Phosphorus Metabolism in Infection with Murine Leukemia Virus. (25574)

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Our previous reports(1,2) indicated that there is increased *in vivo* uptake of P^{32} by spleens of mice infected with leukemia-inducing virus described by Friend(3), as compared with non-infected controls. This increased uptake of P^{32} occurs before the infected spleen shows enlargement characteristic of the disease. At this early stage of infection little or no pathological alterations can be seen, and little or no new virus has been formed. Under conditions in these tests stimulation of P^{32} uptake is to some degree specifically related to this disease, in that it is not seen in some other diseases characterized by spleen enlargement or pathological changes. These other diseases are histoplasmosis, lymphocytic choriomeningitis, and several transplanted leukemias. On the other hand, spontaneous leukemia in AKR mice and a leukemia induced by Gross's virus (4) do show the stimulated P^{32} uptake. As with some human leukemias and lymphomas, the disease produced by the Friend virus undergoes temporary remission on treatment with X-ray. In most cases the spleen in remission shows presence of many abnormal cells characteristic of the disease. Consistent with this latter observation, the P^{32} uptake of spleens in X-ray induced remission is higher than that of spleens from X-ray treated control animals(2). A more detailed examination of phosphorus metabolism of infected spleens was therefore undertaken. The effect of X-ray was also studied.

Methods. Virus and mice. Preparation of virus, method of infection, treatment with X-ray and mice used have been described(1). *Fractionation and analytical methods.* The general plan of experiments was as follows: About 20-40 infected and equal number of control mice were given 5-15 μc P^{32} i.p., and sacrificed 3 hours later, when spleens were removed, chilled and fractionated. Several experiments were done in which biochemical fractions of whole cells were separated by the Schneider, Schmidt-Thannhauser method(5). Results by this procedure were consistent with those obtained by subcellular fractionation combined with biochemical fractionation of subcellular elements, and will not be presented separately. Subcellular fractionation was performed as follows: spleens of mice killed by cervical fracture were perfused *in situ* with cold 0.85% NaCl, about 3-7 ml/mouse, by inserting 27-gauge short needle successively into several places in the spleen and gently expressing perfusing fluid from the syringe. The perfusate contained mostly red cells which otherwise would have formed the bulk of the nuclei preparation. Spleens were collected in ice cold 0.25 M sucrose and fractionated into subcellular particles using 0.25 M sucrose according to the method of Schneider and Hogeboom, as given in Umbreit, *et al.*(6), except that the nuclei were sedimented twice at 1000 rpm for 10 minutes in International rotor No. 253, and once at 2000 rpm for 10 minutes the mitochondria were sedimented in Servall SS-1 centrifuge at 9000 rpm for 10 minutes.*

Results. Experiments were performed on animals infected from 2 to 10 days. The earliest that metabolic changes were detected was 3 days after infection, when spleens had not yet enlarged. The data presented are restricted to 3-day, and 10-day infection, when marked spleen enlargement had occurred.

The effects of 3 days of infection on concentration of phosphorus in lipid and nucleic acid fractions of subcellular elements, and on uptake of P^{32} into these fractions are shown in Table I. With respect to phosphorus up-

* In spite of perfusion of spleen, a great many red blood cells were in the nuclei fraction and some in the mitochondria fraction. It was sometimes necessary to resort to additional high and low speed cycles of centrifugation to reduce number of red blood cells. Judging by loss of DNA this additional washing resulted in loss of 20-40% of the original nuclei. Because of these losses the γ P/mg figures for nuclei and mitochondria represent only minimal values, but they serve to indicate relative values for infected and control tissues.

TABLE I. Alteration in Phosphorus Metabolism Associated with 3-Day Infection with Friend Virus.

	Infected		Control		Ratio: Spec. act. infected/Spec. act. control
	γP/mg	S.A., cpm/γP	γP/mg	S.A., cpm/γP	
Nuclei					
Lipid	.25	24	.24	25	.96
Nucleic acid	1.65	30	1.61	32	.94
Mitochondria					
Lipid	.18	31	.21	29	1.05
Nucleic acid	.02	142	.02	132	1.08
Microsomes					
Lipid	.45	22	.54	18	1.23
Nucleic acid	.36	22	.38	18	1.23
Supernatant					
Lipid	.08	78	.10	44	1.80
Nucleic acid	1.43	29	1.26	20	1.45

take the early effects (3 days) of the virus were restricted to stimulation of the microsome and supernatant fractions. In 2 other experiments the results were qualitatively the same although S.A.'s varied from experiment to experiment.

