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On the dynamics of activation of mammalian *X* chromosomes

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SUMMARY

We have investigated a mathematical model of the process of activation of the X chromosomes in eutherian mammals. The model assumes that the activation is brought about over some definite time interval T by the complete saturation of N receptor sites on an X chromosome by M activating molecules (or multiples of M). The probability λ of a first hit on the receptor site is considered to be very much lower than that of subsequent hits; that is, we assume strong co-operative binding. Assuming further that an incomplete saturation of receptor sites is malfunctional, we can show that for proper activation of X chromosomes in normal diploid males and females, we must have $\lambda MT \geqslant 3$ and $0{\cdot}96 \leqslant N/M \leqslant 1$. An extension of this analysis for the triploid cases shows that under these conditions, we cannot explain the activation of two X's if the number of activating molecules is fixed at M. This suggests that there must be two classes of triploid embryos differing from each other in a step-wise manner in the number of activating molecules. In other words, triploids with two active X chromosomes would require $2M$ activating molecules as opposed to M molecules in triploids with a single active X. This interpretation of the two classes of triploids would be consistent with differing imprinting histories of the parental contributions to the triploid zygote.

1. INTRODUCTION

The mechanism that leads to random activation of one of the two X chromosomes normally present in human and other eutherian females continues to be an unresolved problem (Lyon, 1961, 1974). A model proposed by Brown & Chandra (1973) seems to be consistent with most of the cytogenetic data, but apparent exceptions have since become known (Lyon, 1974; Chandra & Brown, 1975; Cattanach, 1975). It is not yet clear whether these exceptions, nearly all of which are among human triploids, are attributable to vagaries either in the expression of sex chromatin in triploids or in the imprinting process following, for example, fertilization by more than one sperm.

The data on X inactivation are varied, but appear to be highly specific within each cytogenetic category. For example, in basically diploid cases, one X remains

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active irrespective of the total number of X chromosomes or the sex of the individual. Among XXY triploids, some have only one X active, while others have both X's active. Similarly, XXX triploids occur as two classes: those with one X active and others with two X's active. The Brown–Chandra model assumes that in diploids (1) each X chromosome, whether maternal or paternal in origin, has a *receptor site* capable of accepting one *informational entity* or activating molecule; (2) there is a *sensitive site* on each of a pair of autosomes (homologues). When this autosomal sensitive site is paternal in origin, it is inactive because it is imprinted in the egg at the time of fertilization; the maternally derived sensitive site, which is not so imprinted, remains active and releases a single activating molecule sometime in early embryogeny. This molecule binds to the receptor site of one of the two X chromosomes normally present in eutherian females, thus activating that X chromosome. The remaining X chromosome or chromosomes become inactive non-specifically.

The above model assumes that in diploids only one of a pair of homologous autosomal genes (the sensitive sites) is functional in each cell. This is an almost unprecedented requirement because the only autosomal genes for which this appears to be true are those governing the production of immunoglobulins (Pernis *et al.* 1965; Weiler, 1965). Although it is not known whether a single informational entity or molecule is involved in the activation process, the cytogenetic data seem to require a mechanism with a unitary effect. Such an effect could conceivably be brought about by the functioning of more than one activating molecule and receptor sites. In fact, Ohno (Drews *et al.* 1974; Ohno, 1973) postulates that a small number of activating molecules, of unspecified origin, bind co-operatively to a small number of receptor sites on the X chromosome and bring about its activation. He further assumes that parental origin of the autosomes has no bearing on the activation process (Ohno, 1973).

Keeping in mind the qualitative features of the cytogenetic data mentioned earlier, we have attempted to set up in this paper a mathematical model to obtain some insight into the process of activation of X chromosomes. Among other things, the number of activating molecules is treated as an independent parameter of the model. It is not one of the aims of the model to see which of the two possible hypotheses, the single informational entity of Brown and Chandra or the many such entities suggested by Ohno, is to be preferred. Rather, the aim is to see whether, in the framework of a simple mathematical scheme, requirement of consistency with the data gives fairly restrictive conditions on the parameters of the model or allows wide variations in them; and to find out in what way the 'unitary effect' of the activation process could be understood. In other words, this is, among other things, an attempt to put limits on plausible models of the nature of the activation process. Furthermore, it should be emphasized that the dynamics of the process modelled here is independent of the source or origin of the activating molecules. Although we will, for purposes of exposition, refer to the autosomal origin of these molecules as specified under the Brown–Chandra model, they could as well have other origins in so far as the following analysis is concerned.

In Section 1, we describe the model, specifying the independent parameters

involved. Section 2 contains the basic equations of the model and the solution in the case of a normal diploid male, and also in the case of a normal diploid female in an interesting limiting case. The analytical results of Section 2 are compared with the data in Section 3, and fairly stringent conditions on some of the parameters of the model are shown to follow. Making use of these conditions, we analyse the case of triploids in Section 4 and show that the occurrence of two distinct classes of triploids convincingly demonstrates a distinct difference in the number of activating molecules in the two cases. Section 5 summarizes our work, and some mathematical details relating to Section 2 are given in the Appendix.

2. DESCRIPTION OF THE MODEL

We confine our attention to the sex chromosomes and to the as yet unidentified homologous pair of autosomes each of which is presumed to carry the sensitive site. The sex chromosomes will as usual be called X and Y chromosomes, while the autosome of interest will be denoted by A. Each A is assumed to carry a sensitive site which when imprinted becomes desensitized. When not so imprinted a single A releases M activating molecules at the particular developmental stage – after the first division of the zygote but significantly prior to implantation (Lyon, 1974) – when the process of activation takes place. This process is assumed to last for a time interval T. Each X chromosome is assumed to possess N receptor sites. The activating molecules released at time zero may attach themselves up to time T to the receptor sites on the X chromosomes. The activating molecules are assumed to bind to the receptor sites in a definite sequence. When all the sites on an X are unoccupied, the probability per unit time per available molecule that the first site in the sequence will get occupied is λ; this is assumed to be relatively small. If one or more sites on an X are already occupied the probability per unit time per available molecule that the next site will get occupied is μ. We assume the existence of *co-operative binding*, which means μ is much greater than λ. It is implicit in this model that the activating molecules are all alike.

