$$\sigma = \begin{pmatrix} \sigma_{11} & \sigma_{12} \ldots\ldots\ldots\ldots & \sigma_{1n} & 1 \\ \sigma_{21} & \sigma_{22} \ldots\ldots\ldots\ldots & \sigma_{2n} & 1 \\ \ldots & \ldots & \ldots & \ldots \\ \sigma_{n+1,1} & \sigma_{n+1,2} \ldots\ldots\ldots & \sigma_{n+1,n} & 1 \end{pmatrix},$$

$$P = \begin{pmatrix} P_{11} & P_{12} \ldots & P_{1n} & 0 \\ P_{21} & P_{22} \ldots & P_{2n} & 0 \\ \ldots & \ldots & \ldots & \ldots \\ 0 & 0 \ldots & 0 & 1 \end{pmatrix}.$$

Before writing the coefficient γ as a matrix component, we must extend the summation to $n + 1$. The only nonvanishing term which we must add to the summation corresponds to $j = k = n + 1$. Inspection shows that this term is identically unity. Thus, equation (11) becomes

$$\gamma = (\sigma P^{-1}\sigma^*)_{ss} - 1.$$

Now that γ is formulated in standard matrix terminology, the rest of our analysis is trivial. Upon inverting

$$P = \sigma^*\alpha\sigma,$$

we find that $\sigma P^{-1}\sigma^*$ contracts to α^{-1}, and hence $\gamma = \alpha_s^{-1} - 1$.

The identity of the perturbation expansion and the exact expansion is thereby established.

The writer is indebted to R. Duffin for suggesting the matrix approach to this problem.

[1] Zener, C., these PROCEEDINGS, **47**, 537 (1961).

[2] Duffin, R. J., communicated to the *Journal of the Society for Industrial and Applied Mathematics*.

TYPES AND RATES OF X-RAY-INDUCED CHROMOSOME ABERRATIONS IN HUMAN BLOOD IRRADIATED IN VITRO

BY M. A BENDER AND P. C. GOOCH

BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY,* OAK RIDGE, TENNESSEE

Communicated by Bentley Glass, February 7, 1962

Measurement of the rate of chromosome aberration production by ionizing radiation in humans has at least two purposes. It can give us information on human radiation hazards, and it can also provide the necessary "calibration" for using chromosomal aberration production as a biological dosimeter. Such measurements may, of course, be made either on meiotic or mitotic chromosomes. Unfortunately, although the important chromosomes from the point of view of long-term human radiation hazards are those of the germ cells (the meiotic chromosomes), no measure of their radiation sensitivity has yet been made. Neither have any quantitative measurements been made of induced chromosome aberration rates in irradiated

humans. Our lack of information is due in part to our having just recently learned to make satisfactory preparations of human chromsomes; more importantly, it is the result of the difficulty of obtaining tissues from suitable irradiated human subjects.

To provide quantitative dose-effect information on chromosomal aberrations, material must be obtained from normal subjects who have received a known dose of radiation given uniformly to the entire tissue to be sampled. Because some types of aberrations are lost after one cell division, aberration rates must be measured in the first postirradiation division. Needless to say, these restrictions make it very difficult to measure aberration rates in humans *in vivo* and no quantitative information is available. It has, however, been shown that aberrations are, as is to be expected, induced in the somatic chromosomes of irradiated humans.[1-6]

A number of studies have been made of the rates of metaphase chromosomal aberration production by irradiation of diploid human cells cultivated *in vitro*.[7-10] The spontaneous rates observed were quite variable, depending on whether epithelioid cells or "fibroblasts" were used (see *Discussion*). In any case, they are probably much higher than those occurring in human cells *in vivo*.[8] The available data on X-ray-induced chromatid aberrations in human cells are not in good agreement. For epithelioid-type cells the rate is 0.0032 breaks/cell/r,[7, 8] but for "fibroblasts," Puck[9] found a rate of about 0.02 breaks/cell/r, and Chu *et al.*[10] found a rate of 0.019 breaks/cell/r in a mixture containing about 90 per cent of fibroblast-type cells. The only data on chromsome aberrations are those of Chu *et al.*[10] who found 0.009 breaks/cell/r.

