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Radiation and cells; design by Raymond Geary. See page 2.
Reversible and Irreversible Effects of Densely Ionizing Radiations Upon the Reproductive Capacity of Cultured Human Cells*

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Introduction

More than eight years have passed since Puck and his colleagues first developed the now-common laboratory techniques for the continuous cultivation of mammalian cells in the same manner as microorganisms and with the same assays for reproductive capacity (Puck et al., 1956). The widespread use of cultured mammalian cells as radiobiological test objects has steadily improved our understanding of the mammalian radiation syndromes (Elkind, 1961). Detailed experiments under altered environmental conditions and with a variety of ionizing radiations indicate that the physico-chemical basis of radiation sensitivity in mammalian cells is not unlike that of microorganisms when reproductive capacity (colony forming ability) is used as the end-point (Barendsen, 1960; Barendsen and Walter, 1964; Todd, 1964).

The purpose of this paper is to summarize the experimental data that have been accumulated to demonstrate that environmental factors that alter the sensitivity of the reproductive capacity of cultured mammalian cells to the effects of x- and γ-rays fail to alter the effects of densely-ionizing particulate radiations or alter them less effectively. Only work with cultured human kidney “T1” cells will be described, although similar experiments with other cultured mammalian cells have been performed (Deering and Rice, 1962; Skarsgard, 1963), and an excellent paper by Skarsgard discussing the use of Chinese hamster cells in the evaluation of end-points other than colony forming capacity is forthcoming.

Materials and Methods

Cells

Human kidney T1 cells (van der Veen et al., 1958; Barendsen et al., 1960) were cultured in Eagle's Minimum Essential Medium (1959), 10% in fetal bovine serum. Colony survival experiments were performed with four-hour cultures derived from log-phase cultures and growing on a day-old “feeder layer” (Puck et al., 1956) of lethally-irradiated (4000 rads) cells in all experiments.

Irradiation

X-irradiations were performed with highly-filtered 50 kVp radiation (Todd, 1964). Cells were irradiated from above on the bottom surfaces of plastic petri dishes, to which they were attached. Medium was removed during the exposure of cultures under anoxic conditions.

Heavy-Ion Irradiation

The apparatus used for irradiations at the Berkeley Hilac is a modified version of that described by Brustad (1962; Todd and Lyman, manuscript in preparation). It differs mainly in that the experimental beam pipe is much longer, due to the necessity of interposing a long distance between the scattering foils and the sample holder in order to obtain a wide, uniform beam.

Detailed structure of the ionization chamber and sample indexing apparatus is shown in Figure 1. The vacuum window is 38 mm in diameter and is composed of Dural 0.001 inch thick. After emerging from the vacuum, the beam traverses the ionization chamber, which is constructed of two mylar foils 0.00025 inch thick and aluminized on the inside surfaces only. The ionization chamber gas is dry nitrogen. A 0.001 inch mylar mask and 12 mm of air separate the samples from the ionization chamber. The sample wheel is readily removed to facilitate the loading of samples under sterile conditions. The outer electrode of the ionization chamber can be removed to facilitate the mounting of a solid-state detector at the position occupied by the cells and at the position of the center of...
the ionization chamber for the purpose of measuring beam energies at these locations.

Just prior to mounting sample dishes in the apparatus the medium is withdrawn, the cover of the dish is removed, and the dish is inserted into the holder as indicated at a and b in Figure 1, all under sterile conditions. The loaded wheel is mounted on its axis, as indicated. Air, which is 4% in CO₂ and saturated with water vapor (or saturated nitrogen), is admitted at atmospheric pressure, under sterile conditions, at room temperature, at the end of the axis and allowed to circulate as indicated by the arrows in the air path in Figure 1. Entrance of airborne microorganisms is prevented by the 0.001 inch mylar mask indicated at c, held in place by inner and outer rings shown at e and d, respectively. The center line of the beam axis is shown at f. After exposure of the samples to the beam, the gas hose is removed, the wheel is removed, the dishes are removed from the wheel, and medium is added, so that post-irradiation incubation starts immediately.

Heavy-Ion Dosimetry

The instantaneous dose rate of the Hilac is roughly thirty times the average dose rate, as the beam is pulsed. The problems of pulsed-beam dosimetry have been studied by Boag (1951 and 1956), and the ionization chamber used in these ex-
Experiments was designed to cope with these problems. The charge collected on a vacuum-tube electrometer was used to interrupt the beam electronically as soon as the desired dose had been delivered to each sample, so that doses delivered varied less than 1% among samples receiving the same exposure. The estimated accuracy of the ionization chamber is about 4%, due to the uncertainty in the thickness of the sensitive volume.

The foils and gaps in the exposure apparatus absorbed a small fraction of the beam energy, thus the ionization-chamber dose was corrected to cell dose by multiplying by the ratio of the values of $dE/dx$ at the two locations, as determined from energy measurements with a small semiconductor detector. Beam energies were all adjusted to about 6.6 MeV/nucleon at the position of the cells, so that all of the irradiations were performed with ions of the same velocity (and, therefore, the same $\delta$-ray spectrum). The argon beam energy was 5.7 MeV/nucleon.

The cellular effects of pulsed beams do not appear to differ from those due to steady beams (Hood and Norris, 1964).

**Modifying Conditions**

The environmental and biological conditions which modify radiosensitivity were achieved as follows: Anoxia was achieved by the passage of highly purified (hot copper or oil pumped) nitrogen, saturated with water vapor over the cultures in the absence of culture fluid for at least ten minutes before exposure to ionizing radiation. Cells which were allowed to recover from sublethal damage between doses were kept at 36–37°C in the above-described medium and in an atmosphere of air, 5% in CO₂ and saturated with water vapor, as were all cultures at all times except during exposure. In a similar experiment by Barendsen and Walter (1964), chemically-protected cultures were kept in medium which was 0.025 M in cysteamine 30 minutes prior to and during radiation exposure. The radio-protective chemical was washed away immediately afterwards. Cultures were sensitized to the lethal effects of ionizing radiations by growth for several generations in the thymidine analog 5-iododeoxyuridine (IUdR) at a concentration of 20 micromolar (Tym and Todd, 1964). The drug was removed from the cells four hours prior to exposure, so that all that remained was presumably in the DNA of the cells (Djordjevic and Szybalski, 1960). The observed consequences of each of these treatments on the radiation sensitivity of human kidney cells is described under “Results.”

**Results**

**Standard X-ray Survival Curve**

For exposure to graded doses of x-rays 4 hours after plating, the standard survival curve for the colony-forming ability of T1 cells attached to the bottom of plastic petri dishes containing medium is shown for 50 kVp x-rays in Figure 2. In most radiation experiments a set of dishes was ex-

![Fig. 2—Dose-response curve for the colony forming ability (expressed as a fraction of that of unirradiated controls) of T1 cells exposed to 50 kVp x-rays. The growth curve is that of unirradiated cells.](image-url)
posed to three or more different doses of x-rays and compared to the curve of Figure 2 to ascertain that the experimental cells were from a normal culture. The continuous downward curvature of the curve at high doses appears to be characteristic of T1 cells (Barendsen, 1962). On the survival curves, vertical bars represent the propagated standard errors of colony counts on control and irradiated dishes. Standard errors smaller than the plotted points are not indicated. Due to the necessity of pipetting small numbers of cells onto small dishes, the standard errors were seldom less than 3% and occasionally exceeded 5% of the mean survival. Growth curves of unirradiated cultures are determined and recorded in each experiment.

The Effect of Oxygen

The “sigmoid” shape of the mammalian-cell survival curve for colony formation in vitro was first discovered by Puck and associates (1956), and typical examples are shown again in Figure 3, which presents the plots of survival of colony forming ability against dose of 50 kVp x-rays in the presence and absence of oxygen. The cells appear to be about 2.8 times as sensitive to x-rays in the presence of oxygen, in agreement with the observations of D. M. Dewey, who was probably the first to observe this effect in cultured mammalian cells (1960).

Response of Cells to Heavy-Ion Irradiation

Typical dose-survival curves ob-
Fig. 4—Response of the colony-forming ability of T1 cells to irradiation with heavy ions of equal velocity. The ion and its average charge is indicated on each plot. Plotted squares correspond to data obtained under anoxic conditions. Solid points are for x-irradiation, and open circles are for heavy-ion irradiation.

The curves indicated by open squares in Figure 4 were obtained by the passing of moist purified nitrogen gas into the exposure wheel shown in Figure 1. Cells were exposed to the nitrogen atmosphere for at least 10 minutes before exposing to radiation, and nitrogen was passed over them during exposure as well. Due to the nature of the experimental arrangement, most cultures remained in nitrogen for a few minutes after exposure (about 5 minutes is required for the irradiation of a wheel containing 10 samples).

When cultures are irradiated with heavy ions there are three distinct alterations in the dose response of colony forming ability: There is a steady increase in sensitivity up to \( dE/dx = 2200 \text{ MeV-cm}^2\text{-g}^{-1} \); there is a change from “sigmoid” to “exponential” survival curves between \( dE/dx = 1650 \) and 2200 \( \text{MeV-cm}^2\text{-g}^{-1} \); and there is a steady reduction in the effect of the presence of oxygen.
until its apparent abolition by radiation with $dE/dx = 3000$ MeV·cm$^2$·g$^{-1}$ or greater. The exact value at which the oxygen effect becomes undetectable has not been established.

**The Effect of Dose Fractionation**

Elkind and Sutton (1960) were first to show that the subthreshold, or sublethal, radiation injury accumulated by surviving cells is not inherited but is rapidly repaired. The existence of sublethal injury is implicit in the sigmoid shape of the dose response curves for inhibition of colony formation. Cell lethality characterized by an exponential dose response would not be expected to be due to the accumulation of sublethal damage and, hence, would not be subject to repair between two doses of ionizing radiation.

Figure 5 summarizes a series of experiments in which human kidney T1 cells were exposed to x-radiation, heavy-ion radiation, and paired doses of heavy-ion radiation separated by various intervals of time. The increase of survival with time between two doses is taken to indicate that cellular recovery occurred between the exposures. There was evidently no recovery between paired doses of carbon ions ($dE/dx = 2200$ MeV·cm$^2$·g$^{-1}$), for which the dose-response curve appears to be exponential, and for which the survival appears to remain unchanged with the passage of time between paired doses.
IONIZING RADIATIONS AND CULTURED HUMAN CELLS

The Effect of Chemical Protection

Barendsen and Walter (1964) found a marked reduction in the sensitivity to 200 kVp x-rays of T1 cells exposed in the presence of 0.025 M cysteamine. On the other hand, this chemical protected the cells only slightly, if at all, against the lethal action of natural alpha particles having \( \frac{dE}{dx} \) of about 1700 MeV/cm. See Figure 6.

The Effect of a Thymidine Analog

Djordevic and Szymbalski (1960) discovered that IUdR and BUdR (5-bromodeoxyuridine), when incorporated into the DNA of cultured cells, increased their sensitivity to the lethal effects of ionizing radiation. Figure 7 presents dose-survival curves for human kidney T1 cells exposed to various ionizing radiations with and without having incorporated (IUdR) into their DNA. The sensitizing effect is evidently present at low and intermediate values of \( \frac{dE}{dx} \), but not for fast carbon ions (\( \frac{dE}{dx} = 2200 \) MeV-cm\(^2\)-g\(^{-1}\)). There is presumably no sensitization at higher values of \( \frac{dE}{dx} \), although no experiments were performed to verify this point (Tym and Todd, 1964).

Discussion

The effects of densely ionizing radiation are modified negligibly, if at all, by the usual radiation modifying conditions. This knowledge leads to some conclusions which should be useful in understanding the fundamental nature of radiosensitivity.

Mathematical Expression of Data

The dose-survival curves of Figure 4 were analyzed in terms of the relationship

\[
S = e^{-\frac{D_1}{D_2}}[1 - (1 - e^{-\frac{D_1}{D_2}})^n],
\]

in which \( S \) is the surviving fraction, \( D_1 \) is the reciprocal of the initial slope (rads), \( D_2 \) is the reciprocal of the final slope minus the initial slope, \( D \) is the dose, and \( n \) is the extrapolation number, essentially that defined by Alper, Gillies, and Elkind (1960). This form was chosen because the initial slope of the "sigmoid" T-cell survival curves is non-zero (Barendsen, 1962; Todd, 1964), and it is
a functional form which yields values for the parameters \( D_1 \), \( D_2 \), and \( n \) and places the expression of data in terms similar to those suggested by Alper, Gillies, and Elkind (1960). These three survival parameters and their standard deviations were evaluated from the survival curves by the method of Berman and Weiss (1963 and 1964), and inactivation "cross sections" were calculated from \( D_1 \) and \( D_2 \) according to

\[
\sigma_1 = 1.6 \frac{\epsilon}{D_1} \quad \text{and} \quad \sigma_2 = 1.6 \frac{\epsilon}{D_2},
\]

in which \( \sigma_1 \) and \( \sigma_2 \) are the inactivation cross-sections (in square microns) corresponding to \( D_1 \) and \( D_2 \), respectively. \( \epsilon \) is the \( dE/dx \) in MeV-cm\(^2\)/g, taken as the "total mass stopping power", as defined recently by the International Commission on Radiation Units (1962). The calculated cross sections for x-ray and heavy-ion inactivation are given in Tables 1 and 2.

**Extrapolation Numbers**

The value of the extrapolation number \( n \) is, in most experiments determined with large error limits. Examination of the curves of Figure

---

*Fig. 7—Effect of IUdR pretreatment upon the response of T1 cells to irradiation by 50-kVp x rays, 26.3-MeV helium ions, and 79.0-MeV carbon ions. Growth curves are given for cells under both conditions. Composite drawing, based on a number of experiments.*


**TABLE 1**

Inactivation Cross Sections for Aerobic Irradiation of TI Cells with Heavy Ions

<table>
<thead>
<tr>
<th>Radiation</th>
<th>dE/dx (MeV-cm²/g)</th>
<th>D₁(rads)</th>
<th>D₂(rads)</th>
<th>σ₁(µ²)</th>
<th>σ₂(µ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X Ray</td>
<td>45</td>
<td>388 ± 51</td>
<td>200 ± 10</td>
<td>0.186 ± 0.024</td>
<td>0.360 ± 0.018</td>
</tr>
<tr>
<td>D¹</td>
<td>65</td>
<td>358 ± 48</td>
<td>200 ± 15</td>
<td>0.292 ± 0.038</td>
<td>0.600 ± 0.045</td>
</tr>
<tr>
<td>He¹</td>
<td>250</td>
<td>213 ± 32</td>
<td>152 ± 18</td>
<td>1.87 ± 0.27</td>
<td>2.63 ± 0.32</td>
</tr>
<tr>
<td>Li²</td>
<td>550</td>
<td>143 ± 41</td>
<td>91 ± 12</td>
<td>6.16 ± 1.76</td>
<td>9.65 ± 1.27</td>
</tr>
<tr>
<td>B¹</td>
<td>1650</td>
<td>82.6 ± 8.3</td>
<td>100 ± 9</td>
<td>31.9 ± 3.2</td>
<td>26.4 ± 2.6</td>
</tr>
<tr>
<td>C¹⁺</td>
<td>2200</td>
<td>66.3 ± 1.0</td>
<td></td>
<td>53.1 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>N¹⁴⁺</td>
<td>3000</td>
<td>85 ± 10</td>
<td></td>
<td>56.5 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>O¹⁶</td>
<td>3850</td>
<td>92.6 ± 2.6</td>
<td></td>
<td>66.5 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Ne²⁰</td>
<td>5800</td>
<td>103 ± 2</td>
<td></td>
<td>90.3 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Ne²⁰⁺</td>
<td>11600</td>
<td>200 ± 25</td>
<td></td>
<td>92.5 ± 11.5</td>
<td></td>
</tr>
<tr>
<td>A⁴⁰</td>
<td>19400</td>
<td>208 ± 3.5</td>
<td></td>
<td>148 ± 3</td>
<td></td>
</tr>
</tbody>
</table>

* D₁ and D₂ determined by visual estimation from plotted survival curves.

