amount of each type of culture in the mixture, however the data are too sparse to determine a precise relationship.

Several investigators have previously shown that joining 2 existing macroplasmodia in different phases of the cell cycle can alter the timing of mitosis[19-21]. The mixing experiments described here differ in that 1. contact between the 2 types of plasmodia occurs at the time the macroplasmodia are formed rather than after each individual macroplasmodium has established a synchrony of its own and 2. the 2 partners do not have the same cycle lengths.

These experiments permit several conclusions to be drawn. First and foremost is that exposure of *Physarum* to weak electromagnetic fields produces biological effects. This conclusion is also supported by our previous findings that exposure causes the mitotic cycle and protoplasmic shuttle streaming to slow. We may also conclude that the exposed cultures have been altered in some way that immediately manifests itself when control and experimental cultures are brought together. Similar averaging of the cell cycle has been reported by Haugli et al.[22] in mixtures of normal cultures and cell-cycle mutant cultures that have a lengthened cell cycle. A 3rd important conclusion is that the alteration brought about in exposed cultures is not drastic from the point of view of basic cellular processes. When exposed and unexposed microplasmodia are mixed together and allowed to fuse to form a single large macroplasmodia, the fusion takes place and all nuclei behave normally to the extent that they undergo synchronous mitosis. They behave abnormally in that the mitotic cycle time is different from either parent culture. Since all of the processes we have observed to be influenced by EMF require energy, we suspect that weak low frequency EMF may interfere with either energy generating processes or the transport of essential metabolites in *Physarum*.

1 Acknowledgment. This work was supported by the Naval Electronics Systems Command through an Office of Naval Research Contract and a grant from Parkside's Center for the Application of Computers.

2 Abbreviations: EMF for electromagnetic field; QO_2 for rate of oxygen uptake; C for control cultures, E for exposed cultures, and M for a mixture of E and C cultures.
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Activity of the reticuloendothelial system following exposure to electric stress and thymectomy

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Summary. After electric stress stimulation, granulopectic activity is reduced in otherwise normal rats, whereas it appears to be increased in thymectomized animals. The differences between the 2 groups of animals seem to support the hypothesis that the effects of stress upon the overall phagocytic capacity may be mediated by the products of lymphocyte breakdown.

It has long been known that the phagocytic activity of reticuloendothelial cells is affected by exposure to stress. Some conflicting results in the literature can be explained, at last partially, by the use of different stress procedures, many of which obviously possess specific undesirable components like tissue damage, superimposed infections, blood or plasma loss[3,4]. It is mostly agreed that the phagocytic activity is reduced as a consequence of the rise of plasma corticosteroids acting directly on the macrophage[5-8], although the action mechanism of corticosteroids is still under investigation; recent observations seem to indicate that the role of corticosteroids may be questioned[9].

The effects of thymectomy on the reticuloendothelial system have been reported under several experimental conditions[10-15]: evidence was provided that the phagocytic response to repeated stimulation by colloidal suspensions is much higher in thymectomized rats, whereas it is reduced in normal animals[12].

In the present research, experiments were designed to investigate the interference of thymectomy and stress in the phagocytic activity of the reticuloendothelial system and to clarify to some extent the mechanism by which the phagocytic activity is affected in stressed non-thymectomized animals.

All experiments were carried out with Wistar albino rats at 5-6 weeks of age. The granulopectic activity was estimated by measuring the rate of removal from the blood of colloidal carbon (16 mg/100 g b.wt) by the method previously described[12]. Thymectomy was performed 2 days after birth according to the method of Miller[16]. Stress was applied in grid-floored cages according to the method of Hall et al.[3], by electric shocks of 200 V and 2000 cps over

periods of 5 msec at regular intervals of 23 sec: treatment was divided in 2 sessions, of 6 and 8 h respectively, by a pause of 10 h. The advantages of electric shock as a stressor have long been apparent[3,17-19], since the emotional reaction seems to override the response induced by the physical stress alone, and few other mechanisms are likely to interfere with the activation of the hypophyseal-adrenocortical system as the major mediator of the effects of stress[20]. The results are summarized in table 1.

