Table 1. Relative ATP concentrations, expressed as percentages of control concentration ± standard deviation, in tissues of rats exposed for 1½ hours to 100-percent oxygen at 5 atm. The values for the surviving unprotected rats are from Sanders *et al.* (*4*). The numbers in parentheses indicate the numbers of rats.

Cerebral hemisphere	Liver	Kidney
Controls		
100 ± 12 (9)	100 ± 20 (8)	100 ± 14 (12)
Unprotected		
47 ± 11* (8)	56 ± 14* (5)	37 ± 8* (5)
Succinate protected		
100 ± 25 (9)	138 ± 29* (7)	109 ± 20 (7)

* Significantly different from controls ($P < .01$).

(as indicated by adenosine triphosphate, ATP) of the cerebral hemisphere, liver, and kidney of rats that survived exposure to 5 atm of 100 percent oxygen for 1½ hours were reduced 53, 46, and 62 percent, respectively, as compared to tissues from normal animals (*4*). The ATP concentration was determined on the cerebral hemisphere, liver, and kidney of rats from groups 1, 3, and 4 to see whether succinate would prevent the decrease in ATP. The firefly-luminescence technique (*5*) was used to measure concentration of ATP in the tissue.

Table 1 shows the relative concentrations of ATP in three tissues of animals from the controls, from surviving animals given similar hyperbaric exposures without any protective agent (*4*), and from succinate-injected animals. Tissues from the animals treated with succinate had normal or above normal ATP concentration.

The two dextrose-treated animals that survived the exposure and were normal in appearance had ATP concentrations in the liver and kidney within the normal range. The three dextrose-treated animals that exhibited severe symptoms of oxygen toxicity had an averaged reduction of 58 and 63 percent in ATP concentration in liver and kidney, respectively.

Toxicity of high-pressure oxygen (*6*) is generally attributed to the inhibition of tissue respiration and oxidative phosphorylation (ATP production under aerobic conditions) and inhibition of enzyme activities. Brain-tissue slices exposed to high-pressure oxygen had decreased intracellular potassium and increased intracellular sodium (*7*). This observation led to the hypothesis that high-pressure oxygen exerts its toxic effect on tissue "by reducing energy available" for metabolic functions. We have shown (*4*) that toxicity from high-pressure oxygen is accompanied by marked reduction in ATP concentration in rat tissue. Thus protective efforts against oxygen toxicity should be directed toward restoring ATP concentration and metabolic functions in tissues. Among succinate, glutamate, and α-ketoglutarate, succinate has the highest ATP-production capacity in rat brain, liver, and kidney (*5*). When succinate is the substrate, only two molecules of ATP are formed per atom of oxygen used, as compared to three molecules of ATP per atom of oxygen for the other aerobic substrates which lead to ATP formation by oxidative phosphorylation. Thus succinate not only causes high production of ATP but also uses more oxygen than other oxidative phosphorylation substrates; and both processes counteract the local effects of oxygen. King (*8*) has observed that succinate is necessary for the reconstitution and stabilization of electron-transport particles (the fundamental units of the oxidative phosphorylation process). One effect of high-pressure oxygen is the inhibition of the reduction of nicotinamide-adenine dinucleotide (NAD) (*9*). The reduction of NAD is essential for oxidative phosphorylation with all substrates except succinate. Succinate lessens the effect of high-pressure oxygen on the inhibition of NAD reduction, and there is minimum inhibition of electron flow from succinate into the electron-transport chain (thence ATP production) at an oxygen pressure of 12 atm (*9*). Succinate oxidation may monopolize the respiratory chain and inhibit the oxidation of NADH (*10*). This latter action—if present in high-pressure oxygenation—would tend to counteract the effect of high-pressure oxygen in shifting NADH toward NAD. These observations support our hypothesis that succinate should be an effective protective agent against the toxic effects of high-pressure oxygen.

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11. We thank S. Baylin, B. Thomas, and Miss Elaine Goble for valuable technical assistance. Supported in part by UMRF grant No. 123 and NIH grant CA-07581-02. I.H.H. is a postdoctoral fellow, NIH training grant 5-T-1-MH-8394-02.