---

**TABLE 2**

Inactivation Cross Sections for Irradiation of TI Cells with Heavy Ions in the Absence of Oxygen

<table>
<thead>
<tr>
<th>Radiation</th>
<th>dE/dx (MeV-cm²/g)</th>
<th>D₁(rads)</th>
<th>D₂(rads)</th>
<th>σ₁(µ²)</th>
<th>σ₂(µ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X Ray*</td>
<td>45</td>
<td>334 ± 107</td>
<td>226 ± 29</td>
<td>1.19 ± 0.38</td>
<td>1.77 ± 0.23</td>
</tr>
<tr>
<td>D²*</td>
<td>65</td>
<td>236 ± 70</td>
<td>150 ± 28</td>
<td>3.73 ± 1.06</td>
<td>5.85 ± 1.09</td>
</tr>
<tr>
<td>He¹</td>
<td>250</td>
<td>107 ± 16</td>
<td>111 ± 13</td>
<td>24.6 ± 3.7</td>
<td>23.7 ± 2.4</td>
</tr>
<tr>
<td>Li²</td>
<td>550</td>
<td>76 ± 1.7</td>
<td></td>
<td>46.4 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>B¹¹</td>
<td>1650</td>
<td>93 (error</td>
<td>&lt;66.1 (error</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C¹⁺</td>
<td>2200</td>
<td></td>
<td>N.D.)</td>
<td>N.D.)</td>
<td></td>
</tr>
<tr>
<td>O¹⁶⁺</td>
<td>3850</td>
<td></td>
<td>N.D.)</td>
<td>N.D.)</td>
<td></td>
</tr>
<tr>
<td>A⁴⁰</td>
<td>19400</td>
<td>208 ± 20</td>
<td></td>
<td>149 ± 16</td>
<td></td>
</tr>
</tbody>
</table>

* D₁ and D₂ determined by visual estimation from plotted survival curves.
N.D. not determined.

---

4 shows no significant evidence of a continuous decrease of n with increasing dE/dx, but the extrapolation number appears to fall abruptly to unity near dE/dx = 2000 MeV-cm²/g. Large error limits make interpretation of the meaning difficult.

**Inactivation Cross Sections**

The data of Tables 1 and 2 are plotted in Figures 8 and 9 on log-log scales, so that Figure 8 shows the dependence upon dE/dx of σ₁ in the presence and absence of oxygen, and Figure 9 shows the dE/dx dependence of σ₂ in the presence and absence of oxygen.

It is helpful to express σ₁ and σ₂ as analytical functions of dE/dx. One such function is

\[ σ₁(ε) = σ₁(∞)[1 - \exp(-α₁d₁)] \]

where α₁ is a constant coefficient, d₁ is the thickness of the “target”, and σ₁(∞) is the maximum value of the inactivation cross section, σ₁, and similarly for σ₂. Since the nature of the “target” in the present situation is not yet known, a more general analytical function is desirable.

An elaborate statistical analysis of the spatial distribution of ion-pair clusters (not well known in condensed phases), leads to a table of “track-segment” functions that are in reasonable agreement with certain observations on small test objects (Brustad, 1962; Howard-Flanders, 1958). And Kondo (1963) has suggested that the dE/dx dependence of inactivation cross sections take the form

\[ σ₁(ε) = σ₁(∞)[1 - \exp(-d₁ \sum_{n=0}^{N} αₙeⁿ)] \]

with no clear explanation for the existence of higher powers of e in the exponent.

The functional dependence of σ₁ used here is based on the following widely accepted observations:

(a) At low dE/dx, interaction between radiation-produced free radicals is relatively improbable, due to the distance between them, hence they must de-excite or react with a nearby reactive molecule, such as
\( O_2 \) or a thiol. This is a first-order reaction and linearly dependent upon the linear radical concentration.

(b) At high \( dE/dx \), anoxic protection disappears, and free radicals are thought to be produced in such close proximity that they react with one another rather than with other molecules, such as \( O_2 \) or thiols. This is a second-order reaction and dependent upon the square of the linear radical concentration (Kuppermann, 1961).

The linear radical concentration is directly proportional to \( dE/dx \), and this kinetic reasoning is in agreement with the following relationship:

\[ \sigma_1(\varepsilon) = \sigma_1(\infty)[1 - \exp(-\alpha\varepsilon - \beta\varepsilon^2)], \quad (3) \]

where \( \alpha \) and \( \beta \) are coefficients determined from experimental data, and \( \sigma_1(\infty) \) is also determined experimentally. At low \( dE/dx \) equation (3) reduces to

\[ \sigma_1(\varepsilon) = \sigma_1(\infty)[1 - [1 - (\alpha\varepsilon + \beta\varepsilon^2)] + \cdots \text{higher terms}] \]

\[ \sigma_1(\varepsilon) = \sigma_1(\infty)[\alpha\varepsilon + \beta\varepsilon^2]. \]

Thus equation (3) on a log-log plot of cross section against \( dE/dx \) corresponds to a straight line at low \( dE/dx \) that bends upward at intermediate \( dE/dx \) and saturates at high \( dE/dx \) so that

\[ \sigma_1(\varepsilon) \to \sigma_1(\infty), \]

as is obvious from the nature of equation (3) (Tobias and Manney, 1964; Tobias and Todd, 1964).

Fig. 8—Inactivation cross section \( \sigma_1 \) for Tl cells as a function of \( dE/dx \) under aerobic and anoxic conditions of irradiation with ions of constant velocity. The solid lines are plots of equation (4) for aerobic and anoxic irradiation conditions. The coefficient \( \alpha \) has been divided by 3 to give the anoxic curve.

Fig. 9—Inactivation cross section \( \sigma_2 \) for Tl cells as a function of \( dE/dx \) under aerobic and anoxic conditions of irradiation with ions of constant velocity. The solid lines are plots of equation (5) for aerobic and anoxic irradiation conditions. The coefficient \( \alpha \) has been divided by 3 to give the predicted anoxic curve.
Graphical analysis of the plots of Figures 8 and 9 yield the following numerical forms for equation (3):

$$\sigma_1(\epsilon) = 90[1 - \exp(-4 \times 10^{-5} \epsilon - 1.5 \times 10^{-7} \epsilon^2)]$$

(4)

and

$$\sigma_2(\epsilon) = 28[1 - \exp(-3 \times 10^{-4} \epsilon - 6 \times 10^{-7} \epsilon^2)]$$

(5)

in the presence of oxygen, where $\sigma$ is in square microns and $\epsilon$ is in MeV·cm²/g. The value found for $\sigma_1(\infty)$ in equation (4) is 90 square microns, which was also found by direct microscopic measurement to be near the modal cross-sectional area of the nuclei of T1 cells attached to plastic under the conditions of irradiation. It was assumed that the nuclear area was elliptical.

**Dependence of Inactivation Cross Section on $dE/dx$**

Although there is no obvious physical basis for the functional form just presented, it is in agreement with radiation-chemical hypotheses suggested by Howard-Flanders (1958). Consider that radiation-produced free radicals may undergo any of three different reactions:

1. $R^* + A \xrightarrow{K_{1,2}} R - A$  
   
   (6)

2. $R^* + O_2 \xrightarrow{K_{3,5}} R - O - O - H$  
   
   (7)

3. $R^* + R \xrightarrow{K_{4,6}} R - R$.  
   
   (8)

These state that an active radical $R^*$ may react with unknown species $A$, it may react with $O_2$ to form a peroxide, or it may react with a neighboring radical. In each case, the fraction of unreacted molecules is proportional to

$$e^{-K_{1}[R^*][A]}, \quad e^{-K_{3}[R^*][O_2]}, \quad \text{and } e^{-K_{4}[R^*]^2},$$

respectively, so the overall reaction probably is

$$1 - \exp{[-K_1[R^*][A] - K_3[R^*][O_2] - K_4[R^*]^2]}$$

But since the linear concentration of free radicals is proportional to $dE/dx$ (or $\epsilon$), substitution gives, as the overall reaction probability

$$1 - \exp{[-\alpha(O_2)\epsilon - \beta\epsilon^2]}$$

(9)

which is the same as the ratio $\sigma(\epsilon)/\sigma(\infty)$. The dependence of the first-order term on $O_2$ is indicated in equation (9), and it is clear that the quadratic term does not contain the oxygen concentration. Most experiments reported (including those in this paper) indicate that cells are about three times as sensitive to the action of x-radiation in the presence of oxygen (above about 10%) as in its absence. Thus, the values of $\alpha$ in equations (4) and (5) have been divided by 3 to obtain the solid cross-section curves given in Figures 8 and 9, and the equations so obtained appear to predict the value of $\epsilon$ at which no oxygen effect is observed.

**The Usefulness of RBE**

If one wishes to use results of the type presented here for hazards evaluation, particularly at low exposure rates, then relative biological effectiveness ratios (RBE's) should be determined on the basis of the initial negative slopes of survival curves. Although computer analyses have been used to effect an evaluation of the initial negative slopes of these composite survival curves, they are difficult to use for the estimation of RBE's at low doses, due to the changes in curve shape for radiations of high $dE/dx$. For this reason, the whole curves of Figure 4 have been used to estimate RBE's as dose ratios (x-ray/heavy-ion) for a given survival at a variety of survival levels. The result is a family of RBE curves with survival level as parameter, that is, just another way of presenting the data of Figure 4. This family of curves is shown in Figure 10.

The main point illustrated by this figure is the high RBE at low doses and the low RBE at high doses of ions having $dE/dx$ in the range of 1000 to 5000 MeV·cm²·g⁻¹. In the absence of oxygen, of course, these RBE values would be multiplied by another factor of about 3.0. A similar factor would apply in the presence
Fig. 10—Plots of RBE against \(-dE/dx\) for inhibition of colony formation by T1 cells for various levels of survival.

of cysteamine. The RBE's would, of course, be reduced by IUdR, as would survival to low-\(dE/dx\) radiation (Tym and Todd, 1964). On the basis of the preceding paragraph, then, the applicable RBE's at low exposure rates are the highest ones shown on Figure 10. This reflects the immodifiability of the lethal effect by fractionation described under "Results."

Summary

Some aspects of the radiosensitivity of single human cells have been examined in vitro. So far, all factors which tend to modify sensitivity to x-radiation appear not to modify (or to modify less effectively) the effects upon single cells of high-\(dE/dx\) radiation. Whenever the hazards of heavily ionizing radiations are being evaluated, chronic exposure must be considered equivalent to acute exposure, and any attempts to modify the effects of densely ionizing radiations at the cellular level are probably useless.

In the case of cultured human cells, the RBE is dependent upon the endpoint because of the differently-shaped survival curves. Thus, for example, the RBE of fast carbon ions for 50% survival is about 6, whereas, it is only about 2 for 1% survival.

Acknowledgments

I am grateful to Drs. C. A. Tobias, G. W. Barendsen, R. Tym, and J. T. Lyman for sharing in the conceptual and experimental aspects of this work, and to Dr. Barendsen for the initial supply of T1 cells. Work was supported jointly by AEC and NASA.

References


IONIZING RADIATIONS AND CULTURED HUMAN CELLS


Determination of Leukoagglutinin Specificity by In Vivo and In Vitro Studies*

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Antibodies of various specificities may be found in patients receiving multiple blood transfusions. Investigations in the past decade have established that some of these patients may develop antibodies specifically directed against leukocyte antigens and not against those of the red cells (Bridges, Boyd, and Nelson, 1962; Dausset, 1954; Felbo and Jensen, 1962; Killman, 1958; Payne, 1947; van Loghem et al, 1958; van Rood, Leeuwen, and Eernisse, 1959; van Leeuwen, 1963). The transfusion of patients whose sera contain these antibodies, known as leukoagglutinins, frequently results in febrile transfusion reactions. In the past, most febrile transfusion reactions were attributed to the presence of pyrogens in the blood-collecting apparatus. However, with the use of disposable equipment, this source of reactions has been virtually eliminated, and most authors agree that leukocyte-leukoagglutinin incompatibility is a major cause of such febrile reactions today (Bridges, Boyd, and Nelson, 1962; Brittingham, and Chaplin, 1957; Dausset, 1954; Hossaini; Killman, 1958; Payne, 1957; van Loghem et al, 1958; Wilson, Rheins, Naegeli, and George, 1959). This study was undertaken to determine in vivo and in vitro the specificity of leukoagglutinins, and to establish the nature of the pyrogenic reaction.

Materials and Methods

Normal sera were obtained from donors in the blood bank of the Ohio State University Hospital. All pathologic sera were obtained from patients on the hematology service of the department of medicine. Leukocyte Suspensions

Blood was collected from normal donors in the blood bank in 15 ml amounts in silicized tubes containing 0.3 ml of 5% disodium versenate (EDTA). After thorough mixing of blood and anticoagulant, 2 ml of 6% dextran were added, and the contents of the tube were mixed again by inversion. The blood was divided equally and pipetted into three 22 x 1.5 cm test tubes. After 90 minutes of sedimentation, the supernatant plasma, rich in leukocytes and platelets, was transferred to a 15 ml graduated centrifuge tube. The tube was spun in a centrifuge at 800 rpm for 15 minutes. The supernatant fluid was removed and, by the same centrifugation technique, the cells in the sediment were washed twice with saline-serum-anticoagulant (SSA) solution. The latter solution was prepared by mixing 2 ml of inactivated normal blood group AB serum with 0.5 ml of 5% EDTA, and then diluting it to 200 ml with physiological saline. After the second washing, the supernatant was removed as completely as possible, and the button of cells was homogeneously suspended in a fresh 0.5 ml aliquot of SSA solution. A white cell count was made, and additional SSA was added to produce a cell density of 8 to 10,000 per mm³.

Red Cells

The red cell portion remaining after sedimentation was washed in saline and was used for the absorption of erythrocyte isoagglutinins. All erythrocyte isoagglutinins were removed from test sera by absorption with washed red cells of the leukocyte donor before leukoagglutination tests were performed.

Leukoagglutination Test

The leukoagglutination tests were performed by placing 0.2 ml of a 1:8 dilution of the absorbed sera in physiological saline in 10 x 75 ml tubes, followed by one drop of bromelin solution (Dade Reagents, Inc., Miami, Florida) or 0.1 ml of the SSA suspended leukocytes. The
tubes were shaken, left to stand for 15 minutes at room temperature, and then were spun for 30 seconds in a serofuge (Clay-Adams). After tapping the tubes gently to disperse the cells in the sediment, the solutions were examined for agglutination by holding the tubes in the direct beam of a light source over a concave magnifying mirror.

A known positive control and a negative control were included in each series of tests. Each serum was tested against two cell suspensions prepared from randomly selected donors.

**Induction of Febrile Transfusion Reaction**

The following sera were used:
1. Serum from a Group A, Rho (D) positive blood patient with aplastic anemia, whose serum had shown strong leukoagglutinating activity.
2. Serum from an apparently normal individual (negative control) of the same blood group and Rho (D) as that of the patient.

Five ml of both the control and the leukoagglutinin-positive sera were diluted to 30 ml using injectable physiological saline followed by Seitz-filtration.

Basal white cell and differential, red cell, reticulocyte, and platelet counts were obtained by finger stick; temperature, pulse rate, and blood pressure were recorded on a male volunteer (AAH). Two hours after obtaining the basal data, a slow intravenous transfusion of 250 ml of physiological saline was begun. Twenty-five minutes later the control Seitz-filtered serum was injected into the saline bottle and infusion continued. At half-hourly intervals for six hours, counts were taken, and temperature, pulse, and pressure were recorded.

