Eq. 4b, stable but complex oscillations, which appear to be analogous to the stable oscillations of periods 3 and 6 found in finite difference equations, have also been observed over limited parameter ranges (*18*).

The solutions of Eqs. 4a and 4b are especially intriguing when considered in light of the clinical literature, where periodic fluctuations in circulating levels of platelets, red blood cells, and white blood cells have been observed. In particular, normal and pathological granulocyte production has been intensively studied (*16, 19, 20*). In normal healthy adults, circulating levels of granulocytes are either constant or show a mild oscillation with a period of 14 to 24 days (*16*). Cyclical neutropenia is a disease characterized by spontaneous oscillations in granulocyte numbers from normal to subnormal levels with a period of about 21 days (*19*). In some patients suffering from CGL circulating granulocyte numbers display large-amplitude oscillations with periodicities ranging from 30 to 70 days, depending on the patient (Fig. 2a) (*20*). In a number of CGL patients the cellular generation time is significantly increased, which would lead to an increase in τ (*21*). These long-term oscillations (Fig. 2a) occur in the absence of any clinical intervention. The variability in the maxima in Fig. 2a and the irregularities of the white blood cell counts over the last 100 days suggest, but not conclusively, that sequences of bifurcations may occur in patients with CGL.

We have shown how simple mathematical models of two physiological control systems can reproduce the qualitative features of normal and pathological function. We believe there is a large class of dynamical diseases, two of which have been considered here, characterized by the operation of a basically normal control system in a region of physiological parameters that produces pathological behavior (*22*). Our analysis suggests the following approaches: (i) demonstrate the onset of abnormal dynamics in animal models by gradual tuning of control parameters; (ii) gather sufficiently detailed experimental and clinical data to determine whether sequences of bifurcations similar to those found here actually occur in physiological systems; and (iii) attempt to devise novel therapies for disease by manipulating control parameters back into the normal range.

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14. With x_0, V_0, S_0, and V_m known, $n = x_0 S_0 V_m / V_0 (V_m - V_0)$, $\theta^n = x_0 (V_m - V_0)/V_0$, and $\alpha = \lambda / x_0 V_0$. For our numerical simulations we assumed $\lambda = 6$ mm-Hg per minute, $\tau = 0.15$ minute, $x_0 = 40$ mm-Hg, $V_0 = 7$ liter/min, and $V_m = 80$ liter/min, and varied S_0 [see (*9*)].
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17. Equation 4b is a continuous time analog of a number of finite difference equations previously studied (*5–8*). It also arises as a limiting case of a more realistic model for stem cell production (M. C. Mackey, in preparation).
18. A stability analysis of the steady states of Eqs. 4a and 4b indicates that destabilization occurs when n, β_0, and τ are increased or γ is decreased (*15*). In Eq. 4a destabilization is marked by the appearance of a stable oscillatory solution. Extensive numerical simulations of Eq. 4b indicate that the same sequence of bifurcations described in the text for increasing τ may be obtained by increasing n or β_0 or decreasing γ. For the numerical simulations we took $\gamma = 0.1$ per day, $\beta_0 = 0.2$ per day, $n = 10$, and $\theta = 1.6 \times 10^{10}$ cells per kilogram. This gives a total white blood cell density of 1.6×10^{10} cells per kilogram, a steady-state granulocyte turnover rate of 1.63×10^9 cells per kilogram per day, and a maximum granulocyte turnover rate of 3.66×10^9 cells per kilogram per day. See M. M. Wintrobe, *Clinical Hematology* (Lea & Febiger, Philadelphia, 1976).
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23. This research was supported by grant NRC-A-0091 from the National Research Council of Canada and a grant from the Cancer Research Society. We thank J. Milic-Emili, C. Polosa, and B. A. Cooper for helpful discussions.