The independent basic parameters are therefore N, M, λ, μ and T. The activation process is described by a system of differential equations in time for a set of probabilities which are functions of time. If we have one X chromosome we have a set of functions $p_n(t)$, with $n = 0, 1, \ldots, N$ and $0 \leqslant t \leqslant T$, denoting the probability that at time t precisely n sites are occupied. For the case of two X's we have a double string of probabilities $p_{mn}(t)$ for m sites on the first X and n on the second to be occupied at time t; we have $p_{mn}(t) = p_{nm}(t)$, i.e. the two chromosomes are symmetric. For three X's, we have a triple string of probabilities $p_{lmn}(t)$, completely symmetric in l, m and n.

A normal XY male has a single X and $p_N(T)$ for it will denote the probability that all N receptor sites are occupied at time T, the end of the activation period. For normal functioning, $p_N(T)$ must be close to unity; that is, proper activation of X must take place in almost all the embryonic cells. In a normal diploid XX female, the probability of interest is $p_{0N}(T)$ and its symmetric partner $p_{N0}(T)$. *We now*

assume that proper activation of an X *chromosome results from occupation of all* N *receptor sites, while occupation of less than* N *but more than zero sites is malfunctional.* We know that in a normal female, only one of the two X's is active or potentially so, the other remaining inactive. The assumption just stated means that we must require $p_{0N}(T) = p_{N0}(T) \approx 1/2$. This would ensure that one and only one of the X's is properly activated in almost all the embryonic cells, a condition presumably essential for proper functioning of the organism; and that this is achieved without making the cell malfunctional.

It is a corollary of our assumptions that the number of activating molecules, M, must not be less than the number of receptor sites N on a single X chromosome.

3. DYNAMICS OF THE SYSTEM

We now set up the equations of the model and solve them for the cases of the normal diploid male and female.

(i) *Case* (*a*): *normal diploid male*

The quantities to be determined now are $p_n(t)$, $n = 0, 1, \ldots, N$. The number of activating molecules available is M. The condition of the X chromosome at time zero, and the fact that the $p_n(t)$ are probabilities, are expressed by the equations

$$p_0(0) = 1, \quad p_1(0) = p_2(0) = \ldots = p_N(0) = 0,$$

i.e.
$$p_n(0) = \delta_{n,0}; \tag{2.1a}$$

$$p_0(t) + p_1(t) + \ldots + p_N(t) = \sum_{n=0}^{N} p_n(t) = 1. \tag{2.1b}$$

We obtain the $(N+1)$ functions $p_n(t)$ by solving a system of linear first order differential equations for them, subject to the given initial conditions. Denote by λ_n the probability per unit time per available molecule that the $(n+1)$st site gets occupied if the previous n have already been occupied. The words 'per available empty site' are not included in this definition since the receptor sites are to be occupied in a definite sequence. The assumptions of the model show that λ_n depends on n but not on t and has the values

$$\lambda_n = \begin{cases} \lambda & \text{for} \quad n = 0; \\ \mu & \text{for} \quad 1 \leqslant n \leqslant N-1; \\ 0 & \text{for} \quad n = N. \end{cases} \tag{2.2}$$

We now get the differential equations of the model by relating the condition of the X at time $t + \delta t$, δt being small, to its condition at time t:

$$p_n(t+\delta t) = [1 - \lambda_n(M-n)\delta t]\, p_n(t) + \lambda_{n-1}(M-n+1)\,\delta t\, p_{n-1}(t). \tag{2.3}$$

This is easily understood: the probability that n sites are occupied at time $t+\delta t$ is the sum of two terms. The first is the product of the probability that n sites were already occupied at time t and the probability that no further occupation occurs in the small time interval δt. The second is the product of the probability that $(n-1)$

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sites were occupied at time t and the probability that one more site gets occupied in the time span δt. Transposing terms, dividing by δt and passing to the limit $\delta t \to 0$ we get the system of differential equations

$$\dot{p}_n(t) = \lambda_{n-1}(M-n+1)\,p_{n-1}(t) - \lambda_n(M-n)\,p_n(t) \quad (0 \leqslant n \leqslant N). \tag{2.4}$$

With the help of equation (2.2) this system could be displayed more explicitly as:

$$\dot{p}_0 = -\lambda M p_0; \tag{2.5a}$$

$$\dot{p}_1 = \lambda M p_0 - \mu(M-1)\,p_1; \tag{2.5b}$$

$$\dot{p}_n = \mu(M-n+1)\,p_{n-1} - \mu(M-n)\,p_n \quad (2 \leqslant n \leqslant N-1); \tag{2.5c}$$

$$\dot{p}_N = \mu(M-N+1)\,p_{N-1}. \tag{2.5d}$$

A straightforward consequence of these equations is

$$\sum_{n=0}^{N} \dot{p}_n(t) = 0. \tag{2.6}$$

This ensures that the solution to equation (2.5) will automatically satisfy the condition (2.1b) if the initial conditions (2.1a) are respected.

Two methods are available for solving the system of equation (2.5); this system is in any case a well-known one. The first, a 'direct integration' technique, is a step-by-step process in which one first solves for p_0, uses this to solve for p_1, and so on. The second, using a generating function which contains all the p_n's in it, converts (2.5) into a partial differential equation for this function and solves this equation. We briefly describe these in turn, leaving details to the Appendix.