Under similar environmental, temporal and nutritional conditions, the same volunteer was injected one week later with an equal amount of the identically processed positive serum, and similar observations were recorded as above.

**Results**

In all, 395 sera obtained from 375 normal individuals and patients with various pathological conditions were tested for leukoagglutinins. When a patient gave a negative test and subsequently became positive, the reactions were recorded as two specimens; this accounts for the difference in numbers. There were 20 such cases. Many of the pathological groups had received multiple transfusions. Results of the sera tested for leukoagglutinins and the number of transfusions received by each patient prior to the first testing of the serum are shown in Table 1. Table 2 lists the diagnoses of patients whose sera were tested for leukoagglutinins before they received any blood transfusions. None of the seven leukoagglutinin-positive sera was from a woman who had ever been pregnant.

Cytologic studies of agglutinated cells revealed participation of both the granulocytes and mononuclear cells in the clumps. Clumping resulted in complete loss of the amoeboid movement of cells in the positive tubes, whereas cells in the negative sera remained mobile, though sluggish.

Transfused patients were classified by clinical diagnosis. Those showing transfusion reactions, leukoagglutinins, or both, are presented in Table 3.
**TABLE 1**

Distribution of 395 Sera Tested for L.A.*

<table>
<thead>
<tr>
<th>Number of blood units</th>
<th>Number tested</th>
<th>No L.A.</th>
<th>L.A. demonstrated</th>
</tr>
</thead>
<tbody>
<tr>
<td>From patients never transfused prior to testing</td>
<td>247</td>
<td>240†</td>
<td>7</td>
</tr>
<tr>
<td>From patients receiving less than 8 units</td>
<td>63</td>
<td>48</td>
<td>15</td>
</tr>
<tr>
<td>From patients receiving more than 8 but less than 20 units</td>
<td>59</td>
<td>42</td>
<td>17</td>
</tr>
<tr>
<td>From patients receiving more than 20 but less than 50 units</td>
<td>16</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>From patients receiving more than 50 units</td>
<td>10</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>395</td>
<td>342</td>
<td>53</td>
</tr>
</tbody>
</table>

* L.A.: Leukoagglutinins
† Some of these patients were subsequently transfused and re-tested.

**TABLE 2**

Diseases Found in Patients Showing Negative and Positive Leukoagglutinins Prior to Receiving any Transfusion

<table>
<thead>
<tr>
<th>Disease</th>
<th>Number tested</th>
<th>Number positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Chronic pyelonephritis</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Acute monoblastic leukemia</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Healthy</td>
<td>76</td>
<td>1</td>
</tr>
<tr>
<td>Miscellaneous*</td>
<td>133</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>240</td>
<td>7</td>
</tr>
</tbody>
</table>

* Diseases included:
  1. Other acute leukemias
  2. Chronic leukemias
  3. Lymphomas
  4. Hemolytic anemias
  5. Refractory anemias
  6. Lupus erythematosus disseminatus
  7. Coagulation defects
  8. Various forms of non-hematologic diseases

**TABLE 3**

Correlation between Disease, Transfusion Reaction and L.A. Production

<table>
<thead>
<tr>
<th>Disease Group</th>
<th>Reaction only</th>
<th>Reaction and L.A.</th>
<th>L.A. only</th>
<th>Both negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute leukemias</td>
<td>3</td>
<td>7</td>
<td>6</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td>Chronic leukemias</td>
<td>4</td>
<td>10</td>
<td>4</td>
<td>19</td>
<td>37</td>
</tr>
<tr>
<td>Lymphomas</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>Hemolytic anemias</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Refractory anemias</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Thrombocytopenic purpuras</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Coagulation defects</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Non-hematologic diseases*</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Insufficient data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>34</td>
<td>12</td>
<td>78</td>
<td>148</td>
</tr>
</tbody>
</table>

* Six untransfused patients showed leukoagglutinins (L.A.). (See Table 1)
LEUKOCYTE AND PLATELET AGGLUTINATING SERUM
NORMAL RECIPIENT

A.H. 32

TEMPERATURE
HEADACHE, MYALGIA, SHAKING CHILL

PLATELETS

RETICS

NORMAL SERUM INFUSION
SALINE INFUSION
POS. SERUM ADDED COMPLETED

W.B.C.

R.B.C.

R.B.C. IN MILLIONS

8:30 10:30 11:30 12:00 1:45
AM 10:00 11:00 12:00 1:15
PM

8:30 10:45 11:40 1:15 2:15 3:15 4:15 5:30
AM 10:00 11:00 12:00 1:15
PM

Fig. 1.

Figure 1 shows white cell, red cell, platelet, and reticulocyte counts, temperature, and symptoms experienced during the infusion of both the control and positive sera. No change occurred in these parameters following the infusion of the control serum. On the contrary, the following alterations occurred after the infusion of the positive serum. One hour after the start of the infusion of serum, there was a marked leukopenia, lasting for 90 minutes, followed by a gradual return to the basal level, and a subsequent leukocytosis over the next three hours. Only slight changes in the reticulocyte and red cell counts occurred, but a significant thrombocytopenia was observed. Platelet levels remained at the basal value for two hours, but 30 minutes later the count showed a precipitous drop. The thrombocytopenia persisted for two hours, and then the count returned to normal levels. No further counts were made.

Fifteen minutes before the completion of the infusion (40 minutes after starting the serum infusion), the subject developed a frontal headache, which increased in intensity over a period of an hour. Within 15 minutes after the onset of the headache, a shaking chill, a mild tachypnea (28 breaths per minute), and a 2–3° rise in temperature (which reached its peak within three hours) developed.

Table 4 shows the basal red cell, reticulocyte, platelet, white cell, and differential counts and the values obtained over the six hour study period following the injection of the positive serum. Since detailed listing of the hourly values following the injection of the control serum did not contribute additional information, only the recorded range of values were included in this table. The values obtained with regard to the red cell, reticulocyte, platelet, and total white cell counts have been mentioned already in Figure 1. Differential counts obtained after the infusion of the control serum varied slightly from basal values. However, there were marked absolute neutrophilia, eosinopenia, lymphopenia and monocytopenia. The neutrophilia continued for over three hours and was followed by a neutrophilic leukocytosis. Values for the remaining white cell elements remained below normal levels over the six-hour period. However, the monocytes showed signs of reappearance five hours after infusion.
A. A. HOSSAINI AND H. E. WILSON

TABLE 4
Changes Induced in a Human Volunteer by Injection of Leukoagglutinin Positive Control Sera

<table>
<thead>
<tr>
<th>Cells</th>
<th>Control Serum</th>
<th>Test Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range of values over 6 hours</td>
<td>Time in hours after injection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>RBC's in millions</td>
<td>5.2-5.3</td>
<td>5.8</td>
</tr>
<tr>
<td>Reticulocytes %</td>
<td>1.2-2.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Platelets in 1000's</td>
<td>190-208</td>
<td>214</td>
</tr>
<tr>
<td>WBC Total</td>
<td>4150-5000</td>
<td>5000</td>
</tr>
<tr>
<td>Polys. Total %</td>
<td>3050-3071</td>
<td>66</td>
</tr>
<tr>
<td>Eos. Total %</td>
<td>0-250</td>
<td>0</td>
</tr>
<tr>
<td>Baso. Total %</td>
<td>0-0</td>
<td>0</td>
</tr>
<tr>
<td>Lympho. Total %</td>
<td>747-900</td>
<td>1300</td>
</tr>
<tr>
<td>Mono. Total %</td>
<td>125-150</td>
<td>400</td>
</tr>
</tbody>
</table>

Discussion

This study confirms the work of other investigators (Bridges, Boyd, and Nelson, 1962; Daussert, 1954; Felbo and Jensen, 1962; Killman, 1958; van Rood, Euwen, and Eernisse, 1959; van Rood and von Leeuwen, 1963; Wilson, Rheins, Naegeli, and George, 1959) that the mechanism of the leukoagglutination phenomenon is immunologic and is specific for leukocytes. There is a significantly higher frequency of occurrence of leukoagglutinins in patients receiving multiple transfusions as compared to patients who were not transfused. Table 1 shows that only seven out of 247 sera from the latter group gave a positive test. In contrast, 46 out of 148 sera from transfused patients were positive.

Twelve of the leukoagglutinin-positive patients had been tested before any transfusion. All their sera were negative then, but became positive after one or more blood transfusions. Yet other patients, who had been tested at different stages of transfusion therapy, were found to become leukoagglutinin-positive as the number of transfusions was increased. Table 1 shows the direct relationship between the number of leukoagglutinin-positive patients and the number of units of blood they had received. Among the 46 leukocyte agglutinating sera from transfused patients, only three were known to possess a concomitant red cell antibody. Therefore, the increase in the number of leukoagglutinin-positive patients with increasing transfusions may have been caused by further stimulation by the antigen or antigens of transfused leukocytes.

Absence of reaction following the injection of the control serum indicates that all pyrogenic agents had been eliminated from the serum. Variations in differentials, in blood counts and temperatures, in pulses and pressures during the negative control experiment were within the range of physiological variations and experimental error.

Excluding the unlikely possibility of sensitization by the negative control serum, it may be assumed that the reaction to the positive serum was due to the specific effect of the injected leukoagglutinins on circulating leukocytes. The possibility of a pyrogenic reaction must be considered, however, as marked leukopenia is not characteristic of this type of transfusion reaction.

The marked leukopenia in the absence of a change in the red cell
LEUKOAGGLUTININ SPECIFICITY

count indicates that the reaction was not caused by erythrocyte antibodies. The simultaneous onset of neutropenia, eosinopenia, lymphopenia, and monocytopenia is additional evidence suggesting that the antibody is directed against granular and mononuclear leukocytes. This reinforces the findings by microscopic studies that both cell types participate in the clumps of cells seen in positive leukoagglutinin tests. The thrombocytopenia was shown to be associated with the presence of platelet antibodies in the injected serum.

The injection of a serum known to contain leukoagglutinins into a normal individual resulted in the development of a febrile reaction and a mild leukopenia. In an earlier report, one of us (AAH) explained this on the basis of passive transfer of leukoagglutinins.

Using the continuous-flow electrophoresis cell, Wilson, Rheins, and Naegeli (1959) fractionated seven negative sera and ten sera from six leukoagglutinin-positive patients. Results showed a rise in activity of the γ-globulin fraction only in the positive sera. Persistence of leukoagglutinins in positive sera after absorption indicates that the red cells lacked the corresponding antigens.

There was an absence of a marked reduction in the severity of febrile transfusion reactions when packed red cells with a leukocyte count of less than 1000 per mm³ were given to three leukoagglutinin-positive patients who had experienced multiple febrile transfusion reactions. The mild reactions after receiving the processed blood might have been due to the almost inevitable contamination of packed, washed, red cell preparations by a few unremoved leukocytes.

Summary

Results of testing sera from normal individuals and pathological patients, using a bromelin technique for the detection of leukoagglutinins, are reported. These results, supported further by an in vivo experiment, suggested that the phenomenon of leukoagglutination is immunologic in nature and that leukoagglutinins may be the cause of some febrile transfusion reactions. These are specifically directed against leukocytes, and do not involve the red cell series. Furthermore, the antigen (or antigens) is probably present in both the granulocytes and the mononuclear leukocytes. Anti-platelet antibodies could be presumed to be the cause of thrombocytopenia.

References


Hossaini, A. A critical review and a new approach to the serological detection of leukoagglutinins. Dissertation for Ph.D. degree, Ohio State University, Columbus, 1960.


Further Correlations of Cell Metabolism and Resistance to Tuberculosis: Studies on Mononuclear Peritoneal Exudate Cells from Mice and Guinea Pigs*

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The work of Lurie (1944) and Lurie, Zappasodi, and Tickner (1955) suggested that resistance to tuberculosis resides in certain mononuclear phagocytic cells of the reticuloendothelial system. On the premise that the physical activities of these cells might rest on metabolic activity, one of us (M.J.A.) began a series of experiments aimed at correlating resistance to tuberculosis and the level of enzyme activity in the system of intermediate metabolism of these cells.

Initial studies (Allison, Zappasodi, and Lurie, 1961) gave a close correlation between the level of metabolism of mononuclear cells from the peritoneal cavity and the level of native resistance of various inbred rabbit families to tuberculosis. Families of high resistance had a higher rate of metabolism for these cells than did families of low resistance; families of intermediate position in resistance were also intermediate in their cell metabolism. Later, it was shown that BCG vaccination produced a biphasic pattern of resistance associated with a biphasic pattern of metabolism (Allison, Zappasodi, and Lurie, 1962a and b). Shortly following administration of BCG, there was a depression in host resistance that rose considerably above normal about 4 to 5 weeks later. The mononuclear cell metabolism fluctuated with these changes in resistance. Alterations in resistance to tuberculosis, due to administration of triiodothyronine, cortisone, and thyroidectomy, were all associated with alterations in cellular metabolism (Allison and Gerszten, 1962 and 1963). When the host's resistance was depressed, the metabolism of mononuclear exudate cells also decreased.

The aim of this study was to determine if species other than the rabbit also show a correlation in their resistance to tuberculosis with the metabolism of mononuclear cells from the peritoneal cavity. For this purpose, the mouse was chosen as a species that is more resistant than the rabbit and the guinea pig as more susceptible.

Materials and Methods

Three sources of mice were used; a Swiss albino variety, a market albino variety, and the grasshopper mouse. The mice were housed at a constant temperature of 70 F and were fed Purina dog chow checkers and water ad libitum. Market guinea pigs were used. Their aver-
age weight was 400 g, and they were housed under similar conditions as the mice, and fed Purina rabbit chow checkers and water. The mononuclear peritoneal exudate cells from the mice and guinea pigs were studied for metabolic activity. The procedure for obtaining these exudates was as follows. In the mouse, 1 cc of sterile mineral oil was injected intraperitoneally five days before the animals were killed by crushing the vertebral column at the base of the neck. The peritoneum was opened and washed with 5 cc of 0.90% citrated saline. The cells were removed from the peritoneum with a 10 cc syringe and then centrifuged for 10 minutes at 1,500 rpm. The supernatant was drawn off and the cells resuspended in 0.90% saline. The guinea pigs were killed by a blow on the head, having been injected four days previously with 5 cc of sterile mineral oil. Guinea pig cells were counted and adjusted in a similar manner, each animal being studied individually. In removing the peritoneal exudate cells, a 50 cc syringe was used to wash the peritoneum with 60 cc of 0.85% citrated saline. Total cell counts were done immediately, using a hemocytometer, and the saline volumes were adjusted to give a standard number of cells per cc. The mouse cells were pooled using ten animals per group. Slides were prepared for differential counts, and these were stained with Wright's stain and counted at a later date.

