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DOSE-RESPONSE THRESHOLDS FOR PROGRESSIVE DISEASES

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□   Many diseases, including cancers, heart diseases, and lung diseases, can usefully be viewed as arising from disruption of feedback control systems that normally maintain homeostasis of tissues and cell populations. Excessive exposure can destabilize feedback control loops, leading to sustained elevation of variables to saturated levels and clinical consequences such as chronic unresolved inflammation, destruction of tissue (as in emphysema), proliferation of cell populations (as in lung cancer), and increases in reactive oxygen species and protease levels (as in coronary heart diseases and chronic obstructive lung disease). We propose a framework for understanding how exposure can destabilize normally homeostatic feedback control systems and create sustained imbalances and elevated levels of disease-related variables, by creating a new, locally stable, alternative equilibrium for the dynamic system, in addition to its normal (homeostatic) equilibrium. The resulting model, which we call alternative-equilibria (AE) theory, implies the existence of an exposure threshold below which transition to the alternative equilibrium (potential disease) state will not occur. Once this threshold is exceeded, progression to the alternative equilibrium continues spontaneously, even without further exposure. These predictions may help to explain patterns observed in experimental and epidemiological data for diseases such as COPD, silicosis, and inflammation-mediated lung cancer.

Key words: exposure-response threshold, dose-response threshold, mathematical model, crystalline silica, lung cancer, silicosis

1. INTRODUCTION

Many diseases can be viewed as arising from destabilization of physiological feedback control loops that normally maintain homeostasis. For example, several important heart (Eleuteri *et al.* 2009) and lung (Azad *et al.* 2008) diseases are associated with oxidative stress caused by disruption of the normal balance between reactive oxygen species (ROS) and antioxidants. Examples include chronic lung inflammation, fibrosis, silicosis, and inflammation-mediated lung cancer. Chronic obstructive pulmonary disease (COPD) involves failures to maintain protease/anti-protease and apoptosis/replacement balances in the alveolar epithelium, and degradation/repair balance in the extracellular matrix, as well as oxidant-antioxidant balance in alveolar macrophages (AMs) and other lung cell populations (Cox 2011). Chronic inflammation in the lung, heart, and other organs or organ systems arises from failure to maintain the normal balance between influx and clearance of inflammatory cells, such as

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neutrophils and macrophages, in the inflamed areas. Cancer reflects a loss of homeostatic control balancing proliferation and apoptosis rates in cell populations.

When such diseases are associated with preventable occupational or environmental exposures, it is natural to wonder whether there are safe exposure levels, below which exposures do not cause harmful imbalances due to failures of normal homeostatic regulatory processes. This paper proposes a simple mathematical model and conditions for which such protective thresholds exist in disease processes arising from disrupted feedback-control loops. Its focus is on understanding general qualitative mechanisms of disrupted homeostasis that can produce such thresholds, rather than on estimating specific parameter values, which are likely to differ for different individuals.

Our mathematical approach is based on modeling systems of dynamic (changing) variables, organized into networks in which the levels of some variables affect the rates of change (e.g., production or removal) of other variables. This framework is similar to that of Biochemical Systems Theory ("S-theory") (Savageau, 1988), except that we do not assume any specific functional form (e.g., power laws) for the relations among levels of variables and their rates of changes. As in S-theory, we make the realistic assumption that the rates of production and removal of biological substances are saturable (i.e., the maximum possible rates of change are finite). We consider networks with positive feedback loops, and study how the equilibrium levels of variables in these networks change when exposure increases the responsiveness of some variables (e.g., their net rates of production) to the levels of others, since this a commonly observed in inflammation-mediated diseases (e.g., Azad *et al.* 2008, Cox 2011). If there is more than one stable equilibrium for a system, then, following the usual terminology in dynamical systems theory, we call the set of all starting points (i.e., initial values for all variables) from which the system will move to a given equilibrium point its "basin of attraction," and we examine how exposure can change the dynamics of self-regulating systems, creating new equilibria (which we identify as potential disease states) and sending the network of feedback loops from the basin of attraction of the initial healthy equilibrium into the basin of attraction of a potential disease state equilibrium.

2. HOMEOSTASIS AND DISEASE STATES IN A SIMPLE MODEL OF A FEEDBACK LOOP

We model a biological quantity, X, (e.g., a measure of oxidative stress) that is normally kept at a stable equilibrium level, X^*, via homeostatic regulation of inflows and outflows of X. This process may involve an entire network of other variables and processes, not all of which are necessarily known to the risk analyst. Ultimately, however, X affects and is affected by

other variables so that departures from X^* are eliminated. Realistically, we assume that, although the normal background level X^* is always restored following sufficiently small perturbations of X around it, both production and removal rates for X have finite maximum possible (saturated) values. We will explore conditions under which such modest assumptions imply the existence of distinct "normal" and "disease" states, with a tipping-point threshold between them.

Explicit Formulas for a Simple Linear Model

Figure 1 shows a simplified conceptual model of inflammation-mediated lung cancer caused by exposure to agents (such as bacteria, cigarette smoke, chemical or particle pollutants, or radiation) that trigger the production of excess ROS in the lung. In this model, excess ROS disrupts oxidant-antioxidant balance, causing oxidative stress and stimulating chronic inflammation. The biological basis and evidence for this scheme, and many additional details (e.g., which cell populations secrete which cytokines and mediators, and how these attract and activate alveolar macrophages and neutrophils via specific receptors), are discussed in a large recent literature on inflammatory lung diseases (Azad *et al.* 2008, Cox 2011). The simplified high-level version in Figure 1 allows explicit analysis of the input-output behavior of the ROS feedback loop (i.e., *ROS → pro-inflammatory mediators → macrophages and neutrophils → ROS*) when each component is described by a simple (piecewise linear) feedback control model. The resulting analysis can then be generalized to a wide range of nonlinear models. Throughout, we use the arrow notation to mean that an increase in the variable at an arrow's tail increases the variable at its head, other things being held fixed, e.g., by increasing its production rate and/or decreasing its removal rate.

