INTRODUCTION

Recent reports of the production of leukemia or leukemia-like disease in mice subsequent to inoculation of cell-free extracts of infected tissue have reawakened interest in the possibility of a virus etiology for some forms of cancer in addition to those already described (11). The agent used in the present study was described by Friend (1). It produces an enlargement of the spleen of mice within approximately a week, with an accumulation in the spleen of stem cells. Furth (2) has stated that the first cellular change is the production of neoplastic reticulo-endothelial cells about 3 days after infection. This is followed by a benign lymphocytosis and erythroblastosis. Furth (2) and Friend (1) call the disease leukemia, but there is not universal agreement that leukemia is the proper designation for the disease. We will, for convenience, call it the Friend leukemia agent.

Several investigators have reported that there is an increased uptake of $^{32}P$ by tissues of animals with transplanted leukemia, including spleen and liver (4, 10). In most of these investigations one or more inoculations of the isotope were given at the beginning of the experiment. The amount of isotope remaining in the tissues was then determined at several times during the course of the disease. Under these conditions the difference in uptake of $^{32}P$ by two tissues is determined by many physiological factors in addition to the metabolic activity or turnover of the biochemical components of the cell. These factors would include differences in excretion, synthesis, or breakdown of tissue components, changing hormone patterns during the course of the disease, and others.

Previous reports from this laboratory (7-9) have indicated that early metabolic changes can be detected in virus-infected animal tissues by the measurement of the uptake of suitable isotopes after a short exposure to the isotope. By the use of this technic in animals it was felt that the effect of slower physiological changes would be minimized. In continuance of this work the present investigations were undertaken to gain some background about the metabolic alterations induced by infection with the Friend leukemia agent. This report deals with the effect of infection with this agent on the short-term uptake of $^{32}P$ by the spleens and livers of mice as well as the effect of cortisone or x-ray treatment on such uptake. Comparison with certain other diseases involving the spleen, including transplanted and spontaneous leukemia, is also presented.

MATERIALS AND METHODS

Animals.—Most of the work was done with female Swiss mice, random-bred at NIH, approximately 16–18 gm. in weight at the time of infection. When other animals were used, they are specifically mentioned.

Virus.—The virus used was that described by Friend (1), to whom we are indebted for starting material. It was prepared from a 10 per cent homogenate of infected spleens by filtration through a Selas 03 filter. Adequacy of the filtration was tested by the addition of $Serratia marcescens$ before filtration and the establishment of sterility after filtration. The route of inoculation was intravenous (I. V.), and the dose was 0.5 ml. of a filtrate titering approximately $10^4$ ID$_{50}$/0.2 ml. These titers were obtained by serial ten-fold dilutions of the original filtrate. An increase in spleen weight in 50 per cent of the mice to 500 mg., as compared with the normal of 200 mg. or less, was used as the criterion for infectivity endpoint (Reed and Muench Method).

Cortisone.—Cortisone was given intramuscularly at a dose of 1 mg/day, for the times indicated in the experiments.

X-ray.—In the experiments with x-ray, the
mice received a total-body irradiation of 300 r, in a Westinghouse 250-kv dual tube machine, at a rate of 126.5 r/min at a distance of 54 cm. from each tube. The animals were in plastic cages and were in the beam all the time. The machine was operated at 200 kv at 15 ma. with a 0.25-mm. Cu and a 0.55-mm. Al filter.

RESULTS

It was found in preliminary experiments that infected spleens took up more P32/mg dry tissue than did the uninfected ones. Two experiments were then performed as follows to study the time course of infection on P32 uptake: 150 mice were inoculated I.V. with 0.2 ml. of undiluted virus (titering 10^3.8 ID50/0.2 ml. for Exp. 1 and 10^4.3 ID50/0.2 ml. for Exp. 2); 150 other mice were given either nothing (Exp. 1) or 0.2 ml. of a similar extract prepared from normal spleen (Exp. 2). On the days post-inoculation indicated in Charts 1, 2, and 3, six infected and six control mice were selected at random for observation on that day. At 10:30 A.M. each of these six mice received 5 μc. of P32 in 0.5 ml. of 0.15 M sodium chloride solution by intraperitoneal (I.P.) inoculation. After 3 hours the mice were killed by cervical fracture, the spleens rapidly removed, weighed wet, transferred to weighed steel planchets, dried 2 hours at 125°C, reweighed, and the radioactivity counted. Because the larger spleens characteristic of the later stages of the disease could not be satisfactorily handled this way, the whole spleen was weighed wet, and then a longitudinal slice was taken for drying and counting. Charts 1 and 2 summarize the data from the two experiments of this type. The average wet weight of the six infected spleens and of the six control spleens, as well as their specific activities as counts/min/mg dry weight of spleen tissue are plotted as a function of time after infection. Chart 3 shows the percentage increase of specific activities for the infected tissues as compared with the control tissues. It will be seen that the infected spleens took up more P32/mg dry tissue than did the controls and that this stimulation began prior to increased growth rather than simultaneously or subsequently. This early stimulation was seen in two out of two additional experiments designed primarily for other purposes. The statistical reliability of the radioactivity data was high, the
CHART 2 (Exp. 2).—Effect of Friend agent on uptake of $^{32}$P by spleen, with and without x-ray treatment; 300 r of total-body radiation was administered on the 5th day.

