, were devoid of effect on the lymphoma cells *in vivo*.

A direct comparison was made in the experiment of Chart 5. IRS 11:Anti–Lymphoma AKRL1, when given alone on the first day to test mice that had been implanted with Lymphoma AKR17 cells, was only moderately inhibitory; when given together with normal guinea pig serum the inhibitory effect was considerably increased. This was not the case, however, with the other two anti-lymphoma serums employed in the test; both were as inhibitory when given alone as when given in mixture with normal guinea pig serum.

The findings just cited were representative of those obtained in several additional experiments. For in these too normal guinea pig serum regularly failed to render inhibitory immune serums that had been produced with normal mouse liver or kidney cells as antigens, and in the bulk of instances it did not notably enhance the inhibitory effects of anti-lymphoma immune serums. Occasionally, however, the addition of normal guinea pig serum to anti-lymphoma serums definitely enhanced their inhibitory effects *in vivo*, as was also noted in a previous study (20), and in several experiments

TABLE VII

Survival of AKRL1 Cells Held 16 Hours in Vitro in Contact with Anti-Lymphoma Immune Serums in the Absence of Complement

In vitro mixtures	Outcome of tests for viability							
	Mixtures containing unheated guinea pig serum, 1:10.				Mixtures containing heat-inactivated guinea pig serum, 1:10.			
Lymphoma AKRL1 cells (5 million/cc.) and unheated or heated guinea pig serum 1:10, plus an equal part of serum diluted as indicated	Final dilutions of serum							
	1:8	1:16	1:32	1:64	1:8	1:16	1:32	1:64
(1) Normal rabbit serum	++++	++++	++++	++++	++++	++++	++++	++++
(2) IRS 58: anti-Lymphoma AKRL1	0	0	0	0	+++	+++	0*	+++
(3) IRS 61: anti-Lymphoma 6C3HED	0	0	0	0	+++	+++	++++	+++

The anti-lymphoma immune serums were heated at 65°C. for 20 minutes immediately before use. Half of the guinea pig serum employed was heated at 56°C. for 30 minutes prior to use; subsidiary tests showed that it was devoid of hemolytic capacity for sheep cells sensitized with amboceptor.

Following incubation for 16 hours, the various *in vitro* mixtures were centrifuged at 600 R.P.M. for 5 minutes, with resuspension of the sedimented cells in the same volume of BGR. 0.5 cc. of each suspension was implanted at different sites in two AKR test mice.

The outcome of the viability tests was recorded as follows:—

++++, lymphomas in both implanted mice reaching 1.0 cm. across on day 9, enlarging progressively thereafter until death of the host.

+++ , lymphomas in both implanted mice reaching 1.0 cm. across on day 11, enlarging progressively thereafter until death of the host.

0 , negative throughout observation period (18 days).

0* , negative, anomalous result probably resulting from technical error.

TABLE VIII

Tests for Effects of Various Immune Serums on Established Lymphomas of Three Types in Vivo

Serums given intraperitoneally to mice of respective groups on days 6, 7, 8, 9, and 10.	Outcome of implantations: 5,000 lymphoma cells implanted in both groins of four susceptible mice ((*a*), (*b*), (*c*), (*d*)) in each experimental group on day 1											
	C3H mice with 6C3HED lymphomas				C3H mice with E9514 lymphomas				AKR mice with AKRL1 lymphomas			
	(*a*)	(*b*)	(*c*)	(*d*)	(*a*)	(*b*)	(*c*)	(*d*)	(*a*)	(*b*)	(*c*)	(*d*)
(1) Nil-untreated controls	+++	++++	++++	d9	+++	+++	+++	+++	++++	++++	++++	++++
(2) Normal rabbit serum	+++	+++	+++	++++	+++	+++	+++	d24	++++	++++	++++	++++
(3) IRS 59: anti-C3H mammary carcinoma	+++	+++	+++	+++	+++	+++	n22	n22	++++	++++	++++	++++
(4) IRS 62: Anti-Lymphoma E9514	0	0	0	0	0	0	0	0	++++	++++	++++	++++
(5) " 64: " " 6C3HED	0	0	0	++	0	0	++	+++	++++	++++	++++	k18
(6) " 61: " " "	0	0	++	+++	0	0	0	+++	0	++++	++++	h10
(7) " 58: " " AKRL1	0	0	+++	d6	0	0	++	h6	0	0	0*	d6
(8) " 60: " " A1	0	0	+++	h6	0	0	++	+++	0	+++	+++	+++
(9) " 63: " " A2	0	0	0	0	0	+++	+++	+++	++++	++++	++++	k18
(10) " 35: " " AKR17	0	0	++	+++	+++	+++	+++	+++	++++	++++	++++	++++

The serums were heated at 65°C. before use. 1.5 cc. of serum was given to each mouse on the 5 successive days, except with serums IRS 64, IRS 58, IRS 35, IRS 52 in which the amount was reduced to 1.0 cc. or 0.5 cc. on days 8, 9, and 10 because of manifest toxicity.

++++, died before day 20 with subcutaneous tumors and signs of lymphomatosis.

+++ , died before day 32 " " " " " " "

++ , died before day 44 " " " " " " "

0 , remained lively and devoid of palpable tumors or signs of lymphomatosis throughout observation period (120 days).

d9, etc., died on day indicated, not autopsied.

d24, died with ear infection and paralysis, day 24.