Figure 1 is a directed graph model for non-negative variables. (All, other than the exogenous input variable, *exposure*, are positive even in the absence of exposure.) Perhaps the simplest explicit model of self-regulation of each variable, X, in such a system is as follows:

$$dX/dt = b_X - d_X X \tag{1}$$

inhibited apoptosis and increased proliferation of damaged cells ⇾ *lung cancer risk*
⬆
exposure ⇾ ROS from macrophages and neutrophils ⇾ pro-inflammatory mediators
⬆ ⬇
number of activated alveolar macrophages, neutrophils, fibroblasts
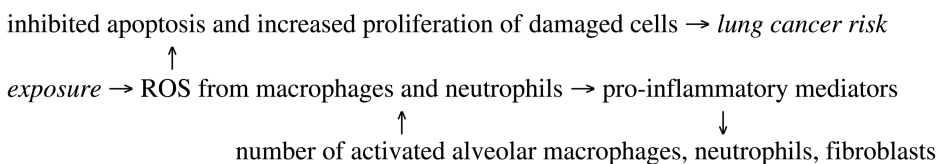
FIGURE 1. Simplified schematic diagram of key events by which exposure can create chronic lung inflammation and increased risk of lung cancer (*Source:* Adapted from Figure 3 of Azad *et al.* 2008).

L. A. Cox, Jr.

where b_X is the birth rate or production rate of X and d_X is the death rate or removal rate of X, per unit of X per unit time. This model implies that there is a unique, globally stable normal (unexposed) equilibrium level of X, which we will denote as X^*, that can be found by setting $dX/dt = 0$ (the definition of equilibrium) and solving for X:

$$X^* = b_X/d_X \qquad (2)$$

If X starts at any other level, then the dynamic process (1) will restore its level to X^*.

The analysis is almost as simple when several such self-regulating variables are linked in a feedback cycle, so that the level of each variable depends partly on the level of its predecessor. Let Y be the predecessor of X, and generalize equation (1) as follows, to let Y affect X:

$$dX/dt = b_X + a_{XY}Y - d_XX \qquad (1a)$$

(This is essentially the same model as (1), except that b_X is extended to become $b_X + a_{XY}Y$. The new parameter, a_{XY}, may be interpreted as a potency parameter, showing by how much a unit increase in Y increases the rate of formation of X.) Now, the equilibrium level of X is:

$$X^* = (b_X + a_{XY}Y)/d_X \qquad (2a)$$

In a stable feedback loop, with X and Y as its only two variables, X would also be a predecessor of Y, and symmetry then implies that the equilibrium level of Y is

$$Y^* = (b_Y + a_{YX}X)/d_Y \qquad (2b)$$

The joint equilibrium values of X and Y can be found by requiring mutual consistency between equations (2a) and (2b). Substituting (2b) into (2a) for Y yields:

$$X^* = (b_X/d_X) + (a_{XY}/d_X)(b_Y + a_{YX}X^*)/d_Y$$

$$d_Xd_YX^* = d_Yb_X + a_{XY}b_Y + a_{XY}a_{YX}X^*$$

$$X^* = (d_Yb_X + a_{XY}b_Y)/(d_Xd_Y - a_{XY}a_{YX}) \text{ if } a_{XY}a_{YX}/d_Xd_Y < 1 \qquad (3)$$

The formula for Y^* is symmetric [i.e., just exchange X and Y throughout (3)]. If exogenous exposure affects the model variables by increasing b_X, then equation (3) implies that each unit of increase in b_X will increase X^* by $d_Y/(d_Xd_Y - a_{XY}a_{YX})$, as long as the system is stable ($a_{XY}a_{YX}/d_Xd_Y < 1$).

The preceding analysis is unchanged if many variables and pathways intervene between X and Y, provided that a unit change in Y eventually produces a unit increase of a_{XY} in the rate of formation of X, and, conversely, a unit change in X eventually produces a unit increase of a_{YX} in the rate of formation of Y. In general, if N such variables are arranged in a feedback loop, with the equilibrium level of each depending on the equilibrium level of its predecessor, then generalizing (3) shows that all N of them will reach stable equilibrium levels if the product of the component factors a_j/d_j around the loop is less than 1, where a_j is the potency factor quantifying how much component j is increased by a unit increase in its predecessor. Explicitly, denote the N variables by X_1, X_2, ..., X_N, and arrange them into a loop: $X_1 \rightarrow X_2 \rightarrow ... \rightarrow X_N \rightarrow X_1$ (that is, each X_j has X_{j+1} (modulo N) as its successor). Generalize equation (1) as follows:

$$dX_j/dt = b_j + a_j X_{j-1} - d_j X_j \qquad (4)$$

(Here, a_j denotes the potency factor linking the inflow to compartment j to the level of compartment $j-1$.) At equilibrium, the inflow ($b_j + a_j X_{j-1}$) must equal the outflow ($d_j X_j$), implying the following flow balance equation:

$$X_j = (b_j/d_j) + (a_j/d_j)X_{j-1}. \qquad (5)$$

This first-order linear difference equation can be solved by repeated substitution. For the equilibrium level in compartment 1:

$$X_1 = (b_1/d_1) + (a_1/d_1)X_N = (b_1/d_1) + (a_1/d_1)[(b_N/d_N) + (a_N/d_N)X_{N-1}]$$

$$= (b_1/d_1) + (a_1/d_1)(b_N/d_N) + (a_1/d_1)(a_N/d_N)[(b_{N-1}/d_{N-1}) + (a_{N-1}/d_{N-1})X_{N-2}]$$

$$= [(b_1/d_1) + (a_1/d_1)(b_N/d_N) + (a_1/d_1)(a_N/d_N)(b_{N-1}/d_{N-1})] + (a_1/d_1)(a_N/d_N)(a_{N-1}/d_{N-1})X_{N-2}$$

$$= ... \text{ (iterate through } X_{N-3}, X_{N-4}, ... X_2, X_1)$$

$$= [(b_1/d_1) + (a_1/d_1)(b_N/d_N) + ... + (a_1/d_1)(a_N/d_N)(a_{N-1}/d_{N-1}) ... (a_2/d_2)(b_1/d_1)]$$

$$+ (a_1/d_1)(a_N/d_N)(a_{N-1}/d_{N-1}) ... (a_2/d_2)X_1.$$

This solution (which is just the usual solution to a first-order constant-coefficient linear difference equation) exploits the fact that X_1 can be

L. A. Cox, Jr.

viewed as its own ancestor to eliminate all of the other variables. To save space and clarify the solution, it is useful to rewrite it as follows:

$$X_1 = c_1 + gX_1,$$

where the two constant coefficients are defined as follows:

$$c_1 = [(b_1/d_1) + (a_1/d_1)(b_N/d_N) + ... + (a_1/d_1)(a_N/d_N)(a_{N-1}/d_{N-1})... (a_2/d_2)(b_1/d_1)]$$

$$g = (a_1/d_1)(a_N/d_N)(a_{N-1}/d_{N-1})...(a_2/d_2)$$
$$= (a_1/d_1)(a_2/d_2)(a_3/d_3)...(a_N/d_N).$$

We will call g the *gain factor* around the loop. By symmetry, since any of the variables in a loop can be arbitrarily numbered as "1," the solution for any of the N variables is

$$X_j = c_j + gX_j, \tag{6}$$

which can be solved explicitly, yielding:

$$X_j = c_j/(1 - g), \text{ for } 0 \leq g < 1 \text{ and for } j = 1, 2, ..., N \tag{7}$$

If exposure increases at least one variable (by increasing any of the birth rates b_j or decreasing any of the death rates d_j, each of which will increase all of the c_j), or if it increases g (by increasing some of the inter-action potency factors a_j determining coupling strengths between vari-ables, or decreasing some of the d_j) then equation (7) implies that the equilibrium levels of *all* variables will increase in response. If g increases until $g \geq 1$, however, then the entire feedback loop becomes unstable, and its variables increase until they become saturated. Equation (7) no longer applies, since we have not yet modeled saturation; a refined model (dis-cussed in the next section) is needed.

Although we have developed equations (6) and (7) for a single stable feedback loop, they hold for more general regulatory networks (e.g., with multiple overlapping feedback loops), with equation (4) generalized to contain multiple predecessors for X. The following sections consider feedback control systems with nonlinear (and possibly unknown or uncertain) input-output relations, for which explicit formulas may be unavailable. Section 4 considers an alternative analysis of the simple lin-ear model analyzed so far, and extends it to allow for saturation in the lev-els of variables. Section 5 applies the same ideas to more general systems, to infer qualitative properties of exposure-response relations.

Dose-Response Thresholds for Progressive Diseases

3. ITERATIVE CALCULATION OF EQUILIBRIUM LEVELS

Although it is easy to compute the equilibrium values of variables using explicit formulas such as (7), in the simple model discussed so far, this section describes a more complicated-seeming iterative numerical computational procedure that will allow immediate generalization to more flexible (and uncertain) models. Suppose that X is the variable whose level we wish to predict. (We drop the subscript j from X, since the analysis leading to equation (7) applies symmetrically to each variable.) Suppose that the equation for the equilibrium level of X is

$$X = c_X + gX, \text{ for } g < 1 \tag{6}$$

This is equation (6), with the subscript j eliminated, and the subscript X for c_X indicating that this constant depends in general on which X we are considering. Now, suppose that a change in exposure (perhaps from 0 to a positive constant) leads to a change in X and in either or both of the two parameters (c_X, g). Let X_0 be the initial value of X when the change in exposure disturbs the system. (Henceforth in this section, subscripts on X will index iterations in a procedure for guessing the new value of X. X_0 is its initial value.) When the system settles down to a new steady-state equilibrium (assuming for the moment that it eventually does so), what will the new value of X be? Of course, we could calculate the answer from equation (7), as $X = c_X/(1 - g)$, by plugging in the new values of (c_X, g). But, instead, we apply the following iterative numerical calculation of values, based on equation (6):

$$X_{t+1} = c_X + gX_t \tag{8}$$

It is instructive to interpret this iteration as follows. Starting from level X_t, the effects of X propagate out into the feedback loop (or more general system) affected by X. We imagine letting all other compartments (i.e., variables) adjust until they are in equilibrium with X_t. As a result of their new levels, new information will feed back to the parameters governing compartment X (e.g.., via equation (5)). However, we can envision clamping X at value X_t until all other variables have finished adjusting to it (to as many significant digits as desired). Then, when all other variables have reached their new values in response to X_t, we hold their values fixed, and let X respond to come into equilibrium with them. This generates a new value of X, denoted by X_{t+1} in equation (8). Now we advance the iteration counter one step (so that the new value X_{t+1} will play the role of X_t in the discussion just given), and repeat.