10 November 1965

Spleen as a Production Site for Erythropoietin

Abstract. *Mice treated with acellular extracts of spleen and liver show a pronounced and significant increase in blood reticulocytes. In control mice treated with saline and extracts of kidney, muscle, and liver of splenectomized mice and rats, the number of reticulocytes remained constant in all the cases.*

In 1926 Krumbhaar (*1*), in his review of the spleen, emphasized the indirect influence of the spleen on blood formation through a stimulating action on bone marrow, possibly after activation of spleen factor by the liver. Later, Gley, Delor, and Laur (*2*) and Ruhenstroth-Bauer (*3*) concluded that the spleen may represent one of the sites of production of a factor that stimulates erythropoiesis.

In 1959, at the conclusion of some research on hypoxilienine (*4*), a suggestion, based on the relationship between the spleen and x-ray mortality, was made by one of us (*5*) that a splenic humoral factor capable of reducing x-ray mortality had a specific

stimulating action on the bone marrow.

We have tested the reticulocyte response pattern of mice given intraperitoneal injections of acellular spleen extracts, using the methods described by Winkert *et al.* (*6*), who investigated the physiological effects of human urinary erythropoietin.

In our previous research, performed on mice subjected to x-irradiation of the total body, it was shown that homogenates of only spleen and liver, among several organs tested from several species, are protective in action against x-ray mortaliy. An acellular preparation from the spleen was also active when tested in animals of another species.

We have now compared the effects on the reticulocyte patterns of acellular homogenates of liver, kidney, and muscle from rats, mice, and calves. The alterations in hematocrit and in red cell number have also been measured. We tested 137 young male mice of the Morini strain (age, 8 to 9 weeks; average weight, 25.6 g). Mice were given free access to food that had been enriched in vitamins and minerals.

The organs to be tested for their erythropoietic effect were removed from killed animals and homogenized in a blender with cold saline in a cold room, and then centrifuged in a refrigerated centrifuge at 15,000*g* for 15 minutes; the supernatant, to which sucrose was added to obtain 0.25*M* concentration, was centrifuged under the same conditions, and the precipitate was discarded.

The resulting acellular homogenate was injected intraperitoneally, daily for 4 days, in an amount equivalent to 150 mg of the fresh organ. Saline and sucrose homogenates of calf, rat, and mouse liver, kidney, and muscle were injected into mice as a control. Reticulocyte counts were made on smears of tail blood stained with brilliant-cresyl-blue. Hematocrit values (Wintrobe tubes) and red cell numbers were determined for 4 days.

Liver sucrose homogenates from rats and mice that had had their spleens removed 15 days before were injected into one group of mice. In another group a further purified homogenate of calf spleen was tested. Ammonium sulfate (40 percent saturation) was added to the supernatant of the sucrose–spleen homogenate, which was centrifuged as described above. The precipitate was resuspended in a minimum amount of cold saline and dialyzed against tap water in a cold room until all the ammonium sulfate was removed, as determined by the Nessler test. The dialyzate was again centrifuged in a refrigerated centrifuge at 15,000*g*, for 20 minutes. The precipitate was discarded.

The mean values of reticulocyte responses to different treatment are given in Fig. 1.

