sion, when separated from the suppressor, is the expected group A lesion. The ability to segregate ts and ts^+ progeny from the cross of clone 101 with wild type indicates that the site of the suppressor mutation can be separated from the suppressed ts lesion, presumably by reassortment of genome segments, and that the suppressor must lie in a second gene.

We have not yet defined the site of the suppressor mutation, or the specific mechanism of this phenotypic suppression. However, the ability to separate the suppressor mutation from the ts lesion by reassortment indicates that the suppression is extragenic rather than intragenic.

In prokaryotic systems extragenic suppressors often are in genes whose products interact physically with the original gene product. These suppressor mutations frequently have temperature phenotypes of their own (*2*) which make them useful in the study of protein interactions, as well as making reversion a useful method for the selection of new mutations. We have been unable to identify a new temperature-sensitive phenotype among the progeny of this backcross. This indicates that the suppressor mutation described does not have an intrinsic temperature-sensitive phenotype.

Our results suggest a previously undetected perhaps general mechanism by which the ts phenotype of eukaryotic virus mutants may be altered. The presence of pseudorevertants in this virus system suggests that reversion in animal viruses can occur by mechanisms similar to those elucidated in other biological systems and that when revertants are examined, standard genetic considerations apply.

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9. We thank Dr. R. K. Cross for discussions. Supported by NIH grant 1 RO1 AI 13178-01, American Cancer Society grant VC-195, and PHS grant 5 T32 CA 09131-01 for fellowship support to R.F.R.

26 July 1976; revised 24 September 1979

Control of Gamete Formation (Exflagellation) in Malaria Parasites

Abstract. *The only stages of malaria parasites capable of establishing an infection in a mosquito are the gametocytes that circulate in the blood of the vertebrate host. Within minutes of ingestion by a mosquito the gametocytes transform into mature gametes in the process of "exflagellation." This process is controlled in vitro solely by the change in* pH *in the blood as it moves from the environment of the circulation to that of the atmosphere, the* pH *rise being mediated by the fall in carbon dioxide tension as the blood equilibrates with the atmosphere.*

All parasitic organisms cyclically transmitted by arthropod vectors experience a profound change in environment at least twice during their life cycle—once as the parasite is transferred from the vector to the vertebrate host and again as the parasite moves back from the vertebrate to the vector. Accompanying these environmental changes most parasites undergo extensive and often rapid biological transformations. Such transformations are well recognized among arthropod-transmitted parasitic protozoa and are especially dramatic in Haemosporidia, including malaria parasites. Although in several instances considerable effort has been devoted to describing the morphological and biochemical developments associated with these changes (*1*), there is little understanding concerning the factors initiating or controlling the transformation of malaria parasites from the forms present in the vertebrate host to those that establish the infection in the mosquito vector—a problem that has vexed malariologists for almost a century.

Malaria parasites multiply in the red blood cells of their vertebrate host by repeated cycles of an asexual process known as schizogony. Certain parasites, themselves the products of schizogony, do not undergo further division in the red cell but become gametocytes, the precursors of the gametes. While in the blood stream the mature gametocytes remain quiescent. Within the stomach of the mosquito following a blood meal, however, the gametocytes undergo rapid transformation. In malarious blood removed from the stomach of a freshly engorged mosquito the membrane of the red cell enclosing each gametocyte disintegrates releasing a macrogamete (female gamete) or a microgametocyte (male gametocyte). This process is specific to red cells containing mature gametocytes; no similar disintegration of the host cell normally occurs in either red cells infected with asexual parasites or immature gametocytes or in uninfected red cells. The microgametocyte completes its development in the dramatic process of exflagellation, releasing eight highly motile, flagellated microgametes. The process is completed 10 to 20 minutes after ingestion of blood; fertilization, in which an entire microgamete enters or fuses with a macrogamete, follows rapidly (*2*).

The intervention of the mosquito is not essential for the initiation of exflagellation. The sequence of events described above may be readily observed in gametocyte-carrying blood directly exposed to air, for example, in a drop of blood on a microscope slide. It is clear, therefore, that changes taking place in blood as it is transferred from the circulation to the atmosphere are capable of initiating the transformation of gametocytes to gametes.

Although the factors controlling its initiation have not been extensively studied, it has been well established that exflagellation is suppressed by maintaining infected blood at CO_2 tensions corresponding to those of circulating blood (*3–5*). Hitherto the role of CO_2 in controlling this process has been obscure, although Bishop and McConnachie (*4*) believed that it was independent of its effect on the *p*H of the blood. Our results, obtained with the chicken malaria parasite, *Plasmodium gallinaceum*, demonstrate that the effect on exflagellation of different CO_2 tensions correlates solely with their effect on the *p*H of the surrounding medium.