The justification for this procedure is familiar from numerical analysis: equation (8) has as its (unique, globally stable) fixed point the same solution as equation (6). Figure 2 shows why. For *any* parameter values
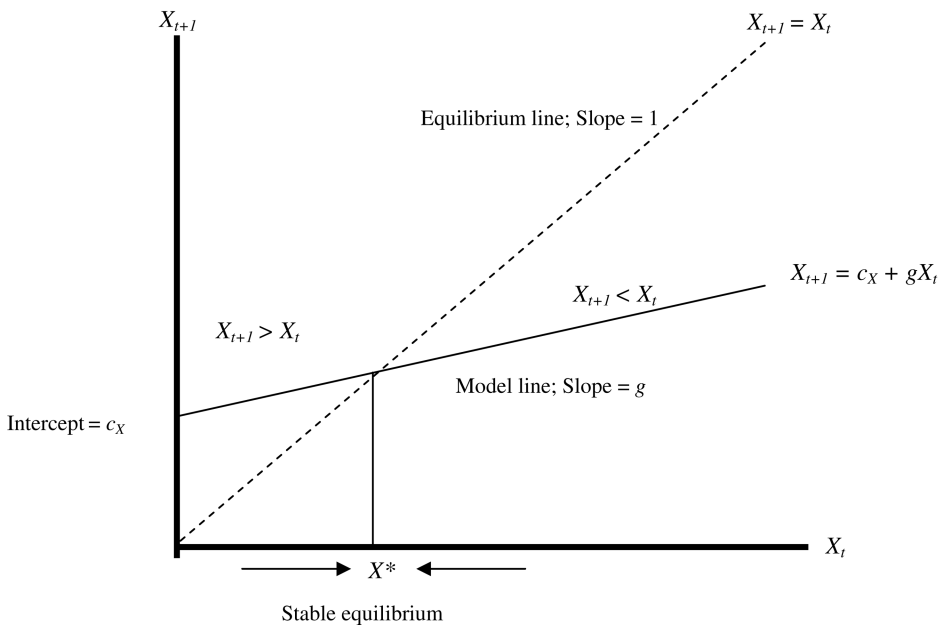
L. A. Cox, Jr.

FIGURE 2. Finding the equilibrium of a stable positive feedback loop.

(c_X, g), with $0 \leq g < 1$, the *model line* representing equation (8) (with slope g and y-intercept c_X) cuts the *equilibrium line* (meaning the 45 degree line through the origin, the collection of all points with $X_{t+1} = X_t$) exactly once, from above. Call the value of X at this intersection point X^*. Then, for all starting values of X less than X^*, the sequence of X values produced by iteration (8) is increasing, since $X_{t+1} > X_t$ to the left of X^*. (Graphically, the iterations from equation (8) can be visualized as a series of steps, moving horizontally across from any starting point (X_t, X_{t+1}) on the model line to the dashed equilibrium line, then moving vertically to a new point on the model line, and continuing via a sequence of smaller and smaller such steps toward X^*.) Conversely, for any starting value to the right of X^*, the sequence of X values produced by iteration (8) is decreasing, since $X_{t+1} < X_t$ to the right of X^*. Equilibrium is achieved only at X^*, where $X_{t+1} = X_t$.

This iterative procedure for computing equilibrium values, known in numerical analysis as functional iteration or fixed-point iteration, generalizes immediately to permit calculation of equilibria for a wide variety of systems with *nonlinear* model curves in place of the model line shown in Figure 2. It can be used to gain insight into the qualitative behaviors of systems for which only some general features of the model curve are know, even if there is not enough knowledge available to calculate exact answers. This is useful for modeling realistically uncertain disease processes.

4. EFFECTS OF EXPOSURES ON MODEL CURVES AND EQUILIBRIA

Exposure can affect the model line and the resulting equilibrium level of each quantity (i.e., X^* in Figure 2) in several ways. Consider first the explicit model developed so far for the linear case.

- If exposure *increases the birth rate* (i.e., the influx rate, b_X) into *any* compartment, then the intercept term (c_X in Figure 2) increases for *all* compartments in the feedback loop (or more general network). The model line shifts up, and the equilibrium value of each X (where the model line intersects the equilibrium line) shifts rightward. Thus, all variables increase.
- If exposure *decreases the death rate* (i.e., the fractional elimination rate, d_X) in any compartment, then not only do the y-intercepts and model lines for all compartments shift upward, but the gain factor g (which has the product of the death rate parameters as its denominator) increases, making the model lines steeper.
- If exposure *increases the coupling constant* describing the increase in the influx or production rate into one compartment per unit quantity in another compartment (i.e., one or more of the a_X coupling constants whose product is the numerator of g), then the slope of each model line, g, also becomes steeper. Again, the equilibrium levels of all variables in the feedback loop increase. If exposure increases $n > 1$ of the coupling constants, each in proportion to exposure, then the slope g will increase in proportion to the nth power of exposure.