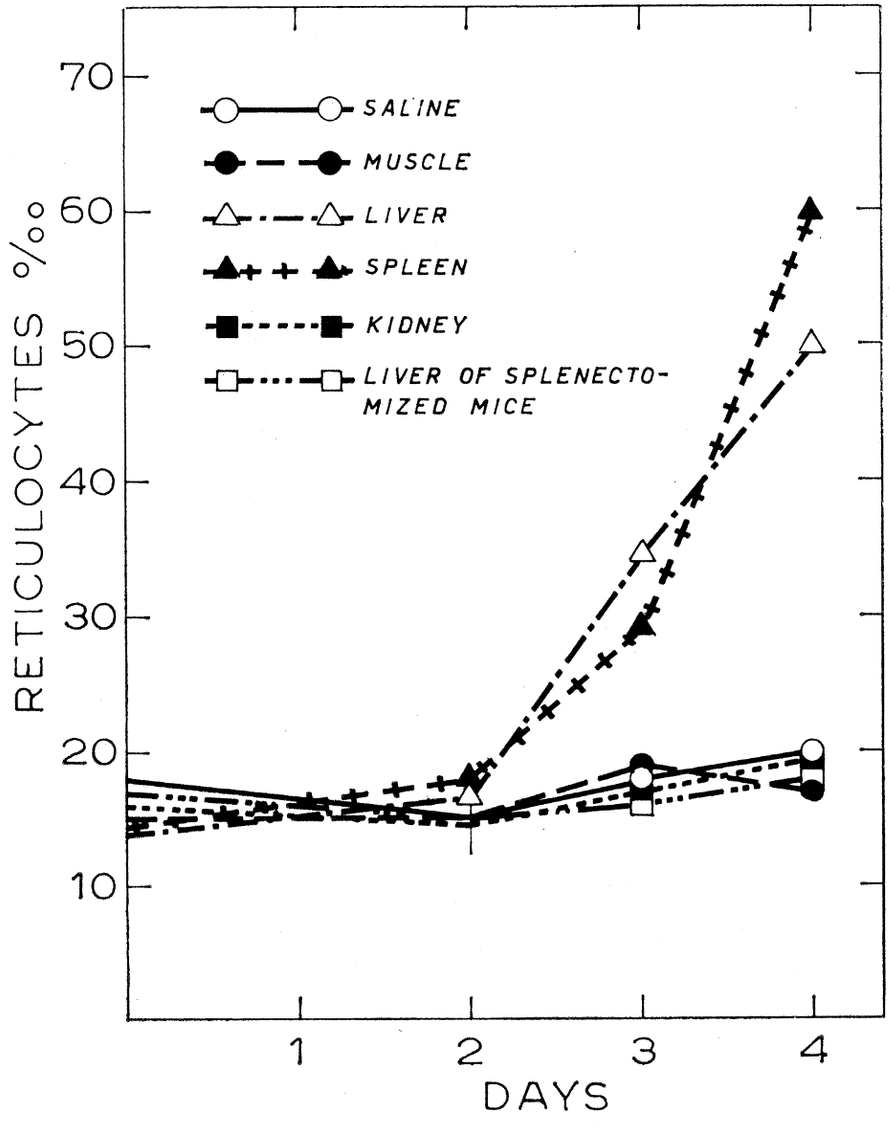

Fig. 1. Time course of the reticulocyte response to intraperitoneal injection of saline and of sucrose homogenate of mice spleen, liver, kidney, and muscle and of liver of splenectomized mice. Ordinate, number of reticulocytes per thousand red cells.

No statistically significant alterations were recorded, for the homologous and heterologous acellular homogenate treatment, in the hematocrit values or in red cell numbers of mice injected with homologous or heterologous acellular homogenates.

It is evident (Fig. 1) that there was no increase in reticulocyte concentration after treatment of mice with saline (10 animals), or with homogenates of kidney (24 mice), muscle (29 mice), or liver of splenectomized animals (18 mice).

There was a pronounced and significant increase of reticulocytes in mice treated with spleen extract (39 mice) and with liver of normal rats (17 mice); this increase was as great as that observed by Winkert and co-workers after they had treated rats with 14 erythropoietic units of human urinary erythropoietic stimulating factor per day.

Because the kidney is generally considered to be the production site of the erythropoietic factor, we have tested the acellular splenic homogenate on

nephrectomized mice. Unfortunately we have been unable to keep bilaterally nephrectomized mice alive long enough to perform a positive experiment.

Our data are in agreement with Rein's (*7*, *8*) hypothesis of "hypoxilienine," the spleen factor which is stored in the liver, and with the suggestion that hypoxilienine acts as an erythropoietic factor.

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9. Research supported by U.S. Army grant N. 91-591-EUC 2785.

10 November 1964

Flora and Fauna on Backs of Large Papuan Moss-Forest Weevils

Abstract. *Large, living, flightless weevils feeding on leaves of woody plants in high moss forest on various New Guinea mountain ranges have plant growth on their backs. Fungi and algae have been found on 11 species of* Gymnopholus, *lichens on six species, and liverworts on one species. In other genera of weevils, on the same mountains, there are additional specific associations with fungi, algae, lichens, and liverworts. The fungi and lichens, at least, are inhabited by oribatid mites of a new family, which may spread the plants from beetle to beetle. Also, nematodes, rotifers, psocids, and diatoms occur among the plants. Specialized scales or hairs, and a secretion, in depressions on the weevils' backs, appear to be associated with encouragement of the plant growth. Mutualistic symbiotic relationships seem to be clearly indicated.*

