Synergism between a Lactate Dehydrogenase–Elevating Virus and Eperythrozoon coccoides

Abstract. *An interaction occurs when a lactate dehydrogenase–elevating virus and* Eperythrozoon coccoides *are present together in the mouse host. The synergism is expressed by a substantial increase in plasma lactate dehydrogenase, spleen enlargement, host anemia, and quantitative alteration of several blood elements.*

A classical example of synergism between two biological entities is that described by Shope for swine influenza, where both virus and bacteria are required for the host to exhibit the acute disease. Infection by either of the contagious agents alone was shown to be relatively harmless (*1*). Influenza pandemics occurring before the advent of antibiotics, such as that of 1918–19 which caused such a high human mortality, probably represent another example of the synergistic potential of viruses and bacteria when jointly infecting an organism (*2*).

Attention has been directed recently to another biological synergism in the form of a virus-virus interaction—presumably at the nucleic acid level. According to Rubin (*3*), the Rous sarcoma virus is defective and requires an additional "helper virus" to fulfill all of the essential synthetic requirements for its maturation. The presence of this second Rous associated virus determines whether the carcinogenic Rous sarcoma virus is infectious or not.

Another transferable factor, of unknown nature, appears to make it possible for a given virus to overcome species barriers. Specifically, according to Stewart (*4*), when such a factor is combined with the Bryan strain of the Rous sarcoma virus, the latter acquires the new ability to induce tumors in species other than the chicken. The possible significance of such potentiating biological combinations is emphasized by the reported ability of one chicken tumor virus strain to induce tumors in a primate (*5*).

This report describes another in vivo interaction, probably of a metabolic type, occurring between a virus and *Eperythrozoon coccoides*. The virus is a lactate dehydrogenase–elevating agent associated with many transplanted mouse tumors, presumably as a harmless non-oncogenic resident (*6*). *Eperythrozoon coccoides* is a Bartonella-like organism, of uncertain classification, which is frequently present in rodents, causing a low-grade or latent infection, but capable under some circumstances of inducing destruction of erythrocytes and a consequent severe anemia (*7*).

Figure 1 illustrates the most conspicuous aspect of this biological synergism, namely a substantial peak increase in the activity or concentration of a common plasma enzyme, lactate dehydrogenase (LDH). The relatively low normal concentration of this enzyme in the plasma is shown by curve *D* near the base line of the chart. The virus alone induces a permanent increase in plasma LDH of five- to tenfold above normal values (*8*) as illustrated by curve *C* and by Table 1. A high concentration of *E. coccoides* can induce a temporary increase of LDH in the plasma under proper circumstances, but the enzyme activity invariably returns to an essentially normal level (*9*) (curve *B*).

When the two infectious agents are injected simultaneously into a normal mouse, a synergistic increase in plasma LDH activity, several times higher than that produced by either agent alone, is observed. Curve *A* indicates the magnitude and the time relations of this effect. At its peak, LDH activity is about 200-fold higher than that of normal mouse plasma.