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Fatty Acids and Their Prostaglandin Derivatives: Inhibitors of Proliferation in Aortic Smooth Muscle Cells

Abstract. *Prostaglandins are synthesized from eicosa-8,11,14-trienoic acid and eicosa-5,8,11,14-tetraenoic acid by smooth muscle cell cultures from guinea pig aorta. Production is inhibited by indomethacin. The precursor fatty acids and their prostaglandin derivatives inhibit proliferation of the cell cultures. The relative availability of fatty acids for prostaglandin biosynthesis may represent a control mechanism for cell proliferation.*

An important characteristic of the early or fatty streak lesion in the development of atherosclerosis is the presence of significant amounts of eicosa-8,11,14-trienoic acid ($C_{20:3}$) in addition to eicosa-5,8,11,14-tetraenoic acid ($C_{20:4}$) within the cholesteryl ester fraction (*1*). Cholesteryl esters of long-chain fatty acids are not surfactants (*2*) and these compounds form relatively inaccessible lipid droplets in the intimal lesions (*3*). $C_{20:3}$ is both an intermediate synthesized in the conversion of linoleic acid to $C_{20:4}$ and a precursor of prostaglandin E_1 (PGE_1) (*4*). The fatty acid $C_{20:4}$ is a precursor of prostaglandin E_2 (PGE_2) (*4*). Since only small amounts of $C_{20:3}$ are found in tissues (*1*), the amount of $C_{20:3}$ that is available for PGE_1 biosynthesis could be markedly diminished by

$C_{20:3}$ accumulation as a cholesteryl ester in an inert lipid droplet. Although larger amounts of $C_{20:4}$ are found in tissues (*1*), the accumulation of $C_{20:4}$ as a cholesteryl ester could also diminish its availability for PGE_2 biosynthesis.

PGE_1 and PGE_2 activate adenyl cyclase in various tissues (*5*). Adenosine 3′,5′-phosphate inhibits cell proliferation in susceptible cell lines (*6*). Several investigators (*7*) have emphasized the importance of the proliferation of aortic smooth muscle cells in atherosclerosis. Other investigators (*8*) have described plaquelike mounds and intertwined multilayered areas in smooth muscle cultures. We propose that accumulation of $C_{20:3}$ and $C_{20:4}$ as cholesteryl esters in fatty streaks decreases their availability for prostaglandin biosynthesis and leads to smooth muscle cell proliferation.

Aortic segments were freed from adventitia (*9*) and a smooth muscle cell culture was produced (*10*). Prostaglandin biosynthesis was measured by a radioimmunoassay procedure (*11*). Proliferative potentials (*12*) of smooth muscle cells were measured in culture media supplemented with 20 percent fetal bovine serum alone and in the same cloning media containing added $C_{20:3}$, $C_{20:4}$, PGE_1, or PGE_2 (*13*).

Radioimmunoassay shows that both $C_{20:3}$ and $C_{20:4}$ yield PGE in smooth muscle cell cultures (Table 1). More PGE is obtained from $C_{20:3}$ than from an equimolar amount of $C_{20:4}$. PGE biosynthesis from $C_{20:3}$ and $C_{20:4}$ is significantly inhibited by indomethacin.

Table 1. Radioimmunoassay of PGE biosynthesis from precursor fatty acids in smooth muscle cells and its inhibition with indomethacin. PGE_1 and PGE_2 show similar cross-reactivities with the antibody (0.7 ng and 0.5 ng, respectively, are required for 50 percent inhibition with [^{3}H]-labeled PGE_2). A false-positive PGE value of 1030 pg/ml was obtained when 160 μM $C_{20:3}$ was incubated with buffer alone. A false-positive PGE value of 125 pg/ml was obtained when 160 μM $C_{20:4}$ was incubated with buffer alone. Data are average values for duplicate determinations.

Incubation	PGE (pg/ml)
Complete media	< 10
+ SM	230
+ SM + 180 μM $C_{18:1}$	280
+ SM + 160 μM $C_{20:3}$	4000
Complete media	18
+ SM	102
+ SM + 160 μM $C_{20:3}$ + 11.2 μM indomethacin	5050
+ SM + 160 μM $C_{20:4}$ indomethacin	650
+ SM + 160 μM $C_{20:4}$ + 5.6 μM indomethacin	1300 540

In low concentrations $C_{20:3}$ stimulates cloning in smooth muscle cell cultures (Table 2). Both $C_{20:3}$ and $C_{20:4}$ in higher concentrations significantly inhibit cloning in smooth muscle cultures (Table 2). In one out of the four smooth muscle cell cultures that were compared $C_{20:3}$ was a better inhibitor than an equimolar amount of $C_{20:4}$.