Immediately upon withdrawal from the heart or wing vein of an infected chicken (parasitemia, 40 to 60 percent; gametocytemia, 0.5 to 2 percent), blood was diluted in not less than 50 volumes of a minimal saline solution (10 m*M* tris, 166 m*M* NaCl, 10 m*M* glucose; *p*H 7.4), which we refer to as suspended animation (SA) solution. In it both emer-

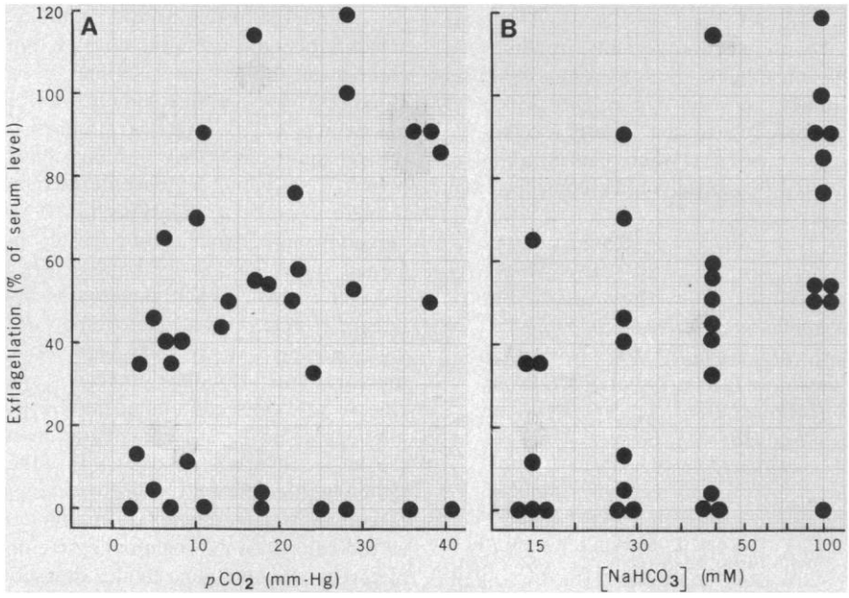

Fig. 1. The relationship between (A) pCO_2 and (B) bicarbonate concentration of the resuspension solution and level of exflagellation. Infected cells were washed in SA solution (see text) and resuspended in one of four bicarbonate saline solutions (added [$NaHCO_3$] = 15, 28, 50, or 100 m*M*) in which the *p*H had been adjusted by equilibration with atmospheres of different CO_2 tensions. The cells were inoculated into an airtight culture chamber and perfused continuously with additional equilibrated bicarbonate saline delivered from a glass syringe through stainless steel tubing. The difference in the *p*H and pCO_2 of the solution determined (Corning blood gas analyzer, model 165) immediately after equilibration and upon leaving the chamber was negligible. Exflagellation was quantified by counting the number of exflagellation events, or centers of agitation in a field of blood cells, and expressing them as the percentage of the events occurring when cells were resuspended simultaneously in serum. Each point shows the level of exflagellation attained in an individual resuspension. The same set of experimental values are successively plotted against pCO_2, bicarbonate concentration, and *p*H in Fig. 1, A and B, and in Fig. 2, respectively.

gence of the gametocytes from the blood cells and exflagellation of the microgametocytes are totally suppressed. The levels of exflagellation obtained after resuspension of the washed cells in a suitable exflagellation-supporting solution were equivalent to those observed when whole infected blood was exposed directly to air. In both types of preparations, Giesma-stained blood smears made 30 minutes after the initiation of exflagellation showed that an average of 85 percent of the microgametocytes completed exflagellation. The use of SA solution, therefore, allowed us to remove all plasma factors while maintaining the gametocytes in the quiescent state. Bishop and McConnachie (*6*) used a similar wash solution that did not contain glucose. We have found that when infected cells are deprived of glucose exflagellation is largely irrecoverable.

After being washed in SA solution the packed cells were resuspended at low concentrations (three volumes of packed cells to 1000 volumes of solution) in the test solutions and introduced into a perfusion chamber with an observation window for microscopic examination (*7*). A slow rate of perfusion over the settled cells was maintained during the course of the experiment. The chamber and entire perfusion system were designed to be airtight (*7*) so that the *p*H and CO_2 tension in the chamber could be accurately determined (see legend, Fig. 1). Experiments were conducted at room temperature.