The data for this figure were obtained

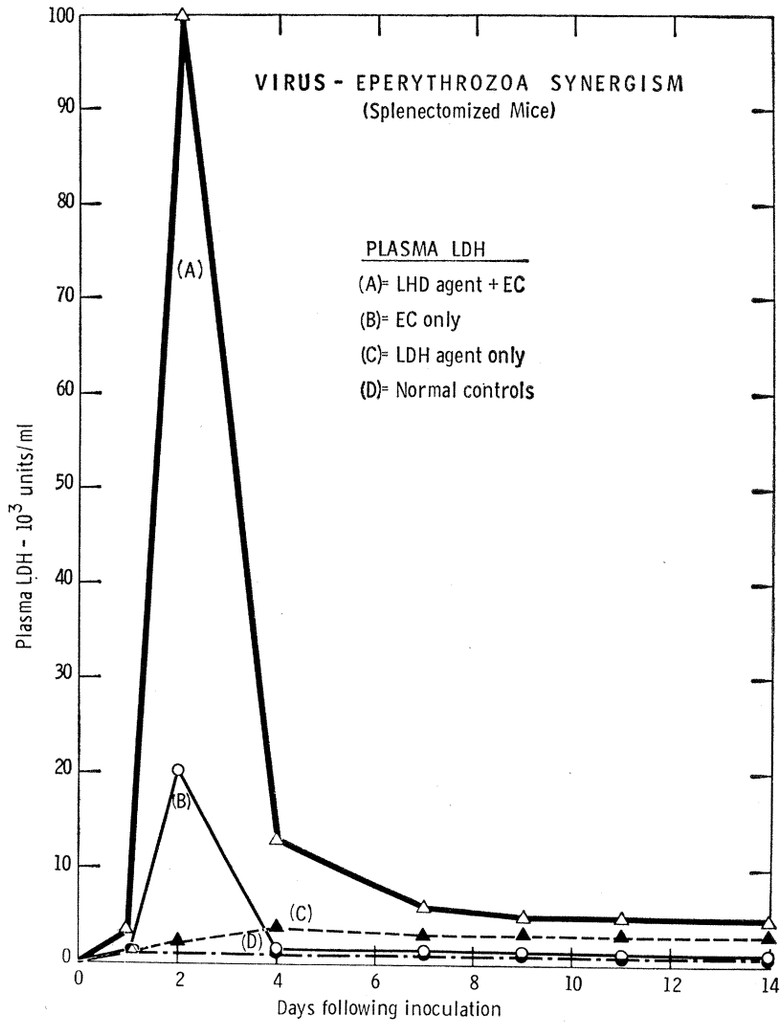

Fig. 1. Synergistic interaction occurring between an LDH-elevating virus and *Eperythrozoon coccoides* (EC) after injection of both agents into Swiss ICR mice. The LDH-elevating agent was injected intraperitoneally as a 0.1 ml dose of diluted plasma containing 10^7 infectious units per milliliter; *E. coccoides* was injected intraperitoneally as 0.1 ml of infected whole blood. A high-titer preparation was obtained by repeated 48-hour passage of whole blood from an infected mouse.

from splenectomized mice, in which the effect is more dramatic than in normal animals. However, the same phenomenon may be observed in nonsplenectomized animals, although the peak LDH concentrations are less. Splenectomy activates latent *E. coccoides* infections, or permits a new inoculation to induce a more virulent destructive effect on the host red cells (*10*). It is presumed that the absence of the spleen permits the eperythrozoa to reach a higher titer, which is responsible for the more severe effects of the disease. The presence of the LDH-elevating virus also appears to assist this organism to increase in titer (Table 1).

The mechanism of the enzyme increase, which results from the dual infection, is not fully understood. However, erythrocytes constitute a logical source of LDH, since they have a high internal concentration of this enzyme and are apparently disrupted during the synergistic disease process. It is perhaps instructive that there is a quantitative inconsistency between the degree of anemia produced and the concomitant plasma LDH observed in the three experimental groups (Table 1).

Other data (*11*) indicate that the LDH-elevating virus reduces the efficiency of the host's enzyme-clearance system. This observation, together with the increased enzyme influx associated with the LDH-elevating virus and *E. coccoides*, is adequate to account for the nonadditive enzyme increase observed with the dual infection. For example, the synergistic phenomenon can be simulated by the injection of equal doses of exogenous enzyme into both normal and virus-infected mice. This is comparable to the extra endogenous enzyme surge which is known to occur during destruction of red cells by either infection with *E. coccoides* or administration of phenylhydrazine (*9*, *12*). Since the time required for removal of the enzyme in the virus-infected host is approximately double that required in the uninfected animal, the excess LDH accumulates temporarily in the infected plasma, whereas it is rapidly removed from the peripheral blood of virus-free animals.

The data in Tables 1 and 2 indicate that the synergistic effect of the two agents is not restricted to the elevation of plasma enzymes. There is evidence that the titer of both infectious entities may be increased when they are present in combination, and this in turn may be responsible for the alteration of the other parameters in the host. With a combination of the two agents, a reduction in the volume of packed red cells (hematocrit) was observed, along with a corresponding decrease in the number of erythrocytes. The number of white cells, however, increased when the animals were infected with both agents; this reflected an absolute as well as a relative neutrophilia. The monocyte population also increased. Comparative values for the various blood elements are given in Table 1.

Table 2 shows the effects of the combined agents on the spleen and the lymph nodes as compared with the alterations caused by either agent alone. Although no synergistic effect was seen in respect to axillary and inguinal lymph node enlargement, a substantial increase in spleen weight was observed in mice in which the two infectious elements were present simultaneously.