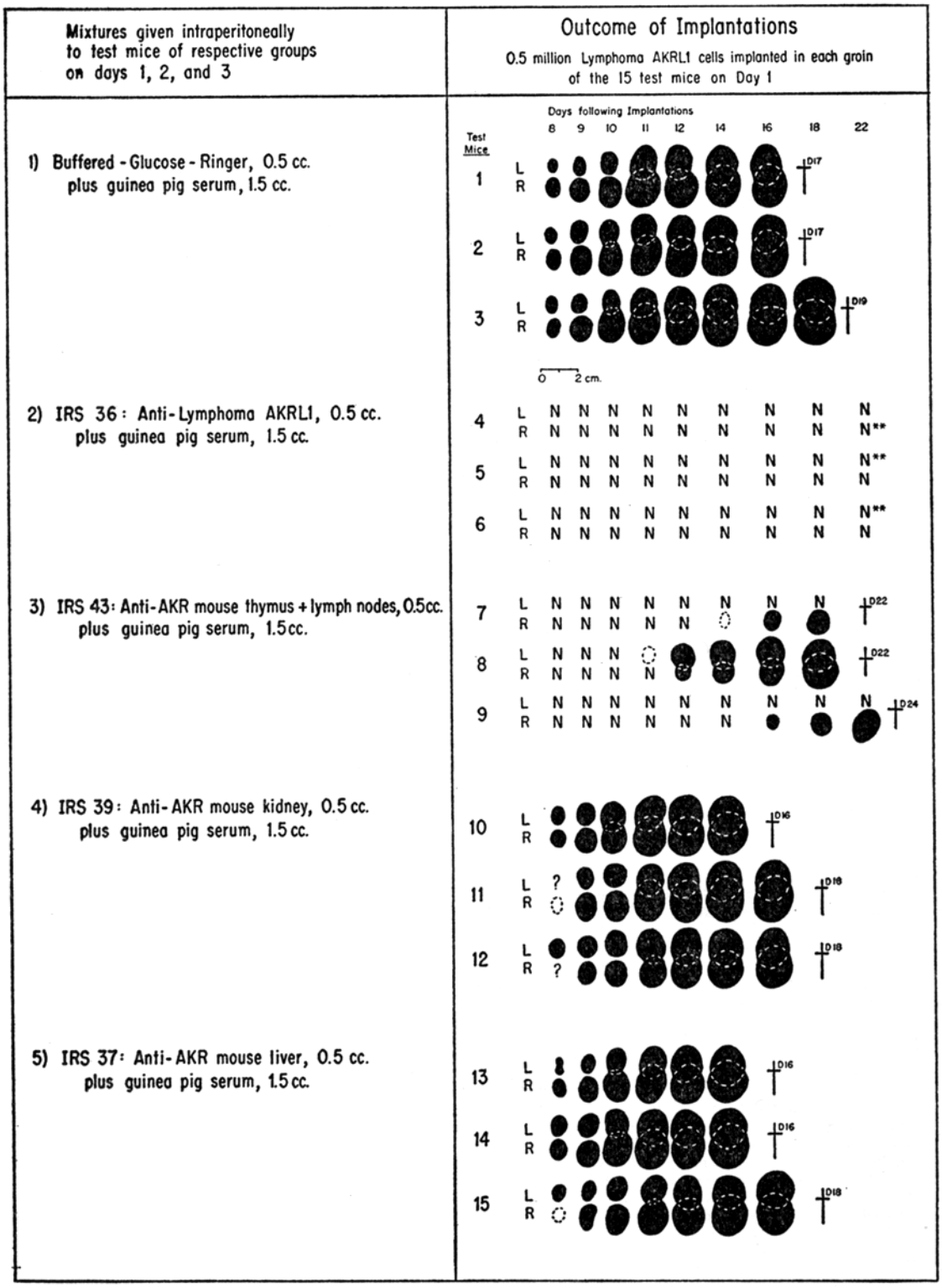
h6, died on day indicated—intraperitoneal hemorrhage resulting from injection, proved at autopsy.

k18, killed and discarded on 18th day.

n22, died with anasarca and other signs of nephrotoxic nephritis on day indicated.

0*, died on day 30—groins negative. No gross signs of lymphomatosis at autopsy.

CHART 4. Tests for effects *in vivo* on mouse Lymphoma AKRL1 cells of normal guinea pig serum given alone and in mixture with various immune serums.*

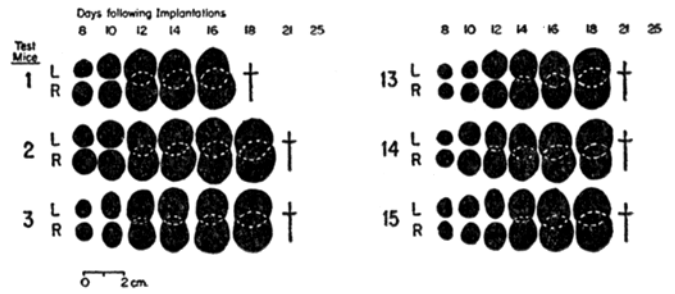
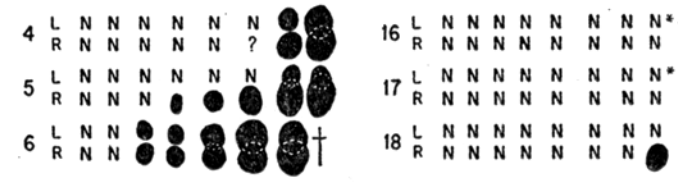
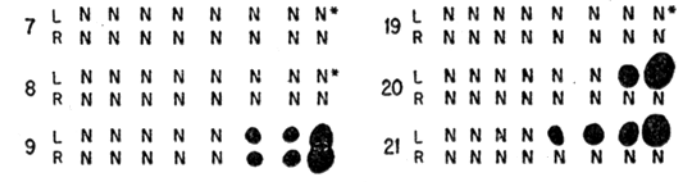
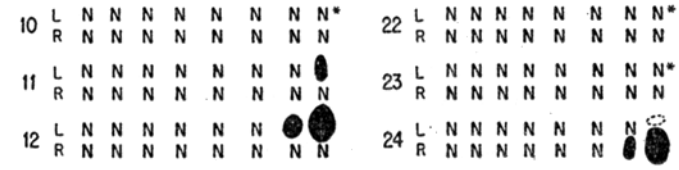


L,R = left and right groins, respectively. So, too, in the charts that follow.

