Some Properties of “RLP”—A Factor from Sheep Spleen Capable of Inhibiting Radiation Leukemogenesis in Mice

By I. Berenblum, G. Cividalli, N. Trainin and M. E. Hodes

After the discovery that injection of syngeneic (isologous) bone marrow or spleen cells can antagonize both the acute lethal effects and the late leukemogenic effects of total body x-irradiation in mice, attempts to determine the mechanisms involved led to some conflicting conclusions. As far as the acute lethal action of a single high dose of total body x-irradiation is concerned, the protective effect of injected bone marrow or spleen cells is generally believed to be dependent on the repopulation of certain vital cellular components of the hematopoietic system destroyed by the irradiation. Protection has, however, been obtained not only with syngeneic but also with allogeneic (homologous and heterologous) bone marrow and spleen cells, and some degree of protection with cell-free extracts of spleen and even with protein-free nucleoprotein fractions of such extracts. In the case of the long-delayed leukemogenic action of repeated, moderate doses of total body x-irradiation, only syngeneic tissues have been found to be effective for protection, whereas cell-free extracts have apparently not been tested for this system.

Another aspect of radiation damage, which has incidentally served as a method of assay both for the degree of damage and the efficacy of protection against it, is the weight loss and recovery back to normal of the thymus. Thymus weight recovery was found to be stimulated by injection of normal bone marrow cells, or by their nuclei, but not by the supernatant fraction, following centrifugation of the suspension. The responsible “factor” has been shown to be radiosensitive in vitro, not to be readily extractable with saline, and to be destroyed by freezing and lyophilization.

A number of important questions, arising from these observations, call for further investigation: (1) Are the mechanisms of protection against the acute lethal action, the early acceleration of thymic involution, and the late leukemogenic effect the same? (2) Does the evidence for cellular repopulation, in the case of protection against acute radiation damage, necessarily preclude the involvement of a cell-free (humoral) factor? (3) May not a humoral factor actually play a predominant role in the protection against radiation leukemogenesis, where the acute radiation damage is less severe, and consequently where cellular repopulation is not essential for survival?

The conflicting interpretations of previous attempts to demonstrate humoral protective factors against radiation damage may partly be ac-
counted for (a) by the fact that these studies dealt with different high doses of radiation, where cellular repopulation may have been essential in some cases only, and (b) by the fact that treatment with cell-free extracts usually consisted of very few or even single injections. Assuming that a humoral factor were implicated, one might suppose that a single injection of a limited number of live cells would be sufficient, the cell, after multiplying in vivo, continuing to produce the hypothetical factor, whereas repeated injections of large doses of material would be needed when using the postulated humoral factor alone.

The present investigation was specifically directed to the problem of possible humoral protection against the leukemogenic effects of x-irradiation, and planned on the assumptions (a) that it might possibly be independent of that involved in acute damage, and (b) that if a cell-free factor were involved, it would have to be administered repeatedly in large doses.

Sheep spleen was chosen as a source of the extracts, to provide sufficient material for large-scale testing. As already reported in a preliminary communication, it was found to contain a factor ( provisionally designated "RLP"—referring to "radiation-leukemia-protection"), capable of inhibiting radiation leukemogenesis in mice. The present communication provides confirmation and extension of these earlier findings, with additional data obtained by further fractionation of the crude material, in an attempt to characterize and isolate the active principle.

**MATERIALS AND METHODS**

The mice used in these experiments were of the C57BL/6/Jax strain, bred in our Institute by brother-sister mating for many generations. The animals were housed in metal cages, 10 per cage, in air-conditioned rooms kept at 21-25 C. and fed Purina Laboratory Chow and tap water ad libitum.

The mice were 5-7 weeks old when submitted to the first of a series of 4 weekly irradiations of 150 r. each. Irradiation was performed with a G.E. Maximar 250-III machine. The physical properties were: 200 kv., 15 MA, with 1 mm. Al and 0.5 mm. Cu filters, and a dose rate of 31-35 r./min. During the irradiation, the mice were placed in Lucite containers, 10 per group, each mouse in a separate compartment.