The solution to (2.5a) subject to (2.1a) is immediate:

$$p_0(t) = \mathrm{e}^{-\lambda M t}. \tag{2.7}$$

To get $p_1, p_2, \ldots, p_{N-1}$, we re-express equations (2.5b, c) as follows. Based on the form of these equations, we set up an auxiliary set of functions q_n as

$$q_n = (M-n)!\,\mathrm{e}^{\mu(M-n)t} p_n. \tag{2.8}$$

Then, after one integration and use of equation (2.1a), equations (2.5b, c) lead to

$$q_1(t) = \lambda \int_0^t \mathrm{d}t_1\, \mathrm{e}^{-\mu t_1} q_0(t_1); \tag{2.9a}$$

$$q_n(t) = \mu \int_0^t \mathrm{d}t_1\, \mathrm{e}^{-\mu t_1} q_{n-1}(t_1) \quad (2 \leqslant n \leqslant N-1). \tag{2.9b}$$

By a process of iteration, this sequence of equations can be solved to yield the various p_n as integrals over p_0 which is known. The steps are given in the Appendix and the result is:

$$p_n(t) = \lambda \frac{M!}{(M-n)!\,(n-1)!}\, \mathrm{e}^{-\mu(M-n)t} \int_0^t \mathrm{d}t' (\mathrm{e}^{-\mu t'} - \mathrm{e}^{-\mu t})^{n-1}\, \mathrm{e}^{\mu(M-1)\,t'} p_0(t') \quad (1 \leqslant n \leqslant N-1). \tag{2.10}$$

One can now expand the first factor in the integrand in a binomial series and integrate each term. On comparing the result with the expression given in the Appendix

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for the hypergeometric function of the form $F(\alpha, -k; \alpha+1; z)$ with k a non-negative integer (see equation A 11), one can write $p_n(t)$ in the form

$$p_n(t) = \lambda(-1)^n \frac{M!}{(M-n)!\,(n-1)!} \frac{\exp\{-\mu(M-1)t\}}{\lambda M+\mu(1-M)} \cdot \times \left[\exp\{-[\lambda M+\mu(1-M)]t\} F\left(\frac{\lambda M}{\mu}+1-M, 1-n; \frac{\lambda M}{\mu}+2-M; 1\right) - F\left(\frac{\lambda M}{\mu}+1-M, 1-n; \frac{\lambda M}{\mu}+2-M; e^{\mu t}\right)\right] \quad (1 \leqslant n \leqslant N-1). \tag{2.11}$$

There is now no need to solve (2.5d) to get $p_N(t)$, since one can use (2.1b); so one gets

$$p_N(t) = 1 - \sum_{n=0}^{N-1} p_n(t). \tag{2.12}$$

The generating function method for solving (2.5) uses the following feature of those equations: the differential equation for $p_n(t)$ involves p_n and p_{n-1} alone, not p_{n+1}, p_{n+2}, ... One can therefore initially ignore the fact that the index n on p_n does not go beyond the value N and that p_{N+1}, p_{N+2}, ... are without meaning, and first solve (2.5a, b, c) alone with no upper limit on n. One would of course use the boundary condition (2.1a), suitably augmented. The results for $p_0, p_1, \ldots, p_{N-1}$ that one gets by solving this extended problem are also valid for the originally posed problem; but one next discards the results one has got for p_N, p_{N+1}, ... in the extended problem, and defines the true $p_N(t)$ via (2.12) again. Even in the extended problem, the presence of the factors $(M-n+1)$ and $(M-n)$ on the right hand side of (2.5c) leads to the fact that quantities $p_{M+1}, p_{M+2}, \ldots$ never appear. Keeping these observations in mind, we define a generating function $\pi(\zeta, t)$ as

$$\pi(\zeta, t) = \sum_{n=0,1,\ldots} \zeta^n p_n(t); \tag{2.13}$$

here ζ is an auxiliary continuous variable and we realize in advance that this series will terminate at $n = M$. Equations (2.5a–c) can now be translated into a partial differential equation for π, after having first agreed that the upper limit on n in (2.5c) is to be ignored. The steps involved, and the equation for π, are:

$$\begin{aligned} \frac{\partial \pi(\zeta,t)}{\partial t} &= \sum_{n=0,1,\ldots} \zeta^n \dot{p}_n \\ &= \sum_{n=0,1,\ldots} \zeta^n [\lambda_{n-1}(M-n+1)p_{n-1} - \lambda_n(M-n)p_n] \\ &= (\zeta-1) \sum_{n=0,1,\ldots} \zeta^n \lambda_n (M-n) p_n \\ &= (\zeta-1)[(\lambda-\mu)Mp_0 + \mu \sum_{n=0,1,\ldots} \zeta^n (M-n) p_n] \\ &= \mu(\zeta-1)\left(M - \zeta\frac{\partial}{\partial\zeta}\right)\pi(\zeta,t) + (\lambda-\mu)(\zeta-1)M\pi(0,t). \end{aligned} \tag{2.14}$$

Here, (2.4) was used to substitute for $\dot{p}_n$ and the values of λ_n were taken from (2.2) (with no upper limit on n). The boundary condition on p_n, (2.1a), appears now as

$$\pi(\zeta, 0) = 1. \tag{2.15}$$

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This boundary condition, plus the vanishing of the right hand side of (2.14) at $\zeta = 1$, ensures that at all times the probabilities p_n sum up to unity (i.e. $\pi(1,t) = 1$).

If we set $\zeta = 0$ in (2.14) we get an equation for $\pi(0,t)$, which is easily integrated using (2.15). The result, which is really the same as (2.7), is

$$\pi(0,t) = \mathrm{e}^{-\lambda M t}. \tag{2.16}$$

This can now be used in (2.14), leading to a definite equation for $\pi(\zeta,t)$ together with a boundary condition at $t = 0$ given by (2.15). The details of solving this equation for $\pi(\zeta,t)$ are given in the Appendix. Here we quote the final result which gives $\pi(\zeta,t)$ again in terms of hypergeometric functions of the same general type as appeared in (2.11):

$$\begin{aligned}\pi(\zeta,t) = {} & [\zeta + \mathrm{e}^{-\mu t}(1-\zeta)]^M + \frac{M(\mu-\lambda)}{\mu - M\lambda}\zeta^{M-1}(1-\zeta) \\ & \times \left[\mathrm{e}^{-\lambda M t} F\left(1 - \frac{\lambda M}{\mu}, 1-M; 2 - \frac{\lambda M}{\mu}\ \frac{\zeta-1}{\zeta}\right)\right. \\ & \left. - \mathrm{e}^{-\mu t} F\left(1 - \frac{\lambda M}{\mu}, 1-M; 2 - \frac{\lambda M}{\mu}; \mathrm{e}^{-\mu t}\frac{\zeta-1}{\zeta}\right)\right].\end{aligned} \tag{2.17}$$

As expected, this is a polynomial of degree M in ζ.