What happens if exposure increases g to some value $g > 1$? Figure 3 suggests the answer. When $g > 1$, the model curve lies above the equilibrium line, and $X_{t+1} > X_t$. However, this increase cannot continue indefinitely: eventually, X reaches its maximum possible (saturated) level, denoted in Figure 3 by X^{**}. We assume that all variables have finite maximum possible (saturated) values. The saturated level for X is depicted by a horizontal line in Figure 3, implying that, once X reaches saturated level X^{**}, it stays there. Thus, X^{**} becomes the new, globally stable equilibrium. We call it a *saturation equilibrium*, since it occurs where the saturation line intersects the equilibrium line. Such an equilibrium may constitute a *potential disease state*, since the normal healthy homeostatic equilibrium level of X (i.e., X^* in Figure 2) has been replaced by one with higher values for X and for all variables in the same feedback loop with X. If X indicates ROS or protease concentration at the alveolar wall, or number of preneoplastic cells in the bronchiolar epithelium, then the new, higher level may cause clinical harm, such as chronic inflammation, emphysema, or increased cancer risk. However, we use the term "potential disease state" to refer to X^{**} itself, rather than eventual clinical consequences,
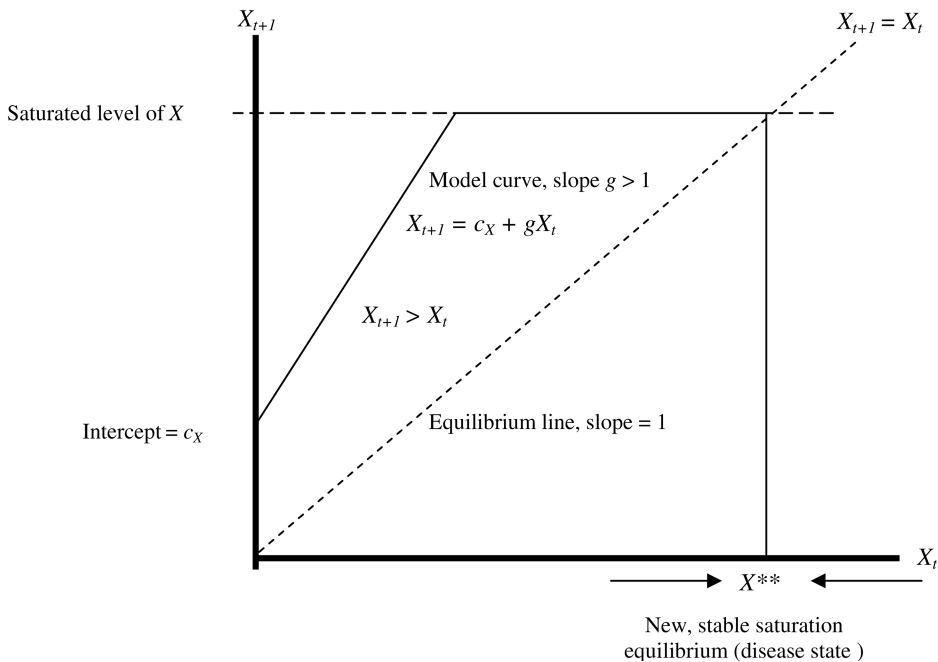
L. A. Cox, Jr.

FIGURE 3. Saturated equilibrium of a stable positive feedback loop with $g > 1$.

since failures of other (e.g., detection and repair) mechanisms may also be necessary for the saturated equilibrium to produce clinical harm.

More generally, exposure can affect dose-response in two different ways, and on two different time scales, as follows.

(i) *Short-run exposures may change the value of X.* For example, inhaling diesel exhaust, cigarette smoke, mineral dusts and fibers, or bacteria, can irritate and inflame the lung, increasing ROS (and other variables in the same feedback loop as ROS in Figure 1) above their usual un-exposed levels (Azad *et al.* 2008). If X is ROS, or increases when ROS increases, then such short-term exposures increase X.

(ii) *Longer-term exposure may change the shape of the model curve.* Exposure that changes cell population sizes and/or their sensitivity and responsiveness to mediators can thereby change the function (i.e., the model curve) mapping each specific value of X, say, X_t, to a corresponding new value, X_{t+1}, that is in equilibrium with the values of other variables when they, in turn, are in equilibrium with X_t. For example, protracted exposure to cigarette smoke or pollutants might induce a long-term shift in alveolar macrophages (AMs) toward phenotypes that release more of certain chemokines or proteases in response to any given level of X (where X could be ROS or RNS, for example). (Alternatively, or in addition, exposure might increase the production of

242

X per unit of such substances produced by the AMs.) Then, if *X* and the AM products form a positive feedback loop or network with each other, exposure will raise the model curve for *X*, as each value of X_t now produces a higher corresponding level of X_{t+1}. If the steepness of the model curve depends on the level of *X*, then a nonlinear model curve, such as "Model curve 2" in Figure 4, results.

Figure 4 provides a graphical framework for discussing both types of exposure effects. A short-term exposure that increases *X* above its usual unexposed level of *X** would be represented by an increase in Model Curve 1, e.g., a steepening of its slope and/or an upward shift in the whole line (not shown in Figure 4). Such a change would shift the unique equilibrium point *X** rightward. If the model curve returns to its initial position after exposure cases, then *X* will eventually return to its initial value of *X**. (Although nothing in our framework or results requires the Model curve 2 to be S-shaped, this is the most common shape observed in detailed S-theory models for networks of dynamic variables (Savageau, 1988), and hence we use it for purposes of illustration.)