CHART 3.—Percentage increase in specific radioactivities of infected spleens as compared with noninfected spleens.
P values associated with the differences between the means for control and infected tissues being 0.005 or lower for almost all sets of observations.

In Exp. 2 the uptake of P³² by liver was also studied. No differences were found between the livers of the infected and the control mice. However, liver pathology is not usually seen until about 60 days after infection, and these experiments were not carried that long.

The time course of the effect on P³² uptake indicates that the infection was responsible for the stimulation of P³² uptake, rather than some non-infectious material contained in the preparation used. Additional confirmatory evidence was obtained through the use of Gradocol membranes. A 520-μm membrane which let through infectious material also let through the material that stimulated uptake. A 220-μm membrane which held back infectious material held back the stimulating material.

1 We are indebted to Dr. Wallace P. Rowe of these laboratories for the animals that had received the Gradacol membrane filtrates.

D R Y W E I G H T/W E T W E I G H T R A T I O

Another alteration that occurred almost without exception was that the ratio of spleen dry weight to wet weight was slightly lower in the infected animals than it was in the controls. This can be seen from Chart 4, where the average percentage dry weight of six animals per point is plotted against time after infection. This increase in water was seen regularly 1–2 days after the virus was given. Similar effects have been reported repeatedly for cancerous tissues (13).

Before any conclusions can be drawn about the specificity of the observed phenomenon it is necessary to consider four additional observations which relate to the increased P³² uptake in the infected spleens.

1. Possible alteration in external spleen permeability.—Since the P³² was given I.P., it was necessary to consider the possibility that the infectious process damaged the capsule of the spleens, that this damage resulted in increased permeability at the surface of the spleen, and that this increased external permeability was responsible for the increased P³² uptake. However, when P³² was given intravenously the same enhancing effect of infection was observed.

2. Effect of spleen growth rate or size increase.—One important question is whether or not this increased P³² uptake is solely a manifestation
of an increased growth rate of spleen. Several pieces of evidence suggest that increased size is not the sole determining factor. In the first place, the increased uptake of $P^{32}$ preceded the increased growth by 1-2 days. In the second place, examination of the individual uptakes of the six spleens in a group more frequently than not showed that the larger ones had a smaller uptake of $P^{32}/$mg than did the slightly smaller ones. These somewhat subjective observations are substantiated by more objective evidence presented below.

The uptake of $P^{32}$ decreased in both the infected and control spleens (Chart 2), but examination of Chart 3 shows that the irradiated infected spleens showed as great a percentage stimulation in $P^{32}$ uptake as did the unirradiated ones. A similar experiment in which 1 mg. cortisone was given daily beginning the day after virus inoculation is summarized in Chart 5. (The data shown for the first 2 days on the curves were obtained with 1 mg. of cortisone immediately after the inoculation with virus; all the other mice received injections of 1 mg cortisone/day beginning the day after virus inoculation.

**Chart 5.—Effect of early treatment with cortisone on increased $P^{32}$ uptake by spleens of animals infected with Friend agent. The mice killed at 1 and 2 days were injected**

**Cortisone and X-Ray**

As adjuncts to Experiments 1 and 2 (Charts 1, 2, and 3), two agents were used which cause temporary remissions in certain leukemias and lymphomas, namely, cortisone and x-ray. Cortisone was given daily to some of the large group of animals used in Exp. 1, beginning on the 8th day after infection. The weights of the spleens decreased to that of the control spleens, and on the basis of this gross criterion disease would not be detected. However, the uptake of $P^{32}$ was as high in these spleens as it was in the enlarging spleens (Charts 1 and 3). Similarly, in Exp. 2 x-ray was given to some of the animals on the 5th day of infection. The spleen weights from mice which received cortisone beginning immediately after virus inoculation.)