Injection of 0.25 ml. of the various sheep spleen fractions (see below) was made intraperitoneally within 4 hours after each irradiation, and then twice weekly over a period of 3 weeks, totalling 10 injections. In some experiments (as indicated below) the injections were continued twice weekly till a total of 20 or 30 injections was reached. Treated mice were examined periodically for signs of thymic and lymph node enlargement, and were killed when signs of leukemia appeared. All the dead animals were autopsied, and suspected tissues examined histologically.

The various extracts for testing were derived from sheep spleens, removed aseptically from animals killed at the local slaughter-house and transported to the laboratory on ice. Working in a cold room at +2 C., the capsules and main blood vessels were removed, and the residual pulp mixed with 1.5 parts of sterile tyrode solution, disintegrated in a "Virtis" homogenizer for 2 minutes, and further ground in a glass homogenizer. (Kidney homogenate, serving as a control, was prepared in a similar fashion from sheep kidney cortex.) All preparations were made up fresh every week (except where otherwise stated) and kept frozen at -20 C. until used.

The crude homogenate, when not directly used, was allowed to stand for 2-4 hours on ice and then centrifuged at 7000 g. in a Sorvall RC-2 cooled centrifuge for 20 minutes.
The resulting precipitate fraction (resuspended in tyrode to attain the original volume) and the supernatant fraction were used separately for testing in series 462 and 466-7. In all later experiments, unless otherwise stated, the upper ½ of the supernatant was removed, centrifuged again at 7000 g., and only the upper ½ of the second supernatant used for testing.

The filtered supernatant fraction was obtained by passing the original supernatant fraction either through a Selas porosity 015 filter (series 462, 466-7) or through "Millipore" filters 0.8 or 1.2 μ pore size (in later experiments). The effectiveness of filtration was tested in some cases by the ability to prevent the passage of E. coli bacilli. The supernatant heated to 100 C. for 5 minutes and to 60 C. for 30 minutes were subsequently centrifuged at 7000 g. and the resulting supernatant used for testing.

In a later experiment (table 2) the following further fractions were tested: Washed precipitate, obtained by centrifuging the resuspended precipitate 4-5 times, until it was nearly colorless (and thus presumably free from contamination with substances present in the supernatant); and supernatant after 80,000 g. centrifugation for 1 hour, prepared in a Beckmann model L preparative ultracentrifuge. The upper half of the supernatant was used for testing, as well as the 80,000 g. pellet resuspended in half the original volume of tyrode. A further control group, composed of animals which received a saturated solution of lactalbumin hydrolysate in tyrode (approximately 5 per cent concentration) was also added.

One group (table 3) was treated with homogenate irradiated in vitro with 30,000 r. (performed in a glass container, with the thickness of the homogenate not exceeding 2.5 cm. Physical factors: 250 kv., 15 MA, without external filters; dose rate 1000 r./min.); another group received homogenate to which penicillin (1000 units per ml.) and streptomycin (1 mg./ml.) were added immediately after preparation.

The isolation of the nucleoprotein fraction from sheep spleen supernatant (table 4) was carried out in a cold room by the phenol extraction method. New preparations were made every 2 or 3 weeks. The supernatant was first diluted with its own volume of tyrode, an equal volume of 90 per cent redistilled phenol added, and the mixture then heated to 60 C. ± 2 C. for 10 minutes (while kept mixed with a magnetic stirrer) to inactivate the nucleases. The aqueous phase was removed and extracted a further 3 times with \( \frac{1}{4} \) and again \( \frac{1}{4} \) its own volume of 90 per cent redistilled phenol. The nucleic acids in the aqueous phase were precipitated twice with 95 per cent ethanol, and then redissolved in half the original volume of tyrode.

Analysis of several preparations by means of the diphenylamine reaction revealed only traces of DNA. The ultraviolet absorption spectra of the preparations was typical for nucleic acid, with an absorption maximum at 258 mg./ml. and a ratio \( A_{290}/A_{230} = 1.8-2.0 \). On the basis of \( A_{260} \) the RNA concentration was calculated to be 0.6-1.8 mg./ml. Small amounts (less than 5 per cent) of protein were present in 3 specimens examined.