While we have been able to solve the equations describing the activation of a single X chromosome quite explicitly, it is considerably more difficult to get such solutions for cases with two or three X's. At any rate, even with the explicit expressions provided by (2.11, 2.17), the dependence of the p_n's on n is not easily seen, unless one evaluates the expressions numerically. In this paper, our aim will be to obtain qualitative properties of the model described in Section 1 and, as far as possible on the basis of analytic expressions alone. To this end, we will henceforth restrict ourselves to a certain limiting case of the model of Section 1, namely that in which *the parameter μ is taken to be infinitely larger than λ*. One can get the p_n's in this limit starting either from (2.11) or from (2.17) for $\pi(\zeta,t)$. Taking the latter course, we easily find:

$$\pi(\zeta,t) \xrightarrow[\mu\to\infty]{} \pi_\infty(\zeta,t) = \zeta^M + M\zeta^{M-1}(1-\zeta)\,\mathrm{e}^{-\lambda M t} F\left(1, 1-M; 2; \frac{\zeta-1}{\zeta}\right). \tag{2.18}$$

(The property $\pi(1,t) = 1$ is retained.) The hypergeometric function appearing here is quite elementary, as one sees by using (A 11), and (2.18) simplifies to

$$\pi_\infty(\zeta,t) = \mathrm{e}^{-\lambda M t} + (1 - \mathrm{e}^{-\lambda M t})\,\zeta^M. \tag{2.19}$$

By the prescription for passing from the solution of the extended problem to the solution for the true problem, we get the result that in the limit $\mu \to \infty$, the functions $p_n(t)$ describing activation of the X chromosome in a normal diploid male are:

$$p_0(t) = \mathrm{e}^{-\lambda M t}; \quad p_1(t) = p_2(t) = \ldots = p_{N-1}(t) = 0; \quad p_N(t) = 1 - \mathrm{e}^{-\lambda M t}. \tag{2.20}$$

The interpretation of this result, quite obviously, is as follows. When none of the receptor sites on an X chromosome are occupied by activating molecules, there is a constant and finite probability per unit time, λM, that an occupation will occur.

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However, once the first site is occupied, the enhanced occupation probability μ comes into play. If we now take the limit $\mu \to \infty$, then before one can say Mary Lyon *all* the remaining $(N-1)$ sites will get occupied, essentially instantaneously. Therefore the only nonzero entries in the string of probabilities p_n are the first and last ones, p_0 and p_N; all the intermediate ones are strictly zero. One also sees why p_0 and p_N do not depend on N. This interpretation makes sense, of course, provided $M \geqslant N$, which must be so.

A justification for taking the limit $\mu \to \infty$ is given in Section 3.

(ii) *Case* (*b*): *normal diploid female*

The number of available activating molecules is M as before, but there are two X chromosomes and so a double string of probabilities $p_{mn}(t)$ to solve for. In place of (2.1) we now have:

$$p_{mn}(0) = \delta_{m,0}\,\delta_{n,0}; \tag{2.21a}$$

$$\sum_{m,n=0}^{N} p_{mn}(t) = 1. \tag{2.21b}$$

Generalizing the derivation of (2.4) we get the system

$$\dot{p}_{mn} = (M-m-n+1)\,(\lambda_{m-1}p_{m-1,n} + \lambda_{n-1}p_{m,n-1}) - (M-m-n)\,(\lambda_m + \lambda_n)\,p_{mn} \quad (0 \leqslant m, n \leqslant N). \tag{2.22}$$

It is easily checked that these equations conserve the sum of all the probabilities p_{mn}.

The direct integration technique can again be used to get the subset of probabilities $p_{m0}(t)$, with not much more difficulty than in Case (*a*). However, its extension to get all the p_{mn} is rather arduous though possible, and will not be attempted in this paper. Similarly the generating function approach entails quite a bit of work, since one has to handle a partial differential equation with three independent variables. Fortunately, *as long as we are interested only in the limit* $\mu \to \infty$, much of this work can be avoided. The interpretation given above of the result in (2.20) shows quite clearly that in the present case the only probabilities that are non-zero in the limit $\mu \to \infty$ are $p_{00}, p_{0N} = p_{N0}$, and p_{NN}. (For the present, we assume $M \geqslant 2N$; modifications needed if $M < 2N$ are given later.) These four non-zero p's must add up to unity. One can easily obtain $p_{00}(t)$ from (2.21*a*, 2.22):

$$p_{00}(t) = \mathrm{e}^{-2\lambda M t}. \tag{2.23}$$

If by some means p_{N0} could now be calculated, then the problem is solved. This can be done. Setting $n = 0$ in (2.22) and summing over m from *0* to N we get:

$$\sum_{m=0}^{N} \dot{p}_{m0} = \sum_{m=0}^{N} (M-m+1)\,\lambda_{m-1}p_{m-1,0} - \sum_{m=0}^{N} (M-m)\,(\lambda_m + \lambda_0)\,p_{m,0}$$
$$= -\lambda \sum_{m=0}^{N} (M-m)\,p_{m0}. \tag{2.24}$$

Here, the vanishing of λ_N was used. Now this result, an exact consequence of (2.22), is fortunately independent of μ which we are allowing to tend to infinity. Since p_{00}

is known, and since only p_{00} and p_{N0} (out of the subset p_{m0}) survive as $\mu \to \infty$, (2.24) gives in this limit a differential equation for p_{N0}:

$$\dot{p}_{N0} = \lambda M \, e^{-2\lambda M t} + \lambda (N - M) \, p_{N0}. \tag{2.25}$$

The solution is immediate; on using (2.21*a*) we obtain:

$$p_{N0}(t) = \frac{M}{M+N} e^{-\lambda(M-N)t}(1 - e^{-\lambda(M+N)t}). \tag{2.26}$$