Measurement in terms of breaks/cell/r implies, of course, that observed breakage is a linear function of dose. Total breakage actually rises as something greater than the first power of dose. The linear measure is therefore useful only at low doses, where the deviation of the dose curve from a straight line is small. A more accurate measure of damage is the "coefficient of aberration production."[11] Coefficients must, of course, be calculated separately for types of aberrations having different kinetics. When these calculations were made for chromatid aberrations in the data collected earlier for human cells *in vitro* the coefficients found were $0.066 \pm 0.10 \times 10^{-2}$ single chromatid breaks/cell/r, $0.246 \pm 0.16 \times 10^{-2}$ isochromatid breaks/cell/r, and $0.55 \pm 0.35 \times 10^{-5}$ chromatid exchanges/cell/r^2 (ref. 12). These values are similar to those previously found for aberrations in plant cells,[11] and to those found for cells cultured from monkeys and from Chinese hamsters.[8] More recently, Chu *et al.*[10] have made a similar calculation from their data. They found coefficients of $0.601 \pm 0.067 \times 10^{-2}$ single chromatid breaks/cell/r, $0.316 \pm 0.186 \times 10^{-2}$ isochromatid breaks/cell/r, and $2.157 \pm 0.496 \times 10^{-5}$ chromatid exchanges/cell/r.[2] The coefficients for chromatid deletions and chromatid exchanges are significantly higher than those calculated by Bender and Wolff.[12] Such calculations have not yet been made for chromosome aberrations in human cells.

While it was impossible to measure chromosomal aberration rates directly on human cells *in vivo*, parallel measurements have been made for a species of monkey (*Ateles*) and for the Chinese hamster.[8, 13] It was found that the spontaneous aberration rates were extremely low, while the X-ray-induced chromatid aberration rates agreed with those found for cultured epithelioid cells from both experimental ani-

mals and humans.[8] While these observations allowed us to extrapolate for human cells irradiated *in vivo*, a more direct estimate is obviously desirable. In addition, no information is available about chromosome-type aberrations induced in human cells *in vivo*.

The development of a practical method for obtaining adequate numbers of scorable metaphase figures from short-term cultures of human peripheral leucocytes[14] has made it possible to obtain the necessary measurements. Using this technique it has been possible to determine the aberration rates in irradiated freshly drawn human blood. There is no reason to suspect that the rates would be very different if the blood were drawn just after irradiation. Further, it has been found that the induced aberrations are, invariably of the chromosome type. Further investigation, using labeled thymidine and autoradiography, has shown that virtually all of the cells that divide in these short-term leucocyte cultures are in the pre-DNA-synthesis G_1 period when the blood is drawn, and that they remain there until the blood is cultured.[15] These circumstances make the use of short-term blood cultures an ideal technique for biological dosimetry in irradiated humans, since the cells scored are all in a period of uniform sensitivity, and since they stay there for a considerable time after irradiation.

Materials and Methods.—Blood samples were obtained from two male and two female volunteers. All had previously been found to have normal chromosomes. The culture method has been described.[15] Five ml aliquots of blood were placed in sterile centrifuge tubes containing 0.1 ml of heparin solution (1,000 units/ml) each. The tubes were centrifuged in a clinical centrifuge for 10 min. The "buffy coat" and serum were placed in culture bottles containing culture medium, consisting of 10 ml of Mixture 199, 4 ml of freshly inactivated type AB-positive human serum, and 0.1 ml of Phytohemagglutinin (Difco), with 700 units and 0.7 mg respectively of penicillin G and streptomycin. The cultures were incubated at 37°C in an atmosphere of 5% CO_2 and 95% air.

The first mitoses occur between 42 and 48 hr under these conditions. Between 66 and 72 hr the mitotic activity reaches a maximum, and all the divisions are still first mitoses. Because some mitotic delay is expected in heavily irradiated cells, a 72-hr fixation time was used for most cultures. Colchicine was added to the cultures 5 hr before fixation to a level of 10^{-7} *M*. The cells were fixed and mounted by the method of Moorhead *et al.*[14] They were stained with aceto-orcein and mounted in Euparal.

The heparinized 5-ml blood samples were irradiated (immediately after drawing) in 50-mm Pyrex Petri dishes. The dishes were placed on a Lucite turntable 60 cm beneath the target of a Philips constant potential X-ray machine, which was operated at 250 kv and 15 ma. One mm of Cu and 1 mm of Al added filtration were used, yielding an HVL of 2 mm of Cu. The dose rate was measured with a Victoreen dosimeter using a Bakelite ionization chamber which was placed in the position to be occupied by the cells and covered with a Petri dish cover. The dose rate was also monitored during delivery with a Radicon integrating dosimeter. No attempt was made to convert the dose measured in air to absorbed dose. The cells were placed in culture immediately after irradiation.

Samples from three individuals were given doses of 50, 100, or 200 r. Five samples from the fourth were all given 200 r. One of these was fixed every 6 hr from 48 to 72 hr, in order to establish whether any chromatid aberrations were induced in cells dividing earlier than the standard 72-hr fixation time, and also to determine the amount of mitotic delay induced by a large dose of X rays.