The data indicate that the increased $P^{32}$ uptake is manifest even after the early administration of cortisone. However, in one experiment in which 300 r of whole-body x-radiation was administered just subsequent (30 minutes) to the administration of virus, there was no stimulation of $P^{32}$ uptake in the infected animals until the weights of their spleens started to increase.

Determination of the amount of virus in the spleens of mice one week after they had received virus and either Cortisone or x-radiation within 1 day after infection indicated approximately 1/10 the amount of infectious virus that was found
in the spleens of mice that had received the virus without treatment. Friend has shown that the virus is stable in vitro to at least 50,000 r of x-radiation (1).

3. Plasma phosphorus levels.—The higher P\textsuperscript{32} concentration in the infected spleens might be attributable to a possibly higher P\textsuperscript{32} in the blood plasma of the infected animals. In Experiment 2 (Chart 2), the P\textsuperscript{32} contents of the plasma and of the washed blood cells (combined red and white) were determined, as well as plasma inorganic phosphate. The results are summarized in Chart 6. At each point the values were obtained from the pooled bloods of six infected and six control animals.

The inorganic phosphate levels were constant during the period of observation and about the same for both groups. The P\textsuperscript{32} of the cells and plasma in the control animals could be expressed as a fairly smooth curve, but those of the infected animals fluctuated widely from day to day, as though the animals' absorption or utilization of P\textsuperscript{32} was not stable. Radioactivities of the plasma and the blood cells tended to run parallel.

4. Effect of other diseases with spleen involvement. —To determine whether increased P\textsuperscript{32} uptake is a general accompaniment of spleen pathology, several other diseases characterized by spleen involvement were studied. These included:

a) Lymphocytic choriomeningitis (LCM). A strain of LCM virus carried by intraperitoneal passage of a suspension of spleen, kidney, and liver was given to groups of six mice, either intraperitoneally, intramuscularly, subcutaneously, or intravenously in doses of 0.1 ml of 10\textsuperscript{5} or 10\textsuperscript{6} dilution. This dose will generally be fatal to mice in about 8 days, with virus growth demonstrable by about 2 days. Pathologic changes in the spleen could be seen at 5 days, so earlier studies were not done (6). The data reported in Table 1 were obtained from animals sacrificed on the 6th day after I.P. inoculation. The results with other routes of inoculation were the same.

b) Histoplasma capsulatum. Random-bred white Swiss mice, of about 19 gm. weight, were injected I.V. with 5 \times 10\textsuperscript{5} organisms, as determined by direct hemocytometer count.

c) Lymphocytic leukemia: Originally received from Dr. Stephen O. Schwartz and carried in this laboratory by cell passage in C3H/P mice (12). The animals were used 14 days after the tumor cells were inoculated.

d) Hodgkin's-like lymphoma, P-195, in DBA/2 mice: These animals were used 14 days after receiving an intraperitoneal inoculation of a 10 per cent spleen suspension from the preceding generation.

e) A lymphocytic leukemia, HE 11764 G-4\textsuperscript{3} in C3H/P mice: Age of tumor and transfer procedure as in (d) above.

f) Spontaneous leukemia in AKR/Lw mice: Beginning at about 7\textsuperscript{1/2}–8 months of age, this strain of mice develops a spontaneous lymphocytic neoplasm, ultimately involving 90 per cent of the population.

g) Virus-induced lymphocytic leukemia: C3H mice (Bittner strain) were inoculated with the cell-free agent described by Gross (3) when less than 2 weeks of age. They were used when definite

\textsuperscript{3} Kindly supplied by Dr. Lloyd Law of the National Cancer Institute.

\textsuperscript{4} Dr. Ludwik Gross of the Bronx Veterans Hospital kindly supplied us with animals that he had previously inoculated.
enlargement of the spleen was detectable, which was within 3-6 months after inoculation of the virus.

In all cases the infected and control animals received P32 intraperitoneally 3 hours before sacrifice. The amount of P32 given was different for some of the diseases and ranged from 2 to 20 \( \mu \)c/animal. Therefore, the absolute levels of radioactivity in the spleens were different from group to group. At least six animals of each type were used to obtain the average values recorded in Table 1.