Enzyme preparations were also added to the sheep spleen supernatant fraction, and to the purified nucleic acid fraction, for \( \frac{1}{2} \) an hour at room temperature, to determine whether this would destroy the protective factor. DNAase (Worthington Biochemical Corporation) was added to the supernatant fraction in the concentration of 50 μg./ml. Both DNAase and RNAase (Worthington Biochemical Corporation) were added to samples of the nucleic acid fraction in the concentration of 2 μg./ml.

Fractionation by salting of the proteins present in the spleen supernatant was carried out in the cold room by using progressively higher concentrations of ammonium sulphate. A 30,000 g. supernatant was used as starting material, and solid ammonium sulfate (puriss., Fluka AG Buchs SG, Switzerland) added, to reach progressively 30 per cent, 40 per cent, 50 per cent, 60 per cent and 100 per cent saturation. The precipitated proteins, collected after centrifugation at 13,000 g., were redissolved in tyrode. After dialysis for 4-8 hours against physiologic saline, to remove the ammonium sulfate, the protein solutions were brought to \( \frac{1}{4} \) of the original volume of supernatant, filtered, and kept at −20 C.
(for up to 2 months) before being used. Protein concentration in each sample was determined by the Biuret reaction.

**Results**

*Detection of RLP Factor*

Since the results previously reported (series 462, 466-67, 473, 476, 480-82, 484-85) were from experiments that had not yet all been completed at the time, the final results are now presented in table 1. These final results differ only slightly from those reported previously, and confirm the fact that the homogenate, the supernatant, the filtered supernatant, and (slightly less effectively) the precipitate fractions, inhibit radiation leukemogenesis in C57BL mice. On the other hand, heat appears to inactivate the principle.

A control group, added to one of these series, consisting of sheep kidney homogenate, prepared similarly to the spleen homogenate, showed only slight evidence of inhibition, of borderline significance.

*Further Fractionation by Centrifugation*

Since the previous fractionation by centrifugation at 7000 g virtually failed to provide separation of the active principle between the supernatant and precipitate fractions, a further experiment was undertaken involving a number of additional steps (resuspension and washing of precipitate, use of only upper portions of the supernatant, etc., as well as further centrifugation at 80,000 g.). The results, summarized in table 2, provide clearer evidence of separation, the highest activity being with the 7000 g. upper supernatant portion (58 per cent inhibition) and with the 80,000 g. supernatant (53 per cent inhibition), and the lowest activity with the precipitate fraction after 80,000 g. (17 per cent inhibition).

In an additional control group in this experiment, 5 per cent lactalbumin hydrolysate was tested, in comparison to sheep spleen extracts. No inhibition whatsoever was obtained, the resulting leukemia incidence being, if anything, slightly higher than in the uninjected, irradiated control. (The values obtained were: 58/60 survivors, of which 35 developed leukemia—i.e., 60 per cent, as compared to 54 per cent in the uninjected irradiated control.)

*Test for Stability of the Active Principle to In Vitro Irradiation and to Antibiotics*

The test for the influence of in vitro irradiation of the homogenate to 30,000 r., was partly to obtain supporting evidence that activity did not depend on the presence of live cells (cf. activity of the filtered supernatant—table 1), and partly to determine whether the active principle (whatever its nature) is radiosensitive, as has been claimed to be the case for the factor stimulating thymus regeneration. The test for the influence of antibiotics on the inhibitory properties of spleen homogenate was to exclude the remote possibil-
Table 1.—Influence of Sheep Spleen Extracts on the Leukemogenic Action of Total Body X-Irradiation in C57BL/6 Mice