Table IIA presents comparable data for a 10-day infection. Specific activities of fractions from 10-day infected tissues are all higher than from uninfected ones, in all 4 experiments. In general, *concentrations* of several P fractions in infected spleens, particularly DNA phosphorus, were higher than those of control spleens, but not always so.

Data of Table IIA were obtained from half of group of mice. The other half was X-rayed on day 8 of infection, and given P^{32} and killed on day 10. Then average spleen weights were the same in X-ray infected and X-ray control animals, 0.065 g, as compared to 1.14 g for unirradiated infected and 0.184 g for unirradiated controls. Spleens of X-irradiated infected animals had about 1 log unit less virus than the non-irradiated ones. Data are summarized in Tables IIB and III.

It is clear that X-irradiation reduces concentration of nuclear nucleic acid in both infected and control tissues, and reduces P^{32} uptake into virtually all fractions in both groups. However, Table III shows that P^{32} uptake into fractions, particularly nucleic acid fractions, from infected tissues is more

TABLE II. Alteration in Phosphorus Metabolism Associated with 10-Day Infection with Friend Virus.

	A. Non X-rayed				B. X-rayed			
	Infected		Control		Infected		Control	
	γP/mg	S.A.	γP/mg	S.A.	γP/mg	S.A.	γP/mg	S.A.
Nuclei								
Lipid	.77	348	.71	127	.44	132	.64	109
DNA	2.3	400	2.7	273	.54	130	.37	168
RNA	1.8	395	1.2	300	.46	114	.43	116
Mitochondria								
Lipid	.46	363	.27	159	.64	219	.72	150
RNA	.22	309	.09	212	.10*	98*	.09*	194*
Microsomes								
Lipid	.56	389	.40	235	.38	237	1.01	154
RNA	.83	394	.31	132	.17	359	.23	322
Supernatant								
Lipid	.37	895	.27	258	.31	245	1.10	175
RNA	3.80	452	.71	277	.26	242	1.10	185

* Sample lost. These data from previous experiment.

TABLE III. Ratio of Specific Activities of Fractions from Infected Spleen to Those from Controls.

	Non X-ray	X-ray	% reduction by X-ray
Nuclei			
Lipid	2.74	1.21	56
RNA	1.32	.98	26
DNA	1.47	.77	48
Mitochondria			
Lipid	2.28	1.46	36
RNA	1.46 (1.82)*	(.51)*	(72)*
Microsomes			
Lipid	1.65	1.53	7
RNA	2.99	1.11	63
Supernatant			
Lipid	3.46	1.40	60
RNA	1.63	1.31	20

* Sample lost. These data from previous experiment.

strongly inhibited than those from controls.

Discussion. If one assumes that on day 3 of infection with Friend leukemia agent, most infected cells are in early phases of virus reproduction, then it appears that re: phosphorus metabolism, the first detectable effect† of the virus stimulates the microsome and subparticulate RNA and lipid. This could mean that virus RNA combines with microsomal particles to act as templates for manufacture of new virus materials, and is consistent with current thoughts on the role of microsomal particles and subparticulate elements in the synthesis of protein and RNA. (Previous work with fluorescent antibody(2) showed viral antigen in the cytoplasm of infected cells.) This hypothesis, however, is but one of several that could be used to explain the findings.

Animals infected for 10 days show increased P^{32} uptake into all biochemical fractions. It should be noted that the physical condition of spleens after 10 days infection was not the same for all experiments. Average wet spleen weights in 4 experiments were 0.766, 0.900, 1.14 and 1.46 g with ratios to control spleen weights ranging from 3½ to 7. There were varying degrees of engorgement with blood. In some experiments there was a 2-fold increase in DNA concentration as compared to controls, in some there was none. It may be that DNA concentration was high in spleens that were still growing rapidly, and decreased when growth reached a plateau, but there were not enough experiments to establish this.