In studies used to measure inhibition of enzyme activity by histamine, the quadriceps femoris muscle from mice, guinea pigs, and rabbits was used. The muscle was ground in a mortar with a small amount of sea sand and distilled water. This triturated muscle was utilized in the studies discussed below. Ten million peritoneal exudate cell aliquots were used in the modified Thunberg technique (described by Allison et al., 1961) to study the dehydrogenase activity. The following substrates were used: lactic acid, sodium succinate, sodium glycerophosphate, L-malic acid, glycerol, α-keto glutaric acid, β-hydroxybutyric acid, and sodium glycerophosphate for the acid phosphatase determination. The lactic dehydrogenase (LDH) activity of the muscle tissue was also studied, using the Macalaster Bicknell coenzometer. The substrate for this LDH test is prepared by dissolving 7 g of sodium pyrophosphate in 250 ml of hot distilled water. The solution is cooled to room temperature and lactic acid (1.5 ml) and diphasphopyridine nucleotide (1 g) are dissolved into this solution. The pH is adjusted to 8.8 with hydrochloric acid (1 N), and the final volume is adjusted to 280 ml with distilled water; 2.8 ml aliquots are placed in individual test tubes, capped, and stored frozen. This preparation will keep for several weeks. The coenzometer's light source is a 4-watt fluorescent lamp that emits a narrow band of ultraviolet light at 3,400 Å units. The change in concentration of reduced diphasphopyridine nucleotide during the enzyme reaction was measured by noting the change in ultraviolet absorption over a 3-minute period. Total nitrogen analyses were done using the Microkjeldahl method adapted from Hawk, Oser, and Summerson (1954). Acid phosphatases were measured with the Barringer and Woodard Harleco phosphate substrate (pH 4.5) and incubated for three hours. The released phosphate was then determined by the method of Fiske and Subbarow (1925).

Results

Table 1 shows the peritoneal exudate cell counts. Strains I and II mice yielded approximately 5 million cells per animal, with 85% mononuclears. Strain III mouse yielded approximately 6 million cells, 90% mononuclear cells. A five-day exudate following the induction of the chemical peritonitis was found to contain the highest percentage of mononuclears. The guinea pig yielded, on the average, 100 million cells per animal after a four-day
chemical peritonitis. The differential counts of these exudates were not statistically different, except that 5% mast cells were found in the peritoneal exudates from Strains I and III mice. These cells were absent in the exudates of Strain II mice. On the average, the total protein values for all three strains of mice were 0.79 mg per 10 million cells, and for the guinea pig, 1.66 mg per 10 million cells. To correct for differences in cell size, the dehydrogenase activity values were adjusted to the rabbit's cell protein (1.12 mg per 10 million cells); all values then are expressed in terms of rabbit protein. Table 2 shows these metabolic values with the adjustment for protein. The guinea pig showed the highest acid phosphatase activity, having a value of 4.60 mg ± 0.96 mg of phosphorus per 10 million cells per hour. The rabbit was next, with a value of 2.4 mg ± 0.33 mg of phosphorus per 10 million per hour. Acid phosphatases were run only on mouse Strains I and III; the values were 0.11 ± 0.02 mg and 0.54 ± 0.10 mg of phosphorus per 10 million cells per hour, respectively. Strain I mouse had peritoneal exudate cells lower in dehydrogenation activity than those of the guinea pig or the rabbit; Strain II and, in general, Strain III also "metabolized higher than the rabbit."

Since the activity of the mononuclears from Strains I and III mice metabolized at a lower level than anticipated, it seemed appropriate to study muscle tissue to determine comparative metabolism in the three species. The coenzometer was used to compare the lactic dehydrogenase activity in the three species. The quadriceps femoris was used as the enzyme source. The normal value for the rabbit was 258,500 LDH units; for the mouse, 625,562 LDH units; for the guinea pig, 100,000 LDH units per gram wet weight. The rabbit muscle LDH activity was intermediate between the guinea pig, which was lowest, and the mouse, which was highest.

Because of the possibility that
METABOLISM OF PHAGOCYTES AND RESISTANCE TO TUBERCULOSIS

TABLE 3

The Effect of Histamine Hydrochloride on the Lactic Dehydrogenase Activity of Quadriceps Femoris Muscle of Mice

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.033 mg. Histamine</td>
<td>0.00013 mg. Histamine</td>
<td>0.000093 mg. Histamine</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6</td>
<td>1365 ± 240</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>1287 ± 159</td>
<td>0</td>
<td>3</td>
<td>711 ± 185</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>1246 ± 108</td>
<td>0</td>
<td>3</td>
<td>446 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

Histamine released from mast cells could alter the metabolic picture of cells (Graham et al., 1955), we decided to repeat the muscle LDH tests after the addition of small amounts of histamine, to see if this altered the LDH activity. Histamine hydrochloride (Sigma Chemical Company) was used to study the effects on mouse muscle LDH activity. Different doses of histamine were incubated for 1 hour with the mouse muscle tissue. The LDH activity was then measured. Table 3 shows the effects of histamine on muscle LDH activity of mice. With a dose of 0.033 mg, there was no LDH activity displayed by the mouse muscle. At a dose of 0.00013 mg, there was a 45% reduction in activity; with a 0.000093 mg dose, there was a 64% reduction in activity.

Discussion

The metabolic activity of mononuclear cells from mice of different strains and from the guinea pig have been studied and compared to those of the rabbit. It was thought that, since the guinea pig is highly susceptible to tuberculosis, the activity of cells from its reticuloendothelial system would be lower than that of the rabbit. Because of its relative resistance, the mouse was expected to show high metabolic activity. The peritoneal exudate cells of the guinea pig followed the expected pattern by being lower than the rabbit in metabolic activity. Results from two of the three strains of mice used were equivocal, but one strain supported our hypothesis linking metabolic level of the mononuclear cells with resistance to tuberculosis. It was noted that the two “equivocal” strains of mice had in their exudates 5% mast cells. These cells were not found in the rabbit, guinea pig, or mouse Strain III. The different values for metabolism in the strains of mice whose exudates contained these cells suggest a possible interfering substance derived from these cells. Among the substances produced by these cells are histamine, serotonin, and heparin (Padawer, 1963). Motta, Da-Silva, and Fernandes (1960) showed that there is a simple exchange reaction between free histamine and the nicotinamide portion of the DPN molecule. This exchange might interrupt the electron transport system (Alivisatos et al., 1960) and could be the means whereby the dehydrogenase activity of the peritoneal exudate cells of the Strains I and III mice were depressed. Graham demonstrated that dog mast cells produce, on the average, 7 µg histamine per cell. A dose
similar to this was incubated for 1 hour with mouse muscle tissue, which is relatively low in mast cells. A definite depression in lactic dehydrogenase activity with the muscle-histamine mixture was noted. The exact mechanisms involved here remain to be investigated.

Our findings regarding acid phosphatase are similar to the findings of Colwell, Hess, and Tavaststjerna (1963) and Colwell and Hess (1963), who found a higher acid phosphatase activity in the susceptible guinea pig than in the resistant rat. The present work showed that the guinea pig has about 40 times as much acid phosphatase activity as the mouse. This suggests that this enzyme cannot be correlated with resistance to tuberculosis. It is clear from this work that there is a great species variation in the metabolism of the substrates used. However, the guinea pig clearly demonstrates that a depressed metabolism of the reticuloendothelial system can be correlated with low resistance to tuberculosis. The findings in the mouse also confirm this idea, if allowance is made for the possible depressing effects of histamine from the mast cells on the electron transport system. It is also interesting that different families of mice have different levels of metabolism, as in Lurie's inbred races of rabbits.

Summary

The metabolic activity of mononuclear exudate cells from mice of different strains, and from the guinea pig, have been compared to the rabbit with the aim of relating metabolic activity of these cells to resistance of these species to tuberculosis. The presence of mast cells in the peritoneal exudates of mice was thought to interfere with the dehydrogenation of certain substrates due to the release of histamine. Some experimental evidence presented by the authors seems to support this thesis.

References


The ninhydrin and alloxan reactions have been criticized as being nonspecific and unreliable for histochemical studies due to the diffusion, solubility, and fading of their reaction products. This paper presents some modifications of the techniques that appear to render the reactions more reliable for the identification of proteins and peptides possessing terminal \( \alpha \)-amino acids. The resulting color complexes are also stable.

In 1862 Strecker demonstrated that a mixture of alloxan and \( \alpha \)-amino acid in aqueous solution reacted with the generation of carbon dioxide and the concomitant development of a deep blue color which could be attributed to the formation of murexide (acid ammonium purpurate). The latter product was isolated as purple-red crystals from the reaction mixture which, in addition, yielded an aldehyde containing one less carbon atom than the parent amino acid. Hence an oxidative deamination of the amino acid was instigated by the alloxan reagent (Greenstein and Winitz, 1961).

The cytochemical application of the alloxan reaction to tissue sections was criticized by Romieu (1925) on grounds of lack of specificity, and by Giroud (1929) because of color diffusibility. Serra (1946) quotes Winterstein (1933) as saying that the test was insensitive with fixed material. Vercauteren (1951) indicated that the reaction products of ninhydrin were, to some extent, soluble in water, and thus may be absorbed on cellular structures which are not the site of the reaction. Hutton (1953) was able to stabilize the reaction product for about a week in unerupted molars of hamsters, fixed only by their incidental embedding in celloidin. Sections sprayed with a 0.25% ninhydrin solution in n-butanol required three to four days incubation at 55 C to develop maximum color.

Notovny and Owens (1960) prevented diffusion of amino acids, and their ninhydrin reaction products, from original sites by fixing and dehydrating the tissues in anhydrous,
peroxide-free dioxane. The color produced by α-amino acids faded within 24 hours, while that produced by protein was stable for seven days or longer. Vainer and Bona (1963) used various fixatives, such as Carnoy and methyl alcohol, and different concentrations of ninhydrin varying from 0.1% to 0.4% to study its reaction on peripheral blood. Their reactions were carried out at 80°C for 5 to 30 minutes, and the staining remained intact for "a long time."

Yasuma and Ichikawa (1953) stained the aldehyde groups by the Schiff reagent in an oxidative deamination reaction brought about by ninhydrin. Alloxan was also used as an oxidative deamination reagent. A serious objection to such use of the Schiff reagent is its tendency to stain other tissue components (direct Schiff reaction), thus confusing the recognition of true protein sites in the sections.

Both the ninhydrin and the ninhydrin-Schiff histochemical reactions have been criticized. Recently, Puchtler and Sweat (1962), reviewed the literature and concluded that the ninhydrin-Schiff method was unreliable for demonstrating proteins. Kasten (1962) took issue with this conclusion and stated that, in the ninhydrin reaction, as distinct from the ninhydrin-Schiff reaction, ammonia reacts with reduced ninhydrin (hydrindantin) to form a violet product which is diffusible, unstable, and offers little possibility for histochemical application.

He observed that acetylating and deaminating agents block the ninhydrin and alloxan reactions by masking the carboxyl or the amino groups of the α-amino acids. This indicates that the action of ninhydrin in tissues is at the amino group, and qualifies the ninhydrin reaction as an histochemical test for proteins and polypeptides with free and reactive amino groups.

Materials and Methods

Pieces of rat kidney, liver, small intestine, skeletal muscle, and cardiac muscle were removed after decapitation. Different concentrations of aqueous and alcoholic solutions of trichloroacetic, phosphotungstic, phosphomolybdic, and acetic acids were used as fixatives for 24 hours at room temperature. Tissues fixed in aqueous solutions were dehydrated in a series of alcohols, cleared in xylene, and embedded in Paraplast. Tissues fixed in 80% alcoholic solutions were dehydrated in 95% and absolute alcohol, cleared in xylene, and embedded in Paraplast.

Both aqueous and alcoholic solutions of phosphomolybdic and phosphotungstic acids proved to be poor penetrants and their use was discontinued.

Sections of tissues fixed in 5% acetic acid in 80% alcohol were treated with ninhydrin, but, only a weak color developed. The following detailed procedure was followed: Tissues were fixed in 5% trichloroacetic acid in 80% alcohol for 24 hours at room temperature. They were then transferred to 95% alcohol and absolute alcohol for one hour each, and after clearing in xylene, were embedded in Paraplast. Paraffin sections were mounted on slides with starch paste, instead of egg albumin, to avoid false localization. Deparaffinization of sections in xylene was followed by going through two changes of absolute alcohol. Sections were then treated in 1% ninhydrin in absolute alcohol at 37°C from one to three hours. Over-treatment tended to weaken the reaction. After rinsing in two changes of absolute alcohol, and clearing in xylene, the sections were mounted in Permount. Both 0.1% and 0.5% stannous chloride were added to the ninhydrin solution.

Pepsin and trypsin digestion procedures were used as well as acetylation and deamination blocking reactions (Pearse, 1961). Alternate sections were treated with 1% alloxan in absolute alcohol instead of ninhydrin in the above procedures.

Comparable sections of the kidney, liver, muscle, and intestine were stained by the ninhydrin-Schiff and alloxan-Schiff methods for protein-bound amino groups (Yasuma and Ichikawa, 1953), the chloramine-T Schiff method for protein-bound amino groups (Burstone, 1955), and the acid solochrome cyanine method for basic proteins (Pearse, 1961).
Photomicrographs of rat tissue sections fixed in 5% trichloroacetic acid in 80% alcohol and treated with alcoholic ninhydrin or alloxan.

Fig. 1—Kidney tubules following alloxan treatment (X 700).

Fig. 2—Liver cells following the ninhydrin reaction (X 700).

Fig. 3—Smooth muscle fibers in the small intestine following the alloxan reaction. Arrow points to darkly colored submucosa (X 700).

Fig. 4—Cardiac muscle colored by the ninhydrin reaction (X 700).

Fig. 5—Striated muscle colored by the alloxan reaction (X 700).

Fig. 6—Striated muscle demonstrating ninhydrin-reaction (X 1400).
Results and Discussion

We observed an intense bluish-violet color in trichloroacetic acid-fixed sections after the ninhydrin treatment. Alloxan stained the tissues a rich red color. Both ninhydrin and alloxan produced intense coloration of proximal renal tubular epithelium and basement membranes (fig. 1). Some parenchymal liver cells (fig. 2) were well-stained while others in the same area failed to bind the color complex. Smooth muscle in the small intestine (fig. 3) stained strongly in contrast to the epithelium. The myofibrils and striations in both cardiac (fig. 4) and skeletal muscles (fig. 5 and 6) were intensely stained. The connective tissue fibers of the muscle, liver, and kidney were poorly stained, except in the submucosa of the small intestine where they were intensely stained.

Pepsin and trypsin digestion, prior to ninhydrin and alloxan treatment, completely blocked the reaction by digesting the proteins. Both acetylation and deamination blocked the ninhydrin and alloxan reactions and prevented color development, thus establishing that the ninhydrin and alloxan act on the terminal amino acids of the protein and peptides chains.

According to Moore and Stein (1948), the addition of stannous chloride to the ninhydrin solution reduced for ninhydrin, thus intensifying the reaction; however, we found that the addition of 0.1% or 0.5% stannous chloride completely abolished the reaction.

The localization and color obtained by the ninhydrin reaction or the alloxan reaction were superior to those obtained by the ninhydrin-Schiff or the alloxan-Schiff reactions.

The chloramine-T Schiff method, where chloramine-T acts as a dicarbonyl compound like alloxan and ninhydrin, stained the tissues with the same intensity and localization as those of ninhydrin-Schiff and alloxan-Schiff, but was less intense than that of alloxan or ninhydrin alone.

The acid solochrome cyanine method stained the tissues rather weakly.

Summary

The ninhydrin (triketohydrindene hydrate) reaction, used extensively in biochemical work, has not been successfully applied cytochemically, due to lack of specificity, fading, and diffusion artifacts. These faults are effectively overcome by avoiding aqueous media and by fixing the tissues in a 5% solution of trichloroacetic acid in 80% alcohol, a good precipitant for proteins possessing free NH$_2$ groups. A fixation of 24 hours is followed by customary embedding in paraffin, and deparaffinized sections are passed through two changes of absolute alcohol, then treated in 1% ninhydrin in absolute alcohol for one to three hours at 37 C. The presence of free NH$_2$ groups in the peptide and protein chains produces a blue-violet color. Treated sections retained their color for up to six months. Alternate sections were used for the alloxan procedure; the resulting red color showed the same reaction sites.

