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Endogenous Pyrogen Release from Rabbit Blood Cells Incubated in vitro with Parainfluenza Virus

Abstract. *Rabbit blood cells incubated in vitro with purified parainfluenza-5 virus (DA strain) released a rapidly acting pyrogen. Spleen and lymph node cells were inactive. The pyrogen resembled in behavior a pyrogen extracted from granulocytic exudates. Similar cells in the blood are believed to be activated by virus in vivo to produce the circulating endogenous pyrogen that mediates virus-induced fever.*

The mechanism by which viruses induce fever is obscure. After intravenous inoculation in rabbits, myxoviruses produce fever indirectly by releasing a circulating pyrogen of endogenous origin (*1*). This pyrogen was presumed to act directly on thermoregulatory centers of the brain. Unlike virus, which caused fever only after 45 to 60 minutes of latency, the pyrogen appearing in the serums of febrile donor animals injected with virus produced immediate transient fevers when transfused into recipient rabbits. Injection of Coxsackie B-1 virus into rabbits has produced similar results (*2*).

There is now considerable evidence that circulating granulocytes, though containing little or no preformed pyrogen, are capable of generating large amounts of pyrogen after contact with specific stimuli (*3*). Two such stimuli, the endotoxins of gram-negative bacteria (*4*) and certain particulate materials that are phagocytized (*5*), liberate pyrogen from granulocytes in vitro. Similar events in vivo are believed to account for fevers caused by intravenous injection of endotoxins (*6*) or various particulate substances (*7*).

Parainfluenza virus was added to rabbit blood cells in vitro to determine whether it would activate these cells to release endogenous pyrogen. In correlated studies, virus was also incubated with mononuclear cells from the spleens and mesenteric lymph nodes of the same animals, because homogenates of spleen and other normal tissues contain a substance or substances with similar pyrogenic properties (*8*).

The DA strain of parainfluenza-5 virus (*9*) was grown in primary monolayer cultures of rhesus monkey kidney and in replicating cultures of rhesus (LLC) and African green monkey kidneys (BSC-1). Culture fluid containing virus was harvested 7 to 10 days after infection and clarified by centrifugation and filtration. The supernatant fluid was then centrifuged in a Spinco model L ultracentrifuge at 34,000*g* for 45 minutes; the pellet was resuspended in saline. An equal amount of 12.5 percent barium sulfate was added, the mixture was kept overnight at 4°C, and the barium plus the adsorbed virus was washed twice in distilled water. Virus was eluted from the barium into citrated saline and the barium was removed by centrifugation. Under pyrogen-free conditions, the virus was then washed 2 or 3 times in saline by centrifugation at 34,000*g* until the supernatant fluid was free of pyrogenic activity when tested in rabbits. Antibiotics were added and cultures made to insure bacterial sterility of all material for injection. Virus preparations showed a single sharp band of hemagglutinin and protein when centrifuged in a potassium tartrate equilibrium density gradient and presented a homogeneous array of particles under electron microscopy (*10*). Procedures used for assay of fever-inducing materials in rabbits have been described (*1*).

Figure 1*a* depicts the mean fevers produced by two different virus samples injected intravenously into two groups of two rabbits each. Duration of the latent period between injection of the virus and onset of fever was inversely related to the dose of virus: more than 30 minutes for the larger dose and extending to more than 75 minutes with the smaller dose.

To determine whether virus could activate blood cells, purified virus was added to heparinized rabbit blood cells that had been washed once and resuspended in saline. The mixture was incubated in vitro at 37°C for 4 hours, and the cells were removed by centrifugation. The supernatant fluid was then injected intravenously into rabbits. The results (Fig. 1*b*) show that samples of supernatant fluid from the virus-blood cell mixture produced immediate monophasic fevers which reached peaks after 45 to 60 minutes and subsided rapidly. The configuration of the febrile responses closely resembled that induced by leucocyte pyrogen obtained from rabbit blood cells incubated with purified endotoxin derived from *Proteus vulgaris* (*8*). Samples of supernatant fluid from similarly incubated mixtures of virus and rabbit spleen or lymph node cells failed to produce immediate fevers.

These results indicate that "purified" DA virus is capable, like endotoxin, of activating cells in the blood to release endogenous pyrogen in vitro. With endotoxins, there is good evidence that the cell releasing pyrogen is the granulocyte. Normal red blood cells and platelets have not been shown to contain a pyrogen, and incubation of virus with mononuclear cells from two different sources—spleen and lymph nodes—fails to release an endogenous pyrogen. It seems likely, therefore, that the granulocyte is also the responsible cell in fevers induced by the intravenous inoculation of DA virus. The results were similar when tuberculin was incubated in vitro with blood cells of rabbits sensitized with

