100 mg/day (Armour Standard) of ACTH in normal people. It is probably significant that Compound B, administered either orally or parenterally in normal people or in Addisonians, fails to reduce the number of circulating eosinophilic granulocytes (*3*), whereas Compound F causes them to disappear completely. The fact that administration of Compound F to a normal woman results in a sharp and progressive increase in the excretion of urinary 17-ketosteroids indicates that one need not postulate cortical elaboration of a separate "androgenic steroid" to account for the rise of 17-ketosteroid excretion that accompanies administration of ACTH to normal people. Although several other steroidal compounds administered orally, at similar levels of dosage, to normal individuals produce increased renal excretion of 17-ketosteroids (*7*), the over-all metabolic activities of Compound F, in terms of intensity and diversity, approximate more closely those observed during administration of ACTH. As with ACTH (*8*), Compound F produces a decrease of red cell reduced glutathion. The present study indicates an 11% decrease from baseline levels (sustained for 3 consecutive days) following 3 days of administration of Compound F.

The only important difference we have been able to measure between the metabolic activities of ACTH and Compound F relates to their different effects upon total and free cholesterol of serum. With ACTH a sharp fall of esterified cholesterol of serum occurs by the fourth day of administration (*9*). Compound F failed to produce a significant change in either the total or free cholesterol of serum. Inasmuch as it is presumed (*9*) that the decrease of esterified cholesterol of serum observed during administration of ACTH represents withdrawal of this substance from serum as a precursor for steroidal hormone synthesis by the adrenal cortex, it should not be expected that Compound F would exert a similar influence upon serum cholesterol.

If the normal response to the administration of ACTH consists of the elaboration by the adrenal cortex of Compound F solely, one is faced with the problem of explaining the differences in over-all metabolic activities observed among normal people given ACTH. It is probable that these differences are due, not to variations in the types of steroidal materials produced or to differences in the relative amounts of specific steroidal substances elaborated, but rather to differences in the receptivity and reactivity of the various end organs to a single steroidal compound (*10*).

Within the limits of this study we are able to discern no significant differences between the metabolic effects of orally administered Compound F and the diverse metabolic effects known to occur in normal people given ACTH. It seems likely that Compound F is the adrenal cortical hormone, and that one observes the metabolic effects of this compound when he stimulates the normal adrenal cortex with ACTH.

Addendum: Since submission of the data reported above we have made several additional observations that should be recorded. Recognition of these facts will avoid in future reports on Compound F what otherwise would appear to constitute conflicting results:

1. Compound F acetate administered orally produces approximately the same metabolic effects as those recorded above for free Compound F given orally.
2. Compound F acetate administered intramuscularly for the same length of time, in the same dosage, to the same person, produces only slight metabolic effects.
3. Free Compound F administered intramuscularly results in at least as intense metabolic activities as those observed when either the free compound or the acetate is given orally.

The statements made above are based upon results obtained on the same normal female subject, J. B., who received free Compound F and Compound F acetate orally, as well as each compound intramuscularly. In each of the 4 separate experiments the dose was 400 mg/day.

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Number of Entities Inactivated by X-Rays in Greying of Hair[1]

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Greying of hair by x-radiation has been observed by several workers and described in some detail by Hance and Murphy (*1*), Murray (*2*), Danneel and Lubnow (*3, 4*), and Chase *et al.* (*5–8*). In the mouse the degree of greying depends on the dose, the dose rate, the stage of follicle at time of treatment, and size of the follicle. For any given dose, follicles in the anagen (active growth stage) develop a lower percentage of white hairs in all ensuing hair generations than do follicles in the catagen or telogen (resting) stages (*5, 6*). The smallest hairs of the regular coat of the mouse, the zigzags, are the most "sensitive" in respect to percentage of greying (*8*). These hairs make up 82% of the coat. The larger auchenes, awls, and mono-

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trichs have a somewhat smaller percentage of all-white hairs, have more mosaic hairs, and show less difference between anagen and telogen treated follicles. The average size of hair (and follicle) in other species would seem to be at least one factor in the greying response due to x-rays. The hamster has a threshold of effect similar to that of the mouse (200 r), the rabbit and cat have a higher threshold, and the guinea pig, with its relatively coarse hair, has a much higher threshold (2,000 r).