* Three untreated control mice were also implanted. They developed tumors like those of the mice in groups 1, 4, and 5, and died on the 17th and 18th days.

** Tumor noted in right groin of Mouse 4 on the 30th day, with death on the 32nd day. Mice 5 and 6 remained devoid of tumors and were lively throughout the observation period (50 days).

CHART 5. Tests for effects *in vivo* on Lymphoma AKRL17 cells of immune serums given as such and mixed with normal guinea pig serum.

Serums given intraperitoneally to test mice of respective groups on days 1, 2, and 3	Outcome of Implantations 0.75 million AKRL17 lymphoma cells implanted in each groin of the 24 test mice on Day 1 a) Mice given 1.0 cc. rabbit serum plus 1.0 cc. Ringer's solution b) Mice given 1.0 cc. rabbit serum plus 1.0 cc. of guinea pig serum 2:1
1) Normal rabbit serum	
2) IRS 11A: Anti-Lymphoma AKRL1	
3) IRS 26: Anti-Lymphoma 6C3HED	
4) IRS 27: Anti-Lymphoma AKRL17	

* Remained negative throughout observation period (42 days).

IRS 35: Anti-Lymphoma AKR17 proved notably potent when given in combination with guinea pig serum but was largely devoid of effect when given alone.

Toxicity of the Immune Serums

As has already become apparent, a number of the immune serums used in the present work proved toxic for young adult mice when given repeatedly in doses of 0.5 or 1.0 cc. intraperitoneally. The serums varied widely in their ability to induce toxic manifestations, however, and their toxicity was in general not proportional to, and in some instances was wholly unrelated to, their ability to kill lymphoma cells *in vitro* and *in vivo*. Furthermore the toxic manifestations caused by different immune serums were quite diverse, and there were wide differences too in the susceptibility of various mice to the toxic effects, young mice in general succumbing much more readily than older weightier ones, while mice of certain breeds, notably AKR/JAX, tolerated the immune serums much less well than did mice of other breeds *e.g.*, C3H/JAX.

Numerous observations provided evidence that hemolysins acting in conjunction with complement were responsible for some of the toxic effects. For example, it was learned early in the course of the work that various immune serums could be largely "detoxified" by heating them at 56 or 65°C. for 20 minutes. This procedure virtually abolished the ability of the serums to induce a more or less transitory shock-like state (characterized by respiratory distress and lassitude, sometimes accompanied by hemoglobinuria) which often followed within an hour or 2 after the injection of the serum intraperitoneally; furthermore when normal guinea pig serum was added to a specimen of heated immune serum, its capacity to induce the shock-like state was restored, as many observations showed. But heating did not by any means completely detoxify the immune serums. Virtually all of these contained hemagglutinins as well as hemolysins, the titers varying from 1:40 to 1:320 or more in the numerous tests made. And anti-erythrocyte serums—produced with either AKR or C3H cells—proved by far the most toxic of the serums used in the present work. Thus, when heated specimens of the anti-erythrocyte serums were given daily in amounts of 0.25 cc. or less to young adult mice of several breeds, the animals almost regularly died 12 to 24 hours after the first or second injection, postmortem examination disclosing as a rule slight icterus together with marked congestion of the lungs and distention of the right side of the heart with dark liquid blood and a very greatly enlarged spleen that was almost black. Mouse erythrocytes from animals of various breeds readily absorbed the hemolysins and hemagglutinins from the various immune serums *in vitro*, as many experiments showed, and the absorbed serums were devoid of ability to induce the toxic manifestations just described. The findings to be given in the next two sections, however, show clearly that attempts to detoxify anti-lymphoma immune serums by means of absorption were not always successful.

Certain of the immune rabbit serums, notably those produced with mouse kidney and mouse brain as antigens, often gave rise to anasarca and other signs of "nephrotoxic" nephritis. These manifestations usually appeared several days after a course of injections had been given, and sometimes the animals survived a week or 2 with extreme ascites and subcutaneous edema, the kidneys being enlarged and pale and having proliferated glomeruli with greatly swollen endothelial and epithelial cells, as microscopic examinations showed. No systematic attempts were made to absorb from the immune serums the antibodies responsible for the nephrotoxic effects.

The toxicity of the anti-erythrocyte, anti-brain, and anti-kidney serums, as just described, has special significance; for these serums were all devoid of ability to inhibit growth of the several types of lymphoma cells *in vivo*, as has been shown. Some of the immune serums that

were effective *in vivo*—notably those produced with the lymphoma cells as antigens—also proved toxic. The toxicity of different anti-lymphoma serums varied widely, however, and absorption tests disclosed that in most instances this toxicity was referable to antibodies that reacted with mouse erythrocytes.

Attempts to Overcome "Established" Lymphoma Cells of Several Types with Anti-Lymphoma Serums

In experiments already given (Charts 3 to 5, Tables V and VI), eight anti-lymphoma serums, made by injecting the cells of five transplanted mouse lymphomas into rabbits, overcame lymphoma cells of several types *in vivo* when they were given on the day of implantation and on the two succeeding days as well. In the experiments referred to, however, the conditions were such as to favor the action of the immune serums; for surely these might be expected to overcome a relatively small number of recently implanted (and perhaps specially vulnerable) lymphoma cells more readily than they could overcome the much larger numbers of proliferating lymphoma cells known from microscopic studies to be present at the subcutaneously-implanted sites and in the viscera of susceptible mice several days after implantation. In the experiments now to be described, conditions were much less favorable for the immune serums; for these were given after the implanted lymphoma cells had had 5 or 6 days in which to establish themselves and proliferate.