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A woman patient came to see me, bringing a letter from her own doctor, a tuberculosis specialist. He is well-known, and I have great esteem for him. He gave me a few details concerning the patient. He had known her for many years. She first suffered from pulmonary tuberculosis ten years ago, and then spent a fairly long time in a mountain sanitorium of which he was the director. She improved there sufficiently to be able to come down to the valley again; however, he continued to keep her under constant supervision. There were moments when her condition improved and she was able to live quite normally for several months. Then things got worse again. She would have a little treatment and, if that did not prove to be enough, he would send her up for three months to the mountains again.

In his letter he wrote, "It is the patient herself who asks to see you, and it is because of this that I recommend her to you, but I am not of the impression that psychological factors play any part in her case. She is a most charming woman. She is well-balanced. She has a husband who is also charming, and I don't think there is any problem so far as the couple is concerned. But it will do her good, no doubt, to see you all the same."

Then she began to tell me something of her life. She was a convinced Roman Catholic. She married a Catholic, and had two daughters by him. Very rapidly, however, this husband took to drinking. She told me at considerable length of her suffering as the wife of a man who had taken to drink and of all the efforts she made to save him. But
little by little, he continued to slip downwards, and in the end he committed suicide. His suicide caused her great religious concern.

After a few years spent as a widow, she married again, this time to a man who was a Protestant, and who, in addition to this, had been divorced. He is the husband who was so delightful. But you can understand how this situation nonetheless confronted this woman with many problems. This second marriage cut her off from her Church. She could no longer receive Holy Communion, and she was considered by her Church to be living in adultery.

The problem had reached an acute stage when she came to see me, because her daughter was just reaching the age when she was about to receive her first Communion. The child asked her mother, “But why can you not receive the Communion with me?” From the start the mother had found this separation from the Church deeply painful. Not only did she feel that she was separated from the Church, but she also felt that she was cut off from God. Several times she repeated to me, “It’s several years now that I’ve not been able to pray.”

She also had grudges and resentments against her charming husband. He was a big industrialist, and big industrialists, just like doctors, are extremely busy men whose wives often have the feeling that they are neglected. In the evening, he frequently telephoned his wife saying there was some industrialist in town for a short time, and he had to take him out to dinner. She described the terrible evenings she spent just waiting for her husband. The husband must have had a slightly uneasy conscience over this, and as a result, he built her a fine villa. The villa was in fact an expiatory villa. It was as if once she had a beautiful villa, she would have no more justification in complaining about him. She said to me, “This villa, I hate it.”

There were after all some problems in her life! My colleague the specialist was perfectly right when he said she was not a psychologically ill person, unless we consider everybody psychologically ill. No, in the specific sense of the word, she was not psychologically ill, and I did not practice psychology in any specific sense in her case. I don’t believe that we even analyzed any dreams. She was not suffering on the psychological level; she was suffering on the moral level. Sometimes a person who is not suffering from any nervous illness has to go to see a psychiatrist. This woman had no one to whom she could speak of her religious and moral problems. She could not discuss them with a Roman Catholic priest, because she was separated from her Church. Nor could she speak of them to a Protestant pastor, because she was not a member of his Church. Nor could she speak of them to the charming husband, for he did not make such dialogue possible, and in addition, he was only Protestant in name. He had deprived her of her own Church without giving her an alternative Church link in exchange.

Quite suddenly one day, she turned up in the consulting room with a radiant expression. I was so astonished by this that I said to her, “But what has happened to you?” She replied, “Just think, yesterday evening my husband telephoned saying he was not coming home, and I was just getting ready to be very irritated with him for the entire evening. Then the idea suddenly came into my mind that I might try to recollect myself and be silent in prayer before God, as you say you do. In the silence before God, it seemed to me that God was saying to me, ‘The empty time that you have this evening you could spend in prayer.’” She added, “I began to pray for my husband.” Prayer took the place of irritation. A change of attitude!

This change of attitude was a major event for her, for she had had the feeling for a number of years that she could no longer pray, perhaps precisely because her heart had been filled with irritation. This event had had very considerable consequences. One could already see this in her face, and the beneficial results in regard to her illness followed extremely rapidly. A few weeks later, my colleague-specialist sent me a delightful letter to say that, clinically speaking, there had been a complete cure. He added with generosity, “And to think that I did not think there was any psychological factor in this case!” I then asked this woman why she had never spoken to her doctor about these factors in her life. She had great confidence and trust in him. She had been in his hands for over ten years, and known him almost as a friend. She replied, “But we never have the time! You know how consultations are! I arrive. He looks at the temperature chart. He listens to my heart; he thumps my chest. We
have an x-ray. He writes a prescription and, if things are not going satisfactorily, I just get sent up to the mountains again! One never has a moment to slip in a word on anything else!"

It is not only a question of time, but also a problem in regard to atmosphere. It is very difficult to shift from the doctor-patient situation to the human situation of contact between man and man. Doctors are, to some extent, victims of their own routine. When a doctor supposes that there is some psychological illness, he sends the patient to a psychiatrist who produces an admirable report. But there are many patients who do not stand in need of a psychiatrist’s help, but who need to be able to give some expression to their feelings so that they can discover a human atmosphere which will help them to resolve their personal problems. Then it becomes possible for the doctor to see the links between the patient’s illness and the history of his or her life. Many illnesses do not occur by chance, but are prepared by years and years of emotional difficulty. There are even patients who desire to fall ill, hoping it will provide them with a kind of way out, or solution, to a problem to which they can see no other issue. This the doctor cannot understand as long as he practices medicine in a purely technical manner, as long as he questions the patient instead of allowing the patient to open himself spontaneously.

There is a London doctor of whom you have perhaps heard, a Hungarian Freudian psychoanalyst, Dr. Balint. He has tried to initiate this form of contact between the general practitioner and the patient. He forms little groups of general practitioners who discuss the patients they have under their care. He, as a psychiatrist, endeavors to enable them to see the link between the patient’s illness and his life history. In other words, he attempts to make it possible for the doctor to see the significance of the illness in the context of the patient’s life. Balint himself explains that it is necessary that a change should come about in the doctor. The doctor is accustomed to adopting an objective attitude. He examines the patient as an object, and as long as he does examine a patient this way, there is no human relationship in the specific sense of the term. If this human interaction is to be achieved, a change is necessary within the doctor himself. Balint tells them how to begin. He says to begin by listening. Just as long as you ask questions, you will only get answers to those questions. You must give your patient the chance of opening himself up spontaneously. All patients have secrets in their lives and they all have great resistances, which make it difficult for them to bring them into the daylight. For a patient to feel able and free to do this, there needs to be a special atmosphere and climate that will give him a feeling of trust and confidence.

When I read Balint, I was enthusiastic. I said to myself, “But that’s what I have been doing for thirty years—making it possible for patients to open themselves up.” It seems such a simple thing that one hesitates to mention it. It is thirty years ago now that I began to be interested in the personal problems of people, because I myself had gone through this change of attitude of which Balint speaks. I was no longer interested exclusively in the illness in itself, but also in the person. It was then that my patients began to open up to me.

However, it seemed to me that this was no longer medicine! I would say to them, “Listen, we cannot allow this kind of thing to continue! We cannot go on in this way in a consultation. Come back this evening. Then we will talk in front of the fire, no longer as doctor to patient, but as two men who meet as two friends.” In this way, over a number of years, I led two lives; classical medicine by day, like all my colleagues, with prescriptions, scalpel, and so on, and in the evening, talks in front of the fire. I thought that these were not medicine; but then, lo and behold, the evening talks became more and more interesting, while my daily business appeared less and less interesting. I began to see that the evening talks were also an important factor in cure and healing. When a patient feels that he is understood, a waking-up of vitality occurs within him, which can play a great role in his cure. As I myself had had spiritual experiences, in a certain sense I went further than Balint. In front of the fire, I also spoke of myself as a person so that there might be an attitude and atmosphere of reciprocity. That is not the technical attitude of the analyst. He always remains an objective doctor, whereas I was entering into more human relationships, more fraternal relationships. I saw how it became possible for patients to solve the problems in their lives.
through this atmosphere. And frequently, when patients do solve their emotional problems, this can contribute to their healing. We cannot create Grace. Jesus Himself says, "The wind bloweth where it listeth." There are many patients to whom one would wish to be able to offer Grace on a tray; but neither doctors, nor pastors, nor Roman Catholic clergy can do this. However, we can create a favorable climate, and this climate comes into being when we ourselves become men again.

A woman once told me of her childhood history. She, too, suffered from tuberculosis. She was a very small girl, a kind of Cinderella. She was tormented by an aunt who suffered from tuberculosis. She was eating with fine appetite. For the first time for months now, she was eating with pleasure. He commented to her, "It's good to see you eating so well! That will be helpful for your treatment." She answered him, "I am eating because I want to be cured. I heard you yesterday, when you told your assistant that I was lost, but God told me last night that I would be cured!" The doctor took this very well. "If you do want to be cured," he said, "you must be obedient." Patients obey what the doctors tell them to do far less than doctors believe. Patients very rarely confess this to their own doctors, but as they are more honest with me than with their own doctors, they tell me. They often go to consult many doctors without obeying a single one of them.

To obey, it is necessary that there should be a certain inner attitude. There are countless patients who see a whole series of doctors, but who do not wish to be cured. We are touching here on a problem of man as a person. It is not a psychological problem in the strictly scientific sense of the term. It is a spiritual problem. The problem is the attitude that we adopt in life. An attitude that is constructive plays a great part in healing. This is the question with which Jesus confronts someone who is ill. "Do you wish to be healed?" Many patients confess to us with truth that they do not wish to be healed, or that they are afraid of cure because to be cured means they will have to confront life, and life is hard. An illness can sometimes represent a species of armistice in the war with life.

I once received a letter from Moscow, from a Western diplomat who was working in Moscow. He wrote, "Would you have a remedy for me? I cannot sleep. There is so much noise in Moscow. I have been to see a doctor. He gave me a pill; it was very effective for a month and then it had no more effect at all. Then I went to see a second doctor. He gave me another pill. Things went very well in the beginning, and then, again in a little while, the pill didn't have any effect anymore. I saw still other doctors. Have you not some other kind of remedy than pills?"

I answered him with a very brief letter. I wrote, "It is not the noise that is disturbing your sleep, it is the irritation you feel towards the noise." A year later, he wrote that he had been furious with my answer. It had seemed to him that I was mocking him. He was a poor, sick man who was seeking help, and I was answering simply with a joke, and a poor joke at that. Then, little by little, a train of thought began in his heart. He realized he was in
fact extremely irritated by the noise. In reality this was an irritation against the Russian government, because the Russian government did not allow him to go to live in the country as he would have liked to do, to escape from the noise. He also came to realize that there were many other things in life that irritated him, and that all these irritations did damage to his health. He finished his letter by saying, “Now I sleep through the night, in the middle of the noise, without any pills.” You see, there had been an evolution in his inner attitude. I would not advise you to practice in this way, by correspondence, because there is a certain lack of human warmth! There is, nonetheless, the lesson that can be drawn from a case of this kind, that our attitude to life can play a role in our health.

To help people change their attitude toward life does not demand much scientific knowledge of psychology. Naturally, for some patients, it is necessary that there be psychiatrists with technical knowledge. But to help reach this attitude toward life, everyone of us can contribute if we have a real interest in the patient; if we understand that each patient has problems in his life and that the vast majority of patients are deeply alone with their problems. This can often be true also of people who go to church or who are members of countless societies, Rotary Clubs, or whatever group it may be. They may have an extremely full social life, and still be radically and absolutely alone with their deep inner suffering. Men need someone to and with whom they can express themselves, and from whom they will find a certain sympathy, or empathy as is said in America, so that they may come to find the climate and atmosphere in which it will be possible for them to find spiritual solutions.

It is this that we define as “the medicine of men as persons.” One must be as capable and knowledgeable a doctor as one can be from the scientific viewpoint. Whether one is a surgeon or internist or some other specialist, one must be a good doctor who knows his medical work through and through; but one must also not forget that men also stand in need of something else. This other thing, this human contact, also plays a role in health and in healing. For this it is not necessary that we should have technical psychological training, but rather, as Balint says, that we should go through a certain change within ourselves. Because personal contact with another man frightens them, patients are afraid of it and flee from it. But we also, on our side, are afraid of it. We are afraid of not being able to provide the answers.

Think of my tuberculous woman patient. She confronted me with religious problems to which I could not find any solution. I am a Protestant, and it was not my business to mix myself up in the attitude of the Catholic church to her remarriage, and I gave her no answer to this. But through communion with me, she did receive help to find communion again with God, and that is not something specific to any one Church. That is a universal thing. All men are seeking this contact with the Sovereign of the world. Either consciously they are seeking it, or unconsciously. Each man is trying to find a way out of his solitude. All are afraid of opening themselves up, but find an amazing liberation when they do. We do not need to be very scientific and erudite psychiatrists in order to be able to give this to our patients. One can, perhaps, even be a psychiatrist who is exclusively a technician or a psychiatrist who is human, without going in for any psychological specialization. The patient feels what the attitude of his doctor is.

The patient senses very well whether he is simply a case in the eyes of his doctor, or whether the doctor sees that there is in him a human being who is suffering, who is thirsty for human communion. First, a patient needs to be able to give expression to his secrets. He desires to become himself through expressing himself, and wishes to express that which he has never dared to express before in his life. My patient, who had all sorts of resentments against her husband, found it particularly difficult to give expression to them, since her husband also happened to be charming. But, to find love, we need first to be able to give expression to our hatred. We must first bring out our aggression, if we are to be able to find afterwards authentic forgiveness. So we must see that there are two phases; and first, a phase that is human and psychological, where men must be able to express their feelings. It is frequently Christians who find it most difficult to give expression to their feelings because they wish to give the impression of being very nice people. They hide their hatred in
the depths of their hearts, and that produces an "ecclesiogenic neurosis." But after we have given expression to everything that is negative within us, we can then find a religion that is far more authentic, a forgiveness that is far more true, a love that is far more true, that love which every man is seeking. This is what we must help our patients find.

When one reads Dr. Tournier's contribution on the "Healing of Persons," one is first impressed by his simple, direct presentation of many matters which on the surface seem obvious. However, through this simplicity one senses more and more a profound appreciation of the human being in troubled circumstances.

Dr. Tournier offers an introduction to the concepts and practices of a movement in European psychiatry which has in recent years attracted attention in American psychiatric circles. This psychiatric development has been influenced by existential philosophic thought, especially that of Kierkegaard, Nietzsche, Dostoievski, Sartre, Heidegger and Jaspers. In Switzerland and other countries of Central Europe, under the leadership of such men as Binswanger and Strauss, this approach in psychiatry is referred to as "Daseinanalyse." The existential analysts have not discarded classical psychoanalytic concepts, but have redirected their attention to personality functions not stressed by the Freudians. They have focussed on such matters as the person in his immediate life situation here and now, the continuing evolving of personality at all stages of life, the subjective experience of individuality, the patient's responsibility for committing himself to his own decisions, that personality to a large extent results from one's own critical decisions and choices, and that mental health derives from wholehearted commitment to one's life and to one's responsibility for that life.

In this paper, Dr. Tournier gives an enlightening sample of such an approach to the matter of "The Healing of Persons."

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A Systematic Approach to the Evaluation and Treatment of Marital Problems

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With the renewed explorations and evaluations of the role of the family in emotional problems and the blossoming of new therapeutic techniques involving the family, there is a need to formulate our knowledge into some systematic order. I have found the schema presented here to be a useful guide in analysis of marital problems.

The collaboration of two persons of the opposite sex is a part of the natural sequence of human existence, stemming out of biological and emotional needs of human beings. The particular form that this collaborative relationship takes is dependent upon the prevailing culture in terms of time and place. Marriage, the legal confirmation of heterosexual collaboration, involves not only psychobiological functions, but also a complex of social roles. What follows is an attempt to describe these multiple factors in a systematic fashion.