(It can be verified that if one calculates p_{m0} for finite μ by the direct integration method and in the result one lets $\mu \to \infty$, then indeed $p_{N0}(t)$ takes the value (2.26) while all p_{m0} for $1 \leqslant m \leqslant N-1$ vanish.) The value of p_{NN} follows from conservation of total probability. Collecting the results, in the case of the normal diploid female with two X chromosomes, in the limit $\mu \to \infty$ the non-zero probabilities are:

$$\begin{aligned} p_{00}(t) &= e^{-2\lambda M t}; \\ p_{N0}(t) &= p_{0N}(t) = \frac{M}{M+N} e^{-\lambda(M-N)t}(1 - e^{-\lambda(M+N)t}); \\ p_{NN}(t) &= 1 - \frac{2M}{M+N} e^{-\lambda(M-N)t} + \frac{M-N}{M+N} e^{-2\lambda M t}. \end{aligned} \tag{2.27}$$

An interesting feature of these expressions is that even with $\mu \to \infty$ they show dependence on both M and N, whereas in the case of a single X chromosome all dependence on N vanishes in the limit. Another point to be mentioned is that (2.27) are fully valid if $M \geqslant 2N$ as in that case the system of (2.22) does really involve all $(N+1)^2$ quantities p_{mn} for $0 \leqslant m, n \leqslant N$. However, if $M < 2N$, one can see that (2.22) break off at the point $m+n = M$: only those p_{mn} with $0 \leqslant m, n \leqslant N$ and *in addition* $m+n \leqslant M$ enter the problem. Thus our calculation of p_{NN} is without meaning in case $M < 2N$, but the results for p_{00} and p_{N0} *are unaffected by this problem.* Some of the probabilities $p_{M-n,n}$ would become non-zero. As we shall see in the following Section, we shall need only the expressions for p_{00} and p_{N0} in our analysis.

4. CONSTRAINTS ON THE PARAMETERS

Before making use of the results of the last section to estimate the values of the parameters, we shall attempt to justify the limiting operation $\mu \to \infty$. The point is that the parameter μ can reasonably be estimated to be about a hundred times as large as λ. This gives one the expectation that one can get a reliable guide to the system by examining the limit $\mu \to \infty$, and that making μ finite but much larger than λ is unlikely to drastically alter the results obtained when $\mu \to \infty$. Another argument is the following. In the extended problem that was set up in the last section in connection with the generating function method, we saw that there was no dependence at all on N. The characteristic shape of the distribution of probabilities p_n must then be fixed by M: one must expect a peaking of probabilities around $n = M$. This is indeed what happens as $\mu \to \infty$, as is seen from (2.19): the only non-zero p_n's are at the beginning and end of the extended chain $p_0, p_1, \ldots, p_M$. Bringing μ down from

infinity to a large finite value will make $p_1, p_2, \ldots, p_{M-1}$ non-zero but small, with p_0 and p_M remaining the most significant ones. In passing next from the extended to the true problem we leave $p_0, p_1, \ldots, p_{N-1}$ unchanged, and just lump the sum of $p_N, p_{N+1}, \ldots, p_M$ in the extended solution into the p_N of the true solution. If anything, then, the true p_N is slightly larger than the extended p_M. (Of course, all the p_n *are* positive in the extended problem.)

The results of (2.20, 2.27) can all be expressed in terms of two dimensionless parameters $\alpha = \lambda M t, \beta = \lambda N T$, where it is known that $\alpha \geqslant \beta$. The expressions of interest are:

$$\text{normal diploid male: } p_0(T) = e^{-\alpha}, p_N(T) = 1 - e^{-\alpha}; \tag{3.1a}$$

$$\text{normal diploid female: } \quad p_{N0}(T) = \frac{\alpha}{\alpha+\beta}(e^{\beta-\alpha} - e^{-2\alpha}). \tag{3.1b}$$

We can now make numerical estimates of α and β by requiring that the process of activation of an X chromosome should occur with a definite efficiency. Considering first the case of the normal diploid male, it is reasonable to demand that there be, say, a 95 % chance that one X chromosome is activated. This means that the early embryo can tolerate a cell death rate of no more than 5 % due to malfunctioning of the activation mechanism. Such a requirement fixes the parameter α, for we must have

$$p_0 = e^{-\alpha} \approx 1/20, \quad p_N = 1 - e^{-\alpha} \approx 19/20. \tag{3.2}$$

This is achieved by making α 'large enough'; it turns out that one must have

$$\alpha = \lambda M T \gtrsim 3. \tag{3.3}$$

If we next demand that the efficiency of activation of *one* X chromosome in each embryonic cell in a normal female be as high as in the male case, the *other* X chromosome having all its receptor sites unoccupied at the end of the time period T, we must have the probability $p_{0N}(T)$ of (3.1*b*) lying somewhere between 0·475 and 0·5:

$$0{\cdot}475 \lesssim \frac{\alpha}{\alpha+\beta}(e^{\beta-\alpha} - e^{-2\alpha}) \lesssim 0{\cdot}5. \tag{3.4}$$

In the expression involved here, the term $e^{-2\alpha}$ may safely be neglected since it is of the order of 1/400 by virtue of (3.2). Having determined α already, β must now be so chosen (remembering $\alpha \geqslant \beta$) as to have

$$0{\cdot}475 \lesssim \frac{\alpha}{\alpha+\beta} e^{\beta-\alpha} \lesssim 0{\cdot}5. \tag{3.5}$$

A plot of the two functions $\alpha/(\alpha+\beta)$ and $e^{\beta-\alpha}$ with respect to β in the range $0 \leqslant \beta \leqslant \alpha = 3$ quickly shows that β *must be very close to* α for (3.5) to be satisfied. The trend can easily be seen from the following list of values compiled for chosen values of β:

β	...	0	1	2	2·5	2·8	2·9	3
$\frac{\alpha}{\alpha+\beta} e^{\beta-\alpha}$	...	0·05	0·102	0·223	0·332	0·425	0·462	0·5

(3.6)

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We can infer that demanding an efficiency rate of 95 % for single X-chromosome activation in the normal diploid female, as in the male, leads to

$$0{\cdot}96 \lesssim \frac{\beta}{\alpha} = \frac{N}{M} \lesssim 1. \tag{3.7}$$

The conditions we have obtained on α and β imply that the number of activating molecules present in the normal diploid male or female cell can only very slightly exceed the number of receptor sites on a single X chromosome; it is in particular ruled out that this ratio be significantly larger than unity, say 1·25 or 1·5. It should be stressed here that these stringent conditions on the parameters of the model have followed from analysis of the diploid cases alone because μ was assumed to be practically infinite, that is, co-operative binding is tremendously efficient. Of course we are unable to estimate the individual parameters λ, M, N and T; only the dimensionless combinations α and β can be fixed. If values of λ and T could be estimated, we could put bounds on N and M. The analysis given above by itself does not rule out either the single informational entity postulate of Brown and Chandra, or the many entities postulate of Drews *et al.* (1974).