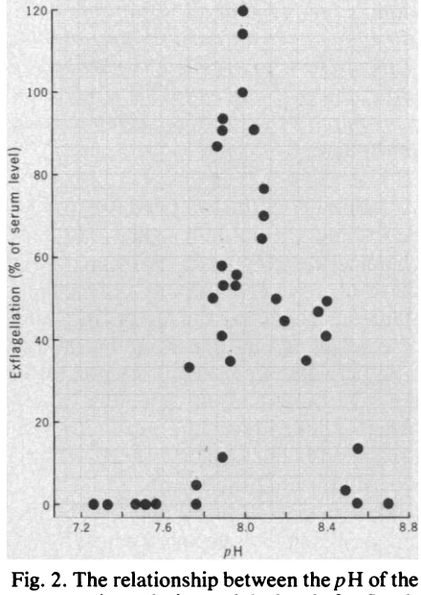

Fig. 2. The relationship between the *p*H of the resuspension solution and the level of exflagellation. See Fig. 1 legend.

Except for the presence of tris, our experimental solutions contained only minimal constituents required to support exflagellation, that is, bicarbonate, NaCl, and glucose. In studies with *P. gallinaceum* Bishop and McConnachie (*6*) reported that bicarbonate ion was the only component of plasma other than NaCl essential for exflagellation in vitro. Our experience supports this conclusion as long as an energy source (10 m*M* glucose) is continuously available to the parasites. The test solutions were prepared by mixing buffered isotonic saline (10 m*M* tris, 166 m*M* NaCl) with isotonic $NaHCO_3$ (170 m*M*) in varying proportions to achieve a range of final bicarbonate concentrations (15 to 100 m*M*). Glucose was added to a concentration of 10 m*M*, and the *p*H was adjusted by equilibration with a predetermined gas mixture of CO_2 and air.

The parameters of *p*H, partial pressure of CO_2 (pCO_2), and bicarbonate concentration are interdependent, being related by the Henderson-Hasselbalch equation

$$pH = pK' + \log [HCO_3^-]/ \alpha \cdot pCO_2$$

where pK' is 6.193 for bicarbonate-containing saline solutions of ionic strength 170 at 21°C, $[HCO_3^-]$ is given in millimoles, and α is 0.044, the solubility coefficient of CO_2 in water (*8*). Using different bicarbonate concentrations, we were able to vary the *p*H and pCO_2 independently of each other. In our experiments the values of these parameters covered the ranges found in chicken blood within the circulation (*p*H 7.3 to 7.4; pCO_2, 35 to 40 mm-Hg) and during equilibration with air ($pH \simeq 8.3$; $pCO_2 < 3$ mm-Hg).

Exflagellation was observed with phase-contrast illumination at a magnification of × 400 and was quantified by counting events (centers of agitation) in standard microscope fields containing a monolayer of approximately 1000 red cells. In each experiment a positive control was made by suspending washed infected cells in a commercial chicken serum (Grand Island Biological) at *p*H 8.0 ($pCO_2 < 5$ mm-Hg). This medium supported exflagellation at or near maximum levels, typically 25 to 40 events per five fields. Experimental values are expressed as a percentage of those exflagellation counts recorded in the control.

Figure 1 shows that there was no clear correlation between the level of exflagellation and the pCO_2 of the resuspension solution within the range of values studied. By contrast, there was a strong correlation between the level of exflagella-

tion and the pH of the solution (Fig. 2). Exflagellation increased sharply from 0 to 100 percent of the control value between pH 7.7 and 8.0 and declined more slowly at higher pH values until no exflagellation occurred above pH 8.6. Thus, regardless of the pCO_2, whether physiological or atmospheric, the level of exflagellation was determined solely by the pH of the medium bathing the cells.

Within the range of values studied the bicarbonate concentration was without marked effect on exflagellation (Fig. 1). In the complete absence of bicarbonate, however, exflagellation is totally suppressed at all pH values (*9*). The relationship between exflagellation and pH is, therefore, entirely dependent upon the presence of bicarbonate. We have, at present, no hypothesis to explain how the bicarbonate-dependent pH control of exflagellation operates at the cellular level.

Our results show that pCO_2 influences the amount of exflagellation in our in vitro system only by its effect on the pH of the medium; similarly above an undetermined minimum concentration bicarbonate ion affects the amount of exflagellation primarily by its control of the pH of the medium. In the light of these findings the natural control of exflagellation could be interpreted as follows. In venous blood taken from the circulation and equilibrated with air the bicarbonate concentration (approximately 25 m*M*) does not change significantly. As the blood passes into the gut of an engorging mosquito through the fascicle, the hairlike food channel in the proboscis, the pCO_2 probably falls to equilibrate with that of the surrounding atmosphere. Such a fall in pCO_2 mediates a rise in pH from that of the circulating blood to one at which exflagellation is initiated. Measurements on the pH of blood in the stomach of mosquitoes taken between 5 and 10 minutes after engorgement (*4*) confirm that these pH values overlap the range at which exflagellation is initiated in the in vitro system. Nevertheless, further experimentation is necessary before it will be possible to say whether the bicarbonate-dependent pH control is alone responsible for initiating exflagellation in the mosquito.