<table>
<thead>
<tr>
<th>Fraction*</th>
<th>462, 466, 467</th>
<th>473, 476</th>
<th>480-82, 484-85</th>
<th>Total</th>
<th>Per Cent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>S</td>
<td>L</td>
<td>N</td>
<td>S</td>
</tr>
<tr>
<td>— (Irradiated control)</td>
<td>50</td>
<td>46 (92%)</td>
<td>36 (72%)</td>
<td>40</td>
<td>39 (98%)</td>
</tr>
<tr>
<td>Homogenate x 10</td>
<td>50</td>
<td>42 (84%)</td>
<td>19 (45%)</td>
<td>80</td>
<td>34 (43%)</td>
</tr>
<tr>
<td>7000 g. supernatant x 20</td>
<td>40</td>
<td>33 (83%)</td>
<td>12 (32%)</td>
<td>40</td>
<td>33 (83%)</td>
</tr>
<tr>
<td>7000 g. precipitate x 10</td>
<td>50</td>
<td>48 (96%)</td>
<td>27 (54%)</td>
<td>40</td>
<td>24 (60%)</td>
</tr>
<tr>
<td>Filtered supernatant x 20</td>
<td>40</td>
<td>35 (88%)</td>
<td>17 (49%)</td>
<td>50</td>
<td>47 (94%)</td>
</tr>
<tr>
<td>Supernatant heated 100 C. x 20</td>
<td>60</td>
<td>56 (93%)</td>
<td>40 (71%)</td>
<td>40</td>
<td>37 (93%)</td>
</tr>
<tr>
<td>Supernatant heated 60 C. x 20</td>
<td>50</td>
<td>46 (92%)</td>
<td>30 (65%)</td>
<td>50</td>
<td>46 (92%)</td>
</tr>
<tr>
<td>Kidney homogenate (control) x 10</td>
<td>55</td>
<td>44 (80%)</td>
<td>25 (57%)</td>
<td>55</td>
<td>44 (80%)</td>
</tr>
</tbody>
</table>

*"x 10" and "x 20" refer to 10 and 20 injections, respectively. In Series 473, 476, the supernatant and boiled supernatant fractions were given x 30.
†Calculated in relation to its own control.
N = number of mice used; S = survivors at time of appearance of the first leukemia in the series ("effective total"); L = number of leukemia-bearing mice: percentage calculated on the basis of effective total, i.e. L/S x 100.
SOME PROPERTIES OF "RLP"

Table 2.—Results from Further Attempts at Fractionation by Centrifugation

<table>
<thead>
<tr>
<th>Fraction*</th>
<th>Number of Mice Used</th>
<th>Survivors†</th>
<th>Leukemia-Bearing Mice†</th>
<th>p.</th>
<th>Per Cent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>— (Irradiated control)</td>
<td>80</td>
<td>71 (80%)</td>
<td>38 (54%)</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Homogenate</td>
<td>60</td>
<td>58 (97%)</td>
<td>16 (28%)</td>
<td>&lt;0.01</td>
<td>49%</td>
</tr>
<tr>
<td>7000 g. supernatant</td>
<td>60</td>
<td>56 (93%)</td>
<td>15 (27%)</td>
<td>&lt;0.01</td>
<td>50%</td>
</tr>
<tr>
<td>7000 g. supernatant (upper 2/3)</td>
<td>60</td>
<td>55 (92%)</td>
<td>12 (22%)</td>
<td>&lt;0.001</td>
<td>59%</td>
</tr>
<tr>
<td>7000 g. precipitate</td>
<td>60</td>
<td>49 (82%)</td>
<td>18 (37%)</td>
<td>&lt;0.1</td>
<td>31%</td>
</tr>
<tr>
<td>Washed 7000 g. precipitate</td>
<td>60</td>
<td>54 (90%)</td>
<td>20 (37%)</td>
<td>&lt;0.1</td>
<td>31%</td>
</tr>
<tr>
<td>80,000 g. supernatant</td>
<td>60</td>
<td>51 (85%)</td>
<td>13 (23%)</td>
<td>&lt;0.01</td>
<td>52%</td>
</tr>
<tr>
<td>(upper ½)</td>
<td>60</td>
<td>55 (92%)</td>
<td>25 (45%)</td>
<td>&lt;0.5</td>
<td>17%</td>
</tr>
</tbody>
</table>

*All injections x 10.
†See table 1.

it that the inhibition was a side effect of any bacterial contamination. As will be noted from table 3, neither in vitro irradiation of sheep spleen homogenate to 30,000 r., nor addition of penicillin plus streptomycin, in any way affected its inhibitory activity.

Test for Inhibitory Activity of Nucleoprotein Components of the Homogenate

Though the results so far pointed to the likelihood that the active principle in sheep spleen, effective against the leukemogenic action of x-irradiation, was a heat-labile protein, the possibility of a nucleoprotein or nucleic acid component being responsible had to be examined, in view of the earlier claims that the inhibiting factor against the lethal effects of irradiation and that involved in the regeneration of the thymus appeared to be associated with nucleic acids.