In scoring, the chromosome count, the presence of obvious aberrations, and abnormal form of the readily identified chromosomes (numbers 1, 2, 3, 13, 14, 15, 21, 22, and the Y, by the generally accepted system of nomenclature),[16] were recorded. In scoring chromcsome number, acentric fragments were not counted.

Results.—*Mitotic delay:* Previously it had been seen that the first *in vitro* mitoses in unirradiated human leucocyte cultures occurred during the period from 42–48 hr after culture initiation.[15] In the series of cultures of blood given 200 r of

X rays, there were only a few mitoses in the culture treated with colchicine at 42 hr and fixed at 48 hr. In the culture colchicine-treated at 48 hr and fixed at 54 hr mitoses were plentiful, as they were in subsequent samples. Thus, a dose as high as 200 r appears to induce only a moderate mitotic delay.

Chromatid-type aberrations: In the control cultures, chromatid aberrations occurred with a frequency of 0.014 aberrations/cell. All were of the single-break type (chromatid and isochromatid deletions). No significant increase in the frequency of chromatid aberrations occurred in the X-ray-treated samples (Table 1). The culture fixed at 54 hr after 200 r of X rays, the earliest sample with sufficient mitoses, plus those fixed at 72 hr were scored for aberrations. Although some increase appeared in some samples, there was no consistent increase in the frequency of chromatid aberrations in the irradiated samples. Since cells in the pre-DNA-synthesis period (G_1) are expected to yield virtually no chromatid aberrations, it is apparent that almost all of the cells which divide in the leucocyte cultures synthesize their DNA after X-ray treatment; i.e., that the cells capable of division in our leucocyte cultures are in the G_1 phase in the peripheral circulation. This observation constitutes independent confirmation of the observations with tritium-labeled thymidine and autoradiography.[15]

Abnormal chromosome number: When cells are prepared by the methods used in the present study, a few per cent are always found that have a chromosome number other than $2n = 46$. As shown in Table 1, however, their frequency does not depend on X-ray dose. In addition, such cells usually contain fewer than 46 chromosomes, suggesting that they result primarily from breakage of cells during cytological preparation.

Chromosome aberrations: The frequencies of chromosome aberrations in control and irradiated blood samples are given in Table 1. It is impossible to distinguish between isochromatid deletions of the "nonunion proximal and distal" type and simple chromosome deletions; we scored all such aberrations as the chromosome type. It is thus possible that the spontaneous chromosome-type aberration rate is as high as 0.23 per cent, but it seems likely that it is much lower. As is expected from their multi-hit kinetics, no rings, dicentrics, or exchanges were seen in control material. A typical control figure appears in Figure 1.

Many aberrations of all of the expected types[11] were seen in the irradiated material. Typical examples are shown in Figure 1. The aberration frequencies are given in Table 1. While deletions (of either the terminal or the interstitial type), rings, and dicentrics are readily scored, symmetrical exchanges are more difficult. In scoring our material no karyotype analyses were made to aid in the detection of this class of aberrations. Thus, only those exchanges that resulted in new chromosomes whose size or shape were obviously abnormal could be scored. Figure 1, *D* shows such a translocated chromosome. Obviously, since we detected only a small fraction of the symmetrical exchanges that actually occurred, this class of aberration is, at least in our data, a poor measure of radiation effect. The values in the "breaks" column in Table 1 were arrived at by adding the number of deletions (chromatid and chromosome) and twice the number of two-hit aberrations excluding the exchanges, which are certainly not determined accurately. Pooling of the data of the subjects at each dose was justified by analysis of the aberration data, which shows no significant difference between individuals.[17] Figure 2 shows the pooled