In contrast, suppose that long-term exposure permanently increases the height of the model curve, at least for relatively high levels of *X*, by increasing the gain factor ($g = dX_{t+1}/dX_t$). (This occurs, for example, if exposure induces a permanent shift in, or selection of, cell phenotypes toward types that produce higher levels of variables in the feedback loop, such as ROS, in response to the same levels of other variables. For lung
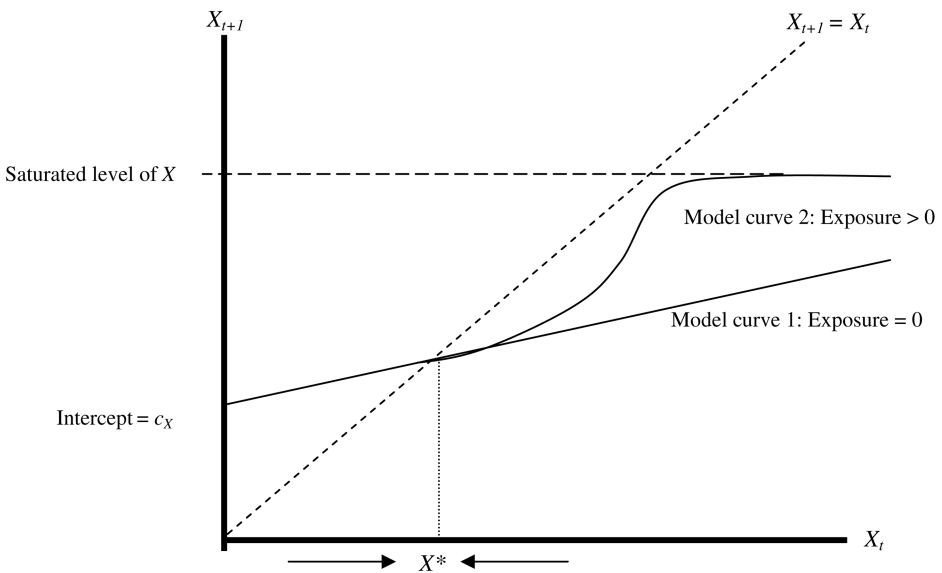

FIGURE 4. Exposure may increase the model curve, by increasing the gain factor around a feedback loop. This increase may depend on exposure, creating a nonlinear model curve.

243

diseases such as COPD, alveolar macrophages are an example of a population that undergoes such a lasting change in phenotype in response to exposure (Azad *et al.* 2008, Cox 2011).) Model Curve 2 in Figure 4 shows such an exposure-related increase in the model curve. Increases are greater at higher levels of X (but approaching a horizontal asymptote of saturated response). They are zero or negligible at sufficiently low levels of X. (This might occur if negative feedback loops maintain tight homeostasis, despite exposure, at these low levels. For example, if low levels of exposure start to increase ROS, this increase could trigger a compensating increase in antioxidants to help decrease net ROS and maintain oxidant-antioxidant balance. If low exposure hastens cell death, this could trigger a compensating increase in replacement rates to help maintain birth-death balance. Thus, Model Curve 2 is significantly elevated, compared to the no-exposure Model Curve 1, only when X levels are high enough to overwhelm such tight homeostatic control.) Although an infinite number of other model curves could be constructed, they all share the qualitative property that long-term exposure that increases the model curve only affects X if it affects the intersection of the model curve with the equilibrium line. Since this does not occur for Model Curve 2 in Figure 4, the effects of exposure are not observed in a change in X^*.

Finally, consider the case in which high, prolonged exposure permanently shifts some of the model curve upward, as in Model Curve 3 in Figure 5. This is very similar to Model Curve 2 in Figure 4, except that
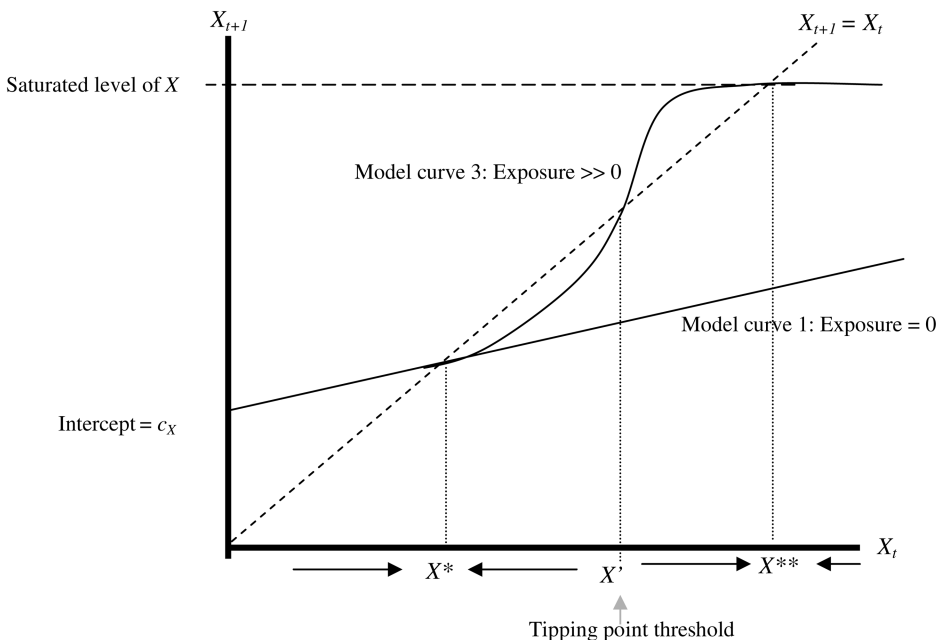

FIGURE 5. Destabilizing a feedback loop creates a disease state and a threshold.

now the increase is great enough to create a saturation equilibrium, in which the saturated portion of the curve intersects the equilibrium line from above and to the left. (In Figure 4, it did not intersect the equilibrium line at all.) Model Curve 3 has both a homeostatic equilibrium (X^*) at the left, and a saturation equilibrium (X^{**}) at the right. Both are locally stable (since both cut the equilibrium line from above and to the left). It is a topological necessity that any continuous model curve that cuts the equilibrium line at two different points from above must also cut it at least once, somewhere between them, from below. If the model curve is "S-shaped" (i.e., is a smooth curve with a slope that is non-decreasing in X up to some point of inflection, and non-increasing thereafter), then the point at which it intersects the equilibrium line from below is unique. This is an *unstable* equilibrium point, and the system will move away from it toward one of the two stable equilibria (since $X_{t+1} > X_t$ to its right and $X_{t+1} < X_t$ to its left). In the example shown in Figure 5, the unstable equilibrium point is denoted by X', and is called the "tipping point threshold." If X starts below this threshold, then it will return to X^*; if it starts above the threshold, then it will progress to X^{**}.