5. CONSEQUENCES FOR TRIPLOIDS

The implications of the results of the previous Section for the triploid cases (69, XXX and 69, XXY) are worth consideration. The cytogenetic data show that the triploids fall into two clear-cut categories, those with one X active and those with two X's active. The occurrence of these two distinct categories implies that there cannot be a single set of parameters describing both. In the first case (one X active) the parameters must be such as to produce

$$p_{00N}(T) \approx 1/3, \tag{4.1}$$

while in the second case (two X's active) it is necessary that

$$p_{0NN}(T) \approx 1/3. \tag{4.2}$$

With three X's and only M activating molecules, we have a triple string of probabilities $p_{lmn}(t)$ completely symmetric in l, m, n and obeying

$$p_{lmn}(0) = \delta_{l,0}\delta_{m,0}\delta_{n,0}; \tag{4.3a}$$

$$\sum_{l,m,n=0}^{N} p_{lmn}(t) = 1; \tag{4.3b}$$

$$\dot{p}_{lmn} = (M-l-m-n+1)(\lambda_{l-1}p_{l-1,m,n} + \lambda_{m-1}p_{l,m-1,n} + \lambda_{n-1}p_{l,m,n-1}) - (M-l-m-n)(\lambda_l+\lambda_m+\lambda_n)p_{lmn}. \tag{4.3c}$$

Equation (4.3c) is a straightforward generalization of (2.4, 2.22), and is easily checked to be consistent with (4.3b). After evaluation of $p_{000}(t)$, we follow the pattern of calculation in the normal diploid female case and go straightaway to the limit $\mu \to \infty$, when only p_{000}, p_{00N}, p_{0NN}, p_{NNN} and their symmetric partners survive. Setting $l=m=n=0$ in (4.3c) we get an equation for p_{000} with the solution (independent of μ)

$$p_{000}(t) = \mathrm{e}^{-3\lambda M t}. \tag{4.4}$$

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Next we set $m = n = 0$ and sum on l in (4.3c) and simplify to get:

$$\sum_{l=0}^{N} \dot{p}_{l00} = -2\lambda \sum_{l=0}^{N} (M-l)\, p_{l00}. \tag{4.5}$$

In the limit $\mu \to \infty$, this gives rise to an equation for p_{N00},

$$\dot{p}_{N00} + 2\lambda(M-N)\, p_{N00} = \lambda M\, \mathrm{e}^{-3\lambda M t}, \tag{4.6}$$

with the solution

$$p_{N00}(t) = \frac{M}{M+2N} \mathrm{e}^{-2\lambda(M-N)t}(1 - \mathrm{e}^{-\lambda(M+2N)t}). \tag{4.7}$$

(As in the case of (2.26) it can be verified that if p_{l00} is calculated for finite μ by the direct integration method and one lets $\mu \to \infty$ in the result, then $p_{N00}(t)$ takes the value (4.7) while all p_{l00} for $1 \leqslant l \leqslant N-1$ vanish.) Moving next to the determination of p_{NN0}, we set $n = 0$ in equation (4.3c), sum over both l and m, and simplify to get:

$$\sum_{l}^{N} \sum_{m=0}^{N} \dot{p}_{lm0} = -\lambda \sum_{l,m=0}^{N} (M-l-m)\, p_{lm0}. \tag{4.8}$$

Now we let $\mu \to \infty$, use (4.4, 4.7) for p_{000} and p_{N00} $(= p_{0N0})$ and end up with an equation for p_{NN0}

$$\dot{p}_{NN0} + \lambda(M-2N)\, p_{NN0} = \frac{2\lambda M(M-N)}{(M+2N)} \mathrm{e}^{-2\lambda(M-N)t}(1 - \mathrm{e}^{-\lambda(M+2N)t}). \tag{4.9}$$

The solution is:

$$p_{NN0}(t) = \mathrm{e}^{-\lambda(M-2N)t}\left[\frac{M-N}{M+N} + \frac{M(M-N)}{(M+N)(M+2N)} \mathrm{e}^{-2\lambda(M+N)t} - \frac{2(M-N)}{(M+2N)} \mathrm{e}^{-\lambda M t}\right]. \tag{4.10}$$

Finally, the value of p_{NNN} in the limit $\mu \to \infty$ follows from probability conservation:

$$\begin{aligned} p_{NNN}(t) &= 1 - p_{000}(t) - 3p_{N00}(t) - 3p_{NN0}(t) \\ &= 1 - 3\frac{M-N}{M+N} \mathrm{e}^{-\lambda(M-2N)t} + 3\frac{M-2N}{M+2N} \mathrm{e}^{-2\lambda(M-N)t} - \frac{(M-N)(M-2N)}{(M+N)(M+2N)} \mathrm{e}^{-3\lambda M t}. \end{aligned} \tag{4.11}$$

So far, the assumption $M \geqslant 3N$ has been made for analytical convenience; certain modifications must be made if $M < 3N$. If $2N \leqslant M < 3N$, then p_{NNN} is without meaning, while the calculations of p_{N00} and p_{NN0} stand. Similarly, if $M < 2N$, then both p_{NNN} and p_{NN0} are meaningless, but again the calculation of p_{N00} is unaffected. In these cases, some terms like $p_{N,N,M-2N}$ or $p_{N,m,M-N-m}$ would become nonzero. These comments relate only to the mathematical analysis of the triploid cases.