In high-altitude moss forests in New Guinea, a symbiotic relationship exists whereby cryptogamic plants (fungi, algae, lichens, liverworts) of a number of species of 12 or more families grow on the backs of large weevils. The plants represent groups normally living on bark and leaves, as far as is known, and the weevils feed on leaves of woody plants in the very damp environment. Shade and humidity requirements of the plants are fairly high, and in this environment rain and fog are very common. Temperatures are normally between 12° and 33°C. The weevils represent a number of species of two subfamilies and are large, heavily sclerotized, flightless, more or less slow-moving, and evidently long-lived. Some of the plant growth appears to have required 3 to 5 years to develop, and this with other factors speaks against a parasitic association. Furthermore, the weevils seem to be specially adapted, by modification over a long evolutionary period, to encourage the plant growth, which may serve the function of protective camouflage for the weevils. The natural enemies of the weevils are not yet known, but may include birds of paradise.

Living in the plants on the weevils are oribatid mites, representing a new family, and nematode worms, rotifers and diatoms, not yet studied. Also there is evidence that psocopterans feed on some of the plants on the beetles. The mites may aid in transfer of the plant spores from weevil to weevil. A secretion, and modified scales and hairs, on the weevils, seem to assist the plant growth in making a start. The newly emerged weevils have the structural modifications (depressions, tubercles, scales, hairs), and the secretion, to encourage the plants, but do not have plant growth initially. The activities of the weevils appear to give minimum disturbance to the plants. Although the animals living with the plants are fairly numerous, their moving environment may be a little less favorable than in the plants on the normal substrates, so that this may benefit the plants, as may some measure of similar protection from other enemies or competitors. Of course the mite may be a symbiote in the sense of serving to transfer the plants to new beetles, at least. The nematodes and rotifers may not directly damage the plants, but may feed upon microorganisms and waste products within the association.

The genus *Gymnopholus* (Curculionidae: Leptopiinae) includes over 40 species (*1*) of large weevils, restricted to the mountains of New Guinea. They have been found in various mountain ranges, almost entirely in eastern New Guinea. The species vary in length from about 20 to 40 mm, and some are quite broad. Those species in the lower mountains are mostly smooth and glabrous, generally black, but sometimes with patches of metallic scales. Only the high-altitude species, and some of the mid-altitude species which live mostly in moss forest and in the border area of the alpine grass zone and alpine shrubberies, have rough or sculptured upper surfaces with special modifications which seem to be correlated with the growth of plants on their backs. These areas on the pronota and elytra, inaccessible to the legs of the beetles, include large depressed areas, series of pits, or depressions between vermiculate or reticulate ridges. Furthermore, there is generally a pair of high tubercles on the pronotum and one or two on the posterior part of each elytron, which further protect the plant growth. A thick waxy secretion which appears to foster the growth of plants on the protected areas is apparently produced by glands in the areas of the depressions. Some of the weevils have the depressed areas lined

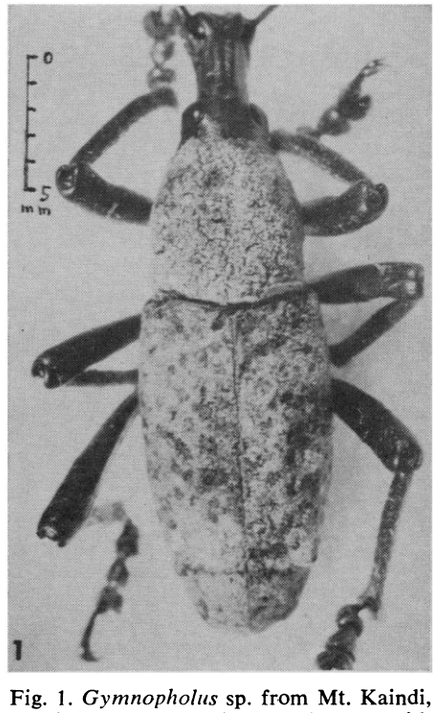

Fig. 1. *Gymnopholus* sp. from Mt. Kaindi, North-East New Guinea, 2300 m., with pronotum and elytra covered with fungal growth; darker spots are deeper pits, possibly with higher concentration of algal growth; shiny dots in these are oribatid mites (× 4).