The general situation illustrated by this example can be summarized as follows:

> THEOREM (Alternative Equilibria): *Any S-shaped model curve having both a homeostatic equilibrium and a saturation equilibrium (both locally stable), must have a unique threshold between them such that the system described by the model curve is attracted to the homeostatic equilibrium from all starting points below the threshold, and to the saturation equilibrium from all starting points above it.*

In other words, there is a unique threshold between the respective basins of attraction for the two equilibria.

> *Proof:* For the model curve to intersect the equilibrium line twice from above, it must cross back from below the line to above it, somewhere between them. This point of intersection is the threshold referred to. It is unique because the S-shape prevents a second crossing from below (as the slope cannot again become steeper than the equilibrium line, starting from below it, at any point to the right of the first such crossing from below, which is therefore the unique unstable threshold). QED

The Alternative Equilibria theorem, though simple, has potentially useful consequences for dynamic disease models and exposure-response modeling under uncertainty. In a system with alternative equilibria, any exposure (even a relatively brief transient one) that sends X above the

245

threshold pushes the system into a basin of attraction that leads to the saturated equilibrium (*X***) as its new equilibrium point, *even in the absence of any further exposure.* It may require sustained exposure to increase the model curve far enough to create a saturation equilibrium (e.g., by converting a large enough fraction of alveolar macrophages to a high-ROS phenotype, in our ongoing example). But, once this has been done, any exposure history that sends *X* above the threshold triggers a self-amplifying escalation of the feedback loop or network. This escalation will continue, even without additional exposure, until saturation is reached. Exposure has destabilized the original system, allowing the values of its variables to escape from their homeostatically controlled levels and to be sent to an alternative, saturated state. Moreover, this state does not depend on the size of the exposure transient that began the self-amplifying escalation in variable values: the final result depends only on their saturated levels. Therefore, many uncertainties about exposure histories are irrelevant for determining the resulting health effects (if any) of exposure. All that matters is when and whether the threshold is exceeded, and, if so, how quickly the system then moves to the new, saturated equilibrium. If elevated levels of the system variables cause harm or risk of clinical diseases, then the time for this harm to manifest itself can be added to the time-to-initiation (when the threshold is first exceeded) and the time for progression to the saturated equilibrium, to obtain the total time until exposure causes observable harm.

5. TESTING ALTERNATIVE EQUILIBRIA (AE) THEORY: CRYSTALLINE SILICA AS AN EXAMPLE

The Alternative Equilibria (AE) theoretical framework in Figures 3-5 makes several testable predictions. It implies that, in susceptible species or individuals (i.e., those whose gain factors or model curves increase enough in response to exposure to cut the equilibrium line from below), sufficiently large and prolonged exposures can create a *threshold for disease progression* (or, more precisely, for permanent increases in the levels of variables) in normally homeostatic systems. Short-term exposures that send a system over this threshold then trigger a self-sustaining increase in variable levels that continues until saturation is reached, even in the absence of further exposure.

How well do these implications correspond to real-world observations? Figure 1 suggests that inflammatory lung diseases may provide a useful empirical testing ground for the AE framework. Several feedback loops regulate lung cell populations and the levels of cytokines and their receptors, ROS and antioxidants, proteases and anti-proteases, apoptotic and proliferating epithelial cells, and destruction and replacement of extracellular matrix (ECM) (Cox 2011). These loops generally reinforce the key ROS-mediated inflammatory loop shown in Figure 1 (Cox 2011),

and exposure to a wide variety of pollutants increases ROS and associated variables (Azad *et al.* 2008). Thus, it is natural to wonder whether lung diseases associated with elevated ROS exhibit the following properties, as predicted by the AE theory:

(i)   *Correlated values.* Levels of variables in the feedback loops are strongly correlated over time, i.e., they increase or decrease together.

(ii) *Susceptible and non-susceptible individuals.* Individuals with relatively low levels of ROS elevation in response to exposure are not susceptible to loop-saturating increases in variables and resulting exposure-related diseases. (Such individuals would be described by the homeostatic equilibrium in Figure 4.)

(iii) *Exposure threshold for disease causation.* Even in susceptible individuals, exposure concentrations and durations that do not push the system over the threshold between the two basins of attraction are not predicted to cause excess disease risk (at least for diseases that are mediated by a transition from the homeostatic to the saturated, high-ROS equilibrium).

(iv) *Progression without further exposure.* Exposure concentrations and durations that do push the system over the threshold will trigger a progressive increase in all loop variables to the saturated equilibrium (causing any damage and diseases or risk associated with these high levels), even without further exposure.

We can test the plausibility of these predictions by examining diseases for which an increase in ROS levels and resulting oxidative stresses in the lung environment are crucial in causing subsequent exposure-associated lung injury and disease. These diseases are thought to include chronic obstructive pulmonary disease, fibrosis (Fubini and Hubbard 2003), silicosis, and lung cancer (Ding *et al.* 2000, Shi *et al.* 1998 and 2001, Schins and Knaapen 2007, Huaux 2007, Azad et al, 2008). Particulate pollutant-related cardiovascular diseases may also follow the same paradigm (Mossman *et al.* 2007).