Cortisone or x-ray reduced the spleen size to normal or below normal. In spleens so treated disease would not be detectable by gross examination. However, these treated spleens still show the phosphorus uptake pattern characteristic of diseased tissue. These data suggest that growth alone is not the sole factor responsible for the increased P32 uptake. Since the spleens from these treated animals sooner or later resumed the growth pattern seen with infected spleens that did not receive therapy, it will be of interest to see what their histological appearance is. Such studies are currently under way. At any rate, the recurrence of gross disease should not be surprising in view of the abnormal metabolic picture.

All the diseases listed in Table 1 were characterized by pathological changes in the spleen, with or without spleen enlargement. However, with the exception of the spontaneous and the virus-induced leukemia, none was associated with more than a slight increase in P32 uptake, if any.

DISCUSSION

The data presented indicate that the uptake of P32 by spleens is increased in mice receiving the virus described by Friend as producing a leukemia-like disease. This increase is detectable even before the spleens show the enlargement characteristic of the disease.

All the diseases listed in Table 1 were characterized by pathological changes in the spleen, with or without spleen enlargement. However, with the exception of the spontaneous and the virus-induced leukemia, none was associated with more than a slight increase in P32 uptake, if any.

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>Av. WET SPLEEN</th>
<th>AV. DRY SPLEEN</th>
<th>PERCENTAGE INCREASE</th>
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<tbody>
<tr>
<td>Lymphocytic choriomeningitis</td>
<td>(mg.)</td>
<td>(mg.)</td>
<td>24.8</td>
</tr>
<tr>
<td>Histoplasma capsulatum</td>
<td>508</td>
<td>247</td>
<td>285</td>
</tr>
<tr>
<td>Lymphocytic leukemia (transplanted)</td>
<td>263</td>
<td>68</td>
<td>640</td>
</tr>
<tr>
<td>Leukemia P-185 (Hodgkin's-like) (transplanted)</td>
<td>613</td>
<td>187</td>
<td>62</td>
</tr>
<tr>
<td>Leukemia HE-11764 G-4 (transplanted)</td>
<td>154</td>
<td>147</td>
<td>49</td>
</tr>
<tr>
<td>Spontaneous AKR leukemia:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Spleen</td>
<td>415</td>
<td>75</td>
<td>123</td>
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<tr>
<td>Thymus</td>
<td>(71)</td>
<td>(19)</td>
<td>174</td>
</tr>
<tr>
<td>Gross-virus-induced leukemia:</td>
<td></td>
<td></td>
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<tr>
<td>Spleen</td>
<td>751</td>
<td>57</td>
<td>548</td>
</tr>
<tr>
<td>Thymus</td>
<td>(21)</td>
<td>(0)</td>
<td>396</td>
</tr>
</tbody>
</table>

At least six experimental and six control mice were used, and in most cases two or three such groups were used. Differences of less than 20 per cent are not considered significant.

* For reasons not relevant to these experiments, differing amounts of P32 were given to the different groups of animals. Therefore, the uptake by controls varied considerably.

† Percentage increase in counts/min/mg in infected tissue as compared with control.

‡ Average thymus dry weight.

It might be pointed out that some human leukemias and lymphomas, the Friend leukemia, and some spontaneous mouse leukemias show a similarity in being sensitive to cortisone and x-ray and in having an accelerated P32 uptake picture (4, 10). This is in contrast to at least those transplanted mouse leukemias reported here, which do not show accelerated P32 uptake under our conditions and which in general are not sensitive to cortisone or x-ray (5). In these respects, at least, the Friend leukemia behaves more like the
This increased rate of $^{32}\text{P}$ uptake was demonstrable even before the spleens showed the enlargement characteristic of the disease. Subsequent treatment with cortisone or x-ray led to a reduction in spleen size to normal. Nevertheless, these treated spleens showed the increased $^{32}\text{P}$ metabolism of untreated infected spleens. Several other diseases characteristically showing spleen pathology were studied in a similar way. Of these only spontaneous leukemia in AKR mice and in other virus-induced leukaemia (Gross) showed a similar effect.

**REFERENCES**


The Effect of a Leukemia Virus on Phosphorus Uptake by Mouse Spleen

Hilton B. Levy and Isadore Brodsky


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