Harmonious, Adjusted, and Disharmonious Marriages

The most basic ingredient for a healthy marriage is the emotional maturity of the partners. Nevertheless, individuals with emotional problems can and do make satisfactory marriages whereas, not infrequently, the marriage of two relatively healthy persons ends in discord and unhappiness. This may seem puzzling at first, but it becomes readily understandable if we recognize that the success of marriage depends not only on the personality of each person, but also on the interaction between the two personalities. An understanding of this interaction is the key to the understanding of marital harmony and conflict. Is the interaction positive or negative? Is it complementary, leading to harmony and stability, or is it uncomplementary, leading to discord and disequilibrium? Marriages can be classified into three general groups: harmonious, adjusted, and disharmonious.

Primarily, a harmonious marriage involves two relatively mature persons who have found realistic satisfactions and creative fulfillment separately in their own lives and by their own efforts, but who have found that individual satisfaction is enhanced and enriched by sharing it with another. This collaboration permits a double satisfaction—individual satisfaction and the participation in the other person’s satisfaction. Collaboration is further enhanced by a continually developing interdependence, the voluntary agreement between two relatively independent individuals to divide the multiple responsibilities of living. One person takes on the economic responsibilities, the other the management of the household. Social relationships, the care of children, educational responsibilities, civic participation, and so on are mutually shared. With the com-
plexity of our present times, there are many subdivisions in which roles are interchanged, and considerable flexibility is required. Conflicts do occur in a harmonious relationship, but these are realistically evaluated and resolved and are not permitted to develop into a struggle for dominance. With the resolution of differences, there is an expansion of collaboration due to the added understanding that has been gained. Sexual intercourse is a mutually satisfactory experience and expresses the intensity of affection for each other. This type of marriage may appear highly idealized, but many people do attain it.

The adjusted marriage involves two individuals who are handicapped in their collaborative efforts by neurotic forces. Collaboration is artificially maintained by limitation and restriction of those areas of interaction which would provoke anxiety. These people bring into the relationship not only the need for a loving relationship with another person, but also the unresolved remnants of earlier needs for tenderness and acceptance. The dependency needs of each limits their interdependence. Responsibilities cannot be fully shared or divided, as each is expecting the other to assume certain fantasy roles so that his (or her) infantile, childhood, or juvenile needs can be satisfied. Owing to their neurotic problems, they are unable to evaluate on a realistic basis the conflicts that arise. Instead, something is done to restore the appearance of harmony, or the basic problem is eliminated from awareness. Collaboration does not expand. Sexual intercourse may be a mutually satisfactory experience or may become one of the areas of limited interaction.

The case of a 29-year-old woman and her 42-year-old husband illustrates this type of marriage. The wife came for treatment at the urging of her husband, although she herself desired help because she was not enjoying life. The initial impression she gave was that of a very capable woman who suffered from low self-esteem, which manifested itself primarily in obsessional-compulsive symptoms. In the second interview she brought out that she felt her problem was resentment of her husband. She then poured out a series of complaints consisting of his belittling a great number of the things she did. He could not tolerate her mother (neither could she), but she was supposed to put up with his senile mother. She wanted to have children, but he did not desire any. Their marital equilibrium was maintained generally by her repressing her feelings and by submitting to and pacifying his childlike needs. Sexually, they had an excellent relationship, although she had recently lost interest. They were sincerely in love and collaborated in many areas where they had mutual interests. The equilibrium of their marriage was maintained by restricting themselves to certain areas of interaction. This equilibrium was now endangered by the presence of both mothers in the home, and it was this factor that brought the wife for treatment at this time.

The disharmonious marriage group is similar to the adjusted group, except that the neurotic concept of emotional needs has become the predominant force; collaborative aspects are pushed into the background. Conflicts are continuously arising and have grown into a power struggle in which the primary focus is who will triumph and be justified. Self-esteem, at the expense of the other person, becomes the goal of the relationship. Most frequently, sexual intercourse is either curtailed or is unsatisfactory. Primarily, it is experienced as a lustful gratification and not as the ultimate expression of mutual affection.

Such a marital relationship was revealed in a recent consultation. The husband, who announced that he would be the first to talk with the therapist, opened the interview by explaining that he had impregnated his wife 2 years before the marriage and that at that time an abortion was performed upon her.
insistence. Contemptuously, he then began to list her deficiencies, which ran the gamut from sheer laziness to belittling him in public. He dismissed her complaints about him by either denial or deflation. He loved their little boy and said that he was the only reason he did not leave. He bitterly complained of her withholding sexual gratification. He stated that the main issue was that he refused to be taken advantage of; he could put up with her if she would go to bed with him regularly and get up in the morning to make his breakfast and take care of the house. He complained that she always ran home to her mother whenever there was any conflict. The wife repeated the same story, but in reverse. Contemptuously, she described his sloppiness, uncontrolled temper, brutality, lack of consideration, selfishness, etc. She dismissed sex as being unimportant. She felt he purposely tormented her; if he would handle himself in an organized fashion and control his temper, she would be willing to continue the marriage. She complained that she always ran home to her mother whenever they had a conflict. All attempts to dissuade them that the therapist was not a judge or arbitrator and to encourage them to focus on what they themselves were doing to contribute to the discord were in vain. They gloweringly left the office, with an extremely poor opinion of psychiatric assistance.

Marital discord can be studied in terms of the individual neurotic personalities and the interplay between two such personalities. It can also be studied in terms of the social roles two individuals assume in their relationship. In reality both the social roles and the neurotic personalities are intertwined, exerting an influence on each other; however, we will artificially separate the two in order to study each aspect.

**Neurotic Marital Interrelations**

There are many ways of classifying the complementary neurotic interactions. They can be described in terms of tensions and gratifications, in psychopathological categories, or in terms of sexual behavior. In marital discord, the neurotic concept of emotional needs displaces collaborative efforts, and the relationship degenerates into a power struggle in which each partner is primarily concerned with the protection of his (or her) own security or self-esteem. Marital discord, therefore, can be grouped according to the type of predominant security operation by which the neurotic concept of the needs of each spouse are manifested. The security operations of one spouse tend to complement the other's security operations. The following is an elaboration of Dr. Bela Mittleman's (1956) classification of neurotic marital interrelations.

1. Predominant security operation in which each partner is aggressively attempting to dominate the other. The second case described above is an excellent example. Each is attempting to force the other to satisfy his dissociated dependency needs; each must have complete dominance or he cannot feel securely loved. This sets up a vicious cycle in which the need for dependent affection keeps them apart and at the same time leaves them unable to let go of each other.

2. Dominant, aggressive security operations evidenced in one, and passive, submissive security operations in the other. The henpecked husband of the comic strips would be an example of this category. The person who assumes the dominant role handles his anxiety as described in the first group. The passive partner handles his anxiety by being the good, suffering one who uses misery as a source of self-esteem. The passive dependency is used as an exploitive attitude.

3. Alternating periods of infantile dependency and exaggerated self-assertion by one member. The other partner assumes a responsible, dominantly supportive attitude alternating with disappointed childish
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desires for affection and support as a reward for his noble efforts.

4. Emotional detachment of one member while the other is self-abso-

eredly demanding affection and support. The detached person dis-

sociates his dependency needs and maintains an artificial self-suffi-

ciency. The other person is dramatically and constantly seeking to

bolster his low self-esteem by seeking acceptance, comfort, and grati-

fication from others. This sets up a vicious cycle; the greater the de-

mands for love, the greater the detachment, and the greater the de-

tachment, the more rejected the other person feels and the more in-

tensified are his affectional demands.

5. Both partners emotionally detached. Here the gulf between the

two widens and both find themselves more and more isolated. Eventu-

ally the marriage becomes meaningless. It may continue in this fash-

ion relatively stabilized, with two strangers living together, or with

one or both seeking extramarital relationships to fill the gap. The dis-

cove" of such an affair by the other may then precipitate intense anx-

iety.

6. One person helplessly depend-

ent and the other assuming an om-

nipotent, supportive role. Marriages

involving either emotionally sick or

physically handicapped persons are

examples of this group. The sick

person expects that the so-called

strength of the other will relieve

him of his suffering and restore his

self-esteem. His partner, because of

his own low self-esteem, hopes to

gain strength by helping the weaker

person and thus prove how capable

he is. Naturally, both are disap-

pointed, which results in either

overt or covert hostility.

7. Both partners’ predominant

security operation one of helpless

dependency, with each expecting or

hoping that the other will omnipo-

tently alleviate his deep sense of un-

worthiness.

One must keep in mind that

these categories are not self-con-

tained but that some overlap is al-

ways present. Within the same cate-

gory, intensity of sexual disturbance

and degree of immature behavior

versus emotionally mature behavior

may vary greatly.

Marital Discord as a Group Phenomenon

In an article on role conflict

within the family, Spiegel (1957) has
classified marital conflicts in terms

discrepancy in roles. He defines a

role as “a goal-directed pattern or se-

quence of acts tailored by the cul-
tural process for the transactions a

person may carry out in a social
group or situation . . . . no role exists

in isolation but is always patterned
to gear in with the complementary or
reciprocal role of a role partner.” All

roles are acquired in accordance

with the cultural values of the exist-
ing society. Roles may also be de-

fined in terms of secondary personifi-
cations and are part of the

self-system. The person automatic-

ally enacts various roles in social

circles which permit an economy of

psychological effort. Spiegel

points out that “. . . the principle of

complementarity is of the greatest

significance because it is chiefly re-
sponsible for that degree of harmony

and stability which occurs in inter-

personal relations.” The breakdown

of complementarity results in dise-

quilibrium of the interpersonal re-

lationship or in marital conflict.

Spiegel lists five causes of failure of

complementarity.

1. Cognitive discrepancy. One or

both persons do not know their re-

quired roles. For example, the

woman does not know what consti-
tutes the role of a wife, or the hus-

band has little comprehension of

the role of a father.

2. Discrepancy of goals. This in-

volves the person’s concepts of se-
curity and gratification in living.
The motivation behind the assump-
tion of a particular role may be

gratification or defense. For ex-

ample, one person seeks gratifica-
tion by expressions of affection,

viewing the lack of affection as re-

jection. The other person views the

seeking of affection as an attempt
to dominate and withholds affection, even though he feels quite affectionate toward his spouse. If he initiates the affectionate gesture, that is an altogether different situation.

3. Allocative discrepancy. This refers to the person's right to the role he wishes to occupy. Spiegel lists four principal ways by which roles are assigned: a) ascribed—age and sex. We are either male or female; any attempt to change roles becomes socially unacceptable. The child who tries to assume an adult role is either ridiculed or criticized. b) achieved—occupational and domestic roles. For example, one must graduate from a school of social work before one can be a professional social worker. Skill in cooking, cleaning, gardening or handyman repairs is required for the housewife or husband. c) adoption—neurotic interaction of projection-introjection. The paranoid person adopts the position of the persecuted victim, assigning to one or more persons the role of the persecutor. d) assumption. Spiegel points out that assumed roles are not serious. They are taken in games or play, as a child does in learning social roles. In adult life, “I was kidding” is frequently used as a means of escaping from impending disequilibrium situations. There are three sources of allocative discrepancy. “First, use of a culturally invalid or inappropriate allocative principle; second, withholding a cue indicating the allocative principle being used; and third, emission of a misleading cue which gives . . . the impression that one allocative principle is in use when in fact another one is actually present.” For example, the basic conflict may be the lack of sufficient affectional expression on the part of the husband, but the wife focuses her complaint instead on his being stodgy and old-fashioned. In addition to assigning the inappropriate stodgy role to the husband, she withholds her adoption of the unloved role and her allocation to him of the role of the unfeeling, cold lover. She may further mislead by adopting the role of the benefactor who is only trying to help her husband achieve a better or fuller life.

4. Instrumental discrepancy. This involves the acquisition of more or less personalized objects—furniture, automobiles, clothing, housing, money, etc. The lack of the object interferes with role transactions; for example, the wife cannot entertain because she does not have a large enough house or a new dress. This discrepancy may be actual or symbolic.

5. Discrepancy in cultural value orientations. This involves concepts of what is of value in life. For example, the husband feels that the wife's place is in the home and the wife feels the husband should help more with the children and the housework. Social position, religious affiliation, recreational activities, civic participation and many, many other areas of life have different cultural values for different individuals.

Discrepancies in the roles just listed are obviously intertwined and partly determined by the emotional structure of the individual. The degree of emotional maturity will determine to what extent the social roles that a person assumes are perceived on a consensually validated basis. Discrepancies in cultural value orientations or of allocative roles will vary from person to person. It is probable that, if two relatively healthy persons clash because of role conflicts, some regression to an earlier infantile level of emotional operation will occur, distorting the relationship and causing a temporary marital discord. Therefore, marriage between two mature persons may not necessarily be successful.

Resolution of Conflict of Roles within the Family

Spiegel uses the term “re-equilibration” to signify the re-establishment of equilibrium in the interpersonal relationships. He divides the various methods of resolution
of conflicts into two general groups. The first is termed role induction, which he defines as a resolution "effected by means of a unilateral decision... one or the other party agrees, submits, goes along with, becomes convinced, or is persuaded in some way." This group includes: 1) Coercing, which he regards as the most universal inductive technique, involving the hostile-aggressive patterns of behavior within the person, used to manipulate present and future punishments. 2) Coaxing, the manipulation of present and future rewards. This involves the individual's wish for gratification, stimulating a wish to gratify in the other person. 3) Evaluating, the manipulation of reward and punishment by placing the person's behavior in a value context. One person punishes the other by associating his behavior with a devaluated class such as fools, or by making a ridiculous comparison. 4) Masking, "the withholding of correct information or the substitution of incorrect information pertinent to the settlement of the conflict. It includes such behavior as pretending, evading, censoring, distorting, lying, hoaxing, deceiving, and so on." 5) Postponing, "the process by which the conflict to be settled is deferred in the hope of a change of attitude."

The aforementioned techniques of resolving conflicts are evident in all marriages. They will be minimal in degree and intensity in harmonious marriages and maximal in disharmonious marriages. A sixth approach is role reversal, in which one partner suggests that the other put himself in his position and try to see his side of the conflict, or one person initiates the reversal, hoping that the other will follow suit. This procedure can be used on either a manipulative or nonmanipulative basis. Spiegel considers role reversal a transition between role induction and the second general group which he terms role modification.

In role modification, "re-equilibration is accomplished through a change in roles of both... complementarity is re-established on a mutu-
situation logically and systematically. In the actual interview, both diagnostic evaluations go on at the same time.

Diagnostic evaluation of personality structure involves participant observation of the person in terms of what he tells you about himself and his relationships to others, now and in the past. A longitudinal or historical account of his past relationships and experiences is essential, and in marital situations we are particularly interested in how the person relates to his spouse, especially in terms of the security operations which he uses. Since the goal is reequilibration, the focus is on the self-system or ego structure, i.e., the degree of maturity and integrative strengths of the person. What level of integrative behavior does he manifest? Are his needs and attitudes mainly infantile, juvenile, preadolescent, etc.? What evidence of emotional maturity is present? We can theoretically divide the diagnostic personality evaluation into two parts: (1) the operative level of the self-system or ego, and (2) the predominant security operations in relationship to the spouse.

Diagnostic Evaluation of the Personality

The operative level of the personality involves what is commonly spoken of as "ego strength" and can be divided into the following six levels.

1. The level of conceptualization. The degree of infantile, childish, juvenile, preadolescent, or adolescent behavior versus the degree of consensually validated or mature behavior present. This tells us how emotionally sick the person is and what inner resources or capacities he might be able to mobilize and constructively utilize in reestablishing a harmonious marriage.