In the present experiment, tests were carried out with 7000 g. supernatant and dialyzed supernatant as controls, and with supernatant treated with DNAase, with separated nucleic acid fraction of the supernatant (i.e. RNA fraction, since most of the DNA had been removed in the precipitate fraction), and with the nucleic acid fraction treated with DNAase and RNAase respectively.

The results of this experiment (table 4) are less clear-cut, because the inhibition with the supernatant control was unusually low—33 per cent, as compared to 48 per cent as the average of the earlier series; though the inhibition produced by the dialyzed supernatant in this experiment was as high as 47 per cent. It is clear, nevertheless, that addition of DNAase to the supernatant did not depress its inhibitory activity, while the nucleic acid fraction of the supernatants showed only borderline inhibition.

Fractionation with Ammonium Sulfate

In this experiment, the precipitated protein fractions, after progressive additions of ammonium sulfate to a sample of sheep spleen supernatant
Table 3.—Influence of In Vitro Irradiation of Sheep Spleen Homogenate and of Addition of Antibiotics on Leukemia-Protecting Action

<table>
<thead>
<tr>
<th>Fraction*</th>
<th>Number of Mice Used</th>
<th>Survivors†</th>
<th>Leukemia-Bearing Mice†</th>
<th>p.</th>
<th>Per Cent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Irradiated control)</td>
<td>60</td>
<td>58 (97%)</td>
<td>38 (66%)</td>
<td>&lt;0.001</td>
<td>69%</td>
</tr>
<tr>
<td>Homogenate</td>
<td>60</td>
<td>56 (93%)</td>
<td>12 (21%)</td>
<td>&lt;0.001</td>
<td>71%</td>
</tr>
<tr>
<td>Homogenate irradiated in vitro</td>
<td>60</td>
<td>59 (98%)</td>
<td>12 (20%)</td>
<td>&lt;0.001</td>
<td>73%</td>
</tr>
<tr>
<td>Homogenate plus antibiotics</td>
<td>60</td>
<td>53 (88%)</td>
<td>10 (19%)</td>
<td>&lt;0.001</td>
<td>73%</td>
</tr>
</tbody>
</table>

*All injections x 10.
†See table 1.

Table 4.—Influence of Nucleoprotein Components of Homogenate

<table>
<thead>
<tr>
<th>Fraction*</th>
<th>Number of Mice Used</th>
<th>Survivors†</th>
<th>Leukemia-Bearing Mice†</th>
<th>p.</th>
<th>Per Cent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Irradiated control)</td>
<td>50</td>
<td>49 (98%)</td>
<td>37 (76%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7000 g. supernatant</td>
<td>60</td>
<td>57 (95%)</td>
<td>29 (51%)</td>
<td>&lt;0.001</td>
<td>47%</td>
</tr>
<tr>
<td>Dialyzed supernatant</td>
<td>50</td>
<td>47 (94%)</td>
<td>19 (40%)</td>
<td>&lt;0.001</td>
<td>47%</td>
</tr>
<tr>
<td>Supernatant plus DNAase</td>
<td>40</td>
<td>36 (90%)</td>
<td>14 (39%)</td>
<td>&lt;0.001</td>
<td>48%</td>
</tr>
<tr>
<td>Nucleic acid fraction</td>
<td>50</td>
<td>42 (84%)</td>
<td>27 (64%)</td>
<td>&lt;0.3</td>
<td>15%</td>
</tr>
<tr>
<td>NA plus DNAase</td>
<td>40</td>
<td>39 (98%)</td>
<td>25 (64%)</td>
<td>&lt;0.3</td>
<td>15%</td>
</tr>
<tr>
<td>NA plus RNAase</td>
<td>40</td>
<td>38 (95%)</td>
<td>24 (63%)</td>
<td>&lt;0.3</td>
<td>16%</td>
</tr>
</tbody>
</table>

*All injections x 20.
†See table 1.

(after 30,000 g. centrifugation), and subsequent dialysis to remove the salt, were tested for inhibition in the standard fashion, and compared with dialyzed whole supernatant. The results (table 5) show partial separation of the active principle, the highest activity being in the precipitate with 30 per cent ammonium sulfate, but with appreciable activity in several of the other fractions.