PGE_1, the prostaglandin obtained from $C_{20:3}$, is a more potent inhibitor than its precursor fatty acid of cloning in smooth muscle cell cultures (Table 3). Thus, inhibition is observed with 2 μM PGE_1 and 80 μM $C_{20:3}$. PGE_2, the prostaglandin obtained from $C_{20:4}$, is also a more potent inhibitor than its precursor fatty acid of cloning in smooth muscle cell cultures (Table 3). PGE_2 is a less effective inhibitor than PGE_1. Indeed, a low concentration of PGE_2 stimulated cloning in one smooth muscle cell culture.

Table 2. Effect of precursor fatty acids on the proliferative potential of smooth muscle cell cultures. All values are corrected for 100 percent plating efficiency.

Incubation	Number of clones (mean ± S.D.)
Control 1	560 ± 51
+ 1.6 μM $C_{20:3}$	690 ± 77*
+ 16 μM $C_{20:3}$	710 ± 65*
+ 80 μM $C_{20:3}$	300 ± 19*
+ 160 μM $C_{20:3}$	21 ± 4*
Control 2	300 ± 31
+ 160 μM $C_{20:3}$	34 ± 8*
+ 160 μM $C_{20:4}$	49 ± 14*
Control 3	680 ± 37
+ 160 μM $C_{20:3}$	84 ± 35*
+ 160 μM $C_{20:4}$	210 ± 40*
Control 4	700 ± 70
+ 160 μM $C_{20:3}$	180 ± 23*
+ 160 μM $C_{20:4}$	180 ± 13*
Control 5	510 ± 35
+ 160 μM $C_{20:3}$	190 ± 43*
+ 160 μM $C_{20:4}$	240 ± 14*

*Results differing from the control at the <.01 significance level.

Table 3. Effect of PGE_1 and PGE_2 on the proliferative potential of smooth muscle cell cultures. All values are corrected for 100 percent plating efficiency.

Incubation	Number of clones (mean ± S.D.)
Control 1	700 ± 70
+ 0.2 μM PGE_1	660 ± 78
+ 2 μM PGE_1	420 ± 77*
+ 20 μM PGE_1	20 ± 8*
Control 2	380 ± 65
+ 0.2 μM PGE_2	510 ± 32*
+ 2 μM PGE_2	390 ± 38
+ 20 μM PGE_2	3 ± 2*
Control 3	640 ± 73
+ 0.2 μM PGE_2	680 ± 52
+ 2 μM PGE_2	560 ± 132
+ 20 μM PGE_2	220 ± 27*

*Results differing from the control at the < .01 significance level.

In this study, we show that prostaglandins are synthesized from $C_{20:3}$ and $C_{20:4}$ fatty acids in smooth muscle cell cultures. Both the precursor fatty acids and their prostaglandin derivatives suppress cell proliferation. Previous studies (*1–3*) have shown that the $C_{20:3}$ and $C_{20:4}$ fatty acids are entrapped in the cholesteryl esters of fatty streaks. We suggest that the relative unavailability of $C_{20:3}$ and $C_{20:4}$ allows the proliferation of smooth muscle cells in the development of the atherosclerotic plaque.

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11. Precursor fatty acids were dissolved in ethanol, diluted with complete media, and added to 75-

cm² flasks containing a confluent monolayer. The final ethanol concentration was 0.04*M*. The monolayer was incubated for 1 hour at 37°C. PGE levels in the media were then determined by radioimmunoassay (*14*) with antiserum to PGE, [^{3}H]PGE_2 (from New England Nuclear), and goat antibody to rabbit γ-globulin (from Clinical Assays, Inc.). In inhibitor experiments, cells were incubated with indomethacin (IM) for 15 minutes.