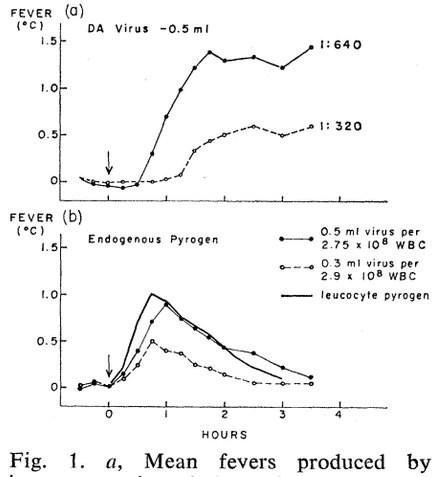

Fig. 1. *a*, Mean fevers produced by intravenous inoculation of two different doses of virus (represented by hemagglutinin titers). *b*, Mean fevers produced by saline supernatants of blood cells incubated with virus at the two concentrations shown. For comparison, responses of five rabbits to leucocyte pyrogen (obtained from 8 to 16 × 10^7 blood leucocytes incubated with 0.003 μg of *Proteus vulgaris* endotoxin) are shown. Figures given for endogenous pyrogen represent individual dosages. Each curve represents the mean response of two or three rabbits.

bacille Calmette-Guerin, whereas mononuclear cells from the spleens and lymph nodes of the same animals were inactive (*11*).

ELISHA ATKINS
MURIEL CRONIN
PETER ISACSON
Department of Internal Medicine and Microbiology,
Yale University School of Medicine,
New Haven, Connecticut

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Muscular Contraction as Regulated by the Action Potential

Abstract. *Lowering the mechanical threshold and, independently, prolonging the duration of the action potential cause an increased rate of tension development, as well as potentiation of the twitch, of frog skeletal muscle. The alterations of the two different features of function of the action potential modify excitation-contraction coupling in essentially identical fashion (probably by increasing the liberation of free Ca^{2+} into the myoplasm), and this results in early intensification as well as later prolongation of the active state.*

Diverse potentiators of skeletal muscle contraction cause their common mechanical effects—prolongation of the active state and thus potentiation of the twitch—by producing independent alterations in two distinct functions of the action potential in excitation-contraction coupling (*1*). Caffeine and the lyotropic anions (which we will call type A potentiators) lower the mechanical threshold (see also *2*) but produce no significant change in the shape of the action potential itself. Conversely, certain heavy metal ions, for example, Zn^{2+} and UO_2^{2+} (type B potentiators), greatly decelerate the repolarization phase of the action potential but have no effect on the mechanical threshold. These results are consistent with the generally proposed, though loosely defined, view that the duration of the active state of the contractile mechanism, and thus the output of the twitch, are determined by some function of the level of the mechanical threshold and the length of time during which the action potential maintains depolarization of the membrane beyond this threshold (*1–3*). Our earlier results were limited, however, in their capacity to elucidate especially the early events of excitation-contraction coupling, because they referred to mechanical features of the twitch that occurred long after excitation was over and that did not show any variation in relation to the different electrical effects of the two types of potentiators. We have extended this work and can now show (i) that the potentiators increase the rate of rise of tension during the earliest indications of contraction; (ii) that such increases differ significantly with the type of potentiator in regard to timing and yet are basically similar; and (iii) that these results in general provide new information about the link in excitation-contraction coupling of both normal and potentiated contractions.

We used excised frog sartorius muscles at 23° to 25°C, equilibrated to oxygenated Ringer solution containing 0.002 percent *d*-tubocurarine hydrochloride and adjusted to *p*H 7.0 with tris buffer. The muscles were set up at an initial tension of 2 g for isometric responses massively stimulated by slightly supermaximal square-wave shocks of 0.3 msec duration. Tensions were recorded by an RCA 5734 transducer tube, and ordinary and differentiating amplifiers and multichannel cathode-ray oscillography gave us traces as a function of time, t, of both the direct tension output, P, and also the first time derivative of the tension, dP/dt (that is, the rate of tension change).

Nitrate, in addition to producing characteristic potentiation (Fig. 1, top), accelerates development of tension. Thus, in the curves for P, the developed tension of the activated muscle 3.5 msec after the start of the shock is 1.0 g for treated muscle but only 0.72 g for untreated muscle. Such effects are more strikingly indicated by the dP/dt curves, especially when the curves are compared (Fig. 1, bottom). The curves show that at each instant the nitrate-treated muscle develops tension at a faster rate than does the normal muscle and that this difference becomes evident as early as 2 msec after the start of the shock—that is, about midway in development of the latency relaxation (*4*). This effect of the nitrate is also found when nitrate-treated and normal muscle are compared at common values of P. The effects of 1 mM caffeine are in all respects identical with those of nitrate.

As shown in Fig. 2, Zn^{2+} in 0.05 mM concentration causes changes generally like those of NO_3^- and caffeine: the usual potentiation and prolongation of the twitch, and increase in dP/dt. But an important difference is that the increase in rate of tension development begins about 3.3 msec after application of the stimulus—that is, about 1.3 msec later than that caused by NO_3^- or caffeine. Other heavy metal potentiators, such as UO_2^{2+} (1 μM) and Cd^{2+} (0.2 mM), act like Zn^{2+} (*5*).

Our most general result is that all potentiators tested cause an increase in dP/dt which at room temperature begins no later than 3.5 msec after stimulation. Similar increases in speed of shortening in isotonic contractions are also caused by potentiators (*6*). Thus we conclude that the present increases in dP/dt during the earliest part of isometric contractions result from comparable increases in velocity of shortening of the contractile component, which in turn effect greater rates of tension development in the series elastic component and thus in the muscle as a whole. Since the velocity of