Table VIII shows the results of attempts to influence established lymphomas of three types—6C3HED, E9514, and AKRL1—with a number of anti-lymphoma serums and with an anti-mammary carcinoma serum. The serums were given intraperitoneally on the 6th, 7th, 8th, 9th, and 10th days to C3H and AKR mice (four animals in each group, all weighing 24 gm. or more initially) that had been implanted in each groin with 5,000 lymphoma cells of one or another of the three types on the 1st day of the experiment. It can be seen that all the untreated control mice developed palpable growths and died with lymphomatosis except for one which died with diarrhea on the 9th day; so too with the mice given normal rabbit serum and those given the anti-mammary carcinoma serum (IRS 59), though one of the former was lost as a result of otitis following trauma and two of the latter died with signs of nephrotoxic nephritis on the 22nd day. The seven anti-lymphoma immune serums had quite different effects, as the table shows. Taken together they brought about a cure—*i.e.*, survival for 120 days without signs of lymphomatosis—in 19 of 26 treated mice in which Lymphoma 6C3HED cells had been implanted, in 14 of 27 treated mice that had been implanted with Lymphoma E9514 cells, and in 5 of the 27 treated mice implanted with AKRL1 cells.

Several of the immune serums had more or less specific effects. For example, IRS 62: Anti-Lymphoma E9514 proved completely effective against the cells of Lymphomas 6C3HED and E9514 *in vivo* but had no observable effect on the cells of Lymphoma AKRL1, while IRS 63: Anti-Lymphoma A2 was effective against the cells of Lymphoma 6C3HED but had little or no effect on the cells of the other two lymphomas. It seems especially noteworthy that the only serum which notably inhibited the AKRL1 cells was IRS 58: Anti-Lymphoma AKRL1, and that this serum was less effective against lymphoma cells of the other two types than were several of the immune serums tested in comparison with it.

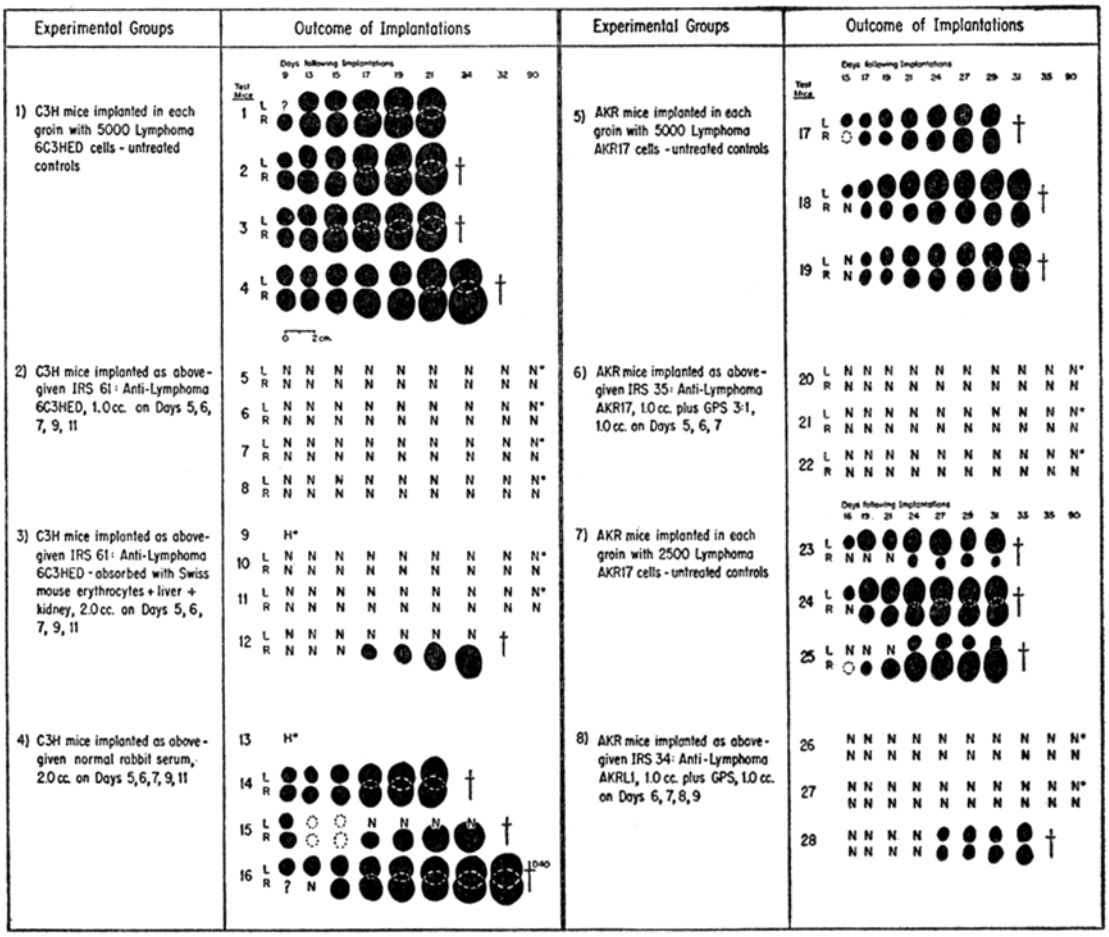
A number of the immune serums, which were given repeatedly in relatively large amounts as the table shows, proved manifestly toxic, bringing about losses in weight of as much as 5.0 gm., together with ruffled fur and sluggishness, during the period of injections and for a few days thereafter. These manifestations proved transitory, however, in the bulk of instances, though they were so marked in four of the experimental groups that reduced amounts of serum were given on days 8, 9, and 10, the details being given in the table. Only one of the anti-lymphoma serums (IRS 58:Anti-AKRL1) proved lethal, however; it brought about death in two of the 12 injected animals within a few hours following the second injection, while a third mouse died on the 30th day without gross evidence of lymphomatosis and perhaps as a result of delayed toxic effects. Mention has already been made of the fact that two of the mice injected with IRS:59 (anti-mammary carcinoma) died with anasarca and other signs of nephrotoxic nephritis on the 22nd day of the experiment; actually most of the mice injected with this serum developed ascites and subcutaneous edema during the period 10 to 17 days, and in some instances the signs were marked; but they proved transitory, except in the two instances already noted, and the hosts manifesting them developed lymphomatosis as did the mice of the control groups. In contrast to the serums just mentioned, IRS 62:Anti-Lymphoma E9514 was notably devoid of toxicity, the animals receiving this serum remaining sleek and lively and maintaining or increasing their respective weights throughout the injection period and thereafter, as daily observations showed; yet this serum completely cured the mice implanted with 6C3HED cells and so too those implanted with E9514 cells, in these respects being the most potent of all the serums used in the experiment. The same was true of IRS 63:Anti-Lymphoma A2, which did not induce weight loss or any other signs of toxicity but cured all the animals that had been implanted with Lymphoma 6C3HED cells, as the table shows.