Whether or not factors within the insect vector are involved in controlling the events of exflagellation, our results explain how equilibration with atmospheric pCO_2 is, in the case of *P. gallinaceum*, sufficient to initiate them. Like the malaria parasites and other Haemosporidia, parasitic protozoa such as the leishmanias and African and American trypanosomes generally transform into forms similar or identical to those found in the insect vector when cultured under atmospheric conditions (*1*). The possibility arises, therefore, that atmospheric equilibration may be an important factor for the initial transformation to the vector form in other arthropod-transmitted parasites.

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12 July 1976; revised 14 September 1976

Coronary Tone Modulation: Formation and Actions of Prostaglandins, Endoperoxides, and Thromboxanes

Abstract. *Exogenous prostaglandin (PGE_2) contracts bovine and human coronary arteries but its precursor, arachidonic acid, relaxes them. The endoperoxides PGH_2 and PGH_3 relax bovine coronary strips, but PGH_1 produces contraction. The primary prostaglandins exert opposite effects to their own endoperoxide precursors, thus, PGE_2 and PGE_3 contract, and PGE_1 relaxes the bovine coronary arteries. The paradoxical coronary dilation produced by the arachidonate or the PGH_2 suggest that little if any coronary isomerase which converts endoperoxide into PGE_2 exists, or that a novel, potent, PG-like substance is produced by the isolated coronary arteries. Although the coronaries do not possess thromboxane A_2 synthetase activity, the vessels are profoundly contracted by exogenous thromboxane A_2. Thromboxane A_2 can be synthesized and released by circulating platelets when they are aggregated by endothelial injury or thrombin. Thus, coronary tone, and possible spasm, in ischemic myocardial zones may be influenced markedly by interplay between prostaglandins, endoperoxides, and thromboxane formed by platelets on the one hand, and endoperoxide products synthesized endogenously in the coronary arteries on the other.*

Isolated or cultured vascular smooth muscles synthesize prostaglandins which may be involved in the regulation of blood vessel tone (*1*, *2*). Isolated bovine (*3*) and human coronary arteries exhibit dose-dependent contractions to prostaglandin (PG) E_2 and $PGF_{2\alpha}$ (*2*). Paradoxically, administration of arachidonic acid, precursor of PG's of the 2 series, caused relaxation of the bovine and human coronary arteries, which was abolished by cyclooxygenase inhibitors (*2*). These results suggest that arachidonate was converted by coronary PG-synthetase to a substance which has a vasodilating effect, and therefore is not PGE_2 or $PGF_{2\alpha}$. The unstable endoperoxides, PGG_2 and PGH_2, are candidates for vasodilating substances produced from arachidonate. However, the existing evidence indicates that the endoperoxides contract vascular smooth muscle including rabbit thoracic aorta (*4*), and isolated pig coronary artery strips (*5*). A further understanding of endogenous regulators of coronary resistance and their potential impact in such conditions as myocardial ischemia, coronary vasospasms, and coronary thrombosis seems of critical importance.

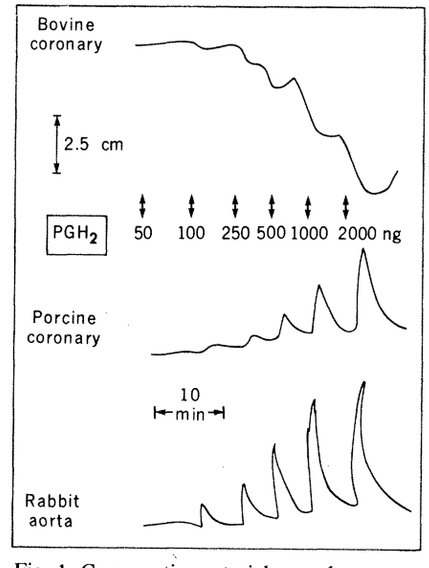

Fig. 1. Comparative arterial vascular responsiveness to the endoperoxide PGH_2.

Bovine and porcine coronary artery and rabbit aorta were excised and handled from freshly removed hearts as previously described (*2*), and spiral strips were prepared (*6*). In experiments designed to compare simultaneously the re-