A recent quantitative risk model of COPD caused by cigarette smoking (Cox 2011) is fully consistent with the AE model and its implications. To test the AE model further, however, we focus here on lung diseases caused by crystalline silica. Compared to cigarette smoke (as well as coal dust, diesel exhaust, soot, PM10 in ambient air, and many other pollutants, including bacteria), crystalline silica lacks organic content, which might potentially trigger diseases via mechanisms different from the inflammatory one in Figure 1. Indeed, crystalline silica has previously been studied as a model for chronic inflammation-mediated lung carcinogenesis (Blanco *et al.* 2007).

247

L. A. Cox, Jr.

The following empirical observations are consistent with the main predictions of the AE model for crystalline silica-associated lung diseases.

- *Correlated values.* Correlations among levels of ROS, pro-inflammatory mediators (such as tumor necrosis factor alpha (TNF-α), interleukin-1, and activation of transcription factors AP-1 and NFκB involved in inflammation), lung cell apoptosis, and lung injury have been observed in silica-exposed animals *in vivo* and in lung cells *in vitro* (e.g., Fubini and Hubbard 2003). For humans, too, levels of ROS and TNF-α released by AMs have been recommended as better predictors of silica-associated lung cancer risk than silica concentration itself (Cocco *et al.* 2007), consistent with our core hypothesis (Figure 1) that escalation of ROS-loop variables creates increased risk of silica-associated lung cancer.

- *Possible progression threshold in humans.* Empirically, as noted by Porter *et al.* (2004), "Human epidemiologic studies have found that silicosis may develop or progress even after occupational exposure has ended, suggesting that there is a threshold lung burden above which silica-induced pulmonary disease progresses without further exposure."

- *Progression threshold in rats.* Experimental results in animals are also consistent with this threshold-like exposure-response pattern for progressive lung disease in humans. Porter *et al.* (2004) found experimentally that "the time course of rat pulmonary responses to silica inhalation as biphasic, [with] the initial phase characterized by increased but controlled pulmonary inflammation and damage. However, after a threshold lung burden was exceeded, rapid progression of silica-induced pulmonary disease occurred." They reported that "During the first 41 days of silica exposure, we observed elevated but relatively constant levels of inflammation and damage, with no fibrosis. Subsequently, from 41 to 116 days of exposure, rapidly increasing pulmonary inflammation and damage with concomitant development of fibrosis occurred. This suggested that pulmonary defense mechanisms were initially able to compensate and control silica-induced pulmonary inflammation and damage, but after a certain threshold lung burden was exceeded, these control mechanisms no longer were adequate to prevent the progression of silica-induced pulmonary disease." This account is consistent with the AE theoretical prediction that sustained exposure that increases the model curve thereby shifts the homeostatic equilibrium rightward (corresponding to increased but controlled levels of loop variables) *and* creates a threshold and a disease state (saturated equilibrium) that will be reached once exposure passes a tipping point threshold (Figure 5).

- *Escalation of ROS as a mechanism of lung disease.* Porter *et al.* (2006) subsequently confirmed that the mechanism of progressive injury in rat lungs following cessation of exposure is indeed continuing increased production of ROS (and also reactive nitrogen species). This is consis-

tent with the AE theory prediction that a loop, once destabilized and pushed over its threshold, will continue to escalate until it locks into a saturated equilibrium. They reported that "even after silica exposure has ended, and despite declining silica lung burden, silica-induced pulmonary NO [nitrogen oxide] and ROS production increases, thus producing a more severe oxidative stress. ...iNOS and NO-mediated damage are associated anatomically with silica-induced pathological lesions."

6. DISCUSSION AND CONCLUSIONS

The foregoing observations suggest the potential practical applicability of AE theory to explaining some observed exposure-response patterns that appear to involve thresholds. In addition to describing important aspects of COPD (Cox 2011), AE theory may be applicable to silicosis and related diseases, with chronic lung inflammation and progressive pulmonary damage, fibrosis, and lung cancer as other possible adverse health outcomes, depending on an individual's damage-detection and repair capabilities. The theory does not attempt to describe all of the necessary and sufficient conditions needed to produce clinically detectable diseases. But it does suggest that, when diseases depend on sustained elevation of one or more variables (such as ROS, or net destruction rates of alveolar tissue in emphysema, or net proliferation rates of altered bronchiolar epithelial cells in lung cancer, or net deposition of collagen and formation of scar tissue in fibrosis), then there are simple conditions under which we should expect both that there are exposure thresholds for disease causation, and also that there will be irreversible progression to a disease state (or to a high-risk state, if events other than escalation of variable levels are also required for disease) once the exposure thresholds are exceeded.

Although more work is needed to further test and refine the theory – ideally, leading to quantitative analysis of exposure thresholds and times-to-disease based on more fully developed models of relevant physiological feedback control loops or networks – available human and rat data support the hypothesis that a range of particulate pollutants (such as cigarette smoke for COPD, or crystalline silica for silicosis) may act through a common high-level dynamic exposure-response mechanism. We have proposed that, despite numerous differences in detailed pathways and cell population responses, particulate exposure-related diseases as diverse as inflammation-mediated lung cancer, coronary heart disease, COPD, and silicosis may all be usefully described as acting by the same high-level process: they create an alternative to the normal homeostatic equilibrium. Exposures that push feedback control systems into the basin of attraction for this new, alternative equilibrium then cause progressive, irreversible diseases. If correct, this unifying description suggests that pre-

L. A. Cox, Jr.

venting such diseases requires keeping exposures low enough so that no alternative equilibrium is formed – or, if one is created, keeping exposures low enough so that passage into its basin of attraction, with its irreversible slide to the new equilibrium, never occurs.

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