From the findings just described (Table VIII) it is obvious that the three types of lymphoma cells differed notably in susceptibility to the effects of the immune serums *in vivo*, the AKRL1 cells being much less susceptible (5/27 cures) than those of Lymphomas 6C3HED (19/26 cures) and E9514 (14/27 cures). The effects in general, it may be noted, paralleled those observed in the *in vitro* studies already given (Table I).

Chart 6 shows the results of two experiments of similar sort.

In the first experiment (left hand columns—experimental groups 1 to 4) sixteen C3H mice, each weighing 28 gm. or more, were implanted with Lymphoma 6C3HED cells, 5,000 in each groin. Four of the mice (numbers 1 to 4) were left untreated as controls, and 3 others (Nos. 14 to 16) were given 2.0 cc. of normal rabbit serum intraperitoneally on the 5th, 6th, 7th, 9th, and 11th day following implantation (mouse 13, originally included in this group, died within an hour following the second intraperitoneal injection with abdominal hemorrhage resulting from a rent in one of the mesenteric veins). From this chart it can be seen that subcutaneous lymphomas up to 1.0 cm. across appeared at both implantation sites in all 7 mice in these groups; furthermore, the lymphomas enlarged progressively and brought about death of the animals within 40 days. The results were very different in the remaining mice. Four of these (Nos. 5 to 8) were given 1.0 cc. of IRS 61:Anti-Lymphoma 6C3HED, again on the 5th, 6th, 7th, 9th, and 11th days following implantation. These animals maintained their respective weights during the period of intraperitoneal injections, as daily weighing showed, and they remained lively and fleshy and devoid of palpable growths or other signs of lymphomatosis throughout the entire observation (120 days); *i.e.*, they were cured. The remaining 4 animals (numbers 9 to 12) were given, on each of the injection days, 2.0 cc. of IRS 61 which had been

CHART 6. Tests for effects of anti-lymphoma immune rabbit serums on "established" lymphomas of two types *in vivo*.



The serums were heated at 65°C. before use. L,R = left and right groins, respectively. H* Died with intraperitoneal hemorrhage immediately following injection.

N, no palpable tumors,—mouse lively.

N*, mouse remained lively and devoid of palpable tumors or other signs of lymphomatosis throughout observation period (120 days).

absorbed successively with mouse erythrocytes, mouse liver cells, and mouse kidney cells. Mouse 9 died with abdominal hemorrhage a few minutes following the final intraperitoneal injection. Mice 10 and 11 did not develop palpable growths and remained lively throughout the observation period (120 days). Mouse 12 developed a palpable tumor in its right groin on the 17th day and died on the 32nd day with lymphomatosis. The facts suggest that, although the absorbed serum was still remarkably potent, its effectiveness was diminished, though whether the diminution was referable to absorption *per se* or to the prolonged handling of the serum during the procedure is not wholly clear.

In the experiment summarized in the right hand columns of Chart 6, six AKR mice, all weighing 26 gm. or more, were implanted in each groin with 5,000 Lymphoma AKR17 cells, three of the animals (Nos. 17 to 19) being left untreated as controls while the rest (Nos. 20 to 22) were given, on the 5th, 6th, and 7th days following implantation 2.0 cc. of a mixture containing equal parts of IRS 35:Anti-Lymphoma AKR17 plus normal guinea pig serum that had been concentrated 3:1 by pervaporation. The control animals all developed palpable growths between the 15th and 17th days and died with lymphomatosis. The treated animals, by contrast, were cured, as the chart indicates. Six additional AKR mice were implanted in this experiment with 2,500 Lymphoma AKR17 cells in each groin. Three of these animals (Nos. 23 to 25) were left untreated as controls; all died with lymphomatosis before the 35th day. The rest were given IRS 34:Anti-Lymphoma AKRL1 plus normal guinea pig serum; two of these treated animals were cured while the third developed palpable growths and died with lymphomatosis on the 35th day.

Susceptibility of Serum-Treated Mice to Reimplantation with Lymphoma Cells

Several experiments were made to learn whether the effects of anti-lymphoma immune serums *in vivo* might be influenced or determined by resistance of the hosts to the transplanted lymphoma cells.

In the first such experiment, 16 AKR mice were implanted in each groin with 0.2 million AKRL1 cells; 8 of the mice were then given 1.0 cc. of heated anti-Lymphoma AKRL1 serum on days 1, 2, and 3, and the others were left untreated as controls. The treated animals failed to manifest growths during an observation period of 30 days, while the controls all developed palpable tumors within 8 days which enlarged progressively and brought about death within 14 days. On the 30th day, the 8 treated animals were reimplanted in each groin with 0.2 million AKRL1 cells from the same suspension, and a second group of control AKR mice were also implanted. 8 days later tumors approximately 1.0 cm. across were palpable in both groins of all the animals; the growths enlarged progressively until death of the hosts during the period 11 to 14 days, the course of events being precisely the same in the controls and in the mice that had previously overcome implanted lymphomas as result of serum treatment.