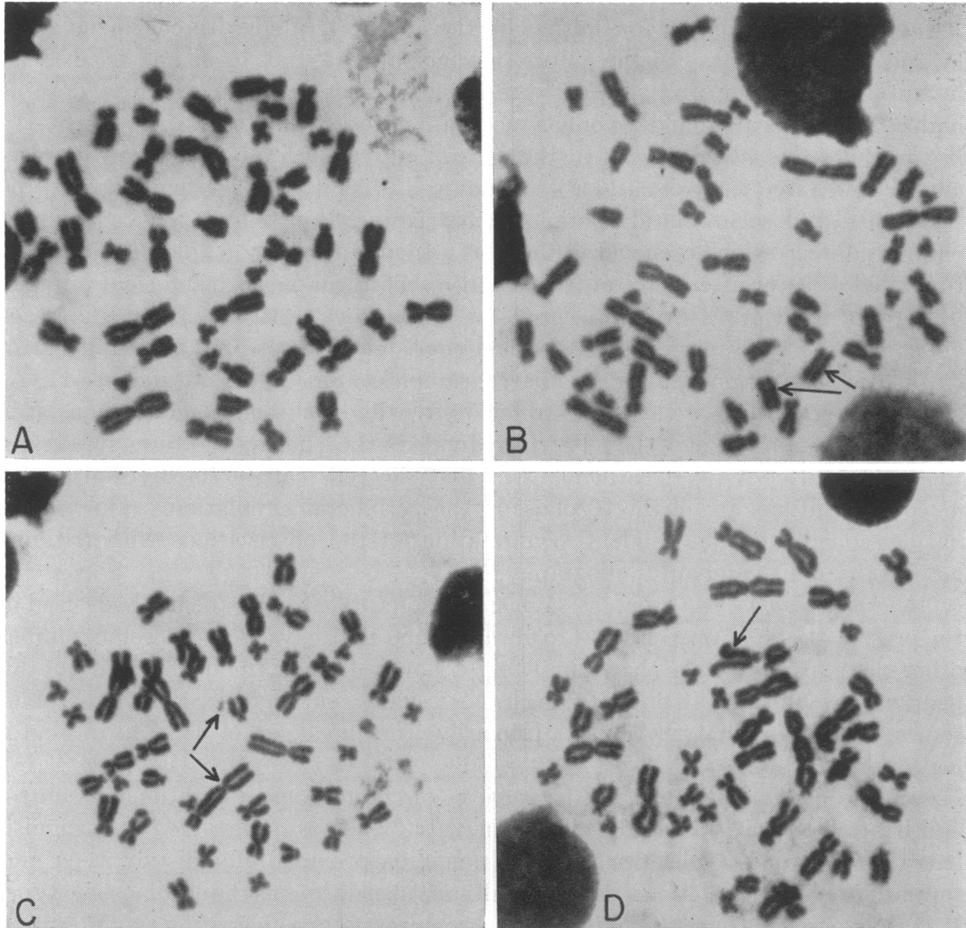

FIG. 1.—Metaphase figures from short-term human leukcocyte cultures. *A*, normal; *B*, chromosome deletions after 50 r; *C*, dicentric and fragment after 200 r; *D*, symmetrical chromosome exchange after 100 r. Arrows mark the aberrant chromosomes.

values for total breaks and rings plus dicentrics plotted graphically. The curve drawn for total breakage is the least-squares fit of the data to the expression

$$Y = a + bD + 2cD^2, \qquad (1)$$

where Y is the yield of breaks, a the control breakage frequency, D the dose of X rays, and b and c the coefficients of production for one- and two-break aberrations, respectively. The curve drawn for rings plus dicentrics is the least-squares fit of the data to the expression

$$Y = cD^2, \qquad (2)$$

the classical expression for two-hit aberration yield where their spontaneous frequency is zero. We have used this expression for simplicity, although the production of such aberrations is actually better described by a more complex expression.[18]

We have calculated coefficients of aberration production from our experimental

TABLE 1

ABERRATIONS IN HUMAN BLOOD IRRADIATED WITH X RAYS *in vitro*

Dose and individual	Cells scored	Cells with 2n ≠ 46	Chromatid aberrations	Chromosome-type Aberrations: Deletions	Rings	Dicentrics	Exchanges*	Breaks† (%)	Rings and dicentrics (%)
				(Analyzed as 72-hr cultures)					
Control									
1 (♂)	100	3	1	0	0	0	0	1.0	0.0
2 (♀)	125	4	2	0	0	0	0	1.6	0.0
3 (♀)	105	2	2	0	0	0	0	1.9	0.0
4 (♂)	100	6	1	1 (?)	0	0	0	2.0	0.0
Total	430	..	..	..	..	..	..	1.6	0.0
50 r									
1	100	3	0	9	0	2	0	13.0	2.0
2	100	2	1	5	0	3	0	12.0	3.0
3	100	6	0	3	0	3	0	9.0	3.0
Total	300	..	..	..	..	..	..	11.3	2.7
100 r									
1	200	8	5	27	1	14	4	31.0	7.5
2	102	4	3	11	1	6	0	27.5	6.8
3	100	5	5	6	0	4	4	19.0	4.0
Total	402	..	..	..	..	..	..	26.9	6.1
200 r									
1	100	6	2	18	1	15	2	52.0	16.0
2	100	5	4	20	3	15	7	60.0	18.0
3	100	4	1	19	3	21	0	68.0	24.0
4	100	3	2	22	5	15	5	64.0	20.0
Total	400	..	..	..	..	..	..	61.0	19.5
				(Analyzed as 54-hr culture)					
200 r									
4	100	2	3	18	6	24	2	81.0	30.0