Our gross observations on the rabbit agree in essentials with those of Danneel and Lubnow (*3*). The effect is permanent, there is a considerable percentage of mosaics along with the all-whites, the threshold is approximately 900 r, and there is little difference between the anagen and telogen areas. Danneel and Lubnow hold that the effect is slightly greater when anagen areas are irradiated; we hold that the effect is slightly greater when telogen areas are irradiated. The principal controversy, however, involves the site of the x-ray effect. Lubnow (*4*) contends that x-radiation prevents the formation of lipochondria in the matrix cells of the bulb by destroying the individual precursors of these mitochondrial types. Melanin granules are presumed to develop from these lipochondria. We contend that x-rays destroy a "reservoir" of pigmentary dendritic cells. We presume that these cells supply the matrix cells with the basic pigment granules (or precursors), possibly by a cytocrine function as postulated by Masson (*9*) for human epidermis. The precise mechanism is unknown, but some form of induction is implied. In the matrix cells there is further development of melanin on these sites, according to the phenotype involved (*8*). The melanogenic dendritic cells may be seen (unstained frozen sections, B minus phase-contrast objective) in early anagen before the matrix cells acquire the granules (*10, 11*). They are few in number, with each one in contact with several matrix cells. The "reservoir" is included in or near the resting hair germ, and the cells are generally in the non-melanogenic phase at this time. Rawles (*12*) has shown in embryonic transplants of mouse neural crest material and epidermis on chick chorioallantois that hairs develop normally but with no pigment unless such neural crest-derived dendritic cells are present in the transplant. Obviously, the controversy as to site of x-ray effect is part of a larger and more basic pigment controversy—i.e., "melanophore" hypothesis of extrinsic origin of pigment granules vs. the hypothesis of intrinsic origin.

An analysis of the greying response as a dose-response survival curve is instructive in this matter. The percentage of survivors is the percentage of fully pigmented hairs found in a given x-rayed area. Such dose-response distributions have been given in a paper by Chase and Rauch (*8*). A pair of these experimental distributions is given here in Fig. 1. Both are the result of x-radiation at 100 kv and 550 r/min; one is on inactive (telogen) follicles, the other is on active (anagen) follicles. There are 300 hairs in each count and there are at least 5 separate counts for

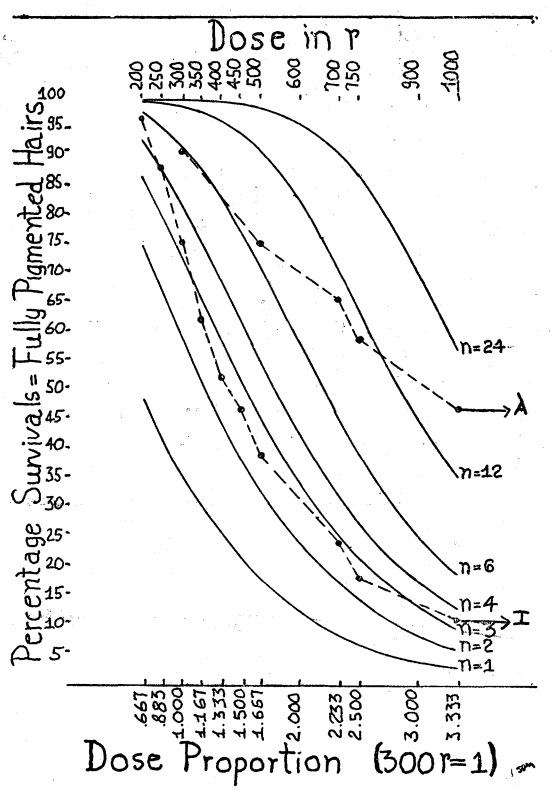

FIG. 1. Experimental dose-response distributions and theoretical curves. *I* is the experimental survival (= fully pigmented) distribution for hairs from follicles in the inactive (telogen) stage at the time of x-ray treatment at 100 kv, 550 r/min. *A* is a corresponding distribution for follicles treated in the active (anagen) stage. Theoretical exponential and sigmoid exponential curves are indicated for $n = 1, 2, 3, 4, 6, 12$, and 24 entities. The formula $y = 1 - (1 - e^{-x})^n$ is used.

each point on these distributions. The standard error for each point is 2.5% or less. All 4 types of hair are counted together, but the smallest hairs, the zigzags, make up approximately 82% of each count. Theoretical survival curves are drawn—exponential and sigmoid exponential—for number of entities that need to be destroyed or inactivated to prevent a fully pigmented hair (survivor). For instance, if there are 4 entities, all of which must be inactivated to prevent pigment formation, the curve where n equals 4 should be obtained. The percentage of survivors is plotted on the ordinate with dose proportions on the abscissa. Empirically, from the experimental distributions and dose rate given here, the dose of 300 r is taken as unity. The formula for the theoretical curves is $y = 1 - (1 - e^{-x})^n$ where x is the proportional dose, n is the number of entities, and y is the proportion of survivors (*cf.* Lea (*13*) for clumped bacteria).