We can now confront the expressions obtained for various probabilities in the triploid case with what we have learnt about α and β in the previous section. Let us to start with suppose that the number of activating molecules present in a triploid

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cell is the same number M as in the diploid cell. The probabilities $p_{N00}(T)$ and $p_{NN0}(T)$ in (4.7, 4.10) when expressed in terms of α and β appear as:

$$p_{N00}(T) = \frac{\alpha}{\alpha+2\beta}(e^{2\beta-2\alpha} - e^{-3\alpha}), \tag{4.12a}$$

$$p_{NN0}(T) = \frac{\alpha-\beta}{\alpha+\beta}e^{2\beta-\alpha} - 2\frac{\alpha-\beta}{\alpha+2\beta}e^{2\beta-2\alpha} + \frac{\alpha(\alpha-\beta)}{(\alpha+\beta)(\alpha+2\beta)}e^{-3\alpha}, \tag{4.12b}$$

We now use the results of the last section: $e^{-\alpha}$ is very small ($\approx 1/20$) and β is very nearly equal to α (i.e. M is only slightly larger than N). This has two consequences for the present case: (1) the expression (4.12*b*) for $p_{NN0}(T)$ is to be discarded since p_{NN0} is itself without meaning; (2) $p_{N00}(T)$ is practically equal to 1/3. Thus if the same parameters are used for the triploid case as in the diploid case, we reach the not-very-surprising conclusion that only that category of triploids with one active X chromosome is understood. For the second category of triploids for which we require $p_{NN0}(T)$ to be very nearly 1/3, *the number of activating molecules in the cell cannot remain* M. The only way to understand this category is for the number of entities in the cell to be $2M$ (which is equal to or slightly in excess of $2N$). With this change in the parameters the expressions for $p_{N00}(T)$ and $p_{NN0}(T)$ may be obtained from (4.12) by simply replacing α everywhere by 2α:

$$p'_{N00}(T) = \frac{\alpha}{\alpha+\beta}(e^{2\beta-4\alpha} - e^{-6\alpha}), \tag{4.13a}$$

$$p'_{NN0}(T) = \frac{2\alpha-\beta}{2\alpha+\beta}e^{2\beta-2\alpha} - \frac{2\alpha-\beta}{\alpha+\beta}e^{2\beta-4\alpha} + \frac{\alpha(2\alpha-\beta)}{(\alpha+\beta)(2\alpha+\beta)}e^{-6\alpha}, \tag{4.13b}$$

(A prime has been added to these p's to distinguish them from the previous ones.) With the values of α and β taken from Section 3, it is immediately seen that $p'_{NN0}(T)$ is essentially 1/3. This describes the second category of triploids satisfactorily.

6. DISCUSSION AND SUMMARY

It is perhaps useful to recapitulate here the essential features of the mathematical model discussed in the preceding sections. The model assumes that the activation of an X chromosome is achieved by complete saturation of N receptor sites. These receptor sites are assumed to receive certain informational entities or activating molecules and that saturation of less than N sites renders the system non-functional. It is further assumed that strong co-operative binding exists such that the probability λ of a first hit of a receptor site is very much lower than the probability of subsequent hits. For the present analysis we have assumed that the probability of subsequent hits is infinitely greater than that of the first hit. On the basis of these assumptions we first found the bounds on the various parameters of the model such that one and only one X chromosome is properly activated in normal diploid males and females. These bounds are $\lambda MT \geqslant 3$ and $0{\cdot}96 \leqslant N/M \leqslant 1$, where M is the

number of activating molecules. As seen above, an extension of this analysis to the triploid cases shows that we cannot explain the activation of two X's if the number of activating molecules is fixed at M. Their number must in this case be double the number in diploids or nearly so. On the other hand, the activation of a single X in a triploid is consistent with the same number of molecules, M, as in normal diploid males and females. The number of activating molecules cannot therefore be constant in all triploid embryos. In other words, this model suggests that there must be two classes of triploid embryos differing from each other in a step-wise manner in the number of these molecules. The two classes could result from two different histories of imprinting of autosomal sets or from other unknown mechanisms.

Our result that M has to be close to N, that is, the total number of activating molecules has to be almost exactly equal to the number of sites to be occupied calls for the following comment. The simplest model satisfying this requirement would be the Brown–Chandra model which postulates a single receptor site per X chromosome. The value of assuming that M may be greater than one is that it can make the activation process more efficient in case there are other reasons to believe that the parameter λ is very small.

We realize that a number of restrictive assumptions have been made in this preliminary investigation. It is possible to solve the equations of our model numerically and probably also analytically without assuming $\mu = \infty$; this is under investigation. Other restrictive assumptions which could be relaxed include (*a*) sequential occupation of the receptor sites, (*b*) simultaneous release of the activating molecules, (*c*) co-operative binding leading to a single-step increase in the probability of hits, and (*d*) a specific kind of activating molecule tailored to a specific kind of receptor site. However, exploration of these modifications does not appear justified until more genetic and biochemical data become available.

During his visit to this Institute in 1974 Professor Spencer W. Brown encouraged us to study this problem. Professor S. K. Srinivasan helped in its mathematical formulation. Dr R. Sundar gave computational assistance during the early phases of this work. Professors S. K. Rangarajan and V. Anantanarayanan helped clarify some aspects of the mathematics and of cooperative binding respectively. We thank all of them. This work was supported in part by the Indian Council of Medical Research.