* Symmetrical exchanges (see text).
† Including chromatid type; calculated as the number of chromatid breaks plus the number of deletions, plus twice the numbers of rings and dicentrics. The exchanges are not included because of the unreliability of scoring this class.

data for comparison with the earlier data on *Tradescantia* microspores. Table 2 gives this comparison. The agreement is good. The values for chromosome deletions are significantly different, but this is probably due to the inclusion of interstitial deletions with the terminal deletions in our data. Although such a measure is of value only at low doses, we have also calculated a total breakage rate for comparison with the earlier data for chromatid aberrations in human cells from tissue cultures. The present data yield a value of 0.0024 breaks/cell/r, which is remarkably close to the value of 0.003 breaks/cell/r found earlier for human epithelioid cells *in vitro*.[8]

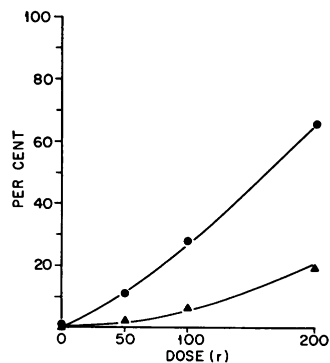

FIG. 2.—Chromosome breakage in irradiated human blood cultured for 72 hr. ●, pooled total breakage values; $Y = 0.0023 + 0.002D + 0.7 \times 10^{-5} D^2$. ▲, pooled total rings plus dicentrics; $Y = 0.52 \times 10^{-5} D^2$. The curves drawn are least-squares fits of the data to the indicated models.

Since the easiest and most reliable aberrations to score are rings and dicentrics, the coefficient of production of these aberrations serves as a useful "calibration" for their use in dose estimation in irradiated humans. The coefficient of ring and dicentric production from the present data (and used for Fig. 2) is $0.52 \pm 0.07 \times 10^{-5}$ rings and dicentrics/cell/r^2.

Discussion.—(1) *Aberration frequencies:* One object of our studies of human chromosomal aberrations is, of course, to obtain a measure of human radiosensi-

TABLE 2

COEFFICIENTS OF CHROMOSOME ABERRATION PRODUCTION

Material	Deletions per cell per r $10^{-2} \times$	Dicentrics per cell per r^2 $10^{-5} \times$
Tradescantia microspores*	0.06 ± 0.01	0.52 ± 0.08
Human leucocytes	0.11 ± 0.012	0.45 ± 0.07

* From Lea,[11] p. 241, Table 64.

tivity. Although it is possible to obtain information directly from experimental animals given whole-body irradiation, such a procedure is obviously impossible in humans. Thus our previous experiments have been limited to human cells grown and irradiated *in vitro*. Although it may be reasoned by analogy with animal experiments that human chromosomes *in vitro* behave, under certain conditions, the same as human chromosomes *in vivo*,[8] a good deal of confusion has arisen over the *in vitro* experiments that have been reported. Our studies of metaphase chromosome aberrations in human-tissue cultures[7, 8] have since been repeated by several workers.[9, 10] In addition, anaphase aberrations have been studied by Fraccaro,[19] Lindsten,[20] Sax and Passano,[21] and Dubinin *et al.*[22] Not only do the reported results differ, but there has been an even greater difference in the way the results were interpered.

(a) *Spontaneous aberrations:* The spontaneous aberration rates found by various workers range from 0.004 to 0.216 breaks/cell for metaphase scoring and from 0.006 to about 0.2 breaks/cell for post-metaphase scoring. These results cannot be said to be in conflict, however, since different types of cells which were grown under different conditions were used. The many different cells grown in primary cultures can generally be classified into two groups: epithelioid cells and "fibroblasts," or fibroblast-like cells. These descriptive terms are based on cell morphology and behavior, and are not completely clear-cut. Under some conditions, in fact, cells of one type can be made to look temporarily like cells of the other, although they always revert when the original conditions are restored. Nevertheless, real differences can be demonstrated between these two cell types. These differences appear to be a reflection of the selective influences of the conditions under which the original cultures were made (*not* of the tissue of origin). In particular, cells from cultures grown in the medium used by Puck,[9] which yields "fibroblast" cultures, seem to have extremely high spontaneous aberration rates.[9, 10, 21] Also, Sax and Passano[21] have shown that the length of time the cells have been in culture has an important influence on the spontaneous aberration frequency. In their "fibroblast" material the frequency of post-metaphase aberrations increased from 3.6 to 20.1 per cent over a period of about 4$^1/_2$ months. This kind of change was not a problem with the epithelioid cells, which is our experiments were used shortly after the cells were put in culture (during the second to the fourth passage).