Five additional experiments were made with mice in which the lymphoma cells had been allowed to "establish" themselves during a period of 4 or 5 days and then were overcome as result of serum therapy. In the first of these experiments, the six mice of groups 2 and 3 in Chart 6, in which Lymphoma 6C3HED cells had been overcome following serum treatment begun on the 5th day, were implanted on the 120th day with 6C3HED cells, 0.2 million in each groin; as controls, 4 normal C3H mice were implanted in the same way. Palpable tumors appeared in all the mice within 8 days, and the growths enlarged progressively thereafter, the course of events again being precisely the same in the serum-treated and control animals. So too, in the second test: five serum-treated mice that had overcome established AKR17 cells (those of groups 6 and 8 of Chart 6) proved fully as susceptible as did 5 control AKR mice to 0.2 million AKR17 cells implanted in each groin.

In the three remaining experiments, the serum-treated mice of the experiments of Table VIII were used. Nineteen of the animals had overcome established 6C3HED cells, 14 had overcome established E9514 cells, and 4 had overcome established AKRL1 cells. All were implanted on the 120th day following the initial implantations, with lymphoma cells of the type previously used, 0.2 million cells being placed in each groin; four control mice were also implanted with each of the respective types of lymphoma cells. Again the serum-treated mice proved fully susceptible, tumors appearing as promptly and growing as rapidly in them as in the respective control animals.

The findings as a whole provide strong evidence that host resistance had no part in the process whereby the lymphoma cells were overcome in the serum-treated mice.

Limitations of the Effects of Anti-Lymphoma Serums on Lymphoma Cells in Vivo: Comparison with Effects of (a) Chemotherapeutic Agents and (b) Normal Guinea Pig Serum

In the experiments of Table VIII and Chart 6, already described, a number of anti-lymphoma serums largely or completely inhibited the cells of Lymphomas 6C3HED, E9514, and AKR17 *in vivo* when they were given to the host animals 5 or 6 days after implantation. These anti-lymphoma serums, however, and others prepared in the same way, had only transitory and on the whole negligible effects in a number of experiments when they were given to mice with palpable tumors of the three types resulting from implantations made 8 to 12 days before therapy was begun. Under such circumstances, the palpable growths often diminished in size during the first few days of serum therapy, but they never disappeared completely, and the animals generally survived only a few days longer than did untreated controls or those given normal rabbit serum. In several attempts to overcome established lymphomas resulting from implantation of E9514, AKR17, and A1 cells, a number of anti-lymphoma immune serums proved no more effective when given in combination with normal guinea pig serum than when given alone.

The effects of anti-lymphoma serums on Lymphoma AKRL1 cells *in vivo* were even more limited. True, a number of the serums regularly and completely inhibited large numbers of implanted AKRL1 cells when they were given to the host mice on the day of implantation and on the 2 succeeding days as well (see Charts 3 and 4 for examples). But in the experiment of Table VIII already cited, six of the seven anti-lymphoma serums employed had comparatively little or no effect when they were injected after the lymphoma cells had become established in new hosts, IRS 58 alone proving notably potent. It should be pointed out, however, that this was the only antiserum prepared with Lymphoma AKRL1 cells as antigen which was used in the experiment of Table VIII; furthermore it was the only such antiserum used in the present work in the attempts to overcome established AKRL1 cells *in vivo*. The results of a further experiment, done concurrently with the experiments of Chart 6 and in the same way, showed clearly that this serum, while acting powerfully against established AKRL1 cells, was toxic for the host animals.

Hence, it is manifest that the anti-lymphoma serums here used had only a limited capacity to overcome mouse lymphoma cells *in vivo*, and furthermore that such serums

were sometimes notably toxic. They share these limitations, it may be noted, with all the chemotherapeutic agents which have thus far been critically tested and fully reported upon, as scrutiny of the literature shows.

For example, Skipper, Chapman, and Bell reported in 1951—in extension of their earlier work and that of others (21), including Burchenal and his associates (22)—that aminopterin and amethopterin, while manifestly toxic, were more effective *in vivo* against the cells of Lymphoma AK4 than were any of a variety of other therapeutic measures, including x-rays, urethan, HN2, colchicine, benzene, potassium arsenite, and 2,6-diaminopurine, given singly and in various combinations; yet all the mice treated by Skipper, Chapman, and Bell with anti-folic acid compounds died with lymphomatosis before the 35th day (23). Subsequently a number of workers have shown that several additional compounds, notably 8-azaguanine, 6-mercaptopurine, and ethionine, given singly and in combinations with one another and with amethopterin, have limited effectiveness against some types of lymphoma cells in mice but no effect at all against other types. For example, Law reported in 1952 that amethopterin and 8-azaguanine, although devoid of effect upon several types of lymphoma cells, prolonged the survival of susceptible mice implanted with one strain of Lymphoma L1210 cells when the two compounds were given on alternate days beginning 24 hours after implantation, and that his most effective regimen brought about a cure (*i.e.* survival for 90 days following implantation without signs of lymphomatosis) in 23 per cent of his treated mice (24). Goldin, Venditti, Humphreys, and Mantel have reported similar findings in mice implanted with L1210 cells and subsequently treated intensively with amethopterin, sometimes in combination with citrovorum factor and other chemical substances, though they have now found therapy with amethopterin, given alone or in combination with other drugs, largely devoid of curative effect on this lymphoma if therapy is delayed until large numbers of the lymphoma cells are proliferating in the implanted host (25).