The results of this analysis (Fig. 1) indicate that the inactive follicles have about 3 entities to be inactivated, whereas the active follicles have approximately 6–12 such entities, presumably after 2 or 3 cell divisions. This number in active (anagen) follicles agrees well with the 4–8 estimated from microscopical examination of such small zigzag follicles (*11, 14*). The

theoretical curves demonstrate why anagen follicles are less "sensitive," why larger follicles are less "sensitive," and why with larger follicles there is less difference between follicles treated in anagen or telogen. The assumption of Lubnow that the individual granule sites or lipochondrial precursors are "hit" by the radiation would appear untenable. Even the small zigzag hair follicles of mice have in the pigmented matrix cone no less than 30 cells with no less than 100 pigment granules (C-57 Black strain of mice) in each. The dose-response distributions and the fact that most hairs of the smallest type are fully pigmented or all-white suggest that the entities to be "hit" must be relatively few and extrinsic to the matrix cells.

Mosaic (partially pigmented) hairs do occur with a somewhat greater frequency in larger follicle types, as might be predicted on the basis of the above analysis. In later hair generations the percentage of mosaics decreases as the percentage of all-whites increases. Lubnow (*4*) makes the same observation in his work on the rabbit. We have seen in active zigzag follicles in the mouse cases of mosaic hair shafts being produced. Only one, or at most 2, pigmentary dendritic cells are present. Some matrix cells apparently receive no granules, some receive a few, and some receive full complements (*10, 11, 14*). It is suggested here that such pigmentary cells have lost the capacity to divide but not entirely the capacity to produce pigment granules and to "inoculate" matrix cells.

The analysis presented in this paper, involving the number and presence of extrinsic entities, is in the nature of a tentative hypothesis and suggests further investigation. Two other lines of work should be mentioned briefly, the first somewhat contradictory, the second, confirmatory. The first is the classic investigation of Packard (*15*) on the effect of comparable moderate x-ray doses on the hatchability of fertilized *Drosophila* eggs. Sigmoid exponential distributions, very similar to ours, were obtained. The explanation offered was not in terms of number of entities but in terms of variations in egg sensitivity. The other line of work is that of Taylor (*16*) on the effect of freezing on the "melanophores" in the rat. He obtained all-white and mosaic hairs, the dendritic cells being relatively easily destroyed by freezing.

Certain conclusions are suggested from the evidence reviewed, from the analysis presented, and from the discussion in this paper. All-white hairs are the result of a complete inactivation (apparently destruction) of the dendritic cells. Mosaics are the result of a complete inactivation of some and a partial inactivation of those remaining—that is, no cell division but the retention of some melanogenic function. The basic pigment sites in the matrix cells are of extrinsic origin, specifically from a small "reservoir" of potentially pigmented dendritic cells.

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Quantum Yields, Ionic Yields, and Molecular Weights of Proteins

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Recently, on the basis of inactivation quantum yield (Φ) data, it has been suggested (*1*) that the larger the molecule (enzyme, or virus) the smaller is the fraction of the absorbed light absorbed by the sensitive volume (the active center?), and hence the lower the quantum yield for inactivation (Fig. 1). If

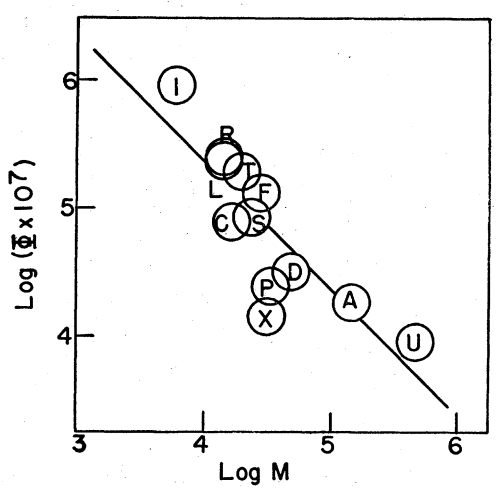

FIG. 1. Plot of log ($\Phi \times 10^7$) vs log M for a number of enzymes and related proteins. The equation $\Phi = Q/M$, where Q is a constant, is approximately obeyed (*1, 3, 4*). (Aldolase, A; carboxypeptidase, X; chymotrypsin, C; triosephosphate dehydrogenase, D; ficin, F; insulin, I; lysozyme, L; pepsin, P; ribonuclease, R; soybean trypsin inhibitor, S; trypsin, T; urease, U.)

this be true, the ionic yields for the indirect effect in aqueous solutions by x- and γ-rays could expectedly show a similar trend; i.e., the larger the molecule the smaller the fraction of reacting ions reacting with the sensitive volume.

A search of the literature reveals two examples (*1, 2*) for which both quantum yields (2,537 A) and