The present study, which is as close to a controlled *in vivo* experiment as it is presently possible to make with human material, shows clearly that the spontaneous chromosome aberration frequency in human leucocytes *in vivo* is very low (not over 0.0023 breaks/cell, and probably much lower). *In vivo* experiments with bone-marrow cells from monkeys[8] and Chinese hamsters[13] also show that the spontaneous chromosome aberration frequency is extremely low. It is impossible to draw any conclusion about the frequency of spontaneous chromatid aberrations *in vivo* from

the present experiments, since the cells scored were virtually all in the pre-DNA-synthesis phase when the blood was drawn,[15] and hence presumably were not capable of chromatid breakage *in vivo*. Those chromatid aberrations seen must have occurred in culture. Our earlier studies with monkey and hamster bone marrow suggest that the spontaneous chromatid aberration rate must be very low *in vivo*.

(b) *Induced aberrations:* The X-ray-induced chromatid aberration rates, reported for human cells grown and irradiated *in vitro* and scored at metaphase, range from 0.0032 to about 0.02 breaks/cell/r. Post-metaphase aberration rates of 0.018 aberrations/cell/r[21] and 0.0024 aberrations/cell/r[22] have been reported. Presumably, these were mainly chromatid-type aberrations. Part of the difference in the rates reported can be attributed to differences in scoring,[12] but even when this is taken into account, a large difference remains. This difference parallels that seen for spontaneous aberrations, in that epithelioid cells have the lowest rates[7, 8] and "fibroblasts" have the highest,[9] while a report on pooled data from experiments with both cell types[10] gives an intermediate value.

Only one study of X-ray-induced chromosome aberration rates for human cells *in vitro* has been reported.[10] Unfortunately, it is impossible to obtain absolute breakage rates from such material. The reason is that the cultures, when irradiated, contain a mixture of cells susceptible to chromatid breakage (with effectively doubled chromosomes) and cells susceptible only to chromosome breakage (with chromosomes still effectively single). Since the information given is insufficient to determine the ratio between these two classes, one can only calculate the chromosome breakage on the basis of the total number of cells scored, which must lead to an underestimate of the breakage rate. The pooled data of Chu *et al.* for epithelioid and "fibroblast" cells fixed 42 hr after irradiation (when 83.2 per cent of the aberrations were of the chromosome type) yield a minimum value of 0.009 breaks/cell/r. This value is roughly half their value for chromatid breakage at 25 hr (when only 4.2 per cent of the aberrations were of the chromosome type).

The present value of 0.0024 chromosome type breaks/cell/r for human leucocytes fixed 72 hr after irradiation is not a minimum estimate. *All* of the cells that were scored had effectively single chromosomes at the time of irradiation, as shown by our failure to induce chromatid aberrations. The material scored at 54 hr (the earliest possible time) yields a value of 0.0039 breaks/cell/r. Since this value is based on a single high dose point, however, there actually may be little decrease over the period up to 72 hr. It will be interesting to compare our results for chromosome breakage with similar data for chromatid breakage, to establish the relation between the sensitivity of pre- and post-doubling chromosomes in human leucocytes.

(2) *Comparison with other organisms:* There exists a large body of data on other material, particularly *Tradescantia*, which was worked on extensively by Sax and his co-workers.[23] It has been shown that chromatid aberrations behave in much the same way, both quantitatively and qualitatively, whether they occur in human epithelioid cells *in vitro* or in *Tradescantia* microspores.[12] This observation has been confirmed by Chu *et al.*[10] for their mixture of cell types *in vitro*. The present results also support the conclusion that there is little difference between the behavior of the chromosomes of normal human cells and those of *Tradescantia* microspores. The observed coefficients of aberration production are, in fact, strikingly similar. Qualitatively, all of the human cell data reported to date, including the present re-

sults (and all of the available data on other mammals), suggest that the kinetics of aberration production are the same as those observed in the classical materials; although none of the published mammalian cell samples are large enough to *prove* that the kinetics are the same, there is no reason to believe that they are not.