X-irradiation reduced spleen size promptly and markedly in both infected and control mice. In experiment reported here in detail, infected spleens were reduced to the same size as controls, even though prior to irradiation, infected spleens averaged 6 times the size of controls. This reduction in infected spleen size after irradiation was a little more marked than usually seen.

It is worthy of mention that increase in P^{32} uptake/mg of tissue occasioned by infection was about as much after irradiation as before, even though irradiation caused a decrease in the ratio:

$$\text{Specific activity of biochemical fraction from } \frac{\text{Infected tissues}}{\text{Control tissues}} \text{ (Table III)} \cdot$$

This is understandable when one realizes that total radioactivity of a tissue depends upon both amount of phosphorus in different fractions, and their specific activities. Consider as an example the following hypothetical situation:

Biochemical fraction	Tissue 1 γP/mg	S.A.	(High spec. act. fractions) cpm/mg tissue
A	20	108	2160
B	10	279	2790
	Total cpm/mg tissue		4950
	Avg S.A.		165

Biochemical fraction	Tissue 2 γP/mg	S.A.	(Low spec. act. fractions) cpm/mg tissue
A	10	80	800
B	20	245	4900
	Total cpm/mg tissue		5700
	Avg S.A.		190

From the above, even with only 2 fractions to work with, distribution of total phosphorus between low and high specific activity fractions can lead the cpm/mg of tissue and average specific activity of Tissue 2 to be higher than those of Tissue 1 even though specific activities of individual biochemical fractions of

† No effects were found prior to day 3, and in some experiments with weaker virus preparations, none were found even on day 3.

Tissue 1 are higher than those of Tissue 2. It is clear therefore that in some cases comparison of uptake of radioisotope by 2 whole tissues may give a misleading idea of their relative metabolic activities. As a corollary it follows that the more detailed the biochemical fractionation, the more meaningful are the data.

Summary. Some aspects of phosphorus metabolism of spleens of mice infected with Friend leukemia agent have been presented. A comparison has been made of concentrations of different phosphorus-containing cellular components as well as uptake of P^{32} into these components. Early in the infection, before significant spleen enlargement occurs, increase in uptake of P^{32} is restricted to microsomal and subparticulate lipid and nucleic acid. Later when spleen enlargement is marked, all phosphorus-containing fractions studied showed increased P^{32} uptake. X-irradiation, which caused marked reduction in spleen size, reduced P^{32} uptake into fractions of both control and infected spleens, with greater reduction in the infected ones.

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Stability Characteristics of Coe Virus.* (25575)

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In preparation for studies of epidemiology of infections due to Coe virus, an agent isolated from the respiratory tract by Lennette and co-workers(1), it was important to investigate stability of this agent and to determine optimum conditions for handling and storing the virus. This paper presents evidence which indicates that Coe virus is relatively stable under varying conditions of pH and temperature.

Materials and methods. Tissue culture. Strain DMB cells derived from nasal mucosa were employed, using methods previously described(2). The nutrient fluid consisted of 40% pooled human serum and 60% Hank's balanced salt solution. Cells were washed 3 times with Hanks salt solution prior to use for virus growth. The maintenance fluid consisted of Scherer's MS 84.5%, chicken serum 8.0%, and tryptose phosphate broth 7.5% (3). Coe virus was supplied by Dr. E. H. Lennette after 6 passages in HeLa cells. Virus pools were prepared following 5 or 6 subsequent passages in DMB cells. *Infectivity Titrations.* Serial 1:3.2 ($10^{-0.5}$) dilutions were prepared in Hanks balanced salt solution, and 0.1 ml of each dilution was inoculated into each of 2 DMB cell culture tubes. Inoculated tubes were incubated in stationary racks at 37°C for 5 days. A culture was considered infected when 50% or more of cells had undergone specific cytopathic changes. Infectivity titer end-point expressed/0.1 ml inoculum was calculated as highest dilution of virus which infected one-half of tubes inoculated. *Alterations of pH.* Viral suspensions were adjusted to the desired pH with 0.5 N HCl or NaOH essentially as described by Ginsberg in a study of adenoviruses(4); in our study pH was measured with Beckman glass electrode meter. After adjustment to various hydrogen ion concentrations, the virus

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