**DISCUSSION**

With the completion of the earlier experiments published in a preliminary communication, and the addition of new supporting data, the presence of a humoral factor ("RLP") in sheep spleen, capable of inhibiting radiation leukemogenesis in mice, seems now well established.

Certain aspects of the work call for special consideration and discussion, as a basis for further studies, namely, (1) the nature of the humoral factor; (2) whether it is identical with, or at least related to, the factor(s) involved in (a) the prevention of the acute lethal action of large doses of x-irradiation and (b) the stimulation of thymic recovery following the sharp loss of weight of the organ resulting from even moderate doses of radiation; and (3) how precisely "RLP" acts in the body.
### Some Properties of “RLP”

#### Table 5.—Influence of Fractions Obtained by Ammonium Sulfate Precipitation

<table>
<thead>
<tr>
<th>Fraction*</th>
<th>Protein Concentration mg./ml.</th>
<th>Number of Mice Used</th>
<th>Survivors†</th>
<th>Leukemia-Bearing Mice†</th>
<th>p</th>
<th>Per Cent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>— (Irradiated control)</td>
<td>...</td>
<td>40</td>
<td>36 (90%)</td>
<td>20 (56%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>30,000 g. supernatant</td>
<td>53.8</td>
<td>40</td>
<td>37 (93%)</td>
<td>9 (24%)</td>
<td>&lt;0.01</td>
<td>56%</td>
</tr>
<tr>
<td>Dialyzed supernatant</td>
<td>50.1</td>
<td>40</td>
<td>37 (93%)</td>
<td>4 (11%)</td>
<td>&lt;0.001</td>
<td>81%</td>
</tr>
<tr>
<td>Ppt. after 30% Am. sulf. saturation</td>
<td>27.6</td>
<td>40</td>
<td>30 (75%)</td>
<td>3 (10%)</td>
<td>&lt;0.001</td>
<td>82%</td>
</tr>
<tr>
<td>Ppt. after 40% Am. sulf.</td>
<td>15.3</td>
<td>40</td>
<td>37 (93%)</td>
<td>9 (24%)</td>
<td>&lt;0.01</td>
<td>56%</td>
</tr>
<tr>
<td>Ppt. after 50% Am. sulf.</td>
<td>15.5</td>
<td>40</td>
<td>40 (100%)</td>
<td>13 (33%)</td>
<td>&lt;0.05</td>
<td>42%</td>
</tr>
<tr>
<td>Ppt. after 60% Am. sulf.</td>
<td>24.4</td>
<td>40</td>
<td>37 (93%)</td>
<td>12 (32%)</td>
<td>&lt;0.05</td>
<td>42%</td>
</tr>
<tr>
<td>Ppt. after 100% Am. sulf. Supernatant after 100% Am. sulf.</td>
<td>41.6</td>
<td>40</td>
<td>38 (95%)</td>
<td>22 (58%)</td>
<td>&lt;0.9</td>
<td>0%</td>
</tr>
</tbody>
</table>

*All injections x 10. Since the ammonium sulfate fractions, after resuspension, were dialyzed to remove the salt, the results obtained should be compared with the dialyzed supernatant.
†See table 1.

Since “RLP” is (1) heat-labile, (2) nondialyzable, (3) not present in significant amounts in the nucleic acid fraction of the crude material, (4) not inactivated by DNAase, and (5) recoverable in active form after precipitation with 30 per cent ammonium sulfate, one may tentatively presume that it is a protein of high molecular weight. (Further fractionation experiments are in progress, in an attempt to characterize the active principle, and to obtain it in a more concentrated, and hopefully more active form.)

Before turning to the question whether “RLP” is identical with, or related to, the factor(s) involved in the reversal of the acute lethal action and the subacute thymic depression, some consideration might be given to the broader aspect of prevention of radiation damage, whether by whole cells or cell-free material, with respect to the genetic compatibility of the sources of material.