Because of the findings noted above, we did not make a detailed and systematic comparison of the effects of immune serums and chemotherapeutic compounds on the lymphomas used in the present study. The results of a number of experiments made it plain, however, that amethopterin, 8-azaguanine, azaserine, 6-mercaptopurine, and ethionine, given singly and in combinations in maximal tolerated (and toxic) amounts, had little or no effect *in vivo* on the cells of Lymphomas 6C3HED, E9514, and AKRL1 when therapy was given according to the dosages employed by Law (24) and instituted 3 days or more following implantation instead of on the following day as in his experiments. In several additional experiments the chemotherapeutic agents mentioned were given in combination with the immune serums, but the inhibitory effects were not notably greater than those brought about by the immune serums alone.

The effects of immune serums on lymphoma cells *in vivo*, and those of chemical substances as described above, deserve comparison also with the ability of normal guinea pig serum to bring about the regression of certain lymphomas *in vivo* (1). It should be noted at the outset that the effects of normal guinea pig serum on transplanted lymphoma cells *in vivo* are singularly specific. For while normal guinea pig serum acted powerfully upon the cells of Lymphomas 6C3HED and A2 implanted in C3H and A/Jax mice respectively, and upon those of the Murphy-Sturm Lymphosarcoma growing in Wistar rats, it had no effect in our original studies on the cells of

Sarcoma 180 or on those of several transplanted mammary carcinomas of C3H mice (1), and in a subsequent study it had little or no effect *in vivo* on the cells of any one of 8 transplanted lymphomas of AKR mice (20). In more recent tests, made with Dr. Michel Haddad, guinea pig serum has furthermore proved essentially devoid of effect *in vivo* on the cells of any one of 12 additional mouse lymphomas, including Mecca and L4946 of AKR mice, L1210 and L5170 of DBA mice, E9514 and L7688 of C3H mice, Lymphoma 1 of Strong A mice, Lymphoma B14 of C58 mice, and 5 lymphomas originating in hybrid AKR × C3H mice and transplanted in this laboratory. Jamieson, Ainhis, and Ryan have lately reported, however, that normal guinea pig serum is active *in vivo* against the cells of a transplanted fibrosarcoma of rats (26). Even so, it is obvious that normal guinea pig serum is sharply limited in its effects by an extraordinary and unexplained specificity. Yet it acts powerfully indeed against lymphoma cells of the sorts that are susceptible to its effects *in vivo*. For in extension of our previous observations (1), recent experience has shown that normal guinea pig serum will regularly bring about regression of subcutaneous lymphomas 2 to 3 cm. across and of generalized lymphomatosis as well which have resulted from the implantation of 6C3HED cells in susceptible C3H mice 12 to 18 days before therapy is begun. It is obvious that under such circumstances the effects of guinea pig serum are far more powerful than are those resulting from the administration of immune serums or chemical substances as described above.

RECAPITULATION AND COMMENT

In the experimental work here described, 43 immune serums were prepared in rabbits with antigens consisting of cells procured from mouse tissues of three sorts: (*a*) normal cells from mouse organs and tissues that were presumably devoid of large numbers of lymphocytic cells—notably liver, kidney, brain, muscle, whole embryos, and erythrocytes; (*b*) non-neoplastic lymphocytic cells from thymuses and lymph nodes of young C3H and AKR mice, and (*c*) the cells of seven different transplanted lymphomas which had originated in C3H, AKR, and Strong A mice. The various immune serums were then tested for capacity to act upon the cells of several of the transplanted mouse lymphomas under controlled conditions *in vitro* and *in vivo*.

Almost all the immune serums promptly killed the various types of lymphoma cells *in vitro* when hemolytic complement was present in the mixtures. The serums varied notably in potency, however, as titrations showed, those in category (*a*) being in general less potent *in vitro* than were those in category (*b*), while those in category (*c*) were generally most potent in the comparative *in vitro* tests. Absorption procedures demonstrated that the immune serums differed qualitatively as well. For mouse erythrocytes, and cells from a variety of non-lymphatic mouse organs also, readily absorbed the antibodies that were responsible for the *in vitro* effects on lymphoma cells from immune serums prepared with antigens of category (*a*), but they did not remove such antibodies from immune serums made with antigens consisting of non-neoplastic and neoplastic lymphocytic cells (categories (*b*) and (*c*)). Non-neoplastic thymus and lymph node cells, however, removed the antibodies responsible for the *in vitro* effects from immune serums of categories (*b*) and (*c*), and so too did the malignant

lymphoma cells of several types, limited observations indicating that the latter were considerably more powerful.

The *in vivo* tests provided further indications of specificity. For the immune serums of category (*a*) were all devoid of effect when they were given intraperitoneally to susceptible mice that had been implanted with the respective types of lymphoma cells. The immune serums produced with non-neoplastic and neoplastic lymphocytic cells, however, often acted powerfully against the lymphoma cells *in vivo*. Furthermore, the antibodies responsible for the *in vivo* effects, like those responsible for the *in vitro* effects, were removed upon absorption with lymphoma cells but not with the cells of normal mouse organs such a kidney and liver, or with mouse erythrocytes. The findings suggest that an antibody which reacts more or less specifically with neoplastic and non-neoplastic lymphocytic cells of mice was largely responsible for the *in vivo* and *in vitro* effects of the anti-lymphoma serums. The findings also disclosed further indications of inherent differences in the various types of lymphoma cells. For in several instances immune serums produced with lymphoma cells of a given type as antigen proved more powerful against lymphoma cells of that type than did immune serums prepared with lymphoma cells of other types as antigens. Furthermore, the cells of Lymphomas 6C3HED, E9514, and AKR17 proved much more susceptible to the effects of various anti-lymphoma immune serums, in comparative *in vitro* and *in vivo* tests, than did the cells of Lymphoma AKRL1.