(3) *"Doubling dose":* The calculation of human "doubling doses" must be approached with considerable caution. Such calculations must be clearly related to the specific effect studied. Doubling doses have been calculated for chromosomal aberrations induced in human cells *in vitro*. The values obtained were 3.3 and 6 r (see refs. 7, 22). The present results suggest that even these estimates are too high, because the spontaneous aberration rates *in vivo* appear to be lower than those *in vitro*, whereas the induced rates are approximately the same. If at very low doses the aberration rate is considered to consist entirely of single-hit aberrations, because the contribution of two-hit aberrations is negligible, the coefficient of aberration production equals b in the expression

$$Y = a + bD + cD^2, \tag{3}$$

where a is the spontaneous rate, b the coefficient of one-hit aberration production, and c the coefficient of two-hit aberration production. The dose required to double the spontaneous rate is a/b or, from the values calculated for our data (see Fig. 2), $0.002/0.0023 = 0.87$ r. Even this estimate may be too high because, as previously pointed out, the single control aberration seen may well have been a chromatid type. Thus the doubling dose for *somatic chromosome breakage* in man may well be considerably less than 1 r.

(4) *Leucocyte chromosome analysis as a "biological dosimeter":* A method for obtaining an estimate of average body exposure within a few days after acute exposure to an unknown dose of radiation would be extremely useful. Aberration analysis of the chromosomes of peripheral leucocytes appears to be a practical method. Because the leucocytes circulate rapidly in the body, a sample taken after exposure should reflect the average exposure. Since the leucocytes that divide in short-term cultures synthesize DNA very slowly, if at all, in the circulation, the blood sample need not be taken immediately after exposure. The blood sample required is small (5 ml is adequate), so there is no obvious reason why it should not be obtained, even from heavily irradiated persons. The culture method is simple and requires a minimum of equipment, and the 2- or 3-day growth period required should not ordinarily be a drawback. Rings and dicentrics can be scored easily, even by those with a minimum of training. The time required to score an adequate sample of cells (perhaps 100), while appreciable, is not excessive. The technique should be effective over a wide range of doses, including those most important from the clinical point of view.

It seems likely that the coefficient of ring and dicentric production by hard X rays (0.52×10^{-5} rings and dicentrics/cell/r^2) would also apply to blood irradiated with hard X- or γ-rays while still in the body. Thus, the dose estimate may be calculated from the yield of rings and dicentrics using the expression

$$D = \sqrt{\frac{Y}{0.52 \times 10^{-5}}}, \tag{4}$$

where D is the dose in roentgens and Y the observed yield of rings and dicentrics per cell.

The coefficients of aberration production by neutron irradiation of human leucocytes are not known. For *Tradescantia* microspores, the coefficient of asymmetrical exchange (dicentric) production is a linear function of dose, with a value of $0.32 \pm 0.02 \times 10^{-2}$ dicentrics/cell/v unit for Li-D neutrons.[11] There is little reason to assume that the value for human leucocytes is very different, in view of the close correspondence of the values for X rays. In any case, measurements of aberration coefficients for neutron irradiation of human leucocytes will be available in the near future. In cases of exposure to mixed γ-rays and neutrons, as in reactor or bomb irradiations, the approximate ratio between the two types usually is either known or may be calculated. With the leucocyte method, the total dose to an individual exposed to such a mixture might be estimated from the yield of dicentrics using the expression

$$Y = \alpha\, aD + (1 - \alpha)\, bD^2, \tag{5}$$

which may be solved for D, $D = -\alpha\, a + \sqrt{(\alpha a)^2 + 4bY(1 - \alpha)}/2b\ (1 - \alpha)$, and the coefficients of aberration production inserted, to give

$$D = \frac{-\,0.0032\,\alpha + \sqrt{(0.0032\,\alpha)^2 + 0.000018\,Y\,(1 - \alpha)}}{0.000009\,(1 - \alpha)}, \tag{6}$$

where D is the total dose in roentgens plus roentgen equivalents, of which α is the fraction of the total dose caused by neutrons, and Y is the observed dicentric yield.

The "biological dosimeter" outlined above obviously suffers from a lack of "calibration checks." Measurements with neutrons and other radiations, and also data obtained from accidentally irradiated people and experimentally irradiated animals is needed. Nevertheless, use of the technique seems justified, if only to serve as a check on the other available methods of dose estimation. When more accurate information about coefficients of aberration production are available the leucocyte technique should become an accurate and reliable means of dose estimation.

Summary.—Metaphase aberrations were scored in first *in vitro* divisions of leucocytes cultured from human blood irradiated with hard X rays immediately after it was drawn. Only chromosome-type, as opposed to chromatid-type, aberrations were found in the irradiated material. Control cells yielded a maximum of 0.0023 chromosome-type breaks/cell. Deletions, rings, dicentrics, and symmetrical translocations were induced in the irradiated chromosomes. Deletions increased linearly with dose with a coefficient of production of $0.11 \pm 0.012 \times 10^{-2}$ deletions/cell/r. Dicentrics increased roughly as the square of the dose with a coefficient of production of 0.45×10^{-5} dicentrics/cell/r^2. These coefficients agree well with those for *Tradescantia* microspores. The results of our studies on irradiated blood, which approximate *in vivo* irradiations as closely as is possible at present, are discussed in relation to previous work on aberrations in human tissue culture cells. The possible use of chromosome aberration analysis of peripheral leucocytes as a "biological dosimeter" is described.

* Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

[1] Tough, I. M., K. E. Buckton, A. G. Baikie, and W. M. Court Brown, *Lancet*, **ii**, 849–851 (1960).

[2] Bender, M. A, and P. C. Gooch, *Radiation Research*, **16**, 44–53 (1962).

[3] Boyd, E., W. W. Buchanan, and B. Lennox, *Lancet*, **i**, 977–978 (1961).

[4] Stewart, J. S. S., and A. Sanderson, *Lancet*, **i**, 978–979 (1961).

[5] Conen, P. E., *Lancet*, **ii**, 47 (1961).

[6] Herschberg, S. N., *Lancet*, **ii**, 104 (1961).

[7] Bender, M. A, *Science*, **126**, 974–975 (1957).

[8] Bender, M. A, in *Immediate and Low Level Effects of Ionizing Radiations*, ed. A. A. Buzzati-Traverso (London: Taylor and Francis Ltd., 1960), pp. 103–118.

[9] Puck, T. T., these PROCEEDINGS, **44**, 772–780 (1958).

[10] Chu, E. H. Y., N. H. Giles, and K. Passano, these PROCEEDINGS, **47**, 830–839 (1961).

[11] Lea, D. E., *Actions of Radiations on Living Cells*, 2d ed. (Cambridge: Cambridge University Press, 1955).

[12] Bender, M. A, and S. Wolff, *Am. Naturalist*, **45**, 39–52 (1961).

[13] Bender, M. A, and P. C. Gooch, *Int. J. Radiat. Biol.*, **4**, 175–184 (1961).

[14] Moorhead, P. S., P. C. Nowell, W. J. Mellman, D. M. Battips, and D. A. Hungerford, *Exp. Cell Research*, **20**, 613–616 (1960).

[15] Bender, M. A, and D. M. Prescott, "DNA synthesis and mitosis in cultures of human peripheral leucocytes," *Exp. Cell Research*, in press (1961).

[16] Denver Classification. *Lancet*, **i**, 1063–1065 (1960).

[17] We are indebted to Dr. M. A. Kastenbaum of the Oak Ridge National Laboratory Mathematics Panel for these calculations.

[18] Wolff, S. personal communication (1961).

[19] Fraccaro, M., Discussion of ref. 8, p. 117 (1960).

[20] Lindsten, J., *Uppsala Läkfören. Förh.*, **64**, 8–9 (1959).

[21] Sax, H. J., and K. Passano, *Am. Naturalist*, **45**, 97–102 (1961).

[22] Dubinin, N. P., M. A. Arsen'eva, and Yu. Ya. Kerkis, "Geneticheskaya Opasnost Malykh Doz Radiatsii dlya Cheloveka i ikh Effekt na Nasledstevennost Obezyan i Gryzunov," *Akad. Nauk S.S.S.R. (Moskva)* (1960).

[23] Giles, N. H., "Radiation-induced chromosome aberrations in *Tradescantia*," in *Radiation Biology*, ed. A. Hollaender (New York: McGraw-Hill Book Co., Inc., 1954).

REVERSAL OF MUTANT PHENOTYPES BY 5-FLUOROURACIL: AN APPROACH TO NUCLEOTIDE SEQUENCES IN MESSENGER-RNA

BY SEWELL P. CHAMPE AND SEYMOUR BENZER

DEPARTMENT OF BIOLOGICAL SCIENCES, PURDUE UNIVERSITY

Communicated February 27, 1962

Genetic information in DNA is apparently expressed via transcription into RNA messengers[1–5, 10] which in turn act as the templates for protein synthesis. Thus, incorporation of base analogues into messenger-RNA could lead to errors in the reading of the message into an amino acid sequence. The effect would be an alteration of phenotype without a permanent change in the DNA genotype. A promising analogue for this purpose is 5-fluorouracil (5FU), which is readily incorporated into RNA, mostly in place of uracil.[6] Modification of proteins by 5FU has been reported by Naono and Gros[7] and by Bussard *et al.*[8] who found that the enzymes alkaline phosphatase and β-galactosidase synthesized in the presence of the analogue are abnormal.

A very sensitive method for the detection of induced errors in the translation of