2. The anxiety threshold. How much tolerance does the person have for frustration, and can he postpone his needs for gratification? In what types of interpersonal relationships is his anxiety threshold higher or lower? For example, a person may have a higher threshold in his professional or business relationships than in social relationships. In relationship to other men he may have a high tolerance for frustration, but in relationship to women he may become extremely anxious when his needs are not immediately gratified.

3. Emotional lability. This involves the types of moods and the rapidity of the change in moods. How stoic, depressed, elated, hostile, or loving is he, and how quickly does he swing from one mood to another?

4. Defensive complexity. This involves the type, number, and intensity of security operations used to handle his selectively unattended and dissociated feelings and thoughts. The multiplicity of defense mechanisms is indicative of a complex motivational system and of a sicker person.

5. Emotional mobility. This involves how free the person is to use his inner resources. A person may have considerable inner capabilities but may be unable to mobilize them constructively, e.g., because of a low anxiety threshold.

6. Intellectual capacities. The collaboration of a person will depend to some degree on his ability to comprehend the various roles which a marriage requires. If his intellectual capacity is low, this may constitute an insurmountable problem in terms of the marriage. Again, we must keep in mind that these six steps are intertwined and that the separation is artificial.

Treatment

With the completion of the diagnostic evaluation, the therapist has a frame of reference by which he can decide whether a family type of therapy can help the marital partners, and whether one or both partners need more intensive psychiatric treatment. If it is determined that the family can benefit on this therapeutic level, then the diagnostic
evaluation is a base from which the therapist can select the appropriate measures to help the marital partners reestablish an equilibrium. The focal point of the treatment is the marital interaction, using the adaptive functions of the ego or the constructive forces of the self to attempt to bring about a modification of security operations or ego defenses. The use of the self means that we aid the individual in reevaluating and clarifying, primarily on a conscious level, his concepts of himself, his roles, and his relationships to others, especially to his marital partner. Again, the emphasis in therapy is focused on reequilibration, not on personality changes.

The treatment of marital problems is to help the partners to increase the areas of collaboration and minimize or restrict the areas of discord, so that they may have a satisfactory and gratifying interpersonal relationship. In all probability, if treatment is successful, some modification of the neurotic problem will also occur.

The treatment itself consists of the mutual collaboration between the therapist and the patient or patients. On the part of the therapist it involves the use of the major psychotherapeutic tools. Dr. Freda Fromm-Reichmann (1950) has listed these as: 1) listening intelligently to the client's communications of his complaints, of factual and emotional biographic data, and of his present and past interpersonal relationships; 2) asking pertinent questions which will promote production of relevant data; 3) offering meaningful interpretations by asking interpretative questions which will stimulate the client's own clarification of his behavior and by piecing together, with and for the client, the seemingly disconnected and disjointed pieces of information which relate to his difficulties; and 4) developing and amplifying repeatedly with the client the new understanding and awareness which he has gained. These therapeutic tools are used to help the patients focus on what it is they are doing that contributes to the marital disequilibrium, pointing out that it is a question not of blame but of awareness and understanding of their emotional attitudes in the marriage. It is especially important to avoid the arbitrator role and to help the patients realize that the therapist is there to help each partner with his own problems. Naturally, there are many variations in therapeutic technique; each case must be approached individually and the treatment tailored accordingly.

Relation between Patient and Therapist

It is important that the therapist realize the manner in which the patient relates to him, both in real and distorted aspects. The distorted aspects we refer to as "transference," which is the repetition of early patterns of interpersonal relations with the therapist, as if the therapist were the person involved in the early experiences. This usually involves the patient's parents or siblings. The therapist, by recognizing the particular role he plays to the patient, can gain insight into the patient's formative years and the manner in which his security operations developed. Also, it permits him to avoid falling into the transference role and reacting in the same manner as the significant persons did in the patient's earlier experiences. The therapist can utilize the transference situation for the patient's benefit. For example, the patient has dependent needs for a "good mother." The therapist, realizing this, can utilize his position to strengthen the person's independent strivings and self-assertive desires, in contrast to the patient's real mother, who encouraged his dependency on her, thereby making the patient feel weak and helpless. It is important that the transference be understood, although generally it is not advisable in the handling of marital problems to interpret the transference. Similarly, it is at least equally important for the therapist to be aware of and to understand
his own reaction to the patient, \textit{i.e.}, countertransference. It is not helpful to the patient that he be confused with early patterns of the therapist's own interpersonal relations. The countertransference can be utilized for the patient's benefit, as it might highlight some aspect of the patient's personality which provokes the countertransference reaction.

**Individual Therapy of Marital Partners versus Family Therapy**

Since the focus is the marital interaction, it is usually advisable that both partners be seen, although at times this may be contraindicated. There is also the technical problem of when the second person should be brought into treatment. It is my opinion that the sooner the spouse is brought into therapy the better. Naturally, the consent of the patient originally seen should be obtained. Depending on the situation, the therapist can request via the patient to see the spouse or contact the spouse himself. The spouse may consent to see the therapist only on the basis of helping the original patient. Although this is not the ideal basis for seeing the spouse, it is nevertheless better to see him on this basis than not at all. If the therapist handles the initial interview skillfully, the person may see the advantage of further counseling. If he does not agree to continue, at least some direct observations of his personality and his concept of the marriage can be made. There is the technical problem of whether the same therapist should handle both partners or whether each partner should have a separate therapist. In my opinion, the main criteria are to what degree the therapist will be placed in the position of the arbitrator or judge and how difficult it will be for the therapist to eliminate this aspect in handling both persons and getting either of them to focus on what is his or her own particular contribution to the discord. Another criterion is the probable intensity of the transference reactions so that each patient feels the need to have a therapist of his own, finding it too difficult to share the same counselor. When two therapists are involved, the degree of collaboration between them and how material obtained from one therapist is used by the other must be considered. It is not advisable, in general, for one therapist to confront the person with information gained from the other therapist. It is better to use this knowledge to help the person focus the examination of himself and of his relationship with others in a more expedient fashion. Occasionally it may be necessary for the therapy of each partner to be completely separate with no communication between the therapists.

In most situations, although it is at times quite difficult, one therapist handling both partners does seem to work out most successfully. This method gives the therapist a more nearly complete picture. At times, if there are considerable exaggerations and distortions by both parties, a joint interview may be necessary to clarify what is really going on; otherwise, it is best that each be seen separately. This last statement might be challenged, particularly with the increasing popularity of family therapy techniques. In spite of that, it is my feeling that, on the whole, it is best to see each client separately, using joint interviews as a special technique. Family therapy is still an experimental procedure which requires systematization. At present, the use of family therapy techniques in marital discord is applicable when 1) individual progress of the partners is blocked; 2) the psychodynamics of the neurotic interaction cannot be clearly discerned; 3) the discrepancy in concepts of roles of the partners cannot be clearly demarcated; or 4) therapeutic progress would be accelerated by a mutual examination and discussion of the neurotic interaction or role discrepancies.

**Summary**

The collaboration of two persons of the opposite sex is a part of the natural sequence of human development and involves the integration of psychodynamic factors and group dynamics. Marriage can be classified into three general types: harmonious, adjusted, and disharmonious. The disharmonious group is classified according to the predominant security operations of the spouse into seven categories. The group dynamics are presented in terms of role functions following Spiegel's (1957) classification of role discrepancies and role resolution, emphasizing the concepts of complementarity and equilibration. The diagnostic evaluation of the personality structure is outlined in terms of the operative level of the self-system and the predominant security operations in relationship to the spouse. The goal of therapy of marital problems is seen as the reestablishment of equilibrium in the marriage. The focus of treatment is on the marital interaction, with the adaptive functions of the ego being utilized to modify the security operations or ego defenses. Personality change is secondary to reequilibration.

**References**


Books


The cardinal manifestations of ocular syndromes and their associated systemic features are described in this manual. As a handy reference book for ophthalmologists, house officers and students it fulfills its purpose admirably.

The wording is so telegraphic in places that the reader may misinterpret the meaning, eg. “nuclear lesion involving the pyramidal tract” (p. 112) is contradictory and should read “lesion involving the nucleus and pyramidal tract.” The phrase “male linkage” (p. 113) should read “more common in males” to avoid the genetic implications of the word “linkage.”

The material is presented in five parts with an exceptionally complete system of cross-references. In the first part a one-page description of each syndrome includes synonyms, general information, ocular findings, other clinical features and bibliography. The ocular manifestations are tabulated according to the anatomical parts affected and the abnormalities of vision, motility and ocular tension. This descriptive formula permits tabulation of the positive and the negative findings and stresses a systematic approach to ocular examination; the empty spaces might have been used better to define more esoteric ophthalmological terms such as “iridodones” (p. 87) for the uninitiated reader.

A glossary of ophthalmological terms would have increased the usefulness of this manual. The author makes no claim to completeness and the book is obviously intended for those who have mastered the basic principles of medicine, genetics and ophthalmology. It deserves a place in the reference library of every internist, pediatrician and ophthalmologist; residents training in these areas will find this manual invaluable as a clinical pocket book.

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Professor Burton studied physics with Helmholtz, physiology with Bazett, and humor with W. Rogers. This text is a marvelously clear, concise introduction to circulatory physiology by an outstanding contributor to knowledge of the physical basis for cardiovascular function. Dr. Burton’s confident mastery of the subject facilitates understanding of the material. Though designed for the medical student, this book is a treasure-store of knowledge for the physician whose cardiovascular physiology needs refreshing, and a source of valuable material for the teacher interested in clear exposition. An additional bonus is humor; the book is hilarious. A minor drawback is the author’s inclusion of deliberately dogmatic statements within arrowheads in the text; considerable burrowing in the preface is required to decipher this secret code. Chapter 1, the introduction, is a delightful summary which should be required reading for all physicians. The book as a whole is good news for everybody.

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This is unquestionably the ideal introductory text on respiratory physiology for all “students of medicine.” What makes this book ideal is that it is written at once by a master teacher and a master investigator. Dr. Comroe can express difficult concepts of physiology in a language that makes excellent sense to medical students and physicians. But this he does not do at the risk of easy generalizations or oversimplifications. On the contrary, throughout the book, he maintains a clear distinction between experimental evidence, clinical observation, and hypothesis, and shows how each new deduction is reached from a given piece of evidence. This approach succeeds in giving the reader an active role in learning.

Over the past two decades, Dr. Comroe, his associates and immediate students, have contributed a considerable portion of present-day knowledge of respiratory physiology, particularly as it applies to disease. The book is therefore not a digest of the literature, but a firsthand account of an exciting and rapidly growing science by one of its foremost leaders.

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Clinicopathological Conference:
Abnormal Sex Characteristics and Intracranial Mass

Discussants:
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Clinical History
A 21 year old white, right handed male was admitted 1/29/65 with complaints of weakness, anorexia and vomiting of 16–18 months' duration. The first symptoms were nocturia (two to three times per night), pain in the right lower quadrant, postprandial vomiting, and weight loss of 16 lbs. A gastrointestinal series in 1963 was negative. In July, 1963, he was admitted to another hospital. The examination revealed his weight to be 108 lbs. There was absence of beard and chest hair and sparse axillary hair. Pubic hair was of female distribution. His voice was high pitched, his arms long, shoulders narrow, hips broad, muscle mass poorly developed, and the external genitalia were small.

In 1954 the patient underwent operative correction of bilateral cryptorchidism. Two years later, bilateral gynecomastia was treated surgically. In 1961, a buccal smear was done and revealed a male sex chromatin pattern. The patient was started on intramuscular testosterone and over the next four months gained approximately 16 lbs.

In October, 1964, he noted the onset of polydipsia and polyuria. Urine specific gravity was 1.007; sugar and protein determination on the urine was negative. He continued to gain weight and remained on testosterone therapy by injections and sublingual tablets, and in April, 1964, his weight was 140 lbs. He had abundant axillary hair, and psychologically he was markedly improved. Despite continuation of the testosterone, by July, 1964, he had lost 10 lbs., had recurrent vomiting and was feeling tired. A gastrointestinal series revealed a duodenal polyp. The vomiting responded to six bland feedings per day. Through the fall of 1964 episodes of vomiting recurred and the patient developed increasing weakness, intolerance to cold and weight loss. He weighed 110 lbs in December, 1964.

On admission to the Medical College of Virginia Hospital his weight was 103 lbs, and his vital signs were a blood pressure of 90/60, pulse 52 per minute, respiration 18 breaths per minute and temperature 98 F. His general appearance was that of a thin, pale, listless, young male who appeared older than his stated age, chronically ill and in no acute distress. There was a surgical scar on each breast. The skin appeared to be warm and dry. There was a female distribution of pubic hair and the testicles were soft and small. Neurological examination revealed a staggering gait which appeared more
related to weakness than to ataxia. He was well oriented, and aside from the listlessness appeared to be alert. Cranial nerve examinations were not remarkable except for a loss of upward gaze. Visual fields were normal, the fundi negative, and the pupils equal and reactive to light and accommodation. There was no sensory deficit. Motor function was intact, but muscles were hypotonic and weak throughout. Deep tendon reflexes could be elicited bilaterally but were markedly depressed. Cerebellar functions appeared to be within the performance of a chronically ill individual.

Laboratory data: Hemoglobin 13.0 gm per 100 ml, white cell count 9,600 per mm$^3$ (polymorphonuclears 70%, lymphocytes 29% and monocytes 1%). The urine was negative for protein and sugar, and had a specific gravity of 1.004. The BUN was 15 mg, calcium 9.8 mg, and phosphorus 4.25 mg per 100 ml. The alkaline phosphatase was 3.0 Bessey-Lowry units (normal 2.9 units). Serum sodium was 143 mEq, chlorides 105 mEq, potassium 4.4 mEq and CO$_2$ 24.3 mEq per liter. Seventeen-hydroxy steroids excretion was less than 1 mg per 24 hours (normal 3–10 mg per 24 hours) and 17 ketosteroids were also less than 1 mg per 24 hours (normal 8–20 mg per 24 hours). Urinary FSH—6 units per 24 hours (normal 10–50 mouse uterine units per 24 hours). There was no alteration in the urinary 17-ketosteroids or 17-hydroxy steroids following Metopirone, but following a standard intravenous ACTH test there was a two-fold increase in both 17-ketosteroids and 17-hydroxy steroids within 24 hours, and a four-fold increase within 72 hours. Protein bound iodine could not be done because of a recent cholecystogram, but a T-3 red cell uptake revealed a 31% uptake. EEG was read as showing an abnormal record with diffuse slowing as well as a specific slow wave abnormality in the right frontal area. This record was interpreted as suggestive of a destructive lesion in the deep right frontal Sylvian region. A brain scan with radioactive mercury revealed an area of increased uptake in the right frontal area. Lumbar puncture revealed normal pressure, protein of 56 mg per 100 ml, with 9 red cells and 46 lymphocytes per mm$^3$.

A chest film showed a small heart with slight demineralization of the bony thorax. Skull series revealed no abnormality. The pineal body was in the midline. The sella appeared smaller than normal. A left carotid arteriogram and a right retrobrachial arteriogram showed no displacement of the vessels and no tumor stain.

After admission the patient continued to vomit, had an urinary output which was slightly higher than his intake, and complained of extreme lethargy and weakness. He was treated with intravenous fluids, and following the studies reported above, was started on replacement therapy with cortisone acetate, 12.5 mg every six hours, Cytomel, 25 micrograms each day, and desiccated thyroid two grains daily. Within 24 hours of starting the replacement therapy, the urinary output was 6,000 cc and from then on the patient required Pitressin for control of his urinary output. His general condition improved rapidly, his appetite was restored, and he was able to sit up without his previous syncopal attacks. He complained more and more of headache and on 2/25/65 a pneumoencephalogram was performed.