In addition to the demonstration of synergistic biochemical and physiological responses in a mammalian host, these observations may have some practical value for investigators working with murine hosts or with their tumor preparations, since the transmissible factors described are widely distributed among rodents, and particularly in association with transplanted mouse tumors (*12*).

In relation to the various synergisms cited, Niven *et al.* (*13*) observed that a fatal hepatitis was produced when *E. coccoides* was present with mouse hepatitis virus. These several observations raise the question of what other synergistic relations may exist between various infectious entities with possible important biological or clinical consequences.

Table 1. Comparison of various blood components of non-splenectomized mice challenged with an LDH-elevating virus, with *Eperythrozoon coccoides* (EC), and with both.

Determination*	Normal controls	Virus	EC	EC and virus
Plasma LDH†	300	3300	1100	10,200
LDH-agent titer‡		$10^{8.5}$		$10^{9.5}$
EC titer§	(–)	(–)	7(+)	11(+)
Packed RBC (% hematocrit)	57	52	45	37
RBC count (10^6)‖	7.9	7.2	7.1	6.0
Reticulocytes (%)	2.8	2.6	5.2	5.0
WBC count (10^3)	8.4	8.6	12.4	21.2
Lymphocytes (%)	80	72	58	47
Lymphocytes (10^3)	7.5	6.2	7.2	9.9
Segmented neutrophils (%)	19	26	39	49
Segmented neutrophils (10^3)	1.8	2.3	4.8	10.4
Relative monocytes (%)	1.6	1.6	3.4	3.2
Monocytes (cells/mm^3)	150	140	420	680

* All determinations were made on day 3 with the exception of those for reticulocytes which were made on day 7, and those for RBC's which were made on day 4. † Plasma LDH determined spectrophotometrically at 340 mμ with $NADH_2$ (DPNH) (reduced nicotinamide adenine dinucleotide) and pyruvate at *p*H 7.4. Values are expressed in conventional Wroblewski units, which equal optical density reduction of 0.001 per minute per milliliter of plasma and may be converted to International Units by dividing by 2 (*9*). ‡ LDH-agent titers were determined by end-point titration and are expressed as the infective dose (ID_{50}) per milliliter of plasma according to the Reed and Muench calculations. The reciprocal of dilution is employed to give number of infectious units per milliliter. § Titer of *E. coccoides* is expressed as relative particle concentration seen microscopically on Giemsa-stained slides. ‖ All absolute blood cell counts are expressed as cells per mm^3, multiplied by the factor indicated.

Table 2. Comparison of weights of spleen, nodes, and carcass of mice injected with an LDH-elevating virus, with *Eperythrozoon coccoides*, and with both. All determinations were made on day 7.

Spleen		Nodes		Carcass
Wt. (mg)	Rel. wt. (mg/g)	Wt. (mg)	Rel. wt. (mg/g)	Wt. (g)
Normal controls				
150	4.5	88	2.7	33
Agent only				
150	5.0	110	3.4	32
EC only				
370	11.6	133	4.2	32
EC + agent				
560	17.5	134	4.2	32

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Phosphorus Excretion and Body Size in Marine Animals: Microzooplankton and Nutrient Regeneration

Abstract. *In marine animals the rate of excretion of dissolved phosphorus per unit weight increases as body weight decreases. As a consequence microzooplankton may play a major role in planktonic nutrient regeneration.*

Animal excretions are a major source of plant nutrients in the sea (*1*). The animals most often studied in this regard are those captured in plankton nets. The smaller species which are not retained by plankton nets, the microzooplankton, are often overlooked by marine ecologists, and their role in nutrient cycles has never been evaluated.

Examinations of unfiltered water samples reveal that microzooplankton may often constitute a considerable fraction of the total animal biomass. Lohmann (*2*), for example, found that protozoa and very small metazoa, including *Rotifera*, copepod eggs, and certain invertebrate larvae, constituted an annual average of over 50 percent of the total zooplankton biomass in the waters off Kiel. A number of investigators have described the abundance of colorless flagellates (*3*) and ciliates (*4*) in the plankton.

It is well known that the smaller the animal the greater the metabolic rate per unit weight. The rate of nutrient excretion of microzooplankton should therefore be higher than that of net zooplankton per unit weight. Accordingly, it seemed worthwhile to attempt to evaluate the relative importance of animals of different sizes in the production of dissolved phosphorus.