Although the lymphoma cells were rapidly killed upon exposure to the anti-lymphoma serums *in vitro* in the presence of complement, they survived many hours' exposure *in vitro* to anti-lymphoma serums that had been heated at 65°C. for 20 minutes—a procedure that inactivated hemolytic complement but did not notably diminish the capacity of the immune serums to act upon the lymphoma cells *in vivo*. The findings suggest that the host organism somehow participates in the process whereby lymphoma cells are overcome in mice given anti-lymphoma immune serum, perhaps by providing some essential "complementary" substance; but they do not disclose the precise nature of this participation. Normal guinea pig serum, given along with anti-lymphoma serums with the aim of providing additional "complement" or perhaps other ancillary factors, occasionally but by no means regularly enhanced the effects of the anti-lymphoma immune serums *in vivo*.

Several anti-lymphoma immune serums, when heated at 65°C. for 20 minutes, were notably effective against lymphoma cells *in vivo* while at the same time being devoid of toxicity for adult mice. Other pools of anti-lymphoma serums, heated in the same way, proved toxic, however, and so too did heated immune serums prepared with antigens made from non-neoplastic mouse cells, notably erythrocytes. In a number of carefully studied instances the toxicity was shown to be due to mouse hemolysins and hemagglutinins which could be absorbed from the immune serums with mouse erythrocytes and other non-neoplastic mouse cells; but in some instances anti-lymphoma immune serums remained toxic following such absorptions.

Under favorable conditions the anti-lymphoma serums exhibited quite powerful effects against several types of lymphoma cells *in vivo*. For example, when given intraperitoneally in suitable amounts on the day of implantation and on the 2 succeeding days as well to susceptible mice that had been implanted with 300,000 or more lym-

phoma cells of one or another of three types (6C3HED, E9514, AKR17), the various anti-lymphoma serums usually brought about death of all the implanted cells, the serum-treated animals being fully susceptible to reimplantation later on. Furthermore, when selected anti-lymphoma serums were given in suitable amounts to mice with established lymphomas resulting from implantations made 5 or 6 days earlier, they frequently brought about a cure—*i.e.*, survival of the hosts without signs of lymphomatosis for 90 days or more, while control untreated mice and those given normal rabbit serum or an anti-mammary carcinoma serum regularly died with lymphomatosis before the 40th day. But the anti-lymphoma immune serums had only transitory and on the whole negligible effects when they were injected 8 to 12 days following implantation of lymphoma cells—at a time, that is to say, when palpable growths were present at the implanted sites and great numbers of proliferating lymphoma cells were widely distributed throughout the organs and tissues of the host mice, as microscopic studies showed.

Scrutiny of the scientific literature showed that the chemotherapeutic agents which have thus far been critically evaluated for effects upon lymphoma cells *in vivo*—notably amethopterin, azaguanine, azaserine, 6-mercaptopurine, ethionine, and others—have proved largely devoid of effect on lymphoma cells of numerous types, and have in each instance displayed sharply limited and, on the whole, quite feeble effects against lymphoma cells of only one or a few selected types. In a number of experiments done in the course of the present work amethopterin, azaguanine, azaserine, 6-mercaptopurine, and ethionine were given singly and in various combinations in maximal tolerated doses to mice that had been implanted several days before with lymphoma cells of the several types here used. Although often toxic for the mouse hosts, the chemical substances had little or no effect on the various types of lymphoma cells growing in them, their effects being obviously less powerful than those exerted by certain of the anti-lymphoma immune serums under comparable circumstances. In contrast, normal guinea pig serum, although entirely devoid of effects on a considerable number of mouse lymphomas *in vivo*, exerts powerful effects *in vivo* against the cells of Lymphoma 2 of Strong A mice and those of the Murphy-Sturm lymphosarcoma of rats, and it regularly brings about regression of Lymphoma 6C3HED, even when given to mice with large subcutaneous lymphomas and generalized lymphomatosis resulting from implantations made 12 to 18 days before treatment is begun. It seems obvious that normal guinea pig serum works far more powerfully *in vivo* against lymphoma cells of the types that are specially susceptible to its action than do any of the immune serums or chemotherapeutic substances described above.

Manifestly, much remains to be learned about the effects of immune serums and of normal guinea pig serum on lymphoma cells *in vivo*. Yet these effects are considerable, and to some extent specific, as has been shown. Whether ways can be found to enhance them remains to be seen.

SUMMARY

Immune serums prepared in rabbits with antigens made from normal mouse organs and tissues that were presumably devoid of large numbers of lympho-

cytic cells (notably kidney, liver, brain, whole embryos, and erythrocytes) proved lethal for the cells of several transplanted mouse lymphomas *in vitro* in the presence of complement; but these immune serums, when given intraperitoneally in large amounts to susceptible mice that had been implanted subcutaneously with lymphoma cells of one or another of several types, failed entirely to inhibit growth of the lymphoma cells *in vivo*. In contrast, immune serums made with cells procured from transplanted mouse lymphomas as antigens, and those made with cells from normal mouse thymus or lymph nodes, acted even more powerfully upon the several types of lymphoma cells *in vitro* than did the immune serums prepared with normal mouse organs, and when given intraperitoneally to implanted mice they brought about death of the lymphoma cells *in vivo*, the effect being to a considerable extent specific and referable to an antibody that reacts with neoplastic and non-neoplastic lymphocytic cells of mice, as absorption experiments disclosed. In comparative tests, furthermore, the anti-lymphoma serums acted more powerfully upon the lymphoma cells *in vivo* than did such chemotherapeutic agents as amethopterin, azaguanine, ethionine, azaserine, and 6-mercaptopurine, given singly or in various combinations in maximal tolerated amounts, though their effects were not so powerful as those exerted by normal guinea pig serum on lymphoma cells of two types that are susceptible to its action *in vivo*.

The significance of the findings was briefly discussed.

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