Clinical Discussion

Dr. Clark T. Randt: This case presents a formidable endocrinological problem. In preparation for this discussion I have learned a number of things and I hope that I shall be able to transmit some of them to you.
This history is of a 21-year-old man whose difficulties began approximately a year and a half prior to his admission to this hospital in January, 1965. His illness started in a nonspecific way with weakness, anorexia, weight loss and vomiting. It is worthy of note that some 21 months prior to this last admission he developed nocturia times two or three and some pain in the right lower quadrant of his abdomen, as well as postprandial vomiting associated with weight loss. On the first hospital admission 18 months prior to January, 1965, it was noted that the patient had absence of the secondary sexual characteristics. He had an eunuchoid configuration and hypoplastic genitalia. He had inability to concentrate his urine (at least on the specimen reported) and there was no sugar in his urine. His symptoms of nocturia some six months prior to this might have signalled the onset of diabetes insipidus. Testosterone therapy benefited him with a significant increase in weight and an increase in the amount of axillary hair. He was also said to be psychologically improved. However, he again began to lose weight and had recurrent vomiting with easy fatiguability. He developed increasing weakness and intolerance to cold. The latter symptom suggests, that in addition to hypogonadism, he had also developed hypothyroidism. The weakness and weight loss might be early signs of adrenal insufficiency. The operation for cryptorchidism at age 10 suggests an association with the subsequent hypogonadism, but, at that age, one could not be certain about gonadal deficiency. At the age of 12 he developed bilateral gynecomastia which was treated surgically. In the absence of liver disease or starvation, one would assume that the interstitial cells of his testes were secreting more estrogens than androgens. The appearance of gynecomastia at puberty is certainly not an unusual event and is thought to be due to a disturbance in the ratio of estrogen to androgen secretion. Again we would not, at that point, be led to a diagnosis of hypogonadism. However, five years later at the age of 17, the patient had definite delayed puberty. A testicular biopsy was done to investigate the possibility of primary hypogonadism. I would assume that Klinefelter's syndrome was suspected and, in light of this, a buccal smear was done. It is reported that the patient had a male sex chromatin pattern. The possibility still remains that he might have a chromatin negative Klinefelter's syndrome. We are not provided with an estimate of his gonadotrophins nor the results of the testicular biopsy which would be necessary to enable us to make such a diagnosis.

At the time of his most recent admission, the patient was found to have a pulse of 52 per minute which, in the absence of evidence of increased intracranial pressure and in the presence of sensitivity to cold, suggests hypothyroidism. He appeared chronically ill and his skin was dry, which also suggests this diagnosis.

The neurological examination was positive only in that the patient had a paralysis of upward gaze. This is known as Parinaud's sign and has a high degree of localizing value. In ablation experiments in animals and in the clinical-pathologic correlation in man, this particular sign has a high correlation with lesions in the region of the superior colliculi in the rostral portion of quadrigeminal plate. The laboratory data again suggested diabetes insipidus since there was no urinary concentration above a specific gravity of 1.010.

He had normal serum sodium and chloride. I mentioned the possibility of hypoadrenalism. In secondary hypoadrenalism findings of normal sodium and chloride might well be due to the fact that aldosterone secretion is routinely affected, but this is not true in secondary hypoadrenalism. The 17-hydroxy- and 17-ketosteroids were markedly reduced, indicative of adrenal insufficiency. Gonadal insufficiency
ABNORMAL SEX CHARACTERISTICS AND INTRACRANIAL MASS

will also cause a reduction in the 17-ketosteroid determination. A Metopirone test was done. Metopirone inhibits the enzyme 11\beta-hydroxylase necessary for the formation of hydrocortisone. The decreased elaboration of adrenal hormone in turn allows an increased secretion of adrenocorticotrophic hormone from the pituitary gland. If this is the case, the urinary ketosteroids should be increased at least two-fold following the administration of Metopirone. It is noteworthy here that there was no alteration in the secretion of the urinary 17-ketosteroids and 17-hydroxy steroids following the administration of Metopirone. This was corroborated by an ACTH test which produced two-fold increase in the 17-keto- and 17-hydroxy steroid secretion. This latter result is indicative of the functional integrity of the adrenal cortex. These tests make it clear that the previously postulated hypoadrenalism was secondary due to pituitary failure, rather than a primary failure of the adrenal gland. The T-3 uptake was 31%, within normal limits for this laboratory.

He had an abnormal EEG with diffuse slowing in the record and a focal slow wave abnormality in the right frontal area. It would be of interest to me to know how much slowing. Slowing below four cycles per second is usually associated with more or less superficial disease, whereas focal slowing to four to seven cycles is more likely to mean a deep seated lesion in the brain. The brain scan corroborated the focal finding in the EEG in that it showed an increased uptake deep in the right frontal area. The chest x-rays showed a small heart which would be compatible with hypoadrenalism. Skull x-rays showed the sella turcica not to be enlarged which militates against a pituitary tumor. The pineal was observed to be in the midline. Bilateral carotid angiography failed to reveal evidence of a space-occupying lesion.

The patient’s course in the hospital was characterized by markedly beneficial response to replacement therapy with cortisone, tri-iodothyronine and desiccated thyroid. His general condition improved rapidly and he was able to sit up and cooperate better than previously. It is noteworthy that his syncopal attacks were seemingly relieved. Syncopal attacks may occur in a wide variety of neurological diseases, but in this particular case having a strong suggestion of hypoadrenalism, I think it would be reasonable to assume that the syncope was related to small blood volume and cerebral anemia due to deficient cerebral circulation and oxygenation. It is noted that he complained increasingly of headache and a pneumoencephalogram was performed. I would like very much to see the films of this patient before proceeding to expose you to my differential diagnoses of his multiple problems.

A brief videotape was shown. As can be seen in figure 1, the patient was a slender individual with female hair distribution and with a healing surgical scar on his head.

Dr. Gerald A. Gildersleeve: Skull films show midline calcification representing the pineal gland, which measures 1 cm in width. This is within the upper limits of normal in size, within normal limits of position in the lateral views. The frequency of pineal calcification varies with age. Twenty percent of people at age 20 have pineal calcification of varying extent. Calcification of the gland is rarely seen in infants and small children. If one sees pineal calcification under 12 years of age, or calcification more extensive than 1 cm in diameter, one should strongly consider pinealoma. Cystic changes within the pineal structure can make the otherwise normal gland appear enlarged.

Views from the pneumoencephalogram study demonstrate the lateral ventricles to be larger than normal (fig. 2). Many attempts actually to visualize the region of the aqueduct were unsuccessful. Air did pass in quantity into the lateral

Fig. 1—Picture of patient four months after surgery showing asthenic habitus, female escutcheon, poor regrowth of scalp hair.
Fig. 2—Pneumoencephalogram showing enlarged lateral ventricles. The aqueduct does not suggest extrinsic pressure. A normal suprapineal recess appears as an air shadow above the pineal calcification.

Fig. 3—Pneumoencephalograms representing "brow-up" and "brow-down" positions respectively. Air is noted in the substance of the right frontal lobe, beneath the anterior horn. A mass is suggested in the anterior part of the suprachiasmatic cistern. The inferior aspect of the right anterior horn is encroached upon.
lack a reaction to light but react to accommodation. These individuals may have some truncal ataxia with a tendency to fall, in the anterior-posterior plane. Occasionally one finds a bilateral decrease in hearing presumably because of extension of the lesion from the superior colliculi to the inferior colliculi, thus interrupting the auditory pathways. Cerebellar signs, as produced by involvement of the cerebellar outflow over the brachium conjunctivum, and, sometimes pyramidal tract signs including spasticity, also accompany lesions in this area. The evidence for a lesion in the supra pineal recess adjacent to the superior colliculi is convincing. There is considerable evidence to indicate hypothalamic involvement with the diabetes insipidus due to interruptions of the supraoptico-neurohypophyseal tract in the hypothalamus proper. Stalk section or ablation of the pituitary does not cause persistent diabetes insipidus. This evidence places the lesion in the hypothalamus.

Hypogonadism might be related to a hypothalamic lesion. In animal ablations one finds that destructive lesions in the posterior portion of the median eminence and the basal tuberal region are likely to produce manifestations of hypogonadism. In man, hypoadrenalinism from hypothalamic lesion is poorly localized although it has been reported that the stimulus for the elaboration of ACTH from the pituitary was selectively depressed in a case of sarcoidosis involving the hypothalamus. Localization of the area in the hypothalamus responsible for stimulating the anterior hypophysis to elaborate ACTH remains not well localized. This lack of hypothalamic localization is also true of the control of thyroid-stimulating hormone elaborated by the anterior pituitary. Bilateral lesions of the ventromedial hypothalamus give rise to adiposity, whereas lesions in lateral portions of the midhypothalamus may produce cachexia. The cachexia here may well be related to such hypothalamic involvement or may be secondary to the hypoadrenalinism, which the patient showed.

One of the most interesting features of this case to me is the question of whether the patient had secondary hypogonadism or primary hypogonadism. I mentioned the possibility of a chromatin-negative Klinefelter's syndrome earlier, and the finding here of a normal FSH (follicle-stimulating hormone) level of 6 units suggests to me that the hypogonadism was primary. As regards the EEG, I believe that the indications are of a superior collicular midbrain lesion extending into the hypothalamus. The EEG and the increased radioactive mercury uptake in the right frontal area probably indicate extension into the deep portion of the right frontal lobe. There were no clinical manifestations that one could identify to corroborate this, but that is not unusual since the non-dominant frontal lobe is a silent area.

As far as the etiology is concerned, I would think that a chromophile adenoma would be extremely unlikely. The presence of midbrain signs with Parinaud's sign and the normal appearance of the sella turcica by x-ray, probably exclude this diagnosis. A craniopharyngioma is unlikely for the same reasons, plus the fact that about 85% of craniopharyngiomas would be expected to show some suprasellar calcification. These are the two most common tumors to produce endocrine disturbances with secondary effects in the gonads, the thyroid and the adrenals. The dogma is that the gonads are the first involved and the thyroid and the adrenal follow. In this instance I believe there is an exception to that rule in that it is likely that primary hypogonadism will be demonstrated. The localization is better in the pineal recess and adjacent regions. Lesions residing in this location are the pineoloma, pineoblastoma, teratomas and gliomas of the astrocytic variety. Of these four, I would think that the extension into the hypothalamus and into the deep portion of the right frontal lobe would be more in favor of pineoblastoma. The findings of 46 lymphocytes per mm$^2$ in the cerebrospinal fluid would suggest this variety of pineal neoplasm. The pineoblastoma may give rise to implants at distant sites, so one may, indeed, have cells which look very much like lymphocytes introduced into the cerebrospinal fluid and misidentified. In any case, lymphocytes may be attributed to some irritative process close to the ventricular surface or close to the subarachnoid space. Here our attention centers on the intraventricular area. This would account for the pleocytosis in the cerebrospinal fluid. The pineoblastoma is one of the few tumors found exclusively in the brain which have a tendency to disseminate in the subarachnoid space or throughout the ventricular system.

Clinical Diagnosis
1. Klinefelter's syndrome
2. Pineal tumor
3. Pituitary tumor

Dr. Randt's Diagnosis
1. Pineoblastoma
2. Chromatin negative Klinefelter's syndrome

Pathological Discussion
Dr. Julio H. Garcia: We have been able to obtain and review tissue from earlier surgery and biopsies. In the breast there was hyperplasia of the ductal epithelium with periductal edema and lymphocyte infiltrates, all considered to result from estrogen stimulation. The testicular biopsy revealed hyalinization of seminiferous tubules, absence of germinal cells, and a relative hyperplasia of interstitial cells. The changes were consistent with those expected with the Klinefelter syndrome. The original buccal smear for sex chromatin study was reported on your protocol as negative, that is "male," or "XY." This is how it was reported from another hospital where it was done. On reviewing their slide and our new
CLINICOPATHOLOGICAL CONFERENCE

Fig. 4—Typical microscopic field of the pineal tumor showing the two cell types (400X).

ones, it was determined the patient was actually chromatin positive or "female," that is, genotype XX or XXY probably, although chromosome studies have not yet been done. You will recall that Barr and co-workers (1949, 1950 and 1951) noted that the nuclei in cells from females usually contained a mass that distinguished them from the cells of males. This was a startling and valuable discovery.

Dr. Randt has related well the clinical signs and symptoms with his concept of the brain lesions. He referred to the comparable or analogous experimentally-induced lesions in laboratory animals. I shall attempt to correlate briefly clinical abnormalities observed in our patient with similar abnormalities in animals reproducible by stimulation and/or destruction of hypothalamic nuclei.

1. Diabetes insipidus has been experimentally obtained by destruction of paraventricular and supra-

optic nuclei (Bailey and Bremer, 1921).

2. Dysgonadism, by which I mean the coexistence of male somatic features with high estrogen levels producing gynecomastia, testicular atrophy, etc., could perhaps be equated with the continuous estrus noted in rodents with destruction of the median eminence (Hillarp, 1949).

3. Appetite disturbance, that is anorexia and other eating disturbances, are symptoms that can be reproduced through excitation of lateral hypothalamic nuclei in monkeys and other animals (Delgado and Anand, 1953).

4. Paralysis of upward gaze is well known to occur with compressive lesions of the superior colliculi (Kulenebeck, 1949).

5. Headaches, projectile vomiting and syncopal attacks are more difficult to account for on the basis of a single morphological abnormality, but, in my opinion, may result from increased intracranial pressure, perhaps secondary to intermittent occlusion of the Sylvian aqueduct.

We can, therefore, arrive at the conclusion, as Dr. Randt did, that we are dealing with a mass that, from the clinical and radiological points of view, extends from the quadrageminal plate, affects the hypothalamic nuclei and reaches the right frontal lobes (ventricular cavity), since this is the area from which the biopsy was obtained.

Microscopic examination of the tissue revealed neoplastic structure basically composed of two cell types (fig. 4): one a small cell that closely resembles lymphocytes, and larger cuboidal cell elements that display hyperchromatic nuclei, frequent atypical mitotic figures, and a scanty clear cytoplasm with well-defined cell membranes. Vascular and connective tissue stroma are very abundant and no argyrophilic fibers can be demonstrated. The overall appearance of groups of these cells in this tumor has been referred to as a mosaic pattern.

We have designated this neoplasm, pineal teratoma (atypical),
TABLE 1

Presenting Signs and Symptoms with Pinealomas (58 Patients)*

1. Disturbance of vision, papilledema 90%
2. Headaches, nausea and vomiting 70%
3. Paralysis of upward gaze 50%
4. Diabetes insipidus 26%
5. Obesity and/or dysgonadism 18%
6. Precocious puberty 5%


according to the classification of tumors of the pineal body area offered by Russell and Rubenstein (1963).

From a report by Russell and Sachs (1943), in which 58 cases of patients with a similar histological diagnosis are analyzed, we have extracted data expressing the frequency of signs and symptoms percent of the total number (table 1).

Following the craniotomy, our patient has experienced some improvement, particularly of his visual acuity. Much of the evidence of intracranial pressure has disappeared but most of the other symptoms and signs remain to some degree. At home with his parents, he is ambulatory and able to take care of himself.

Pathological Diagnosis

1. Pineal teratoma (atypical)
2. Klinefelter's syndrome

References

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George Kriegman (A Systematic Approach to the Evaluation and Treatment of Marital Problems) earned a B.A. in psychology, M.S. in juvenile behavior, and M.D. from the University of Illinois. Later he interned and took a residency in psychiatry at St. Elisabeth's Hospital, in Washington, D.C. Dr. Kriegman came to the Medical College of Virginia in 1950, as assistant professor of mental hygiene at the school of Nursing. In 1959, he joined the department of psychiatry where he is now clinical professor. He has acted as consulting psychiatrist for various welfare and service organizations.

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