For marine animals larger than 1 mg (dry weight), phosphorus excretion rates were determined by measuring spectrophotometrically the total phosphorus content of the animals and their soluble excretions (*5*). Excretion rates of animals weighing less than 1 mg were determined with the radioisotope P^{32} (*6*). Excretion rates are reported here as the time it takes an animal to release an amount of dissolved phosphorus equal to its total phosphorus content. This will be referred to as the body-equivalent excretion time (BEET).

A marked decrease in the body-equivalent excretion time (Fig. 1) is associated with decreasing animal size. The method of least squares leads to the following linear regression equations: For the upper line, log BEET (hours) is equal to 0.67 log dry weight (g) plus 3.2 ($r = 0.96$). For the lower line, log BEET = 0.33 log dry weight plus 2.6 ($r = 0.98$). Both correlations and the difference between the two slopes are significant at the 1 percent level. An analysis of the phosphorus excretion rates of 24 lots of differently sized (0.05 to 0.1 g without shells) mussels, *Modiolus demissus* (*7*), produced the regression equation: log BEET = 0.51 log dry weight plus 3.5 ($r = 0.53$). There was no significant difference between the slope of this regression line and the upper line in Fig. 1.

Whereas a 12-g lamellibranch released an amount of phosphorus equal to its total phosphorus content every 438 days, the body-equivalent excretion time of a 0.6-mg amphipod was 31 hours, and that of an 0.4×10^{-3} μg ciliate was 14 minutes. Excretion-time of an animal the size of a 1-μ^3 phagotrophic microflagellate is estimated to be about 2 minutes (*7*), based on extrapolation of the lower regression line in Fig. 1 (*8*). It is difficult, if not impossible, to determine the excretion rates of these fragile forms directly.

When the data used in Fig. 1 were compared with data on oxygen consumption as related to body weight in marine animals (*9*) it was found that the ratio of oxygen consumed to phosphorus excreted decreases markedly with decreasing animal size. An animal weighing 1 μg releases approximately 50 times as much phosphorus per unit weight as a 100-mg animal, while the smaller animal consumes only 5 to 8 times as much oxygen per unit weight. Two other workers (*10*) have likewise noted that the "O/P ratio" was significantly lower for mixed zooplankton species than for several larger benthic invertebrates. The explanation of the marked lack of parallelism between these two physiological processes deserves investigation.

A recent example serves to demonstrate the importance of considering size distribution of fauna when computing excretion rates of faunal communities. Rigler (*11*) calculated the rate of release of dissolved phosphorus for lake zooplankton, assuming that rotifers excrete the same amount of phosphorus per unit weight as cladocerans 1000 times heavier. He acknowledged the possibility of error arising from this assumption. My results suggest that this error is indeed significant. On the basis of the lower regression line in Fig. 1 the rotifers would be expected to excrete dissolved phosphorus about ten times as fast as the cladocerans per unit weight.

Figure 1 can be used to demonstrate the relative importance of microzooplankton and macrozooplankton for

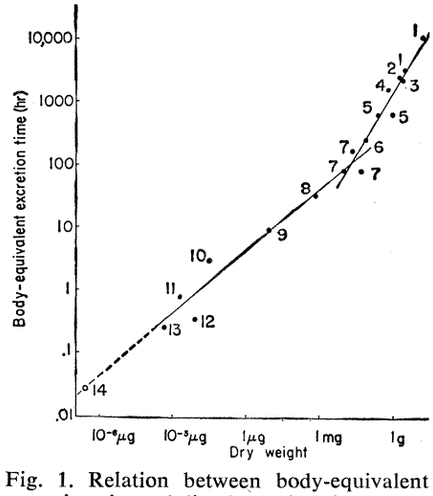

Fig. 1. Relation between body-equivalent excretion time of dissolved phosphorus and body weight of marine animals. 1, *Tridacna crocea*; 2, *Penaeus setiferus*; 3, *Crassostrea virginica*; 4, *Modiolus demissus*; 5, *Uca pugnax*; 6, *Salpa fusiformis*; 7, *Littorina irrorata*; 8, *Lembos intermedius*; 9, *Artemia salina* (nauplii); 10, *Euplotes crassus*; 11, *Euplotes trisulcatus*; 12, *Euplotes vannus*; 13, *Uronema* sp.(?); 14, hypothetical 1-μ^3 (2.5×10^{-7} μg dry wt.) phagotrophic flagellate.