It has been shown that for protection against the acute lethal action of x-irradiation, not only syngeneic but also allogeneic bone marrow cells or spleen cells, and even cell-free extracts of these, are more or less effective, whereas in the case of stimulation of thymic recovery from radiation damage, or inhibition of radiation leukemogenesis, only syngeneic cells or their nuclei have been reported to be effective. This difference in degree of specificity would seem to be due to the fact that the large doses of irradiation, needed to produce the lethal effect, cause at the same time...
depression of the immune response of the animal, thus permitting foreign cells to survive in vivo, which presumably continue to produce the hypothetic humoral factor. This is not so with the moderate doses of irradiation involved in thymic regeneration or in leukemogenesis. It was therefore to be expected that extracts of a tissue foreign to the animal (sheep spleen), administered repeatedly in large doses, might be effective in inhibiting the leukemogenic response. This was indeed found to be the case. It is also conceivable that, using a similar technic, recovery of thymic damage might also have been facilitated with cell-free extracts of allogeneic origin. This assumption would not mean, however, that the factors involved in the different forms of radiation damage are necessarily identical. Therefore it leaves the question of identity or nonidentity of “RLP” with the other cell-free factors still unanswered.

There is little evidence, from the present series of experiments, to indicate the probable mode of action of “RLP.” The fact that removal of the low molecular weight, dialyzable (and relatively toxic) portions of the supernatant fraction did not diminish the inhibitory activity, and that a lactalbumin hydrolysate was devoid of inhibitory activity, argue against a nonspecific “stress-mediated” mechanism. The fact that sheep kidney homogenate showed only borderline activity, is also in favor of a specific type of action on the part of sheep spleen homogenate and extracts. On the other hand, it is noteworthy that, in spite of all efforts to obtain a clean separation, slight activity persisted in almost all the fractions. The possibility remains, therefore, that in addition to strong inhibition by a specific component, slight inhibition may also result from nonspecific effects.

It is important to emphasize once again that the “leukemia” in question, in the C57BL mice used in these studies, is of a distinctive type, in which the pathologic lesion first appears in the thymus as a localized lymphosarcoma, subsequently disseminating to other organs (lymph nodes, spleen, liver, etc.), and only later developing into a typical blood-borne lymphatic leukemia in some of the mice that survive long enough.

We have evidence (as yet unpublished) that “RLP” has no inhibitory effect on spontaneous leukemia or on chemically induced leukemia in mice. Thus, whatever its precise mode of action, “RLP” is not an antileukemia agent, but one which acts specifically on that phase of radiation damage which eventually leads to thymic lymphosarcoma (and/or leukemia) formation. Whether it would be effective in preventing leukemia development in man after excessive exposure to ionizing radiation, is still an open question.

**Summary**

A cell-free factor, “RLP,” derived from sheep spleen, which is capable of inhibiting radiation leukemogenesis in mice, has been shown to be heat-labile, nondialyzable, not present in significant amounts in the RNA fraction of the crude material, not inactivated by DNAase, and recoverable in active form after precipitation with 30 per cent ammonium sulfate. From the evidence so far available, it thus appears that “RLP” is a protein of high molecular weight.
Only borderline inhibition was obtained with extract of sheep kidney, and no inhibition with lactalbumin hydrolysate, thus supporting the view that the inhibitory effect of sheep spleen extracts is probably the result of a specific action and not brought about by a nonspecific, "stress-mediated" mechanism.

**SUMMARIO IN INTERLINGUA**

Le abbreviation "RLP" representa le phrase “protection contra leucemia per radiation.” Le abbreviation es usate como designation de un factor acellular que es derivate ab le splen de oves e es capace de inhibir leucemio-genese radiatori in muses. Il ha essite demonstrate que le factor es thermodabile, non dialysabile, non presente in quantitates significative in le fraction de acido ribonucleic del material crude, non inactivate per acidase deoxyribonucleic, e recovrabile in forma active post precipitation con 30 pro cento sulfato de ammonium. Le datos colligite usque nunc indica assi que "RLP" es un proteina de alte peso molecular.

Un inhibition de grado solmente marginal esseva obtenite con un extracto de ren ovin e nulle del totol con hydrolysato lactalbumini. Isto presta stmpporto al conception que le effecto inhibitori del extracto de splen ovin es probablemente le resultato de un action specific e que illo non es attribuibile a un nonspecific mechanismo de initiation per stress.

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Some Properties of "RLP"—A Factor from Sheep Spleen Capable of Inhibiting Radiation Leukemogenesis in Mice

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