APPENDIX

Here we outline some steps involved in the mathematical analysis of the normal diploid male case in Section 2. We first consider the passage from (2.9) to (2.10). For a given value of n, it is permitted to iterate (2.9*b*) up to $(n-1)$ times. Doing so, we get q_n expressed in terms of q_1. Then, use of (2.9*a*) gives q_n in terms of q_0:

$$q_n(t) = \mu^{n-1}\lambda \int_0^t \mathrm{d}t_1 \int_0^{t_1} \mathrm{d}t_2 \ldots \int_0^{t_{n-1}} \mathrm{d}t_n \exp\{-\mu(t_1 + \ldots + t_{n-1} + t_n)\}\, q_0(t_n). \quad \text{(A 1)}$$

Here the integrand is symmetric in $t_1, t_2, \ldots, t_{n-1}$. The range of integration of these variables can also be made symmetric, with each of them going from 0 to t, if we

divide by the factor $(n-1)!$ and take the upper limit for t_n to be the least of the variables $t_1, t_2, \ldots, t_{n-1}$:

$$q_n(t) = \frac{\mu^{n-1}\lambda}{(n-1)!}\int_0^t \mathrm{d}t_1 \int_0^t \mathrm{d}t_2 \ldots \int_0^t \mathrm{d}t_{n-1} \int_0^\theta \mathrm{d}t_n \exp\{-\mu(t_1+\ldots+t_{n-1}+t_n)\}\, q_0(t_n), \quad \text{(A 2)}$$

$$\theta = \min(t_1, t_2, \ldots, t_{n-1}).$$

Next we interchange the t_n integration and the symmetric integrations over $t_1, t_2, \ldots, t_{n-1}$. This gives:

$$q_n(t) = \frac{\mu^{n-1}\lambda}{(n-1)!}\int_0^t \mathrm{d}t_n\, \mathrm{e}^{-\mu t_n} q_0(t_n) \left[\int_{t_n}^t \mathrm{d}t_1\, \mathrm{e}^{-\mu t_1}\right]^{n-1}$$

$$= \frac{\lambda}{(n-1)!}\int_0^t \mathrm{d}t'\, \mathrm{e}^{-\mu t'}\,(\mathrm{e}^{-\mu t'} - \mathrm{e}^{-\mu t})^{n-1} q_0(t'). \quad \text{(A 3)}$$

This result is valid for $n = 2, 3, \ldots, N-1$ since that is the range of validity of (2.9*b*); but comparison with (2.9*a*) shows that (A 3) is true for $n = 1$ as well. With the help now of (2.8), the result (2.10) of the text is immediate.

The solution (2.17) for the generating function $\pi(\zeta, t)$ is obtained as follows. If we use (2.16) in (2.14), the partial differential equation for π reads:

$$\frac{\partial \pi(\zeta,t)}{\partial t} = \mu(\zeta-1)\left(M - \zeta\frac{\partial}{\partial \zeta}\right)\pi(\zeta,t) + M(\lambda-\mu)(\zeta-1)\,\mathrm{e}^{-\lambda M t}. \quad \text{(A 4)}$$

The change of variable $\zeta \to x$ according to

$$\zeta = 1/(1+\mathrm{e}^{\mu x}) \quad \text{(A 5)}$$

brings about some simplification since then

$$\mu\zeta(\zeta-1)\frac{\partial \pi}{\partial \zeta} = \frac{\partial \pi}{\partial x}. \quad \text{(A 6)}$$

In terms of x and t, (A 4) is:

$$\left[\frac{\partial}{\partial t} + \frac{\partial}{\partial x} + \frac{\mu M \mathrm{e}^{\mu x}}{1+\mathrm{e}^{\mu x}}\right]\pi = M(\mu-\lambda)\,\mathrm{e}^{-\lambda M t}/(1+\mathrm{e}^{-\mu x}). \quad \text{(A 7)}$$

Multiplying through by the Mth power of $(1+\mathrm{e}^{\mu x})$ gives

$$\left(\frac{\partial}{\partial t} + \frac{\partial}{\partial x}\right)(1+\mathrm{e}^{\mu x})^M \pi = M(\mu-\lambda)\,\mathrm{e}^{\mu x - \lambda M t}(1+\mathrm{e}^{\mu x})^{M-1}. \quad \text{(A 8)}$$

Since M is an integer not less than one, the right-hand side can be expanded as a binomial series and written in terms of $x \pm t$:

$$\left(\frac{\partial}{\partial t} + \frac{\partial}{\partial x}\right)(1+\mathrm{e}^{\mu x})^M \pi = M(\mu-\lambda)\sum_{r=0}^{M-1}\frac{(M-1)!}{r!(M-1-r)!}$$

$$\times \exp\{(\lambda M + r\mu + \mu)(x-t)/2\}.\exp\{(r\mu+\mu-\lambda M)(x+t)/2\}. \quad \text{(A 9)}$$

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Integration of this is immediate; with $f(x-t)$ an as yet unknown function to be fixed with the use of (2.15), we find:

$$(1+\exp\{\mu x\})^M \pi = f(x-t) + (\mu-\lambda)\sum_{r=0}^{M-1} \frac{M!}{r!(M-1-r)!} \times \exp\{(\lambda M + r\mu + \mu)(x-t)/2\} \frac{\exp\{(r\mu+\mu-\lambda M)(x+t)/2\}}{r\mu+\mu-\lambda M}$$

$$= f(x-t) + \frac{(\mu-\lambda)}{\mu} \exp\{\mu x - \lambda M t\} \sum_{r=0}^{M-1} \frac{M!}{r!(M-1-r)!} \frac{\exp\{r\mu x\}}{r+1-\lambda M/\mu}. \tag{A 10}$$

Before fixing f, we note that this result can be neatly expressed in terms of the hypergeometric function of type ${}_2F_1$. For k a positive integer, we have:

$$F(\alpha, -k; \alpha+1; z) = \sum_{r=0}^{k} \frac{\alpha}{\alpha+r} \frac{k!}{r!(k-r)!} (-z)^r. \tag{A 11}$$

Using this, (A 10) takes the form:

$$(1+e^{\mu x})^M \pi = f(x-t) + \frac{M(\mu-\lambda)}{\mu-M\lambda} e^{\mu x - \lambda M t} F\left(1-\frac{\lambda M}{\mu}, 1-M; 2-\frac{\lambda M}{\mu}; -e^{\mu x}\right). \tag{A 12}$$

The form of f is now determined using the condition (2.15) at $t = 0$:

$$f(x) = (1+e^{\mu x})^M - \frac{M(\mu-\lambda)}{\mu-M\lambda} e^{\mu x} F\left(1-\frac{\lambda M}{\mu}, 1-M; 2-\frac{\lambda M}{\mu}; -e^{\mu x}\right). \tag{A 13}$$

Putting this into (A 12) after having replaced x by $(x-t)$, and then reverting to ζ in place of x, (2.17) of the text is obtained.

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