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Premenstrual Symptoms: A Reinterpretation

Abstract. *Conclusions regarding the physiological basis and disruptive effects of premenstrual symptoms may be biased because of the reliance on self-report questionnaires as a source of data. In order to examine this possible bias, women's perceptions of their cycle phase were separated experimentally from actual cycle phase. Women who were led to believe that they were premenstrual reported experiencing a significantly higher degree of several physical symptoms, such as water retention, than did women who were led to believe they were intermenstrual. Thus, because of these psychosocial influences on symptom reports, it seems necessary to reexamine previous conclusions regarding the magnitude of menstrual-related changes as well as their physiological basis.*

A variety of physical and psychological symptoms, such as cramps, painful breasts, irritability, and depression have been associated with the premenstrual and menstrual phases of women's reproductive cycles (*1–4*). These uncomfortable symptoms have generally been interpreted as reflecting underlying physiological changes which accompany the menstrual cycle (*2, 3*). However, a major source of evidence regarding cyclic changes has been women's self-reports of symptoms experienced at various phases of the menstrual cycle (*1*). The data presented in this report suggest that self-report studies may have led to exaggerated conclusions regarding the kinds of symptoms experienced, the magnitude of cyclic changes, and the physiological basis of premenstrual symptoms.

Although studies based on women's self-reports of symptoms have found cyclic differences, studies based on less subjective measures have frequently found no differences. For example, in spite of strong beliefs that women gain weight and retain water premenstrually (*4*), carefully controlled observations have shown little cyclic variation in these symptoms (*5*). Furthermore, investigators who find a premenstrual increase in these variables usually also report a midcycle peak (*5, 6*). In addition, according to a recent review (*7*), most objective measures of performance (such as athletics or tests of reasoning) fail to show an impairment associated with the menstrual cycle, even though 8 to 16 percent of the women themselves believed that their performances are affected negatively by their cycles.

In view of the inconsistent findings regarding menstrual-related symptoms, it becomes necessary to question the validity of self-report studies. That is, self-report measures are susceptible to various kinds of biases and may reflect cultural beliefs concerning the kinds of symptoms women experience at various phases of the cycle. This report presents a study in which a woman's actual cycle phase was separated experimentally from her belief concerning her cycle phase. Women were told that it was possible, through new scientific techniques, to predict the expected date of menstruation. In this way, it was possible to assign them to "premenstrual" and "intermenstrual" groups on a random basis. It was hypothesized that the different groups of women would report experiencing different levels of menstrual-related symptoms even though they were all tested at about a week before the onset of menstruation.

Subjects were 44 women undergraduates at Princeton University, aged 18 to 24, who were not taking oral contraceptives at the time of the study nor had taken them within the previous 3 months. Variability in the length of their cycles did not exceed 2 weeks. Upon initial telephone contact, subjects were told they were participating in contraception-related research in which a new technique for predicting the expected date of menstruation from an electroencephalogram (EEG) was being surveyed on young women, having been successfully tested with older women. Brief menstrual histories were also obtained. Later, subjects were telephoned to arrange an appointment. Unknown to the subject, the scheduled day of testing was chosen specifically to correspond to the sixth or seventh day (as estimated from her menstrual history) before her next menses.

The research was conducted in the university infirmary in two connecting rooms, one of which contained an examining table and a large oscilloscope with EEG electrodes attached to it. Subjects were greeted by the first experimenter, given a sheet explaining the purpose of the study, and asked to complete a short medical history. The experimenter then took the temperature and blood pressure of the subject and explained the EEG procedure. Electrodes were attached to the subject's forehead with beautician's tape, and the experimenter proceeded to "run" the simulated EEG machine. After 4 minutes, the electrodes were removed, and the experimenter pretended to read the output. She then informed the subject, according to the experimental group to which she had been randomly assigned, that (i) the subject was "premenstrual" and her period was due in 1 or 2 days (premenstrual group) or that (ii) she was "intermenstrual" and her period was not expected for at least a week to 10 days (intermenstrual group), or (iii) she was given no information at all about the expected date of menstruation (control group). The subject was then instructed to go into an adjoining room, where a second experimenter, who did not know to which experimental group the subject belonged, administered the Moos (*2*) Menstrual Distress Questionnaire (MDQ), consisting of 48 items, 46 of which form eight clusters of symptoms (*8*). Subjects were asked to rate the extent to which they had experienced any of the symptoms in the last day or two. Immediately afterward, subjects were given information describing the true intent of the experiment and were questioned concerning any suspicions