The analysis of rank data was performed according to Scheirer et al.[21]. In normal and thymectomized animals, which were not subjected to the stress procedure, the velocity of carbon removal was different according to the previously reported data[12]: the difference was significant (180 vs 138; $\chi^2 = 4.11$; d.o.f. 1; $p < 0.05$). Immediately after the stress treatment, the granulopectic activity decreased significantly in the normal rats (180 vs 120; $\chi^2 = 10.17$; d.o.f. 1; $p = 0.01$), whereas it did not change appreciably in the thymectomized rats. 24 h later the granulopectic activity decreased further in the normal rats; an appreciable though not significant increase was detected in the thymectomized rats. The difference between the normal and the thymectomized rats 24 h after stress was highly significant (99 vs 168; $\chi^2 = 13.34$; d.o.f. 1; $p = 0.001$). Interaction between thymectomy and stress was highly significant ($\chi^2 = 16.22$; d.o.f. 2; $p < 0.001$).

In order to evaluate the role of liver in the granulopectic activity, a number of animals was injected immediately after the stress treatment with 0.9% trypan blue in saline, 2 ml/100 g b.wt, and sacrificed 1 h later. The liver was fixed in the Susa solution, imbedded in paraffin and stained with haematoxylin and eosin. The percentage of sinusoidal cells containing the injected dye was counted in both principal lobes, on 400 cells in each animal. The results are summarized in table 2.

The number of vitally stained cells was much larger in the liver of the thymectomized rats: the difference was highly significant ($p < 0.01$ by the Mann-Whitney-Wilcoxon test on the ranked data[22]).

As far as non-thymectomized animals are concerned, the present results are in agreement with the results obtained in animals subjected to trauma[5,7]. The question now arises: why is the effect of stress different after thymectomy? Obviously the problem is related to the debated question by which mechanism the reticuloendothelial activity is depressed by stress in otherwise normal animals. It seems to us reasonable to suggest that the effect of stress on the reticuloendothelial system may be mediated by lymphorrhexis, in that the products of lymphocyte breakdown may involve some degree of blockade of the reticuloendothelial system. It is well established that stress induces the regression of lymphatic organs, and most observations point to the conclusion that the regression is a consequence of the raised levels of corticosteroids, operating largely by karyorrhexis in lymphocytes[8,23,24]. The role of corticosteroids in the depression of the reticuloendothelial function was questioned by recent experiments showing that, in animals subjected to trauma, the corticosteroids reached a normal level earlier than the phagocytic activity[9]. However, it is worth noting that the enhanced lymphocyte destruction after administration of cortisol persists for some hours when the particular active form of the hormone apparently is no longer present in the lymphocytic tissue[25]. This hypothesis concerning the effect of stress on the reticuloendothelial system is in agreement with the present observations in the thymectomized animals. The different response after thymectomy might be accounted for both by the paucity of the lymphatic system and by the peculiar changes occurring in the reticuloendothelial system[12]. Our hypothesis is not incompatible with the view that the RES depression after massive injury might be related to the depletion of opsonic plasma factors consumed by various breakdown products, although further data are needed on these factors[9].

At the present state of our knowledge, there seems to be no indication supporting an alternative hypothesis, that the hypophyseal-adrenocortical system may be changed by thymectomy.

Table 1. Colloidal carbon removal from blood of normal and thymectomized rats without previous stress treatment (controls) or after stress

	Normal	Thymectomized
Controls	180±9 (8)	138± 8 (8)
Immediately after stress	120±7 (8)	144±15 (8)
After 24 h	99±7 (8)	168±13 (8)

Numbers are K mean values (multiplied by 10^4) ± SE. The figures in brackets refer to the numbers of animals: 48 animals were used, each receiving only 1 injection of carbon.

Table 2. Percentage of vitally stained sinusoidal cells in the liver of normal and thymectomized rats injected with trypan blue after the stress treatment

Normal	Thymectomized
43.12±1.02 (6)	53.32± 1.67 (6)

Numbers are mean values ± SE. The figures in brackets refer to the numbers of animals.

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2 The collaboration of Prof. A. Colombi for the statistical analysis of the experimental results is gratefully acknowledged.

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